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Advances in Animal Biosciences

Proceedings of the XIIIth International Symposium on Ruminant Physiology (ISRP 2019), 3–6 September 2019, Leipzig, Germany

advances in animal biosciences

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Advances in Animal Biosciences

Aims and Scope

Advances in Animal Biosciences is an associated publication to the journal *animal*. It aims to publish high-quality conference, symposium and workshop proceedings about animal-related aspects of the life sciences with emphasis on farmed and other managed animals. These can be in the form of a book of abstracts, summaries or complete papers. The format will highlight the title of the meeting and organisations involved but the publications will have the added advantage of forming a series under *Advances in Animal Biosciences.*

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However, due to the integrative nature of biological systems, monographs and conference proceedings dealing with the translation of basic and strategic science into the whole animal and farming system and the impact on Productivity, Product Quality, Food Security, the Environment, Climate Change and Humans will be particularly welcome.

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The Proceedings of the XIIIth International Symposium on Ruminant Physiology constitute summaries of papers presented at the ISRP congress 2019 held at the KONGRESSHALLE am Zoo Leipzig, Germany, 3-6 September 2019.

The summaries have been edited. Views expressed in all contributions are those of the authors and not those of the organisers of the ISRP 2019.

This publication contains all the summaries that were available at the time of going to press.

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Keynote lectures

Reproduction, lactation and growth

Impact of protein and energy supply on the fate of amino acids from absorption to milk protein in dairy cows

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Making dairy farming more cost effective and reducing nitrogen environmental pollution could be reached through a reduced input of dietary protein, provided productivity is not compromised. This could be achieved through balancing dairy rations for essential amino acids (AA) rather than their aggregate, the metabolizable protein (MP). This review revisits the estimations of the major true protein secretions in dairy cows, milk protein yield (MPY), metabolic fecal protein (MFP), endogenous urinary loss and scurf, as well as associated AA composition. The combined efficiency with which MP (Eff_{MP}) and EAA (Eff_{AA}) are used to cover the estimated protein secretions is calculated as the sum of true protein secretions (MPY + MFP + scurf) divided by the net supply (adjusted to remove the endogenous urinary excretion: MP_{adj} and AA_{adj}). Using the proposed protein and AA secretions, Eff_{MP} and Eff_{AA} were estimated through meta-analyses (807 treatment means) and validated using an independent database (129 treatment means). The effects of MP_{adj} or AA_{adj}, plus digestible energy intake (DEI), days in milk (DIM) and parity (primiparous vs. multiparous) were significant. Models using [MP_{adj}, MP_{adj}×MP_{adj} and DEI] or [MP_{adj}/DEI and MP_{adj}/DEI× MP_{adj}/DEI] were similar but the model using MP_{adj}/DEI gave a better fit in the validation database. This model was therefore used to derive equations to predict Eff_{AA}. These equations estimated well Eff_{AA} in the validation database except for Arg which had a strong slope bias. Using the predicted Eff_{MP} (based on MP_{adj}/DEI, MP_{adj}/DEI×MP_{adj}/DEI, DIM and parity) yielded a better prediction of MPY than a direct prediction (based on $MP_{adj,}$ MP_{adj}×$MP_{adj,}$ DEI, DIM and parity). Predictions of MPY based on each Eff_{AA} yielded similar results among AA, likely because of the limited number of studies where the supply of a single AA varied. It is proposed to ponder the mean of MPY predictions obtained from each Eff_{AA} by the lowest prediction to retain potential limitation of the AA with the shortest supply. Overall, the new estimations of endogenous urinary and MFP, revised AA composition of protein secretions and inclusion of a variable combined Eff_{AA} (based on AA_{adj}/DEI, AA_{adj}/DEI×A_{adj}/DEI, DIM and parity) offer the potential to improve predictions of MPY, identify which AA are potentially in short supply and, therefore improve the AA balance of dairy rations.

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Reproduction, lactation and growth

Environmental Impact on early embryonic development in the bovine species

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Assisted Reproduction Techniques (ARTs) provide access to early stage embryos whose analysis and assessment deliver valuable information. The handling of embryos, including the *in vitro* production of bovine embryos, is a rapidly evolving area which nonetheless exposes the embryos to unnatural conditions for a period of time. The Fallopian tube provides innumerable quantitative and qualitative factors, all of which guarantee the successful development of the embryo. It is well known that the Fallopian tube can be bypassed, using embryo transfer, resulting in successful implantation in the target recipient animal and the birth of calves. However, the question arises as to whether such circumvention has a negative impact on the embryo during this sensitive development period. First crosstalk between the embryo and its environment confirms mutual recognition activities and indicate bilateral effects. Nowadays, *in vitro* production of bovine embryos is a well-established technology. However, it is still evident that *in vitro* generated embryos are not qualitatively comparable to embryos obtained ex vivo. To counteract these differences, comparative studies between *in vitro* and ex vivo embryos are advantageous, as embryos grown in their physiological environment can provide a blueprint or gold standard against which to compare embryos produced *in vitro*. Attempts to harness the bovine oviduct were sometimes very invasive and did not result in wide acceptance and routine use. Long-term development and refinement of transvaginal endoscopy for accessing the bovine oviduct has meanwhile been routinely applied for research as well as in practice. Comparative studies combining *in vitro* development with development in the cattle oviduct revealed that the environmental conditions to which the embryo is exposed before activation of the embryonic genome can have detrimental and lasting effects on its further development. These effects are manifested as deviations in gene expression profiles and methylation signatures as well as frequency of whole chromosomal or segmental aberrations. Furthermore, it was shown that hormonal superstimulation (MOET), varying progesterone concentrations as well as metabolic disorders caused by high milk production, markedly affected embryo development in the postpartum period. Assisted reproductive techniques that allow the production and handling of extra numbers of generated embryos promise to have a very high impact on scientific and practical application. Any influence on the early embryonic life, both in animals and *in vitro*, is accompanied by a sensitive change in embryonic activity and should be assessed *in vivo* on the basis of physiological conditions before being used for ART.

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Absorption and Secretion

Regulation of gastrointestinal and renal transport of calcium and phosphorus in ruminants

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In comparison to monogastric animals, ruminants show some peculiarities in respect to the regulation of mineral homeostasis which can be regarded as a concerted interplay between gastrointestinal absorption, renal excretion and bone mobilisation to maintain physiological Ca and phosphate (P_i) serum concentrations. The intestinal absorption of Ca or P_i is mediated by two general mechanisms: paracellular, passive transport is in the front when luminal Ca or P_i concentrations are high and transcellular, active transport takes over when dietary Ca or P_i supply is restricted or the demand increased. Both pathways are modulated directly by dietary interventions, influenced by age and regulated by different endocrine factors. Similar transport processes are observed in the kidney. After filtration, Ca and P_i are resorbed along the nephron. However, as urinary Ca and P_i excretion is very low in ruminants the regulation of these renal pathways differs from that described for monogastric species, too. Furthermore, salivary secretion, as part of the efficient endogenous P_i recycling, and bone mobilisation participate in the maintenance of Ca and P_i homeostasis in ruminants. Saliva contains large amounts of P_i for buffering rumen pH and to ensure optimal conditions for the rumen microbiome. The skeleton is a major reservoir of Ca and P_i to compensate for discrepancies between demand and uptake. But alterations of the regulation of mineral homeostasis induced by other dietary factors like a low protein diet were observed in young ruminants. In addition, metabolic changes for example at the onset of lactation have pronounced effects on gastrointestinal mineral transport processes in some ruminant species. As disturbances of mineral homeostasis do not only increase the risk of the animals to develop other diseases, but are also associated with protein and energy metabolism, further research is needed to improve our knowledge of its complex regulation.

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Absorption and Secretion

Short-term and long term adaptation of SCFA absorption from the rumen

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Short-chain fatty acid (SCFA) absorption across the ruminal epithelium is critical for energy balance of the animal and for maintaining intraruminal pH. Adaptation for SCFA absorption occurs acutely for proliferative and retrogressive responses. For example, marked reductions for the rates of SCFA absorption occur within 48 h of feed deprivation and when exposed to 5 d of low-feed intake. On the other hand, exposure of lambs and calves to an abrupt increase in dietary fermentability increases SCFA uptake into the epithelium and net flux of SCFA across the epithelium. The increases in uptake and flux occur within 1 week of the dietary change and prior to the detection of changes in absorptive surface area. As increases in uptake and flux can be measured ex vivo, they must occur independently or additionally to changes in blood flow and represent direct adaptation of the epithelia. Interestingly, of the pathways for SCFA absorption, passive diffusion appears to be acutely responsive promoting SCFA flux during proliferative adaptation and the increases in passive diffusion support previously reported increases in Na uptake and greater sodium-hydrogen exchanger expression. Adaptation to allow for greater passive diffusion must be mediated via changes in the permeability of the apical membrane. Supporting the concept that permeability of the epithelium may limit apical uptake of SCFA, altering dietary fatty acid supply and composition to favor greater dietary fat concentration and greater proportions of long-chain saturated fatty acids relative to long-chain polyunsaturated fatty acids increases passive apical uptake of propionate and tends to increase passive apical uptake of butyrate. Dietary composition such as greater dietary fermentability may promote longer-term adaptation through increases in absorptive surface area, greater blood flow, and by altering pathways for SCFA absorption. For example, increasing the dietary sugar concentration does not appear to have a major effect on rates of SCFA absorption, but increases bicarbonate-dependent SCFA flux. Transition from a hay diet to a highly fermentable diet stimulates both SCFA absorption and bicarbonate secretion. However, if highly fermentable diets induce ruminal acidosis, SCFA absorption is rapidly reduced with recovery occurring within ~1 week depending on severity. Thus, the ruminal epithelium adapts to modulate SCFA absorption using both short-term and long-term mechanisms.

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Ruminant intermediary metabolism

Relationships between metabolism and innate immune function in dairy cows in the peripartum period

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Aspects of neutrophil function are diminished or dysregulated in dairy cows in the weeks just before and after calving, which appears to be an important contributor to the occurrence of retained placenta, mastitis, metritis, and endometritis. The timing and mechanisms by which specific elements of neutrophil function are impaired are only partially understood. Oxidative burst capacity is the element of neutrophil function most consistently shown to be impaired in the week after calving, but that observation may partially be biased by it being the most-studied functional step. There is sufficient evidence to conclude that the availability of calcium and glucose, and exposure to elevated concentrations of non-esterified fatty acids or beta-hydroxybutyrate affect some aspects of neutrophil function. However, these factors have mostly been studied in isolation and their effects are not consistent. Social stressors such as a competitive environment for feeding or lying space should plausibly impair innate immune function, but when studied under controlled conditions such effects have generally not been produced. Similarly, treatment with recombinant bovine granulocyte colony-stimulating factor consistently produces large increases in circulating neutrophil count with modest improvements in function, but this does not consistently reduce the incidence of clinical diseases thought to be importantly attributable to impaired innate immunity. Research is now needed that considers the interactions among known and putative risk factors for impaired neutrophil function in dairy cows in the transition period.

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Gastrointestinal Digestion: Mechanisms and Regulation

Fifty years of research on inhibition of rumen methanogenesis: lessons learned and future challenges

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Meat and milk from ruminants provide an important source of protein and other nutrients for human consumption. Although ruminants have a unique advantage of consuming forages and grazing lands not suitable for arable cropping, 2 to 12% of the gross energy consumed is converted to enteric CH_4 during ruminal digestion. This loss contributes approximately 6% of global anthropogenic greenhouse gas emissions. Thus, the ruminant industries need to find cost-effective ways to reduce emissions while meeting consumer demand for food. This paper provides a critical review of the substantial amount of ruminant CH_4 research published in past decades, highlighting hydrogen flow in the rumen, the microbiome related to methanogenesis, current and future prospects for CH_4 mitigation, and insights into future challenges for science, governments and the livestock industries. Methane emission intensity, measured as emissions per unit of meat and milk, has continuously declined over the past decades due to improvements in production efficiency and animal performance, and this trend is expected to continue. However, continued decline in emission intensity will be insufficient to offset the rising emissions from increasing demand for animal protein. Thus, decreases in both emission intensity (g CH_4/animal product) and absolute emissions (g CH_4/day) are needed. Providing livestock producers with cost-effective options for decreasing CH_4 emissions is therefore imperative, yet few cost-effective approaches are currently available. Future abatement may be achieved through animal genetics, early life programming, dietary formulation, use of alternative hydrogen sinks, chemical inhibitors and fermentation modifiers, and vaccine development. However, these strategies alone are expected to have only moderate effects (< 20% reduction), with the exception of the experimental inhibitor 3-nitrooxypropanol for which responses have been greater (20 to 40% consistent decreases). If approved by the regulatory authorities, this inhibitor (and potentially others in the future) offers substantial potential for CH_4 mitigation, although many issues such as optimum dose for different diets and ways of supplementing it to grazing animals still need to be resolved. Further research is needed to determine whether combining anti-methanogenic strategies will have additive effects, as combining strategies may help attain the sizable decreases in CH_4 needed. It is also not clear whether CH_4 decrease leads to improvements in animal performance, which will be necessary for adoption by producers unless governments impose or subsidize CH_4 mitigation targets. A major constraint for decreasing global enteric CH_4 emissions is the difficulty of applying mitigation strategies to grazing ruminants, a challenge that also needs further investigation.

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Gastrointestinal Digestion: Mechanisms and Regulation

Nutritional Regulation of Intestinal Starch and Protein Assimilation

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Pregastric fermentation along with production practices that are dependent on high energy diets means ruminants rely heavily on starch and protein assimilation for a substantial portion of their nutrient needs. While the majority of dietary starch may be fermented in the rumen, significant portions can flow to the small intestine. The initial phase of small intestinal digestion requires pancreatic α-amylase. Numerous nutritional factors have been shown to influence pancreatic α-amylase secretion with starch producing negative effects and casein, certain amino acids, and dietary energy having positive effects. To date, manipulation of α-amylase secretion has not resulted in substantial changes in digestibility. The second phase of digestion involves the actions of the brush border enzymes sucrase-isomaltase and maltase-glucoamylase. Genetically, ruminants appear to possess these enzymes; however, the absence of measurable sucrase activity and limited adaptation with changes in diet suggests a reduced capacity for this phase of digestion. The final phase of carbohydrate assimilation is glucose transport. Ruminants possess Na^+-dependent glucose transport (SGLT1) that has been shown to be inducible. Because of the nature of pregastric fermentation, ruminants see a near constant flow of microbial protein to the small intestine. This results in a nutrient supply which places a high priority on protein digestion and utilization. Comparatively, little research has been conducted describing protein assimilation. Enzymes and processes appear consistent with non-ruminants and are likely not limiting for efficient digestion of most feedstuffs. The mechanisms regulating the nutritional modulation of digestive function in the small intestine is complex and coordinated via the substrate, neural, and hormonal effects in the small intestine, pancreas, peripheral tissues, and the pituitary-hypothalamic axis. More research is needed in ruminants to help unravel the complexities by which small intestinal digestion is regulated with the aim of developing approaches to enhance and improve the efficiency of small intestinal digestion.

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Biosensors and Big data management

Rumen sensors: data and interpretation for key rumen metabolic processes

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Rumen sensors provide specific information to help understand rumen functioning in relation to health disorders and to assist in decision making for farm management. This review focuses on the use of rumen sensors to measure ruminal pH, and discusses variation in pH in both time and location, pH associated disorders, and data analysis methods to summarize and interpret rumen pH data. Acids may accumulate and reduce ruminal pH if acid removal from the rumen and rumen buffering cannot keep pace with their production. The complexity of the factors involved combined with the interactions between the rumen and the host that ultimately determine ruminal pH, result in large variation among animals in their pH response to dietary or other changes. Although ruminal pH and pH dynamics only partially explain the typical symptoms of acidosis, it remains a main indicator and may assist to optimize rumen function. Rumen pH sensors allow continuous monitoring of pH and of diurnal variation in pH in individual animals. Substantial drift of non-retrievable rumen pH sensors, and the difficulty to calibrate these sensors, limit their application. Significant within day variation in ruminal pH is frequently observed, and large distinct differences in pH between locations in the rumen occur. The magnitude of pH differences between locations appears to be diet dependent. Universal application of fixed conversion factors to correct for absolute pH differences between locations should be avoided. Rumen sensors provide high-resolution kinetics of pH and a vast amount of data. Commonly reported pH characteristics include mean and minimum pH, but these do not properly reflect severity of pH depression. The area under the pH × time curve integrates both duration and extent of pH depression. Use of this characteristic, as well as summarizing parameters obtained from fitting equations to cumulative pH data, is recommended to identify pH variation in relation to acidosis. Some rumen sensors can also measure the redox potential. This measurement helps to understand rumen functioning, as the redox potential of rumen fluid directly reflects the microbial intracellular redox balance status and impacts fermentative activity of rumen microorganisms. Taken together, proper assessment and interpretation of data generated by rumen sensors requires consideration of their limitations.

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Biosensors and Big data management

Sensor techniques in ruminants: More than fitness trackers!?

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The estimated global value of animal biosensor technologies is expected to exceed 20B$ by 2020. That statement might make your heart rate increase and, if it did, a sensor worn on your wrist as part of a smart watch (for instance), could detect that. In the future, heart rate sensors might be incorporated into animal ear tags along with the electronic id and activity sensor (accelerometer) that can already be located there. In this review we will consider the history of animal sensor technologies with emphasis on dairy ruminants and on sensors that are worn, as opposed to those that are swallowed and function in the rumen, or those that are located “off-animal”, i.e. somewhere in the immediate or remote environment. Heart rate can, in theory at least, be measured by Doppler radar from a distance of several meters, and we shall include some speculation regarding possible future developments in animal biosensors, as well as consideration of how the industry might develop in order to best serve the needs of farmers, the broader livestock communities and consumers. From a practical point of view the history of worn sensors is relatively simple, based on the biological observation that cattle increase their activity during estrous. For the farmer, the ability to detect estrous has significant value, hence a number of commercial products have become available that track activity using an accelerometer and provide an alert signifying estrous. As one might imagine, the information coming from an accelerometer (which detects changing motion in three axes) is complex, requiring the development of algorithms to “tease-out” the number of steps or other activity feature. What was initially a complication has now become a virtue, and more advanced algorithms can deduce other features such as rumination, eating and posture (lying/standing) from the same data, especially if the accelerometer is combined with a gyroscope. A single “device” can incorporate several sensors, a notable example being temperature and activity in a rumen bolus, and appropriate biological knowledge can allow sensors to provide information about diverse physiological phenomena; drinking behavior from rumen temperature fluctuations and lameness from lying time, for instance. The next transition in sensor-based husbandry support will likely be from estrous detection to true health management, and is more likely to be constrained by economics than by the performance of the technology. Nevertheless, as dairy herds become ever larger, the role of technology is almost certain to expand such that 20B$ may soon prove to be an underestimate.

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Ruminants and Comparative Physiology

Comparative methane production in mammalian herbivores

Marcus Clauss[1], Marie Dittmann[1], Catharina Vendl[2], Katharina Hagen[1], Samuel Frei[1], Sylvia Ortmann[3], Dennis Müller[4], Sven Hammer[5], Adam Munn[2], Angela Schwarm[6], Michael Kreuzer[7]

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Methane (CH_4) production is a ubiquitous, apparently unavoidable side effect of fermentative fibre digestion by symbiotic microbiota in mammalian herbivores. We present a data compilation of *in vivo* CH_4 measurements in individuals of 37 mammalian herbivore species fed on forage-only diets from the literature and from hitherto unpublished measurements (resulting in a dataset of n=707, from which different subsets were used depending on the information available in addition to body mass and CH_4 emisson). In contrast to previous claims, absolute CH_4 emissions scaled linearly to dry matter intake and CH_4 yields (per dry matter or gross energy intake) did not vary significantly with body mass. CH_4 physiology hence cannot be construed to represent an intrinsic ruminant or herbivore body size limitation. The dataset does not support traditional dichotomies of CH_4 emission intensity between ruminants and nonruminants, or between foregut and hindgut fermenters. Several rodent hindgut fermenters and nonruminant foregut fermenters emit CH_4 of a magnitude as high as ruminants of similar size, intake level, digesta retention or gut capacity. For example, a 2 kg-rodent, the nutria (*Myocastor coypus*), and a 2 kg-ruminant, the dikdik (*Madoqua saltiana*) both produce 1.5 litres CH_4 per day. By contrast, equids, macropods (kangaroos) and rabbits produce less CH_4 and have low CH_4:CO_2 ratios for their size, intake level, digesta retention or gut capacity, ruling out these factors as explanation for interspecific variation. These findings lead to the conclusion that host-specific factors other than digesta retention characteristics, or the presence of rumination or a foregut, must be responsible for CH_4 physiology. Measurements of CH_4 yield per digested fibre indicate that the amount of CH_4 produced during fibre digestion varies not only across but also within species, possibly pointing towards variation in microbiota functionality. Recent findings on the genetic control of microbiome composition, including methanogens, raise the question about the benefits methanogens provide for many (but apparently not to the same extent for all) species that prevented natural selection for low-methanogenic microbiota across mammals.

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Ruminants and Comparative Physiology

Seasonal differences in the physiology of wild ruminants

Walter Arnold

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Ruminants living in seasonal environments face during winter a two-fold challenge. The energetic cost of maintaining a high body temperature is higher at lower ambient temperatures while food availability and quality is poor. We found in several species of wild ruminants that they acclimatize to the change of living conditions by reducing food intake, changing the digestive strategy, and switching to fat reserves as the major metabolic fuel. Metabolic rate, approximated by the continuous measurement of heart rate, is during winter remarkably reduced, down to more than half of the summer level. Contributions to lower energy expenditure come from lesser foraging activity, heat increment of feeding, and shrinking of organs. However, the most important determinant is reduced endogenous heat production and abandoning the maintenance of a high body temperature, particularly in peripheral body parts. Therefore, the transformation from the summer into the winter phenotype, visible in the change from a summer fur into a better insulating winter fur, is in fact a profound change of the whole physiology of the animal.

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Lifespan and Productivity

Overview of factors affecting productive lifespan of dairy cows

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The average productive lifespan is approximately 3 to 4 years in countries with high producing dairy cows. This is much shorter than the natural longevity of dairy cattle. Dairy farmers continue to cull cows primarily for reasons related to poor health, failure to conceive or confirmation problems prior to culling. These reasons may indicate reduced welfare leading up to culling. Improvements in health care, housing and nutrition will reduce forced culling related to these welfare reasons. However, productive lifespan has remained similar in decades, despite large improvements in cow comfort and genetic selection for the ability to avoid culling. On the other hand, genetic progress for economically important traits is accelerating within the last decade which should slightly shorten the average economically optimal productive lifespan. A major driver of productive lifespan is the availability of replacement heifers that force cows out when they calve. The average productive lifespan could be extended by reducing the supply of dairy heifers, which would also have benefits for environmental sustainability. Modeling shows that extending productive lifespan is economically advantageous if the main health and fertility related reasons for culling can be reduced. Improvements in culling decision support tools would strengthen economically optimal replacement decisions. In conclusion, major factors of the relatively short productive lifespan of dairy cows are welfare related, but other, economic factors like supply of heifers, genetic progress, and non-optimal decision making also play important roles.

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Lifespan and Productivity

Nutrient partitioning and the sphingolipid ceramide in dairy cattle: Considerations for optimum health and performance

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The physiological control of lactation through coordinated changes in nutrient partitioning and utilization is of critical importance for neonatal life. These homeorhetic adaptations involve reduced peripheral insulin sensitivity and responsiveness, and enhanced adipose tissue lipolysis to provide glucose and fatty acids for milk production. However, severe insulin antagonism and body fat mobilization may compromise liver health and lactation in dairy cattle. The mechanisms that limit insulin-stimulated glucose utilization in the early lactation cow are not entirely defined but appear to involve the sphingolipid ceramide. In non-ruminants, ceramide is a potent mediator of saturated fat-induced insulin resistance that defines aspects of obesity, type 2 diabetes and nonalcoholic fatty liver disease. In the ruminant cow, ceramide has emerged as an associative and causative antagonist of insulin signaling. Initially, ceramide accumulation was observed in periparturient cows with enhanced prepartum body condition. Subsequent work demonstrated that ceramides accumulate postpartum in plasma, liver, and skeletal muscle independent of adiposity phenotype. Research also demonstrated the ability of feed-restriction or intravenous triglyceride infusion to promote ceramide accrual. In bovine differentiated adipocytes, the inhibition of de novo ceramide synthesis activates insulin signaling and glucose uptake. Because ceramide may influence insulin action in the cow, dietary approaches that influence ceramide synthesis represent potential strategies to influence health and lactation. Repeated investigations have demonstrated the ability of dietary palmitic acid supplementation to stimulate ceramide synthesis in the cow, relative to other fatty acids (e.g., stearic acid) or no-added fat controls. In addition, the abomasal infusion of insulin-sensitizing docosahexaenoic acid suppresses circulating ceramide concentrations when compared to palmitic acid. The past five years have broadened our understanding of nutrient partitioning and sphingolipid biology in the dairy cow. Future work will need to define the influence of endocrine control on ceramide synthesis, define alternative roles for ceramide, and determine whether modulation of ceramide production is a means to influence productive lifespan in dairy cattle.

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Inflammation, immunology

Do inflammatory signals play a role in metabolic homeostasis and homeorhesis of dairy cattle?

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Inflammatory cascades are a critical component of the immune response to infection or tissue damage, involving an array of signals, including water-soluble metabolites, oxylipids, and several classes of proteins. Early investigation of these signaling pathways focused largely on immune cells and acute disease models. However, more recent findings have highlighted critical roles of both immune cells and inflammatory mediators on tissue remodeling and metabolic homeostasis in healthy animals. In dairy cattle, inflammatory signals in various tissues and in circulation change rapidly and dramatically at the onset of lactation. Furthermore, several observations point to homeostatic control of inflammatory tone in healthy cows, which may be a mechanism to keep downstream effects under control. Recent evidence suggests that peripartum inflammatory changes influence whole-body nutrient flux of dairy cows over the course of days and months. Inflammatory mediators can suppress appetite, even at levels that do not induce acute responses (e.g., fever), thereby decreasing nutrient availability. On the other hand, inhibition of inflammatory signaling with non-steroidal anti-inflammatory drug (NSAID) treatment suppresses hepatic gluconeogenesis, leading to hypoglycemia in some cases. Over the long-term, though, peripartum NSAID treatment substantially increases peak and whole-lactation milk synthesis by multiparous cows. Inflammatory regulation of nutrient flux may provide a homeorhetic mechanism to aid cows in adapting to the rapid changes in metabolic demand at the onset of lactation, but excessive systemic inflammation has negative effects on metabolic homeostasis through inhibition of appetite and promotion of immune cell activity. Overlapping regulation of immune responses and metabolism by inflammatory mediators, therefore, may provide a mechanistic underpinning for links between infectious and metabolic diseases in transition dairy cows, and points to novel approaches to management of this challenging phase of the production cycle.

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Global change and ruminants

Challenges for dairy cow production systems arising from climate changes in Europe

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The so called “gobal change” refers to changes on a planetary-scale. The term encompasses various issues like resource use, energy development, population growth, land use and cover, the carbon and nitrogen cycle, pollution and health, and the climate change. The paper deals with challenges for dairy cow production systems in Europe arising from global changes with a focus on climate change. Global warming is increasing and therefore ecosystems, plant and animal biodiversity, and food security and safety are at risk. The potential and already measurable effects of climate change will be discussed in the paper. In particular the challenges of global warming, on grassland production, fodder quality, and nutrition in general, cow welfare, health and performance as well as economy of dairy production will be reviewed. It is already commonly accepted knowledge that the direct and indirect effects of global warming in combination with an increasing frequency of weather extremes is an issue serious of livestock production, even in areas with temperate zones. Direct effects on health and welfare and performance for example are already proven by the change of temperature and humidity and the increased frequency of heat waves. Indirect effects arise from changes in the quality and quantity of feedstuff and water available, and the spread of new pathogens and vectors. The impact of the effects will correlate with the performance of the animals. There are clear indications that with selection for high yielding dairy cows the susceptibility to environmental challenges of these genotypes did increase. Cumulative effects (e.g. higher temperature plus increased no. of pathogens) do increase the challenge. To cope with the consequences of climate change several possible adaptation and mitigation strategies are needed. These include changes in production systems (including management, barn, feeding), breeding and health management.

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Ontogenesis of the newborn ruminant

Importance of colostrum supply and milk feeding intensity in gastrointestinal and systemic development in calves

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Feeding management of the postnatal and preweaning calf not only has an important impact on calf growth and development during this critical period but also affects the health and well-being of calves. After birth, a sufficient colostrum supply is a prerequisite for successful calf rearing. Colostrum provides high amounts of nutrient as well as non-nutrient factors that promote the immune system and intestinal maturation of the calf. The maturation and function of the neonatal intestine enables the calf to digest and absorb the nutrients provided by colostrum and milk. Therefore, colostrum and milk feeding supports the start of anabolic processes in several tissues, resulting in enhanced postnatal body growth and further organ development. Insufficient milk intake may delay postnatal growth and may have detrimental effects on organ development, e.g., the intestine and the mammary gland. The stimulation of the somatotropic axis as the main postnatal endocrine regulatory system for body growth and development indicates the promotion of anabolic metabolism in calves when high amounts of colostrum and milk are fed. The development of the forestomach is an important issue during the preweaning period in calves, and forestomach maturation is best achieved by solid feed intake. Unfortunately, intensive milk feeding starting immediately after birth compromises solid feed intake during the first weeks of life. In the more natural situation for beef calves, namely, when milk and solid feed intake occurs at the same time, calves may benefit from the high level of milk intake as evidenced by enhanced body growth and organ maturation without impaired forestomach development during weaning. To realize this feeding concept, it is recommended that the weaning process should not start too early and that solid feed intake should be at a high level despite intensive milk feeding during the preweaning period. A feeding concept based on intensive milk feeding also prevents hunger and abnormal behaviour of the calves and fits the principles of animal welfare during preweaning calf rearing. Studies on milk performance in dairy cows indicate that feeding management during early calf rearing influences lifetime performance. An enhanced growth rate during early life stimulates milk production during the production lifespan. Therefore, an intensive milk-feeding programme affects immediate as well as long-term performance, probably by programming metabolic pathways during the preweaning period.

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Regulation of feed and water intake

Control of feed intake by hepatic oxidation: integration of homeostasis and homeorhesis

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The control of feed intake is complex with mechanisms to ensure an adequate supply of nutrients to meet requirements as well as to prevent their overconsumption. These include short-term controls to maintain physiological equilibrium (homeostasis) as well as coordinated changes necessary to support alterations in physiological state (homeorhesis). Feed intake is a function of meal size and meal frequency, which are controlled by short-term mechanisms. However, certain elements of the short-term mechanism are likely modulated by long-term signals.

Whereas multiple signals are integrated in brain feeding centers to ultimately determine feed intake, the liver is likely a sensor of energy status integrating long and short-term controls. The signal from the liver may be both inhibitory (affecting satiety) and stimulatory (affecting hunger) and is related to hepatic oxidation of fuels. Signals from the liver are transmitted to brain feeding centers via vagal afferents. Feeding behavior is affected by the firing rate of the nerve; increased oxidation decreases the firing rate, inhibiting feeding, whereas decreased oxidation increases the firing rate, stimulating feeding. Fuels extracted from the blood by the liver can be converted to acetyl CoA and oxidized in the tricarboxylic acid (TCA) cycle. Hepatic oxidation of fuels varies over minutes to affect feeding behavior and is dependent upon the supply of acetyl CoA, the pool size of TCA intermediates (determined by the balance between anaplerosis and cataplerosis), and the speed at which TCA cycles spin (dependent upon enzyme concentration and activity).

Fuel supply to the liver is dependent upon absorption of fuels derived from the diet as well as those supplied to, or extracted from the blood by extra-hepatic tissues. Homeorhetic mechanisms affect concentrations of insulin, somatotropin, growth factors, and leptin as well as additional factors affecting insulin sensitivity of tissues, all of which interact with diet to affect fuel supply to the liver and feeding behavior. The complex interplay of multiple metabolic pathways that affect energy intake and partitioning are entwined and inseparable and are affected by both diet and physiological state. The liver is likely a primary sensor of energy status integrating homeostatic and homeorhetic mechanisms offering the unique advantage of sensing not just energy availability, but energy balance relative to nutrient demands. Feeding centers in the brain integrate all signals to determine feeding behavior and dominant mechanisms controlling feeding change with diet and physiological state.

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Regulation of feed and water intake

Pro-inflammatory cytokines and hypothalamic inflammation – implications for insufficient feed intake of transition dairy cows

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Improvements of feed intake of dairy cows entering the early lactation period potentially decrease the risk of metabolic disorders, but before developing approaches targeting the intake level, mechanisms controlling and dysregulating energy balance and feed intake need to be understood. This review focuses on different inflammatory pathways interfering with the neuroendocrine system regulating feed intake of periparturient dairy cows. Subacute inflammation in various peripheral organs often occurs during shortly before or after calving and is associated with increased pro-inflammatory cytokine levels. These cytokines are released into the circulation and sensed by neurons located in the hypothalamus, the key brain region regulating energy balance, to signal reduction in feed intake. Besides of these peripheral humoral signals, glia cells in the brain may produce pro-inflammatory cytokines even independently of peripheral inflammation. Preliminary results show intensive microglia activation in early lactation, implying their involvement in hypothalamic inflammation and the control of feed intake of dairy cows. On the other hand, pro-inflammatory cytokine-induced activation of the vagus nerve transmits signalling to the brain, but this pathway seems not exclusively necessary to signal feed intake reduction. Yet, less studied in dairy cows so far, the endocannabinoid system links inflammation and the hypothalamic control of feed intake. Distinct endocannabinoids exert anti-inflammatory action but also stimulate the posttranslational cleavage of neuronal proopiomelanocortin towards b-endorphin, an orexigen promoting feed intake. Plasma endocannabinoid concentrations and hypothalamic b-endorphin levels increase from late pregnancy to early lactation, but less is known about the regulation of the hypothalamic endocannabinoid system during the periparturient period of dairy cows. Dietary fatty acids may modulate the formation of endocannabinoids, which opens new avenues to improve metabolic health and immune status of dairy cows.

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The rumen as mediator between diet and host metabolism

Ruminal microbiome and microbial metabolome: Effects of diet and host

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The rumen contains a great diversity of prokaryotic and eukaryotic microorgnisms that allow the ruminant to utilize ligno-cellulose material and to convert non-protein nitrogen into microbial protein to obtain energy and aminoacids. However, rumen fermentation also has potential deleterious consequences associated with the emissions of greenhouse gases, excessive nitrogen excreted in manure and may also adversely influence the nutritional value of ruminant products. Whilts several strategies for optimizing the energy and nitrogen use by ruminants have been suggested, a better understanding of the key microorganism involved and their activities is essential to manipulate rumen processes suscesfully. Diet is the most obvious factor influencing the rumen microbiome and fermentation. Among dietary interventions, the ban of antimicrobial growth promoters in animal production systems has led to an increasing interest in the use of plant extracts to manipulate the rumen. Plant extracts (eg saponins, polyphenol compounds, essential oils) have shown potential to decrease methane emissions and improve the efficiency of nitrogen utilization; however there are limitations such as inconsistency, transient and adverse effects for their use as feed additives for ruminants. It has been proven that the host animal may also influence the rumen microbial population both as an heritable trait and through the effect of early life nutrition on microbial population structure and function in adult ruminants. Recent developments have allowed phylogenetic information to be upscaled to metabolic information; however research effort on cultivation of microorganisms for an in depth study and characterization is needed, The introduction and integration of metagenomic, transcriptomic, proteomic and metabolomic techniques is offering the greatest potential of reaching a truly systems level understanding of the rumen; studies have been focused on the prokaryotic population and a broader approach needs to be considered.

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Short communications

O 01

BFH12 cell line: A promising tool for studies on drug-metabolizing enzymes and efflux transporters of bovine biotransformation

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Introduction Cattle are frequently exposed to xenobiotics such as antimicrobial drugs and environmental pollutants. Xenobiotic bioaccumulation poses a serious threat to both animal and human health. Therefore, it is important to study the mechanisms of persistence and clearance of these compounds using a suitable in vitro model. In this study, we aimed to characterize the novel hepatocyte-derived cell line BFH12 with respect to gene expression of drug-metabolizing enzymes and efflux transporters as well as functional induction of cytochrome (CYP) P450 enzymes.

Methods BFH12 were cultured in Williams' Medium E containing 5 % heat-inactivated FBS, 1 % penicillin/streptomycin, 2 mM L-alanyl-L-glutamine, 100 nM dexamethasone and 0.2 U/mL insulin. Total RNA from BFH12, fetal hepatocytes and adult liver tissue was isolated, reverse transcribed and amplified by PCR using gene-specific primers. Several genes of phase I – III enzymes/transporters were selected for validation. Benzo[a]pyrene was used as inductor of CYP1A activity, which was determined by ethoxyresorufin-O-deethylase (EROD) assay. Efflux transporters were assessed by indirect immunofluorescence using transporter-specific antibodies.

Results Gene expression pattern of BFH12 was very similar to that of fetal hepatocytes. We observed expression of the phase I and II enzymes CYPs 1A1, 2C19, 3A4, UDP glucuronosyltransferase 1 family polypeptide A1 (UGT1A1), UGT1A6, glutathione S-transferase M1 (GSTM1), as well as of phase III efflux transporters P-glycoprotein (PGP), sodium-taurocholate cotransporting polypeptide (NTCP), ATP-binding cassette sub-family G member 2 (ABCG2) and multidrug resistance-associated protein 1 (MRP1). Strong expression of ABCG2 and MRP1 was also confirmed by immunofluorescence microscopy. Benzo(a)pyrene treatment induced CYP1A1 and 1A2 gene expression and dose-dependently increased their activity.

Conclusion The results suggest that BFH12 has retained biotransformation capacity which allows functional studies on drug metabolizing enzymes. Therefore, BFH12 may provide an alternative to current biotransformation models.

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O 02

Ruminal and intestinal calcium absorption in growing sheep: acute and long-term effects of a menthol-containing feed additive

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Introduction: A considerable amount of calcium is absorbed in the rumen although the classical calcium transport proteins TRPV5 and TRPV6 are not expressed. Recent studies suggested the involvement of TRPV3 in ruminal calcium absorption and the TRPV3 agonist menthol indeed increased calcium absorption in isolated ruminal epithelium. The present study investigated if either pre-feeding with a menthol-containing feed additive or direct application of menthol influences calcium absorption across ruminal and intestinal epithelia of growing sheep ex vivo.

Material and methods: Twenty four growing sheep were allotted into three groups in a randomized block design. They received hay ad libitum and 600 g concentrate feed. The latter contained either no additives (control diet) or plant bioactive lipid compounds (PBLC) with menthol as lead compound (feed additive OAX17, PerformaNat GmbH) in a low or high dose (PBLC low, 80 mg/d or PBLC high, 160 mg/d, respectively) for at least 28 d. Ruminal and jejunal tissues were mounted in Ussing chambers for measurement of unilateral calcium flux rates in the presence and absence of 50 µM menthol. Net flux rates were calculated and analyzed using mixed model procedures of SAS.

Results: The basal net flux rate of calcium was about 7 times higher in rumen than in jejunal tissue. In jejunum, PBLC feeding decreased net calcium flux rate before and after menthol application ($p<0.05$), and the acute menthol application did not alter net calcium flux rates within feeding groups ($p>0.1$). Ruminal net calcium flux rate was highest in the PBLC low group (quadratic effect, $p<0.05$). Menthol addition increased calcium net flux rate in all groups ($p<0.05$) but epithelia from sheep pre-fed with PBLC showed a stronger increase compared to control sheep ($p<0.05$).

Discussion: The present study confirmed that menthol increases ruminal calcium absorption, possibly by activating the ruminal TRPV3 channel. Pre-feeding with PBLC increased ruminal calcium absorption and responsiveness to menthol in a dose dependent manner. In jejunum, PBLC feeding decreased calcium absorption, which may be linked to different calcium transporting channels as the intestinal calcium-absorbing channels, TRPV5 and TRPV6, are regulated by vitamin D3. The results suggest that menthol-containing PBLC effectively modulate calcium absorption in rumen.

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O 03

Dietary P restriction induces bone mobilization, downregulates fibroblast growth factor 23 expression and interferes with vitamin D metabolism in sheep

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Recently, we could demonstrate that in late gestating dairy cows, restricted dietary P supply resulted in bone mobilization and increased 1,25-dihydroxyvitamin D (1,25-(OH)2D) serum concentrations at parturition despite of a markedly lower peak in serum parathyroid hormone (PTH) compared to cows on adequate P supply. Therefore, we hypothesized that a decline in serum phosphate (Pi) concentration ([Pi]) stimulated osteoclastic activity and resulted in a downregulation of fibroblast growth factor 23 (FGF23), a phosphatonin synthesized by osteocytes that was shown to modulate vitamin D metabolism.

To test this hypothesis 11 female, non-pregnant, non-lactating sheep, four to nine years of age were either fed a diet with adequate (AP; 0.25% P, 0.93% Ca) or low P content (LP; 0.11% P, 0.93% Ca) over a period of 6 weeks. Blood samples were analyzed for [Pi] and serum concentrations of Ca [Ca] and the bone resorption marker CrossLaps® [CL]. Tissue samples from bone, kidney and parathyroid gland were obtained for analysis of RNA expression of Receptor Activator of NF-κB Ligand (RANKL), a crucial factor for osteoclast activation, osteoprotegerin (OPG), a glycoprotein inhibiting bone mobilization by binding RANKL, FGF23, its receptor FGFR1, PTH receptor (PTHR), vitamin D receptor (VDR), its co-receptor retinoid X receptor (RXR) and the enzyme crucial for the activation of vitamin D to 1,25-(OH)2D, the 1α-hydroxylase (CYP27B1).

After six weeks on the experimental diets, sheep on LP diet had significantly increased [CL] with unchanged [Pi] and [Ca]. RNA expression of FGF23 was significantly down-regulated in LP sheep. The ratio of RANKL to OPG expression in bone was negatively associated with serum [Pi]. Interestingly, renal expression of CYP27B1 was negatively correlated with renal expression of PTHR, FGFR1 and RXR in LP- but not in AP sheep. The linear down-regulation of parathyroid VDR expression with decreasing serum [Pi] may also be indicative of an interference of P homeostasis with the interplay of the parathyroid gland and the kidney in regulating vitamin D metabolism by altering their responsiveness to FGF23, PTH and 1,25-(OH)2D.

The results presented here show that dietary P restriction induces bone mobilization in sheep as this was described to occur in dairy cattle. The modulation of key regulators of vitamin D metabolism and the reduction of the inhibitory effect of FGF23 on 1,25-(OH)2D-production might additionally facilitate the adaptation of Ca homeostatic mechanisms to the sudden increase in Ca demand at the onset of lactation.

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O 04

Effects of GLP-1 on expression of functional genes in rumen epithelium and its association to concentrate intake

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Background: GLP-1 exerts multiple roles in gastrointestinal and metabolic physiology. Apart from its action as "ileal brake", GLP-1 stimulates both pancreatic β-cell proliferation and inhibits β-cell apoptosis. Furthermore, GLP-1 improves endothelial dysfunction. Dietary nutrients induce GLP-1 release. Our previous studies showed diet-SCFA effects on gene expression in rumen epithelium. However, little is known about the role of GLP-1 in this process. The present study aimed at understanding the modulating effects of GLP-1 on the expression of functional genes in the ovine rumen epithelium and its relation to concentrate intake.

Materials and methods: Two experiments were performed. In Exp1 ovine ruminal epithelia were incubated in Ussing chambers for 7 h with 25 nM or 250 nM of GLP-1 administered to the serosal side to test the mRNA abundances in three functional gene groups: 1) genes related to SCFA absorption, i.e., AE2, PAT1, MCT1, MCT4, NHE1, NHE3, and Na/K ATPase; 2) genes involved in the regulation of cell cycle progression, i.e., cyclin D1, cyclin E1, CDK2, CDK4, and CDK6, and genes involved in the regulation of cell apoptosis, e.g., Bax, Bcl2, caspase3, caspase8 and caspase9; 3) genes of cell connection, i.e., ZO-1, DSG-1 and Connexin-43. In Exp2, goats received a diet of dried hay:concentrate at 70%:30% (ML group) or 90:10 (LL group) for 28 d. Samples of rumen epithelium, ileum and colon were collected for mRNA analysis using real-time quantitative PCR.

Results: Exp1: A concentration of 25 nM GLP-1 did not change the abundance of 73% of tested genes, but reduced the abundance of 23% of them. A concentration of 250 nM of GLP-1 enhanced the abundance for 71% of tested genes and inhibited 29% of them. These effect patterns appeared in each of three gene groups.

Exp2: The blood concentration of GLP-1 was higher in ML than LL group. The expression of gene in rumen epithelium, including those 21 genes, being same as in Exp1, plus GLP-1 receptor increased in the ML group. The mRNA abundance of GLP-1 precursor proglucagon and prohormone convertase 1 was greater in the ML than in the LL group in ileum and colon.

Conclusion: Concentrate feeding induces GLP-1 synthesis in the ileum and colon and GLP-1 receptor in the rumen epithelium. Concurrently, changes of functional gene expression occur in the rumen epithelium of goats. The stimulating effects of high-dose GLP-1 application to isolated ruminal epithelia ex vivo suggested that GLP-1 may contribute to these changes.

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O 05

Ex vivo application of *Scrophularia* extract and Monensin to determine effects on ruminal barrier function and nutrient uptake in sheep

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Previous research has shown that extract from the Iranian plant Scrophularia striata had similar impacts on rumen fermentation and microbiota as monensin. The objective of this study was to determine the impact of both monensin and S. striata, specifically with regards to the acute (15 min) and chronic exposure (6 h) of the ruminal epithelia, on tissue permeability and nutrient uptake rates. Ovine ruminal epithelia from six female Merino landrace sheep were mounted in Ussing chambers under short-circuit conditions. The apical uptake of radioactively labelled 14C-acetate and 3H-propionate were measured as indicators of nutrient uptake transcellular absorption. The serosal-to-mucosal flux rate of fluorescein, an indicator of barrier function, was also measured over a 6-h period when tissues were subjected on their mucosal side to either a control treatment (ethanol (70% (v/v); CON), monensin (3µg/mL in ethanol; MON) or S. striata (600µg/mL in ethanol; SCRO). In addition, tissues were incubated with either a bicarbonate containing buffer (COF), a bicarbonate-free buffer (HEF) or a bicarbonate-free/nitrate-supplemented buffer (HEF-NO). The mucosal buffer solution had a pH of 6.1 and the serosal buffer pH 7.4. Tissue conductance (Gt) and short-circuit current (Isc) were monitored to assess epithelial integrity and active electrogenic ion transfer, respectively. The change of Isc (relative to baseline) tended to be highest for MON ($P = 0.07$) in the acute but not in the chronic phase of exposure. However, the Gt for MON was lower than CON and SCRO ($P < 0.001$) after 6-h exposure. There was a significant effect of buffer in the acute phase for Isc and Gt. However, only Gt was significant ($P < 0.001$) when corrected for baseline with the lowest tissue conductance measured in the HEF-NO buffer. There was no effect of supplementation on the apical uptakes of 14C-acetate and 3H-propionate. 3H-propionate had the highest total uptake at both the acute and chronic exposures and the bicarbonate-independent, nitrate-insensitive uptake (HEF – HEF-NO) also increased at 6 h for 3H-propionate but not for 14C-acetate. The fluorescein flux was significantly impacted by treatment throughout the 6 h measurement period with monensin maintaining a reduced flux (nmol/h/cm2) over time compared to both SCRO and CON ($P < 0.001$). These results indicate that monensin increases the epithelial integrity and the barrier function of ruminal epithelium under normal pH conditions, whereas luminal exposure to nitrate modulates the uptake of major nutrients across rumen epithelium.

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O 06

Role of fatty acid receptors in the regulation of transporters for short chain fatty acids and pH-homeostasis in sheep rumen

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Recent studies in the intestine of monogastric animals point to the ability of the epithelium to sense luminal content by G-protein-coupled receptors (GPRs), which can modify levels of cyclic adenosine monophosphate (cAMP). Influence of cAMP on transport proteins for short chain fatty acids (SCFAs) as well as pH homeostasis like monocarboxylate transporter1 (MCT1) and Na+/H+ exchangers (NHEs) was shown in in vitro studies. In sheep ruminal epithelium, we detected two GPRs (GPR109A and FFAR2) immunohistochemically. Our study aimed to investigate, whether MCT1 and NHEs could be influenced by activation of GPRs in ruminal epithelium.

To test if cAMP level is modulated by SCFAs, isolated ovine ruminal epithelia were mounted in Ussing chambers and incubated with 10µM forskolin (an activator of adenylyl cyclases which raises cAMP level), 10mM butyrate or 1mM niacin (a GPR109A agonist). After incubation, cAMP concentration was analysed in lysed epithelia. Forskolin administration provoked a significant rise of cAMP level, which was significantly lowered when butyrate was applied subsequently to forskolin. This effect was even more pronounced, when tissues were incubated at a more acidic mucosal pH and butyrate was administered only mucosally. Hardly any reduction of cAMP level in comparison to forskolin-stimulated epithelia was observed after niacin application.

MCT1 extrudes SCFAs and their metabolites into the blood on the basolateral membrane of the ruminal epithelium. To evaluate the impact of cAMP on MCT1 activity, unidirectional 14C-acetate-fluxes were analysed under the influence of forskolin and an MCT1-inhibitor. The MCT1-inhibitor p-hydroxymercuribenzoic acid (1.5mM) led to a significant reduction of 14C-acetate-fluxes. High cAMP levels provoked by application of 50µM forskolin did not exert any effect.

To assess the capacity of acidification recovery with modified cAMP levels, primary cultured ruminal epithelial cells were generated. Intracellular pH was determined as a parameter of NHE activity. Cells were incubated with 10µM 5-(N-ethyl-N-isopropyl)-amiloride (EIPA), an NHE-inhibitor and/or 10µM forskolin. EIPA induced a significant inhibition of counter-regulation after acidification, indicating NHE activity. Forskolin alone, on the other hand, tended to worsen the recovery capacity.

Our data indicate that modulation of cAMP level by butyrate is not mediated by GPR109A. It might be rather likely that FFAR2 is the key factor, inducing changes in intracellular pathways. Nonetheless, under physiological conditions, regulation of MCT1 and NHEs does not seem to occur by cAMP (via GPRs), hence other mechanisms remain to be revealed.

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P 162

Evaluation of digestibility discrepancy of different parts of corn stover by in vitro fermentation

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Objective： The structural components and nutritional value of different crop straws are different, even the structural components and nutrient utilization rates of different parts of the same straw are significantly different. There are differences in the content and structure of cellulose and hemicellulose in the stem bark, leaf blade and stem pith of corn stover. Therefore, the potential of these components to produce feed is different. The purpose of this experiment was to study the differences in the degradation and utilization of different parts of corn stover by anaerobic fungus and methanogen co-culture, and to provide a scientific basis for the comprehensive utilization of corn stover.

Methods: The corn stover was collected and separated as the leaf blade, stem pith, and stem bark, to be used as substrates in the present study divided into three groups. The co-culture of anaerobic fungus and methanogen (*Pecoramyces* sp. F1 & *Methanobrevibacter thaueri*) was previously isolated from goat rumen. The co-culture was grown at 39°C for 72 h in 90 ml media containing of the different substrates. The gas and methane production were measured every 6h. The pH was determined at the end of fermentation and supernatant was collected for the analysis of fermentation metabolites and carboxymethyl cellulase and xylanase activity. The substrates were collected for the analysis of idry matter, neutral detergent fiber, and acid detergent fiber. The water-soluble metabolites were determined using gas chromatography. Data were analyzed using SPSS 20.0 and the significance was declared at $P < 0.05$.

Results: The stem bark of corn stover had the lowest digestibility and methane production as it had lowest contents of neutral detergent solution and hemicellulose, and had the highest contents of cellulose and lignin. Stem pith and leaf blade of corn stover had similar gas and methane production, though their digestibility and chemical composition were significantly different. The pH, activity of fiber degrading enzymes, water-soluble metabolites were significantly different among three groups.

Conclusions: The substrate characteristics significantly affected the fiber degradation and methanogenesis of the co-culture of anaerobic fungus and methanogens. The degradation rate of stem pith was significantly higher than that of leaf blade, but its methane yield was similar to that of leaf blade. Therefore, the stem pith with high degradation rate and low methanogenesis might be more suitable for feed utilization of ruminants, but its specific mechanism still needs to be further studied.

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P 163

Influence of protein intake on ammonia emissions and nitrogen secretions of Belgian Blue heifers

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Reducing dietary crude protein (CP) intake might be a cost-effective and source-oriented measure to reduce ammonia (NH_3) emissions from beef barns. We evaluated the effect of dietary CP level and rumen degradable protein balance (RDPB) on NH_3 emissions, urinary and fecal N-excretions in a 20-week trial with 28 Belgian Blue heifers, divided into a low (LCP) and high protein group (HCP). In the control period, both groups were fed the same TMR with 13.1% CP and an RDPB of +2 g/kg DM. In the treatment period, a LCP and a HCP TMR were fed, equal in energy (105% daily requirement) and intestinal digestible protein (105% daily requirement) content but varying in CP level (11.6 % versus 14.2%; 13% is theoretical requirement) and in RDPB (-15 vs +15 g/kg DM). In addition, a fixed amount of concentrate feed containing 5% of Cr_2O_3 marker was fed to estimate the fecal production using the indirect marker method. Fecal grab samples were taken twice daily (5 consecutive days), pooled and analysed for N content. Total collection of urine was done using a Foley catheter over 5 consecutive days. An acidified urine sample was analysed for urinary N (UN) and urinary urea N (UUN) concentrations. NH_3-emissions were measured in the mechanically ventilated ILVO emission barn. Outgoing air was analysed using a cavity ring-down spectroscope (Picarro G2103) and the airflow rate was constantly logged.

In the control period, the DMI and intake of CP and RDPB were not different between the HCP and LCP. In the treatment period, the LCP and HCP group had a DMI of 7.7 vs 7.4 kg, a CP intake of 865 vs 1062 g/day and a RDBP of -34 vs 178 g/day respectively. The differences in dietary protein intake were reflected in the UN- and UUN-excretions, with significantly higher UN- (64.6 versus 39.2 g/day; $P<0.001$) and UUN-excretions (55.5 versus 21.1 g/day; $P<0.001$) in the HCP group. Fecal N-excretions tended to be higher in the LCP group (66.7 versus 62.0 g/day; $P=0.02$). The NH_3-concentrations measured in the ILVO emission barn were on average 49% higher in the HCP groups (1385 ± 261 vs 2759 ± 487 ppm; $P<0.001$) (Goossens et al., 2019). This study shows that reduction in dietary protein intake has a significant impact on the urinary N-excretions and subsequent NH_3-emissions. Long term effects on performances of growing Belgian Blue heifers and steers are under investigation.

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P 164

Effects of concentrate feeding on expressions of RhBG and RhCG in omasum epithelium and their association to pH and ammonia

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Background: The ammonia-specific transporters Rhesus glycoprotein B (RhBG) and C (RhCG) play critical roles in ammonia transport in mammalian renal, liver and intestine. In ruminants the ammonia transport in rumen and omasum influences urea utilization and N-salvage. Proton and SCFA are main rumen fermentation products. Dietary-pH and -SCFA have effects on ammonia absorption and multiple functional gene expression in omasum. However, little is known about the presence of RhBG and RhCG in the omasum and the effects of dietary factors on the expression of RhBG and RhCG. Our paper studied the modulating effects of concentrate feeding on RhBG and RhCG expression and its mechanism.

Materials and methods: Two experiments were performed. In Exp1 goats received a diet with 35% (M group) or 15% concentrate (L group) for 28 days. At the end, the samples of omasal fluid and epithelia were collected for analysis. In Exp2 the isolated cells from goat omasal epithelium were incubated by the method of primary cell culture under different conditions: 1) pH 7.4 or 6.8; 2) pH 7.4 + or 6.8 + SCFA; 3) pH 6.8 + NH_4Cl; 4) pH 7.4 + urea. After 24h incubation, cells were collected for analysis.

Results: Exp1: Comparing with L group, M group had significantly higher concentrations of SCFA and NH3-N, but lower pH in omasum fluid. The distribution of RhBG and RhCG were observed in omasal tissue. The mRNA expression and protein abundance of RhBG and RhCG were higher in M than in L group. Exp2: Reducing pH from 7.4 to 6.8 in the medium caused significant increase of mRNA expression of RhBG and RhCG. Treated cells with pH 6.8+NH4Cl promoted mRNA expression of RhBG and RhCG compared to pH 6.8 alone. We found pH and ammonia exerted synergistic effects on expression of RhBG and RhCG. However, with the presence of SCFA, both the neutral and acidic pH led to decline of mRNA expression. In addition, pH 7.4 + urea inhibited RhBG and RhCG mRNA expression.

Conclusion: Increase of concentrate feeding caused decline of pH but increase of concentrations of ammonia and SCFA in omasal fluid, and synchronously greater mRNA expression of RhBG and RhCG in omasal epithelium. Acidic pH and ammonia promoting, but SCFA inhibiting mRNA expression of RhBG and RhCG. Taken together, our study indicates that concentrate feeding caused expressions of RhBG and RhCG in omasum epithelium is predominantly associated to changes of omasal pH and ammonia.

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P 165

Assessing the bioavailability of rumen-protected lysine using the plasma lysine response method

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Lysine (Lys) is a limiting amino acid in high-lactating dairy cows, especially those fed a corn grain/silage-based diet. Increasing metabolizable Lys may increase milk protein and yield. Rumen-protected lysine (RPL) products are made of L-Lys-HCl, which has a high hydrophilicity. It is important to know how much of the product, when consumed under conditions similar to normal feeding practices, escapes rumination and microbial fermentation, and consequently available for absorption within the small intestine. The purpose of this study was to compare Lys bioavailability in 3 RPL products commercially available in Japan (A: AjiPro®-L, B: FeedtechTM, C: LysiGEMTM). Nine multiparous late-lactating Holstein cows (613 ± 27 kg of BW; 226 ± 35 DIM) housed in a tie-stall facility were used in a replicated 3 × 3 Latin square design in 14 d experimental period. Cows were fed a corn silage-based TMR ad libitum with free access to water and mineralized block. Cows were fed products A, B, or C coupled with the same intake of additional Lys (7.6 g/100 kg of BW/d) at 0930 h and 1830 h during the treatment period. Blood samples were collected from the jugular vein of each cow at 0800 h, 1300 h, and 1700 h on d 0 (the day before first treatment) and d 14 during the experimental period and samples obtained on the same day were pooled as one day-plasma sample. The dry matter intake (DMI) was measured for the last five days of each period. Milk yields were measured every day and milk samples for measurement of milk composition (fat, protein, lactose, MUN, and SNF) were obtained on d 14. Plasma samples were analyzed for concentrations of amino acids (AA), metabolites, and hormones. Data were analyzed using the MIXED procedure of SAS and considered significant at $P \leq 0.05$ using the Tukey's test for multiple comparisons among the days and treatments. The DMI, milk yields and milk composition did not significantly differ among the treatments. Ratio of Lys to total AA- Lys (Whitehouse et al., 2014) in plasma on d 14 was significantly greater than that at d 0 for product A group (3.9 vs 4.6, $P \leq 0.05$), but no significant differences were observed for product B (3.8 vs 4.1) and C groups (3.9 vs 4.3). These results suggest that the Lys bioavailability may vary depending on the RPL products.

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Effect of kraft pulp inclusion in calf starter on plasma concentration of glucagon-like peptide 2 in calves.

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Glucagon-like peptide 2 (GLP-2), which plays a role in stimulation of intestinal growth, has been shown to be secreted in response to dietary fiber ingestion. Kraft pulp (KP), an intermediate product obtained when wood chips are converted to paper, contains high digestible neutral detergent fiber (NDF). The objective of this study was to evaluate the effect of KP inclusion in calf starter on plasma concentration of GLP-2 in calves. Holstein heifer calves (n = 25) were raised on a high plane of nutrition program using milk replacer [MR; 28.0 % crude protein (CP) and 15.0 % fat] until weaning at 49 d after birth. Calves were fed calf starter containing KP at 0 (CON; n = 14) or 12 % (KPS; n = 11) on a dry matter basis. Calf starters and timothy hay were offered *ad libitum* from the beginning of this study. All calf starters were formulated for 20.7% CP, and NDF content of CON- and KPS-starter were formulated for 16.4 and 22.6 %, respectively. Blood samples were collected at 4, 14, 21, 35, 49, 70, and 91 d after birth to measure plasma GLP-2 concentration. Data were analyzed by ANOVA of JMP® 14 using fit model procedure. Dry matter intake (DMI) of MR and calf starters did not differ among treatments, but hay DMI was lower (Treatment $P = 0.02$) for KPS (0.31 ± 0.06 kg/d) than for CON (0.44 ± 0.06 kg/d). Whereas, NDF intake was higher for KPS compared with CON from 56 to 84 d after birth (Treatment × time $P < 0.01$), which was due to higher NDF content for KPS-starter caused by KP inclusion. Body weight and average daily gain were not affected by KP inclusion. Plasma GLP-2 concentration was not affected by dietary treatment at pre-weaning period but higher (Treatment $P = 0.04$) for KPS (0.6 ± 0.13 ng/mL) compared with CON (0.41 ± 0.13 ng/mL) at post-weaning period. These results indicate that KP inclusion in calf starter increases plasma GLP-2 concentration, which may be associated with greater fiber intake.

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P 167

The TRPV3 channel: a pathway for the uptake of Ca^{2+} from the rumen

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Although it is well-known that up to 50% of calcium absorption occurs in the rumen via an electrogenic pathway, the molecular mechanisms have not been clarified. In particular, attempts to demonstrate expression of classical epithelial calcium channels such as TRPV5 and TRPV6 have failed (1). Instead, it has recently been shown that the ruminal epithelium expresses mRNA for the bovine representative of TRPV3 (bTRPV3), a non-selective cation channel with a conductance to various cations including Ca^{2+} (2). Furthermore, functional agonists of bTRPV3 have been shown to stimulate the uptake of Ca^{2+} both by the ruminal epithelium (2) and by cells overexpressing bTRPV3 in vitro (3), suggesting that this channel may play an important role in the uptake of this mineral. However, demonstrating the expression of bTRPV3 by the rumen remains problematic due to a lack of antibodies for the bovine species.

To select a suitable antibody, we aligned the binding sites of commercial antibodies for the human TRPV3 channel (hTRPV3) with the sequence of the bovine homologue. For validation, two expression systems were used. *Xenopus* oocytes were either injected with RNAse-free water (control) or linearized strep-tagged bTRPV3-cRNA or hTRPV3-cRNA. Likewise, HEK-293 cells were either transiently transfected with a pIRES2-AcGFP1 vector (control) or with the same vector construct including strep-tagged bTRPV3 or hTRPV3 sequence. Immunohistochemical staining with the commercial antibody showed staining of the cellular membranes of cells overexpressing bTRPV3 or hTRPV3, but not of controls. Additionally, the antibody was used in western blots of both the expression systems and bovine ruminal protein. In native bovine ruminal tissue, staining patterns suggest expression of bTRPV3 primarily by the stratum spinosum and granulosum of the epithelium.

In conjunction with previously obtained data, bTRPV3 emerges as an uptake pathway for the absorption of monovalent and divalent cations. Modulation of the channel by naturally occurring herbal agonists invites new approaches in ruminant nutrition (4).

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P 168

The effect of industrial processing and abomasal methionine infusion on utilisation of faba bean protein in dairy cows

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Faba bean (FB, *Vicia faba*) is an ancient grain legume, the utilization of which is hampered by higher protein rumen degradability and lower Met content relative to soybean or rapeseed meals (RSM). We hypothesized that industrial processing of FB and supplemental Met improves milk production to a comparable level with RSM.

Five rumen-fistulated multiparous Finnish Ayrshire cows averaging 74 d in milk were allocated to experimental diets according to a 5x5 Latin square with 21-d periods. Treatments consisted of total mixed rations (TMR) containing isonitrogenously milled or processed FB with or without abomasal Met infusion (15 g/d) or RSM as a positive control. Industrial processing of FB comprised dehulling, flaking and heat treatment. Concentrates (400 g/kg TMR dry matter (DM), crude protein (CP) 189-196 g/kg concentrate DM) contained also barley, oats and molassed sugar-beet pulp. Forage was grass silage (11.2 MJ ME/kg DM, 146 g CP/kg DM). Data were analysed by ANOVA.

Processing of FB substantially decreased the N solubility in borate-phosphate buffer (672 vs. 233 g soluble N/kg N) without an increase in acid detergent insoluble N (< 92 g ADIN/kg N). Commercial RSM contained high amounts of ADIN (297 g/kg N). The DM intake was on average 1.5 kg higher on FB diets relative to RSM ($P<0.05$), but this had no effect on milk yield. Milk production level of this physiological study averaged 27.7 kg/d. Processing of FB tended to increase milk yield by 1.4 kg/d ($P=0.07$), but abomasal Met infusion on FB diets had no effect. Milk content of lactose, fat, protein and urea were unaffected by treatment except for higher milk protein content on FB diets relative to RSM ($P<0.05$). Protein source had no major effect on rumen fermentation pattern. Rumen fluid ammonia-N concentration averaged 7.69 mmol/L and processing of FB tended to decrease it ($P=0.09$). Replacing RSM by FB had no effect on arterial essential amino acids, but processing of FB increased or tended to increase arterial Arg, Leu and Val ($P\leq0.10$). Furthermore, abomasal infusion of Met increased arterial Met (19.7 vs. 26.7 µmol/L, $P<0.01$).

Interestingly, replacing RSM with FB resulted in similar milk yield. Processing of FB decreased N solubility and improved milk yield, whereas Met supplementation of FB diets was ineffective. Further milk production trials are needed.

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O 07

Effect of the inclusion of coffee grounds in concentrate on productive performance, feeding behavior and ruminal fermentation of Latxa sheep

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Coffee grounds (CG) represent one of the main residues derived from restoration and hostelry. Due to their polluting capacity, especially when there are poured directly to the ground, revalorization and reutilization of this sub-product is a key factor in the search of the circular economy. The antihypertensive, antimicrobial and antioxidant properties of CG, related to some of its components such as melanoidins and phenolic compounds, makes them a potential functional ingredient to be incorporated in the formulation of a concentrate for ruminants. The aim of this trial was to evaluate the effect of CG, included in different concentrations (3%, 5% and 10%) in the concentrate, on milk yield and quality, feeding behavior, dry matter intake and apparent digestibility and ruminal short chain volatile fatty acid profile. In this trial of 51 days of duration, 48 Latxa dairy sheep were used. Ewes were divided in four groups according to parity, milk yield (1918 ± 287g) and days in milk (35.7 ± 8.9 days). All of the concentrates were formulated to be isoenergetic (1.01 UFL), isoproteic (16.6 %), isofat (7.6 %) and to meet the production needs. The concentrate was given in two doses of 450 g of dry matter per milking and fescue hay was offered ad libitum. Increasing doses of CG in the concentrate up to 10%, resulted in a linear ($P<0.001$) increase in the iso-valeric and iso-butyric acid proportions, due to a possible depressor effect of CG in the deamination of proteins. This could cause a greater flow of protein to the small intestine and could explain the observed quadratic increase ($P<0.001$) in milk yield and a linear increase ($P<0.001$) in milk protein. A linear increase ($P<0.001$) in milk fat was found, which could be explained by the observed linear increase ($P<0.001$) in the ruminal acetic acid proportion. Increasing doses of CG in the concentrate linearly decreased ruminal ($P<0.001$) propionic acid proportion, resulting in a concomitant linear decrease ($P<0.001$) in the propionic: acetic ratio. Furthermore, no differences were found in apparent digestibility and feeding behavior. In conclusion, the formulation of CG up to 10% in the concentrate modified the ruminal fermentation pattern towards more isoacids and acetic acid proportions in the rumen with a concomitant improvement in milk production and composition, without impairing feeding behavior or apparent digestibility.

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O 08

Composition of milk fatty acids as an indicator of negative energy balance of dairy cows in early lactation

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Most dairy cows experience negative energy balance (NEB) in early lactation because the energy demand for milk synthesis is not met by energy intake. Excessive NEB may lead to metabolic disorders and impaired fertility. Therefore, it is useful to detect NEB in early lactating cows to optimize herd management. However, direct calculation of NEB is not feasible in commercial herds. Alternative methods rely on fat-to-protein ratio in milk or on concentrations of non-esterified fatty acids (NEFA) and B-hydroxybutyrate (BHB) in blood.

Here, we demonstrate a novel method to assess cow's energy balance based on the fatty acid (FA) composition in milk. Short-chain fatty acids (primarily, C14.0) are typically synthesized de novo in the mammary gland and the proportion of these FA in milk fat decreases during NEB. Long-chain C18.0 and C18:1C9 are typically released from body fat depots during NEB and the proportion of these FA increases. These FA can be routinely determined by mid-infrared (MIR) spectroscopy of individual milk samples.

We performed an experiment on 86 dairy cows in early lactation at the Swedish Livestock Research Centre (Uppsala, Sweden) between February and July 2016. Cows were given the same concentrate ration of up to 5 kg per day and forage ad libitum. Milk yield and feed intake were automatically recorded on a daily basis. Milk samples for MIR spectroscopy and blood plasma samples were collected in lactation weeks 2, 4 and 6 relative calving. We estimated net energy content in feed, net energy required for maintenance and lactation, and, subsequently, derived energy balance, which was used as a reference to compare alternative indicators.

We fitted a number of generalized additive models (GAMs) and found that deviance explained for NEFA, BHB and fat-to-protein ratio were 0.155, 0.159 and 0.147, respectively. Values for concentrations of individual fatty acids C14.0, C18.0 and C18.1C9 in milk fat were significantly higher: 0.235, 0.234 and 0.162. This indicates that composition of milk fatty acids indeed could be used as an indicator of energy balance in early lactation cows. Furthermore, this method is non-invasive and does not require individual measurements of feed intake, which makes it suitable for use on most of commercial dairy farms.

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O 09

Maternal nutrition and stage of early pregnancy in beef heifers: Influence on glucose transporter GLUT3 in utero-placental tissues

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The facilitated diffusion transporter, GLUT3 (SLC2A3), is a high efficiency, high capacity glucose transporter localized in tissues with a high glucose demand, such as the placenta and brain. We hypothesized that maternal nutrition and day of gestation would influence the abundance of GLUT3 in bovine-uteroplacental tissues during days 16 to 50 of gestation. Angus-cross heifers (n = 43) were synchronized, bred via AI, assigned to nutritional treatment (CON = 100% of requirements for 0.45 kg/d gain and RES = 60% of CON), and ovariohysterectomized on d 16, 34, or 50 of gestation (n = 6 to 9/group). Cross sections were taken from the uterine horn ipsilateral to the CL, fixed in Carnoy's solution, embedded, sectioned at 5 µm, and stained for GLUT3. Images of fetal membrane (FM; chorioallantois), endometrium (ENDO), superficial glands (SG), deep glands (DG), and myometrium (MYO), were taken and analyzed for relative intensity fluorescence to provide relative abundance of GLUT3. Fetal membrane analysis was only conducted on d 34 and 50. Abundance of GLUT3 was not influenced ($P \geq 0.21$) by a day × treatment interaction in any tissue. Maternal nutrition decreased GLUT3 in DG of RES heifers ($P = 0.04$) compared with CON heifers. Lastly, GLUT3 in FM was greater ($P = 0.03$) on d 34 compared with d 50 of gestation. Previously reported results from our laboratory found that expression of GLUT3 was influenced by day of gestation in caruncular and intercaruncular endometrium. In jugular serum of heifers or histotroph, previously published data showed that glucose concentrations from the same study were not affected by maternal nutrient restriction; however, glucose concentrations in allantoic and amniotic fluid were greater in CON compared with RES heifers. Additionally, recently published transcriptomic analysis of fetal liver on d 50 of gestation from the same study using RNA-seq indicated altered carbohydrate metabolism pathways; for example, upregulation of gluconeogenic enzymes in fetuses from RES dams compared with CON. Taken together, results of the current report and previous research suggest that a moderate maternal nutrient restriction of beef heifers during early pregnancy alters the concentration of glucose in fetal fluids. These changes may not be due to the ability to transport glucose across the maternal-fetal interface, but rather differences in glucose concentrations in fetal fluids may be due to altered metabolism by either placental or fetal tissues and consequently should be the focus of future research.

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O 10

Effects of feeding systems on gonadal development, scrotal fat accumulation and semen quality of rams of different Merino breed types

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This study investigated the effects of finishing systems for young breeding rams of Merino breeds (5-12 months old) on gonadal development, scrotal fat accumulation and semen quality.

The effects of extensive feeding (C; 200 days), extensive followed by intensive feeding (TR1; 133 days) and intensive feeding (TR2; 77 days) were evaluated in Merino, Döhne Merino and South African Mutton Merino rams. Growth, size, scrotal measurements, semen quality and post mortem gonadal measurements were recorded. Data were analysed by GLM ANOVA procedures and differences between treatment means were tested by Bonferroni's multiple range test ($P<0.05$). Regression analyses and partial correlations were calculated by controlling for initial weight.

Rams in the C-system were larger and heaviest, while TR2 rams were smaller and lightest ($P<0.05$). Finishing systems influenced weight gained ($P<0.05$), with C rams gaining most and TR2 least. Average daily gain (ADG) differed between finishing systems ($P<0.05$), but here TR2 had the highest ADG and C had the lowest. TR2 rams had less subcutaneous fat (SubFat) compared to C and TR1 ($P<0.05$). Testes weight, length and volume were greater ($P<0.05$) in rams in C and TR1 compared to rams in TR2. Rams in C and TR1 had thicker ($P<0.05$) scanned scrotal neck fat (SSNF) than TR2. Rams in TR1 deposited more scrotal fat ($P<0.05$) than rams in C and TR2. Rams in TR1 had higher semen volumes (SV) ($P<0.05$) than C rams. Rams in TR2 had the lowest percentage normal sperm (%NS) ($P<0.05$) compared to those in TR1 and C. Regression analyses confirmed that higher weight gains resulted in more SubFat ($r^2=0.59$, $P<0.001$) and more SSNF ($r^2=0.43$, $P<0.001$). Weight gains exceeding 30kg caused a decrease in SV ($r^2=0.16$, $P<0.01$; $y=-5.9+0.6x-0.01x2+9.4x3$), while gains above 55kg resulted in decreased %NS ($r^2=0.14$, $P<0.01$; $y=71.5-0.96x+0.05x2-0.001x3$). In turn, increasing SubFat caused more SSNF ($r^2=0.40$, $P<0.001$) and more scrotal fat ($r^2=0.06$, $P<0.05$). SubFat exceeding 1.5cm caused a decrease in SV ($r^2=0.08$, $P<0.05$; $y=0.2+2.3x-0.7x2$) while SubFat exceeding 2cm resulted in lower %NS ($r^2=0.19$, $P<0.001$; $y=36.5+41.8x-9x2$). Scrotal fat exceeding 30g reduced mass motility ($r^2=0.07$, $P<0.05$; $y=2.8+0.1x-0.001x2$).

Increased weight gain resulted in more subcutaneous and scrotal fat, of which SubFat is a more accurate measure. Although fat accumulation is a normal feature of animal growth, once a certain threshold is breached (excess SubFat or scrotal fat), it may adversely affect semen quality and quantity. Thus, intensive feeding of younger rams should not occur for extended periods of time.

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O 11

Fusarium mycotoxins deoxynivalenol and fumonisins affect milk production, nutrient digestibility and liver health in dairy cows

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The Fusarium mycotoxins deoxynivalenol (DON) and fumonisins (FUM) are commonly found in dairy cow rations. Despite the rumen's ability to bind or degrade these mycotoxins to some extent, especially high producing dairy cows are sensitive to Fusarium mycotoxins. Studies investigating the effect of lower dietary mycotoxin concentrations on dairy cows are scarce. Therefore, we investigated the effect of dietary DON and FUM concentrations below European Union guidance levels on the performance, dry matter digestibility (DMD), neutral detergent fiber digestibility (NDFD) and liver enzyme activities in blood of dairy cows. In a randomized design, 12 Holstein cows in mid- lactation (114 ± 16 days in milk) were fed either a negative control TMR (CTR) (447 µg/kg DON, 96 µg/kg FUM, 7 µg/kg Zearalenone ZEN, 4 µg/kg T-2 toxin) or a TMR with a low mycotoxin contamination level (959 µg/kg DON, 948 µg/kg FUM, 37 µg/kg ZEN, 25 µg/kg T-2 toxin) (MTX). The trial consisted of a 3-week challenge period followed by a 2-week clearance period. Milk production was recorded daily and blood samples were collected from the jugular vein at the beginning (day 0), on day 14 and day 21 of the experimental period. Fecal samples were taken at days 0 and 21. Acid-insoluble ash was used as an internal marker to calculate digestion coefficients. Data were analyzed using the MIXED procedure of SAS (SAS 9.4 TS, 2018). The mycotoxin treatment had a significant negative effect ($p < 0.05$) on the milk production (CTR 37.94 kg/d vs. MTX 36.37 kg/d). The DMD as well as NDFD were lower ($p < 0.05$) in the MTX group (DMD: CTR 71.0% vs. MTX 67.3%; NDFD: CTR 52.3% vs. MTX 42.8%). Aspartate amino transferase (AST) activity was significantly higher ($p < 0.05$) in MTX treated animals (117.1 U/L) compared to CTR animals (106.6 U/L) and Ɣ-glutamyl transferase (GGT) activity tended to be higher ($p = 0.059$) in MTX-treated animals (30.7 U/L) compared to CTR animals (29.1 U/L). Other parameters related to liver functions, such as alkaline phosphatase (ALP), paraoxonase, total bilirubin or albumin, were not affected by the MTX treatment. In conclusion, dietary DON and FUM concentrations that comply with legal limits showed a negative effect on the milk yield, DMD and NDFD. Furthermore, dietary DON and FUM increased liver enzyme activities in blood of dairy cows suggesting a moderate increase in cytolysis.

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O 12

Effects of low-fat corn distiller grains on carcass traits and muscle cell growth genes of crossbreed bulls

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Introduction: Brazil's ethanol production from corn has increased over the past 5 years, enabling the use of distiller grains in rations of feedlot cattle, especially due to its nutritional value. Thus, the aim of this study was to evaluate carcass traits and expression of genes involved in muscle growth in crossbred cattle fed low-fat wet distiller grains (LFWDG).

Materials and Methods: One-hundred crossbred Angus-Nellore bulls with initial average BW of 369 ± 48 kg were fed for 129 d. The animals were allocated in group pens (n = 5), with five pens per treatment in a completely randomized blocks design. Diets contained increasing levels of LFWDG (0, 150, 300 and 450 g/kg DM) and were composed as follow: 148, 161, 191 and 220 g of CP/kg DM; 627, 540, 434 and 328 of non-fibre carbohydrates (g/kg DM); and 162, 234, 308 and 382 of NDF g/kg DM; and 34.2, 35.7, 36.3 and 36.9 EE g/kg MS, respectively. Animals were slaughtered with an average BW of 601.8, 616.5, 630.1 and 615.3 ± 18.8 kg, respectively. At the abattoir, backfat thickness (BFT), rib eye area (REA), pH, carcass weights (HCW and CCW) and yield (CY) were measured. Immediately after slaughter, LT muscle samples were collected and placed on RNA storage reagent. Subsequently, genes of cell growth such as eIF4, IGFR1, mTOR, GSK3B, and P7056K were investigated by reverse-transcription-quantitative real-time PCR (RT-qPCR). Data were analyzed using the MIXED procedure of SAS with linear, quadratic and 0 (control) vs. LFWDG contrasts.

Results: No linear or quadratic effects of levels of LFWDG were observed on carcass variables. LFWDG increased HCW, CCW, CY, and REA compared to control diet ($P<0.05$). In addition, lower cooling loss was observed in animals finished with LFWDG ($P<0.05$), whereas BFT and pH were not affected by the treatments ($P>0.05$). The genes IGFR1 and mTOR mRNA were linearly downregulated as increasing levels of LFWDG ($P<0.05$), while eIF4, GSK3B, and P7056K mRNA expression were similar among treatments. When comparing control versus LFWDG diets, eIF4 IGFR1 and mTOR mRNA expression were downregulated ($P<0.05$). Although increased levels of LFWDG influenced carcass weights and REA (an indicator of muscle deposition) in response to feeding, it did not produce concerted changes in genes of LT muscle hypertrophy. mTOR and IGF1 have a central function in integrating a variety of growth signals, resulting in protein synthesis. The physiological significance of observed changes in select genes remains to be determined.

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O 13

Effect of Proteolytic Enzymes on Cytokine Concentrations, Uterine Inflammation and Fertility in Postpartum Water Buffalo with Cytological Endometritis

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Proteolytic enzymes are used in human medicine due to their anti-bacterial, anti-inflammatory and fibrinolytic properties. Our objective was to evaluate intrauterine proteolytic enzyme therapy for treatment of subclinical endometritis (SCE) in buffalo. Buffaloes (n=38) diagnosed with SCE (≥18% polymorphonuclear cells in endometrial cytology) on day 21 postpartum (pp), were randomly allocated to the procedural control (PC; n=19; intrauterine infusion of 20 ml sterile saline on day 21 pp) and treatment (TR; n=19; intrauterine infusion of proteolytic enzymes namely Trypsin 8 mg, Chymotrypsin 8 mg, Papain 4 mg compounded in 20 ml normal saline) groups. Non-endometritic buffaloes (<18% PMN cells, day 21 pp) were kept as control group (NC; n=30). All the buffaloes were examined (transrectal ultrasonography for endometrial thickness and uterine horn diameter) and sampled [endometrial cytology using cytobrush for PMN%; low volume uterine flush as well as blood serum for PGF2α metabolite (PGFM), pro- (IL-1β, IL-8, TNF-α) and regulatory (IL-6 and IL-10) cytokine concentrations using direct-ELISA] on day 21 (before-treatment) and 28 pp. One-way ANOVA, paired sample t-test and Chi-square were used for statistical analyses. A significant (P<0.05) reduction in PMN% and horn diameter from day 21 to 28 pp was observed in NC and TR groups only, although PMN% in PC and TR remained always higher than of NC. Significant (P<0.05) reduction in endometrial thickness of TR group was observed. Pre-treatment uterine concentrations of only IL-1β, IL-8 and TNF-α were higher, while IL-10 and PGFM were lower (P<0.05) in TR and PC compared to NC group. Within groups, cytokine profiles in uterus and peripheral blood changed over the time. Uterine concentrations of IL-1β, IL-8 and TNF-α declined (P<0.05) both in TR and NC groups; whereas PGFM decreased in NC and IL-6 increased in TR group only. Treatment did not affect uterine IL-10 concentrations. Post-treatment serum concentrations of TNF-α and IL-8 in TR, whereas PGFM and IL-1β in NC group were increased. The first service conception rates, similar in PC (15.8%) and TR (31.6%), whereas higher in NC (43.3%) than PC (15.8%) were recorded. Pregnancy rates at 150 days pp were higher (P<0.05) in NC (66.7%) and TR groups (63.2%) compared to PC (31.6%). Further, comparison of TR and PC groups revealed reduction (P<0.05) in median days to conception by 27 days. In conclusion, our preliminary trial on intrauterine infusion of proteolytic enzymes recorded differential changes in uterine and circulatory cytokines and reduction in days open in endometritic cows.

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O 14

The role of unfolded protein response in mammary gland development and milk production

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The endoplasmic reticulum (ER) has a crucial role in quality control during the folding and secretion of secretory proteins. The accumulation of misfolded proteins in the ER provokes ER stress by increasing the demand for energy, chaperones, and other proteins that are needed to fold client proteins or to degrade unfoldable secretory cargo. Cells possess a defense mechanism against functional abnormalities in the ER, which is referred to as unfolded protein response (UPR). Under excessive or persistent stress, cells undergo apoptosis and die. Mammary glands are exocrine tissues that synthesize large amounts of proteins during lactation. Because UPR controls protein synthesis in cells and regulates secretory cell differentiation, it is believed to be involved in the homeostasis of lactation and development of the mammary gland. However, at present, the roles of UPR in the mammary gland tissue of dairy cattle are unknown.

To investigate the roles of UPR during the differentiation of mammary epithelial cells, we used MAC-T cells. We performed a biopsy to collect samples of mammary gland tissue in dairy cows during late gestation and lactation periods and examined the expression of UPR-related genes by quantitative real-time PCR. We found that UPR regulate the differentiation of mammary epithelial cells by controlling the expression of lactogenic hormone receptors. Moreover, we found that the expression levels of C/EBP homologous protein (CHOP), an apoptosis-related protein induced by ER stress, gradually increased prior to delivery and achieved significantly higher levels immediately after delivery, and a strong negative correlation was observed between the expression of CHOP and the initial milk yield. In order to know the trigger of UPR in mammary epithelial cells, we examined the effect of heat or fatty acid treatment on the expression of UPR related genes in MAC-T cells. Heat treatment (42 °C) caused an increase in the transcriptional level of CHOP. In addition, short- and middle-chain fatty acids decreased the transcriptional level of CHOP, but long-chain fatty acids increased it conversely.

These results suggest that the UPR plays a pivotal role in mammary gland development and the homeostasis of lactation. Furthermore, UPR in mammary epithelial cells may be affected by changes in temperature environment and lipid metabolism.

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O 15

Energy balance during the transition period of purebred Holstein and Simmental cows and their crosses

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The aim was to compare the energy balance through body condition score (BCS) and beta-hydroxybutyrate (BHBA) concentration in blood of purebred Holstein and Simmental cows and their crosses during the transition period at the Livestock Center of the Ludwig Maximilians University (Munich, Germany). The herd was composed of 128 lactating cows of the Holstein and Simmental breeds as well as their crosses. Cows were divided into 5 genetic groups (GG) according to their theoretic proportion of Holstein and Simmental genes as follows: Holstein (≥ 87.5% Holstein), R1 Holstein (between 62.5 and 87.4% Holstein), "F1" crossbreeds (between 37.5 and 62.4 Holstein), R1 Simmental (between 62.5 and 87.4% Simmental) and Simmental (≥87.5 % Simmental). The research was carried out from April 2018 until February 2019. Multiparous (n = 88) and primiparous (n = 51) cows were evaluated 3 weeks before calving until 8 weeks after calving. In a weekly routine, BCS (1 = very thin to 5 = very fat) and BHBA (in mmol/l) were evaluated. A mixed model variance analysis (SAS 9.4) with fixed effects GG, week, the interaction of GG * week, lactation (1, >1) and the repeated measure of the co-variance effect "cow" resulted in significant ($p<0.05$) GG and week effects for BCS and BHBA but no interaction effects. Cows with a high Simmental proportion (≥62.5 %) showed higher BCS (>4.07 ± 0.05) combined with lower BHBA levels than cows with lower (≤37.5 %) Simmental proportions (BCS <3.77 ± 0.05). The F1 crossbreed cows showed an intermediate BCS (3.82 ± 0.05). Generally, the average BCS declined continuously by ca. 0.05 points from the first week after calving (4.02 ± 0.05) until week 8 after calving (3.51 ± 0.06). Holstein and R1 Holstein cows presented significantly higher BHBA values (0.93 ±0.05 and 1.00 ±0.05) than Simmental (0.81±0.04), R1 Simmental (0.88±0.03) and F1 crossbreed cows (0.84±0.04). BHBA level increased to a maximum level in the first week after calving (1.02 ± 0.04) and then decreased to a level between 0.86 ± 0.05 and 0.93 ± 0.05 during week 6, 7 and 8 after calving. Three weeks before calving, however, the BHBA level started at 0.75 ± 0.05 mmol/l. Genetic composition of the cow and, expectedly, the transition status of the cow (time difference to calving) affect body condition and energy metabolism.

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O 16

The relationship between blood metabolites and milk fatty acids in early lactating dairy cows

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The periparturient period is associated with negative energy balance and increased risk of metabolic disease. Blood plasma nonesterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) are frequently used as indicators of compromised metabolic status in dairy cows. Milk fatty acids (MFA) has been launched as noninvasive and inexpensive biomarkers of compromised metabolic status and fertility in dairy cows. Relationships between blood NEFA and BHB and specific MFA have been described before. However, in contrast to previous studies, the present study included 19 commercial farms, which makes it possible to determine if the farm has a significant effect on the correlations. Specific MFA were analyzed with the aim to investigate if individual MFA or ratios of them can be used to predict NEFA and/or BHB by linear regression or other statistical models.

Milk and blood samples were collected simultaneously from 200 primiparous early-lactation dairy cows (2-30 days in milk) representing 19 commercial farms in southern Sweden. The blood samples were analyzed for NEFA and BHB and milk samples were analyzed for myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1 cis-9). Milk analysis was done by Fourier transform infrared spectroscopy (FTIR) with a CombiScope FTIR 300. Statistical analysis included correlation matrices and linear regression of relevant parameters.

Preliminary statistical analysis of the correlation matrix of BHB, NEFA and the milk FA specified above has shown a correlation of 0.52 ($P < 0.0001$) between LogNEFA and the ratio C18:1 cis-9 to C16:0. No other correlations above 0.5 were found in the correlation matrix.

The statistical model used (a general linear model) describes how NEFA changes in response to the ratio C18:1 cis-9 to C16:0, in other words how much of the variation in NEFA that can be predicted by the C18:1 to C16:0 ratio. It showed an adjusted R2-value of 0.37. ($P < 0.001$). The results also showed that farm had a significant ($P < 0.001$) effect on NEFA.

Conclusion: This study is apparently the first to show a correlation between MFA and NEFA in samples representing different herds with different management and feeding strategies. The correlation was modest. The fact that farm was significant in the model further indicates that these parameters vary between farms.

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O 17

Insulin mediates mTORC1 regulation of milk protein translation by essential amino acids in bovine mammary epithelial cells

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Essential amino acids (EAA) signal on the mechanistic target of rapamycin complex 1 (mTORC1) to regulate mRNA translation. In bovine mammary epithelial cells (BMEC), lactogenic hormones are required for synthesis of milk proteins. Therefore, we hypothesized that lactogenic hormones are also required for mTORC1 activation of mRNA translation in response to EAA in BMEC. To test our hypothesis, first, we investigated the effect of lactogenic hormones on mTORC1 signaling by Western blotting. Confluent, overnight starved primary BMEC were incubated in EAA-restricted (0.2 mM) DMEM/F12 supplemented with the lactogenic hormones insulin (100 nM), prolactin (100 ng/mL) and hydrocortisone (100 ug/mL) individually or as triple cocktail. Data were analyzed by ANOVA with Dunnett post-hoc test in R. Insulin stimulated the phosphorylation of mTORC1 substrate and mRNA translation regulator S6K1(T389) , with no additional effect of prolactin and/or hydrocortisone. Following, we investigated if insulin was required for EAA stimulation of mTORC1 activity. Primary BMEC were incubated in treatment medium containing none or DMEM levels of EAA, and insulin (0 or 100 nM) in a factorial arrangement. Intriguingly, insulin not only increased S6K1(T389) phosphorylation ($P<0.05$), but also doubled ($P<0.05$) the effect of EAA on that mTORC1 substrate. Finally, in four independent factorial experiments, we tested the effect of insulin (0, 10, or 100 nM) on individual EAA (Leu, Ile, Met, and Arg) stimulation of mTORC1 in immortalized BMEC (MAC-T). At each level of insulin, serum-starved cells (16 h) were incubated with four levels of each EAA (0, 0.5X, 1X, or 3X of reference plasma level in lactating dairy cows). Data were analyzed with the lm function in R. Astonishingly, insulin was required for maximum stimulation of mTORC1 by each of the four EAA, significantly ($P<0.05$) affecting linear and quadratic parameters of predicted equations for S6K1 and 4E-BP1(S65). Our results challenge the conventional limiting nutrient theory used to represent the interaction between metabolizable protein (MP, or individual AA) and energy in nutrient requirement systems, in which efficiency of one (e.g. MP) goes from its maximum to zero if the other (i.e. energy) is limiting but does not accommodate partial changes in efficiency or synergistic effects between the two. These results also entertain the idea of energy beyond calories, in which insulinemic and non-insulinemic energy sources would be playing different roles in production outcomes.

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O 18

Lipidomic profiles of milk from cows with consistently small or large milk fat globule size distributions suggest underlying metabolic differences

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Naturally occurring differences in milk fat globule size distributions within a dairy herd have been well established and milk fat globule size is emerging as a potentially valuable milk production trait. However, to aid the selection of cows for this trait, it is important to understand the underlying metabolic reasons for the characteristic milk fat globule size distributions in individual cows. Therefore, the aim of this study was to use milk, which contains a proportion of the apical plasma membrane of the mammary epithelial cell, as a window into the lipid metabolism of cows that consistently produce milk with predominantly small or large milk fat globule size distributions. To select the cows for the present study, milk fat globule size was repeatedly measured during a pre-trial observation period and the selected Holstein-Friesian cows fell into their respective size groups over at least three independent measurements. The cows' diet was pasture-based and supplemented with concentrate, which was fed during milking. Furthermore, the selected cows did not differ in other milk production traits and were in similar stages of lactation.

We applied a targeted lipidomics approach using triple quadrupole LC-MS/MS analysis to compare the lipidomic profiles of milk from cows presenting the small or large milk fat globule size phenotype and found several important differences. The milk from cows with the large milk fat globule phenotype contained an increased relative abundance of plasmenyl-ethanolamine by 33.08 ± 0.04 % (±SED, $p < 0.001$) and an increased relative abundance of plasmanyl-ethanolamine by 24.79 ± 0.08 % ($p = 0.045$) compared to cows with small milk fat globule size distributions. The small milk fat globule phenotype on the other hand was characterised by a 20.19 ± 0.02% increased PC/PE ratio ($p < 0.001$). Plasmenyl-ethanolamine has been suggested to promote lipid droplet fusion. Therefore, increased relative abundance of plasmenyl-ethanolamine in the large compared to the small milk fat globule group could impact the propensity of lipid droplets to fuse prior to secretion in the mammary epithelial cell and explain the shift towards larger milk fat globules. Increased relative abundance of PC and increased PC/PE ratios, observed in the small compared to the large milk fat globule phenotype, can protect lipid droplets from fusion, because PC is a more efficient surfactant than PE. This study suggests, for the first time, a potential role for ether phospholipids, particularly plasmenyl-ethanolamine, in the determination of milk fat globule size in the mammary epithelial cell.

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O 19

Essential amino acid profile of metabolizable protein affects mammary gland amino acid metabolism in dairy cattle

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Mammary gland metabolism in response to incomplete essential amino acid (EAA) profiles was examined using 5 rumen-fistulated, Holstein-Friesian dairy cows (2.8 ± 0.4 lactations; 81 ± 11 d in milk) subjected to continuous 5-d abomasal infusions (according to a 5 x 5 Latin square design) of saline (SAL) or 562 g/d of EAA delivered in 4 profiles: 1) complete EAA mixture (EAAC), 2) Ile, Leu, and Val (BCAA), 3) His, Ile, Leu, Met, Phe, Trp, Val (GR1+ILV), and 4) Arg, His, Lys, Met, Phe, Thr, Trp (GR1+ALT). Individual AA content in each profile corresponded to their relative content in casein. A corn silage-based total mixed ration was formulated to meet 100 and 83% of net energy and metabolizable protein (MP) requirements, respectively, and fed at 90% of individual cow ad libitum intake. Arterial and venous blood samples with respect to the mammary gland were collected at 5 time points on d 4 of infusion. Data were subjected to ANOVA using a mixed model (treatment and period as fixed effects; cow as random effect). Multiple comparisons between treatment means were made using the Tukey-Kramer method. Differences were considered significant at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$. Milk protein yield did not differ between EAAC, GR1+ILV, and GR1+ALT, or between SAL and BCAA, and increased over SAL with EAAC and GR1+ILV. Mammary plasma flow increased with BCAA infusion compared with EAAC and GR1+ILV. Infusion of EAAC tended to increase mammary net uptake of total EAA, in particular that of group 2 AA (Arg, Ile, Leu, Lys, Thr, Val) and decreased the mammary uptake to milk protein output ratio (U:O) of non-EAA compared with SAL. Infusion of BCAA increased uptake and U:O of branched-chain AA over all treatments. Uptake of branched-chain AA tended to be higher on GR1+ILV compared with GR1+ALT, and uptake of non-branched-chain group 2 AA was higher on GR1+ALT compared with GR1+ILV. The U:O of Lys decreased with GR1+ILV infusion compared with EAAC. During GR1+ALT infusion, U:O of non-branched-chain group 2 AA was greater than that during EAAC infusion. In conclusion, when Ile, Leu, and Val or Arg, Lys, and Thr were absent from abomasal EAA infusions, intramammary catabolism of present group 2 AA compensated in support of milk protein synthesis. These findings illustrate flexibility in mammary metabolism of group 2 AA when the EAA profile of supplemented MP is incomplete with respect to casein.

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Poster presentations

P 01

High-fiber by-product feedstuff as a substitute for corn in feedlot diets: dry matter intake, cattle growth and carcass traits

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Introduction: Feedlot diets normally contain high proportions of non-fiber carbohydrates, especially starch. This nutrient intake may cause digestive disorders such as ruminal acidosis. Consequently, the use of high-fiber byproducts can reduce the risks of acidosis and optimize performance. Dry corn gluten feed (DCGF) seems to present less palatability than corn, so researches on the inclusion levels are necessary. The purpose of this trial was to evaluate dry matter intake, performance and carcass traits of bulls fed dry corn gluten feed.

Materials & Methods: A hundred twenty Nellore bulls with average initial body weight of 361±15 kg were used. Animals were blocked by weight and allocated to pens with 5 animals each and 6 pens per treatment and fed 113 days. Four treatments with increasing levels of DCGF were used (T1 – Control, T2 – 180 g/kg, T3 - 360 g/kg and T4 - 540 g/kg of DCGF). The experimental diets contained 135, 135, 139 and 166 g/kg of crude protein and 557, 486, 408 and 306 g/kg of non-fiber carbohydrates, respectively. All diets contained 112 g/kg of roughage NDF (132 g/kg of sugarcane bagasse). Animals were slaughtered in a commercial abattoir. Fat content of L.dorsi muscle was obtained according to AOAC (2007.04) protocols. Statistical analyses were performed through SAS, using MIXED procedure with linear, quadratic and Control vs. DCGF contrasts.

Results: Inclusion of increasing levels of DCGF had a quadratic effect ($P<0.05$) on dry matter intake (9.60; 10.85; 10.42 and 10.16 kg/day). On average, the inclusion of DCGF increased average daily gain by 0.15 kg/d in comparison to the control (1.51 vs. 1.66 kg/day), with quadratic tendency ($P=0.06$) according to the inclusion levels (1.51; 1.66; 1.69 and 1.65 kg/day) Feed conversion was not affected by the treatments ($P>0.05$). The use of DCGF promoted a linear increase ($P<0.05$) in slaughter weight (531.5; 548.0; 553.4 and 548.8 kg) and carcass weight (294.3; 301.6; 307.4 and 304.9 kg, $P<0.01$). Other carcass traits such as carcass yield, ribeye area and backfat thickness were not affected by the treatments ($P>0.05$). However, the intramuscular fat content in L.dorsi had a quadratic tendency ($P=0.055$) in response to the treatments (2.52; 2.90; 2.66 and 2.58%).

Conclusion: DCGF inclusion up to 540 g/kg in feedlot diets improves dry matter intake, live weight gain, carcass weight. Small to moderate inclusion of DGCF can improve intramuscular fat in Longissimus muscle.

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P 02

Effects of rumen-protected folic acid supplementation on reproductive performance in ewes

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Uptaking folic acid in human before and during early pregnancy reduced the risk of neural tube defects, such as anencephaly and spina bifida. However, the influences of FA on reproductive performance of ewes is still unclear. The aim of this study was to evaluate the effects of rumen-protected FA (RPFA) on fertility rate of ewes, lamb mortality, lamb weight and litter weight at birth, and the pre-weaned average daily gain of lambs. One hundred twenty Hu ewes (24 ± 4.2 months of age and 44.6 ± 5.43 kg of BW) by estrus synchronization and artificial insemination, were assigned to one of three groups in a randomized experimental design, 40 ewes for each group. During the pregnant period, ewes were fed a total mixed ration with 0 mg (control), 16 mg and 32 mg FA from RPFA per kg dry matter (DM), respectively. The results showed that fertility rate was higher for 16 mg group (82.5%) than for control (67.5%) and 32 mg group (65.0%), whereas lamb mortality rate was the lowest for 16 mg group (2.63%) followed by control (3.44%), the highest for 32 mg group (10.53%). There was no significant influence of RPFA on lambing ratio and litter weight at birth. The lambing ratio for control, 16 mg group and 32 mg group were 220%, 237% and 219%, and litter weight for control, 16 mg group and 32 mg group were 8.24, 8.38 and 7.94, respectively. However, the lamb weight at birth quadratically decreased and was lower for 16 mg group (3.67 kg) than for control (3.91 kg) and 32 mg group (3.87 kg). Average daily gain during the first 30 days of age and subsequent 20 days quadratically increased with RPFA supplementation, and was higher for 16 mg group than for control and 32 mg group. The results showed that supplemented 16 mg/kg of FA from RPFA in the pregnant ewes diets improved fertility rate and lambing ratio, decreased lamb mortality and lamb weight at birth, increased average daily gain of lambs during pre-weaned, supplemented 32 mg/kg of FA from RPFA in the pregnant ewes diets was not benifical for fertility rate, lamb survival rate and average daily gain of lambs.

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P 03

Ascorbic acid inhibits oxidative stress of mastitis induced by LPS in mice

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Introduction: Mastitis is one of the most severe diseases in daily cows, leading to enormous economic losses in daily industry. Most cases of mastitis are caused by bacteria, especially E. Coli, a gram negative bacteria, triggers strong immune responses through lipopolysaccharide (LPS) on bacterial cell walls in the mammary gland. Ascorbic acid (AsA) is the most abundant and probably most important water-soluble antioxidant in mammals (Sauberlich, 1994) and is recently reported to be related to mastitis (Weiss & Hogan 2007), but the mechanism is still unknown. In the present study, we investigated the effect of AsA on LPS-induced mastitis using mouse mastitis model.

Materials and Methods: Lactating ICR mice were divided into two groups: control and AsA. Mice on the AsA regimen received fresh daily L-ascorbic acid (330mg/100ml) substituted for drinking water for 7 days. On the 10th day of lactation (7th day of AsA treatment), LPS solution (5 μg/50 μl) was injected into fourth inguinal mammary gland (left side) through teat duct. Phosphate buffered saline (PBS) was injected into the right side mammary gland of the same animal. Six or 24 hour after LPS and PBS injection, blood, the mammary glands and liver were collected under anesthesia. Aliquots of the mammary gland and liver tissue were homogenized and the supernatants were used the following analyses. TBARS, ascorbic acid and sulfhydryl contents of tissue homogenates and plasma were determined according to Ding et al. (2004), Okamura (1980), and Ellman (1959), respectively. For the histological analysis, the mammary glands were fixed with 10 % formalin and dehydrated in ethanol. After paraffin embedding, 10 μm sections were cut and strained with hematoxylin and eosin (H&E).

Results and Discussion: The AsA-supplemented mice had higher plasma ascorbic acid levels than control ($P<0.05$). Plasma TBARS concentrations were not affected by the AsA treatment. AsA concentrations in the liver were not changed by the treatment. In the mammary gland, TBARS concentrations were increased by LPS and decreased by AsA treatment. AsA and sulfhydryl contents of the mammary gland were decreased by LPS. The control group showed uniform structural integrity and no pathological change in both control and AsA treatment. After LPS challenge, abundant inflammatory cells were observed in the tissue. However, these histopathological changes were ameliorated by AsA treatment.

In conclusion, oral administration of AsA exerts anti-inflammatory effects in LPS-induced mastitis by altering redox status in the liver and mammary gland.

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P 04

The impact of phantom training systems to habituate heifers to automatic milking systems

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The automatic milking system (AMS) is growing in popularity as it is based on individual voluntary milking visits and supports animal welfare. The first contact of heifers with AMS is associated with stress, because they are not familiar with that system at their first milking contact shortly after the stressful parturition. This stress can negatively affect the milk yield and influence animal health. The objective of this study is to examine if training at an AMS-phantom before birth will support the health of the heifers in the future, aiding increased animal welfare.

77 Holstein Friesian heifers were divided into two experimental settings: group 1 (control group; n=34) and group 2 (phantom group; n=43). The heifers of group 2 were introduced to the phantom four weeks before calving and given free access to explore the phantom by positive conditioning with concentrate. The phantom is similarly built as the AMS (Lely Astronaut A4) to familiarise heifers with their future milking situation. In contrast, heifers of group 1 had absolutely no contact to the phantom; hence, their first contact with the AMS was at the first milking. In order to assess the calving process, faecal samples were collected every other day from 28 days prepartum till 42 days postpartum to determine the cortisol metabolites. Additionally, milk yield, milking frequency, milk flow and milk composition were recorded for each animal. Failed and refused milkings were also registered. Furthermore, the scope of backfat thickness by ultrasonographic measurement was estimated to obtain body conditions of the heifers.

65.7% of group 1 and 79.1% of group 2 had a normal calving process. Heifers of group 1 had a higher percentage of calvings classified as moderate (31.4%) than heifers of group 2 (20.9%). 14.7% of group 1 had stillbirths, whereas group 2 had only 4.7% stillborn calves. In general, group 2 had higher cortisol levels before calving than group 1. The cortisol profile of the phantom group showed a typical progress with a clear prepartal increase followed by a drastic decline throughout and after birth, whereas this was not obvious in group 1. No statistical differences were found in milk compounds, milk yield and backfat thickness between the two groups. Showing more milking visits in the first few days of lactation indicates that group 2 is better adapted to the AMS.

In summary, training at the phantom has a positive impact on animal welfare.

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P 05

A phytogenic feed additive altered the fatty acid profile of beef

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Phytogenics, botanicals or phytobiotics, are plant extracts or mixtures of plant-derived compounds, which are proposed as natural growth promotants. This study assessed the effect of a phytogenic feed additive (Digestarom® (DA), Biomin, Getzersdorf, Austria) containing primarily licorice, caraway, vanilla and clove oil, salt and silicon dioxide on growth performance, feed intake, and fatty acid composition of finishing steers. One hundred and twenty Angus × Charolais crossbred steers (initial BW 488 ± 26.5 kg) were blocked by weight and randomly assigned to 12 pens with 10 steers per pen. Each pen was allocated to 1 of 3 diets and steers were fed for a total of 110 d. The basal diet (Control) contained 86.5% barley, 10.0% barley silage and 3.5% vitamin and mineral supplement (dry matter basis). In the two of the diets, DA was pelleted in the vitamin and mineral supplement at 1.41 and 2.82 g/kg, to achieve mean daily DA intakes of 0.5 (LowDA) and 1.0 g (HighDA) per steer. All three diets were prepared once daily and provided *ad libitum* (targeting 2% refusals). Steers were weighed every 28 d. At slaughter, samples from the *pars costalis diaphragmatis* were collected from 20 steers within each treatment. Fatty acid methyl esters (FAME) were quantified using gas chromatography. Dry matter intake (average: 9.34 kg/d) did not differ ($P>0.05$) among diets, but average daily gain (ADG) tended ($P<0.09$) to increase in response to DA (1.82, 1.87 and 1.95 kg/d, respectively, for control, LowDA and HighDA). Supplementation of DA tended to result in a quadratic response in palmitic acid (C16:0; $P=0.07$) and total proportion of saturated fatty acids (SFA; $P=0.06$); with steers fed LowDA containing the lowest proportions (% total FAME). Steers fed LowDA had higher C18:1 t9 ($P<0.05$) and C18:1 t10 ($P<0.05$) compared with HighDA and control steers, resulting in a tendency for a quadratic response in C18:1 t6-8 content ($P=0.06$). In contrast, trans vaccenic acid (TVA; C18:1 t11), linolenic acid (C18:3), CLA c9t11, and CLA+TVA linearly ($P<0.05$) decreased with increasing level of DA in the diet. The share of unsaturated (USFA) and monounsaturated fatty acids (both % of total FAME) tended ($P=0.06$) to respond quadratically to DA. Percentage of polyunsaturated fatty acids was low (average of all diets: 2.72 ± 0.1 %) and not affected by feeding DA. These results demonstrate that LowDA in particular, reduced SFA, while increasing USFA content, suggesting that DA potentially modulates biohydrogenation in the rumen.

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P 06

Effects of centrifugation and cholesterol-loaded cyclodextrin in the soybean lecithin -based extender on quality of the post-thaw Ghezel ram sperm

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The goal of this research was to clearly elucidate the effects of centrifugation and cholesterol-loaded cyclodextrin (CLC) on a soy lecithin-based extender on post-thaw ram sperm quality. Sperm samples of 5 adult ram of Ghezel were collected for 3 weeks. After the initial assessment, the approved semen samples were combined and divided into 8 equal parts in the Falcon tube. Four samples were combined at 30°C and the seminal plasma was then removed by centrifugation. The semen from the other four tubes was not centrifuged and was diluted with Tris buffer + different concentrations of CLC (0, 0.75, 1.5, and 3 mg / 120 x 106 spermatozoa) and 7% glycerol, similar to the first group. The sample was then cooled to 5°C and frozen in a 0.25 ml of straws. After thawing, total motility (TM) and progressive motility (PM), straight-line velocity (VSL), curvilinear velocity (VCL), beat-cross frequency (BCF), total antioxidant capacity (TAC), superoxide dismutase (SOD), Malondialdehyde (MDA), glutathione peroxidase (GPX), the integrity of the acrosome membrane and the integrity of the plasma membrane were significantly ($P < 0.05$) higher in the CLC group 1.5 mg compared to other groups. The integrity of TM, PM, VCL, TAC, SOD, GPX and plasma membrane was significantly ($P < 0.05$) higher in the group without centrifugation than in the centrifugation groups. Overall, the results suggested that 1.5 mg CLC was better than the other groups in most of the sperm parameters measured in vitro.

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P 07

Circulating prolactin concentrations are decreased by reduced energy intake but not by reduced milking frequency in dairy cows around dry-off

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Thirty-two Holstein cows were used in a randomized design to study the effect of the management factors energy intake and milking frequency on blood prolactin concentrations around dry-off. During the last seven days before dry-off, cows received a diet equivalent to either 100% or 50% of energy requirements for cows in late lactation, and were milked either twice or once daily in a 2 × 2 factorial arrangement (Adlib×2, Adlib×1, Red×2, and Red×1). After last milking at day 0, all cows received the same diet fulfilling requirements for dry cows. Feed intake and milk yield were recorded daily. Blood samples were collected on day -9, -6, -5, -2, 0, 1, 2, 5 and 7 relative to dry-off. Plasma prolactin concentrations were determined by radioimmunoassay. The MIXED procedure from SAS was used and the model included energy intake, milking frequency, sampling day, and the interaction between them as dependent variables, sampling at day -9 as covariate and animal as repeated subject. Values were significant when $P<0.05$. As expected, the Adlib×2 and Adlib×1 groups had higher energy intake than the Red×2 and the Red×1 groups ($P<0.05$). No differences in energy intake were observed between either the Adlib×2 and the Adlib×1 groups (126.9 ± 4.20 and 118.6 ± 4.20 MJ/d, respectively) or the Red×2 and the Red×1 groups (75.9 ± 4.20 and 71.1 ± 4.20 MJ/d, respectively). Milk yield was affected by the interaction between energy level and milking frequency ($P<0.05$). The highest milk yield was recorded in the Adlib×2 group (24 ± 0.84 kg/d) and the lowest in the Red×1 group (12.8 ± 0.84 kg/d). No differences in milk yield were detected between the Adlib×1 and the Red×2 groups (16.3 ± 0.84 and 16.1 ± 0.84 kg/d, respectively). Prolactin concentrations in blood were affected by energy intake ($P<0.05$), but not by either milking frequency ($P>0.05$) or the interaction between energy level and milking frequency ($P>0.05$). Thus, the Adlib×2 and Adlib×1 groups showed higher prolactin concentrations (21.05 ± 2.71 and 23.34 ± 2.71 ng/mL, respectively) than the Red×2 and Red×1 groups (14.81 ± 2.71 and 11.09 ± 2.71 ng/mL, respectively). In conclusion, prolactin concentrations decreased around dry-off when energy intake is reduced to 50% of the energy requirements. Despite the fact that reduced milking frequency decreased milk yield before dry-off, prolactin concentrations were not affected.

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P 08

Involvement of Cyclin B1 and Cyclin B3 in testicular development promoted by Vitamin E in prepubertal sheep

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With regard to Vitamin E, it is characterized that it is able to promote testis development. Some studies have uncoverd that Vitamin E can increase testicular size and diameter of seminiferous tubules, as well as improve histological feature of the seminiferous tubules in sheep and goat. However, specific mechanism on Vitamin E regulating testis development in prepubertal sheep remain poorly understood. Our prior studies demonstrated that Vitamin E was able to change cell cycle phase. To further identify key candidate genes on cell cycle, we isolated the testicular cells in 2 months of age Dorper×thin tailed Han crossbred sheep (♂×♀) by collagen digestion, seeding into 100-mm plastic culture dishes. After 85% confluence of the cells, which were divided into 2 groups, including control and Vitamin E group, the cells in Vitamin E group are treated with Vitamin E for 24h. Then, all the cells were collected and used to q-PCR and Western blot analysis. The q-PCR results showed that Vitamin E significantly increased Cyclin B1 ($P < 0.05$) and Cylin B2 ($P < 0.01$) mRNA expression level. Simultaneously, it had an increasing trend for Cyclin A1($0.05 < P < 0.1$). Vitamin E had no significant impact on Cyclin B3 ($P = 0.127$) and Cyclin A2($P = 0.130$) although mRNA expression level rised. As for protein expression level, Vitamin E strikingly enhanced Cyclin B1 ($P < 0.01$) and Cyclin B3 ($P < 0.05$) expression. Overall, Cyclin B1 and Cyclin B3 are both crucial mediator for regulation of testicular cell cycle in response to our previous results that Vitamin E increased testicular cell proportion in G2/M phase.

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P 09

Application of principal component analysis and non-hierarchical clusters to study crossbreed Angus-Nellore bulls feedlot finished

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In beef cattle production, some carcass and meat quality traits (fatness, tenderness, and marbling) are difficult to measure consistently. In addition, the relationship among them is not usually clear when studied separately. Thus, multivariate statistical tools such as the principal component (PCA) and the clustering analysis can be applied to identify the most important variables. To study the influence of carcass weight on meat quality traits of crossbreed Angus-Nellore bulls, data from 98 animals aged 24 months in feedlot finishing were used. The PCA was performed to characterize variables in carcass and longissimus thoracis (LT) muscle. The variables comprised hot carcass weight (HCW), cold carcass weight (CCW), carcass dripping losses (DL), total losses (TL), carcass yield (CY), pH (48h postmortem), rib eye area (REA), backfat thickness (BFT), shear force (SF), cooking losses (CL), color (lightness, yellowness, redness, chromaticity and hue), intramuscular fat (IMF), protein, collagen, moisture and ash. Considering HCW as a separation criterion, the K-means cluster analysis was applied to classify animals in three groups (low, moderate and high HCW). The five first PCs explained about 64.5% of total data variability and meat color (yellowness, chromaticity, and redness) was more effective to define the first PC. The CCW and CY were more effective to define the second and third PCs, respectively. In this study, beef samples with higher values of SF (tough meat) commonly showed lower chromaticity values. The PCA results also showed that BFT was positively associated with IMF. Color had a high weight to characterize PC 1 and consequently, chromaticity, yellowness, and redness were the correlated variables. The three HCW groups were projected in the plane defined by PC 1 (23.8 % variance) and PC 2 (13.5 % variance). The PCs were named as “Meat color aspect” and “Low carcass weight plus high losses”, respectively. HCW means in the clusters were 301.37±16 (I), 348.31 ± 12 (II) and 386.62 ± 15 (III) kg with 24, 48 and 25 animals in each group, respectively. The projection of HCW groups I, II and III in the plane allowed to distinguish carcass weights in relation to meat color aspect and carcass weight losses. Animals with lower HCW and CCW showed higher DL and TL values, and also presented lower moisture values. PCA and K-means clustering analysis were useful multivariate techniques to identify crossbreed bulls regarding carcass and meat quality.

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P 10

Factors affecting birth weight and growth rate of lambs from the Icelandic sheep breed

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In Iceland, the sheep production year is characterized by lambing in May, weaning and slaughtering in September and October. There is considerable variation in the lamb weaning weight, which influences profitability. Our hypothesis is that variation in weaning weight can be controlled by better management and planning of the production. The aim of this study was therefore to define the main factors affecting variability in weaning weight. The Hestur sheep experimental farm was established in 1943 and has since then been a hub for breeding work and nutritional studies of the Icelandic sheep breed. We analyzed data from twelve production years in that farm, 2002 to 2013, with total records of 9938 lambs born to 1851 dams. In the whole dataset, 7.5% of the lambs were born as singles, 77.4% as twins, 14.4% as triplets and 0.8% as quads. Surrogate mothers fostered 5.5% of the lambs.

We fitted multilevel models to data on lamb birth weight and growth rate. The nested error term represented by the ewe population was included as a grouping random effect. This model gave a significantly better fit than a model with simple error term. Fixed group effects that significantly influenced birth weight were (presented as least square means, kg): litter size (singles 4.60; twins 4.01; triplets 3.35; quads 3.02), lamb sex (male 3.84; female 3.65), dam age (2 year old dams gave significantly lower birth weights than older dams), and production year (3.68 to 3.84). The model describing birth weight, also included a positive linear relationships with ewe weight in the 1st month of pregnancy, ewe weight gain from 1st to 3rd month of pregnancy, and a 2nd degree relationship with body condition score in 3rd month of pregnancy. Fixed group effects significantly influencing growth rate from birth to weaning at 4-5 months of age were: rearing type (as singles 264.0; as twins 235.9 g d-1); lamb sex (males 263.6; females 237.4 g d-1), dam age (growth rate highest for 3 and 4 year old dams), production year (238.8 to 262.5 g d-1), time of weaning (late October 240.0; late September 254.5 g d-1). There were also positive linear relationships with lamb birth weight, ewe weight in 3rd month of pregnancy, and body condition score in last month of pregnancy. In conclusion, multilevel models on lamb birth weight and growth rate provide an opportunity to predict and control the variability in lamb weaning weight.

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P 11

Effects of different dietary NFC/NDF and niacin addition for regulate hepatic gluconeogenesis of perinatal ewes

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The purpose of this study was to investigate the effects of different dietary NFC（Non-fibrous carbohydrates）/NDF （Neutral detergent fiber）and niacin addition for regulate hepatic gluconeogenesis of perinatal ewes. Forty 58.9±4.55 Kg perinatal 3-year-old Erdos fine wool ewes were randomly divided into 4 groups with 10 animals in each group. With a 2×2 completely randomized trial design. The first factor was the NFC/NDF ratio, which was 0.44 and 0.84 respectively. The second factor is the amount of niacin in the diet, which is 2g/d and 4g/d each. The diet was divided into four groups: group I (NFC/NDF=0.44+2 g), group II (NFC/NDF=0.44+4 g), group III (NFC/NDF=0.84+2 g), and group IV (NFC/NDF=0.84+4 g). The trial period was prenatal 40 days to postpartum 21 days. During feeding, blood and milk samples of ewe were collected, and milk composition and blood physical and chemical indexes of ewe were determined. Liver samples were collected in vivo to determine the expression of key genes and glycogen related proteins in liver.The results showed that: 1) When the NFC/NDF ratio was 0.84 and niacin content was 4g/d each, the blood GLU concentration significantly increased(P<0.05), while the NEFA concentration significantly decreased(P<0.05); 2) The expression of PCK1, PC, FOXO1, PI3K and INSR mRNA of hepatic gluconeogenesis had significant effects in prenatal 21 days by different NFC/NDF diets (P<0.05). The expression of PCK1, PC, SIRT6 and FOXO1 mRNA of hepatic gluconeogenesis were significant affected in postpartum 9 days by different NFC/NDF diets(P<0.05). The expression of PC mRNA in prenatal 21 days and PC, SIRT6 and FOXO1 mRNA in postpartum 9 days were significant affected by the different NA levels(P<0.05). So, the different NFC/NDF diets and niacin addition can activate FOXO1 protein activity to regulate PCK1 and G6P activities through INSR-AKT/PI3K-FOXO1 pathway which can effectively improve NEB（Negative balance of energy）and production performance of perinatal ewes. In addition, FOXO1 protein activity can be regulated by the P53-SIRT6-FOXO1 pathway to regulate hepatic gluconeogenesis. In conclusion, dietary addition of niacin with a NFC/NDF of 0.84 was 4g/ d, which could only promote liver glycoeogenesis of perinatal ewes and alleviate NEB.

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P 12

Extended view on young stock losses in Holstein dairy cattle - First results from the EIP-project "Die Entwicklung des KUH-mehr-WERT Navigators"

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The EIP-project "Die Entwicklung des KUH-mehr-WERT Navigators" („The development of the COW-more-VALUE") is a multicentric cooperative five-years project in which different partners from the dairy industry in Brandenburg (Germany) are working together to develop an economical risk analysis tool for dairy cow production systems. One aspect which is intensively analysed within the project are the losses that occur between calving and the onset of first lactation and the risk factors in the respective young stock rearing systems.

Twelve dairy farms were analysed, with a dataset containing 41´178 calvings between Jan 2012 and June 2018. Farm characteristics: average size of 553 cows (235 - 1383), German Holstein breed, total mixed ration (TMR)-based confinement system, an average milk production of 10´561 kg (9´977 - 11´234 kg) per cow and lactation, 29.6 % (15.9 - 38.2 %) culling rate, age at first calving of 25.4 (23.9 - 29.2) months and stillbirth rates in cows and heifers of 7.9 % (2.5 - 11.1 %) and 9.7 % (4.5 - 16.3 %), respectively (mean (min.-max.)), data from 2017/2018). The proportion of number of cows and young stock ranged between 0.78 and 1.06 (av. 0.93).

The young stock losses were analysed in four distinct phases: 1. Birth, 2. Living newborn to insemination, 3. Insemination to first calving, 4. First calving to 30 days in milk (young stock sold for breeding purposes excluded, only female offspring included). The overall loss rate is devided across the four periods as follows: 1. 7.9 % (5.8 - 9.2 %), 2. 15.3 % (7.6 - 26.2 %), 3. 4.9 % (2.6 - 10.3 %) and 4. 6.4 % (2.4 - 10.4 %), accumulating to 34.4 % (22.1 - 49.2 %) in total. The proportion of animals that died or were euthanised (vs. sold for slaughter) was: 2. 65.0 % (43.1 - 77.8 %), 3. 10.7 % (2.1 - 27.3 %) and 4. 17.2 % (6.2 - 54.5 %).

In summary, approximately one third of the potential offspring is lost between birth and the first month in lactation and this total loss is 1.7 times higher than when just the phase from living newborn to calving is considered.

Different influences for the respective phases were evaluated and following attributes were identified as risk factors: 1. and 2. birth weight, twins, gestation length, parity, 3. number of inseminations and bulls, age and weight at first insemination, 4. calving ease, twins, stillbirth, sex of calf. Further, in-dept analysis of housing and management aspects as influencing factors are planned in the scope of the project.

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P 13

Effects of adding different levels of α-tocopherol on post- thaw sperm quality of ram semen

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Ram sperm is particularly sensitive to oxidative stress, due to the high percentage of polyunsaturated fatty acids present in the membrane, which can be oxidized and lead to lipid peroxidation. Antioxidants may ameliorate the effects of the freeze-thawing stress on cryopreserved spermatozoa. We evaluated the effect of different levels of α-tocopherol on sperm motility and lipid peroxidation. Sperm samples of 5 adult Ghezel rams were collected for 3 weeks. After the initial assessment, the approved semen samples were pooled and divided into 4 equal parts in tubes. The semen from the other four tubes was diluted with Tris buffer + different concentrations of α-tocopherol (0, 1, 2, and 3 mM) and 7% glycerol. The sample were then cooled to 5 ° C and frozen in a 0.25 ml of straws. In the group receiving 2 mM α-tocopherol, the results indicated a significant improvement in total motility and progressive motility when compared to control group. The 2 mM α-tocopherol showed a lower percentage of MDA levels when compared to control group. The results showed that addition of 2 mM curcumin improved most of the evaluated parameters compared to other groups.

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P 14

Effect of curcumin on post-thaw variables and oxidative status of ram semen

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Reactive oxygen species cause damage to mammalian sperm during freezing. Curcumin is a known antioxidant compound against oxidative stress. The aim of this study was to investigate the effects of curcumin in soybean lecithin-based extender on various parameters of sperm motility, and lipid peroxidation of Ghezel ram semen. Sperm samples of 5 adult Ghezel rams were collected for 3 weeks. After the initial assessment, the approved semen samples were pooled and divided into 4 equal parts in the tubes. The semen from the other four tubes was diluted with Tris buffer + different concentrations of curcumin (0, 1, 2 and 3 mM) and 7% glycerol. The results indicated a significant improvement in total motility and other parameters of sperm motility and decreased lipid peroxidation in the 2mM curcumin group after the thawing process compared to other groups ($p < 0.05$). Generally, the results showed that addition of 2 mM curcumin improved most of the evaluated parameters compared to other groups.

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P 15

The dynamics of oxidative stress development at the final stage of pregnancy in cows

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The present study assessed oxidative stress during pregnancy, characterized by changes in the peroxide and antioxidant status of high-yielding dairy red-motley cows (n=154) during the physiological and pathological progression of pregnancy and the postpartum period. The study was conducted at farms, located in the Voronezh region of the Russian Federation. The experiment included the cows of 7 - 8 months of pregnancy and 2 - 1 week before calving. The obtained data reveals that during the seventh to the eighth month of pregnancy period, the content of malondialdehyde in the cows' blood increased by 8.7% (1.03 ± 0.06 vs 1.12 ± 0.04 μM/l). During the two to one week before calving period, its concentration kept on growing by 12.6% and 16.5% (respectively 1.16 ± 0.06 and 1.20 ± 0.03 μM/l). Such an increased content of peroxidation products in blood may indicate the stress state of animals, and intense of metabolic processes occurring in the body of pregnant cows. In relation to the non-enzymatic component of the antioxidant system in the studied animals, we have determined that from the seventh to the eighth month of pregnancy, the concentration of vitamin E had decreased by 4.6% (from 32.4 ± 2.40 to 30.9 ± 1.92 μM/l, with P <0.01), and at two weeks and at one week before parturition – by 20.7 - 33.9% respectively. The same trend is observed of carotene content. The enzymatic component of the antioxidant system of pregnant animals changed in a similar way – from the seventh month of pregnancy to two and one weeks before calving, catalase, glutathione reductase and ceruloplasmin activity decreased by 7.3-9.5, 3.6-3.9% and 9 1-15.5%, respectively (P <0.01). The exhibited differences in the enzymatic and non-enzymatic components of the antioxidant defense system indicate a high level intensity of the lipid peroxidation processes at the final stage of pregnancy, and consequently, their role in preventing the development of oxidative stress of pregnancy and parturition.

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P 16

Sex steroids increase skeletal growth rate in Bos indicus-crossbred steers but do not affect histological changes in the growth plate

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The maximum skeletal elongation rate (SER) for Bos indicus-crossbred steers has not been determined. Skeletal elongation rate drives muscle growth through a passive stretch mechanism. Previous studies found that changes in hip height (HH), as a proxy for SER, could be increased at a high metabolisable energy intake (MEI) of a high crude protein (CP; 200 g/kg DM) diet but not when ME intake was restricted, regardless of the CP content of the diet. Hormonal growth promotants (HGP) have anabolic effects promoting increased protein deposition in muscle and an increase in height and skeletal growth of steers and lambs. The current experiment examined changes in liveweight and skeletal dimensions of Bos indicus-crossbred steers from 167 ± 7 kg liveweight (mean ± standard deviation) to 601 ± 53 kg liveweight. Steers were allocated to one of three HGP treatments [Control (sham implant), E2 (Compudose100 implant; 21.1 mg oestradiol 17β; Elanco, Australia) or TE2 (Component TE200; 200 mg trenbolone acetate and 20 mg oestradiol 17β; Elanco) with n=8/treatment) and had ad libitum access to a 11.6 MJ ME, 165 g CP/kg DM pellet from self-feeders in a single 1.4 Ha paddock. Skeletal dimensions were measured every two weeks and bone biopsies were collected from the tuber coxae when the average liveweight of steers were 239 ± 15 kg, 356 ± 32 kg, 506 ± 49 kg and 583 ± 52 kg. HH was used to describe the growth curve and the equation of Brody (1945) was fitted to obtain parameters of growth. The results showed that there was no significant difference in change in HH for Control and E2 steers but TE2 steers had a higher maturation rate (P=0.01) and attained a mature HH earlier than the other treatment groups (P<0.01). Hip height growth of all steers slowed as they approached maturity. The overall growth plate length was shorter (P<0.01), the height of the hypertrophy zone (P<0.01), proliferative zone (P<0.01) and the diameter of terminal hypertrophy chondrocytes (P=0.07) all declined as steers approached maturity. The trabecular bone showed a significant decline in bone volume fraction (P=0.01), bone surface (P<0.01) and trabecular thickness (P<0.01) and showed a greater trabecular separation (P<0.01) as steers approached maturity. However, implant treatment did not significantly affect any of the growth plate and trabecular bone measurements. In conclusion, this information will enable meat producers to develop feeding strategies to increase the performance of cattle based on their growth phase.

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P 17

Insulin and magnesium increase the activity of GPDH in bovine adipocytes

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Purpose: Lipomobilization at the onset of lactation is linked to insulin deficiency and/or insulin resistance. Magnesium is known to increase sensitivity to insulin and could thus potentially alleviate lipomobilization. The aim of the present study was to measure the activity of glycerol-3-phosphate dehydrogenase (GPDH) in bovine adipocytes at different concentrations of magnesium and insulin to investigate the potential role of magnesium for insulin sensitivity. GPDH is an adipogenic differentiation marker that catalyzes the reaction of dihydroxyacetonphosphate and NADH to glycerol-3-phosphate and NAD+.

Material and methods: Bovine subcutaneous adipose tissue was collected from five young calves. Preadipocytes were cultivated as an explant culture. After 48 h, preadipocyte differentiation was induced and differentiating cells were subsequently incubated with various concentrations of magnesium (0.1 mM, 0.3 mM, 1 mM, and 3 mM) and insulin (25 pM, 250 pM, and 25 nM) in a two-factorial design for 7 days. GPDH activity was measured in 1 x 106cells by a colorimetric GPDH activity assay kit. One unit of GPDH represents an enzyme activity required to generate 1.0 µmole of NADH per minute. Results are given as percentage relative to preadipocyte GPDH activity (GPDH%).Statistics were conducted by two-way repeated measures ANOVA.

Results: Following incubation over 7 days, GPDH% was affected by both insulin ($P = 0.045$) and magnesium concentration ($P = 0.037$). The effect of insulin was based on a trend towards lowest GPDH% at intermediate insulin concentration (125 ±13 % at 250 pM), especially when compared to the lowest insulin concentration (188 ±17 % at 25 pM; $P = 0.054$). A concentration of 3 mM magnesium induced a trend for higher GPDH% (214 ±21 %) compared to all other magnesium concentrations (138 ±15 % at 0.1, 135 ±15 % at 0.3 mM and 136 ±15 % at 1 mM; $P < 0.062$ each).

Conclusion: Insulin affects GPDH activity of cultivated bovine adipocytes. Surprisingly, however, a trend for lowest activity was observed at concentrations that would reflect high insulin levels *in vivo* (250 pM). A high magnesium concentration of 3 mM increased GPDH activity independently of insulin. The latter may point to the fact that the magnesium effect on GPDH activity may not necessarily be a direct consequence of increased insulin sensitivity.

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P 18

Protein fraction distribution and amino acid content in milk of midlactating dairy cows fed hybrid rye grain

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New hybrid varieties of rye are available and characterized by certain nutritional benefits over wheat, such as higher mineral, lysine and fiber content. The effects of cereal grains typically used in diets for dairy cows have been widely investigated, but information on the effect of hybrid rye grain on milk composition and its technological suitability is limited. The aim of this study was to determine the effect of partial or full replacement of wheat grain with hybrid rye grain in the diet for dairy cows on milk yield and composition, amino acid (AA) composition, and protein fraction structure (PFS).

Thirty midlactating (112±41 DIM) Holstein-Friesian dairy cows were randomly allocated to 3 groups of 10 cows each and fed diets with concentrate mixture containing wheat grain (W; 100%); wheat and hybrid rye grains (WR; 50%/50%) or hybrid rye grain (R; 100%). Diets were fed as TMR for 56 days and during the last 28 days, individual milk samples were collected weekly. Chemical composition of milk, including AA and PFS were determined using standard laboratory procedures. Data were analyzed using one-way ANOVA and the Tukey's test, with dietary rye level as the main factor.

Milk yield tended (P=0.08) to be lower for W compared to WR but tended (P=0.07) to be higher for W compared to R. Significant differences were found for milk yield between WR and R (31.5 *vs.* 29.9 kg/d; P<0.05). The milk contents of total solids, protein, lactose, fat and urea, as well as proportion of β-casein, α-lactalbumin and lactoferrin in total protein content (TPC), were not affected by treatments. The highest proportion of α-casein in TPC (40.02%; P<0.05) was observed in R group. The lowest proportion of k-casein in TPC, highest β-lactoglobulin, immunoglobulins and serum albumin was for WR. These features indicate that WR milk is more suitable for cheese production than for highly heated products such as UHT milk or milk concentrates. With the exception of milk concentration of methionine, arginine, leucine and glutamine that tended to be lower for R compared to W and WR, contents of other AA were not affected by treatment. Lower milk yield and changes in milk composition when R fully replaced W in the diet suggests that hybrid rye grain should be offered to lactating cows in a combination with other sources of grain, such as wheat.

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P 19

Can infrared ocular thermography monitor growth in dairy calves?

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For efficient, successful rearing of replacement dairy heifers we need to monitor their health and growth in a reliable and practical way. Infrared thermography (IRT) is a non-invasive remote method that can help detect fever or localised inflammation and which has shown potential to monitor productive attributes. As such, ocular surface temperature (OST) measured by IRT has been associated with residual body weight gain in dual purpose calves in the tropics (J. Dairy Sci. 2018,101:5060-68). There is a strong correlation between OST and body core temperature in several species including cattle. Furthermore, the eye provides a suitable body surface for automatic IRT measurements on farm (Res. Vet. Sci. 2012,93:928-35). The aim of this study was to determine whether OST measured by IRT is associated with growth in housed dairy calves (n=30) under temperate conditions. Starting in the week after birth, OST and body measurements were collected every two weeks for three months by the same operator using a standardised protocol. Three IRT images were taken per animal at each recording, along with body weight (BW), girth circumference (GC), wither height (WH) and environmental parameters including wind speed, ambient temperature, relative humidity, and level of light. The average OST of the three IRT images was calculated using the maximum temperature from each IRT image and was used for analysis with linear regression. OST ranged from 32.5 °C to 38.5 °C with a mean (±standard deviation) of 36.3 (±0.85) °C. The coefficients of determination using OST as predictor of BW (r^2=0.031, P=0.011), GC (r^2=0.008, P=0.122) and WH (r^2=0.002, P=0.252) were low. OST measurements were not affected by wind speed, ambient temperature, relative humidity, light levels, nor time of day at which measurement was taken (r^2 of the model=0.181). In conclusion, OST measured by IRT does not explain much of the variation observed in growth variables of dairy calves during their first three months of life and thus appears to have limited value as a non-invasive method for monitoring growth. Whether this lack of association between growth performance and infrared ocular thermography will change later in life remains to be determined.

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P 20

Can high immune response sires reduce disease in a small UK dairy herd?

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Reducing production diseases is a major goal in the dairy industry and one strategy might be selective breeding for robustness, using sires with superior estimated breeding value for antibody and cell-mediated immune responses. Data from large dairy herds in the US and Canada suggested that high immune response (HIR) sires (i.e. *Immunity*+® bulls from SEMEX) can decrease lameness and mastitis incidence in their progeny (Cattle Pract. 2017,25:74-81) with potential benefits for reproductive performance as well (Jap. J. Vet. Res. 2015,63,Suppl.1:S37-S44). Given that herd-level disease prevalence usually increases as herd size increases (Prev. Vet. Med. 2009;88:264-77), the usefulness of *Immunity*+® bulls needs to be examined in small dairy herds, where the occurrence of production diseases might be low. This is relevant for countries such as the UK, where the average herd size is 146 cows (AHDB Dairy, 2018). Furthermore, the impact of *Immunity*+® bulls is also unknown on farms using other technology to improve herd health such as automatic milking systems (AMS) (J. Dairy Sci. 2018,101:9599-9607). The aim of this study was to examine the impact of *Immunity*+® bulls on disease occurrence and reproductive performance of their progeny in a small dairy herd managed by AMS. Cows sired by *Immunity*+® bulls (n=53) were compared to matched controls (n=53) in terms of number of lactations (1-4 lactations). Data were analysed by logistic and linear regression models including type of bull (*Immunity*+®, controls), bull, age, lactation number, and milk yield. *Immunity*+® bulls did not influence the cases of mastitis (17% in *Immunity*+® vs. 13% in controls) and lameness (34% in both groups). Likewise, the variation in somatic cell count (89,334 ± 18,046 cells/ml in *Immunity*+® vs. 120,981 ± 25,050 cells/ml in controls) and services per conception (1.9 ± 0.13 in *Immunity*+® vs 2.1 ± 0.11 in controls) was not affected by the use of *Immunity*+® bulls. Lactation number influenced the likelihood of lameness, with most cases observed in the first three lactations. Bull, cows' age, and milk yield did not influence any of the variables tested. Overall, our data suggest that the use of *Immunity*+® bulls may not be an effective way of decreasing the occurrence of production diseases in small dairy herds with good health management. However, the possible benefits in subsequent lactations (>4 lactations) need to be investigated.

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P 21

Investigations on the influence of energy concentrations in rations of fattening bulls on different carcass characteristics

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The current German recommendations on the energy and nutrient supply for fattening bulls are based on experiments which were conducted more than 30 years ago. These recommendations cover a range of average daily gains from 600 to 1400 g/d for animals of the German Holstein breed. The proportion and amount of fat accretion during the fattening period influences the energy utilization and requirement. The present study was conducted to provide data for animals of the current fast-growing fattening bulls. A total of 48 fattening bulls of the German Holstein breed were used for the experiment. The animals were equally divided into two feeding groups receiving a ration with either a low (20%) or a high (60%) concentrate proportion on dry matter basis. Maize silage was used as the roughage component in both rations. Over the experimental period of 9 months sub-groups of bulls were slaughtered at three different time points approximately three, six and nine months after start of the trial. In course of the experiment and before slaughter the live weight was recorded. During the slaughter process the fat depots (retroperitoneal, omental, mesenteric and subcutaneous fat) and all other parts of the carcass were weighed. The meat of one carcass half was manually separated from the bone and weighed. By adding the masses of the corresponding carcass parts, the mass of the empty body (mass of the animal without contents of the digestive tract as well as the urinary and gall bladder) was calculated. The average daily gain of the animals was in a range of 1700 to 1900 g/d. The sum of the fat depot mass was higher when the ration with the enhanced concentrate proportion was fed ($P = 0.045$) and increased in the course of the study. Between the slaughter time points a doubling of the fat depot masses was observed. Relative to the empty body mass, it was found that the meat mass did not differ between the feeding groups and slaughter time points. However, the percentage of total fat depot mass increased with progressive slaughter time points ($P < 0.001$). The data from the present study suggest that the fat accretion increases with advancing age and the average daily gain of the current fast-growing fattening bulls could be higher than the ranges in the recommendations for energy and nutrient supply.

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P 22

Comparison of prepartum diets varying in dietary cation anion difference and K and Ca contents for prevention of hypocalcemia

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Feeding rations low in dietary cation anion difference (DCAD) prepartum is a common strategy to improve peripartum calcium homeostasis. However, several studies reported negative effects on dry matter intake (DMI). Results on the suitability of decreasing the potassium content without lowering the DCAD are inconsistent. As a low calcium intake prepartum has been shown to be beneficial, too, we aimed at comparing the effects of two rations low in potassium but differing in calcium content and two different rations low in DCAD (based on mineral salts or hydrochloric acid) on calcium homeostasis and performance. We fed the following rations during the last 3 weeks of gestation to 48 multiparous dairy cows allocated to four groups: LCLK: 0.24% Ca, 1.14% K, DCAD: +86 mEq/kg DM, HCLK: 1.23% Ca, 1.17% K, DCAD: +95 mEq/kg DM, AMS (supplemented with anionic mineral mixture): 1.21% Ca, 1.21% K, DCAD: -112 mEq/kg DM, and CAS (supplemented with SoyChlor®): 1.28% Ca, 1.16% K, DCAD: -115 mEq/kg DM. All animals received the same diet after calving and data were collected until day 21 postpartum. Prepartum DMI was lowest in the AMS group and highest in the LCLK group. Prepartum serum concentrations of Ca, P, and Mg as well as postpartum serum concentrations of P and Mg did not differ, while postpartum Ca was lower in the HCLK group compared to LCLK, AMS and CAS, especially 24 h and 48 h after parturition. Postpartum DMI was significantly higher in LCLK and CAS cows than in animals fed the HCLK and AMS ration. Serum concentrations of insulin and glucose were not affected by the different treatments. Although prepartum serum concentrations of non-esterified fatty acids were higher in the AMS group, postpartum concentrations were not altered. Energy-corrected milk yield was highest in cows fed the LCLK ration prepartum. Interestingly, milk protein concentration was lower in CAS cows compared to LCLK, HCLK and AMS. This might be related to a significant treatment effect of the CAS ration that was observed for postpartum serum concentrations of total protein, albumin and globulin. Furthermore, serum urea was significantly lower in the CAS group pre- and postpartum. As both prepartum and postpartum DMI were higher in comparison to two commonly used anionic diets and no negative effects on milk yield or milk protein and fat content could be observed, feeding a LCLK ration prepartum might have some advantages over other feeding regimes.

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P 23

The effect of prepartum treatment with vitamin D_3 on mineral homeostasis and energy metabolism in dairy cows

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At the onset of lactation, calcium homeostasis is severely challenged in dairy cows. As both the clinical and the subclinical form of hypocalcaemia are known to negatively affect feed intake, insulin secretion, the function of smooth muscles and immune function, they are associated with an increased risk of developing various other production diseases. One of the strategies to prevent hypocalcaemia is the administration of vitamin D before calving. However, the production of the biologically active form of vitamin D, 1,25-dihydroxycholecalciferol (1,25-$(OH)_2D_3$), is stimulated by a decrease in blood calcium that results in the secretion of parathyroid hormone (PTH) from the parathyroid gland. As 1,25-$(OH)_2D_3$ acts via the genomic pathway, about 24 hours are required before gastrointestinal Ca transport is increased significantly. In addition, high plasma concentrations of 25-hydroxycholecalciferol (25-OHD_3) that are observed after vitamin D treatment, might directly interfere with vitamin D metabolism, PTH secretion and mineral homeostasis. This might be the reason why data on the suitability of this approach are inconsistent.

In the present study, eighteen cows entering their 2nd or later lactations were randomly allotted to a control group (Con, N = 8) or treated intramuscularly with 10,000,000 I.U. vitamin D_3 (Vit D, N = 10) 3 to 10 days before parturition, at day 273 of gestation. Blood samples for determination of ionized calcium (Ca^{2+}), and plasma samples to be analyzed for total calcium (Cat), phosphate (Pi), magnesium (Mg), parathyroid hormone (PTH), 25-OHD_3, non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), insulin and glucose were taken from the tail vein between 14 and 17 hours and between 51 and 54 hours postpartum.

Vitamin D treatment resulted in significantly higher Ca^{2+}, Cat and Pi concentrations and significantly lower Mg concentrations. While 25-OHD_3 levels were substantially increased, plasma PTH concentrations were significantly reduced in the Vit D group in comparison to Con cows. NEFA, BHB, insulin and glucose remained unaffected.

Taken together, prepartum treatment with vitamin D seems to have beneficial effects on Ca homeostasis during the first two days after calving. However, further research is needed to evaluate whether the changes observed for Pi, Mg, PTH and 25-OHD_3, factors that are known to interfere with bone mobilization and vitamin D metabolism, exert any side-effects on mineral homeostasis and energy metabolism later on.

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P 24

Dietary amino acid regulation of murine lactation is mediated by mTORC1

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Dietary amino acids (AA), the building blocks of proteins, drive synthesis of milk protein in the mammary gland. The mechanistic target of rapamycin complex 1 (mTORC1) is an ubiquitously expressed, highly conserved kinase complex that integrates nutrient and endocrine signals to regulate metabolic functions, including synthesis of milk components. We hypothesized that mTORC1 is indeed crucial in the regulation of murine lactation by dietary AAs.

To test this hypothesis, C57BL/6J dams were randomly assigned to 1 of 3 treatment groups (n=7 dams, litters standardized to 5 pups/dam) on lactation day (LD) 1: adequate protein (AP, 18% crude protein, CP), protein restricted (RP, 9% CP), or AP plus every other day intraperitoneal injection of 4 mg/kg rapamycin (AP-R). On LD13, mice were sacrificed and tissues harvested for analysis. Live animal data, including litter and dam weights, milk production, and dam daily food consumption were analyzed by two-way ANOVA with repeated measures in RStudio. Tissue western blot data were analyzed by one-way ANOVA with Dunnett post hoc multiple comparisons against AP treatment.

By LD13, milk production was 66% less for RP and 48% less for AP-R compared to AP ($p<0.05$). Similarly, litter weight was 31% less for RP and 28% less for AP-R ($p<0.05$). Dam weight remained unchanged despite differences in lactation performance. Phosphorylation of mTORC1 substrates S6K1(T389), S6(S240/244), and 4E-BP1(S65) in the mammary glands respectively decreased 30, 37, and 66% for RP, and 61, 85, and 33% for AP-R ($p<0.05$) compared to AP. Substrate phosphorylation in the liver was differentially affected, with only S6(240/244) decreased by 40% for AP-R ($p<0.05$).

Overall, pharmacological inhibition of mTORC1 closely mimicked the effects of severe dietary AA restriction on lactation performance, suggesting a central role of mammary mTORC1 in the regulation of milk and milk protein production by dietary AA.

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P 25

The propylene glycol usage in the prevention protocol of subclinical / clinical forms of ketosis in dairy cows

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This study is intended to be a method of early detection of ketosis, rapidly, since maternity of newly-born cows (5-6 days postpartum) in order to stop or reduce the metabolic stress of specimens prone to this metabolic condition. The study was conducted at the farm of the Research and Development Station for Bovine, ARAD, in a herd of 116 cows in maternity. We considered that 5-6 days after the birth, the amount of ketone bodies that can be detected in urine harvested from each parturient and is relevant to both existing metabolic problems and potential future problems.

To highlight ketone bodies in the urine, we used the strip-based Dirui reagent immerse for 40 seconds in containers where fresh urine was harvested. The stripping substance impregnated on the strip is sodium nitroprusside. Revealing colors are increasing from pink to dark purple, depending on the ketone body co-centering in the test sample. For instance, light pink is the colour corresponding to urine with 0.5 mmol / L ketone bodies and violet with dark shade for 16 mmol / L.

In the evaluations of our experiment, the maximum incidence of clinical ketosis occurred in the cold months of January and February in a number of 11 from 39 cows postpartum.

In these cows 6 were detected with 16 mmol per L and we applied treatments with KETOMIX POLMASS product 1L per day administered in 2 halves followed by another 5 days each 500ml in a single intake.

The results are favorable and encouraging, so in 5 of them, the average interval between the date of birth and the date of the first heat occurred from 83.8 days, and the interval between fertilization and fecundation was 132 days. The other 5 ketosis cows, where the test results were 8 mmol / L, the average time interval of the first heat was 81.2 days and the interval between fertilization and fecundation was 171 days on average. Concluding, in our experiment the administration of propylene glycol had good results, primarily by the decreased number of cows with ketosis and secondary by the optimization of intervals between birth to the first heat and fertilization to fecundation.

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P 26

Effects of apelin stimulation on cell proliferation and differentiation of myoblast cells from Japanese Shorthorn cattle

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Introduction: Apelin, a peptide isolated from bovine stomach extracts, appears to act as an endogenous ligand for the previously orphan G-protein-coupled APJ receptor. Apelin mRNA is expressed in the brain, spinal cord, pituitary, heart, lung, testis, adipose tissue, placenta and mammary gland. In previous studies, we have reported some of the effects of apelin in ruminants. It has been shown that administration of apelin to sheep increases growth hormone secretion, suggesting that apelin may act as a growth factor. Therefore, the aim of this study was to investigate the effects of apelin stimulation on cell proliferation and differentiation using myoblast cells from Japanese Shorthorn cattle.

Materials and Methods: 1) Effect on cell proliferation: Myoblast cells isolated from *the longissimus muscle* of one Japanese Shorthorn cattle (male, 8 months old) were used. Cells were seeded in cell culture plates and cultured for 14 days while measuring the number of cells at intervals of 48 hours. As growth medium, Dulbecco's Modified Eagle Medium (DMEM, high glucose) containing fetal bovine serum (10%) was used. In addition, a medium for apelin stimulation was prepared by adding apelin to a growth medium at a concentration of 1.0×10^{-7} M as a stimulation group. 2) Effect on differentiation: The cells were cultured to subconfluency, differentiation induction stimulation was performed using DMEM (low glucose) containing horse serum (2%). In the same manner as in 1), an apelin stimulation medium was prepared in which apelin was added at a concentration of 1.0×10^{-7} M to a differentiation induction medium as a stimulation group. After 4 days differentiation induction, the expression levels of myoD and myogenin mRNA were examined using real-time PCR. The results were considered significantly different when $P<0.05$, and a "tendency" was defined when $P<0.10$. The *t*-test was used to identify significant differences between the control group and the stimulation group.

Results and conclusion: 1) From the 4th to the 10th day of the culture start, the number of cells in the apelin-stimulated group was significantly higher than the number of cells in the control group. 2) In real-time PCR, the expression level of myoD was significantly higher in the apelin-stimulated group, and the expression level of myogenin tended to be larger in the apelin-stimulated group.

These results suggest that apelin may promote cell proliferation and differentiation into muscle fibers in myoblast cells derived from Japanese Shorthorn cattle.

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P 27

The antilipogenic effect of t10c12-CLA does not explain marine lipid-induced milk fat depression in dairy ewes: Insights from a meta-analysis

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Diet-induced milk fat depression (MFD) is presumably caused by the effects of ruminal biohydrogenation intermediates with antilipogenic activity. However, in marine lipid-induced MFD the involvement of *t*10*c*12-CLA, which is the only intermediate shown unequivocally to inhibit milk fat synthesis in ruminants, has been questioned, particularly in sheep. Thus, we conducted a meta-analysis to summarize the relationship between milk *t*10*c*12-CLA concentration and milk fat traits in lactating ewes. A database comprising 23 trials conducted by our team has been used. Fifty-five dietary conditions were characterized and grouped in two major categories (experimental treatments): non-MFD and marine lipid-induced MFD. The non-MFD category included 40 diets without supplementation or supplemented with plant oils and extracts, which modified the fatty acid profile of milk without detrimentally affecting milk fat synthesis. The marine lipid-induced MFD group comprised 15 diets supplemented with fish oil or microalgae. To ensure stable responses to diets, only the data collected after 21 or more days of adaptation were considered. Differences in diet formulation and chemical composition between the two groups of experimental treatments (non-MFD and MFD) were analyzed by one-way ANOVA. Linear and quadratic relationships between milk fat traits (yield and concentration of fat and yield of de novo synthesized and preformed fatty acids) and milk *t*10*c*12-CLA levels were examined using the MIXED procedure of SAS. Diet characteristics that may influence the response to lipid supplementation (e.g., forage:concentrate ratio, fibre concentration or starch content) did not differ between marine lipid-induced MFD and non-MFD groups. Prediction models showed an inverse linear relationship between *t*10*c*12-CLA and milk fat concentration in both treatments (R^2=0.78; P<0.05), which was unexpected, particularly in the non-MFD conditions. Nevertheless, this relationship was equivalent in the two experimental treatments and, therefore, the difference in milk fat concentration between non-MFD and MFD (on average, -19% in the latter) remained constant irrespective of *t*10*c*12-CLA levels (P>0.10 for the treatment × *t*10*c*12-CLA level interaction). Similarly, milk fat yield was 23% lower in MFD than in non-MFD conditions (P<0.001), regardless of *t*10*c*12-CLA proportions. In both treatments, the linear decrease in the yield of de novo synthesized fatty acids with incremental *t*10*c*12-CLA levels was counteracted by increases in the secretion of preformed fatty acids derived from plasma uptake (P<0.001). In conclusion, our results might support a relationship between increases in *t*10*c*12-CLA levels and decreases in milk fat concentration that, however, does not explain the marine lipid-induced MFD in lactating ewes.

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P 28

Effects of dietary energy concentration on feed intake and growth performance of fattening Fleckvieh and Braunvieh bulls

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The objective of this study was to evaluate the effect of dietary energy concentration on feed intake and growth performance of growing Fleckvieh and Braunvieh bulls of modern type.

Methods: Data from three feeding trials involving a total of 104 Braunvieh (BV, age: 153 d, body weight (bw): 222 kg) and 105 Fleckvieh (FV) bulls (age: 159 d, bw: 233 kg) were evaluated. In each trial bulls were equally distributed to two feeding groups receiving maize silage and concentrates based Total Mixed Rations with either a high or a norm energy concentration (12.1 vs 11.5 MJ Metabolizable Energy (ME)/kg Dry Matter (DM) on average) for ad libitum intake. Individual feed intake was automatically recorded daily while BW was recorded every four weeks. The bulls were slaughtered at an average age of 465 d, respectively. Data were evaluated using a two factorial model using SAS. Data are presented as LSMEANS and pooled standard error (s.e.).

Results: There was no effect of breed or dietary energy concentration on DM intake but ME intake was higher ($P<0.05$) in group ME high compared to group ME norm. Final BW, daily gains and carcass weight were higher ($P<0.05$) in FV compared to BV bulls. Increasing dietary energy concentration increased final body weight, daily gain and carcass weight in FV bulls (750 vs. 772 kg, s.e.: 9; 1701 vs. 1766 g, s.e.: 22; 418 vs. 435 kg, s.e.: 5) but not in BV bulls (725 kg; 1611 g; 392 kg). Energy intake per kg of BW gain was higher ($P<0.05$) in group ME high compared to group ME norm and higher in BV compared to FV bulls ($P<0.05$). Most parameters of body fat concentration where higher in BV compared to FV bulls and higher in ME high compared to ME norm group.

Conclusions: Results of the present study confirm the potential for high growth rates in BV bulls, which is, however, lower than in FV bulls. Increasing dietary energy concentration led to significant increase in indicators of body fat concentration with a comparable extent in both breeds. Growth rate and carcass weight was, however, increased by higher energy concentration in FV but not in BV bulls. Therefore it is concluded that increasing dietary energy concentration up to the high level of 12.1 MJ ME/kg DM may be reasonable in feeding FV but not in feeding BV bulls.

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P 29

Effects of rumen-protected choline, propylene glycol and monensin sodium on blood parameters of ghezel ewes during late gestational feed restriction

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Due to coincidence of sheep late pregnancy with low forage availability, sheep undergo different levels of feed restriction in this period which results in imbalanced metabolic status and finally metabolic disease. In order to study the effects of administration of ketogenic and glucogenic additives in the ration of feed restricted ewes during last six weeks of pregnancy, 48 Ghezel ewes (body weight 60.21±1.39 kg) were randomly divided into six treatments (n=8) as follows: 1) control (CON); 2) restricted feed (RES- 60% of the control diet); 3) restricted feed+ propylene glycol: 30 g/d (RES-PG); 4) restricted feed+ propylene glycol+ monensin sodium: 30 mg/d (RES-PG-M); 5) restricted feed+ propylene glycol+ rumen protected choline chloride: 15 g/d (RES-PG-RPC); 6) restricted feed+ propylene glycol+ monensin sodium+ rumen protected choline chloride (RES-PG-M-RPC). Blood samples were taken from the jugular veins of the ewes on days 55, 28, 21, 10, 5 prior to parturition, and 2 hours and days 7 and 17 postpartum. Metabolic characteristics like plasma concentration of glucose, non-esterified fatty acid (NEFA), ß-Hydroxybutyrate (BHBA), triglyceride, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined for the assessment of the nutritional status of animals. Statistical analyses were performed using the Statistical Analysis System (SAS 9.3.1). The results showed that RES-PG-M-RPC and RES ewes had totally the highest and the lowest ($P<0.05$) concentration of glucose during whole experimental period respectively (72.96±3.26 vs 55.39±3.36). Ewes in RES-PG-RPC treatment had higher ($P<0.05$) concentration of triglyceride compared with RES ewes (39.40±3.70 vs 28.65±3.54) with RES-PG-M-RPC, RES-PG-M, RES-PG and CON ewes being intermediate. Feed restriction resulted in elevated concentration of BHBA ($P<0.05$) and NEFA ($P<0.05$) (0.77±0.06 and 0.8±0.04 for BHBA and NEFA respectively) compared to other treatments while RES-PG-RPC and RES-PG-M-RPC resulted in the lowest concentration of NEFA (0.51±0.05) and BHBA (0.55±0.05) respectively. Serum AST and ALT were not affected by treatments significantly; however, RES-PG-RPC ewes had a lower concentration of AST ($P=0.75$) and ALT ($P=0.80$) compared to other treatments. In conclusion, results showed that late gestational undernutrition negatively affects maternal physiological and biochemical reactions and administration of propylene glycol and choline chloride individually or in combination with monensin sodium in the ration of pregnant ewes during prepartum period preventing negative effects of negative energy balance or metabolic disease.

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P 30

Effect of prepartum administration of propylene glycol, monensin sodium and choline during food restriction period on lamb growth and development

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An increased energy supply during late gestation is required to support conceptus growth and maintenance requirements of the ewe which results in sharp conflict between the high nutrition requirements of ewes and shortage of feed supply from farmland. Maternal undernutrition not only restricts production performance of ewes but also affects lamb birth weight and postnatal growth. In order to investigate the effect of maternal nutrient restriction during late gestation and specific additives for reducing negative effects resulting from nutrient restriction, 48 Ghezel ewes (body weight 60.21±1.39kg) were randomly divided into six treatments (n=8) during the last six weeks of their gestation as follows: 1) control (CON); 2) restricted feed (RES- 60% of the control diet); 3) restricted feed+ propylene glycol: 30 g/d (RES-PG); 4) restricted feed+ propylene glycol+ monensin sodium: 30 mg/d (RES-PG-M); 5) restricted feed+ propylene glycol+ rumen protected choline chloride: 15 g/d (RES-PG-RPC); 6) restricted feed+ propylene glycol+ monensin sodium+ rumen protected choline chloride (RES-PG-M-RPC). Immediately after parturition, placentas of ewes were gathered, washed and then maintained at 4ºC to process in the laboratory. The placentas were weighed, the placentomes were counted, and the placental efficiency was calculated as the relation between the average weight of the newborn lambs and the average weight of the placenta from each ewe. After parturition all lambs were weighed and average daily gain of lambs was recorded weekly until 30 days. Results were analyzed statistically by Statistical Analysis System (SAS 9.3.1) according to a completely randomized design. RES-PG-M ewes had a lower ($P<0.05$) placenta weight compared with CON ewes (781.64±23.17 vs 477.29±24.98) with RES, RES-PG, RES-PG-RPC, RES-PG-M-RPC ewes being intermediate. Total placentome number was the highest and the lowest ($P<0.05$) in CON and RES ewes respectively (75.87±2.11 vs 57.55±1.82). The placental efficiency was higher ($P<0.05$) in RES-PG-RPC compared with RES ewes (12.85±0.53 vs 8.16±0.52). Lambs from RES-PG-RPC ewes were heavier ($P<0.05$) and had a higher average daily gain ($P<0.01$) than those from RES ewes (4.97±0.09 vs 3.69±0.1 and 189.58±6.92 vs 115.89±5.81) with lambs from CON, RES-PG, RES-PG-M and RES-PG-M-RPC being intermediate. The results of this study indicate that administration of propylene glycol and rumen protected choline to restricted feed of ewes during late gestation can have beneficial effects on average birth weight and average growth rate of lambs.

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P 31

Comparison of milk yield and somatic cell count of German and New Zealand dairy cows

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The objective of the study was to compare milk yield (MY) and somatic cell count (SCC) of dairy cattle breeds or crossbreds mainly used in New Zealand (NZ) and Germany (GE). NZ is the world´s 8th largest milk producer and exports 95 % of its milk. Therefore it is the largest and most important exporter of dairy products. Besides NZ, the EU is second largest exporter and within the EU GE produces and exports high amounts of milk. Hence, those two countries were chosen to be compared. Six NZ farms and eight GE farms were visited and the MY and SCC were recorded. A total of 713 lactating cows entered the study. A mixed model variance analysis was performed by using SAS 9.3 with a restricted maximum likelihood (REML) estimation method. SCC was transformed into somatic cell score (SCS=log2(SCC/100) +3) to obtain a normal distribution. Fixed effects were: country (NZ, GE), "breed/crossbred (NZ Holstein Friesian = NZ HF, NZ Jersey = NZ J, NZ Kiwi Cross (NZ HF x NZ J) = KiCr, GE Holstein Friesian = GE HF, GE Fleckvieh = GE FV) x country", and number of lactation (1-5), "country x milking frequency (1-3)" in combination with the covariate "days in milk x days in milk". Concerning MY, cows in GE and NZ differed significantly ($p<0.05$). At the beginning of the last third of lactation, GE HF reached daily milk yields of about 33.34 (±2.15) litres in comparison with 12.74 (±2.61) litres of NZ HF. NZ J reached the lowest daily milk yield with 9.61 (± 2.70) l/day, while KiCr performed slightly below NZ HF. GE cows are allowed to higher concentrate intakes contrary to mainly grass feeding in NZ, and GE cows contrary to NZ cows are selected for high milk yield. Unexpectedly, the SCS did not differ between NZ (3.40 ± 0.26) and GE (2.92 ± 0.33) although milk yield in NZ is lower. Commonly, milk yield is negatively correlated with SCS regarding physiology. The different dairy systems might be the reason. In NZ, most of the cows are kept on pasture throughout the year exposing them to all weather conditions; while, additionally, farmers do not pre clean the teats. In GE, instead, cows are mainly kept indoors, and climate does not influence them as much. In conclusion, though GE cows produce more milk than NZ cows the SCS does not differ between GE and NZ.

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The effects of in-feed resin acid inclusion on milk production responses of dairy cows

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The objective of this study was to investigate the effects of in-feed resin acids on milk production of dairy cows during early lactation. Thirty-six Nordic Red cows were used in a continuous feeding trial starting 3 weeks prepartum and lasting for 10 weeks into lactation. The cows were loose-housed with individual feed intake measurements. The diet consisted of grass silage ad libitum and the amount of pelleted concentrate given from automated feeders was gradually increased from 3.5 kg at parturition to 12 kg per day. The three dietary treatments were: Control with basal concentrate (CON),CON supplemented with tall oil fatty acids (TOFA; 9% resin acids) at 7 g/cow/day or CON supplemented with resin acid concentrate (RAC; 37.5% resin acids) at 1.68 g/cow/day. TOFA and RAC are natural mixtures of resin acids from Norway spruce and Scots pine consisting mostly of abietic acid and dehydroabietic acid. They were produced by Forchem (Rauma, Finland) and TOFA is a commercial product Progres® (Hankkija, Hyvinkää, Finland). Feed intake and milk production were measured continuously and milk samples were collected at weeks 2, 3, 6 and 10.

The proportion of concentrate during lactation averaged over all diets was 0.40 of dry matter (DM) intake ($P>0.10$). The average energy corrected milk (ECM) production of the cows on diets CON, TOFA and RAC were 43.2, 43.5 and 43.6 kg/day and respective total DM intakes 23.9, 24.3 and 23.9 kg/day resulting in no differences in feed efficiency presented as kg ECM production per kg DM intake (1.82, 1.80 and 1.84). Milk fat concentration was 46.3, 47.9 and 44.7 g/kg while that of milk protein 39.7, 39.2 and 39.0 g/kg for diets CON, TOFA and RAC, respectively. The time was statistically significant for all reported performance parameters ($P<0.01$), but diet and diet×time interaction were not statistically significant ($P>0.05$), although ECM production on diets TOFA and RAC were numerically 0.29 and 0.40 kg/day higher during the 10-week period. It seemed that both supplements resulted in faster and higher milk production peak during the first weeks of lactation as e.g. during weeks 2&3, the milk production on RAC diet was on average 2.08 kg/day higher than on CON ($P<0.075$). Sensory analysis of milk revealed no differences between treatments. Further studies on rumen fermentation and blood analyses will provide clearer insight into the mode of action of resin acids.

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Relationship between milk yield and body and udder characteristics in Bedouin goat reared under the Sahara desert conditions

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Bedouin goat is one of the most adapted ruminants to arid conditions of the Saharan environment, because of its heat resistance, water economy and energy balance performance. To study body and udder characteristics of this breed and their correlation with milk yield we used 11 multiparous goats in their second month of lactation which were maintained in a sheepfold. The mammary morphology was assessed by nine-point linear scale to evaluate udder depth, udder and teats form, teats inclination and orientation and udder susponsory system strength. Results showed a shallow udder, not favorable to high milk production, but adapted to desert rangelands which represent a risk of injury and contamination for deep udders. Udder and teats morphology was not entirely favorable to milking. In fact, the udder was generally globular with long thin teats, vertically inclined, which was desired. However, the teats have a bottle or funnel shape and divergent orientation and the supernumerary teats were frequents which can hinder the milking. The suspensory system was of a medium force with a well-marked medium ligament, a balanced rear attachment width and a loose fore attachment. Body weight, trunk length, withers height, rump height, chest depth, chest width, rump width and ear length were respectively 19.5 ± 0.8 kg, 61.1 ± 1.3 cm, 64.2 ± 0.9 cm, 66.3 ± 1.2 cm, 26.9 ± 0.5 cm, 8.1 ± 0.2 cm, 8.6 ± 0.4 cm and 14.4 ± 0.5 cm. Udder length, width, circumference and depth measured 20.6 ± 2.2 cm, 6.3 ± 0.3 cm, 26.1 ± 0.8 cm and 12.9 ± 1.1 cm respectively. Also, teats measured 2.5 ± 0.2 cm of length, 0.7 ± 0.1 cm of width and 3.4 ± 0.3 cm of circumference and were distant of 5.7 ± 0.5 cm and inclined by 46.8 ± 8.8° from the vertical. Mean daily milk yield (0.54 ± 0.06 kg) was significantly correlated with body weight, withers height, rump height and udder width and circumference with coefficients of 0.75, 0.64, 0.68, 0.70 and 0.66 respectively. This result must be confirmed by analysis of genotypic correlation to use these traits as selection markers in a breeding program, which aims to improve the productive potential of this breed while preserving its adaptive qualities.

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Effect of season on scrotal temperature, semen characteristics and testosterone in zebu bulls

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The objectives of the study were (1) to evaluate the effect of seasons on the surface temperature of scrota and eyeballs, semen quality, and plasma testosterone concentration in extensively bred Nellore bulls, (2) to assess the protein composition of seminal plasma in each season and identify the most common proteins, and (3) to investigate the relationships of these proteins with semen parameters, scrotum surface temperatures, and Temperature-Humidity Index (THI). Infrared thermography (FLIR E40®) of Nellore bulls (n = 20) with image analysis for spermatic cord (SCT), proximal pole of the testis (PPT), distal pole of the testis (DPT), epididymis tail (TeT) and scrotal temperature gradient (TG), and semen collected and analyzed. Blood samples were collected to obtain the plasma concentration of testosterone by radioimmunoassay (RIA). The seminal plasma proteins were identified by SDS-PAGE. The values of ambient temperature (AT), black globe temperature (Ttg), and relative humidity (RH) were measured in pasture and recorded every hour during the time of semen collection, using a portable black globe thermometer device (Instrutemp, São Paulo, SP, Brazil). The THI of each season (spring, summer, autumn and winter) was estimated according to the equation described by Thom (1959): $THI = 0.8 \times T_{tg} + RH (T_{tg} - 14.4) + 46.4$ where T_{tg} is the black globe temperature (ºC) and RH is relative humidity in decimal form. The bulls were kept exclusively with access to pasture. The TG was higher ($P < 0.05$) in autumn (5ºC) and winter (4.4ºC). The THI of spring (73.5) and summer (72) differed ($P < 0.05$) from autumn (64.5) and winter (59.6) and there were correlations ($P < 0.01$) with SCT (0.54), TeT (0.74), PPT (0.71), DPT (0.72) and TG (-0.35). Similarly, MOT (61.5%) and VIG (2.7) in spring were lower in relation to autumn and winter ($P < 0.05$). The plasma concentration of testosterone was higher ($P < 0.05$) in the autumn. Seminal plasma proteins of 20, 55 and 66KDa contributed positively to seminal quality. The results indicates that thermal stress at a THI above 72 occurs in spring and summer and negatively affects fertility of Nellore bulls raised in the tropics.

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Towards the identification of milk fat globule size as a beneficial milk production trait: small and large phenotype characterisation

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Milk fat globule size distribution varies between individual animals of the same breed. This milk production trait, which has received little attention from the dairy industry, can have important effects on milk and milk products. A naturally occurring shift in milk fat globule size towards smaller size distributions can change the processing properties of milk and increases the amount of milk fat globule membrane material, which has beneficial effects on human health.

To characterise the small and large milk fat globule phenotypes, we selected two groups of Holstein-Friesian cows in established lactation (57 to 230 days in milk) that produced milk with small or large size distributions in a pre-trial observation period. An aliquot of the entire milk volume obtained during morning milking was sampled. Duplicate samples per cow were taken on separate days and a second cohort of cows was sampled in a different season. Gas chromatography was used to examine the fatty acid profiles of the milk fat globule core, which was separated from the milk fat globule membrane by manual churning. The abundance of the major milk fat globule membrane proteins was measured using densitometry analysis of the main protein bands on SDS-PAGE gels.

Although the selected cows did not differ in other milk production traits and were fed the same pasture and concentrate-based diet, cows presenting the small milk fat globule phenotype produced milk with higher proportions of mono- and polyunsaturated fatty acids. This resulted in a proportion of 33.1% compared to 27.5% (± 0.6 SE, $p < 0.001$) unsaturated fatty acids in the small and large groups, respectively. Furthermore, the milk fat globule membrane from the small phenotype contained higher concentrations of perilipin 2 (32.9 compared to 22.7 ± 4.0 µg/mg total protein in the large phenotype, $p = 0.072$). Perilipin 2 can bind fatty acids and could provide intracellular binding sites for an improved fatty acid flux from the bloodstream into the mammary epithelial cell. Indeed, cows in the small milk fat globule group (39.4%) contained higher proportions of fatty acids ≥ C18 compared to the large milk fat globule group (34.7%) and (± 0.9, $p = 0.004$). This study shows that in depth characterisation of the small and large phenotype could lead to on-farm selection of animals based on this trait and could be exploited for a more targeted use of milk from individual cows for distinct purposes, such as butter manufacturing or direct consumption.

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P 36

Energy partitioning and body weight change in early lactation dairy cows fed canola meal- or soybean meal-based diets

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Parturition marks a shift in energy demand as a result of lactogenesis. Energy requirement for milk production exceeds what is provided by intake, resulting in body weight loss post-partum. The objective was to formulate diets with canola meal (CM) or soybean meal (SBM) at two isonitrogenous concentrations [(16% or 18% CP (DM basis)] during early lactation on energy intake and production measures with respect to BW_{change} over time. Multiparous Holstein cows (n=79; mean ± SD; 2.76 ± 0.87 parity) received 1 of 4 treatments beginning on d 1 of lactation. Cows were blocked by calving date and received the assigned treatment for 16 wk. Energy equations were based on the NRC (2001). Cows fed CM-based diets had greater milk energy outflow ($NE_{L(Milk)}$, Mcal/d) than cows fed SBM-based diets (39.6 vs. 36.8 ± 0.69 Mcal/d; $P<0.006$). Energy intake ($NE_{L(Intake)}$, Mcal/d) increased weekly until plateau was reached at wk 10 ($P<0.001$). While there was a marked difference in $NE_{L(Milk)}$, no difference in $NE_{L(Intake)}$ was observed among treatment groups. Therefore, a greater energy efficiency [($NE_{L(Milk)}/NE_{L(Intake)}$)*100] was observed for CM-fed cows compared to SBM-fed cows (94.7 vs. 90.0 ± 1.58; $P<0.035$). The disparity in energy efficiency was compensated by BW loss. All cows had a similar wk 1 BW (735 ± 12.8 kg). Maintenance energy requirement ($NE_{L(Maintenance)}$, Mcal/d) decreased as body reserves were converted to meet the demand for milk production. Body reserves were restored as $NE_{L(Intake)}$ increased. BW_{change} from wk 1 to nadir was more severe ($P<0.006$) for cows fed 16SBM and 18CM (-85.7 and -81.5 ± 6.99 kg) than cows fed 18SBM (-58.7 ± 6.99 kg). All three treatments had BW_{change} similar to cows fed 16CM (-69.7 ± 6.99 kg). This interaction suggests CM-fed cows had greater $NE_{L(Milk)}$ in early lactation compared to SBM-fed cows, but compensated with BW loss differently at 18% CP vs. 16% CP concentrations. At 18% CP, there was more BW loss for CM-fed cows than SBM-fed cows (-22.8 ± 6.99 kg; $P<0.021$). However, at 16% CP, SBM-fed cows tended to lose more BW than CM-fed cows (-16.0 ± 6.87 kg; $P<0.097$). Inclusion of CM in early lactation diets was effective in increasing $NE_{L(Milk)}$ compared to diets using SBM. Greater $NE_{L(Milk)}$ produced by CM-fed cows could be attributed to greater BW_{change} when feeding 18% CP diets; however, for 16% CP diets, CM-fed cows may have higher nutrient use efficiency.

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P 37

Supplemental bovine lactoferrin and probiotic in ghezel lambs during the pre-weaning phase

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This study was designed to evaluate the potential of bovine lactoferrin and probiotic on health status and growth performance of Ghezel lambs during the pre-weaning phase. Thirty six Ghezel suckling male lambs (3.9±0.65 kg body weight (BW)) were selected for the experiment from the 3rd day of age and housed in individual pens. Lambs were assigned randomly to 6 Groups included: 1) control (without bLF and probiotic), 2) 1g/d probiotic, 3) 0.25 g/d bLF, 4) 0.25 g/d bLF and 1 g/d probiotic, 5) 0.5 g/d bLF, 6) 0.5 g/d bLF and 1 g/d probiotic. Bovine lactoferrin (Shangqiu Kangmeida Bio-Technology Co. Ltd) and probiotic (Primalac™) were given orally every day (0900) in 56 days. Suckling lambs were fed fresh milk from ewes by nipple bottle three times per day (06:00, 14:00 and 22:00). Starter (Alfalfa Hay (17.54%), Soybean Meal (17.54%), Barley Grain (29.24%), Wheat bran (31.11%), Sodium Bicarbonate (1.00%), Mineral and vitamin premix (1.50%), Calcium carbonate (1.17%), Salt (0.90%)) and water were available ad libitum beginning on two weeks age. Feed intakes (FI) and BW were recorded daily and weekly respectively. Feces were scored 3 days in week on a scale of 1 through 7(1 = separate hard lumps and 7 = liquid consistency without solid space diarrhea Rectal temperatures (≤37.50 and 39.50≤) were determined in lambs that appeared languid, listless to eat and had diarrhea. Days medicated were recorded as each days that a lamb received drug. Statistical analysis was performed as repeated measures data using the mixed proc model of SAS software 9.2. Results showed that despite the same initial BW among the lambs, the final BW was more significantly in bLF and bLF plus probiotic groups ($P<0.05$) (11.89, 12.24, 13.73, 14.65, 13.86 and 14.74 respectively for the 6 groups). The bLF and probiotic could increase and promote FI and feed conversion ratio (FCR) respectively, but the results showed that different levels of bLF and bLF plus probiotic were more effective comparing probiotic alone ($P>0.05$). Although rectal temperature decreased during the experimental period, there was no significant change in rectal temperature with the treatments. Lactoferrin supplementation resulted to lowered medicated days ($P<0.05$). In conclusion, our study revealed that healthier lambs had higher FI which caused to improved average daily gain and health indices

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Effect of feeding cold-pressed sunflower cake in the concentrate on ruminal fermentation and rumen bacterial community composition

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Cold-pressed sunflower cake (CPSC) is a cheap by-product of oil-manufacturing which can be obtained on-farm after mechanical extraction of the oil. CPSC has higher crude fat than those of conventional solvent and expeller meals and contains significant amounts of linoleic acid, which makes it a promising lipid supplement to change rumen and milk fatty acid (FA) profile. Some studies, however, indicated that using vegetal oils could result in impaired rumen fermentation due to a negative effect of dietary unsaturated FA on rumen microorganisms. Further research demonstrated that these disturbances largely depended on the amount, the type and the physical form of lipid supplement. The aim of this study was to study the effect of CPSC on ruminal fermentation and bacterial community composition. Ten dairy cows were used in a crossover design with two treatments (control: CTR; sunflower cake: CPSC) and two 63-day experimental periods. Cows were fed a diet with 60:40 forage-to-concentrate ratio, forage was group fed *ad libitum*. Concentrates, CTR and CPSC (230 g/kg CPSC replacing totally palm fat and DDGs and partially soybean meal), were individually fed, and were formulated to provide similar amounts of energy (1 UFL), protein (190 g kg-1) and fat (60 g kg-1). Ruminal samples were collected 4 times over two consecutive days using an oesophageal tube and analysed for FA analysis using gas chromatography and volatile fatty acids (VFA) using HPLC. DNA was extracted and subjected to paired-end Illumina sequencing of the V4 region of the 16S rRNA. Data processing was performed using QIIME (v.1.9.0). Data were analysed using the MIXED procedure of SAS, with fixed effects of concentrate, sequence of treatments, period and breed, and the random effect of pair (cow), treatment means were separated using a Bonferroni adjustment. Feeding CPSC did not significantly affect molar proportions of ruminal VFA, except for a slight decrease in molar proportion of butyrate (13.2 vs. 12.7 mmol/100 mmol; P=0.005). Feeding CPSC did not affect ruminal total saturated FA, but decreased C16:0 (21.9 vs. 15.7 g/100gFA; P<0.001) and increased C18:0 (44.0 vs. 51.3 g/100g; P<0.001). Regarding unsaturated FA, CPSC increased total monounsaturated FA by increasing mainly trans-monounsaturated FA and specially trans-11 C18:1 (4.83 vs. 5.56 g/100gFA; P=0.023), but no differences in total polyunsaturated FA were observed. There were no differences between treatments on bacterial community composition. In conclusion, feeding CPSC under these feeding conditions modifies ruminal fermentation and FA profile without changing the composition of bacterial community.

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Potential use of milk Fourier transform mid-infrared spectra on predicting heat production of dairy cows

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Heat production (HP) is a major factor determining the efficiency of energy use by an animal. Measurement of O_2 consumption, and CO_2 and CH_4 production using open-circuit respiration chambers (RC) allows the estimation of HP. In dairy cows, the level of HP is primarily determined by maintenance energy and energy secreted into milk. Fourier transform mid-infrared spectroscopy (FTIR) is a fast and cost-effective approach to depict major components determining milk energy including fat, protein and lactose. This study aimed to predict HP of dairy cows by milk FTIR spectra, exploiting the data obtained in RC. The data from 4 different experiments with a total of 138 individual observations from 65 Holstein-Friesian and 12 Fleckvieh (dual purpose Simmental) cows were used for establishing the prediction model. Animals were transferred into RC, and gas exchange measurements were conducted every 6 min for 2 consecutive days. The daily CH_4, O_2 and CO_2 volumes were 576 ± 80.5, 6878 ± 703 and 7177 ± 905.3 L/d, respectively. Daily HP was calculated using the Brouwer equation: HP(kJ)= (16.18 V_{O2}(L) + 5.02 V_{CO2}(L) – 2.17 V_{CH4}(L) – 5.99 N_u(g)) and normalized per unit metabolic bodyweight ($kg^{0.75}$). Daily urine nitrogen excretion was assumed to be constant (150 g/d). Cows were milked at 0700 and 1630 h. Milk yield was determined and samples from each milking were analysed by FTIR. Partial least squares regression (PLSR) on 288 selected wavenumbers was applied to predict HP using the R package "pls". PLSR is particularly suitable when high number of, possibly correlated, predictor variables and relatively few observations exist. The recorded milk yield was taken into consideration for developing the model. The model was trained by randomly keeping 70% of the observations and using the others (30%) as the validation data-set for prediction. Optimal number of PLSR components, i.e. latent variables (LV), was selected by visual inspection of cross-validated root mean squared error of prediction (RMSEP). Three LV yielded RMSEP of 101 $kJ/kg^{0.75}$ and relatively similar RMSEP in the validation data-set (91.35 $kJ/kg^{0.75}$), suggesting the model to be fairly robust. Applying the developed model on the validation data-set indicated fair potential of predicting cow's HP from FTIR milk spectra (R^2=0.45). Overall, this study showed the possibility to predict HP in dairy cattle using the milk spectra. The increase in number of observations could further improve the robustness and accuracy of the prediction model.

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O 20

Effects of a metaphylactic butaphosphan and cyanocobalamin treatment on liver, blood and urine metabolome of transition dairy cows

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Dairy cows in modern production systems are at a high risk to develop metabolic disorders during the transition period. Reasons for individual differences in susceptibility and underlying pathomechanisms are still only partially understood. The identification of prognostic markers and the development of metaphylactic treatment protocols are needed. The aim of the present study was to analyze the metabolome of transition dairy cows to characterize the metabolic alterations in the liver, blood and urine in relation to clinical and production data to identify possible prognostic markers and the effect of a metaphylactic treatment with butaphosphan and cyanocobalamin.

An on-farm prospective double-blinded randomized study involving 80 German Holstein dairy cows (lactation number: 3.9 ± 1.8, 305 d milk production in previous lactation: 10.944 ± 2.013 kg, mean ± SD) was performed between November 2015 and November 2016. The trial involved exact documentation of clinical and production state, as well as liver biopsies, blood and urine sampling at day -14 prepartum, and day 7 and 28 postpartum. Two groups (n = 20) received a treatment at either a low or high dosage (5 ml and 10 ml / 100 kg body weight (BW) 10% butaphosphan and 0.005% cyanocobalamin, Catosal ®, Bayer Animal Health) and two placebo-groups (n = 20, 5 ml and 10 ml NaCl 0.9% / 100 kg BW). The animals were treated at six time points: -7/-6/-5 days prepartum and 1/2/3 days postpartum. Mass spectroscopy-based metabolomics analysis of blood and liver samples were performed using the AbsoluteIDQ p180 kit (Biocrates Lifes Sciences), whereas the urine samples were analyzed by NMR-spectroscopy. Statistical analysis was performed applying multivariate data analysis.

Multivariate data analysis of the three different matrices (liver tissue, plasma and urine) show a clear time effect: the three different sampling points cluster distinctively, indicating large metabolic alterations over the course of the six weeks. Especially in the metabolic profile of the liver tissue influences of feeding periods due to alterations in grass silage quality were observed. The animals were therefore clustered into a “high risk” (= poor silage quality; calving between February-June 2016; all treatment groups equally affected) and “low risk” group. The treatment effect was observed in all matrices and risk groups at various time points. Notably, in the “high risk animals” the treatment effect was observed up to 28 days postpartum in the liver. Further analysis using traditional statistical analysis methods are needed to identify the metabolites involved and to assign a biological relevance.

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O 21

Clay mineral-based mix reduces liver damage in early lactation dairy cows switched to a high-concentrate diet

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During early lactation, dairy cows are typically switched to a concentrate-rich diet to meet their high-energy requirements. However, these diets also increase the risk of rumen disorders, such as subacute ruminal acidosis (SARA). SARA might induce disturbance in the systemic metabolism. The aim of this study was to evaluate if a clay mineral-based mix (CM) can counteract negative effects of switching cows from a moderate to a high-concentrate diet.

Twenty-four lactating Simmental cows (51±23.8 DIM, 8 primiparous, 16 multiparous) were fed a baseline diet for two weeks (BASE1-2, 40% concentrate, 60% roughage; DM base) and then switched to a high-concentrate diet for 4 weeks (HC1-4, 60% concentrate, 40% roughage; DM base). Cows were either assigned to the group receiving the diet with CM addition (n = 12 cows) from BASE2 onwards, or to the group with no additive (CON; n = 12 cows). Blood samples were collected weekly. Liver enzymes aspartate-aminotransferase (AST), glutamate-dehydrogenase (GLDH) and gamma-glutamyltransferase (GGT) as well as blood metabolites were measured in the plasma. Haptoglobin concentration in the plasma was measured with a commercially available ELISA kit. Statistical analysis was performed using the MIXED procedure of SAS, including diet, group and parity, as well as a possible interaction between them as fixed effects.

High concentrate diet caused an increase in liver enzymes (HC1-HC4) and haptoglobin (HC1) in all cows ($P < 0.05$), indicating liver damage and systemic inflammation. During HC1, the concentration of non-esterified fatty acids was lower but triglycerides and albumin was increased in the plasma of cows receiving CM ($P < 0.05$). However, this effect was more pronounced in primiparous cows. Furthermore, primiparous cows receiving CM showed a decrease of GLDH during HC1 and GGT during HC1 and HC2 ($P<0.05$). In addition, supplementation of CM resulted in a decreased Hp concentration in multiparous cows during BASE2 ($P<0.05$).

Data suggest that high-concentrate feeding negatively affected liver health. Primiparous cows seemed to be more prone to the negative effects of high-concentrate diets compared to multiparous cows. Furthermore, CM holds potentials to reduce liver damage induced by high-concentrate feeding.

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O 22

Abomasal infusion of ground corn and anions in early lactating Holstein-Friesian cows to induce hindgut and metabolic acidosis

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The study aim was to induce hindgut and metabolic acidosis via abomasal infusion of ground corn and anions, respectively, and to determine their effect on performance and energy balance of early lactating dairy cows. In a 6 × 6 Latin square design, six rumen-fistulated, second-lactation Holstein-Friesian dairy cows (48 ± 17 days in milk) were subjected to 120 h of isonitrogenous, continuous abomasal infusions of water as control (CONTROL), or solutions of 2.5 mole equivalent ammonium chloride / d (low dietary cation-anion difference; DCAD), 5.0 mole equivalent ammonium chloride / d (high DCAD), 3.0 kg ground corn / d (STARCH), or the combination of ground corn with one of the two ammonium chloride levels (low DCAD + STARCH and high DCAD + STARCH), followed by 48 h of rest. A total mixed ration consisting of 70% grass silage and 30% concentrates (on dry matter basis) was fed at 95% of ad libitum intake of individual cows. The experiment was conducted in climate respiration chambers to determine feed intake, lactation performance, and energy balance. Preliminary results show that blood pH was affected by STARCH × DCAD interaction ($P < 0.01$, SEM = 0.016), with a mean blood pH of 7.50, 7.44, 7.30, 7.45, 7.36, and 7.33 for CONTROL, low DCAD, high DCAD, STARCH, low DCAD + STARCH, and high DCAD + STARCH, respectively. Faecal pH was not affected by DCAD ($P = 0.15$) or STARCH × DCAD interaction ($P = 0.51$), but decreased with STARCH ($P < 0.01$, SEM = 0.120), with a mean faecal pH of 6.94, 6.88, 6.77, 6.11, 5.91, and 5.98 for CONTROL, low DCAD, high DCAD, STARCH, low DCAD + STARCH, and high DCAD + STARCH, respectively. These results indicate a hindgut acidosis can be induced via abomasal infusion of ground corn, and a metabolic acidosis can be induced via abomasal infusion of anions. Dry matter intake (DMI; total mixed ration + abomasal infusion; kg/d), fat- and protein-corrected milk (FPCM; kg/d), and total energy retention were not affected by DCAD ($P > 0.10$) or STARCH × DCAD interaction ($P > 0.23$), whereas STARCH increased both DMI and total energy retention ($P = 0.03$ and $P = 0.02$, respectively) and tended to increase FPCM ($P = 0.09$). In conclusion, the results suggest that metabolic acidosis does not affect performance or energy retention in early lactation dairy cows. Additionally, despite experiencing hindgut acidosis, early lactation dairy cows partitioned extra energy into milk and had a more positive energy balance.

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O 23

Dietary protein influences the hepatic signalling pathway of the somatotropic axis in young goats

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A decrease in protein intake caused massive changes in mineral homeostasis and vitamin D metabolism in goats. Blood calcium (Ca), calcitriol, insulin and insulin-like growth factor 1 (IGF1) concentrations decreased during such a dietary treatment. IGF1 is synthesized by the liver after growth hormone (GH) stimulation. GH binds to the hepatic GH receptor (GHR) dimer and initiates the Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway leading to IGF1 secretion. The JAK-STAT signaling pathway is controlled via negative feedback by suppressor of cytokine signaling (SOCS) proteins. Therefore, it was hypothesized that components of the somatotropic axis were modulated by a protein reduced diet. In this study, male goats were divided in two groups, receiving either an adequate (20% crude protein (CP); n = 8 animals) or a reduced protein supply (9% CP; n = 9 animals) for 6 weeks. Ionized Ca concentrations were measured using an ion-sensitive electrode. Glucose levels were measured by mutant Q-GDH-based blood glucose monitor. Concentrations of serum calcitriol and plasma insulin were determined using an ELISA. Concentrations of serum IGF1 and plasma GH throughout 24h of blood sampling were analyzed using in-house ELISA and RIA in the Clinic for Cattle, Endocrinology Laboratory, University of Veterinary Medicine, Hannover. The mRNA and protein expression levels of GHR, JAK2, STAT5B, SOCS1, SOCS2 and SOCS3 were determined by qPCR and Western blot analyses, respectively. Data were analyzed by unpaired Student's t-test. Concentrations of ionized Ca, calcitriol, insulin and IGF1 decreased due to the protein reduced feeding. Glucose levels were not affected by dietary treatment. Plasma GH levels remained unchanged throughout 24h. The expression of hepatic GHR was diminished in the protein reduced fed animals while expression levels of JAK2, STAT5B and SOCS1 were not modulated. Additionally, SOCS2 and SOCS3 expression levels increased due to the protein reduced feeding. Due to the non-physiological relationship between unaltered concentrations of GH and decreased IGF1 concentrations, a disruption of the somatotropic axis mediated by reduced GHR expression levels seems possible. Decreased GHR expression occurred in response to reduced insulin levels that regulate GHR biosynthesis. The reduction in insulin might be due to decreased levels of extracellular Ca which is essential for pancreatic exocytosis of insulin while blood glucose levels were not modulated. Increased expression levels of SOCS2 and SOCS3 contributed to reduced levels of IGF1. In conclusion, a dietary protein reduction influences the hepatic signaling pathway of the somatotropic axis in young goats.

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O 24

Impact of ethanol administration on insulin sensitivity in wethers

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Background: Chronic alcohol intake enhances insulin resistance in human. The relationship between alcohol intake and insulin sensitivity in ruminants is unclear. Silage alcohols may induce insulin resistance. And, changes in insulin sensitivity induced by silage feeding during periods of fluctuating energy balance are unclear in ruminants. Therefore, insulin responsiveness to ethanol administration was investigated in sheep.

Materials and methods: Four mature wethers (84.0±2.6 kg BW) fitted with a rumen-cannula were assigned to either high energy (HE: 1.7 ME of maintenance) or low energy (LE:0.5 ME of maintenance) diet with intraruminal infusion of either 240 ml water (control) or 96 g ethanol (equivalent to 5% of energy fed in HE) in a cross-over design for 14 d for HE and 7 d for LE. Diets and infusates amounts were divided into two equal portions and offered simultaneously twice daily (0900 and 2100 h). Hyperinsulinemic-euglycemic clamp (EGC) was performed on 13th and 14th d of HE and on 6th and 7th d of LE. Preliminary blood samples were collected 1 h before the morning feeding to determine basic blood glucose level. Afterward, insulin infusion (1 mμ/kg.BW/min) started at 0815 h and continued for 2 h. Glucose infusion (20% glucose) continued for 2 h to maintain the basic blood glucose level. Blood samples were collected at 5- and 15-min intervals to monitor blood glucose and insulin concentration, respectively. The amount of glucose infused was recorded at 10 min intervals and glucose infusion rate (GIR) was calculated (mg/kg.BW/min).

Results and conclusions: Plasma insulin concentration was greater in the control (88.9 ng/ml) than ethanol (68.4 ng/ml) in LE ($P=0.012$), but the reverse was observed in the HE where ethanol group showed higher insulin (77.9 ng/ml) than the control (51.0 ng/ml) ($P=0.002$). Plasma glucose concentration did not differ between control and ethanol in either energy plan, but the GIR showed the same trend of plasma insulin where GIR was greater in the control (1.78) than ethanol (1.04) in LE ($P=0.004$) but was higher in ethanol (1.448) than in control (1.11) in HE ($p=0.008$). Plasma ethanol was higher in LE (53.5 mg/dl) than in HE (40.9 mg/dl) ($P<0.05$). In conclusion, ethanol administration may alter the systemic insulin action based on energy intake which affected ethanol turnover. However, ruminal metabolism of ethanol to acetate and consequently alterations in rumen fermentation products could be partly implicated in these findings.

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P 100

Impact of induced negative energy balance on the feed sorting behavior of dairy cows

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The objective of this study was to determine how dairy cows alter their feed sorting behavior in response to diet-induced negative energy balance (NEB). Holstein cows (n=30; DIM=59±5) producing 44.6±1.2kg/d of milk were fed a total mixed ration (TMR) (NE_L=6.99MJ/kg; 68% forage) during a 2-wk baseline period. To induce NEB, cows were exposed for 3 wk to 1 of 2 TMR, each formulated for a 12% reduction in energy available for milk (NE_L=6.49MJ/kg; 73% forage including 9.5% straw). These diets only varied in straw chop length: 1) LS: straw chopped with a 10.2cm screen, or 2) SS: straw chopped with a 2.54cm screen. Blood samples were taken for non-esterified fatty acids (NEFA) analysis 1x/wk during baseline and every 4d on the treatment diets. TMR samples (fresh and orts) were collected every 3d and separated into: long (>19mm), medium (<19,>8mm), short (<8,>4mm), and fine (<4mm) particles. Feed sorting was calculated as: % of actual/predicted intake of each particle fraction. Data were analyzed in repeated measures, mixed-effect linear regression models. During baseline, NEFA averaged 0.27±0.02mmol/L. During the experimental period NEFA increased to 0.32±0.04mmol/L, across treatments, with a peak of NEFA (0.59±0.06mmol/L) occurring 4d after dietary change. Cows consumed 25.6±0.3kg/d during the baseline period; dry matter intake (kg/d) decreased (P<0.01) similarly on the experimental diets to 22.8±0.5kg/d. During baseline, cows sorted against long particles (95.3±0.6%), did not sort medium particles (99.8±0.1%), and sorted for short (101.1±0.1%) and fine (101.9±0.2%) particles. Cows did not change sorting on SS, but on LS they increased (P<0.01) sorting against long (89.4±1.1%), and for short (103.2±0.4%) and fine (104.5±0.6%) particles. During baseline there was no association between sorting and blood NEFA. During the treatment period, greater blood NEFA were associated with greater sorting of short particles for both the LS (% short sorting=4.6*NEFA(mmol/L)+101.7; R^2= 0.28; P=0.04) and SS (% short sorting=2.8*NEFA(mmol/L)+100.0; R^2= 0.10; P=0.1). Furthermore, greater NEFA levels were associated with greater sorting against the longest particles for both the LS (% long sorting=-12.8*NEFA(mmol/L)+93.4; R^2=0.26; P=0.05) and SS (% long sorting =-13.7*NEFA(mmol/L)+99.5; R^2= 0.17; P=0.1). Overall, in response to a diet-induced period of NEB, cows increased sorting against long, and for short and fine, dietary particles. Further, the severity of NEB experienced was associated with the extent of that change in feed sorting.

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The cell line BFH12 as an in-vitro model for bovine hepatosteatosis

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Objective: Fatty liver is a metabolic disorder of dairy cows in early lactation. It is associated with decreased health and reproductive performance. Fatty liver develops when the hepatic uptake of fatty acids exceeds the oxidation and secretion capacity of the liver. The excess lipids are then stored mainly as triglycerides (TG). The molecular pathogenesis of the disease is not completely understood yet.

The aim of the current work was to establish a suitable in-vitro model for bovine hepatosteatosis, because cellular models may help to provide a more profound understanding of molecular mechanisms. In order to address this issue, we established the new fetal bovine hepatocyte cell line BFH12. In our current study, we hypothesize that this cell line is a suitable model for hepatosteatosis contributing to understanding and prevention of liver-associated health problems in dairy cows.

Methods: BFH12 cells were cultured in Williams' Medium E containing 5% FCS, 1% penicillin/streptomycin, 2mM L-alanyl-L-glutamine, 100 nM dexamethasone and 0.2U/mL insulin. In order to induce a steatosis-like phenotype, cells were supplemented with increasing concentrations of one of the following fatty acids (FA): palmitate (PA), stearate (SA) or oleate (OA). After a 24 h incubation cytotoxicity was measured. 60µM PA, 50µM SA or 100µM OA were used for the following experiments, because these were the highest possible concentrations without cytotoxic effect. Intracellular fat accumulation was assessed by Nile red or Oil red O staining. Lipid composition and fatty acid pattern of BFH12 was determined by thin-layer chromatography followed by gas chromatography (GC).

Results: Treatments with FA in the concentrations used had no effect on cell viability. After FA supplementation lipid droplets were observed, indicating an uptake of the fatty acids. OA induced the formation of larger droplets compared to PA and SA. Furthermore, all treatments resulted in higher levels of major lipid classes in BFH12, including phospholipids, TG and non-esterified fatty acids. GC analysis showed that OA and also PA accumulated in a dose-dependent manner, while SA was largely oxidized. OA is extensively incorporated into TAG. .

Conclusion: BFH12 acquire a steatotic phenotype by incorporating and accumulating different fatty acids. Therefore, the BFH12 cell line is a useful in vitro model to study bovine hepatic steatosis and its underlying molecular mechanisms.

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A reduced protein diet modulates enzymes of vitamin D and cholesterol metabolism in young ruminants

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A reduction in dietary protein caused massive changes in mineral homeostasis of young goats. A decrease in blood calcium (Ca) along with reduced concentrations of serum 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$), a diminished RNA expression of renal 1-α-hydroxylase (CYP27B1) and decreased intestinal Ca absorption was observed (1). Interestingly, serum concentrations of 25-hydroxyvitamin D_3 (25-OHD_3) were greater in animals kept on a protein reduced ration although the expression of renal cytochrome P450 family 24 subfamily A member 1 (CYP24A1) was stimulated. Therefore, we hypothesized that the expressions of hepatic sterol 27-hydroxylase (CYP27A1), vitamin D 25-hydroxylase (CYP2R1) and cytochrome P450 3A24 (CYP3A24), enzymes possessing 25-hydroxylase activity, are modulated during protein reduction in young goats. Additionally, we addressed the hepatic expression of cholesterol 7 alpha-hydroxylase (CYP7A1) and cytochrome P450 2J2 (CYP2J2) because both are involved in the metabolism of cholesterol which is a precursor of steroid hormones. Two groups of male German colored goats aged three months were kept on a control diet (20% crude protein (CP); n = 8 animals) or a protein reduced (9% CP; n = 9 animals) but isoenergetic diet for six weeks. Ionized blood Ca levels, serum concentrations of 1,25-$(OH)_2D_3$, 25-$(OH)D_3$, plasma total cholesterol and triglycerides were measured. In line with our previous studies, protein reduction resulted in a decrease in blood Ca, an increase in 25-$(OH)D_3$ levels and a reduction in 1,25-$(OH)_2D_3$. Hepatic expression of CYP2R1 and CYP3A24 was not affected, while the expression of CYP27A1, CYP7A1 and CYP2J2 was significantly diminished during this dietary treatment. Plasma cholesterol was significantly elevated in goats kept on the protein reduced diet and negatively correlated with serum 1,25-$(OH)_2D_3$ as well as hepatic expression of CYP27A1 and CYP2J2. The concentrations of triglycerides were not affected. The results of the present study show that a dietary protein reduction is associated with changes in both mineral and cholesterol metabolism in young goats. Therefore, further investigations are needed before dietary interventions aiming at diminishing nitrogen excretion can be implemented.

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Influence of glucose and macrophages on growth hormone receptor expression in primary bovine hepatocytes

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The hepatic growth hormone receptor (GHR) is an actuator of the somatotropic axis. During catabolism or severe sepsis the responsibility of the GHR is impaired. In order to study the mechanism of GHR insensitivity in bovine species, the aim was to develop a hepatocyte model with a functional GHR expression. Primary hepatocytes were gathered using a three-step-collagenase perfusion from cows euthanized in the clinic for cattle. Viable hepatocytes +/- kupffer cells (KC) were separated with Easycoll™ and seeded on collagen coated plates with a collagen overlayer (n=6 wells per each experiment). Williams' medium E was amended by 100 nM insulin, 100 nM dexamethasone, or both, and glucose (0/+5mmol/+10mmol). KC were seeded in direct contact (KC*D*C) or above the collagen overlayer in indirect contact (KC*I*C). Medium urea and lactate dehydrogenase (LDH) concentrations were determined, as well as albumin, HNF4α, vimentin, GHR, IGF1 mRNA expression. Insulin increases albumin, HNF4α, GHR1A and IGF-1 expression whereas dexamethasone suppresses albumin, HNF4α, vimentin, GHR1A and IGF-1 ($P<0.05$). Interestingly, high glucose concentration increased LDH leakage and vimentin expression ($P<0.05$). Culturing of hepatocytes with KC*I*D increased albumin and KC*D*C reduced GHR1A expression probably due to cytokine expression by KC in culture. In conclusion, the lowest dedifferentiation rate and LDH leakage were achieved by culturing hepatocytes without glucose but with propionate and pyruvate as physiological glucose precursors in polygastric species. Moreover, the functional GHR expression was possible for 4 days by retaining dexamethasone from the medium.

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Exposure of primary bovine hepatocytes to physiologically relevant fatty acid profiles have altered gene expression

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It is well established that during the transition to lactation period in dairy cows, adipose tissue is mobilized by releasing fatty acids (FA) into the bloodstream to the liver, often exceeding the capacity of hepatic metabolism. These mobilized FA released into circulation are known regulators of some hepatic genes, which may be mediated by transcription factors. During the transition to lactation period, shifts in serum FA profile occur at three distinct points: at calving (CALV), postpartum during accumulation of liver lipids (ACCUM), and recovery from fatty liver (RECOV). The objective of this research was to determine the effect of these distinct FA profile shifts on hepatic gene expression in vitro with administraion of FA cocktail. Primary bovine hepatocytes were isolated from bull calves (n=4, <7 d of age) and cultured in monolayers. After 24 h, cells were treated in triplicate with each cocktail comprising of varying percentages of C14:0, C16:0, C18:0, C18:1, C18:2, C18:3 (only present in CALV), C20:5, and C22:6 to mimic circulating FA in dairy cows. Treatments were control (1% bovine serum albumin) or 0.25, 0.5, 0.75, or 1 mM of each distinct FA cocktail. Cells were incubated for an additional 24 h and then harvested in TRIzol for subsequent RNA isolation. Gene expression of carbohydrate (Ch) regulatory element-binding protein 1 (REBP1), sterol (S) REBP1c, and liver X receptor (LXR) α and β were quantified and made relative to reference genes. Data were analyzed (PROC MIXED, SAS, 9.4) with fixed effect of concentration and FA and random effect of calf; means were separated with Tukey adjustment. Expression of LXRα and LXRβ were not affected (P>0.1) by FA profile or concentration. There was an interaction (P=0.009) of FA cocktail and concentration on ChREBP1 expression. Expression of SREBP1c was increased (P=0.003) with exposure to RECOV (CALV and ACCUM vs. RECOV: 0.53 and 0.68 vs. 1.04 ± 0.2 arbitrary units). The presence of an effect of FA on SREBP1c but not LXRα and LXRβ suggests that FA regulation of SREBP1c in this case may not be mediated through LXR. These data indicate that hepatic transcription factors associated with carbohydrate and lipid metabolism are influenced by FA cocktail, concentration, or combination of both in primary bovine hepatocytes. Regulation of transcription factors through FA profile shifts may influence downstream gene regulation which could influence hepatic metabolism during the transition period.

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The effect of fatty acid profiles mimicking timepoints across the transition period on lipolytic protein abundance in primary bovine hepatocytes

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Lipolysis of hepatic lipids stored peripartum may allow for recovery from fatty liver during the transition period in dairy cows; however, regulation of bovine lipolytic proteins is not yet understood. In other species, lipolytic proteins are responsive to fatty acid (FA) regulation, which may be a potential mode of regulation in bovine. Shifts in circulating serum FA profile occur at three distinct time points peripartum: at calving (CALV), postpartum during accumulation of liver lipids (ACCUM), and recovery from fatty liver (RECOV). The objective of this research was to evaluate the effect these FA profile shifts have on hepatic lipase abundance in vitro. Primary bovine hepatocytes were isolated from bull calves (n=3, <7 d of age) and cultured in monolayers. After 24 h, treatments were control (1% bovine serum albumin) or 0.25, 0.5, 0.75, or 1 mM of each distinct FA profile as a cocktail comprising of varying percentages of C14:0, C16:0, C18:0, C18:1, C18:2, C18:3 (only in CALV), C20:5, and C22:6 to mimic circulating FA in vivo. Cells were incubated for 24 h and then harvested in TRIzol for subsequent protein isolation. Protein abundance of abhydrolase domain containing 5 (ABHD5), adipose triglyceride lipase (ATGL), phosphorylated hormone sensitive lipase (P/HSL), phosphorylated perilipin (P/PLIN), patatin-like phospholipase domain-containing protein 3 (PNPLA3), and sterol regulatory element-binding protein 1c (SREBP1c) were determined through Western blot analysis, normalized to total lane protein, and expressed relative to control treatment. Data were analyzed (PROC MIXED, SAS, 9.4) with fixed effect of concentration and FA and random effect of calf, with preplanned contrasts of CALV and ACCUM vs. RECOV and linear effect of FA concentration. Abundance of ABHD5, a coactivator of ATGL, and ATGL, the rate-limiting lipase in humans, were not affected (P>0.15) by any concentration nor CALV, ACCUM, and RECOV (ABHD5: 0.59, 0.62, and 0.75 ± 0.16 arbitrary units (AU); ATGL: 1.13, 1.32, and 1.50 ± 0.39 AU). No evidence of change was observed for PLIN (P>0.15); conversely, FA cocktail tended to influence (P=0.09) HSL abundance. Phosphorylation of HSL and PLIN indicate active protein, yet no change (P>0.15) in PHSL nor PPLIN was observed. Abundance of SREBP1c was unaltered (P>0.15). Abundance of PNPLA3 tended to be greater (P=0.11) with RECOV compared to CALV and ACCUM (1.15 vs. 0.81 and 0.87 ± 0.25 AU) as observed at corresponding in vivo timepoints. Regulation of hepatic lipolytic proteins postpartum should be further examined, but FA may contribute to regulation of HSL and PNPLA3.

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The influence of short chain fatty acids and ß-hydroxybutyrate on gluconeogenic processes in the bovine liver cell line BFH12

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In dairy cows, the energy metabolism is an important factor in different metabolic diseases. The short chain fatty acids (SCFA) acetate, propionate and butyrate are produced in the rumen of cattle and serve as the main energy source. The ketone body ß-hydroxybutyrate (ßOH) is produced in the rumen wall and in the liver to provide energy, especially when there is a lack there of. In ruminants, gluconeogenesis is essential, as feed carbohydrates are almost completely fermented in the forestomach. Free Fatty Acid Receptor 2 and 3 (FFAR2/3) have recently been identified as receptors for SCFA in the liver. ßOH is the endogenous ligand for the Hydroxycarboxylic Acid Receptor 2 (HCA2). The key enzymes of gluconeogenesis in the liver are pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase 1 and 2 (PEPCK1+2), and glucose-6-phosphatase (G6P). Propionate is the main substrate for gluconeogenesis in dairy cows. Therefore, propionyl-CoA-carboxylase (PCCB) is the key enzyme for gluconeogenesis from propionate. In our investigations, we address the question whether SCFA and ßOH affect the gene expression of their specific receptors and key enzymes of gluconeogenesis in the bovine liver cell line BFH12. Methods We evaluated primer sets for the genes of interest and five housekeeping genes (ß-Actin, GAPDH, HPRT, RPL13, SDHA) with conventional polymerase chain reaction (PCR) and assessed different basic conditions for quantitative PCR (qPCR), including primer efficiency. BFH12 cells were cultured for 4 hours in a medium containing 3.3 mmol/l glucose without insulin and treated separately with 1000 µmol/l acetate, 250 µmol/l propionate, 20 µmol/l butyrate or 1500 µmol/l ß-hydroxybutyrate. For comparison we also carried a control group. qPCR was performed using the qPCRBIO SyGreen Mix Separate-ROX kit from PCRBiosystems. Statistical analyses were performed with the REST© software. Results PCR analysis showed that the genes for PCCB, PC, PEPCK2 and the housekeeping genes are expressed in untreated BFH12. In qPCR studies, GAPDH and SDHA were identified as suitable reference genes. All genes of interest were expressed in untreated and treated cells. Under the chosen experimental conditions, SCFA and ßOH treatment resulted in higher gene expression levels. FFAR3 expression was induced by all tested treatments while PC was up regulated by acetate and butyrate. Conclusion In our study, we demonstrate that the liver cell line BFH12 regulates gluconeogenesis during exposure to SCFA and ßOH by inducing enzyme and receptor expression. Future studies will be directed at investigating the energy state of BFH12 under the different treatment conditions.

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Nutri-proteomic effects of conjugated linoleic acid on the phospho-proteome of abdominal and subcutaneous adipose tissues from transition dairy cows

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Dietary conjugated linoleic acid (CLA) has many metabolic effects in dairy cows. Also, it reduces energy output in milk, which could increase fat storage, but CLA is also known to promote lipolysis in adipose tissue (AT). Phospho-proteomics is a new frontier in omics-research that reveals the full repertoire of phosphorylation sites in proteins. Our objective was to elucidate the effect of CLA on protein activation in AT of transition cows by phospho-proteomics.

Ten rumen cannulated Holstein cows were fed a corn silage-based TMR with low fat content and daily abomasally supplemented from wk 9 prepartum until slaughtering at 63 d postpartum with coconut oil (CTL, n=5; 45.5% C12:0; 16.9% C14:0; 76g/d) or Lutalin® (CLA, n=5; *c*9, *t*11 and *t*10, *c*12, 10g/d). Subcutaneous (S) and abdominal (A) AT were collected and frozen (-80°C). Proteins were extracted for global quantification of the phospho-proteome by enrichment of phospho-peptides (PP) by HPLC based Immobilized Metal Affinity Chromatography (IMAC) with Fe(III) followed by discovery analysis. Data was analyzed for the effect of fat depot (S vs. A), treatment (CLA vs. CTL), and their interaction by 2-way ANOVA, and bioinformatics analysis was conducted by Ingenuity (Qiagen).

Overall, CLA cows had lower fat and energy output in milk compared to CTL, and had more total and abdominal AT than CTL ($P<0.005$). A total of 5,919 PP were identified. The abundance of 854 PP (14.4%) was different between CLA and CTL ($P<0.05$, FC ± 1.5). In addition, the abundance of 470 PP (7.9%) differed between A and S ($P<0.05$, FC ± 1.5). Regarding the effects of CLA vs. CTL, increased protein phosphorylation, i.e. higher abundance of several PP, was found in lipid-metabolism proteins: 7 PP were more abundant in acetyl-CoA carboxylase 1 (ACACA); 9 PP in fatty acid synthase (FASN); 4 PP in hormone sensitive lipase (HSL); and 3 PP in perilipin (PLIN). Increased total abundance of FASN and HSL was found by immunoblots in CLA vs. CTL AT ($P<0.02$). Top canonical pathways enriched in CLA AT were protein kinase A (PKA) signaling and insulin receptor signaling. Top canonical pathways according to differential PP in A vs. S AT were tight junction signaling, signaling by Rho family GTPases and PKA signaling.

Our findings add new information on the phospho-proteome of AT in dairy cows, and provide insight to the molecular mechanism by which CLA might stimulate protein phosphorylation in A and S AT towards both lipogenesis and lipolysis.

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Effect of milk replacer feeding intensity on energy metabolism in dairy calves

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Milk replacer (MR) allowance influences solid feed intake and energy metabolism in growing calves, but its short- and long-term effects on feed efficiency have not been investigated. To address this gap, 28 female newborn Holstein calves were equally allocated to 2 feeding groups either fed with 10% or 20% of their body weight (BW) with MR after a 3 days period of feeding the same colostrum. MR feeding of the 20%-MR group was gradually reduced to 10% of the BW from week (wk) 9-10. Both groups were gradually weaned from wk 11-12. Starter and hay were offered ad libitum until wk 12 and 14, respectively. Starter was limited to 2 kg per day in wk 13-14. A total mixed ration was offered ad libitum from wk 11-23. Feed intake was measured daily and BW weekly. Energy metabolism and physical activity (PA) were analyzed in open-circuit respiration chambers 2 times pre-weaning and 2 times post-weaning for 48 h in wk 6, 9, 14 and 22. Daily heat production (HP), carbohydrate oxidation (COX) and fat oxidation (FOX) were calculated and normalized to metabolic body weight (mBW) or metabolizable energy intake (MEI). Data was analyzed by repeated measures ANOVA of SAS with the fixed effects of feeding group, time and their interaction. The BW increased in both groups over time but to a greater extent in the 20%-MR group with higher values in wk 5-23 ($P<0.05$). The average daily gain was higher in wk 1-6 and 8 of the 20%-MR group ($P<0.05$). Daily MEI and MEI/mBW were higher in the 20%-MR group pre-weaning ($P<0.05$) but not post-weaning. Daily COX and FOX increased over time and tended to reach higher values in the 20%-MR group in wk 6 and 9, respectively ($P<0.1$). Metabolic efficiency, expressed as FOX/mBW, COX/MEI or FOX/MEI, was not different between groups, whereas FOX/MEI and FOX/mBW decreased, while COX/MEI and COX/mBW increased over time ($P<0.05$). Total HP increased over time and was greater in the 20%-MR group ($P<0.05$). However, HP/mBW did not differ among groups. HP/MEI was lower and COX/mBW higher in the 20%-MR group in wk 6 ($P<0.05$), but these differences did not persist over time. PA also increased over time ($P<0.05$) without differences between groups. The results indicate that a higher MR feeding intensity improves energy efficiency in calves due to a lower HP/MEI in wk 6.

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Effect of zearalenone treatment on lipid metabolism in primary culture of ketotic bovine hepatocytes in vitro

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Introduction: Ketosis is one of the major metabolic disorders among dairy cows in early lactation resulting in significant economic losses in dairy farming. In ketotic liver hepatocytes must cope with symptoms of energy imbalance by altering gene expression of enzymes involved in energy metabolism. The aim of this study was to determine if the presence of the zearalenone mycotoxin impairs the ability of hepatic cells in primary culture to cope with negative effects of ketosis.

Animals, materials and methods: Bovine hepatocytes were isolated from 0.5 g liver biopsies taken from healthy animals (control group, n = 3 cows) and from animals with clinical ketosis (level of ketone bodies in blood > 3 mmol/L; n = 3 cows). Isolation of hepatocytes was conducted by manual perfusion method followed by incubation of minced tissue in collagenase solution. Cells were seeded on collagen coated plates at a density of 4.5–1x105 viable cells/cm2, in growth medium with 10% of FBS and exposed to zearalenone in doses 10-100 μM for 1, 3, 6, 12 and 24 hours. Expression of genes involved in lipid metabolism (ACOX1, HMGCR, ACACA, FASN, FADS2) was determined by the qPCR method.

Results and discussion: Our data revealed upregulation of ACOX, involved in peroxisomal oxidation, and downregulation of HMGCR (cholesterol synthesis), ACACA and FASN (de novo fatty acids synthesis) and FADS2 (fatty acid desaturation) in the culture of ketotic hepatocytes compared to hepatic cells isolated from healthy animals. Addition of zearalenone to cultures from non-ketotic animals did not affect levels of mRNA for HMGCR, ACACA, FADS2 while gene expression of ACOX1, FADS2 was decreased. In cell cultures obtained from ketotic cows we observed a stronger effect of zearalenone on the inhibition of gene expression of all genes studied.

Conclusion: The results indicate that in hepatocytes isolated from ketotic animals, zearalenone inhibited the expression of the genes involved in lipid metabolism, while hepatocytes from healthy animals were not affected. The increase of peroxisomal oxidation (ACOX1) allowing the production of short-chain fatty acyl-CoA intermediates is one of the adaptation mechanisms of the ketotic cow's liver to energy imbalance, but under tested condition, we observed an inhibition of its gene expression. It leads to the conclusion that mycotoxins such as zearalenone may severely impair the ability of the ruminant liver to cope with symptoms of energy imbalance.

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Early lactating primiparous cows have stronger acidosis index and liver damage as multiparous cows fed the same high concentrate diet

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Dairy cows in early lactation are switched from a fiber-rich close-up diet to a concentrate-rich lactation diet, regardless of parity differences. However, primiparous cows (PPC) are more susceptible to high-energy diets compared to multiparous cows (MPC) due to the fact that PPC are exposed to more stress at the onset of lactation. Therefore, the aim of this study was to investigate parameters associated with rumen, metabolic and liver health of PPC and MPC transitioned from a moderate to a high concentrate diet with the potential of inducing subacute ruminal acidosis (SARA).

Twenty-four lactating Simmental cows (51±23.8 DIM; 8 PPC, 16 MPC) were used for this study. Animals received a baseline diet for two weeks (60% roughage; 40% concentrate; DM basis) followed by a SARA-challenge diet for 4 weeks (40% roughage, 60% concentrate; DM basis). Rumen pH was measured using indwelling sensors. Blood metabolites as well as liver enzymes aspartate-aminotransferase (AST), glutamate-dehydrogenase (GLDH), and gamma-glutamyltransferase (GGT) were measured once a week in the plasma. Haptoglobin (Hp) and serum amyloid A (SAA) concentrations in the plasma were measured with ELISA kits. Statistical analysis was performed by using the MIXED procedure of SAS, including diet, parity and their interaction as fixed effects.

As expected, the SARA diet led to an increased time of rumen pH below 6.0 ($P < 0.01$). However, in PPC, the SARA-index, which serves as severity indicator of SARA, was higher than in MPC ($P < 0.01$). During the SARA diet all cows produced more milk than in the baseline period ($P < 0.05$), but milk yield was lower in PPC than in MPC ($P < 0.01$). SARA diet increased the blood glucose level ($P < 0.01$), with PPC having a higher blood glucose level compared to MPC ($P < 0.01$). All three measured liver enzymes were significantly increased by the SARA diet ($P < 0.01$), whereby a stronger increase in GLDH and GGT was observed in PPC compared to MPC ($P < 0.05$). SARA challenge diet increased Hp level of PPC ($P < 0.01$). Furthermore, there was a trend for an increased SAA concentration in MPC compared to PPC ($P = 0.08$).

In conclusion, PPC seemed to be more susceptible to a high-concentrate challenge diet during early lactation, which was reflected in the stronger severity of SARA as well as in the increase of parameters associated with liver damage of these cows.

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The relationship between hair fatty acid profile and energy balance in early lactating multiparous cows

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From our previous studies we concluded that the hair fatty acid profile, especially C12:0, could be used to assess the energy availability of early lactating primiparous German Holstein cows. The aim of the study was to examine the relationship between the hair fatty acid profile and energy balance in multiparous cows.

For the study we used 64 German Holstein and 54 Simmental multiparous cows, which were housed in two experimental stations. After calving, the cows were assigned randomly to one of four feeding groups, which received either (1) a moderate energy concentration of roughage (6.1 MJ NE_L/kg DM) and moderate amounts of concentrates (150 g/kg ECM), (2) a moderate energy concentration of roughage (6.1 MJ NE_L/kg DM) and high amounts of concentrates (250 g/kg ECM), (3) a high energy concentration of roughage (6.5 MJ NE_L/kg DM) and moderate amounts of concentrates (150 g/kg ECM), or (4) a high energy concentration of roughage (6.5 MJ NE_L/kg DM) and high amounts of concentrates (250 g/kg ECM). Daily measurements of feed intake and milk yield as well as weekly determinations of milk composition and body weight were used to calculate the weekly energy balance. To characterize the energy status during early lactation, we calculated the cumulative energy balance for the lactation weeks 1 to 6. A hair sample was taken in week 8 of lactation. For assessing the relationship between fatty acids in hair and energy balance, Spearman's correlation coefficients were calculated for each feeding group within each breed

In both breeds, we found positive correlation ($0.41 \leq r \leq 0.63$; $P<0.15$) between the cumulative energy balance in lactation weeks 1 to 6 and the content of C12:0 and C14:0 in hair of week 8 in cows that were fed a diet of high energy concentration of roughage and high amounts of concentrates. No significant correlation was found in cows fed moderate energy concentrations of roughage and moderate amounts of concentrates.

To further confirm that the content of C12:0 and C14:0 in hair depends on the energy status of a cow during early lactation, the study has to be expanded with additional cows of both breeds as well as parameters of body condition and blood.

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Reducing milking frequency from three to twice daily during the first month postpartum improves the metabolic status and reduces stress

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Reducing the milk production during early lactation might be of interest to improve the energy balance (EB) of high yielding dairy cows; therefore, the objectives were to test the effects of reducing the milking frequency from three to twice a day during the first 30 DIM, on yields, metabolic status, reproduction, and stress markers. Forty-two multiparous cows were divided into 2 groups according to previous lactation performance, parity and body weight. Control cows were milked 3 times a day (3ML), and treatment cows were milked twice a day until 30 DIM (2ML), and then 3 times a day. Both groups were followed until 100 DIM. Milk samples were taken twice a week from 3 or 2 consecutive milkings until 45 DIM for milk solids analysis. Individual DMI, milk yields and BW were recorded daily. Blood samples were taken 3 times weekly from 14 d pre-partum until 45 DIM. Data were analyzed using the PROC MIXED model of SAS for the first 30 DIM, and then from 31-to 100 DIM for carry-over effects. Milk yields during the first 30 DIM were 9.4% higher (44.3 and 40.5 kg/d, respectively; $P < 0.01$), milk-fat percentage was lower (4.12 and 4.45%, respectively; $P < 0.003$), and yields of all milk solids were higher in the 3ML cows than in the 2ML cows. DMI and FCM (4%) were similar between groups and the EB during the first 30 DIM was better in the 2ML than in the 3ML cows (1.64 and -1.44, respectively; $P < 0.01$). Blood glucose concentrations between 0-30 DIM were higher ($P < 0.0003$), β-hydroxybutyrate were lower ($P < 0.02$), NEFA were higher ($P < 0.002$), and insulin were lower ($P < 0.08$) in the 2ML than in the 3ML cows. Blood malondialdehyde (MDA) concentrations, a marker of oxidative stress, during the first 2 weeks in lactation were 44% lower in 2ML than in 3ML ($P < 0.05$), and the cortisol concentrations tended to be lower in the 2ML compared to the 3ML ($P < 0.1$). A lower proportion of cows that ovulated until 15 DIM was found in the 3ML cows (10%), than in the 2ML cows (40%; $P < 0.02$), with no beneficial effects on pre-ovulatory follicle characteristics. In conclusion, reducing milking frequency to twice a day during the first 30 DIM improved EB and the metabolic status and reduced blood stress markers, with minor effects on production.

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P 113

Dietary fatty acids effects on the fatty acid composition of erythrocytes in dairy cows fed a corn based ration

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In humans, the fatty acid (FA) composition of erythrocyte membranes is used as an indicator to analyze medium-term to long-term effects of dietary FA. Essential n-3 and n-6 FA are incorporated in cell membranes and affect different physiological functions like inflammatory processes. We used erythrocytes of FA supplemented dairy cows, which received a corn silage based ration providing less essential n-3 FA compared to grass silage. Mead acid was utilized as a potential marker of the essential FA status in cows, and we explored the FA composition of erythrocyte in response to the supplementation with different FA in cows fed a corn-based diet.

Rumen fistulated Holstein cows (n = 38, 11.000 kg milk in 2nd lactation) were fitted with abomasal infusion tubes, and fed a corn silage based total mixed ration. Cows were infused twice daily with either coconut oil delivering medium-chain fatty acids (CTRL: 76 g/d), linseed-safflower oil mix, delivering mainly n-3 fatty acids (EFA: 78 + 4 g/d, respectively), LUTALIN (content of c9,t11 and t10,c12 conjugated linoleic acids (CLA) in equal amounts; 10 g/d; BASF SE, Ludwigshafen, Germany), or EFA + CLA from 9 wk antepartum to 9 wk postpartum. Blood samples were collected 8 wk postpartum. After centrifugation, FA methyl esters from washed erythrocytes were prepared. Fatty acid analysis was performed using capillary gas chromatography with a 100 m CP-Sil 88 CB column. Relative FA data were analysed by the mixed model of SAS using treatment as fixed effect, and calving interval in addition to milk production during the second lactation as covariates.

EFA increased certain n-3 FA, including linolenic acid, eicosapentaenoic acid and docosapentaenoic acid. The relative amount of linoleic acid was lowest in the CTRL group and highest in the EFA+CLA group. Highest values of arachidonic acid were detected in the CTRL and CLA group. The mead acid concentration was highest in the CTRL and CLA group and lowest in the EFA+CLA group. Oleic acid, the precursor for mead acid, was highest in the CTRL group and lowest in the EFA+CLA group. Partly EFA and CLA changes were additive.

These data provide information on FA incorporation in erythrocytes after supplementation of functionally different saturated and poly-unsaturated FA for 17 weeks in dairy cows fed an n-3 reduced diet. Mead acid was increased in the CTRL and CLA group but did not reach a level, which indicates essential fatty acid deficiency in other species.

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P 114

Days on feed and dietary starch may impact pancreatic islet morphology and plasma insulin concentrations in feedlot steers

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The objective of this study was to evaluate pancreatic insulin production and insulin sensitivity as affected by dietary energy source (starch vs. lipid and fibre) and days on feed (DOF) for finishing steers. Angus crossbred steers were randomly assigned to one of two isocaloric/isonitrogenous treatments consisting of 10% haylage, 77% high-moisture corn, 11% soybean meal, and 2% vit/min pre-mix including monensin on a DM basis (CON; n = 24) or high-fat, high-fiber pellet that replaced 30% of the high-moisture corn (HLHF; n = 24) relative to the CON diet. The HLHF contained 29.8% wheat shorts, 26.2% corn DDGS, 18.8% soy hulls, 19.2% corn gain, and 6% tallow). At d 1 (n=12), 42 (n=11), 126 (n=12), and 168 (n=13) DOF, steers from each treatment were slaughtered and the pancreas was collected. Immunohistochemistry was conducted on paraffin-fixed pancreas samples to quantify the percent of islets in pancreatic tissue, percent positive insulin staining cells within the islets, average cells per islet, and average islet size. Glucose and insulin concentrations were analyzed from plasma obtained 3 to 5 d prior to slaughter. All data were analyzed using the GLIMMIX procedure of SAS with DOF, dietary treatment, and their interaction as fixed effects. Treatment means were compared using the Tukey-Kramer test and significance declared at P 0.05. HFHF steers had a greater proportion of positive stained insulin cells within islets than CON (P =0.01; 58 vs 41% ±4.6, respectively); however, DOF had no effect ($P \geq 0.71$; 57.7, 47.7, 44.2, 53.5% ±6.7, for d 1, 42, 126, 168, respectively). The average number of cells per islet tended (P = 0.054) to be higher at d 168 than d 1, but was not influenced by dietary treatment (P =0.28). Plasma insulin was not affected by treatment (P =0.51); however, plasma insulin was greater at d 126 and 168 DOF than d 1 (P while d 45 was intermediate. Plasma glucose was not affected by treatment or DOF (P ; however, the insulin to glucose ratio was greatest at d 1 (P and similar across all other time points (P This study indicates that dietary energy source may influence insulin production in the pancreas, independent of DOF, but DOF may decrease insulin sensitivity. These results suggest that insulin signalling may contribute to declining feed efficiencies often observed in steers towards the end of the finishing period.

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P 115

Gene expression in the skeletal muscle of *Bos taurus* and *Bos indicus* steers undergoing compensatory gain

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Cattle in northern Australia undergo stasis or loss of liveweight during the dry season when pasture crude protein (CP) and metabolisable energy (ME) content decline (35 g CP and 5 MJ ME/kg DM). This is followed by a period of accelerated liveweight gain during the subsequent wet season when pasture quality increases (100 g CP and 9 MJ ME/kg DM). The extent of compensatory liveweight gain during the wet season is variable and will influence annual liveweight production and enterprise profitability. The objective of this experiment was to identify genes involved in compensatory growth of muscle in steers.

Holstein (n=15; 230±34 kg liveweight) and Brahman crossbred (n=15; 178±6 kg) steers were equally allocated to one of three nutritional treatments during a 104-day restriction period [high CP-high ME intake (HCP-HME), low CP-low ME intake (LCP-LME) and high CP-low ME intake (HCP-LME)] followed by HCP-HME intake during a 105-day realimentation period. Biopsies were collected from the *semitendinosus* muscle of steers at the end of the restriction period and early (day-35) and late (day-103) in the realimentation period. The relative abundance of the following mRNA were determined in muscle biopsies using real-time PCR: insulin-like growth factor-1 (IGF1), -2 (IGF2) and the type-1 receptor (IGF1R) and binding proteins-3 and -5 (IGFBP3, IGFBP5), transforming-growth factor-1 (TGFβ1), -2 (TGFβ2) and the type-1 receptor (TGFβ1R) and Kinesin Family Member 21A (KIF21A). Statistical analysis of data was performed using a general linear model.

At completion of the restriction period HCP-HME steers had significantly higher IGF1, IGFBP5, KIF21A, TGFβ1, TGFβ2 and TGFβ1R mRNA in the *semitendinosus* muscle compared to steers with restricted ME intake. In contrast, IGF1R and IGFBP3 mRNA abundance were significantly lower in the *semitendinosus* muscle of HCP-HME steers compared to steers with restricted ME intake. Early during the realimentation period steers previously consuming the HCP-HME treatment had more IGFBP3, IGFBP5, KIF21A, TGFβ2 and TGFβ1R mRNA and less IGF2 mRNA in the *semitendinosus* muscle than steers previously with restricted ME intake. At the end of the realimentation period there was no difference in abundance of any mRNA in the *semitendinosus* muscle. Across all time points, IGFBP3 and IGFBP5 mRNA were more abundant in the *semitendinosus* muscle of Holstein compared with Brahman steers, while KIF21A, TGFβ1 and TGFβ2 mRNA were more abundant in Brahman steers. It is concluded that IGF and TGF genes have key roles in compensatory gain in cattle.

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Metabolic variables and DNA polymorphism of leptin and IGFBP-3 gene in relation to residual feed intake in buffalo calves

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Evaluation of the relationship between efficiency of feed utilization and blood variables permits the identification and selection of efficient and productive animals. Residual feed intake (RFI) can be used to identify those animals that deviate from their expected level of feed intake and they can be of high efficiency (negative residual intake) or low efficiency (positive residual intake). The present experiment was conducted to study the relationship of residual feed intake with blood plasma metabolic hormones and DNA polymorphism of Leptin and IGFBP-3 gene in fifteen buffalo calves of seven to nine months age. The animals were given green fodder and concentrate mixture as per ICAR, 2013 feeding standard. Residual feed intake was computed for each animal and was assumed to represent the residuals from a multiple regression model. The mean values of T3 and T4 were found to be non-significantly different between the two RFI groups and were negatively correlated (r = -0.37 and -0.30 respectively) with RFI. The Concentration of IGF-1 and Leptin in blood plasma were found to be higher significantly ($P<0.05$) in low RFI group and were negatively correlated (r = -0.49, r= -0.23) with RFI. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) was performed in our studied population to identify candidate point mutations in Leptin and IGFBP-3 gene. Leptin PCR amplified product of 522 bp after BsaAI digestion revealed only two genotypes; an intact 522 bp fragment as AA genotype in RFI +ve animals and 522, 441 and 81 bp fragments as GA genotype in RFI –ve animals. In our study, Leptin allele A was prominent with allele frequency 0.75 compared to 0.25 FOR G allele. The 651 bp fragment of IGFBP-3 gene was digested with HaeIII restriction enzyme AND revealed monomorphic banding pattern indicating BB genotype i.e215, 164, 154, 56, 36, 18 and 8 bp. The present study gives an insight of variation at particular locus with respect to Leptin gene and highly conserved targeted sequence of IGFBP-3 gene in low and high efficient Murrah buffalo calves. However, validation of results on large number of animals is warranted to identify physiological indicators that could be predictive of residual feed intake in buffalo calves.

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P 117

Deduction of reference values for parameters in blood and urine of dairy cows

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The object of this multi-site study was to establish the metabolic profile for dairy cows considering breed, number of lactations, stage of lactation and energy supply.

Methods: For our project „optiKuh"[1)], blood and urine samples from almost 1,500 dairy cows were taken during a period of 2 years. The cows were of breeds German Holstein (GH), German Simmental (GS), and Brown Swiss (BS). They were housed in 12 experimental farms distributed over Germany. About one third was primiparous. Blood samples were taken on day (d) 8, 28, and 100 postpartum (p.p.), at the beginning of the dry period and around d 14 ante partum. We used a standardized procedure and analyzed all samples in one laboratory. To investigate the influence of the feeding intensity on metabolic parameters, cows were grouped in two feeding groups (A and B). In group A the cows were fed with up to 4 different feeding intensities (Group 6.1N: Roughage 6,1 MJ NEL / kg DM (dry mass) + 150 g / kg ECM CS (concentrated supplementation); 6.1H: Roughage 6,1 MJ NEL / kg DM + 250 g / kg ECM CS; 6.5N: Roughage 6,5 MJ NEL / kg DM + 150 g / kg ECM CS; 6.5H: 6,5 MJ NEL / kg DM + 250 g / kg ECM CS) . The cows in variation B received routine feeding. Individual feed intake was recorded by using automatic feed troughs. Energy balance was calculated from energy intake and energy requirement for milk production, growth, gestation and maintenance. Statistical analyses were performed using function lme and predict.lme of R (version 3.4.2).

Results: There was no effect by day of sampling on glucose, calcium and adiponectin, but on beta-hydroxybutyrate (BHB), insulin, insulin-like-growth-factor 1 (IGF-1) and non-esterified fatty acids (NEFA). Between primiparous and multiparous cows we found different values for BHB, Insulin and IGF-1. BHB values from heifers are lower, Insulin and IGF-1 are higher than the values of multiparous cows. The feeding intensities influenced BHB, Insulin and IGF-1, but only in the beginning of lactation.

Conclusions: The differences in the metabolic parameters between heifers and cows are an evidence for the need of more detailed reference values. The observed changes during lactation in BHB, insulin, IGF-1 and NEFA concentrations suggest that elaborating reference values specific for defined stages of lactation could be useful to detect metabolic disorders earlier.

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Effects of excess and limited dietary nutrition during whole period of gestatorgans development in Wagyu cattle.

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Background/Aims: Fetal and neonatal programming based on the concept of Developmental Origins of Health and Disease (DOHaD) would have potential to contribute not only medicine but also livestock production. In reference to Wagyu (Japanese Black cattle) which is a fatty type of beef cattle, it has not been experimentally verified that how maternal nutrition affects fetus development during the whole period of gestation. The objective of this study is to investigate the effects of excess and limited maternal nutrition during the whole gestation on several organs development of fetus in Wagyu cattle

Method: Wagyu cows were allocated into high-nutrition (HN: n=5) group to meet 120% of nutrition requirements and low-nutrition (LN: n=6) group to meet 60% of it before fertilization. All the experimental cattle sequentially have been artificially inseminated (AI) with male-sorted semen of an identical sire. In each group, the fetuses were taken by Caesarean section at 260±8.3 days of fetal age and killed with bleeding after anesthesia and dissected. The weights of brain, pituitary, thyroid, liver, kidney, thymus, pancreas, spleen, heart, lung, rumen, reticulum, omasum, abomasum, testis, small intestine and large intestine of fetus were measured.

Results: The weight of liver, kidney, thymus, spleen, heart, lung, omasum and large intestine were significantly larger in HN group than in LN group ($P<0.05$, $P<0.05$, $P<0.05$, $P<0.05$, $P<0.05$, $P<0.01$, $P<0.05$ and $P<0.05$, respectively).

Conclusions: Maternal nutrition during whole period of gestation would strongly affect parts of some organs development of fetus in Wagyu. These things might indicate that the control of maternal nutrition during the whole period of gestation has quite great potential of several organ development of Wagyu fetus, which might be closely related to the future productivity of meat quantity and quality in cattle.

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O 25

Instability versus Acidosis: Is ruminal pH associated with milk production?

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Ruminal acidosis in its subacute or chronic form is believed to be detrimental to animal health and productivity in dairy cows. While clinical models have shown various effects of non-acute forms of ruminal acidosis on metabolism, field data linking ruminal acidosis to production are scarce. The introduction of ruminal pH sensors has helped to understand the course of ruminal pH, however the large volume of data produced by these systems is not easy to interpret. This study aimed to correlate pH patterns derived from ruminal pH-sensors with milk production observations.

24 lactating Holstein cows on a German research farm were each equipped with a continuous ruminal pH measuring system. Milk production and dry matter intake were recorded daily, and pH-values were recorded every 10 minutes for a 50-day period. The observed daily patterns in pH values were fit to a modified sine wave using a series of statistical models, and the daily 'unpredictability' was estimated for each 24-hour period of pH observations relative to the typical daily patterns for that cow.

There were a total of 160,265 pH readings, with the majority being above a pH of 5.8. The statistical model identified an individual strong circadian pH pattern that was broadly consistent, but with a mean pH that differed significantly between animals. Periods of unpredictability in pH (deviations from the standard diurnal patterns) were significantly associated with decreased productivity in terms of both dry matter intake and milk production. However, abnormally low pH observations were not associated with any change in productivity in themselves.

The results indicate that disruption of the usual diurnal patterns in pH are associated with decreased productivity, but there is no evidence for a simple pH threshold that causes a depression of milk yield in these clinically healthy animals. Extreme fluctuations in pH will likely reduce the effectiveness of ruminal fermentation, which may consequently lead to the observed production loss. However, this was observed with both relatively low and high pH values. We therefore conclude that reduced productivity is associated with ruminal instability rather than acidosis. The model is believed to help in the analysis of ruminal pH data as deriving from intraruminal pH-sensors.

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O 26

Validation of a monitoring system of individual drinking behavior for dairy cows housed in loose housing barn

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Individual monitoring of drinking behavior of dairy cows can be a promising tool to predict individual rupture in daily dry matter intake (DMI) and events such as estrus or health problems. Systems allowing this monitoring could also be easier to install in loose housing barns than DMI individual monitoring systems and they do not add constraints on diet delivery to cows. A new individual monitoring system of drinking behavior consists in connected constant level drinking troughs, equipped with electronic flow meters (EFM) and RFID antennas, allowing a centralized storage, for each individual drinking bout, of time, duration and ID of drinking cow. Twenty-four connected drinking troughs were installed at INRA experimental farm (Méjusseaume) in a loose housing barn containing 92 dairy cows. The validation process consisted in 5 steps. First, parametrization of flow of water per impulsion of the 24 EFM was checked by weighting volume of water flowing in water troughs during 1 minute. Second, repeatability and reproducibility of EFM measurements were characterized (4 measurements/day, 4 days). Third, robustness of EFM measurements was tested in situations of low water pressure in the farm water network. Fourth, sensibility and specificity of recognition of cows during each bout by the RFID antennas were calculated by recording drinking behaviors of 23 cows during 3 days by both individual monitoring system and video recordings. Fifth, error risk linked to the fact that EFM indirectly measured water intake by filling of water troughs was characterized by positioning the necessary time to refill water troughs on a frequency diagram of inter-bout durations of all drinking troughs during 3 days of recording. Average measured flow of water per impulsion of EFM was 0.00249 ± 0.000025 l for a default parametrization of 0.00250 l. Average repeatability and reproducibility RSD were 0.27 ± 0.16 % and 0.34 ± 0.15 % for the 24 drinking troughs. Trough filling rate decreased from 12.3 ± 0.5 l/min to 7.6 ± 0.2 l/min when water pressure dropped from 2 to 1.4 bars (maximum water draw). It remained in the range assuring a constancy of flow per impulsion of EFM. Sensibility of detection of cows at drinking troughs was 98.5% and specificity was 98.9% (1012 drinking bouts). More than 96% of inter-bouts intervals lasted more than 15 s, which is the filling time of troughs. A conclusion is that the tested system is reliable for individual drinking behavior recording in a 92 cow loose housing barn.

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O 27

SmartCow: an integrated infrastructure for increased research capability and innovation in the European cattle sector

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The sustainability of cattle production requires improved resource use efficiency, reduced GHG emissions, improved animal health and welfare. The European Strategy Forum on Research Infrastructures roadmap clearly identified the need for improved coordination, harmonisation and access to European research infrastructures (RIs) on farm animals. To face these challenges, the SmartCow project (www.smartcow.eu), answering the call H2020-INFRAIA2016-2017, was selected by the European Commission for 4 years funding starting from 1st February 2018. Networking and joint research activities, as well as transnational access to RIs are being developed.

Networking activities have started to create, thanks to an inventory and an interactive map, a unique portal to key European cattle RIs. The project works towards the use of unified measurement methods through common standards and guidelines. A book of methods in cattle research currently in development will provide guidelines for measurements of metabolic, digestive, anatomic, behavioural traits. The development of the cattle ontology of traits (ATOL and EOL; www.atol-ontology.com) through SmartCow will help to unify research methodologies and link trait definition with standardized methods. A cloud-based database platform will ensure integration, sharing and interoperability of data generated by the project leading to an open European database on cattle traits and phenotypes.

Joint research activities work towards the use of less-invasive methods and high-throughput phenotyping. Refining in vivo methods to evaluate feed efficiency and environmental emissions (N and CH_4) will generate innovations in experimental design and planning for further accuracy. The development of new biomarkers (proxies) that can be easily measured in milk, faeces, urine, or blood through rapid analytical methods (e.g. NIRS) will bring new phenotyping capacities. The development of tools to generate new and improved information from animal sensors and other routinely collected data (e.g. prediction of individual cow status in terms of health and welfare) will enable a more efficient phenotyping and genetic selection of cattle.

Finally, the project organizes transnational access to major RIs: INRA (France), Scotland's Rural College and University of Reading (UK), Wageningen University and WUR/DLO (the Netherlands), FBN-Leibniz (Germany), Teagasc (Ireland), Aarhus University (Denmark) and IRTA (Spain). It provides access to around 2500 dairy and 1000 beef cattle and will facilitate up to 30 research projects to be financed by the SmartCow project after selection through specific calls. Eleven projects have already been selected after the first call. Two other calls are planned in the course of the project.

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Post-partum energy balance of Estonian Holstein cows – practical experiences from on-farm estimations based on body weights and BCSs

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Monitoring of post-calving energy balance (EB) of individual cows is critical for optimizing their health and reproduction. As the direct measurement of EB is not possible, indirect estimation algorithms are needed. Thorup et al (2012) presented one based on frequent body weight (BW) and body condition score (BCS) measurements. The task of the study was to estimate the daily BW, BCS and EB of individual cows, based on their fluctuating on-farm measures, using different statistical models and to study the effect of pre-calving BCS on post-partum EB. Automatic daily weighing and BCS estimation data from 43 multiparous cows from the experimental farm of the Estonian University of Life Sciences were used.

The average weighing period was from the 5.5th to the 142.7th day after calving, the average number of weighings per cow was 113.5. Two trained persons registered BCSs at approximately two-week intervals until the 12th week after calving; the average number of BCS observations per cow was 8.0. To achieve daily BW and BCS of individual animals', quantile regression models as well as general linear models with different polynomial or spline functions of days in milk (DIM) were fitted. In the models, fixed or random effects of animal, fixed effects of parity and pre-calving BCS and interactions with DIM were all considered. Models' Akaike information criterion values, standard errors (SE) and visual inspection of fitted curves and prediction errors were used in model selection. SAS 9.4 procedures HPMIXED and QUANTREG were applied. The best model to predict daily BW of cows considered cubic spline function of DIM with nonequidistant knots at 14, 21, 70 and 112 DIM, parity and pre-calving BCS and their interactions with cubic spline of DIM, and random cubic spline of DIM for each cow. For BCS a similar model with spline of degree 2 and one knot at 70 DIM was used. The SE of the models were 19.1 kg for BW and 0.15 units for BCS. The changes in post-partum EB and overall energy deficit were the highest in animals over-conditioned (BCS>3.5) pre-partum.

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Thorup, V. M., Edwards, D., & Friggens, N. C. (2012). On-farm estimation of energy balance in dairy cows using only frequent body weight measurements and body condition score. Journal of dairy science, *95*(4), 1784-1793.

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O 28

Lipogenic gene expression and chemical composition of longissimus muscle in cattle fed wet distillers grains

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Introduction: Cattle fed grain-based diets for extended periods may develop higher marbling levels. However, there is no consensus about the effects of dietary starch content of feedlot diets on marbling and lipogenic gene expression. This trial evaluated the expression of genes involved in lipid metabolism and chemical composition of longissimus muscle (LM) in cattle fed increasing levels of wet distillers grains (WDG).

Materials and Methods: A hundred F1 Angus-Nellore bulls with initial average body weight of 369 ± 48 kg were fed for 129 days in a completely randomized block design. The animals were allocated to group pens with 5 animals each, with five pens per treatment. The diets presented: 148, 161, 191 and 220 g of CP/kg DM; 627, 540, 434 and 328 g of NFC/kg of DM; and 162, 234, 308 and 382 g of NDF/kg DM, according to the level of WDG (0, 150, 300 and 450 g/kg DM). Immediately after slaughter, samples were taken from the LM between the 12th and 13th ribs for chemical composition (AOAC 2007.04) and gene expression analyses (RT-qPCR). Data were analyzed using the MIXED procedure of SAS with linear, quadratic and 0 vs. WDG contrasts.

Results & Discussion: The inclusion of increasing levels of WDG caused linear decreases in intramuscular fat content of LM (2.67; 2.32; 2.40 and 2.03%; $P<0.05$). Moisture, protein and mineral contents were not affected by the treatments ($P>0.05$). Stearoyl-CoA Desaturase (SCD1), Peroxisome Proliferator-Activated Receptor Gamma (PPARy), Fatty Acid Synthase (FASN) and Carnitine Palmitoyltransferase II (CPT2) expressions were linearly downregulated according to increasing levels of WDG ($P<0.05$). Sterol Regulatory Element Binding Transcription Factor 1 (SREBP1), Lipoprotein Lipase (LPL) and Acetyl-CoA Carboxylase (ACACA) expressions were not affected by the treatments ($P>0.05$). PPARy is the main regulator of fatty acid storage and adipogenesis, thus its downregulation could explain the reduction in intramuscular fat content. Nutritional treatments used in this trial contained decreasing levels of starch (increasing levels of WDG), then, cattle fed high-starch diets had higher LM SCD1 expression. The intramuscular fat content is one of the most important traits in meat quality and influences consumer decisions. Therefore, replacing corn by high proportions of WDG can decrease the beef added value.

Conclusion: The inclusion of by-products such as WDG in feedlot diets decreases intramuscular fat in LM and downregulate lipogenic genes expression.

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O 29

MitoCow – Short-term adaption of metabolite profiles after parturition in L-carnitine supplemented Holstein dairy cows

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Background: This work is part of the MitoCow project, which aimed to elucidate the functionality of the cow's mitochondria during individual and standardized stress situations, interlinking a broad variety of metabolic, physiologic and genetic data. Metabolite profiling by a metabolomics approach provides novel insights into metabolic pathways associated with mitochondrial functionality of individual dairy cows and could open new perspectives in dairy sciences. The acute adaptation of metabolite profiles within a few hours after parturition of adult dairy cows has not been determined yet. However, in this early time period metabolism already switched to onset of lactation.

Objective: Blood metabolite profiles and insulin concentrations were determined during a small time window after parturition in L-carnitine supplemented dairy cows.

Methods: Blood samples were collected from 59 multiparous German Holstein-Friesian cows during the transition period from late pregnancy to early lactation. The cows were divided into a control (n=30) and a L-carnitine supplemented group (25 g rumen-protected carnitine/ d/animal; n=29). The dietary carnitine supplementation started on day 42 ante partum after the first blood collection. For metabolome analysis 4 time points were chosen: calculated day 42 prior to calving and 6, 12 and 72 h after calving. Metabolite profiles were determined in EDTA-plasma samples by liquid chromatography and mass spectrometry using the AbsoluteIDQ p180 Kit (Biocrates Life Science AG, Innsbruck, Austria). Data were analysed by bioinformatic tools (https://www.metaboanalyst.ca). Blood insulin levels were measured by a bovine-specific insulin ELISA (Mercodia AB, Uppsala, Sweden) at day 42, 14 and 3 *ante partum* and 0.5, 1, 2, 3, 4, 6, 9, 12, 24, 48, 72 h *postpartum*.

Results: Dietary carnitine supplementation provoked changes in the blood acylcarnitine levels. Most prominent was the difference in short-chain acylcarnitines ($p < 0.001$) in the small window after calving. Furthermore, medium ($p < 0.001$) and long-chain acylcarnitines ($p < 0.001$) were higher in carnitine-supplemented cows after calving. Blood insulin concentration decreased from 14 days prior to parturition until 1 h after calving and increased 3 h after parturition. Changes in acylcarnitine concentration were discussed in the context with classical biochemical indicators such as non-esterified fatty acids, beta-hydroxybutyrate, glucose and insulin.

Conclusion: Dietary carnitine supplementation potentially led to a stimulation of mitochondria functionality as indicated by higher acylcarnitine concentrations in plasma. However, insulin-glucose metabolism was not affected by dietary carnitine.

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O 30

Contrasted status in B vitamins between dairy cows and goats fed various lipid supplements

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B vitamin status and metabolism are still known in ruminants in spite of their importance for the nutritional value of dairy products (Coudray et al., 2011), productive performance (Girard & Matte, 2005) and apparent link to feed efficiency (Meale et al., 2017, Li & Guan, 2017). In ruminants, B vitamins are from dietary and ruminal origins except B12, exclusively synthesized by rumen bacteria (Graulet, 2014). A better understanding of the factors modulating B-vitamin status in ruminants would help to improve performance of livestock systems. We compared the B-vitamin status in 12 Holstein dairy cows and 12 Alpine dairy goats receiving the same diets supplemented or not with lipids for 28 d-periods in 2 species distinct (4 × 4) Latin square designs. Diets were based on hay (45 %) plus concentrates (55%) containing no additional lipid (CTL), or supplemented with corn oil and wheat starch (COS), marine algae powder (MAP), or hydrogenated palm oil (HPO) (Fougère et al., 2018). Vitamins were analyzed by liquid chromatography for B_2 and B_6 (Meale et al., 2017; Laverroux et al., unpublished) and radioassay for B_9 and B_{12} (Duplessis et al. 2015) in plasma and milk at the end of each period.

Cows had higher B_2 (x2), B_6 (x2 to 3) and B_9 (x5) plasma concentrations than goats ($p<0.001$) whereas B_{12} concentration was 3.4-fold higher in goats ($p<0.001$). In milk, B_6 concentrations were higher in goats (+21%, $p<0.001$) than in cows. Riboflavin (B_2) concentration was similar between the 2 species whereas folates (B_9) and vitamin B_{12} concentrations were 10- and 16-fold higher in cow milk, respectively. The COS diet increased plasma B-vitamin concentrations in both species ($p<0.001$), and cow milk concentration of B_2 and B_9 ($p<0.001$). The MAP diet also induced significant increases in plasma B vitamin concentrations, especially B_2 in goats and B_6 and B_9 in cows. Milk B_6 concentrations were lightly reduced and B_9 was increased in cows fed MAP diet. The HPO diet slightly increased vitamin B_{12} secretion in cow milk. This original study compared plasma and milk B vitamins in dairy cows and goats fed the same diets. Species-specific responses observed in ruminants fed COS or MAP diets vs CTL suggest distinct mechanisms acting on B-vitamin supply, likely their dietary intake and the modulation of rumen bacterial activities. Discrepancies in the pattern of response between plasma and milk also suggest the existence of regulatory mechanisms of vitamin B mammary uptake and milk secretion.

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O 31

Comparing the protective effects of butyrate on ovine rumen and porcine colon epithelium under hypoxia

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Introduction: Ruminal and colonic epithelium vary greatly regarding their morphology. However, they have outstanding similarities in their functional characteristics. Due to the microbial fermentation, both rumen and colon epithelium are exposed to a high intraluminal concentration and inflow of n-butyrate. Butyrate is known to modulate differentiation, proliferation and gene expression in various cell types. Therefore, its effects on the epithelial integrity and gene expression profiles are of special interest. We aimed to compare these effects in ruminants and monogastric animals, i.e. ovine rumen and porcine colon epithelium. Additionally, changes induced by n-butyrate were compared with the effects of hypoxia, because metabolite accumulation after O_2 depletion is at least partly comparable to the accumulation of metabolites after n-butyrate exposure and protective effects of butyrate have been reported under pathologic conditions like inflammation and hypoxia (Gill et al. 2018).

Material & Methods: We incubated isolated ovine rumen epithelium and porcine colon epithelium in Ussing chambers in a buffer solution containing 50 mM Na-butyrate or 50 mM NaCl instead. The solutions were gassed with either 100% O_2 or with 1% O_2 and 99% N_2 to simulate hypoxia. Throughout the incubation, electrophysiological parameters (short circuit current, I_{sc}, and tissue conductance, G_t) were measured. After the incubation, tissues were dismounted and the mRNA and protein expressions of proteins involved in the transmembrane transfer of short-chain fatty acids and their metabolites were measured by RT-qPCR and Western Blot.

Results: In both epithelial types, electrophysiological measurements showed hypoxia-induced damage in the epithelia. N-butyrate-incubation seemed to improve the epithelial integrity throughout the incubation time. In rumen epithelium, the mRNA expression levels of monocarboxylate transporter (MCT) 1 and 4 and glucose transporter 1 were upregulated after both n-butyrate exposure and hypoxia. Upregulation of both MCT isoforms after n-butyrate incubation could be detected on protein level as well. In colon epithelium, the effects were less pronounced, but we could also observe an upregulation of MCT1 gene expression.

Conclusion: Our study provides evidence for a protective role of n-butyrate in both rumen and colon epithelium, indicating a common mechanism. The nature of this mechanism, likely a manipulation of the NFκB-pathway or simple nutritive effects of butyrate, needs further investigation.

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O 32

MitoCow - Effect of dietary L-carnitine on performance and selected blood parameters indicative for energy metabolism in dairy cows

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Dairy cows are metabolically challenged during transition period by a drastic increase of energy demand for milk production on one hand and restricted feed intake capacity on the other hand. Parturition represents an inflammatory challenge. The degree of energy deficit depends, amongst others, on the efficiency of mitochondrial ß-oxidation, in which carnitine plays an important role. The present experiment was designed to test whether carnitine supplemented dairy cows are less affected by negative energy balance during transition period because of an increased efficiency of ß-oxidation.

Pluriparous German Holstein cows were assigned to a control (CON, n=30) or a carnitine group (CAR, n=29). Experimental feeding started six weeks *ante partum* (ap) with a ration consisting of 80% roughage and 20% concentrate (dry matter basis) until day one *ap*. Then, concentrate proportion was increased from 30% up to 50% within two weeks *postpartum* (pp) and kept constant thereafter. Carnitine supplementation (25g/day*cow of rumen-protected L-carnitine) was realized via concentrate feed. Milk yield was determined twice daily, milk composition twice a week and blood samples were taken at the following time points: day/s(d) 42, 14, 7, 3 ,1 ap; 0.5, 1 , 2, 3, 4, 6, 9, 12, 24, 48, 72 hour/s pp and d 7, 14, 21, 28, 42, 56, 100, 110 pp. Carnitine, its precursors (N^{ε}-trimethyllysine [TML], y-butyrobetaine [yBB]) and acetylcarnitine were measured via HPLC-MS. Non-esterified fatty acids (NEFA), ß-hydroxybutyrate and triglycerides in plasma were determined spectrophotometrically. Data were statistically analyzed using a MIXED-Model procedure (SAS 6.1) with time, group and their interaction as fixed factors, time as repeated measurement and day 42 ap as covariable.

Carnitine supplementation did not have a significant impact on net energy balance, NEFA and ß-hydroxybutyrate. In CAR daily milk yield was ~ 2 kg higher in the first five weeks of lactation ($p_{time*group}$ = 0.008). In the first week of lactation milk fat was 10 % higher, whereas milk protein was 6 % lower in the first two weeks of lactation ($p_{time*group}$ < 0.05) compared to CON. Triglycerides concentration in CAR was significantly higher (0.06 mmol/l) at 3 and 1 d ap in comparison to CON ($p_{time*group}$ < 0.002). Carnitine, yBB and acetylcarnitine concentrations were significantly higher in CAR throughout supplementation. Only for TML carnitine supplementation had no impact.

The influence of carnitine on milk yield and milk fat might hint at an enhanced efficiency of energy utilization in supplemented cows.

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O 33

Cattle and sheep – a hypothesis for a basic difference in digestive physiology

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Ruminants have been described to vary in their strategy to influence ruminal fermentation via the ratio of ruminal particle and fluid passage rate („gut-wash"). High fluid outflow in relation to particle retention has been interpreted as a strategy to maximize microbial growth, while slow ruminal fluid throughput is assumed to minimize effects of plant deterrents/toxins in the lower gastro-intestinal tract (GIT). Cattle appear to be the ruminant most distinctively following the former strategy (microbial output), even if compared to other dominantly grazing species like sheep. If rumen microbial output is maximized, a lower apparent crude protein digestibility (aCPD) can be expected to result from increased faecal losses of residual microbial protein. In a meta-analysis, the ratio of gut mean retention times of particles and fluid (MRTparticle/MRTfluid) (10 studies; 37 values per species), aCPD and overall diet digestibility (both 25 studies; 167 values per species) were compared between cattle and sheep; only studies reporting values for both species on identical diets were used. Data was investigated using a mixed linear model in SAS 9.4 with animal species as fixed factor and study and diet as random factors. For the MRT comparison, part of GIT - rumen or whole tract - was included as random factor. Since only either DM or OM digestibility was given, type of digestibility was included as further random factor in the model for overall diet digestibility. All values are given as LSmeans. The ratio of MRTparticle/MRTfluid was larger for cattle than for sheep (2.10 vs. 1.70, SEM = 0.255; $p < 0.0001$); for aCPD, a lower value was present for cattle compared to sheep (n = 334; 59.5 % vs. 62.8 %, SEM = 2.67; $p < 0.0001$). Results for overall diet digestibility showed no difference between species (cattle vs. sheep: (n=334) 66.4 % vs. 66.8 %, SEM = 4.84; species: $p = 0.48$). Data support a hypothesis of cattle maximizing microbial output from the rumen via increased MRTparticle/MRTfluid ratio, most likely by increased saliva flow. Higher microbial growth increases faecal N from microbial residues and in consequence decreases aCPD in cattle. Further points to be investigated will be the influence of factors like feed intake (potentially increasing the effect seen for aCPD) and higher selectivity in sheep (potentially decreasing the effect for aCPD).

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O 34

Evaluating the accuracy and precision of conceptual models for predicting the nitrogen excretion of cattle in tropical environments

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To assess effectiveness in reducing cattle nitrogen (N) excretion in tropical environments, quantifying urine-N and feces-N is required. Adopting N-excretion conceptual models might help to simulate feeding strategies to improve N use efficiency. Temperate conceptual models have been proven to accurately predict N-excretion from temperate cattle systems (AFRC,1993; GfE, 2001; INRA, 2018). However, it is unknown how accurate temperate models can be in husbandry systems under tropical conditions. Hence, the objective was to evaluate the accuracy and precision of four conceptual models for predicting cattle N-excretion under typical tropical feeding situations using data from in-vivo trials conducted by our institute.

The conceptual models build on the feeding recommendations by AFRC (1993; Model A), GfE (2001; Model G), and INRA (2018; for temperate and warm climates; Model It and Iw, respectively). Predicted total, urinary, and fecal-N-excretions were then evaluated using a dataset of 6 studies including 28 dietary treatments (i.e. N under- and oversupply) and 388 cattle observations fed typical tropical feedstuffs. The concordance correlation coefficient (CCC) was used to evaluate model accuracy and precision using NCSS (2019). The database included information (arithmetic mean±standard deviation) on body-weight (419 ±133.5 kg), dry matter intake (13.3±6.4 kg/animal and day), N-intake (345±190.6 g/animal and day), rumen microbial N production (199±132.4 g/animal and day), fecal-N-excretion (102±53.8 g/day), and urinary-N-excretion (140±89.3 g/animal and day).

Model I (CCC=0.92) predicted fecal-N slightly more accurately than models A (CCC=0.90) and G (CCC=0.83), mainly because model A was more sensitive to extreme values and model G tended to underestimate fecal-N-excretion. For urinary-N-excretion, model G (CCC=0.76) and A (CCC=0.65) resulted in highest CCC compared with model It (CCC=0.51) and Iw (CCC=0.12). Low CCC in models I were due to the fact that they overestimated urine-N-excretion (e.g., cattle fed at or below maintenance energy requirements) and underestimated (in case of model It) it at high N-excretion levels (e.g., high-lactating-dairy-cows).

Fecal-N-excretion of cattle under tropical conditions can be accurately estimated using model I. Model G and A have potential on estimate urine-N of cattle under tropical conditions. However, models I, require further adjustments to improve accuracy and precision of predicted urinary-N-excretion in tropical cattle.

AFRC (Agricultural and Food Research Council), 1993. Energy and Protein Requirements of Ruminants. 159pp.
GfE (Gesellschaft für Ernährungsphysiologie), 2001. Empfehlungen zur Energie- und Nährstoffversorung der Milchkühe und Aufzuchtrinder. 135pp.

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O 35

The effect of dietary sugars on ruminal fermentation, stomach measurements and blood parameters in Reeves's muntjac (*Muntiacus reevesi*)

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Increased intake of mono- and disaccharides (MD) by captive ruminants raises concerns, due to possible negative impact on the gastrointestinal tract (GIT). MD are rapidly fermented in the rumen, which may result in a rapid short-chain fatty acids (SCFA) increase and a pH drop below the optimum. The aim of this study was to determine the effect of addition of a glucose, fructose and sucrose mixture (on as fed basis: 25% of glucose, 25% of fructose and 50% sucrose in the mixture) into the diet on feed intake, ruminal fermentation, stomach measurements and selected blood parameters of Reeves's muntjac (*Muntiacus reevesi*), a browsing ruminant. Eighteen male Reeves's muntjacs were kept individually and fed diets consisting of dehydrated chopped lucerne (*ad libitum*), high-fiber pellet (100 g/day) and wheat bran (30 g/day) without (MD0) or with addition of 10 or 20 g of glucose, fructose and sucrose mixture/day (MD10 and MD20, respectively). Doses of mixture of MD were set to increase intake of these saccharides by 25 and 50% relative to MD0, which resulted in a range of water soluble carbohydrates content in consumed dry matter from 7.5 to 12.1%. Blood, GIT tissue and digesta samples were collected after 14 days of adaptation to the experimental diets, two to three hours after feeding. MD20 group tended to be heavier at the end of the study than MD10 (P = 0.07). Total feed intake (g/day) did not differ between MD0, and MD10 and MD20 (P = 0.22), but was lower for MD10 compared to MD20 (P = 0.02). MD supplementation did not affect ruminal pH ($P \geq 0.33$) nor ruminal SCFA ($P \geq 0.15$), except for higher butyrate concentration for MD10 and MD20 compared to MD0 (P = 0.02). Relative ruminal tissue mass (g/kg of body weight) tended to be lower for MD0 compared to MD10 and MD20 (P = 0.09) and MD supplementation decreased relative mass of full omasum and omasal tissue ($P \leq 0.05$). Relative mass of full abomasum and abomasal digesta were lower for MD0 compared to MD10 and MD20 ($P \leq 0.02$). Plasma glucose, cholesterol, triglycerides and non-esterified fatty acids concentrations tended to be higher for MD10 and MD20 compared to MD0 ($P \leq 0.10$). In conlusion, the increase of dietary MD may affect feed intake, measurements of stomach and blood parameters in Reeves's muntjac, without impacting ruminal pH.

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Dietary and faecal Ca/P ratios in hindgut and foregut fermenters

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Introduction: The relation of dietary calcium/phosphors (Ca/P) ratio to faecal Ca/P ratio differs between species (1,2). Hindgut (HF) and foregut fermenters (FF) could be distinguished by this parameter (1). Ruminants as FF can be classified according to their diets into browsers (B), intermediate feeders (IF) and grazers (G). These types have a specialised gastrointestinal physiology, including putative differences in saliva production (3). The aim of this study was to investigate whether dietary and faecal Ca/P ratios of FF show differences that can be attributed to feeding type.

Animals, materials & methods: Samples of feedstuffs and faeces were obtained from 16 herbivorous species (HF: *Equus ferus przewalskii, Equus ferus ferus*; camelids: *Camelus ferus, Lama glama, Vicugna pacos, Vicugna vicugna*; B: *Alces alces, Giraffa camelopardalis reticulata*; G: *Bos mutus grunniens, Bos primigenius taurus*; IF: *Tragelaphus angasii, Capra capra, Nanger dama mhorr, Bos javanicus, Boselaphus tragocamelus, Bison bison athabascae*) in the Munich Zoo (ethical approval obtained). Faecal samples were analysed for Ca and P (flame emission spectrometry, photometry). Feed samples were either analysed, or data was taken from feed tables (e.g. vegetables). The daily rations were estimated. Dietary and faecal Ca/P ratios were calculated. The difference Δ = dietary Ca/P – faecal Ca/P was compared between groups (ANOVA, $p<0.05$, software SigmaPlot).

Results: HF had a significantly higher Δ than FF (mean±SD 0.67±0.34 vs. -1.76±1.48, $p<0.05$). There was no significant difference between Δ of camelids (-1.07±0.35), B (-1.63±0.85), IF (-2.11±1.76) and G (1.49±1.31; $p>0.05$). The variation of Δ was largest in IF with a range of -6.9-0.1, smallest in HF with a range of 0.1-1.1.

Discussion: The differentiation of HF and ruminants (1) by Δ could be confirmed. Camelids as non-ruminant foregut fermenters did not differ significantly from the ruminant groups investigated and the groups B, IF and G did not show systematic differences. The exotic species investigated show Δ values much lower than those of domestic ruminants in literature (1). High variation of faecal Ca/P ratios was observed in species with a higher proportion of non-roughage P-sources in their diets, such as monocalciumphosphate from supplements. This could cause variation of availability and P metabolism.

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P 83

Diets supplemented with various lipid sources differently affect selected milk metabolites concentrations in cows and goats

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Previous research has shown that animal husbandry factors such as diet composition and intake can alter milk content of selected metabolites (Larsen et al., 2016). Our objectives were to determine the effects of species (cow vs goat) and dietary lipid supplements known to largely impact milk fat content at least in cows, on selected metabolites and enzymes in milk. Twelve Holstein cows and 12 Alpine goats, all multiparous, nonpregnant and at 86 ± 24.9 and 61 ± 1.8 DIM respectively, were fed a basal diet (45% forage + 55% concentrate) not supplemented (CTL) or supplemented with corn oil plus wheat starch (COS; 5% diet dry matter (DM)), marine algae powder (MAP; 1.5% diet DM), or hydrogenated palm oil (HPO; 3% diet DM) in a replicated 4×4 Latin square design with 28-d experimental periods. Data on intake and milk production were reported in Fougere et al. (2018). B-hydroxybutyrate (BHBA), isocitrate, glucose, glucose-6-phosphate, glutamate, cholesterol, choline, free amino acid group, urea, alkaline phosphatase and lactate dehydrogenase were analysed (Larsen et al., 2016, 2017) on morning milk samples collected on d24 of each experimental period.

Production data from this experiment is reported elsewhere (Fougère et al., 2018). In cows, milk fat content was lowered by COS and MAP (-45% and -22% respectively; $p<0.001$) and increased by HPO (+13%; $p<0.001$) compared with CTL, whereas in goats only MAP decreased milk fat content (-15%; $p<0.001$) compared to CTL. Energy and protein balance were positive for all treatments in both species.

Regarding milk metabolites and enzymes, irrespective of diet, cow milk was richer in alkaline phosphatase and glucose compared to goats (16 and 3 times more respectively, $p < 0.01$), whereas goat milk contained more urea and glucose-6-phosphate compared to cows (1.9 and 5.3 times more respectively, $p < 0.01$). In cows, COS decreased BHBA and choline (- 25 and -24% respectively; $p < 0.001$) compared to CTL whereas no effects were observed in goats. COS and MAP increased milk isocitrate compared to CTL in cows, but decreased isocitrate concentrations in goat milk. In cows, milk choline was correlated with milk fat content ($r=0.693$, $p=0.001$), and lactate dehydrogenase was correlated with milk somatic cells count ($r=0.839$, $p=0.001$) but not in goats.

We provide evidence of different milk metabolite responses according to species and diets. Metabolites or enzymes secreted in milk may be indicators of specificities of lipid metabolism among ruminant species, and may contribute to a better understanding of mechanisms regarding milk fat secretion processes.

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Milk molecular species of triacylglycerols characterization by lipidomic approach in cows and goats fed diets supplemented in various lipid sources

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Lipid fraction is a major determinant of milk nutritional quality and efficiency of production that can be modulated by nutritional factors such as lipid supplementation.

In a comparative study between dairy cows and goats fed similar diets supplemented with various lipids, we characterized the milk composition regarding molecular species of triacylglycerols (TAG) by a lipidomic approach. This study was part of a trial aiming at characterizing animal performances and milk lipid responses to diets inducing either milk fat depression (MFD) or increase in milk fat secretion, with species-specific responses (Fougère et al., 2018). The effects of diets containing no additional lipids (CTL) or supplemented with corn oil (5% dry matter intake (DMI)) and wheat starch (COS), marine algae powder (MAP) (1.5% DMI), or hydrogenated palm oil (HPO) (3% DMI), on milk fat content and composition were studied in cows and goats (n=12 per species). Animals of each species were conducted simultaneously in a replicated 4x4 Latin square design. Milk samples were collected over 2 consecutive milkings on d24 of each experimental period. Individual samples were pooled by period and diet (4 x 4 = 16 subsamples by species) and lipid extracted (Bligh & Dyer, 1959) before TAG determination by LC-HR/MS. Data were subjected to ANOVA using dedicated R software. Animal performances and milk FA composition were reported elsewhere (Fougère et al., 2018); in cows, milk fat content was significantly lowered by COS (-45%) and MAP (-22%) and increased by HPO (+13%) compared with CTL, whereas in goats, only MAP decreased milk fat content (-15%) compared to CTL. Lipidomic analysis revealed 48 molecular species of TAG from TAG (18:0) to TAG (64:3) with differences among the 2 species: 1/irrespective of diets, 16 were more abundant in cows (TAG (48:0) to (64:3)) and 23 in goats (TAG (18:0) to (46:3)); 2/on COS, 28 were modulated in cows compared to CTL, but not in goats and 3/on MAP, 13 were modulated in cows and 8 in goats, compared to CTL. Principal component analysis (PCA) performed with milk TAG molecular species and individual FA strongly suggests that the main FA candidates for MFD (*trans*-10 18:1, *trans*-10,*cis*-12 CLA and *trans*-10,*trans*-12 CLA; Shingfield et al., 2010) are carried by specific TAG molecular species that differ between cows (TAG (52:3), (54:3), (54:4)) and goats (TAG (60:4)). These new data demonstrate the species specificities of molecular species of milk TAG in cows and goats and their nutritional regulation.

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P 85

Effects of dietary supplementation of rumen-protected L-tryptophan on growth performance and physiological responses in steers during cold environment

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We previously found that steers fed rumen-protected L-tryptophan (RPT) have a greater body weight via increasing blood cholecystokinin concentration and pancreatic α-amylase activity in duodenal fluids, as well as the starch digestion rate in the small intestine. However, studies of its uses in steers under thermal environment factor are limited. Thus, we evaluate the effects of RPT on growth performance and physiological changes in beef steers during exposure to the cold environment in South Korea. Eight Korean native steers (249 ± 21.6 d-old) were randomly assigned into one of 2 groups according to their initial body weight (279 ± 16.6 kg BW) to test 2 dietary treatments: no RPT total mixed ration (TMR; control, n = 4) and RPT TMR (RPTT; 0.1% RPT supplementation in dry matter basis, n = 4). The steers were housed in individual pens and were fed their assigned diets every morning at 0800, while water was provided *ad libitum* for 7 wks. Average temperature and humidity throughout the experiment were 1.1 ± 0.58℃ and 52.3 ± 4.35%, respectively. We measured body weight before feeding at 0, 4 and 7 wks. Sequently, blood samples collected from the jugular vein of each steer were used to assay metabolic (glucose, total protein, blood urea nitrogen, non-ester fatty acid) and hematological (white blood cell, lymphocyte, monocyte, granulocyte, monocyte) parameters. At 7 wk, loin muscles of each steer were sampled by using biopsy tool to determine the gene expressions related to adipogenic (PPARγ, CEBPα, FABP4) and muscle (MYF6, MyoD, Desmin) developments via quantitative reverse transcription-PCR analysis. ANOVA was conducted using the Proc Mixed procedure of SAS for a completely randomized design with repeated measures. Compared with the control, feeding steers with the RPTT resulted in increased feed intake ($p = 0.011$). Supplementation of RPT to a TMR did not promote an increase in BW in steers but had a positive effect on the elevation of average daily gain (RPTT = 0.766 vs TMR = 0.263, $p < 0.001$) compared with the control. In the blood metabolic parameters, glucose concentration in the RPTT group was greater than that in the control group ($p = 0.041$). Throughout the experiment, there was no difference in the hematological parameters in steers between the control and RPTT groups. In addition, RPT supplementation reduced all adipogenic gene expressions, which may contribute to improving a carcass composition of steers experiencing cold environment by reducing fat deposition.

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Clustering based on liver and blood metabolite concentrations suggests cows are susceptible or resistant to early postpartum metabolic disorders

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Ketosis and fatty liver are prevalent metabolic disorders (MD) in dairy cows associated with maladaptation during the transition to lactation. However, the underlying mechanisms and potential for resistance to these comorbid MD remain elusive. We hypothesize that metabolite concentration differences between cows could infer MD susceptibility and resistance. The objective of this research was to cluster cows based on postpartum metabolites associated with MD, in order to suggest MD susceptibility (onset in the absence of challenge) or resistance (delayed onset, despite challenge). Multiparous Holstein cows were randomly assigned to a control (n=13) or fatty liver induction (FLI; n=12) treatment. Control cows were fed ab libitum peripartum, while FLI cows were offered a daily 6 kg cracked corn top-dress prepartum and their diet was restricted to 80% *ad libitum* intake at +14 days relative to calving (DRTC) until blood β-hydroxybutyrate (BHB) ≥3.0 mM. Liver biopsies and blood samples were collected at -28, -14, +1, +14, +28, +42, +56 DRTC. Sample analysis included liver triglyceride (TG; %DM), serum BHB (mM), and plasma fatty acids (FA; mEq/L). A K-means algorithm clustered cows within original treatment (R, v. 3.5.2), using liver TG, serum BHB, and plasma FA contents at +1, +14, and +28 DRTC. From the two largest clusters per treatment, 4 cows were randomly selected per cluster (n=8 per treatment). Clusters from control cows are labeled A and B, while FLI clusters are C and D. Dependence of clusters on the treatment required statistical analysis (SAS 9.4) to be stratified by treatment. Metabolite and performance data were analyzed using the GLIMMIX procedure, with fixed effects of cluster, DRTC, and cluster by DRTC interaction. A random intercept of cow within cluster and repeated measures of a cow across DRTC were included. The DRTC when BHB ≥3.0 mM was tested between FLI clusters using the LIFETEST procedure. Compared to cluster A, cluster B had greater liver TG (P=0.02), greater serum BHB (P=0.01), similar plasma FA (P=0.30), and similar energy balance (P=0.47). Relative to cluster C, cluster D had greater liver TG (P=0.03), greater serum BHB (P=0.04), and similar plasma FA (P=0.43). In addition, cluster D tended to achieve BHB ≥3.0 mM at earlier DRTC (P=0.10) and tended to have a more negative energy balance at +14 and +42 DRTC (P≤0.09) when compared to cluster C. Therefore, A may reflect cows less susceptible to MD compared to B, while C could represent cows more resistant to MD compared to D.

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"MitoCow" - Effect of dietary L-carnitine on haematological profiles in dairy cows with special emphasis on parturition

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The transition period is the most challenging time for dairy cows due to metabolic changes and inflammatory stimuli. It is characterised by a high incidence of metabolic disorders, partly because of an imbalance between demand and supply of nutrients and energy. The haematological profile and its regulation by nutrients might provide information about adaptation and future outcome of diseases in an individual. The objective of this study was to characterise the changes of the haematological profile and to investigate the effect of dietary L-carnitine on blood cell populations in dairy cows, particularly regarding parturition.

For the feeding experiment 59 pluriparous German Holstein cows were assigned into a control (CON, n=29) and an L-carnitine group (CAR, n=30). L-carnitine supplementation (25g of rumen-protected L-carnitine/day*cow) started six weeks *ante partum* (ap) via concentrate. EDTA blood samples were taken on days 42, 14, 3, 1 ap, 0.5, 1, 2, 3, 4, 6, 9, 12 h *postpartum* (pp) and days 1, 2, 3, 7, 14, 21, 28, 42, 56, 100 and 110 pp relative to calving. White blood cell counts, platelets and related parameters were determined using an automatic cell analyser. Statistical analyses were performed using the MIXED procedure of the Software package SAS (9.4) with time, group and their interaction as main factors, time as repeated measurement and day -42 as a covariable.

L-carnitine supplementation resulted in a 15 % higher platelet count in CAR cows compared to CON within the first 48 h pp. In both groups platelet count and plateletcrit varied slightly between time points within 72 h after calving and started to increase significantly from day 7 until day 28 *pp*. Total leukocyte count increased significantly from day 42 *ap* until 4 h *pp* and returned to initial values after 48 h. This progression mainly reflected changes in granulocyte count. The number of lymphocytes showed only minor shifts over time.

The present study documented changes in white haemogram and platelet count during late pregnancy and early lactation in dairy cows. The most marked changes in white blood cell count occurred within the first 12 h after parturition. Increased leukocyte levels were associated with physiologic stress induced by pregnancy and parturition and were not affected by dietary L-carnitine. Supplementation of L-carnitine led to increasing numbers of platelets shortly after parturition. This might indicate that carnitine stabilized the platelet membrane integrity, which has been documented in in vitro studies.

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Hormones and bone turnover in nutritionally restricted growing cattle

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Circulating bone biomarkers are often used to assess bone formation and resorption in human medicine. In growing cattle, there is no information available about the association of hormones or bone markers with trabecular bone parameters during nutritional restriction. In order to assess these relationships, Brahman (n=15) crossbred and Holstein-Friesian (n=15) steers were allocated within breed and fed one of three diets (n=5/treatment). The control diet had high-protein and high-energy (HCP-HME; 199 g CP and 9.7 MJ/kg DM) content, while the two limiting diets low-protein and low-energy (LCP-LME; 38 g CP and 5.1 MJ /kg DM) or had high-protein and low-energy (HCP-LME). The HCP-HME and LCP-LME groups were offered ad libitum access to the diet, while HCP-LME group was offered the same diet as HCP-HME but was restricted to the same metabolizable energy intake as LCP-LME. Diets were fed for 93 days (d; Phase 1) followed by a period of 103d (Phase 2), when all steers had ad libitum access to HCP-HME diet. Tuber coxae bone biopsies and blood sampling for hormone and biomarkers concentration were conducted at the end of Phase 1 and 2. Trabecular bone samples were processed and histomorphometric measurements were obtained using the software ImageJ. Pearson's correlation coefficient (r) was calculated and a regression analysis was performed for all highly ($r \geq 0.7$) correlated variables. Restricted diets significantly decreased bone volume and trabecular thickness, but these differences no longer existed by the end of Phase 2. Plasma insulin-like growth factor 1 (IGF-1; r=0.6 and r=0.6), insulin (r=0.5 and r=0.5) and total triiodothyronine (T3; r=0.6 and r=0.7) concentrations showed significant ($P<0.001$) positive correlations with trabecular bone volume and thickness respectively. The relationship between T3 concentration and trabecular thickness was independent of cattle genotype through regression analysis. Plasma leptin, total tyroxine and adiponectin hormone concentrations were not correlated ($P>0.05$) with these bone measurements. The bone formation marker bone-alkaline phosphatase (BALP) showed a positive (r=0.5 and r=0.5, $P<0.001$), while bone resorption marker pyridinoline exhibited a negative correlation (r=-0.3 and r=-0.3, $P<0.05$) with trabecular bone volume and thickness respectively. Plasma osteocalcin and total deoxypyridinoline crosslinks concentrations were not correlated ($P>0.05$) with any trabecular bone measurement. Overall nutritional restriction resulted in decreased IGF-1, insulin, T3 and BALP, and increased pyridinoline concentrations, indicative of increased bone turnover resulting in lower trabecular bone volume and thickness. BALP and pyridinoline appear to be useful bone markers to assess trabecular bone metabolism in cattle.

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Impact of starch and sugar addition into the diet on feed intake, body weight and digestibility in addax (*Addax nasomaculatus*)

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It is recommended that grains and fruits should be excluded from diets for captive ruminants. An important rational for this recommendation, beside the fact that these feeds are likely not a part of wild ruminants' diet, is that increased intake of nonstructural carbohydrates reduces intake of roughages and may have a negative impact on the gastrointestinal tract of these animals. The aim of the study was to determine impact of addition of starch and sugar on feed intake, body weight and digestibility in addax. Four adult female addax were used in a 4 × 4 Latin square design and fed a basal diet consisting of a small portion of wheat bran (100 g/day/animal; diet A), wheat bran and wheat (100 and 400 g/day/animal; diet B), wheat bran and sucrose (100 and 50 g/day/animal; diet C) or wheat bran, wheat and sucrose (100, 400 and 50 g/day/animal; diet D). Ground wheat was used as a source of starch and sucrose was used as a source of sugar in the diet. The amounts of wheat and sucrose were set to account for 15 and 2% of dry matter (DM), respectively, consumed by addax prior to the study, thus, reflecting starch and sugar intake with grain and fruits by addax in some zoos. Meadow hay was fed ad libitum. Each experimental period lasted 19 days: 14 days of adaptation and 5 days of data and sample collection. Feed intake was controlled daily. Body weight was controlled at the initiation of the study and at the end of each experimental period. Representative samples of feces were collected during the last 5 days of each experimental period and total tract nutrient digestibility was calculated using indicatory method. Wheat bran, wheat and sucrose were always consumed. Addition of wheat to the diet reduced DM intake of hay ($P < 0.01$) but addition of sucrose had no effect ($P = 0.96$). Total DM intake was not affected by treatment ($P \geq 0.21$) as well as total tract nutrient digestibility, with exception to crude protein digestibility that was greater when wheat was fed in the diet ($P = 0.05$). Body weight was also not affected by treatment ($P \geq 0.31$). There was no interaction between wheat and sugar addition to the diet in the current study. In order to ensure high intake of roughages by captive ruminants, grain (starch) supplementation should be limited.

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Rapid field-test for the quantification of vitamin E, β-carotene, and vitamin A in whole blood of dairy cattle

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The blood concentration of fat-soluble vitamins such as vitamin E (VitE), vitamin A (VitA), and ß-carotene (ßCar) can be used to assess the vitamin status in dairy cattle. However, the determination of these blood components requires time-consuming, multistep, and labor-intensive procedures. The objective of this study was to validate a field-portable fluorometer/spectrophotometer assay for rapid quantification of these vitamins in whole blood and plasma of dairy cows and calves. The concentrations of VitE and βCar in whole blood and plasma from 28 dairy cows and 11 calves were measured by iCheck test and the results compared with the current analytical standard, i.e., high-performance liquid chromatography (HPLC) in two independent laboratories at the University of Potsdam (Potsdam, Germany, Lab DE) and at DSM Nutritional Products Ltd (Kaiseraugst, Switzerland, Lab CH), respectively. For VitA the HPLC measurements were limited to Lab DE. The whole blood concentrations of VitE as determined by iCheck™ (blood hematocrit corrected) ranged from 1.82 to 4.99 mg/L in dairy cows and 0.34 to 3.40 mg/L in calves. They were moderately correlated (R^2 = 0.66) with the values assessed by HPLC in dairy cattle (cows + calves). When excluding the calves, the correlation was R^2 = 0.961. The ßCar and VitA values obtained by the HPLC were highly correlated with the iCheck methods in whole blood with R^2 = 0.99 and 0.88, respectively. In plasma, strong correlations were observed between the concentrations assessed by iCheck and HPLC for VitE (R^2 = 0.97), for ßCar (R^2 = 0.98) and for VitA (R^2 = 0.92) in cattle (cows + calves). Overall, comparison of the iCheck fluorometer with the HPLC method as a reference method for measuring VitE, VitA, and ßCar in whole blood and plasma of dairy cattle showed a very good agreement, precision, and high accuracy. Based on this new development, the concentrations of VitE, ßCar, and VitA in the blood can be used as a nutritional biomarker to directly optimize nutritional interventions at farm side together with relevant stakeholders such as veterinarians, farmers, nutritional advisors, and feed consultants.

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Diet supplemented with various lipid sources similarly modulate methane emissions in dairy goat and cow

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Data on methane (CH4) emissions in goat compared to ovine and mostly bovine species are scare. The objective of the work was to assess, in a direct comparative study, the response of dairy goat and cow to diets known to modulate digestive process in cow, including methanogenesis. This work is part of a larger trial aiming at characterizing animal responses (performance, milk composition) to diets involved in milk fat depression or increase in ruminants. Four Holstein cows and 4 Alpine goats were fed limited (95% ad libitum) a basal diet (45% forage + 55% concentrate) not supplemented (CTL) or supplemented with corn oil plus wheat starch (COS; 5% dry matter (DM)), marine algae powder (MAP; 1.5% DM), or hydrogenated palm oil (HPO; 3% DM) in a replicated 4×4 Latin square design with 28-d experimental periods. Methane emissions, total-tract digestibility, eating and ruminating time measurements occurred during 5 days (d-21 to d-26) when animals were in open-circuit respiration chambers. Volatile fatty acids (VFA), ammonia and protozoa concentrations were determined in rumen fluid sampled before feeding by stomach tubing (d-27). Methane emissions (g/kg DM intake, g/kg milk) were similar between goat and cow and were lower with COS (-25% and -29% on average, respectively; $P<0.01$) than with others diets. DM intake and milk production were similar among diets for each species. Digestibility of DM, organic matter and fibre was similar between goat and cow. Compared to other diets, COS decreased fibre digestibility in both species (-18% on average; $P<0.05$). Irrespective of the diet, large differences in VFA concentration (-45%) and composition (+30% butyrate), ammonia (+80%) and protozoa (+1.1 log10) concentrations were observed in rumen fluid of goat compared to cow ($P<0.01$). Compared to other diets, rumen fermentation parameters were altered ($P<0.05$) with COS in both species (+26% propionate, -30% butyrate, -42% ammonia, -2.1 log10 protozoa), and with MAP in goat (+25% propionate). Eating and rumination times (min/kg DM intake/kg BW) were 2 times lower in goat ($P=0.03$) and similar among diets for both species. Methane yield and total tract digestibility were similar between dairy goat and cow despite rumen fermentations and feeding behaviours differed between species. Mitigating effect of COS on methanogenesis for both species are in accordance with changes in rumen fermentations and total tract digestibility. In our experimental conditions, digestive process responses to diets supplemented with various lipid sources were similar in dairy goat and cow.

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Development of the erythrocyte phenotype and blood biochemistry in dairy calves during the first ten weeks of life

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Hematologic parameters of bovines are subject to important changes during the first months of life. Investigating these changes is of interest as some parameters are used in calves to diagnose metabolic disturbances. The objectives of this study were to describe physiological developments of calf erythrocytes and to investigate potential underlying mechanisms.

Blood from 30 dairy calves obtained weekly between birth and the tenth week of life was assayed for hematological, plasma biochemical parameters and erythrocyte electrolyte contents. Associations of hematological parameters with plasma and erythrocyte biochemical parameters were investigated.

The mean corpuscular volume (MCV) declined from 43.6±3.7fL to 35.6±3.2fL between the 1st and 7th week while the red blood cell count (RBC) increased from 7.2±1.1x10^6/L to 9.3±1.0x10^6/L until the 5th week. The blood hemoglobin concentration (Hb) increased from 9.6±1.6g/dL to 11.6±1.1g/dL in the first three weeks of life. Erythrocyte potassium content (K_{ERY}) declined from 91.9±13.5 to 24.6±7.2mmol/L while the erythrocyte sodium content increased from 37.8±35.6 to 102.7±26.5mmol/L. MCV was associated with K_{ERY} from the 6th and with Hb from the 8th week of life, when K_{ERY}, and hematological parameters already approached levels of adult cattle. Plasma iron concentration was not associated with any of the studied parameters.

MCV reduction in bovine neonatal erythrocytes is a physiological development of the postnatal period and is not associated with sideropenia or anemic development in healthy calves. The mechanism driving the observed MCV change was not identified. Results of the statistical analyses indicate that changes in intra-erythrocyte osmotic or oncotic pressure are improbable underlying causes. K_{ERY} is an unreliable indicator for the intracellular K-homoestasis in neonatal calves and a postnatal decrease in MCV per-se is an unreliable indicator for the development of microcytic anemia.

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A study on Relationship of Blood Metabolites and Carcass Traits in Hanwoo Steers

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The blood metabolites have been used as an indicator of the nutritional and metabolic status of livestock and the studies on these metabolites have been conducted primarily on dairy cows. In case of Hanwoo (Korean native cattle), there are not many precedent studies on blood metabolites and only few studies have been conducted to investigate the correlation between the blood metabolites and carcass traits. Therefore, this study was conducted to investigate the relationship between blood metabolites and carcass traits in Hanwoo steers. A total of 1,280 Hanwoo steers were used as experimental animals. The number of growing (5 - 12 months of age), early fattening (13 - 21 months of age), and late fattening (22 - slaughter age) Hanwoo cattle used was 206, 604, and 470, respectively. The steers were raised with 3 - 5 animals per 5 × 10 m pen. The supply of each nutrient was calculated to meet the requirements of Hanwoo steers at each growth stage, based on the Korean Feeding Standard for Korean Cattle (2007). Blood samples were collected from the jugular vein of the experimental animals in plain vacuum tubes between 10:00 and 12:00, which was 2 to 3 hours after morning feeding.

The nonesterified fatty acid (NEFA) concentrations of the growing Hanwoo steers was negatively correlated with the back fat thickness ($r = -0.26$, $p < 0.01$). The glucose concentration of the early fattening steers was negatively correlated with carcass weight ($r = -0.25$, $p < 0.01$) and the albumin concentration were negatively correlated with the meat quality grade ($r = -0.34$, $p < 0.01$). During the late fattening period, the correlation coefficients between the blood metabolites and the carcass traits were lower than what was observed during growing and early fattening periods. In addition, the total protein, albumin, and BUN concentrations of the growing steers increased with increasing meat quality grade. The serum cholesterol concentration of the late fattening steers tended to be higher at quality grade 1+ or higher. The total protein and BUN concentrations tended to be higher at yield grade B or higher. In the present study, the correlations between blood metabolites and carcass traits differed depending on the growth stage and the correlation was highest in growing steers. In addition, this study suggests that the blood metabolites concentration could be used as an index to diagnose the nutritional status and to predict productivity of livestock.

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Study on the relationship between metabolic profile test and performance of Holstein cows according to lactation stage and parity

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This study was conducted to evaluate the relationship between metabolic profile test (MPT) and performance of Holstein cows according to lactation stage and parity. 885 of Holstein cows were used in this study and they were classified into four lactation stages which were EL (early lactation stage, 208 cows), ML (mid lactation stage, 206 cows), LL (late lactation stage, 330 cows) and DP (dry period, 114 cows). The cows were categorized into four groups: <30 kg/d, 30-35 kg/d, 36-40kg/d and >40 kg/d based on the milk yields. In addition, the cows were also divided into five parity groups (parity 1, parity 2, parity 3, parity 4, and parity 5). All blood serum used for MPT was collected from the jugular vein of the cow before morning feeding. The glucose concentrations in EL, ML, LL, and DP were 45.80 mg/dL, 44.23 mg/dL, 48.38 mg/dL, and 50.57 mg/dL, respectively. The NEFA concentration in EL, ML, LL, and DP were 248.68, 168.36, 161.66, and 263.75 μeq/L, respectively ($p<0.05$). The total protein concentrations in EL, ML, LL, and DP were 7.26, 7.24, 7.25, and 7.25 g/dL, respectively, and tended to decreased throughout the lactation stage ($p>0.05$). There were no difference between albumin and globulin concentrations among all lactation stages ($p>0.05$). The cholesterol, TG, AST, GGT concentrations were decreased in higher lactation stage. The Ca and IP concentration in all lactation did not significantly different ($p>0.05$), while the Mg concentration differ significantly by lactation stage ($p<0.05$). The glucose concentration was higher in <30 kg/d group than the other groups ($p<0.05$). The NEFA, albumin, and cholesterol concentrations had the highest concentration at >40 kg/d group with 249.92 μeq/L, 3.17 g/dL, and 293.84 mg/dL, respectively ($p<0.05$). The Ca concentrations tended to decreased at higher milk yield groups ($p>0.05$). The cows with higher number of parity showed higher concentration of NEFA, TP and globulin concentration with 245.02 μeq/L, 7.48 g/dL, and 4.44 g/dL, respectively ($p<0.05$). The glucose, Mg, and IP concentrations were decreased throughout the higher parity number ($p<0.05$). In this study, MPT is highly valuable in diagnosing the nutritional and health status of cows; however, it is necessary to consider the lactation stage, milk yields, and parity for more accurate diagnosis.

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Oleic acid and PPARγ-agonist ciglitazone alter expression of adipogenic genes and convert bovine satellite cells to myoblasts and adipogenic cells

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Oleic acid is the most abundant fatty acid in bovine muscle and i.m. adipose tissue and increases in i.m. adipose tissue mass are accompanied by corresponding increases in oleic acid. Ciglitazone is a member of thiazolidinediones (TZD) class and TZDs specifically bind to PPARγ and increase the expression of PPARγ and promote adipocyte differentiation. We hypothesized that oleic acid and/or would affect expression of adipogenic and myogenic genes and promote trans-differentiation of bovine satellite cells (BSC) to myoblast and adipogenesis. Primary cultures of satellite cells were isolated from the muscle tissues of six 18-mo-old Yanbian yellow cattles. The cells were cultured in differentiation medium containing 10μ*M* ciglitazone (CI), 100 μM oleic acid (OA), and 100 μM oleic acid with 10μ*M* ciglitazone (CI-OA), respectively. Controls (CON) were cultured only in differentiation medium, and the entire experiment was performed in triplicate. The CI, OA and CI-OA treatments increased the area of lipid droplet compared to the CON ($P < 0.0001$). Consistent with significant increases in lipid droplet areas, the concentration of cellular TAG, were also significantly elevated in bovine satellite cells by CI, OA and CI-OA treatments, respectively. Expression of PPARγ, C/EBPα, C/EBPβ and SREBP1 were increased by the treatments of CI, OA and CI-OA relative to control cells. Ciglitazone and/or oleic acid also increased expression of lipid droplet formation related genes in the bovine satellite cells. Expression of PLIN2 and PLIN3 were increased by the treatments of CI, OA and CI-OA relative to control cells. Relative ACSL3 and ACAT2 gene expression decreased with CI, OA and CI-OA treatments, and expression of GPAT3 mRNA was increased by the treatments of CI, OA and CI-OA. In conclusion, this study demonstrated that oleic acid and PPARγ agonist ciglitazone could alter expression of adipogenic genes and promote BSC differentiate into both myoblasts and adipogenic cells.

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Impact of fructose supplementation on feed intake, nutrient digestibility and retention time of digesta in Reeves's muntjac (*Muntiacus reevesi*)

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Increased intake of mono- and disaccharides by captive ruminants classified as browsers raises concerns, due to possible negative impact of high intake of sugars on the gastrointestinal tract (GIT) of these animals. The aim of the study was to determine the effect of fructose supplementation in the diet on feed intake, total tract nutrient digestibility and fluid and particle retention time in the digestive tract of Reeves's muntjac (*Muntiacus reevesi*), a browsing ruminant. Six adult males of muntjac were used in a cross-over design and fed a diet consisting of dehydrated chopped lucerne (*ad libitum*), high-fiber pellet (120 g/day) and wheat bran (30 g/day) without (F0) or with addition of 12 g fructose/day (F12). Dose of supplemental fructose for F12 was set to increase water soluble carbohydrates (WSC) intake by 25% relatively to F0. Each experimental period lasted 21 days. Feed intake was controlled daily whereas fecal samples were collected during the last 7 days of each period. Apparent total tract nutrient digestibility was determined using acid insoluble ash as a marker. Co-EDTA, Cr-mordanted fiber and Ce-mordanted fiber were used as pulse-dose markers of fluid, small and large particles, respectively. Mean retention times (MRT) in the GIT and the reticulorumen (RR), and selectivity factors (SF; the ratio of the MRT of Co and Cr markers) were calculated from fecal marker excretion. Total dry matter intake was not affected by fructose supplementation ($P > 0.59$). WSC intake increased from 46 g/day for F0 to 55 g/day for F12, resulting in a WSC content in consumed DM of 9.9% and 12.2% for F0 and F12, respectively. Total tract nutrient digestibility did not differ between treatments ($P \geq 0.17$). In general, the MRT of particles in the GIT was longer than MRT of fluid, but SF were relatively low and typical for browsing ruminants. MRT of fluid and particles in RR, as well as SF did not differ between treatments ($P \geq 0.22$). MRT of big particles in the whole GIT tended to be longer for F12 compared to F0 (by 1.7 h; $P = 0.09$). Results of this study indicate that moderate increase of WSC intake by Reeves's muntjacs (and possibly other browsing ruminants) does not affect feed intake, total tract nutrient digestibility, but may affect retention time of the digesta in the GIT.

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Hepatic gene expression of fatty acid oxidation and carnitine metabolism in lipopolysaccharide challenged dairy cows receiving a L-Carnitine supplement

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L-carnitine is involved in lipid metabolism and dietary L-carnitine supplements ameliorate fatty liver. L-carnitine is essential for fatty acid oxidation (FAO) since it enables FA uptake into the mitochondrial matrix. L-Carnitine has also antioxidant properties (Sener et al., 2006) which might be particularly relevant in inflammatory conditions. With these backgrounds, our objectives were: (1) assessing the hepatic mRNA expression of key genes of carnitine metabolism (CM) and mitochondrial FAO pathways in dairy cows without or with carnitine supplementation, and (2) comparing their response to an intravenous challenge with lipopolysaccharide (LPS).

In total, 51 multiparous Holstein cows were allocated either to a control group (CON, n=24) or to a supplemented group (CAR) receiving a rumen-protected L-carnitine product (Carneon 20 Rumen-Pro, Lohmann Animal Nutrition, 25 g/cow/day, n=27) from 6 weeks ante partum (ap) until 18 weeks postpartum (pp). Liver biopsies were collected at about 42 days (d) ap and at 100, 110 and 126 d pp. On d 110 pp, LPS (*E. coli* O111:B4, Sigma-Aldrich, 0.5 ug/kg BW) was intravenously injected. The mRNA abundance of the target genes in liver was assessed by the Fluidigm BioMark HD system and analyzed using the MIXED procedure of SAS. The abundances at d42 ap were considered as covariate.

From 5 genes considered in the CM, the mRNA abundance of CROT (carnitine O-octanoyltransferase) and TMLHE (trimethyllysine hydroxylase, epsilon) changed with time ($P < 0.05$). For genes involved in mitochondrial FAO, there was an interaction of supplement and time after LPS at some time points: CAR cows had less FABP6 (FA-binding protein 6) mRNA at d100 and more ACOX1 (Acyl-CoA oxidase 1) at d110 than CON cows ($P < 0.05$). However for the other 19 genes investigated for FAO no effect of supplement, time or their interaction was observed ($P > 0.05$).

The inflammatory reaction towards the LPS challenge was hardly affecting the mRNA abundance of the genes related to FAO and CM in liver at the time points investigated. The differences and interactions found should be further investigated in context with protein expression and metabolite profiles.

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Long-term differential implications of pre- and early postnatal malnutrition on developmental and functional traits of adipose tissues in adult sheep

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We aimed to test the hypothesis that malnutrition in late prenatal and early postnatal life have differential and gender-specific long-term implications for developmental traits in adipose tissues and hence health outcomes later in life. Twin-pregnant ewes were exposed to NORM (fulfilling dietary requirements), LOW (50% of NORM) or HIGH (150/110% of energy/protein requirements) diets during the last trimester (term~147 days). Twin lambs were fed each their diet, moderate low-fat (CONV) or a high-carbohydrate-high-fat (HCHF), from 3 days until 6 months of age (~puberty). Thereafter, they were all fed the same CONV diet ad libitum until 2½ years of age (adulthood), at which time all sheep (particularly females) were obese. Four fat tissues were sampled at autopsy for histomorphometric and mRNA expression analysis. In subcutaneous fat, similar gender-specific (♂<♀), upper-limits for obesity-induced adipocyte sizes and cell-number-indices were reached irrespective of early life nutrition. Perirenal fat mass was higher in sheep fed the postnatal HCHF diet. All females (particularly LOW♀ with highest amount of fat and largest adipocyte cross-sectional-area) and HIGH♀ had substantially larger percentages of large adipocytes (> 6400µm^2) compared to the corresponding CONV♀or♂ and LOW♂ (lowest average adipocyte cross-sectional-area). Interestingly, compared to CONV, LOW♂ had decreased percentages of large adipocytes, whereas they were increased in LOW+HIGH♀. HIGH♂ had larger adipocytes than other males, and resembled HIGH♀. Mesenteric fat was not affected by the early postnatal diet, but early life nutrition had similar gender-specific impacts on cell size distribution as in perirenal fat. In none of the three tissues were altered cellularity or expandability traits associated to gene expression patterns, except that most of genes were up-regulated in LOW in perirenal fat. Epicardial fat was exclusively affected by the early postnatal diet with enlargement of adipocytes in HCHF compared to CONV. This was associated with upregulated expressions for adipogenic and lipogenic genes. In conclusion, subcutaneous fat had a gender-specific, upper-limit for expandability. Perirenal and mesenteric fats were major targets of prenatal malnutrition with marked increased/decreased adipocyte hypertrophic capacity attained in LOW♀/LOW♂, and increased perirenal expandability particularly in HIGH♂. This explains why prenatal undernutrition may be a particular risk factor for ectopic fat deposition and adverse impacts in males, whereas prenatal overnutrition may offer protection.

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A meta-analysis of the impact of the *Aspergillus oryzae* fermentation product on dairy cow performance

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Feed additives produced via microbial fermentation are capable of enhancing the innate ability of animals to degrade substrates such as fiber, and increase the harvest of nutrients from consumed feeds. These additives are valuable tools in modern animal production. A fermentation product based on fungus Aspergillus oryzae (AO) (Amaferm®, BioZyme Inc.) has a prebiotic-like action and is used to enhance milk yield, feed intake, and digestibility in dairy cows. Our objective was to run a meta-analysis from published literature of AO in dairy cows to evaluate the effects of this prebiotic-like additive on dry matter intake (DMI) and fat corrected milk (FCM) yield. A database was constructed from experiments involving AO supplemented to lactating dairy cows. Only in vivo experiments of selected peer review papers published in English from 1983 to 2018 were included to build the database. These experiments must have contained at least individual least squares means (LSM) and standard error of the mean (SEM) or means and standard deviation (SD) data of DMI and FCM in dairy cows. A total of 18 studies comprising 31 treatment means were pooled in a database. Data were analyzed by the means procedure of SAS (SAS 9.0, SAS Institute Inc., Cary, NC). Results from meta-analysis showed significance differences at all evaluated variables. The DMI and FCM average effect sizes were higher for AO treatments (0.390 and 1.028 for DMI and FCM respectively; $P < 0.05$). As AO is known to improve fiber digestion, results on DMI and FCM are sound. In conclusion, adding an AO prebiotic-like action additive to dairy cows diets have positive effects on animal performance.

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O 36

Relationship between rumen microbiota and enteral methane emissions of dairy cows

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Relationships between enteral methane emissions and abundances of bacterial taxa in rumen fluid of dairy cows were determined to assess if these abundances may be used to predict these methane emissions. Six mature non-lactating Holstein dairy cows on diets with forage (alfalfa/grass hay) to grain ratios of 100:0, 75:25, and 50:50 were used during 5-wk experimental periods in a replicated 3x3 Latin Square Design. Dietary NDF and starch concentrations ranged from 38.7 to 56.0 % DM and from 0.5 to 19.5 % DM among diets, respectively. Methane outputs were measured using an open-hood calorimetric system during two 24 h periods on two separate days during the fifth week of experimental periods. Daily methane emissions ranged from 288.5 to 588.5 L/d among cows and diets, and averaged 413.4 L/d. Rumen fluid was sampled twice daily on the days preceding methane measurements. Compositions of the microbiota in rumen fluid were determined using Illumina 16S rRNA sequencing. Linear regression models were developed using the MIXED procedure of the SAS to determine the relationships between daily methane emissions (L/d) and the abundances of bacterial taxa in rumen fluid (%). Relative abundances of taxa that were significantly correlated with methane emissions and that were present in at least 30% of rumen fluid samples were included in the initial model. Abundances with a significance level greater than 0.30 were stepwise removed from the model. Taxa with high correlation coefficients ($r > 0.75$) were not placed together in models. The final model included and intercept and the abundances of 5 bacterial families in the rumen fluid, including Bacteroidaceae, Paraprevotellaceae, Succinivibrionaceae, Dethiosulfovibrionaceae, and Anaeroplasmatacea with regression coefficients of 444.3 (+/- 49.0), 104.5 (+/- 91.1), 21.1 (+/- 17.2), -11.5 (+/- 7.9), -983.5 (+/- 694.7), and -47.8 (+/- 23.0) (estimate +/- SE) L/d, respectively. The final model had an R2, CV, and root MSE values of 0.65, 9.83, and 40.4, respectively. These results show the limitations of predicting enteral methane emissions from the bacterial composition of rumen fluid. Model validation with different data sets and diets is needed.

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O 37

Roles of Toll-like receptor 5 ligand in the innate immune system in primary bovine rumen epithelial cells

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Objective: The rumen epithelial layer consists of stratified squamous epithelium (SSE), which comprises the stratum basale, stratum spinosum, stratum granulosum, and stratum corneum. The tight junction (TJ) of SSE is the most potent in stratum granulosum, and this physical barrier defends the host from bacterial penetration. The rumen epithelial TJs and barrier function were decreased at low pH in the presence of short chain fatty acids (SCFA). However, how rumen epithelial cells (REC) adapt to increased amounts of ruminal SCFA is unclear. Toll-like receptor 5 (TLR5) is known to play a key role in the innate immune system by recognizing bacterial flagellin. TLR5 expression was higher in the rumen papillae of high-grain-fed ruminants. Further, we previously found that rumen epithelial TLR5 was localized in the stratum basale and stratum spinosum (unpublished data). These findings suggested that REC can detect increments in ruminal SCFA levels and upregulate TLR5 to protect against disruption of the ruminal epithelial TJ or barrier function. Therefore, here we aimed to evaluate the role of TLR5 in the innate immune system and SCFA absorption, and elucidate whether SCFA upregulates TLR5 in bovine REC (BREC).

Materials and Methods: (Exp 1) Primary BREC were stimulated by the TLR5 ligand, Flagellin of Salmonella Typhimurium (FliC: 10 or 100 ng/ml) for 6 and 24 hours. Expression of TLR5, ten types of inflammatory cytokines, beta-defensin (DEFB), and monocarboxylate transporter 1 (MCT1) was analyzed by q-RT-PCR. (Exp 2) Primary BREC were incubated in medium at pH 7.4 or 5.6 without Na-SCFA (control) or with 100 mM Na-SCFA (60 mM sodium acetate; 30 mM sodium propionate; 10 mM sodium butyrate) for 3 hours. TLR5 expression was analyzed by q-RT-PCR. Data from Exp 1 and 2 were analyzed using one-way analysis of variance and Tukey's HSD or Dunnett's test.

Results and Discussion: (Exp 1) Interleukin-1 beta and tumor necrosis factor-alpha expression was increased by FliC treatment for 6 and/or 24 hours ($P < 0.05$). There were no differences in the expression of TLR5, other inflammatory cytokines, DEFB, and MCT1 upon FliC treatment. (Exp 2) TLR5 expression was not changed upon Na-SCFA treatment at lower pH. These results suggest that TLR5 selectively upregulates several inflammatory cytokines and activates immune cells around the REC, thus preventing penetration of ruminal bacteria.

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O 38

Effect of rumen nitrogen balance and dietary protein source on intake, milk yield, and milk fatty-acid composition of dairy cows

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Lowering rumen-degradable protein intake may increase nitrogen (N) use efficiency. However, kinetics of feed N supply to rumen microbes and efficiency of microbial protein synthesis may vary with diets, affecting dry matter (DM) intake, and thereby milk yield (MY), composition, and fatty acid (FA) profile. Hence, the aim was to study effects of rumen nitrogen balance (RNB), dietary protein source, and their interaction on intake, MY, and FA composition of dairy cows.

Four diets were tested in 24 lactating Holstein cows during 4 periods (12-d adaptation, 8-d sampling) in a complete Latin square design. Diets were fed *ad libitum* and comprised of grass silage, maize silage, grass hay, second-cut grass hay, and barley straw as forages and four different concentrate mixtures at a forage:concentrate ratio of 55:45 (DM basis). The concentrate mixtures contained barley grain, sugar-beet molasses, feed sugar, and either faba-bean grains (FB) and RaPass®, or SoyPass® (SP) and rapeseed cake, with FB and SP as two main protein sources (>35% of total dietary crude protein). Composition of concentrate mixtures were adjusted to create diets with RNB of 0 g N/kg DM (i.e. FB0, SP0) or -3.2 g N/kg DM (i.e. FB-, SP-). Daily DM intake and MY were measured and samples analysed for their chemical composition. Milk FA profile was predicted using Fourier-transform infrared spectroscopy. Data were analysed using mixed-model analysis with RNB, protein source, their interaction and period as fixed effects and animal as random factor, at significance of $P<0.05$.

Mean daily DM intake ($P<0.01$) and fat-energy-corrected MY were lower for FB- than for FB0 diet ($P<0.01$). Milk urea concentrations were lowest ($P<0.01$) and N use efficiencies highest ($P<0.01$) for FB- and SP- diets, with greater differences between RNB levels for FB than for SP diets ($P<0.01$). The *de novo* synthesized milk FA proportions (except C4:0) were higher for SP- than for SP0 diet ($P<0.01$), and for FB than for SP diets ($P<0.01$). Proportions of long-chain FA ($P<0.01$) and unsaturated FA ($P<0.01$) were higher, while saturated FA were lower ($P<0.01$) for SP than for FB diets, possibly due to high dietary fat content in SP diets. Microbial derived odd-branched-chain FA was lowest in FB- diet ($P<0.01$), hinting towards low microbial protein synthesis. In conclusion, reducing dietary RNB can improve N use efficiency, and possible negative effects of low RNB on feed intake and cow performance appear to be more pronounced in diets with rapidly degradable protein source.

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O 39

Duodenal infusions of starch with casein or glutamic acid increase post-ruminal a-glycohydrolase activities in cattle

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Small intestinal starch assimilation in ruminants is potentially limited by inadequate retention time, reduced glucose absorption, or deficient production of a-glycohydrolases. Previous research has demonstrated that small intestinal starch digestion can be improved by post-ruminal supply of casein or glutamic acid. However, the mechanisms by which casein and glutamic acid increase starch digestion are not well understood. Therefore, the objective of this experiment was to evaluate the effects of duodenal infusions of starch with casein or glutamic acid on post-ruminal a-glycohydrolase activities in cattle. Twenty-two steers (178.2 ± 5.1 kg BW) were surgically fitted with duodenal and ileal cannulas and fed a soybean hull-based diet devoid of starch at 1.5 ´ NEm. Approximately 1500 g/d of raw cornstarch was infused into the duodenum with water (control), 120 g/d glutamic acid, or 400 g/d casein. Treatments were infused continuously for 60 d and then steers were slaughtered for tissue collection. Activities of pancreatic (a-amylase) and intestinal a-glycohydrolases (maltase, isomaltase, glucoamylase) were assayed. Data were analyzed as a randomized complete block design using the MIXED procedure of SAS. Small intestinal data were analyzed for effects of site, treatment, and the site × treatment interaction. Casein increased final BW (P=0.003) and ADG (P<0.001) greater than control or glutamic acid. Pancreatic mass, protein concentration, and trypsin activity were unaffected by treatment. Casein increased a-amylase activity (P<0.05) and the a-amylase:trypsin ratio (P<0.001) compared to control. Mass of the small intestine was greatest (P<0.001) in the ileum. Small intestinal mass, length, and mass:length were not affected by treatment. Casein increased small intestinal protein concentration (P=0.05). In comparison to starch infusions alone, isomaltase activity tended to increase (interaction P=0.07) with glutamic acid in the ileum and with casein in the jejunum and ileum. Glutamic acid increased (interaction P=0.01) maltase activity in the ileum and casein increased (interaction P=0.01) maltase activity in the jejunum and ileum compared to control. Glucoamylase activity was greatest (P<0.001) in the ileum. These results suggest that small intestinal starch digestion may be improved with increased small intestinal flow of casein or glutamic acid through increases in post-ruminal a-glycohydrolase activities.

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O 40

Fermentation of diets for milking cows including clay minerals (zeolite, bentonite and sepiolite) measured in a semicontinuous in vitro system

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Clays are widely used as feed additives in ruminant diets. This in vitro study evaluated the effect of the inclusion of three types of clays on rumen fermentation of diets for milking cows. Maize, barley, soybean meal and wheat bran were mixed at 0:30, 0.30, 0.30 and 0.10 proportions to simulate a typical concentrate mixture, that was combined with alfalfa hay as forage source at either 65:35 or 35:65 proportions, considered as reference feeds used in diets for milking cows. Three different clay sources (zeolite, Z; bentonite, B; and sepiolite, S) were added at 0.01 of total incubated substrate. Unsupplemented concentrate (C) and forage (F) substrates were also included as control. Four in vitro incubation series were carried out for each forage:concentrate ratio, in a semicontinuous system, using rumen fluid from six ewes fed on a 50:50 forage:concentrate diet. Fermentation kinetics were determined under a poorly buffered medium from 0 to 6 h, allowing pH to rise from 8 to 24 h. Gas production and incubation pH were recorded from 2 to 24 h, and dry matter disappearance (DMd) was determined at 24 h. With both 35:65 and 65:35 forage:concentrate ratios, pH dropped to 6.06 □ 0.05 at 8 h, increasing thereafter around 6.5 from 16 h onwards. With the high concentrate ratio, Z recorded the highest incubation pH ($P<0.05$) throughout all the incubation period, and with B it was higher than C up to 8 h. No differences were recorded in gas production between C and B ($P>0.05$). However, it was lower with Z than C ($P<0.05$), whereas S recorded the lowest volume throughout the incubation period ($P<0.05$). With the high forage ratio, Z also promoted a higher ($P<0.05$) pH than F at 2 h, and both Z and B at 6 and 8 h incubation, although the magnitude of differences were below 0.05 pH units. From 4 to 24 h, the volume of gas produced with B was the lowest ($P<0.05$), and with Z it was lower than F. No treatment differences were recorded on DMd at any ratio ($P>0.05$). Zeolite maintained a more stable pH pattern, especially with the high concentrate ratio. Sepiolite and bentonite reduced the fermentation kinetics in low and high forage:concentrate ratios, respectively. The effect of adding mineral clays in diets for milking cows is determined by the forage:concentrate ratio. In terms of pH, zeolite may contribute to a more stable ruminal environment.

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O 41

Rumen protein kinetics of novel grazing systems – why is microbial protein production so high when grazing low protein fodder beet?

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Four yearling Charolais steers (286 ±9 kg) with rumen fistula were used in a four-by-two treatment comparison (experiment 1 and 2) to establish the rumen N kinetics and microbial crude protein production (MCP) in beef steers ad libitum grazing either ryegrass (RG) or fodder beet (FB) + 1 kg DM of lucerne silage. Each experiment consisted of a 9d in vivo digestibility trial, with individual pen total faecal and urine collection, and MCP was estimated via urinary purines. Rumen pH was then measured in situ for 24 hours via indwelling sensor, and then for 24 hours rumen and urine were sampled every 2h for N, urea, NH_3, and SCFA assessment. After a 72h interval, total rumen evacuations were undertaken every 16h three times on all steers for the same assessments and rumen liquid passage rates estimation using polyethylene glycol marker.

Mean MCP increased from 56.4 g to 104.7 g microbial N /day from the RG to FB treatment ($P<0.01$). The mean (kg) DM intake increased from 4.5kg on RG to 6.6 kg on FB, and the efficiency of MCP also increased from 12.5 to 15.5 g microbial N /kg DMI ($P<0.05$). Mean rumen NH_3 concentrations on FB at 2h diurnal intervals ranged from 2.8-48 mg NH_3 /L, significantly lower ($P<0.001$) than the RG (33-188 mg NH3 /L). In contrast, rumen urea significantly increased ($P<0.001$) with FB (5-13mmol /L) compared with RG (1-5mmol /L). Mean rumen pH was significantly higher ($P<0.01$) for FB compared with RG in all but one 2h interval diurnally. Mean rumen SCFA concentration in RG was higher for all but one 2h interval diurnally ($P<0.05$).

Rumen digesta volumes were similar, but DM and N pools were greater ($P<0.05$) in the RG compared to FB at all three intervals. Urine production was two-fold higher for the FB treatment (103.7 vs 49.2ml/ kg LWT), however rumen outflow rates (%/h) were not different for RG and FB (20.5 vs 21.5).

We suggest the similar rumen outflow rates, in conjunction with the two-fold increase in urine production, the high rumen pH values and lower SCFA concentrations with FB represent an increase in rumen epithelial transfer of water, N and SCFA. The anomalous rumen urea and NH_3 concentrations also suggest a novel mechanism of N supply for MCP. We postulate that FB diet induces both ruminal and extra-ruminal adaptions to concurrent low protein, high water load and high fermentable energy supply.

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P 40

Effects of wet distillers grains on dry matter intake, digestibility and ruminal fermentation in beef cattle

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Introduction: Wet distillers grains (WDG) are corn by-products which stand out due to their high protein and digestible fiber content. The aim of this trial was to evaluate the effect of increasing levels of WDG on dry matter intake, ruminal parameters, DM and nutrients digestibility in cattle.

Materials & Methods: Eight castrated Nellore steers with ruminal cannula and average initial body weight of 453 kg ± 32 kg were used. The study lasted for 112 days with four periods of 28 days, adopting a replicated Latin Square (4x4) experimental design. The treatments were: 0, 15, 30 and 45% of WDG in dietary DM. The diets contained 14.8; 16.2; 19.1 and 22% of CP. The contents of non-fiber carbohydrates were 62.8; 54.0; 43.4 and 32.8%. Diets were provided once a day and daily intake was obtained by weighing the offered diet and leftovers. Diets and leftovers were sampled to obtain Digestibility Coefficient (DC) and total faeces collection was performed for 5 days in each experimental period. DC was calculated by the following equation: DC = (total nutrient consumption - total nutrient excretion)/(total nutrient consumption)*100. Data were analyzed using PROC MIXED SAS with linear, quadratic and cubic contrasts.

Results: There was a quadratic effect of treatments on dry matter intake (8.01; 8.75; 8.77; 7.92 kg of DM, $P<0.01$), and a linear effect on crude protein intake (0.99; 1.17; 1.38; 1.44 kg of CP, $P<0.01$). The digestibility of DM (74.8; 70.4; 71.6 and 71.8.%), NFC (83.2; 80.8; 80.1 and 79.2%) decreased linearly ($P<0.01$) and the digestibility of NDF (51.9; 55.8; 57.3; 63.2%) and CP (71.9; 72.8; 77.5 and 80.2%) increased linearly. The inclusion of increasing levels of WDG reduced linearly the concentrations of SCFA in the ruminal fluid (91.05; 84.97; 67.39 and 80.13 mmol/L, $P<0.05$). However, the inclusion of WDG increased linearly the propionate proportion (18.39; 21.34; 20.39 and 21.89%, $P<0.05$) and decreased linearly the butyrate proportion (13.88; 12.76; 11.53; 10.95%, $P<0.01$). Valerate proportion in ruminal fluid presented linear increase (1.17; 1.12; 1.15; 1.32%, $P<0.05$) according to WDG levels. The Ruminal pH six hours after feeding was lower in the control treatment than in the others (5.85 vs. 6.13; 6.19 and 6.27, $P<0.05$). There was a quadratic tendency to a decrease in ruminal N-NH3 (17.06; 15.79; 15.14 and 16.41 mg/dL, $P=0.09$).

Conclusion: The inclusion of WDG increases fiber and protein digestibility, reduces ruminal ammonia-nitrogen and improves ruminal pH.

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P 41

Effect of clay minerals on the bioavailability of dietary zinc in rumen fluid and duodenal chyme *in vitro*

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Clay minerals from soils reflect an important source of crude ash in roughages. These compounds are known to potentially bind metallic cations and might thus affect bioavailability of essential trace minerals. The present in vitro studies aimed to assess the zinc binding capacity (ZBC) of different clay sources in rumen fluids and duodenal chyme. The 1st study used 3 different native clay mineral mixtures (dominating fraction: (a) kaolinite, (b) smectite, (c) smectite/illite) and 3 different liquid media (a) aqueous solution of Zn sulfate (20µg/ml), (b) rumen fluid (RF), and (c) duodenal chyme (DC) derived from non-lactating, fistulated dairy cows fed hay exclusively. Each one clay mineral was suspended at amounts of 0.2 g with 30ml of the respective liquid media in a rotating incubator for 24h at 39°C. Incubated samples as well as liquid media without clay addition were centrifuged (10.000g, 15min). Supernatants were analyzed for Zn. ZBC of clay minerals were calculated by relative reductions in Zn concentrations in the respective media's liquid phase with and without clay addition. The 2nd study continued with smectite as this clay mineral showed strongest ZBC. The clay mineral was incubated at amounts of 0.2 g in 30 ml of (a) RF, (b) RF acidified to pH 2 using HCl in order to mimic abomasal conditions, and (c) DC. Each of the 3 incubations was modified by adding 5 levels of extra Zn (0, 0.04, 0.08, 0.12, 0.16 mg Zn from Zn sulfate), respectively. ZBC was assessed by regression analysis. Incubation, sample preparation and analysis were the same as in the 1st study. In study 1, smectite showed numerically the strongest ZBC compared to smectite/illite and caolinite (55 %, 53% 37 %). Zn binding was most pronounced in aqueous Zn solution, followed by RF and DC, respectively (92%, 52%, 21%; $p<0.05$). In study 2, smectite adsorbed added Zn completely in rumen fluid irrespective of the total Zn dosage (on average 97%). Zn binding was significantly lower ($p<0.05$) in acidified RF (34%) and intermediate in duodenal fluid (58%).

In conclusion, ZBC differs between clay mineral sources. It was highest in RF and lowest under abomasal conditions, probably due to changes in pH. An increase of the Zn binding in DC may indicate rearrangements of Zn complexes inside the duodenum. In total, clay minerals may affect ruminal bioavailability of dietary Zn, and, to a smaller extent, also bioavailability along the entire digestive tract.

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P 42

Glyphosate does not affect rumen fermentation, nutrient digestion or mineral metabolism of non-lactating dairy cows under practical feeding conditions

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Glyphosate may be widely found in livestock feeds as a residue of herbicide treatment. Through inhibition of the shikimate pathway, it harms amino acid synthesis in plants but also in some bacteria that occur in the rumen. This mode of action as well as the particular chemical structure of glyphosate raises the suspect about potential effects on rumen fermentation and complexation of dietary cations *in vivo*. In order to clarify these questions, 9 non-lactating dairy cows equipped with rumen and duodenal fistulae were fed individually on a total mixed ration (TMR, 6.5 kg dry matter per head and day) based on corn silage, grass silage, soybean cake, and wheat meal. Dietary nutrient contents including minerals reflected practical conditions (according to or slightly above recommendations). All dietary compounds were free of glyphosate. Animals were submitted to a metabolism trial according to a Latin Square design including 3 treatments and 3 periods of each 7 weeks. Treatments included CON (control, no glyphosate), GLY (pure glyphosate), and RU (glyphosate administered via a commercial formulation). In both GLY and RU, glyphosate was added on top of the individual meals at each feeding event (morning:evening 50:50) at doses of 100mg glyphosate per head and day. This dose reflected around 10 times higher intake of glyphosate than levels derived from a preceding study where glyphosate was applied according to legal farming practices. Measurements included rumen fermentation parameter (time profiles of rumen pH, ammonia, volatile fatty acids), rumen degradability of TMR and its compounds (nylon bag method), duodenal flux as well as total tract digestibility of nutrients including cationic minerals (Ca, Mg, K, Na, Fe, Cu, Zn, Mn), urinary excretion of minerals, and concentrations of minerals in blood serum directly before feeding. Neither GLY nor RU modified any of the analyzed parameter ($p>0.05$). In conclusion, dietary intake of glyphosate at levels to be expected at practical feeding conditions in cattle neither affects rumen fermentation or nutrient digestion, nor exerts complexation of cationic minerals that would lead to quantitatively relevant changes in mineral metabolism under practical feeding conditions.

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P 43

Effect of mild dietary Cu excess from different Cu sources on Cu metabolism and rumen fermentation characteristics in cannulated cows

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Copper is regularly supplemented to ruminant diets due to risk of Cu deficiency. But excessive Cu is also known for its antimicrobial property in monogastric livestock feeding. The present study aimed into assessing potential effects of mild dietary Cu excess from different Cu sources around the borderline of legally permitted dietary Cu contents on rumen fermentation and Cu metabolism compared to recommended Cu supply. Six non-lactating Holstein cows equipped with rumen and duodenal fistulae were submitted to a metabolism trial according to a Latin Square design including 3x2 treatments and 6 periods of each 3 weeks. Treatments consisted in 2 types of Cu sources (copper sulfate ($CuSO_4 \cdot 5H_2O$); tribasic copper chloride (TBCC; $Cu_2(OH)_3Cl$)). Each source was supplemented at 3 levels that produced total dietary Cu contents of 10, 35, and 50mg/kg dry matter (DM). Animals were fed restrictively (6.5 kg DM/day) a total mixed ration (TMR) (corn and grass silage, wheat meal, soybean extracts). Measurements included time profiles of rumen pH, ammonia, and volatile fatty acids, rumen degradability of feedstuffs (nylon bag method), as well as contents of selected microorganisms in rumen fluid (total bacteria, *Ruminococcus flavefaciens, Fibrobacter succinogenes, Streptococcus bovis*, archaea, protozoa, anaerobic fungi). Further samples of rumen and duodenal contents were collected from morning until evening feeding, pooled and separated into solid and liquid fractions prior to Cu analysis. Fecal samples were analyzed for Cu and nutrient contents and respective total tract digestibilities were calculated using TiO_2 as external marker. Blood serum was analyzed for Cu, activity of superoxide dismutase and ceruloplasmin, respectively. Rising dietary Cu increased Cu concentrations in almost all samples from the digestive tract. Compared to TBCC, $CuSO_4$ resulted in less Cu levels in rumen solids and higher values in the liquid fraction of the rumen fluid as well as in the duodenal fraction of bacteria ($p<0.05$). Also apparently digested Cu responded steeper to added Cu with $CuSO_4$. Rising dietary Cu promoted the speed of DM degradability of TMR ($p<0.05$), particularly with Cu from $CuSO_4$. Although high Cu from $CuSO_4$ induced some small, isolated changes in the rumen microbiota, other traits of rumen fermentation remained unaffected by either source or dose of supplementary Cu. In total, Cu from $CuSO_4$ showed to be more soluble and hence bioavailable than from TBCC. Cu levels up to 50 mg/kg DM did not negatively affect rumen fermentation irrespective of the Cu source.

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Effects of supplementing amylase and protease to ruminant diet on rumen fermentation characteristics and the rumen microbiota

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Recent studies demonstrated beneficial effects of exogenous enzyme addition to ruminant diets on nutrient digestion, for example amylase on NDF digestibility. Proteases might support such effects by accelerating the release of substrates, e.g. from protein-starch-matrices in maize. In order to test this hypothesis, 8 non-lactating, rumen fistulated Holstein cows were submitted to a metabolism trial according to a double 4x4 Latin Square design including 4 treatments and 4 periods of each 40 days. Treatments consisted in CON (no enzymes), AMY (amylase, 300 KNU/kg dry matter (DM)), PRO (protease, 15000 PROT/kg DM), and AMY·PRO (amylase+ protease, (150 KNU + 7500 PRO)/kg DM). Animals were fed restrictively a total mixed ration (TMR) based on maize grain, maize and grass silage, hay, and soybean extracts. Measurements included time profiles of rumen pH, ammonia, and volatile fatty acids, rumen degradability of DM, starch, crude protein (CP), and neutral-detergent fibre (NDF) in feedstuffs (nylon bag method), as well as contents of selected microorganisms in rumen fluid (total bacteria, archaea, protozoa, anaerobic fungi, *Prevotella spp., Fibrobacter succinogenes, Ruminococcus flavefaciens,* and *Streptococcus bovis*). Total tract digestibility of nutrients was determined using TiO_2 as external marker. While AMY remained ineffective, PRO accelerated DM degradability in maize silage ($p<0.05$) and to a quantitatively small extent also CP degradability in soybean ($p<0.05$). AMY·PRO increased degradability of DM and of starch in maize grain as well as DM degradability in maize silage ($p<0.05$). Degradability of other feedstuffs remained unchanged. Rumen fermentation parameters, analyzed microbiota in rumen fluid, as well as total tract digestibility were not affected by either enzyme (combination). In conclusion, our results support the hypothesis that exogenous protease may support access of rumen microbiota and of exogenous amylase to feed-born starch through degradation of coating protein structures, particularly in maize.

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Development of salivary IgA secretory ability in weaned calves

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Objective: Weaning plays one of the key roles to establish rumen microbiota. While the transition from milk to solid feed is thought to change gut microbiota dramatically, immunoglobulin A (IgA) is also expected to affect the microbiota establishment simultaneously, according to previous studies in monogastric animals. Because cattle secrete large amount of IgA in saliva which can bind to rumen bacteria, salivary IgA is expected to shape rumen microbiota. The concentration of IgA in calf saliva increases from 4 to 52 weeks of age. However, it remains to be elucidated in which salivary gland the ability of IgA secretion increases after weaning. To clarify the developmental mechanism responsible for the increase of IgA secretion in calves, we investigated the change in IgA secretory ability before and after weaning in three major salivary glands.

Materials and methods: 1) Saliva was collected from 3 Holstein calves from 1 to 13 weeks of age (weaned at 4 to 5 weeks of age) to compare IgA levels by Western blotting. 2) The submandibular gland, the sublingual gland and the parotid gland were collected from other Holstein male calves at 2 to 3 weeks of age (suckling, n=5) and 12 to 13 weeks of age (weaned, n=6) to investigate the development of IgA secretory ability. Total protein was extracted from each gland to compare the amount of IgA by Western blotting. Total RNA was isolated from the same tissues to compare expressional levels of IGA, and IgA secretion-related factors (PIGR, CCL28 and CCR10) by real-time PCR.

Results and conclusion: 1) The salivary IgA level tended to be higher in weaned calves. 2) The amount of IgA was significantly higher in the submandibular gland. The expression of IGA and PIGR were significantly higher in the submandibular and sublingual glands than parotid gland. The expression of IGA and its secretion-related factors in the submandibular and sublingual glands were compared between suckling and weaned calves. As a result, only in the submandibular gland, IgA and all of the secretion-related factors expression were higher in weaned group. In conclusion, the activation of plasma cell migration, IgA production and its secretion in the submandibular gland contribute to the elevated salivary IgA level in weaned calves which potentially affects rumen microbiota development.

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Effect of phytogenic feed additives with Performizer® application on milk performance of lactating dairy cows

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The aim of the present study was to assess the effects of a phytogenic feed additive Actifor® Pro (Delacon Biotechnik GmbH; Steyregg, Austria) on milk performance of dairy cows when applying the additive according to the Performizer® concept.Therefore, a feeding experiment with 45 mid-lactation Holstein cows was conducted. The animals were randomly assigned to one of the 3 treatments: Negative control (CON; diet formulated at 93% of metabolizable protein (MP) requirements with soybean meal as protein source), positive control (SBT; diet formulated to cover 100% of MP with soybean meal protected with formaldehyde) and an experimental group (ACP; diet supplemented with Actifor® Pro according to the Performizer® concept and reduced in MP, since Actifor® Pro potentially improves the metabolizable protein production in Dairy cows by 7%) . This diet was designed to have similar feed cost compared to SBT. The three-month trial was split into a 3-week pre-period and a 9-week main period. Animals were fed on partial mixed rations (PMR). Residuals were collected and weighted daily. Forages and meal mixtures were sampled weekly and pooled per batch for analysis. Grass silage was sampled every two weeks for near infrared spectroscopy (NIRS) analysis to check if the targeted MP content had been reached. Ambient temperatures were very high relative to optimum temperature, resulting in a Temperature Humidity Index (THI) above 65 during nearly the entire period. In week 7 of the experiment, maximum air temperature exceeded 35°C. Due to these challenging conditions, the cows reduced feed intake and decreased milk production later in the experiment. As expected, throughout the whole experiment, milk production was numerically highest for the positive control, however, differences were not statistically significant. The milk production within ACP was higher compared to the negative control group (+1.1 kg/d) but lower compared to the positive group (-0.4 kg/d). Feed efficiency and protein efficiency within ACP also tended to be higher (respectively +0.04 kg/kg DMI and + 0.7%) compared to the negative control. However, the large standard deviation shows that there was a large individual variation between cows within treatments during this period. Supplementation of Actifor® Pro according to recommendations of Performizer® matrix values, compensated a lower level of MP (-80 g/day) and numerically improved both, the milk production by 1.1 kg/d and the energy corrected milk by 0.6 kg/d, compared to the negative control.

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Ruminal fermentation and enteric methane production of legumes containing condensed tannins fed in continuous culture

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Plant secondary metabolites such as condensed tannins (CT) have been shown to alter ruminal fermentation while reducing enteric methane (CH_4) production. However, no studies exist that directly investigate both ruminal fermentation and CH_4 production of perennial legumes containing various concentrations of tannins. A continuous culture fermentor study was conducted to assess nutrient digestibility, volatile fatty acid (VFA) concentration, microbial protein synthesis, bacterial nitrogen (N) efficiency and CH_4 production of 4 legumes containing different CT concentrations. Legumes were fermented in a 50:50 diet with orchardgrass (Dactylis glomerata L.): 1) Alfalfa (Medicago sativa L.; ALF no CT); 2) Birdsfoot trefoil (Lotus corniculatus L.; BFT, low CT); 3) Crown vetch [Securigera varia (L.); CV, moderate CT]; 4) Sericea lespedeza [Lespedeza cuneata (Dum. Cours.); SL, high CT]. Diets were randomly assigned to 4 fermentors in 4 periods in a 4 × 4 Latin square design, with 7 d for adaptation and 3 d for sample collection. Temperature, pH and CH_4 concentrations were recorded. Effluent samples were analyzed for pH, VFA, and dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations for determination of apparent (DM, OM, NDF and ADF) and true (DM and OM) nutrient digestibilities. Microbial protein synthesis and bacterial efficiency were estimated by analysis of N flows and purines. Forage samples were analyzed for DM, OM, CP, NDF, ADF, minerals and CT concentrations. Data were analyzed using the GLIMMIX procedure of SAS. The CT concentrations (g/kg DM) were 3, 21, 38 and 76 in ALF, BFT, CV and SL, respectively. Apparent and true DM and OM digestibilities were lower ($P < 0.01$) in SL than in ALF and BFT. Bacterial N efficiency per kg of truly digested DM and OM was lower ($P = 0.05$) in SL than in BFT and CV. The lowest ($P < 0.001$) CH_4 production per unit of digestible nutrients was found in SL. There was a negative correlation ($P < 0.001$) between CT concentration and CH_4 production. However, tradeoffs in ruminal fermentation (reduced nutrient digestibility, VFA concentration and bacterial N efficiency) must be considered when SL is used.

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Effects of different levels of rapeseed cake in the ration of beef cattle on nutrient digestion and nitrogen utilization

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Introduction: Rapeseed cake (RSC, Brassica napus) is an important protein feed for cattle (Stasiniewicz et al., 2000). However, glucosinolate limits the feeding value of RSC (Tripathi and Mishra, 2007). A trial was carried out to study the effects of RSC in the ration of beef cattle on nutrient digestion and nitrogen (N) balance.

Materials and methods: Eight growing Simmental castrated cattle (219±14 kg) and four rations containing RSC [glucosinolate 226.1 μmol/g dry matter (DM)] at 0.00, 2.65, 5.35 and 8.00%DM with the same levels of net energy (5.67 MJ/kg DM) and crude protein (CP, 11.94%DM) were randomly assigned in a replicate 4×4 Latin square design. Each experimental period lasted 20 days, including 15 days for adaptation and 5 days for sampling. The DM, organic matter (OM), ether extract (EE) of feeds and faeces were analyzed according to AOAC (1998). The N of feeds, faeces and urine were analyzed using the Kjeldahl method. The CP was calculated as N×6.25.

Results and conclusions: Increasing the RSC level linearly decreased the CP digestibility ($P < 0.001$) from 58.2 to 56.8, 57.0 and 54.5%, increased the EE digestibility from 46.2 to 52.1, 58.1 and 61.3% ($P < 0.001$) and the faecal N excretion ($P< 0.001$) from 27.90 to 29.05, 29.08 and 30.48 g/d ($P< 0.001$) while did not affect the digestibilities of other nutrients ($P > 0.05$) and the urinary N excretion and the N balance ($P > 0.05$). The inhibitory effect of RSC on the CP digestibility could be resulted from the glucosinolate in RSC while the reason for the increase of EE is unclear. In conclusion, including RSC in ration decreased the CP digestibility but did not affect the N balance of beef cattle.

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Evaluation of supplemental autolyzed yeast on nitrogen excretion and apparent digestibility of high starch diets in Holstein cows

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Excess N diets and fecal N excretion can have negative implications on the environment and animal production. The aim of this study was to investigate if the addition of an autolyzed yeast (AY; *Saccharomyces cerevisiae*) supplementation in a high starch diet would affect urinary and fecal N excretion and, subsequently, nutrient intakes and apparent digestibility. Fifteen multiparous, rumen-cannulated Holstein cows were assigned to 1 of 5 treatments in a replicated 5 × 5 Latin square design balanced to measure carryover effects. Treatments were: low starch diet without AY (LS0, control), high starch diet without AY (HS0), high starch diet with either 15 g (HS15), 30 g (HS30), or 45 g (HS45) of AY supplementation. The trial period (21 d) was divided into an adaptation phase (d 1 to 14) and a measurement phase (d 15 to 21). TMR samples were collected on d 18-20 and orts were collected d 18-21 and analyzed for DM, NDF, CP, starch, ash, total nitrogen, and uNDF 120 h. Urine samples were collected by manually stimulating urination at 08:00, and 20:30 h on d 20 of each period. Fecal samples (120 mL, wet weight) were collected from the cow's rectum on d 18, 19, and 20. Data were analyzed using the MIXED procedure of SAS. Cows in HS0 treatment had greater N intake (719.58 vs. 583.66 g/d; P = 0.003), milk protein N (165.99 vs. 140.29 g/d; P = 0.038) and fecal N (268.36 vs. 219.78 g/d; P = 0.014). The addition of AY tended to have a quadratic treatment effect on allantoin (P = 0.09), and uric acid (P = 0.03) when compared to cows in HS0. Cows in HS0 had greater nutrient intakes for DM (24.06 vs. 20.09 kg/d; P = 0.005), OM (22.57 vs. 18.55 kg/d P = 0.0006), CP (4.50 vs. 3.65 kg/d; P = 0.003), and starch (6.56 vs. 4.67 kg/d; P < 0.0001) when compared to cows in LS0. Supplementing AY tended to have a quadratic treatment effect on OM (P = 0.08), and NDF (P = 0.09) nutrient intakes. Apparent digestibility tended to be greater in LS0 for starch (95.05 vs. 93.71 %; P = 0.08) and NDF (51.95 vs. 45.65 %; P = 0.08), when compared to HS0. The AY tended to positively increase CP (P = 0.07) digestibility, being the most digestible at HS45. In conclusion, cows receiving AY had improved N utilization

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Effects of maternal supplementation on intestinal gene expression in progenies fed diets with or without high inclusion of rumen-protected fat

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Maternal nutrition during pregnancy has potential effects on fetal growth and development. Considering that both moderate nutrient restriction during pregnancy and lipid supplementation during feedlot can improve the efficiency of energy utilization, the last stage of beef production is one opportunity to investigate these coupled effects on progeny development later in life. The objective was to evaluate the effects of Nellore cows' supplementation, during mid to late gestation, on the expression of genes involved in the intestinal glucose and fatty acid uptake in progenies fed diets with or without high inclusion of rumen-protected fat. Forty-eight Nellore steers, with an initial average body weight of 340±9.38 kg and 21±0.7 months, were housed in individual pens and distributed in a completely randomized design using a 2 x 2 factorial design. The following treatments were assessed: nutritional management of cows without supplementation (NS); or with supplementation after 124 ± 21 d of gestation (SUPP: 330 g CP and 2.11 Mcal/cow/day); and progeny feedlot diets without rumen-protected fat (NFAT) or with rumen-protected fat (RPF: 6% calcium salts). Duodenal and jejunal samples were taken to analyze the expression of SLC5A1, CD36, and CCK genes. CD36 is a transmembrane protein that binds to long-chain fatty acids, mediating fatty acid uptake and incorporation into esters for chylomicron production, while CCK acts as a short-term regulator of feed intake by stimulating gallbladder contractions and pancreatic secretion in the presence of dietary fat. Both are associated with fatty acid metabolism in the small intestine. Data were analyzed using the GLM procedure of SAS, and the model included the fixed effects of rumen-protected fat supply, maternal supplementation, and their interaction, with steers as the random effect. Duodenal expression of SLC5A1 ($P < 0.001$), CD36 ($P < 0.01$), and CCK ($P < 0.06$) increased in progeny from not supplemented cows. Maternal supplementation did not affect jejunal expression of SLC5A1 ($P = 0.47$), CD36 ($P = 0.90$), and CCK ($P = 0.49$) genes in progeny. The inclusion of rumen-protected fat did not influence SLC5A1 ($P = 0.22$; $P = 0.41$), CD36 ($P = 0.47$; $P = 0.44$) and CCK ($P = 0.52$; $P = 0.70$) expression at the duodenal and jejunal sections, respectively, of the small intestine of progeny. In conclusion, progeny from nutrient-restricted dams have higher expression of membrane transporters genes in the small intestine.

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Effect of *Pseudoramibacter boviskoreani* sp. nov. supplementation on sub-acute ruminal acidosis induced by feeding high level of concentrate diet

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Pseudoramibacter boviskoreani sp. nov. (GenBank registration number: MF926250) is a lactate-utilizing bacterium recently isolated from Hanwoo, a Korean native cattle. With respect to its capability of lactate utilization, it is known to alleviate sub-acute ruminal acidosis (SARA). This study was conducted to examine the effect of *P. boviskoreani* on SARA both in vitro and in vivo. *P. boviskoreani* was prepared by mixing 1% of freeze-dried *P. boviskoreani* with 99% zeolite as delivery medium (4.3×109 CFU/g). For the in vitro study, two levels of *P. boviskoreani* supplementation (0.5% and 1%, w/w feed) were compared with the control (1% of zeolite). Using serum bottles, one gram of feed (1:9 of forage:concentrate ratio) was incubated with a 100 ml of rumen fluid-McDougall buffer mixture (1:4) for 3, 6, 12, and 24 hours in anaerobic condition at 39°C. For the in vivo study, four castrated goats (Korean native × Boer) were allocated to either the control (10 g of zeolite) or the treatment (10 g of *P. boviskoreani*, 8.67×109 CFU/g) group in a cross-over design experiment. SARA was induced by feeding high level of concentrate (1:9 of forage:concentrate ratio). Each period consisted of a 7-day concentrate challenge followed by 3 days of sampling. Rumen fluid was collected at 2 and 5 hours after feeding. The results from the in vitro study showed that 0.5% *P. boviskoreani* presented higher ($P<0.05$) pH (6.48) than the control after 3 hours of incubation (pH 6.45). At 12 hours, both 0.5% and 1% of *P. boviskoreani* supplementation showed higher ($P<0.05$) pH (6.28) than the control (6.22). Total volatile fatty acids (VFA) and lipopolysaccharide (LPS) concentration were not affected by *P. boviskoreani*. Ruminal pH of goats did not differ between the control and treatment groups at 2 hours after feeding. However, at 5 hours, the ruminal pH of the animal supplemented with *P. boviskoreani* tended to be higher (pH 6.29; $P=0.078$) than the control (6.59). There was no difference in total VFA and LPS concentration between the treatment and control groups In conclusion, the supplementation of *P. boviskoreani* may have a potential to improve ruminal pH during a SARA challenge; however, further study is required to monitor the variation in ruminal pH and lactate concentration when *P. boviskoreani* supplementation is used over a longer period.

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The rate of disappearance of Hydrocyanic Acid (HCN) from cassava plant parts under drying or silage processing methods

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Cassava has potential as a livestock feed. Roots are high in fermentable energy, while the foliage is a good protein source. The use of cassava as a livestock feed is limited due to the presence of cyanogenic glycosides which, when hydrolysed, yield hydrocyanic acid (HCN). Sundrying and ensiling are processing methods used to reduce HCN levels. Standard protocols for ensiling and drying cassava parts are required to ensure safe livestock feeding. The decline in HCN under sundrying or ensiling was investigated.

Cassava cultivar MAus7 was grown at The University of Queensland, Gatton, Australia. The experiment was a 2x3 factorial (two processing treatments with three plant part types) in a completely randomised design. After harvesting, roots were washed and peels removed. Peels, peeled roots and foliage (top 50 cm) were chopped to 1-2 cm. Cassava parts were immediately either ensiled using vacuum bags (Johnson et al. 2005) or sundried. Samples were analysed in triplicate at each of the following timepoints: 0, 1, 3, 7, 10, 14, 17, 21, 24 and 28 days, with foliage silage further tested at 9 months. At each timepoint, samples were analysed for dry matter, HCN using the picrate paper kit method (Bradbury et al. 1999), and pH for silages. An acceptable limit of HCN is 40 mg HCN/kgDM (Damardjati et al. 1993).

Sundrying was effective in reducing HCN. Drying foliage reduced HCN levels from 277 to 112, 56 and 32 mg HCN/kgDM on days 1, 10 and 14 respectively. One day of drying peeled roots reduced HCN from 53 to 9 mg HCN/kgDM. Drying peels reduced HCN from initial levels of 894 mg HCN/kgDM to 777, 215, 65 and 46 mg HCN/kgDM on days 1, 3, 14 and 17 respectively.

All silages exhibited low pH (4.0-4.5), but this was not effective in reducing HCN content in foliage or peels. After 28 days, ensiled foliage remained high at 296 mg HCN/kgDM, ensiled peels were 604 mg HCN/kgDM while peeled roots were considered safe at 30 mg HCN/kgDM. After 9 months, the HCN content of foliage silage was 448 mg HCN/kgDM. It was concluded that sundrying foliage, peels and peeled tuber should occur for 1-10, 14-17 and 1 day respectively depending on final level of HCN considered safe. Ensilage did not reduce HCN levels in foliage and peels but peeled tubers were safe as they were low initially.

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Precision and additivity of organic matter digestibility obtained with an in vitro multi enzymatic method

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EDOM is an in vitro multi-enzymatic method for the estimation of organic matter digestibility (OMD) of feeds (Weisbjerg and Hvelplund, 1993). The aim of this study was to evaluate the precision of the EDOM method and determine its additivity as compared to the long-assumed additive property of chemical components of compound feeds. 118 samples, 49 compound and 69 ingredients were analyzed by EDOM (AU laboratory, Foulum, Denmark) with two repetitions separated in space and time. Samples were further analyzed for OMD by EDOM, dry matter (DM), ash, crude protein (CP), NDF and starch on a commercial laboratory. The EDOM method resulted in an intra-laboratory correlation of 99% and an inter-laboratory correlation of 92%, with no significant mean bias between the two laboratories tested.

Formulation of compound feeds, TMR and mixtures in general assumes that their nutrient content can be calculated by adding together the nutrient supply of individual ingredients. Additivity of the EDOM method for the compound feed samples was evaluated by comparing the sum of the digestible organic matter (DOM) of the ingredients (predicted), estimated by EDOM, with DOM estimated directly in the compound feed (observed). Regression of predicted on observed showed a coefficient of regression of 0.93, with no linear bias, but a mean bias (0.87 g DOM/100 g organic matter in DM, $p<0.001$). The mean bias was lower than the standard error (1.49 g DOM/100 g organic matter in DM), therefore practically insignificant. Additivity of NDF, CP and starch showed a coefficient of regression of 0.93, 0.95 and 0.98, respectively, with no mean bias but a linear bias. Linear bias for CP and starch although significant (-0.07 and -0.04 respectively, $p<0.05$), resulted in a maximum bias (0.92 and 1.20 g/ 100g DM respectively) lower than the standard error (1.59 and 1.93 g/100 g DM), thus practically insignificant. NDF linear bias (-0.12 $p<0.01$) showed a maximum bias (2.01 g/100 g DM) higher than the standard error (1.49 g/100 g DM).

Results demonstrate high precision of the EDOM method and its additive property, being an advantage for the estimation of OMD in compound feeds. Moreover, results of CP and starch confirm the additive property of these. Although NDF did not show clear additivity, it should be evaluated if the magnitude of this difference is of importance for the feed industry.

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P 54

A menthol-containing feed additive induces transcriptomic changes in sheep ruminal epithelium

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RNA-sequencing is rapidly evolving and becoming the new gold standard to more accurately and comprehensively measure gene expression during transcriptome analysis. So far, only a few studies have been conducted regarding the global expression analysis in the ruminal epithelium of sheep. Therefore, MACE-seq (Massive Analysis of cDNA Ends), an improved variant of 3´ single end mRNA sequencing, was used to identify differentially expressed genes (DEG) and clusters within the ruminal epithelium of sheep fed either a control diet or supplemented with a menthol-based feed additive. In previous studies, both topical application of menthol to the ruminal epithelium or a menthol-supplemented diet were shown to modulate epithelial nutrient transport in the rumen. In the present study, fifteen female and nine male growing Suffolk sheep were evenly distributed to three dietary treatments including a randomized block design based on initial body weight and sex. The diets consisted of either no plant bioactive lipid compounds (PBLC; Control), a low dose of PBLC (80 mg/d, menthol-based OAX17, PerformaNat GmbH, Germany) or a high dose of PBLC (160 mg/d). After RNA isolation, DNase treatment, cDNA synthesis, PCR, and subsequent MACE-seq, raw reads were mapped and then annotated to the ENSMBL genome version 3.1 of *Ovis aries* (Version 3.1.9.23). Raw reads were normalized and p-values for the likelihood of differential gene expression were calculated. GO (Gene Ontology) enrichment analysis was performed to compare the control group with the combined and averaged PBLC-treated groups. Results showed 409 DEGs in Control *versus* both PBLC groups (209 upregulated, 200 downregulated) and GO enrichment analysis unveiled 132 significantly enriched GO terms among “Biological Process”, 29 among “Molecular Function” and 18 among “Cellular Compartment” ($p < 0.05$). Within the group of DEGs, genes related to intra- and extracellular transport, cell-cell adhesion, Ca^{2+} regulation, or cell cycle, among others, were strongly up- or downregulated ($-1 < log2FC > 1$), e.g. SLC6A17, SLC12A9, SLC25A46, SLC38A7, SCN7A, LRRC8A, CLDN3, S100A16, CDK8, and CUL4B. These results indicate that menthol seems to influence a vast number of genes and signaling pathways that might be beneficial to ion transport, energy homeostasis, and the desquamation of the ruminal epithelium. To our best knowledge, this is the first study evaluating changes in the ruminal transcriptome upon PBLC feeding. The identified DEGs and GO terms may help to understand previously described effects of menthol on gene expression and may provide further insights into the underlying mode of action.

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Do methane emissions per unit of digested fibre vary with fibre digestibility?

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Herbivore diets differ in CH_4 emissions they usually trigger. High fibre feeds generally lead to higher CH_4 emissions than low fibre feeds. The intuitive and often reported explanation is that per unit of cell wall, a relatively fixed CH_4 amount is produced. However, ruminants and their microbiota can differ in the amount of CH_4 they produce when fed the same diet. *In vitro* assays indicate that, depending on the dilution rate, variable CH_4 amounts are produced per unit digested substrate. Still, to our knowledge, the concept that CH_4 per unit digested fibre is statistically constant has rarely been challenged except in a previous study from our group with the dataset of one study. Therefore, the aim of the present study was to collect individual animal data from additional respiration chamber experiments where animal groups received the same diet, and to explore whether the same negative relationship between fibre digestibility and CH_4 yield per unit of digested fibre can be established. We repeated the overall analysis two times: with datasets accepting a minimum either six or 12 animals per feeding group. The first analysis included 11 cattle and 25 sheep feeding groups with a total of 112 and 162 individuals, respectively. For the second analysis, four cattle feeding groups (total n=70) and 2 sheep feeding groups (total n=24) were used. Datasets were analysed separately by species using mixed models in SPSS, with yield per unit digested fibre as the dependent variable, diet as a random factor, relative food intake level, dietary fibre concentration and fibre digestibility as covariables, and confirming normal distribution of residuals. In sheep, CH_4 per digested NDF declined with NDF digestibility ($p<0.001$). CH_4 per digested ADF (in 15 diets with n=6 animals each) also declined with ADF digestibility ($p<0.001$). In cattle, the magnitude of the CH_4 per digested NDF was also negatively related to NDF digestibility ($p<0.001$) and CH_4 per digested ADF (8 diets) with ADF digestibility ($p<0.001$). When accepting only a minimum of 12 animals per diet within experiment, similar results were obtained. Accordingly, CH_4 yield per unit digested fibre is not constant, as a more efficient fibre digestion appears to be linked to a lower production of CH_4. The observation that the same pattern was evident for ADF as for NDF indicates that the variation is not only due to how easily fermentable fibres are digested, but also to the less well degradable cell wall carbohydrates.

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Exploration of undifferentiated cells in bovine rumen epithelial tissue

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Objective: Tissue stem cells are considered as essential cellular population for tissue development and maintenance. This could lead to an idea that they are closely involved in the developmental process of bovine rumen which is induced by the onset of solid feed intake. Esophageal tissue, which has stratified epithelia as well as rumen, is reported to possess undifferentiated stem cells with proliferative potential in its basal layer, and these cells can provide differentiated cells to maintain tissue structure and function. Considering the similarity of tissue structure, rumen is expected to have tissue stem cell-like undifferentiated cellular population. Marker genes and proteins of esophageal stem cells are already found in non-ruminants and could be applied to identify undifferentiated cells in rumen epithelium. Therefore, this study aimed to reveal the presence of ruminal undifferentiated cells, and to obtain initial evidence indicating their involvement in rumen tissue development.

Methods: Totally 11 Holstein male calves and steers at 4, 13 or 40 weeks of age (weaned at 6 weeks) were employed to collect rumen epithelial tissue. Total RNA was extracted from frozen tissue and subjected to RT-PCR and qRT-PCR to investigate the gene expression of esophageal stem cell markers: CD73, SOX2, TP63, ITGB1, ITGB4, ITGA6 and CSPG4, and the expressional changes among different ages. Tissue sections were also prepared from frozen rumen sample for immunostaining of a selected marker protein (SOX2) and a proliferation marker (Ki67) to visualize their location in epithelial structure.

Results: Gene expression of all the investigated esophageal stem cell markers was detected in rumen tissue. Their expressional levels were lower in 13 weeks of age, when compared to 4 weeks of age. Particularly, ITGB1, ITGB4, ITGA6 and CSPG4 expressions were also lower in 40 weeks of age. This suggests the proportion of ruminal undifferentiated cells was decreased by the increase in differentiated cells provided from undifferentiated cells, associated with rumen tissue development. The result of immunostaining indicated that SOX2 was strongly stained in basal layer of stratified epithelium, but also detected in spinous and granular layer with weaker staining. Ki67 was specifically stained in basal layer. This observation suggests that there might be SOX2/Ki67 double positive cells in basal layer. In conclusion, it was suggested that rumen epithelium possesses certain cellular population with stemness and proliferative ability which might contribute to the development of rumen starting from weaning period.

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Changes in the nitrogen fraction of three tropical forage legumes as affected by ensiling length and temperature

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Rapid and extensive degradation of proteins occurs in legume forages after harvest and during ensiling, which results in a decreased efficiency in protein utilization. A significant constraint to silage making in the Tropics is elevated temperatures, and this might cause changes in the characteristics of the forage, particularly of proteins. Therefore, an experiment was conducted to assess the effects of ensiling length and ambient temperature on the nitrogen (N) fraction of three tropical forage legumes. Soybean (*Glycine max*), jack bean (*Cannavalia ensiformis*) and lablab (*Lablab purpureus*) were individually planted, harvested, wilted, chopped, inoculated with silage additive and ensiled in triplicate (a 400 g wilted matter) in polythene vacuum bags for 30 or 75 days. A set of laboratory-scale silos were stored inside a room (indoor), and another batch was stored on the roof of a building (outdoor) with exposure to direct sunlight to induce high temperatures within the bags. HOBO Pro v2 temperature and humidity loggers were used to monitor the ambient temperature of the storage locations at 1 hour-interval during the entire period. At each ensiling time, silages were opened, sampled and analyzed for total N (TN), ammonium-N, neutral-detergent-insoluble N (NDIN) and acid-detergent-insoluble N (ADIN). All data were analyzed using the GLIMMIX procedure of SAS. Least squares means were compared using the Tukey Kramer test with significance considered at $P<0.05$. The average daily ambient temperature for outdoor and indoor storage ranged from 23oC-37oC vs. 20oC-31oC respectively. The TN concentration decreased with advancing ensiling length in soybean and jack bean ($P<0.01$, for both forages) but increased in lablab ($P<0.01$). Ammonium-N concentration increased in all silages ($P<0.01$) with ensiling length and with slightly highest ammonium-N concentrations in silages stored indoor at 75 days. Variable effects of ensiling length were observed for NDIN and ADIN concentrations, but outdoor ensiling resulted in greater ADIN and NDIN concentrations, compared with silages stored indoor ($P<0.01$). The results indicate an effect of high ambient temperatures typical of tropical regions on protein degradation and partially on fiber-bound N during ensiling of forage legumes. Ensiling length has more variable effects and seems to be dependent on the quality of the original material to be ensiled.

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Effect of abomasal infusion of exogenous starch-digesting enzymes on small intestinal starch digestibility of lactating dairy cows

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The aim was to investigate the effect of supplementing starch-digesting enzymes on small intestinal starch digestion in lactating dairy cows in an experimental model preventing confounding effects of rumen starch digestion.

Six lactating Holstein cows (primiparous: n = 3, 493 ± 53 kg BW; multiparous: n = 3, 696 ± 75 kg BW), surgically fitted with ruminal, duodenal, and ileal cannulas were used in a replicated 3×3 Latin square design with 7 day periods. All cows were fed a basal grass-clover silage based diet without starch containing feedstuffs (≤14 g/kg DM dietary starch) added TiO2 for faecal output determination. All cows were abomasally infused with native maize starch (15% w/v suspension; 56.0 ± 0.76 g starch/h). Treatments were abomasal infusion of three different starch-digesting enzymes. Each period consisted of 4 days of blank infusion (enzyme carrier solution) and 3 days of enzyme infusion. Ileal digesta samples were collected for 7.5 h with 1.5 h intervals at the last day of each blank and enzyme infusion sequence. Ileal digesta flow was measured using Cr-EDTA, infused abomasally for at least 16 h before first sampling. Venous blood samples and faecal digesta samples were collected at each sampling day. Starch content was determined by an enzymatic method using immobilised glucose oxidase electrode technique for glucose determination. The MIXED procedure of SAS was used for statistical analysis and model included treatment, parity, and their interaction, experimental period; animal as a repeated effect.

Ileal starch flow did not differ among treatments (P = 0.84), but was higher in primiparous as compared to multiparous cows (P = 0.03; 13.4 ± 0.8 vs. 8.9 ± 0.7 g/h). Accordingly, small intestinal starch digestibility did not differ (P = 0.84), but was lower in primiparous as compared to multiparous cows (P = 0.03; 76.0 ± 1.5 vs. 84.2 ± 1.3 %). Plasma glucose concentrations did not differ (P = 0.35) and averaged 3.78 ± 0.02 mmol/L. The total tract digestibility of starch was not affected (P = 0.58), but was lower in primiparous as compared to multiparous cows (P = 0.03; 96.9 ± 0.3 vs. 98.4 ± 0.4 %). In conclusion, the three starch-digesting enzymes tested did not increase small intestinal starch digestion. The observed differences between primiparous and multiparous cows were unexpected, and warrants further investigation. The low variation in small intestinal starch digestibilities with n = 3 indicate that the used experimental model was robust.

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Effects of including increasing amounts of cauliflower in the concentrate of a dairy sheep diet on in vitro ruminal fermentation

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Reutilization of agroindustrial by-products in animal feeding could contribute to reduce the environmental problems associated with their accumulation and help to achieve an effective circular economy. The objective of this study was to investigate the potential of using market wastes of cauliflower as replacement for cereals and soybean meal in the concentrate of dairy sheep diets. Five cauliflowers were obtained at local markets, cut into pieces, dried (45ºC), ground (1 mm), and mixed before analyses. Cauliflower had low dry matter (DM) content (5.08%), but the DM was rich in crude protein (27.5%) and total sugars (25.7%) and had medium-content in neutral detergent fiber (27.5%) and acid detergent fiber (20.9%). Four concentrates were formulated: a control concentrate for dairy sheep (without cauliflower) and 3 concentrates that included dried cauliflower at 8, 16 and 24% (CA8, CA16 and CA24, respectively) of the concentrate replacing different amounts of cereals and soybean meal. The four experimental diets had 40:60 alfalfa hay:concentrate, and had similar content (DM basis) of crude protein (16.1%) and neutral detergent fiber (31.5%). Samples (400 mg) of each diet were incubated *in vitro* with buffered rumen fluid (40 ml) from sheep, and there were four replicates per diet. Gas production kinetics was determined in 120-h *in vitro* incubations, whereas the main fermentative parameters were measured after 24 h of incubation. Increasing the amount of cauliflower in the diet increased the potential gas production (quadratic; $P = 0.017$) and reduced the *lag* time (linear; $P = 0.043$), but did not affect ($P = 0.385$) the DM effective degradability. Compared with control diet, total VFA production increased ($P < 0.05$) by 6.4, 7.0 and 7.6% for C8, C16 and C24 diets, respectively. Molar proportions of acetate increased (linear; $P = 0.030$) and those of propionate decreased (linear; $P = 0.042$) as the amount of cauliflower in the concentrate augmented, resulting in increased acetate:propionate ratios (3.31, 3.35, 3.43 and 3.53 mol/mol for control, CA8, CA16 and CA24, respectively). There were no differences ($P \geq 0.142$) among diets in NH_3-N concentrations and CH_4 production, indicating a high degradability of cauliflower protein and the absence of antimethanogenic compounds. In conclusion, replacing cereals and soybean meal by up to 24% of dried cauliflower in a concentrate for dairy sheep increased ruminal fermentation and VFA production. These *in vitro* results suggest a potential of cauliflower wastes as ruminant feed that should be further explored.

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Dissociation of the liquid digesta markers CoEDTA and CrEDTA under in vitro ruminal conditions

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Liquid digesta markers are essential tools for evaluating nutrient digestion and passage from the rumen. An ideal marker is inert and unreactive. Cobalt (Co, III) and chromium (Cr, III) salts of ethylenediaminetetraacetic acid (EDTA) are commonly used liquid markers, but, their integrity under ruminal conditions has not been sufficiently evaluated. As colored compounds that have wavelengths (λ) of maximum light absorption when they are complexed (at acidic pH: 535 nm for CoEDTA, 541 nm for CrEDTA), the degree to which the metal and EDTA remain bound can be evaluated spectrophotometrically. In one experiment, CoEDTA and CrEDTA at 40 mg of Co or Cr/L were incubated at pH 6.0 and 39°C in autoclaved clarified rumen fluid (ACRF) from 2 cows or in water (CrEDTA) or buffer (CoEDTA) in replicated 24 h fermentations. Anaerobicity was not maintained. Absorbances of markers incubated in water or buffer were maintained over time, whereas those of CoEDTA in ACRF declined by 4% in 24 h (diluent x time, $P<0.01$), and for CrEDTA decreased by up to 9% at 0 h and 14% by 24 h (diluent x time, $P<0.01$). Effects differed by the cow source of ACRF ($P<0.01$). These results indicate that CrEDTA dissociated to a greater extent than CoEDTA, possibly as organic or mineral compounds in ACRF competed to bind Cr and Co. In a second experiment, approximately 26 mg Co or Cr/L from CoEDTA and CrEDTA or water as a reagent blank were incubated at pH 6.9 and 39°C for 0.5 h in Goering and Van Soest medium with 0, 0.25, 0.50, 0.75, or 1.00 mL of reducing solution (RedSol) added to a 26 mL reaction volume in replicated incubations. Reduction status was determined in samples with resazurin, and absorbance was measured on solutions without resazurin at the peak absorbance λ at pH 6.9 of 535 and 465 nm for Co(III) and Co(II) EDTA and 560 nm for CrEDTA. As RedSol increased, CoEDTA showed a cubic absorbance decline of 75% at 535 nm ($P=0.03$), and a quadratic increase then decline at 465 nm ($P<0.01$). Absorbance of CrEDTA at 560 nm was unaffected by RedSol (linear $P=0.14$). Co(III)EDTA apparently became reduced and dissociated to some extent under in vitro conditions. This suggests that Co(III)EDTA is not a stable marker for use in studies under reducing conditions that can occur in the rumen.

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Effect of supplemental sodium butyrate on p53 expression in the gastrointestinal epithelium of sheep

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The aim of the study was to determine the effect of dietary sodium butyrate supplementation on p53 expression, a marker of DNA damage and repair, in the epithelium of omasum, abomasum, proximal jejunum and ileum of sheep. Eighteen rams (30.8 ± 2.1 kg; 12 to 15 months of age) were fed diet without (CTRL) or with sodium butyrate (BUT; 3.6 g/kg of dry matter). The rams were allocated to the study in four blocks of 6, 4, 4 and 4 rams, within block sub-blocked by body weight, and within sub-block randomly allocated to treatments (9 rams/treatment). Diet consisted of 65% of chopped meadow hay, 19.5% of concentrates and 15.5% of ensiled ground high moisture corn grain (on dry matter basis). Dry matter intake was limited to 2.75% of initial body weight. Feed was offered in two equal meals at 0700 and 1500. Sodium butyrate (unprotected) was mixed with concentrates and high moisture corn grain and fed 10 min prior to hay feeding. Experimental diets were fed for 2 weeks and rams were killed 3 h after morning feeding, and digesta samples from abomasum and proximal small intestine and epithelia samples from the pyloric region of the abomasum, omasal lamina, proximal jejunum and ileum were collected. p53 protein expression was determined using scanning cytometry SCAN^R system in the tissue cross-sections labelled with anti-p53 FL-393 antibodies. The statistical model included the fixed effect of treatment and the random effect of block and animal within sub-block. Sodium butyrate supplementation increased butyrate concentration in the digesta of abomasum (2.0 vs. 1.3 mmol/L; $P = 0.01$) and proximal small intestine (0.68 vs. 0.22 mmol/L; $P = 0.05$). In general, number of p53 positive cells was less in abomasal (6.83%) and omasal epithelium (6.67%) compared to jejunum (10.56%) and ileum (10.95%). Expression of p53 in the epithelium of investigated regions of the gastrointestinal tract did not ($P \geq 0.24$) differ between treatments. Under conditions of the current study unprotected butyrate supplementation in the diet had no effect on p53 expression in the abomasal, omasal and intestinal epithelium of sheep.

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Response of growing bulls to increasing levels of cassava meal in a concentrate maize stover diet

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Cassava meal (*Manihot esculenta* Cranz) is a high metabolisable energy product which is readily available in Indonesia and could be used in a concentrate mix to promote high live weight gain in fattening cattle. However high levels of cassava intake caused a depression in intake and live weight in Madura cattle perhaps due to high HCN and low rumen or caecal pH (Cowley et al 2018). Thirty growing crossbred Limousine x Ongole bulls were used in a Randomised Block Design consisting of 5 treatments with 6 animal replications per treatment. All treatments used 20% maize stover and 80% of a concentrate mixture that contained 30, 40, 50, 60 or 70% cassava meal with the remainder being a mixture (1:1 DM basis) of copra meal and palm kernel meal. The composition of cassava meal was 2.13 % CP and 7.3% NDF, copra meal (24.6 % and 50.7%), palm kernel meal (17.7% and 66.9%) and maize stover (9.5% and 56.9%) respectively. Urea was added at 2% (DM basis) to the cassava meal with mineral mix was added at 2.0% of total ration. DM intake declined curvilinearly (y=2.556+0.2435x-0.003131x2, where x is percentage of cassava inclusion in concentrate meal). With increasing cassava inclusion bulls grew at 1.27, 1.35, 1.05, 0.76 and 0.30 kg/day at cassava inclusion of 30, 40, 50, 60 and 70%, respectively. HCN concentration of cassava meal used in this study was 20.7 ppm which is considered safe. DM digestibility was 76, 72, 73, 70, and 69% and NDF digestibility was 70, 63, 59, 46, and 42%, respectively for low to high cassava inclusion. Rumen parameters (pH 6.34-6.91, ammonia 73 - 94 mg N-NH3/L and VFA concentration 84.8-152.1 mmol/L with the molar percentage of propionic acid varying from 32-39 molar %) were significantly different between treatments ($P<0.05$), but all values were within a normal range. It can be concluded that the use of 40% cassava meal in the concentrate mixture (or 32% of the final ration) was an optimum level for ADG (1.35 kg/day) but that high levels of inclusion at 70% of concentrate mix led to marked depression in intake and average daily gain.

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P 63

The effects of varying zinc dosages from different feed-grade sources on ruminal gas production ex vivo

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We studied how Zn dosages from feed-grade sources affect ruminal gas production ex vivo. Rumen fluid was collected from castrated fistulated rams fed on a standardized diet (intake 1.2 kg DM/d) consisting of 50% hay, 37.5% barley, 11.5% soybean meal and 1% mineral blend (NRC, 2006). Subsequently, it was used for the Hohenheim Gas Test to quantify the ruminal gas production from feed ex vivo (VDLUFA, 2012). For incubation, a total mixed ration (TMR) was used including all mentioned raw components excluding mineral blend. TMR was divided into 13 batches, which were supplemented with varying amounts of Zn (+0, +20, +70, +120 mg Zn/kg DM) from feed-grade sources ($ZnSO_4$*$7H_2O$ (ZS), ZnO-A, ZnO-B, ZnO-B). Data collection comprised TMR Zn contents (KED-ICP-MS) and gas production after 24h (39°C, 1 m/min). Data analysis comprised linear regression and non-linear mixed models. Zn in control was 23.0 ±1.22 mg/kg DM. Varying addition of Zn induced in any case a significant straight linear increase in total Zn (R^2 = 1.0, 0.97, 0.99 and 0.99 for ZS, ZnO-A, ZnO-B, ZnO-C, p_{slope}<0.0001, respectively). However, analytical recovery rates were quite different between Zn sources (38%, 87%, 86% and 104% for ZS, ZnO-A, ZnO-B, ZnO-C), hence, TMR Zn dose ranges were different especially comparing ZS to the ZnOs (28.4-66.6, 44.6-132.0, 45.8-131.6, 40.0-144.2 mg/kg DM for ZS, ZnO-A, ZnO-B ZnO-C). Hence, Zn sources express varying susceptibility to be associated to the mixing container walls by static electricity. Ruminal gas production increased in any case compared to control (168 ±4.44 mL/250 mg) over first dosing steps reaching peak values of 170 ±3.51, 173 ±3.21, 177 ±3.62 and 172 ±5.48 mL/250 mg at 48, 102, 46, 40 mg Zn/kg DM from ZS, ZnO-A, ZnO-B, ZnO-C. Further increase in Zn supply led in any case to a decrease in gas production with lowest levels of 167 ±5.77, 169 ±3.40, 166 ±3.89 and 166 ±3.18 mL/250 mg DM at 67, 132, 132 and 144 mg Zn/kg DM from ZS, ZnO-A, ZnO-B, ZnO-C. These response patterns were statistically significant as indicated by non-linear mixed model analysis ($y=a+bx+cx^2$, P<0.0001). In summary, the data indicates a certain demand for dietary Zn by ruminal microbes >23 mg/kg DM but too high dosages may lead to adverse effects. Critical dosages seem to differ between sources with ZnOs and especially ZnO-A allowing safer application over a wider dose-range.

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High-concentrate diet-induced change of cellular metabolism leads to decreases of immunity and imbalance of cellular activities in rumen epithelium

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Background: In animals, the immune and cellular processes of tissue largely depend on the status of local metabolism. However, in the rumen epithelium, how the cellular metabolism affects epithelial immunity, and cellular processes, when the diet is switched from energy-rich to energy-excess status, with regard to animal production and health, have not as yet been reported.

Materials and methods: Nine male goats received a diet of hay plus concentrate of 65% (HC), 35% (MC), or 10% (LC) for 29 d. RNA-seq was applied to get the expression of genes in the rumen epithelium. RNA was quantified and its integrity was evaluated. All libraries were sequenced via paired-end chemistry (PE125) on an Illumina HiSeq2500 platform. DESeq2 and KEGG enrichment analysis were used. The highly correlated genes were identified by the spearman correlation coefficient, and their functions were identified by the KO annotation. Correlations were considered to be significant when $p<0.05$ and SCC>0.8.

Results: Changing the diet from LC, MC to HC, the ruminal SCFA concentration increased ($p< 0.05$) and the rumen fluid pH decreased in turn ($p< 0.05$). A total of 12, 400, 11, 833, and 11, 471 genes were detected in the rumen epithelium of the LC, MC, and HC group, respectively. Compared with the gene expression in the LC group, 403 genes were significantly upregulated (log2(MC/LC) >1, q value<0.05) and 521 genes were significantly downregulated (log2(MC/LC) <-1, q value<0.05) in the MC group. Compared with the gene expression in the MC group, 273 genes were significantly upregulated (log2(HC/MC) >1, q value<0.05) and 295 genes were significantly downregulated (log2(HC/MC) <-1, q value<0.05) in the HC group. With regard to metabolism, lipid metabolism and amino acid metabolism were most affected. Both innate and adaptive immune responses were promoted by the metabolism genes enriched under the 65% concentrate diet. However, the majority of immune responses were suppressed under the 35% concentrate diet. Moreover, the exclusive upregulation of cell growth and dysfunction of cellular transport and catabolism were induced by the metabolism genes enriched under the 65% concentrate diet. On the contrary, a balanced regulation of cellular processes was detected under the 35% concentrate diet.

Conclusions: These results indicated that the alterations of cellular metabolism promote the alterations in cellular immunity, repair, and homeostasis in the rumen epithelium, thereby leading to the switch of concentrate effects from positive to negative with regard to animal production and health.

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P 65

Effect of dietary SCFA on microbial protein synthesis and urinal urea-N excretion is related to microbiota diversity in rumen

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Background: Urinal urea-N excretion (UUN) increases the cost of livestock production and causes environmental pollution. In ruminant animals the fractions of urea entry transferred to the gut or excreted in the urine are related in a reciprocal manner. The present study aimed to 1) understand the effects of dietary NFC-SCFA on urea-N utilization and UUE; 2) elucidate the relationship between ruminal SCFA and microbiota diversity.

Materials and methods: Two experiments were performed in this study. In Experiment 1, twenty goats were fed with an isonitrogenous diet, containing 28% Non-Fiber Carbohydrate (MNFC group) or 14% NFC (LNFC group). The blood, rumen fluid and epithelium were sampled. The UUN and the microbial protein synthesis were measured. In Experiment 2, an additional eight goats were assigned into the MNFC and LNFC groups. 16S rRNA Amplicon Sequencing was used to analyze the structure of the ruminal microbiota community in relationship to SCFA.

Results: In the MNFC group, the ruminal concentration of SCFA increased, but pH declined. Compared with those in the LNFC group, the microbial protein synthesis in rumen and mRNA abundance of urea transporter B (UT-B) in rumen epithelium increased in the MNFC group. Simultaneously, UUE was reduced in the MNFC group. Significant correlations were found between rumen SCFA and UT-B and between UT-B and UUE. Furthermore, the abundances of SCFA receptor of GPR41 and GPR43 increased in the rumen epithelium of the MNFC group. These results suggest that increases of serum urea-N transported into the rumen and incorporated into MCP and, decreases of UUE are related to ruminal SCFA. This is supported by data from our previous study in which added SCFA on the mucosal side caused increases of urea transport rate (flux J_{sm}urea) from the blood to the ruminal lumen side. In Experiment 2, CCA analysis revealed NFC promoted the expansion of microbiota diversity, particularly of SCFA-producing microbes. The function prediction of 19 upregulated KEGG ortholog groups showed an NFC-induced increase of the types and abundances of genes coding for enzymes catalyzing N and fatty acid metabolism.

Conclusion: Based on our present and previous investigations, our results indicate that, in goats consuming NFC-rich diet, the facilitated urea transport in the rumen and improved urea N salvage are triggered by an expansion of ruminal microbiota diversity and are signaled by ruminal SCFA. This study thus provides new insights into the microbiota involved in the dietary modulation of urea-N salvage in ruminant animals.

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Ruminal *in vitro* protein degradation, anti-nutrient reduction, and *in vivo* digestibility of energy and nutrients in ensiled + toasted pea grains

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Pea grains may be suitable to partially replace soybean or rapeseed meals, and cereals in ruminant diets, but substitution is limited by high protein solubility. The effect of ensiling of pea grains and hydro-thermic treatment (toasting) on protein solubility, trypsin-inhibitor activity, starch morphology, and apparent digestibility of organic matter (OM), energy, and nutrients was examined. Grains were re-moistened to 749 g/kg dry matter (DM), crushed, and ensiled for 9 months. Lactic acid bacteria were used as inoculant. Ensiled grains were toasted with 100 kg/h throughput rate, and 90°C grain temperature. DM, proximate nutrients, gross energy (bomb calorimetry), protein fractions (CNCPS), and trypsin-inhibitor activity were analyzed. True protein (B1+B2+B3+C), protein solubility (A+B1), metabolizable energy (ME), and net energy lactation (NEL) were calculated. Rumen-undegraded protein (RUP) was measured using *S. griseus* protease. Scanning electron micrographs were used to examine morphological alterations of starches. Aerobic stability of silage was determined according to the method of Honig; organic acids and alcohols by HPLC-RID. Eight wethers (78±9.7 kg body weight) were used in the digestion trial, fed close to maintenance. Statistical analysis was performed using pooled t-test and a linear model at $P<0.05$ significance level. Peas had 186 g CP, 533 g starch, and 77 g sugars/kg DM. Pea silage had 2.3 g lactic acid, 0.3 g ethanoic acid, and 9.4 g ethanol/kg DM. It was stable under aerobic conditions ≥ 7 days. Despite best practices, pH was not reduced below 6.1. Peas had 38 g RUP/kg DM (20% of CP), which increased 3-fold after ensiling + toasting ($P<0.001$). Acid detergent insoluble protein increased 5-fold. Protein solubility decreased from 74 to 16% of CP. Inhibited trypsin was 3.8 g/kg DM, and was reduced by ensiling (2.5 g/kg DM; $P=0.079$) and ensiling + toasting (1.4 g/kg DM; $P<0.05$). Morphological changes of pea starches were not visible. Apparent digestibility was 0.94 (OM), 0.91 (energy), 0.89 (CP), and above 0.99 for starch and sugars after ensiling + toasting ($P>0.05$). Neutral detergent fiber digestibility apparently increased from 0.69 to 0.81, confounded by 30%-point increased neutral detergent insoluble CP. ME was 13.9 MJ and NEL 8.9 MJ/kg DM in native and ensiled + toasted peas, respectively. Ensiling and toasting of pea grains decreased protein solubility in the rumen without effects on total-tract digestibility. They reduced trypsin inhibitor activity. Pea starches remained unaffected.

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P 67

Effect of ensiling and toasting of field pea grains on formation of Maillard polymers from lysine and arginine

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Ensiling and hydro-thermic treatment (toasting) may limit degradation of soluble pea protein in the rumen, and make more of it available for small intestinal digestion. Effects of ensiling and toasting on lysine and arginine concentration in peas, and formation of associated Maillard polymers were examined. Crushed and re-moistened peas (749 g/kg dry matter (DM)) were ensiled. Lactic acid bacteria were used as inoculant. Ensiled grains were toasted with 100, 70, or 50 kg/h throughput and 100–190°C supplied temperature (60–110°C grain temperature). DM, proximate nutrients, and protein fractions (CNCPS) were analyzed. Protein solubility (A+B1) was calculated. Lysine-associated CML and pyrraline, and arginine-associated MG-H1 were analyzed by HPLC-ESI-MS. Fructoselysine was calculated from furosine. Furosine, lysine, and arginine were determined after acid hydrolysis and ninhydrin post-column derivatization. Aerobic silage stability was analyzed according to Honig; organic acids and alcohols by HPLC-RID. Statistical analysis was performed using a model with fixed treatment effect at $P<0.05$ significance level. Peas had 190 g crude protein (CP)/kg DM. Pea silage had 2.3 g lactic acid, 0.3 g ethanoic acid, and 9.4 g ethanol/kg DM, and was aerobically stable 7 days. Despite best practices, pH was not reduced below 6.1. Protein solubility decreased from 73 to 33% of CP after ensiling and 11% of CP after toasting ($P<0.001$). Acid detergent insoluble protein increased from 0.7 to 18.8% of CP ($P<0.001$). Fructoselysine increased from 141 to 3650 mg/kg DM after ensiling, 8187 mg/kg DM after toasting (70°C grain temperature), and decreased to 1939 mg/kg DM with rising temperature ($P<0.001$). CML, pyrraline, and MG-H1 increased after ensiling from 0.8, 0.4, and 1.2 to 21.8, 2.8, and 3.9 mg/kg DM, respectively ($P<0.05$ in CML; $P>0.05$ in pyrraline and MG-H1). Toasting increased CML to 80°C (59.9 mg/kg DM; $P<0.001$), and decreased it to 33.8 mg/kg DM ($P<0.01$). Pyrraline and MG-H1 increased to maximal 648.5 and 91.9 mg/kg DM, respectively ($P<0.001$). Lysine decreased from 11.6 to 9.6 g/kg DM after ensiling and 4.7 g/kg DM after toasting ($P<0.01$). Arginine was not affected by ensiling, but decreased during toasting from 7.6 to 4.3 g/kg DM ($P<0.001$). Ensiling and toasting of peas decreased ruminal protein solubility. Amino acid concentrations can be reduced. Toasting temperature should not exceed 100°C to avoid protein damage.

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Use of combinations of commercial extracts from quebracho, oak and grape tannins to modulate in vitro ruminal fermentation

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Despite their bad reputation among some nutritionists, it is nowadays widely accepted that tannins can be used as additives to modulate rumen fermentation. However, there is a void of information about which of the many types of tannins and at which doses may be more useful for a particular purpose. Furthermore, little is known about potential synergistic effects of different combinations of tannins. An *in vitro* assay with batch cultures of rumen microorganisms, using cannulated ewes as donors of rumen inocula, was performed to try to move forward on this subject. Commercial extracts of quebracho, oak and grape tannins, at doses selected from a previous study were combined following a simplex centroid design (in total, 10 combinations plus 1 control without tannins) and added to the incubation substrate (a total mixed ration based on alfalfa hay and concentrates, with 27% of starch and a F:C ratio of 40:60). Results suggested that there might be a synergistic effect of the combinations. For instance, none of the tannin extracts alone altered methane production, while some mixtures (mentioned below) did it, and the reductions of NH_3-N, which would support a protection of dietary protein against ruminal degradation, were higher when combinations were used. Considering all the results together, we selected the next 3 treatments as the most convenient (doses are reported as % of incubated DM; real amounts after considering the simplex centroid design). Combination 1: 1.7% oak + 0.85% grape. This treatment induced lower gas and methane productions, ammonia-N concentration and molar proportion of minor volatile fatty acids (VFA; namely isobutyrate, isovalerate, valerate and caproate). Combination 2: 1.13% quebracho + 1.13% oak + 0.57% grape. Although this treatment affected negatively the ruminal disappearance of NDF (-8%), which would challenge its selection, it reduced gas and methane productions, ammonia-N concentration and molar proportion of minor VFA, as well as the acetate/propionate (A/P) ratio, and also increased the molar proportion of propionate. Combination 3: 0.57% quebracho + 2.27% oak + 0.28% grape. It reduced gas and methane productions, ammonia-N concentration, molar proportion of minor VFA, and the A/P ratio. This was the treatment with the strongest effect on NH_3-N concentration (-22%). It also increased molar proportions of propionate and acetate, and it did not exert non-desirable effects on the in vitro ruminal fermentation parameters that were investigated. In closing, an appropriate combination of tannins may improve ruminant diet utilization while reducing methane emissions.

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Pre-ensiling treatments affect the *in vitro* rumen fermentation profile, microbiota composition and fibre degradability of lucerne silages

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Reducing the import of protein-rich feedstuffs in favour of using inexpensive on-farm produced protein supports sustainability in livestock production. Lucerne (*Medicago sativa* L.) silages (LS) have a great potential, as they include high concentrations of crude protein and fibre. However, LS are also characterized by a high non-protein nitrogen content, which is rapidly degraded to ammonia in the rumen and ultimately lost in excreta.

Pre-ensiling treatments, i.e. varying dry matter (DM) concentration, wilting intensity and sucrose addition have affected LS quality and increased true protein preservation in high-intensity wilted silages with sucrose addition. However, it is unknown whether these beneficial effects are reflected in the rumen fermentation. Therefore, these LS were isonitrogenously incubated in a rumen-simulation technique system for nine days. Two and seven days after first incubating LS, gas production was measured and liquid phase samples were taken 2, 4, 12 and 23 hours after feed bag exchange for measuring pH, short chain fatty acids (SCFA) and microbial community composition. Additionally, feed residues were sampled for microbial community analysis and determining degradability of: (i) neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash (aNDFom), (ii) acid detergent fibre expressed exclusive of residual ash (ADFom) and (iii) organic matter (OM). The four liquid phase samples were pooled for each vessel and run, respectively, to obtain robust mean values. Gas production was determined using water displacement technique, pH by potentiometry, SCFA by gas chromatography and microbial community composition by 16S rRNA gene sequencing and quantitative PCR. Fibre fractions and OM were determined according to standard procedures of VDLUFA. Data were analysed using the MIXED procedure of SAS with pre-ensiling treatments and sampling day as fixed effects and vessel and run as random effects.

At both sampling days, sucrose addition increased gas production as well as SCFA concentrations, particularly propionate and isovalerate, whereas it decreased ADFom degradability and pH. The DM concentration and wilting intensity affected the aNDFom and OM degradability, but showed less impact on SCFA profiles. The degradabilities of OM and fibre fractions declined from day two to day seven, which contradicted the stable total SCFA concentrations. A reduction in anaerobic fungal concentrations and prokaryotic alpha diversity on the feed residues after seven days may partly explain this observation. The extraordinary high isovalerate concentrations were unexpected, but the often suggested fibrolytic stimulation by isovalerate could not be confirmed in this study.

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Effect of rye and wheat grain processing on in situ degradability and intestinal digestibility of protein and starch in ruminants

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The degree and rate of nutrient degradation of rye grain in the rumen has a significant influence on the effects and profitability of ruminants. The decisive role in this respect is played by rye variety and mechanical treatment of grains. However, excessive defragmentation of rye contributes to the intensive formation of organic acids and rumen acidosis. Therefore, the aim of the study was to determine the effect of processing of traditional (TRG) and hybrid rye grain (HRG), in comparison to wheat grain (WG), on effective rumen degradation (ERD) and intestinal digestibility (ID) of protein and starch.

Three cereal grains: TRG (Dańskowskie Opal), HRG (Bono) and WG (Jantarka cultivar) were processed to obtain four degrees of fragmentation: none (whole grain), crushed, and milled to 4.0 or 1.5 mm. Three Holstein-Friesian cows fitted with ruminal and duodenal cannulas were used for the determination of rumen degradation and intestinal digestibility of starch by *in situ* and mobile nylon bag method. Data were analyzed using the PROC MIXED of the SAS (v.9.2) and pre-planned contrasts were used for scientific hypothesis verification (TRG vs HRG and TRG&HRG vs WG).

Processing of grain, regardless of the cereal type, had significant effect ($P<0.01$) on the ERD of protein and starch. The highest ERD of protein was observed in crushed grains of WG (92.05%), then in TGR (85.68%) and HGR (83.80%) grains milled to 1.5 mm. The highest ERD of starch were reported for TGR grains milled to 1.5 mm, then for crushed grain. Crushed grains of rye had the highest content of fraction A of starch (TGR: 79.25% and HRG 65.77%), which is a fraction assumed to be immediately degraded in the rumen. In case of wheat, the highest A fraction content was found for grains milled to 1.5 mm (74.51%). Wheat grains milled to 1.5 was characterized by higher ID of starch than grains of TGR and HGR.

The degree of grain processing is a main factor modifying the effective rumen degradability and intestinal digestibility of protein and starch. Rye grains have similar susceptible to fermentation of starch in the rumen to wheat, but the way of grain processing may significantly alter the site and extent of its digestion. Further studies are needed to determine in detail the structural factors that affect the digestibility of protein and starch in the processed grain of different cereal species.

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Effect of different types of fibre substituting barley straw on *in vitro* rumen fermentation of high-concentrate diets for beef calves

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Intensive beef production generally implies the use of diets based on high (up to 0.90) concentrate proportions, supplemented with chopped straw, which is expensive and laborious. In contrast, low proportions of short fibre in the concentrate mixture may negatively affect rumen pH. Substitution of barley straw (BS) by soybean hulls (SH) and oat hulls (OH) in concentrate diets was studied in a semicontinuous *in vitro* incubation system, in four runs with duplicate bottles per treatment. The BS chopped at 2 cm and SH and OH ground to 1 mm were added to a concentrate mixture (maize, barley and soybean meal at a 41:41:18 ratio) at two neutral detergent fibre (NDF) levels (20 or 24% in the final substrate). Liquid outflow rate was fixed at 0.08 h^{-1}, and pH was allowed to drop to below 6.0 for 8 h, then gradually buffered around 6.5 to simulate concentrate feeding conditions. Total gas and methane production as well as pH were recorded up to 24 h, and 24 h dry matter disappearance (DMd) was determined. Incubation pH dropped to 5.9 at 6-8 h, then increased to reach 6.5 at 20 h. Among fibre sources, pH was the highest with BS in the first 4 h incubation, but differences were below 0.03 pH units and disappeared thereafter. Among fibre levels, pH was higher with 24% NDF at 12 and 16 h ($P<0.05$). Up to 8 h, the inclusion level of SH and OH was positively related with gas production (interaction source x level, $P<0.05$), but not with BS. From 10 h onwards, the higher NDF level promoted more gas ($P<0.05$). Overall, gas production was higher ($P<0.05$) with SH up to 10 h, but differences disappeared thereafter resulting 157, 158 and 152 mL gas/g organic matter for SH, OH and BS at 24 h. From 0 to 6 h methane proportion dropped with the level of inclusion of OH, but increased with that of SH. However, fibre level did not affect thereafter, and methane proportion in total gas was lower with BS than OH from 6 to 12 h, and it was lowest with BS from 12 to 24 h ($P<0.05$). Substitution of chopped barley straw by ground soybean hulls or oat hulls does not greatly affect medium pH nor gas production, so potential differences must be related more with animal parameters such as digestibility or rate of intake rather than with rumen fermentation.

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In vitro study of the effect of the particle size of fibrous substrates on microbial rumen fermentation

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The amount and physical characteristics of dietary fibre determine rumination and rate of intake in ruminants, therefore affecting environmental conditions and transit time. Besides, particle size of fibre sources may also affect microbial fermentation rate. Thus, the rate and extent of fermentation of fibrous materials with different particle sizes was studied *in vitro*. Feeds of small (dehydrated alfalfa meal, AD; weighted mean 0.8 mm), medium (palm kernel cake, PC; soybean hulls, SH; sugarbeet pulp, SB; 1.5, 1.9 and 2.1 mm), large (oat hulls, OH; 5.8 mm) and very large (barley straw, BS; chopped to 2 cm) size were incubated in their original presentation or ground to 1 mm. Four 48h incubation series were conducted, at pH of 6.40-6.70, with duplicate bottles per treatment. Rumen inoculum from five ewes fed on a 50:50 forage:concentrate diet was used. Gas production was recorded at different incubation times, methane (CH_4) concentration was measured on every 12 h interval, and dry matter disappearance (DMd) was determined at 48h. Throughout the incubation, the volume of gas produced with SB and SH was higher than with PC and AD ($P<0.05$), whereas OH and BS recorded the lowest volumes ($P<0.05$). Grinding all substrates to 1 mm increased gas production in the first 24 h, but the presentation x substrate interaction ($P<0.05$) from 6 to 12 h indicates that differences were only significant regarding SB (from 0.19 to 0.33). Ground substrates ranked the same as at their original presentation. Gas production results were supported by DMd for both presentation forms. Up to 12 h, CH_4 proportion of total gas was the lowest with OH and BS ($P<0.05$), and this proportion increased onwards, resulting a higher proportion with AD, OH and SH, whereas PC was the lowest ($P<0.05$). When expressed as volume per unit of incubated substrate, CH_4 production from 0 to 12 h was the highest with SB, and the lowest was recorded with OH and BS ($P<0.05$). At 48 h incubation, accumulated CH_4 with SH was the highest ($P<0.05$), whereas it was the lowest with OH ($P<0.05$). No differences ($P>0.05$) were recorded between presentation sizes of substrates in methane production throughout the period. Despite the particularities among fibrous substrates, the fermentation differences when ground to a common size were not significant, except for SB, and in any case fermentation followed the same trend. Therefore, the size of fibrous substrates is no determinant in their fermentation pattern.

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Effect of supplemental rumen-protected Methionine on zootechnical performance of bulls for fattening

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Improving protein efficiency is a major aim in feeding ruminants. The general strategy comprises reduction in dietary crude protein (CP) at simultaneously improving amino acid quality, e.g. through supplementation of rumen-protected, essential amino acids. While Lysine and Methionine are considered to be the first limiting amino acids in lactating dairy cows, the respective relevance to bulls for fattening is still widely unknown. This project focused on the effect of supplemental rumen-protected methionine (RPM) in a CP deficient diet on dry matter intake (DMI) and DWG (daily weight gain) in fattening German Fleckvieh bulls.

The study involved three diets, a control diet (CON) with sufficient CP (13.5% of DM), a protein-deficient diet containing 8.5% CP of DM (NEG) and a NEG diet supplemented with RPM (NEGM). All diets were composed at an isoenergetic level (11.6 MJ ME/kg DM) and both NEG and NEGM were supplemented with rumen-protected Lysine (0.18% Lys in DM) in order to hold the Lysine content constant to the control diet and to further exclude Lysine from acting as first limiting amino acid. RPM was added to the NEGM diet with 0.12% in DM. A total of 69 young bulls of 238 days age and 367 kg of body weight were allotted to the three diets (n=23 per group) and were fed ad libitum for up to 342 days on average. Starting 57 days after the onset of the study, subgroups of bulls were slaughtered weekly. Statistical evaluation included ANOVA, multiple comparisons of means (SNK test) as well as linear contrasts (SAS 9.4).

Reduction of CP supply depressed DMI (9.24 kg/day of CON vs. 8.38 kg/day as average of both NEG and NEGM, $p<0.0001$), and DWG (1580 vs. 1228 g/day, $p<0.0001$), respectively. Within the protein-deficient diets, addition of rumen-protected methionine did not alter DMI or DWG to a statistically significant extent (8.49 vs. 8.27 kg/d, $p=0.44$; 1256 vs. 1199 g/day, $p=0.45$).

Depressed DMI and DWG demonstrated efficacy of protein restriction in feeding group NEG. Lack of effect of supplemental rumen-protected Methionine suggests that methionine was not the first limiting amino acid under condition of elevated supply with Lysine.

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Effect of decortication of oat on chemical composition and in situ rumen and total tract disappearance of protein

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The aim was to study the effect of decortication of oat on chemical composition and ruminal degradability and post ruminal digestibility of dry matter (DM) and crude protein (CP). Four Danish oat varieties (Dominik, Poseidon, Symphony, Zorro) were decorticated using a mobile decorticator with three Bühler dehuller MHSA (Bühler AG, Uzwil, Switzerland), mounted on a truck. Intact oat and decorticated forms were investigated. Three rumen fistulated cows were used for *in situ* rumen degradability study. Two lactating cows equipped with T-shaped duodenal cannula were used to determine total tract and intestinal disappearance of CP. Decorticated oat showed higher (P = 0.01) concentrations of CP (134 vs. 108 g/kg DM) and crude fat (71.6 vs. 53.1 g/kg DM). The proportion of linoleic acid (C18:2 n6) in total fatty acids increased (P < 0.05) by decortication. Decortication increased (P = 0.01) the concentration of amino acids (AA), but the proportion of lysine in total AA decreased (P < 0.01). In decorticated oat, degradation rate of insoluble potentially degradable DM (b) was higher (0.35 h^{-1}) compared to intact oat (0.09 h^{-1}). The effective degradability of DM was higher (P < 0.001) in decorticated oat (931 g/kg DM) than in intact oat (726 g/kg DM). High particle loss was found in decorticated and intact oat for both DM and CP, and it was 678 and 699 g/kg CP in decorticated and intact oat, respectively. Effective degradability of CP was 934 and 903 g/kg CP in decorticated and intact oat, respectively. Total tract disappearance of CP in decorticated oat (919 g/kg CP) was higher than for intact oat (958 g/kg CP). Similarly, the small intestinal disappearance of rumen ungradable CP (P < 0.001) in decorticated oat (366 g/kg CP) was higher than for intact oat (181 g/kg CP). In conclusion, decortication can be used as a practical approach to increase the nutritional value of oat. However, results of *in situ* degradability of oat should be interpreted with caution due to high particle loss.

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Effect of oat decortication, toasting and in combination on protein metabolism of dairy cows

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Effect of oat decortication, toasting and combination of decortication and toasting on protein digestibility and metabolism of dairy cows was investigated. Four lactating Danish Holstein cows fitted with ruminal, duodenal, and ileal cannulas were assigned to 4 diets containing either whole oat (as control), decorticated, toasted, or combination of decorticated and toasted oat in a 4 × 4 Latin square design. Cows were fed TMR diets containing 607, 217, 165, and 87 g/kg DM of grass clover silage, oat, toasted faba beans, and mineral supplements in the experimental diets, respectively. Energy corrected milk of cows fed control, decorticated, toasted, and combination of decorticated and toasted treatments was 33, 32, 32, and 32 kg/d, respectively, with no significant difference between different treatment groups. Similarly, DMI was not affected by different processing methods of oat and DMI was 21, 20, 22, and 21 kg/d in cows receiving control, decorticated, toasted, and combination of decorticated and toasted treatments, respectively. No significant difference was observed in milk composition between different treatment groups. Ruminal, small intestinal and total tract digestibility of crude protein were not affected by different treatments. Cows fed diets containing toasted oat had a higher duodenal flow of amino acids (AA, $P < 0.04$). Microbial protein synthesis was 1.4, 1.5, 1.5, and 1.7 kg/d in cows fed control, decorticated, toasted, and combination of decorticated and toasted oat diets, respectively and was affected by decortication ($P < 0.01$) and toasting ($P < 0.01$). Proportion of lysine in microbial AA was increased by toasting ($P < 0.03$), while for methionine an interaction between decorticating and toasting was found ($P < 0.01$). Total digested AA in the small intestine was 2.20, 2.29, 2.39, and 2.61 kg/d in cows fed control, decorticated, toasted and combination of decorticated and toasted oat diets, respectively, and was affected by decortication ($P < 0.02$) and toasting ($P < 0.002$). Both toasting ($P < 0.05$) and decortication ($P < 0.01$) increased the methionine proportion in duodenal AA. In conclusion, oat decortication, toasting or a combination of decortication and toasting can be used as an appropriate method for increasing the microbial protein synthesis and digested AA in small intestine of dairy cows.

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The effect of a sudden dietary starch inclusion increase on metabolic status and milk production in dairy cows

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Six lactating Holstein cows with ruminal cannulas and permanent catheters in an intercostal artery were used to study the effect of sudden changes in starch levels on the metabolic status and performance. Animals were used in replicated 3 × 3 Latin square design. Animals were fed ad libitum with a control diet containing 20% starch (DM basis) during the adaptation period (7 days). On d 8, cows received one of the experimental diets containing either 28, 35 or 42% starch (DM basis). Milk yield was recorded twice a day during the experimental period (8 days). On d 8 rumen, blood, urine and milk samples were collected -0.5, 1, 2.5, 4, 5.5 and 7 h relative to morning feeding. The pH was measured in all samples. On milk samples, glucose, glucose-6-phosphate and β-hydroxy-butyrate concentrations were measured using an enzymatic-fluorometric determination. The MIXED procedure of SAS was used with a model including starch level, sampling time and the interaction between both as dependent variables, and time within animal as a repeated effect. Milk yield was not affected by treatment (30.5 ± 1.35 kg). Both ventral and medial ruminal, and urine pH were not affected by treatment ($P>0.05$), but were affected by sampling time ($P<0.05$), decreasing from -0.5 h (6.31 ± 0.19, 5.70 ± 0.17 and 8.17 ± 0.05, respectively) to 7 h relative to feeding for ventral and medial ruminal pH (5.75 ± 0.19 and 5.30 ± 0.17) and to 4 h relative to feeding for urine (8.01 ± 0.05). Blood and milk pH were not affected by either treatment or sampling time (7.48 ± 0.01 and 6.77 ± 0.02 respectively, $P>0.05$). Milk glucose and milk glucose-6-phosphate were not affected by treatment ($P>0.05$), but they were affected by sampling time ($P<0.05$), decreasing from -0.5 h (30.0 ± 5.70 and 5.40 ± 0.20 μM respectively) to 4 h relative to feeding (12.10 ± 5.70 and 4.80 ± 0.20 μM respectively). Milk β-hydroxy-butyrate was not affected by treatment ($P>0.05$), but it was affected by sampling time ($P<0.05$), increasing from -0.5 h (66.9 ± 13.2 μM) to 4h relative to feeding (107 ± 13.2 μM). According to our results, dairy cows are able to cope with sudden and large changes in dietary starch. However, further variables such as creatinine, isocitrate or malate will be analysed in the future to further confirm these results.

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Effects of garlic and cinnamon on *in vitro* and *in vivo* rumen adaptation

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Previous studies have shown positive effects of phytogenic feed additives (PFA) on reducing methane production. However, often these positive effects are seen in *in vitro* studies in which rumen fluid is not adapted to PFA. The aim of this experiment was to study effects of supplementing a blend of garlic and cinnamon (GC) on rumen adaptation, in a combined *in vivo* and *in vitro* study.

Two times per day, 2.5 grams of GC was directly dosed into the rumen of three fistulated non-lactating dairy cows. Trial period was 43 days. Animals were used as rumen fluid donors for an *in vitro* experiment to determine the cumulative gas (GP, ml/g organic matter) and methane production (MP, ml/g organic matter) at week 0 (W0) and week 4 (W4) of adaptation to GC. Grass silage (0.5 g/ bottle) was incubated in buffered rumen fluid (60 mL/ bottle) in duplicate with GC added at inclusion levels of 0 mg/g (control), 0.03 mg/g, 0.30 mg/g, and 3.00 mg/g. After 72 hours of incubation, volatile fatty acids (VFA) and ammonia (NH_3) were measured. GP and MP were measured in real time using an automated gas production system and were fitted using a biphasic and monophasic model, respectively. Furthermore, over the total testing period samples of rumen fluid were taken from the animals and analysed for VFA. Data were analysed with the mixed procedure of SAS. Means were compared after adjustment for Tukey.

Results showed no differences in GP between W0 and W4. The proportion of methane in total gas (CH_4%; MP/GP*100) at W0 was lower for inclusion of 3 mg/g as compared to control (14.2% vs. 18.7% respectively, P=0.0221). At W4, differences between control and 3 mg/g GC inclusion were observed for CH_4% in total gas (19.5% and 16.7%, resp.). No differences were shown between W0 and W4 for total VFA, branched chain proportion VFA and NH_3 concentration. At d3 of the in vivo study, a lower average of the total VFA was observed (P<0.0001). In conclusion, the *in vitro* experiment showed that CH_4% in total gas was reduced as a consequence of GC inclusion. An adaptation of rumen microbes to GC was not shown. The average of total VFA on d3 *in vivo* was lower as compared to the average of total VFA at other time points. No adaptation effects to GC were observed during the whole testing period.

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Effects of garlic and cinnamon supplementation on ruminal methanogenesis and rumen fermentation kinetics determined with the gas production technique

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The aim of this study was to investigate the effects of a blend of garlic and cinnamon (GC) on ruminal methanogenesis and rumen fermentation kinetics. An *in vitro* experiment was conducted to test the effect of GC on cumulative gas production kinetics, (GP, ml/g organic matter), cumulative methane production kinetics (MP, ml/g organic matter) and fermentation end products. Grass silage (0.5 g/ bottle) was incubated in buffered rumen fluid (60 mL/ bottle) in duplicate with GC added at inclusion levels of 0 mg/g (control), 0.03 mg/g, 0.15 mg/g, 0.30 mg/g, 1.50 mg/g and 3.00 mg/g. After 72 hours volatile fatty acids (VFA) and ammonia (NH_3) were measured to test effects on rumen fermentation. GP and MP were measured in real time using an automated gas production system and were fitted using a biphasic and monophasic model, respectively. Data were analysed with the mixed procedure of SAS. Means were compared after adjustment of Tukey.

Results showed a comparable biphasic response for GP of control and all levels of GC. Total gas organic matter corrected volume (OMCV) (ml/g OM) showed a linear increase (P=0.02) with increasing levels of GC. The concentration of CH_4 in total gas produced (CH_4%) showed a linear decrease (P=0.005) at increasing inclusion levels of GC from 18.7% (control) to 14.2% (3.0 mg/g GC). No effects were observed in NH_3 (mmol/L) concentrations and total VFA (mmol/L). The molar proportions of acetic and butyric acid were not affected by GC inclusion, whereas the proportion of propionic acid showed a linear increase (P=0.001) with increasing inclusion levels of GC (18.3% for control vs. 22.0% for 3.0 mg/g GC). The non-glucogenic to glucogenic VFA ratio (NGR) showed a linear decrease (P=0.002) at increasing GC inclusion levels.

In conclusion, GC was able to change rumen fermentation characteristics. The inclusion of GC reduced *in vitro* CH_4 production with no negative effects on total gas OMCV and total VFA production. Inclusion of GC linearly increased the proportion of propionic acid and linearly decreased NGR. These data suggest GC is able to reduce ruminal methane production by stimulating production of propionic acid which acts as an hydrogen sink and which is a precursor for gluconeogenesis.

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Fatty acid profile in EU 'HealthyHay' sainfoin (*Onobrychis viciifolia*) germplasm and the effect of their tannins on *in vitro* biohydrogenation

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Introduction: Condensed tannins modulate the activity of rumen microbiota and affect rumen bacteria like *Butyrifibrio fibrisolvens* and *B. proteoclasticus*, associated with ruminal biohydrogenation. Variable effects of tannins on rumen functioning are observed and may be related to the huge diversity in tannin structures. However, little information is available on the relation between tannin structure and its efficacy to modulate microbial processes.

Method: An *in vitro* study was conducted to investigate the effect of sainfoin (*Onobrychis viciifolia*) tannins on the extent of *in vitro* measured ruminal biohydrogenation. From the EU 'HealthyHay' sainfoin germplasm collection 20 accessions were selected based on a difference in the activity of key-enzymes involved in the polyphenol metabolic pathways; phenylalanine ammonia-lyase (PAL), chalcone synthase/chalcone isomerase (CHS/CHI), flavanone 3-hydroxylase (FHT), dihydroflavonol 4-reductase (DFR), flavonol synthase (FLS) and flavonoid glucosyl-transferase (FGT). Accessions were divided into two groups showing either higher enzymatic activity (HEA; n=10) or lower activity (LEA; n=10). All 20 accessions were analysed for their fatty acid composition by gas chromatography. From each group 2 accessions (LEA accessions: 1017, 1213; HEA accessions: 1043, 1179) were selected to study the extent of ruminal biohydrogenation by *in vitro* batch culture incubations. Samples (1 g) of the sainfoin accessions were incubated in duplicate in 100-mL bottles in phosphate buffered rumen fluid (50 mL) either with or without polyethylene glycol (PEG6000) and maintained under anaerobic condition at 39°C for 0, 12 and 24h. After incubation, bottles were removed, placed on ice, and bottle content was directly quantitatively transferred into aluminium trays, frozen at -20°C, freeze dried, pulverized and analysed for fatty acid composition.

Results: The average fatty acid composition of the HEA accessions, C18:0 (2.66%), C18:1c9 (2.21%), C18:2n6c (17.37%) and C18:3n3 (53.72%), did not differ from the LEA accessions (2.58, 2,28, 17.61, 53.62%, respectively; $P>0.147$). Fatty acid composition changed during prolonged incubation time and was affected by tannins. Addition of PEG gave a stronger decrease in C18:2n6c and C18:3n3 concentration after 12h ($P\leq0.052$) of incubation compared to substrates without PEG, with concurrent accumulations of C18:0, C18:1tr11 and C18:1c9. After 24-h incubations the effect of tannins were only numerical. Differences in *in vitro* biohydrogenation did not show a clear relationship with the activity of plant enzymes involved in polyphenol metabolism (HEA vs. LEA).

Conclusion: Our results suggest that sainfoin tannins alter the extent of *in vitro* ruminal biohydrogenation, however, activity of key-enzymes involved in polyphenol metabolism does not seem to explain this bioactive response.

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Rumen pH and redox regulation in cattle grazing forages by learned intake behaviours

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In New Zealand, high production from cattle grazing forage is achieved with sophisticated ryegrass (RG) grazing management or with specialist forage crops (1.5-2kg/d gain; 6000L/yr). Rumen function, however, is starkly different from ration fed stock.

Ten sequential studies of *in situ* rumen function were undertaken with groups of cattle grazing highly managed RG or fodder beet (FB), with rumenally fistulated cows in early lactation grazing RG under management with allocations of 4.1% LWT/d but strict area restriction. Intakes were 3.5-4% LWT/d, total water intakes >150ml/ kg LWT/d, rumen liquid passage rates >20%/h, concentrations of NH_3 and SCFA were twofold higher than published comparisons of ration fed cows. Lactic acid concentrations were very low (<1mmol/L), rumen volumes were 21-25% of LWT, osmolarity was above 280mOsmol and redox beyond -400mv, and a rumen pH pattern observed below 5.5 for almost 10% of the day.

FB with unrestricted *ad libitum* intakes at similar energy/$LWT^{0.75}$ but with almost threefold non-structural carbohydrate concentrations of RG and 65% of the protein concentration resulted in similar rumen liquid passage rates, but lower rumen NH_3 and SCFA concentrations (approximately 60% of RG), lower rumen volumes (10-12%), similar redox values and markedly greater pH (6.1-6.7) across the diurnal period. This was associated with high $LWT^{0.75}$ gain (0.013-0.015%/d), and a dispersed diurnal distribution of intake.

However, preventing *ad libitum* grazing intakes of FB (to 75%) to produce competitive grazing behaviour dramatically altered diurnal NH_3, SCFA, osmolarity, redox and pH profiles. The greatest change was rumen pH, with a mean reduction of 0.6 in the 8h after initiation of grazing compared to *ad libitum* diets, and significant increases in both diurnal minutes and bout number below 6.0, 5.8, and 5.5. These were associated with reduced daily grazing time and increased grazing bout length.

Typical rumen function in high intensity feeding of cattle on low protein forages is characterised by extremes of liquid passage rates, high osmolarity, and unusually high rumen pH and magnitude redox with low SCFA concentrations, despite high intakes. However, with restricted allocations rumen pH is dramatically lowered while redox remains broadly similar. We suggest competitive grazing overrides the intake behaviours otherwise observed in *ad libitum* fed cattle, which induces rumen 'loading' patterns that push rumen function toward the boundary of normal operation. We conclude that in high metabolisable energy forage systems, feed allocation is the key determinant of the rumen pH, via intake behaviour.

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Effect of harvest time and shredding of grass-clover on feed intake and chewing time in dairy cows

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The effects of physically treating grass-clover at early and late harvest time prior to ensiling were investigated in two cuts in spring growth with a 14 days interval using a grass-clover mix (91.3 and 8.7 % on DM-basis of grass and clover, respectively). Both cuts were wilted before baling (Control, Early: CE; Control, Late: CL) or wilted and shredded prior to baling (Shredded, Early: SE; Shredded, Late: SL). Wilted grass-clover was channelled in between a rotating drum and a curved shell, both equipped with metal ridges in a mobile shredder. Four cows and one spare cow for the last two periods, all primiparous ranging from 282 to 404 days in milk, were used in a 4 x 4 Latin square design. Twice daily, experimental silages were fed ad libitum with supplementation of vitamins and minerals. Chewing time was recorded using the RumiWatch system (ITIN+HOCH GmbH, Switzerland) and the corresponding DMI and milk yield were averaged per cow over three consecutive days starting on day 13 in each period. A model containing harvest time, shredding, their interaction, and period as fixed effects and cow as random effect was used for data analyses.

Silage DM content and milk yield averaged (± SE) 455 ± 15.5 g/kg and 10.5 ± 1.28 kg/day, respectively. At early and late harvest time, in vitro organic matter digestibility averaged 76.7 ± 0.23 and 62.6 ± 0.41 %, respectively, and NDF concentration averaged 464 ± 11.9 and 595 ± 1.92 g/kg DM, respectively. Early compared to late harvest time increased DMI ($P < 0.01$) and an interaction between harvest time and shredding ($P = 0.06$) gave higher intake for shredded grass-clover at late harvest time compared to non-shredded (11.7, 10.5, 8.0, and 9.1 kg DMI/day for CE, SE, CL, and SL, respectively). For CE, SE, CL, and SL, rumination time, with mean 47, 51, 71, and 63 min/kg DMI, respectively, and total chewing time, with mean 92, 107, 131, and 111 min/kg DMI, respectively, were increased for late harvest time ($P < 0.01$ and $P = 0.01$, respectively) and the interaction ($P = 0.02$ and $P = 0.03$, respectively) showed reduced chewing time with shredded grass-clover at late harvest compared to non-shredded. Eating time was not affected by harvest time or shredding.

Physical treatment by means of shredding of grass-clover at late harvest time prior to ensiling increased DMI and reduced both rumination and total chewing time.

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O 42

Methane intensity and residual feed intake of lactating dairy cows

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World-wide, the search is on for dairy cows that have low enteric methane production (MP, g/d), high milk yield and feed efficiency without excessive mobilisation of body reserves. Ideally, cows will have a low methane intensity (MI, g methane/kg energy corrected milk) and low residual feed intake (RFI, kg/d). Energy corrected milk (ECM, kg/d) is the daily milk production expressed as an amount of milk with the same energy content but containing 3% protein and 4% milk fat. Residual feed intake (RFI, kg/d) is a measure of feed efficiency and is calculated as the actual dry matter intake (DMI) minus the estimated DMI required to account for maintenance, body weight change and milk production. Methane intensity (MI, g/kg) is then expressed as MP/ECM. To investigate the relationship between MI and RFI, we measured the individual feed intakes, milk production, body weight changes and methane production of 460 Holstein dairy cows.

Each year during late spring (October to December, 2013 to 2017), cows of 3 to 6 years of age, 40 to 100 days in milk and producing 25.5 ±4.1 kg/d (mean ± standard deviation) of ECM were measured for MI and RFI. The cows were individually offered feed cubes of 70% lucerne hay and 30% crushed barley ad libitum. The cubes contained 190 g/kg DM of crude protein and 10.8 MJ metabolizable energy/kg DM. Residual feed intake was measured over 28 days. Methane emission was estimated using the modified SF6 technique over the last 5 days of RFI measurement. Milk yield was recorded at each milking, and milk composition was analysed three AM and three PM milkings each week. Body weight was measured every day. Dry matter intake was 23.5 ±2.73 kg/d, MP 468 ±80.8 g/d, ECM yield 25.8 ±4.10 kg/d, RFI -0.0082 ±1.243 kg/d, methane yield 20.0 ± 3.55 g/kg DMI and MI 18.4 ± 3.63 g/kg ECM. There was a slight positive correlation (r = 0.118) between RFI and MI (P= 0.011).

MI = 18.4 ±0.168 + 0.34 ±0.135 x RFI $R^2 = 0.012$

In conclusion, RFI and MI are not antagonistic traits. Meaning it should be possible to select dairy cows for feed efficiency combined with low methane intensity. Further research should focus on identifying the appropriate trait(s) to achieve this objective from a genetic selection perspective.

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O 43

Feeding behaviour and methane emissions while eating from a feed bin in growing cattle fed lucerne silage in respiration chambers

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Recently, methods have been developed to estimate methane emissions from cattle while they are visiting feed bins. However, little is known about the effect of feeding behaviour on these estimates of methane emissions. The objective of the current study was, using respiration chambers data, to determine the effect of feeding behaviour on methane emissions during feeding, and on 24-h methane emissions. Data from 8 growing cattle (Hereford × Holstein-Friesian) were used for the current analysis. The animals were individually housed in 4 respiration chambers for 3 (first group of 4) or 2 days (second group of 4) and fed *ad libitum* lucerne silage delivered in Insentec feed-bins on loadcells. The 4 chambers are linked to 1 analyser, which measures CH_4 in each chamber approximately 3 min; each 3 min measure was expressed as g/d and average per 24 h or per time during feeding. Animals had on average (± standard deviation) 13±2.7 meals/day, lasting 35±9.7 minutes/meal and consuming 799±115 g DM/meal at a rate of 30.4±7.3 g DM/minute. Methane emissions averaged 276±20 g/d during feeding (on average 7.3±1.5 h/day) and was statistically similar to the 24 h CH_4 emissions of 261±24 g/d (P = 0.24). There were strong correlations (r = 0.94-0.96) between CH_4 emissions (g/d) or yield (g/kg dry matter intake; DMI) during feeding at the feed bin and measured over 24 h. The difference between CH_4 emission during feeding and CH_4 emission over 24 h averaged 13.6±8.6 g/d and this difference correlated moderately positive with average meal duration (r = 0.72) and moderately negative with number of meals/day (r = 0.61), average DMI rate (r = 0.51) and total daily DMI (r = 0.52). Similar correlations were observed with the difference between CH_4 yield (g/kg DMI) during feeding and determined over 24 h. In summary, in the current analysis CH_4 emissions measured during visits to the feed bin were similar to 24 h emissions, however, there were some correlations with feeding behaviour indicating that the difference between emission rate at the time of feeding and 24 h increases with increasing meal duration and when animals eat their feed in less meals per day.

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Quantitative joint evaluation of sheep methane emissions and nitrogen excretion based on dietary variables and animal characteristics

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Specific nutrition and management practices at farm level may determine the associated greenhouse gas emissions emitted from sheep into the environment. Interventions to reduce enteric CH_4 production may not reduce N excretion, of which the latter contributes to nitrous oxide production. The objective was a) to explore the relationship of dietary variables and animal characteristics with both enteric CH_4 emission and N excretion from sheep fed forage diets, and b) to jointly predict enteric CH_4 emission and N excretion from sheep based on feed intake, dietary nutrient contents and animal characteristics. A database containing 211 indirect respiration calorimetry records of seven New Zealand sheep studies was used.

All data records were assigned to four different groups based on CH_4 emission and N excretion being either lesser or greater than their means of the entire database. A principal component analysis, for which the first three principal components explained 77% of the variation, clearly separated the low and high N excretion records along the second principal component. Dietary crude protein (CP) and ether extract content had the highest loadings of this principal component, which were 0.60 and 0.59, respectively. Assigning the records based on the mean plus or minus the standard deviation of CH_4 production, indicated separation of the high and low CH_4 emission group along the fourth principal component, although this component explained only 10% of the variation. Dry matter intake (DMI) and dietary neutral detergent fiber (NDF) content had the highest absolute loadings of -0.59 and -0.57, respectively.

A bivariate multiple regression mixed-effects model for jointly predicting CH_4 emission and N excretion was developed. This model contained dry matter intake, dietary nutrient composition and animal characteristics covariates, and accounted for study effect. Study effect and residual error were modelled using bivariate normal distributions. Bivariate prediction of CH_4 emissions and N excreta (g/d) indicated DMI, dietary NDF and CP, and age to be the main covariates. However, the regression coefficient of dietary CP for CH_4 emission contained zero in its credible interval, indicating that dietary CP is a better predictor for N excretion than for CH_4 emission. The root mean square prediction errors, obtained using a leave-one-out cross-validation, were 22.5 and 25.6% of the observed mean for CH_4 emission and N excretion, respectively. A trade-off between CH_4 emission and N excretion was indicated based on the regression coefficients of age, with older sheep emitting more CH_4.

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Identification of heat shock protein gene expression in hair follicles as a novel indicator of heat stress in beef calves

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Two studies were carried out to investigate the effect of heat stress on heat shock protein (HSP) gene expression in the hair follicles of beef calves. In study 1, hair follicles were harvested from three Korean native male calves (172.2 ± 7.20 days old) six times from April 10 to August 9 (2017) under different external temperature-humidity index (THI) (THI = 62.6 to 83.0). In study 2, sixteen Korean native male calves (169.6 ± 4.60 days old, with a body weight of 136.9 ± 6.23 kg; four calves per experiment) were investigated in environmentally controlled chambers. Four THI treatments were assigned according to threshold (22 to 24 °C, 60% humidity; THI = 68.7 to 71.6), mild (26 to 28 °C, 60% humidity; THI = 74.4 to 77.2), moderate (29 to 31 °C, 80% humidity; THI = 81.4 to 84.6) and severe (32 to 34 °C, 80% humidity; THI = 86.2 to 89.4) stress levels. The experimental design was a 2 x 2 factorial (period x THI treatment). Calves were subjected to ambient temperature (22 °C) for 7 days (thermoneutral; TN), after which chamber temperature and humidity were raised to each THI level for 21 days (heat stress; HS). Hair follicles were collected from tails of individuals every three days (1400 h) during the TN and HS periods. In addition, heart rate (HR) and rectal temperature (RT) were measured every three days prior to hair follicle sampling (1400 h). In study 1, high variation ($P < 0.0001$) in THI indicated that the external environment influenced HS to different extents. HSP70 and HSP90 gene expressions were higher ($P < 0.05$) at high THI levels (THI > 82.3) than at the low THI levels (THI < 70.4). In study 2, during the TN period, there were no differences in THI ($P = 0.2638$), HR ($P = 0.2181$), and RT ($P = 0.3846$) among all groups. However, during the HS period, there were increases in THI ($P < 0.0001$), HR ($P < 0.0001$), and RT ($P < 0.0001$) among the groups. HSP70 and HSP90 gene expressions were higher ($P < 0.05$) in the severe HS group than in the mild-moderate group. It is concluded that HSP gene expression in hair follicles could be used as a novel indicator of heat stress in Korean native calves under ambient conditions and in climate-controlled chambers.

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Impacts of humidity during heat stress on physiological indicators, blood hematology, feed and water intake in dry Holstein cows

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The ability of Holstein cows to maintain high levels of milk yield and quality is dependent upon genetic, climatic, and environmental factors, particularly in Korea where high relative humidity is an influential factor during the hot and humid summer. For dairy cows, the dry period is a crucial time for strengthening resistance against diseases such as post-partum mastitis, body tissues for parturition, and the health of offspring. For this reason, we investigated the effect of various levels of humidity during heat stress on physiological indicators, blood hematology, and feed and water intake in dry Holstein cows. The experimental site was designed to have regulators in individual chambers to properly maintain the temperature and humidity. Each chamber maintained the temperature at two levels of 25 ºC and 31 ºC, where the relative humidity levels were 35% to 50% (low humidity, LH), 50% to 65% (low-medium humidity, LMH), 65% to 80% (medium-high humidity, MHH) and 80% to 95% (high humidity, HH). The design was a 2 x 4 factorial arrangement, and data were analyzed using the GLM and MIXED procedure of SAS. The results showed that there was no difference in terms of feed and water intake between temperature levels ($P>0.05$). Heart rate, rectal temperature, and surface temperature were higher in the HH group than in the other groups at both temperature levels ($P<0.05$). There was no significant difference in blood characteristics such as granulocytes, hemoglobin, plateletcrit, and platelet among all groups with various levels ($P>0.05$). The HH group showed higher red blood cells than the other groups at both temperature levels ($P<0.05$). The MHH group had higher contents of mean corpuscular hemoglobin concentration, mean platelet volume, and platelet distribution width than the other groups at both temperature levels ($P<0.05$). Conclusion is drawn suggesting the higher importance than the temperature as an influential factor to alter some physiological indicators and blood hematology without any adverse effects on feed and water intake in dry Holstein cows when comparing two levels of temperature at 25°C and 31°C.

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Dose-dependent effects of a garlic-citrus powder on methane production and fermentation parameters of rumen microbial metabolism

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Methane emissions from ruminants contribute to greenhouse gas emissions and result in a loss of digestible energy for the ruminant. Therefore, feeding strategies which reduce methane production are of high environmental and economic interest. Here we investigated dose-dependent effects of a natural compound consisting of a standardized blend of garlic powder and citrus extracts (Mootral™), which has already been proven to be effective in reducing methane production.

For investigating the effects of different doses of Mootral™ the Rumen Simulation Technique (RUSITEC) was applied. Mixed solid and liquid rumen contents from two rumen-fistulated cows were used as inoculum for 12 RUSITEC fermentation vessels. The daily feed supply consisted of 7 g hay and 3 g concentrate. After an equilibration period of 7 days, 3 days of control period (CP, day 8 to 10) were used for daily assessment of pH, redox potential, SCFA production and NH_3-N concentration. Percentages and daily production rates of methane and carbon dioxide were assessed every second day. The CP was followed by an 8 days experimental period (EP, day 11 to 18), where all parameters were assessed every second day. During the EP, four treatments were applied (each n = 3): control, 0.22 g Mootral, 0.88 g Mootral, 1.76 g Mootral, corresponding to 2.2 mg, 8.8 mg and 17.5 mg, respectively, of allicin. The compound was added daily in the feed bag. Data were analyzed statistically using a two-way ANOVA for repeated measurements.

During CP all fermentation parameters remained unchanged. In EP, the high dose of Mootral (1.76 g) led to a significant reduction in pH, elevated NH_3-N concentrations and an increase in total SCFA production. This increase was mainly based on higher production of butyrate, valerate and isovalerate. In fermentation vessels treated with 0.88 g Mootral, the increased production of butyrate and isovalerate compared to the control group was transient. The percentage of methane in the fermentation gas and the daily production of methane were strongly reduced in the 1.76 g treated vessels reaching significance on days 12 to 18. In the 0.88 g group, the percentage of methane was significantly reduced from day 12 to day 16, but increased again at day 18. The 0.22 g group did not differ from the control.

Based on these results the 1.76 g dose should be used for further evalution of long-term effects and impacts on the microbial community.

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Does water limitation following feeding during thermal-humidity exposure alter mineral excretions via various body matrices in ewes?

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This study aimed to determine mineral excretion via wool, serum, urine, and feces in Corriedale ewes under water limitation and during thermal-humidity exposure. Nine non-pregnant non-milking ewes (3 yrs old, ave. BW = 41±3.5 kg) were divided to three main groups of a control group given free access to water (FAW), and treatment groups with 2 and 3 hrs of water limitation (2hWL and 3hWL) following feeding. Feed was provided as a commercial TMR (70% concentrate and 30% forage) based on maintenance requirements (728.3±82.0 g/day dry matter basis, CP=16.1± 0.1 and TDN=69.1±0.5) and was weighed and offered twice daily at 09:00 hr and 18:00 hr. The ewes were kept in a controlled environment at an experimental house and individual metabolic crates (0.75 m × 1.45 m). House temperature was automatically controlled and a data logger was used to record the temperature and humidity at one-hour intervals. A triplicate 3×3 Latin Square design for 3 periods of 21 days (n = 9) was designed and the data obtained were analyzed using the GLM procedure of SAS, while the temperature-humidity index data were analyzed using the MIXED procedure of SAS. Blood was collected by jugular venipuncture in vacutainer tubes (no additives) for collecting serum at 13:00 h on day 21 of each period. Wool was shaved from the posterior vertex region of the neck of each individual. No differences were observed in wool mineral contents (re-grown wool of period 2 and period 3) including Na, Mg, P, Cl, K, Ca, Mn, Cu, Fe, and Zn among the treatment groups ($P > 0.05$). Serum and urine mineral contents were not different among the groups ($P > 0.05$). In feces, K showed lower content in the 2hWL group than in the other groups ($P < 0.05$); however, other mineral content did not show a difference among the groups ($P > 0.05$). The conclusions reached were that water limitation under thermal-humidity exposure could not affect the excretions of minerals in wool, urine, feces (except for K), and serum from ewes. This implied that no special care needed to be provided during the aforementioned stress conditions for ewes. However, to provide more insights in this study area, more research needs to be performed with longer periods of time of depriving water while sheep are searching for water during THE.

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Comparison of feed intake, feeding behavior and ruminal fermentation characteristics of grazing cows categorized as low and high methane emitters

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Reducing methane emission from dairy farming contributes to the achievement of the UN 2030 Agenda for Sustainable Development. As methane emission was shown to be a heritable trait in ruminants, genetic selection may be one possible mitigation strategy. However, the characteristics of a low emitting dairy cow are not yet fully identified. The aim of the present study was to compare feed intake, eating and rumination time and ruminal fermentation traits in groups of grazing dairy cows differing in methane emission. The study was carried out with 20 multiparous lactating Holstein cows that grazed full-time in a rotational pasture system. The categorization of the cows into LOW and HIGH emitters was based on estimates of methane emission from milk mid-infrared spectra (MIR) and measurements using the GreenFeed system. Determination of methane emission was done during 1 month before the first of 2 collection periods started. During the 7-d collection periods, feed intake was estimated using the n-alkane double-indicator technique and feeding behavior was recorded with RumiWatch halters. Ruminal fluid was sampled twice per collection period in the morning directly after milking using a stomach tube. Compared to HIGH cows, LOW cows produced on average 10% and 11% less methane per d or 18% and 19% g methane per kg energy corrected milk (ECM) determined with MIR or GreenFeed, respectively. On average, LOW cows were initially 1 % lighter than the HIGH cows, produced 4 % more ECM and were 10 d earlier in lactation. Across both collection periods LOW and HIGH cows did not differ in dry matter intake and ruminating time. The LOW cows spent less time eating than the HIGH cows ($P < 0.05$). Total ruminal volatile fatty acid concentration and proportion of butyrate were similar between LOW and HIGH cows. The proportion of acetate tended to be lower ($P < 0.10$) and those of propionate higher ($P < 0.10$) for LOW compared to HIGH cows. Ruminal ammonia concentrations did not differ between LOW and HIGH cows. In conclusion, the collected data did not reveal a clear differentiation of high and low methane emitting dairy cows in the intake and digestive variables. Although eating time of HIGH cows was greater, estimated DM intake did not differ between category which otherwise could have been an explanation for higher methane emission.

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Effects of a forage and by-product-based diet on production, blood metabolites and hormones of dairy cows over an entire lactation

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Ruminants produce meat and milk from fibrous feed and by-products not suitable for human consumption. However, high yielding dairy cows are generally fed a high proportion of cereal grain and pulses, which can be consumed directly by humans. If high production and good health of dairy cows can be maintained with ingredients of low human interest, then the sustainability of dairy production would improve. In the present study 37 multiparous (Holstein (n = 13) and Swedish Red (n = 24)) dairy cows were followed over an entire lactation. A low concentrate (LC) diet of 6 kg concentrate per day was fed to 27 cows, while 10 cows were fed a high concentrate (HC) diet of 12 kg concentrate per day. The groups were unbalanced due to a parallel genetic study on the LC cows. The concentrate (12.5 ME MJ/kg dry matter (DM)) was largely based on by-products of low human interest. The grass/clover silage (11.6 ME MJ/kg DM) was offered *ad libitum*. Individual feed intake, body condition score (BCS) and body weight (BW) were recorded automatically. The cows were milked in an automatic milking station. Milk samples were collected every other week and blood plasma was collected in lactation week 2, 4, 6 and once in lactation week 19-21. Plasma was analysed for glucose, insulin, non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB) and insulin-like growth factor 1 (IGF-1). Over the entire lactation cows on the LC diet had lower DM intake (25.2 vs. 27.1 kg DM/d; P=0.02) and higher forage intake than cows on the HC diet (21.0 vs. 19.2 kg DM/d; P=0.03). The LC cows tended to yield less energy corrected milk (34.2 vs. 37.5 kg/d; P=0.05). There were no effect of diet on milk composition. The energy balance was not influenced by dietary treatment. The result is supported by the fact that neither feed efficiency (as ECM/DMI), BW change, BCS change, plasma NEFA, glucose nor BHB were affected by diet. The observed higher plasma concentrations of IGF-1 (85.6 vs. 69.4 ng/ml; P=0.04) and insulin (0.16 vs. 0.09 µg/L; P=0.01) among cows fed the HC diet might reflect a different digestion pattern of the carbohydrates in the concentrate. This study shows that cows can adapt to a high forage diet virtually without human-grade ingredients without compromising feed efficiency or energy balance, thereby contributing to sustainable food production.

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3-nitrooxypropanol blocked postprandial enteric methane emissions from lactating dairy cows

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The antimethanogenic compound 3-nitrooxypropanol (3NOP) has been proven efficient to reduce methane (CH_4) emissions from ruminants but it has been tested mainly on high-producing dairy cows fed high-concentrate diets. The aim of this work was to test the effect of 3NOP on CH_4 emissions from medium-yielding cows fed a high-forage diet. Twenty-eight lactating dairy cows were recruited within 7-11 days after calving and distributed in 2 balanced groups in a randomized block design. The treated group received 3NOP (60 mg/kg DM basis) in a total mixed ration during 105 days and the control group received a placebo. The diet had 75% forage (52% corn silage, 17% hay, 6% straw) plus 25% concentrate and was distributed once a day after the morning milking. Individual daily kinetics of CH_4 emissions were quantified using the GreenFeed system (C-Lock Inc., Rapid City, SD, USA). Intake and milk production were recorded daily, and milk composition (fat, protein, lactose, urea) twice a week. Intake was reduced with 3NOP (-1.6 kg DM on average; $P = 0.01$) whereas milk production was similar between groups (34.7 kg/d of energy corrected milk on average; $P = 0.28$). Milk composition did not vary between groups. Feed conversion efficiency in animals tended to increase with 3NOP (1.37 vs. 1.46 kg milk/kg DM intake on average; $P = 0.07$). Methane emissions, CH_4 yield and CH_4 intensity were lower in the treated group throughout the 105 days (on average -31% g/d, -25% g/kg DM intake, and -30% g/kg energy corrected milk, $P < 0.0001$). When monitoring daily kinetics of CH_4 emissions, we observed that the major mitigation effect of the 3NOP occurred after feeding by preventing the post-prandial peak of CH_4 emissions ($P < 0.001$). In conclusion, the CH_4-reduction effect persisted for the duration of the study in early lactation dairy cows fed a 75% forage-based diet. The additive consistently prevented the CH_4 production peak after the main meal of the day ($P < 0.001$). In systems without a total mixed ration, 3NOP should still have a significant antimethanogenic effect if provided throughout the main daily feeding period.

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Impact of a garlic-citrus powder on methane emissions and performance on dairy cows in real farm conditions

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The reduction of enteric methane in ruminants is a key objective to mitigate greenhouse gas emission in agriculture. Under research conditions, the impact of different feed additives on enteric methane emissions have been already tested in-vitro or in-vivo but there is a lack on studies that directly measure the effect on methane emissions in real farm conditions. Therefore, the objective of this study was to evaluate the effects of the natural feed supplement, containing organosulphur compounds from Garlic (Allium sativum) and flavonoids, on methane emissions and performance in Dairy Cows under real farm conditions. In total, 275 Holstein Friesian (HF) and 121 Jersey (average days in milk: 150) were housed in a sand-bedded free stall barn. Cows were milked twice a day. The basal diet (control) was a total mixed ration (TMR) with grass silage as primary forage source. The treatment diet (treatment) had the feed supplement in form of a pellet (500g/head/day (3% garlic-citrus powder)) mixed in the TMR. The trial was conducted using the complete herd in a 23 weeks feeding trial, where the first 7 weeks and the last 4 weeks served as control (no garlic-citrus powder supplementation) (control). Milk yield, milk composition and dry matter intake (DMI) was monitored weekly. Estimations of methane production were obtained on 15 HF and 15 Jerseys from approximately 6:15 am after milking during 3 consecutive days in week 12 of treatment and 4 days in week 4 post treatment (control) using the hand-held laser methane detector. The milk yield increased by 7.8 % ($P < 0.05$) in the HF herd and by 5 % in the Jersey herd ($P < 0.05$) comparing the pre-treatment period with the treatment period. In the post-treatment period, the milk yield remained consistent. No significant differences were detected in milk composition except for a decrease in somatic cell count during the treatment period. The DMI decreased slightly during the treatment period in Jersey herd and increased in HF. A significant methane reduction of 20.7 % and 38.3 % respectively in HF and Jersey was detected in the treatment period ($P < 0.05$). Overall, these results demonstrate efficacy of the garlic-citrus powder in reducing enteric methane emissions and increasing performance in Dairy Cows in real farm conditions.

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Effects of high ambient temperatures in lactating dairy cows – a field study

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Heat stress in dairy cows is a topic of global importance for animal health and welfare as well as for economic success of dairy farms. Therefore, a field study (herd of about 75 lactating cows) was performed to gather quantitative data regarding temperature of feed [silages, partial mixed ration (PMR) and feed refusals] and feed intake (on herd basis), milk yield (on herd basis) and physiological parameters (respiration rate and rectal temperature, measured at about 17 p. m.; n = 10) of lactating dairy cows during summer in 2018. Average daily ambient temperature varied between 9.1 (09/25/2018) and 27.9 °C (08/07/2018), the temperature-humidity index (THI) in the barn was between 53.4 (09/29/2018) and 76.1 (07/31/2018). Temperature of the grass silage was always higher than temperature of the maize silage (average temperature: 37.2 ± 5.57 vs. 22.6 ± 3.68 °C; n = 68). Temperature of the fresh mixed ration reached values of 33 °C whereas maximum temperature of feed refusals was 53.3 °C. Daily feed intake (PMR, kg DM/cow) was negatively correlated with THI (in the barn, daily mean value; Spearman´s correlation coefficient: -0.77, $p < 0.0001$) and temperature of the mixed ration when offered (Spearman´s correlation coefficient: -0.70, $p < 0.0001$). Correlation coefficients were slightly higher when a one-day time lag was considered. During a period of particular high ambient temperatures (07/24-08/13/2018) mean daily DM intake (PMR) decreased by about 2.5 kg/cow compared to the previous period (07/16-07/23/2018, mean daily DM intake: 17.7 ± 0.693 kg/cow). Milk yield was reduced by about 2.3 kg to 25.8 ± 1.28 kg/cow/day in this hot period. Under the condition of this study a high proportion of cows had respiration rates above established reference range (“panting”) and rectal temperatures above 39 °C without showing signs of clinical disease, indicating hyperthermia and non-compensated heat stress during periods of high ambient temperatures. As many severe and long-lasting effects of heat stress on animal health and performance – especially in high yielding cows – are known from literature, these results should be taken seriously. These relationships and especially the high prevalence of hyperthermia in this study are of particular interest for veterinary practitioners (hyperthermia vs. fever). From an economic point of view the decrease in milk yield due to heat stress is probably the most important short time effect.

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Estimating methane production from dairy cows using information on milk, ruminal fluid, and blood components

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It is well known that methane (CH_4) is a greenhouse gas commonly arising from agricultural activities, and that cattle are one of the main sources of its emission. Because global warming is increasing rapidly, new technology to reduce CH_4 emissions from cattle is urgently needed. Recently, breeding has been suggested as one possibility for reducing CH_4 emissions, but on the farm, it is difficult to measure CH_4 emissions from such large animals accurately enough to analyze the effects of breeding. Therefore, the present study aims to investigate the possibility of estimating traits related to CH_4 emission using information on body fluids, i.e. milk, ruminal fluid, and blood.

A total of 115 Holstein lactating dairy cows were used, and CH_4 was measured for 5 to 7 days by the spot method using an automatic milking system. The fat, protein, and lactose contents of the milk samples and their fatty acid composition were analyzed using mid-infrared spectroscopy. The dry matter intake (DMI) being estimated using the Japanese feeding standard for dairy cows (2017 version). Equations for estimating CH_4 emission (L/day), CH_4/FCM (fat corrected milk) (L/kg), and CH_4/DMI (L/kg) were then created using multiple regression analysis with all the biological fluid parameters as dependent variables.

The average days in milking of the cows was 167 ± 82, average bodyweight (BW) 692 ± 76 kg, and average FCM yield 34.7 ± 7.1 kg. The average CH_4 emission was 523.6 ± 73.3 L/day, average CH_4/FCM 15.4 ± 2.6 L/kg, and average CH_4/DMI 22.6 ± 2.9 L/kg.

The prediction equations obtained were as follows:

$CH_4 = 161.7 + 0.2712 \times BW + 5.014 \times FCM$ (Adjusted $R^2 = 0.420$)

$CH_4 = -458.3 + 0.2385 \times BW + 6.825 \times FCM + 7.304 \times C_2\% + 134.3 \times C_{8:0} - 234.9 \times C_{20:4n6}$ (Adjusted $R^2 = 0.678$)

$CH_4/FCM = 21.93 - 0.7388 \times DMI + 0.01542 \times BW$ (Adjusted $R^2 = 0.466$)

$CH_4/FCM = 0.1999 - 0.6320 \times DMI + 0.01429 \times BW + 0.2749 \times C_2\% + 3.600 \times C_{8:0} - 9.024 \times C_{20:4n6}$ (Adjusted $R^2 = 0.725$)

$CH_4/DMI = 31.43 - 1.337 \times DMI + 0.01462 \times BW + 0.3516 \times FCM$ (Adjusted $R^2 = 0.218$)

$CH_4/DMI = 2.50 - 2.056 \times DMI + 0.02171 \times BW + 0.6982 \times FCM + 0.3664 \times C_2\% + 6.406 \times C_{8:0} - 11.76 \times C_{20:4n6}$ (Adjusted $R^2 = 0.582$)

C_2%; acetic acid content in ruminal fluid (%), $C_{8:0}$, $C_{20:4n6}$; milk fatty acids.

The information on the rumen fluid and milk fatty acid components was shown to be effective for improving the accuracy of the equations for estimating the traits related to methane production.

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Heat stress effects in primiparous and multiparous lactating cows under a warm environment

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In high yielding cows heat stress (HS) sharply decreases milk yield (MY), even beyond decreases in dry matter intake (DMI), due to changes in carbohydrates and protein metabolism, where glucose is preferentially used for functions other than milk synthesis. Multiparous cows are more susceptible to HS than primiparous, due to the much larger MY by multiparous (~10 kg more) and the lower weight of primiparous (~100 kg less). However, due to their long-term exposure to HS, cows in warm environments may react differently to HS and its alleviation.

To prove the hypothesis that in warm climates primiparous are more susceptible to HS than multiparous, a herd of 60 cows was exposed to four 15-days-long experimental periods, where two Control periods (no HS alleviation) were alternated with two cooling periods (fans and sprinklers in 1-hour cycles at 10:00, 12:00, 14:00 and 16:00 h). Six primiparous and six multiparous cows (3/4 Holstein, 1/4 Brahman; 520±35 kg BW; 16.6±1.2 kg/d MY) were monitored for rectal temperature (RT) and respiration rate (RR) at 09:00, 11:00, 13:00, 15:00 and 17:00 h, and for MY.

Contrary to high yielding cows, primiparous were more susceptible to HS than multiparous, with a higher RT (39.46 vs. 39.16ºC) and RR (76 vs. 66 breath/min), likely due to smaller differences in MY (Δ=1.5 kg/d) and body weight (Δ=35 kg) between primiparous and multiparous compared with those differences in high yielding animals. Cooling reduced RT and RR in all cows leading to higher MY in both primiparous (+9.7%) and multiparous (+6.5%). However, contrary to the expectations, HS alleviation did not improve feed efficiency (0.87 and 0.88 kg milk/kg DMI, with or without cooling, respectively), due to a proportionally-equal increased DMI. Reasons for this additional discrepancy with effects observed on high yielding cows may be related to the lower level of production and smaller size of cows in hot environments, as well as their better adaptation to HS compared with cows in temperate regions. But it could also indicate that HS was not alleviated to the point of improving nutrients metabolism, as evidenced by an overall low feed efficiency, and a similar RT and RR with or without cooling before the first cooling cycle of the day. The study shows that contrary to the expectations from high-yielding cows in temperate climates, primiparous cows are more susceptible to HS that multiparous, a phenomenon worth of further research.

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Can nordic hemisphere macroalgae reduce emission of methane from ruminant livestock?

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We aimed to investigate whether cultivable Northern hemisphere macroalgae possess less harmful anti-methanogenic compounds. The impacts of three brown macroalgae species (*Alaria esculenta* (AE), *Ascophyllum nodosum* (AN), and *Saccharina latissima* (SL)),a commercial seaweed mix (OceanFeed^{TM}Swine, OFS, Ocean Harvest Technology, Ireland) and two seaweed extracts (Extract-I and Extract-II) on methane formation and rumen feed degradability were studied *in vitro*. Buffered rumen fluid (90 ml) was added to 120 ml Schott bottles containing dried, ground (1 mm sieve) macroalgae material (0.500 g) and to bottles where macroalge (0.100 g) or macroalge extracts (in quantities equivalent to the content in 0.100 g of the original macroalgae material) were added to 0.500 g of basal feeds (maize silage or sugar beet pulp). Total accumulated gas production (TGP) was detected continuously by automatic wireless pressure sensors in the Ankom^{RF}(Ankom Technology, Macedon, NY, USA) system. Gas was collected in gasbags from two of triplicates to determine end-point methane concentration. Only small (AE=85.2 ml/g OM; SL=76.1 ml/g OM) or negligible (AN=11.9 ml/g OM; OFS=19.6 ml/g OM) amounts of gas were produced when macroalgae were incubated alone compared to maize silage (224 ml/g OM) and sugar beet pulp (245 ml/g OM) consistent with the low rumen degradability of macroalgae. Addition of AE, AN, SL and OFS to either basal feeds significantly reduced TGP ($p < 0.05$), with AN exhibiting maximum inhibition of TGP (18 - 21%) when added to either basal feeds. The AN also exhibited maximum inhibition of *in vitro* methane production (21%) when incorporated in MS. Moreover, methane and TGP also decreased with increasing doses of Extract-I added to 0.5 g of MS (0%, 4.8%, 9.1% and 16.7% of 0.5 g the basal diet MS), whereas Extract-II (0%, 1.96%, 3.85% and 7.41% of 0.5 g basal diet MS) had no impact on gas production or feed degradability. Dry matter degradability of either basal feeds were not affected by addition of macroalgae or derived extracts. The digestibility of MS increased with the addition of SL with no increase in methane production. In conclusion, Northern hemisphere macroalgae (AE, AN, OFS and SL) can suppress enteric methane formation without negative impacts and possibly with positive impacts on rumen feed degradability. *Ascophyllum nodosum* appears to be the most promising macroalgae of the tested species, products and extracts with respect to methane reduction. One of the two extracts tested partly mimicked mimic this anti-methanogenic effect.

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Genetic parameters for multiple definitions of residual methane production in Australian dairy cattle

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Modern breeding objectives in dairy cattle are focused on improving profitability, animal welfare and environmental impact. Although a lot of progress has been made in selecting for farmer profit and animal welfare, breeding objectives for environmental traits are in their infancy. For the dairy industry, the goal is to decrease methane production while maintaining a constant improvement in milk production and functional traits. This may be achieved by including a methane trait in the national index that is independent of feed intake, especially considering that in Australia, feed efficiency is already part of the overall breeding objective. Residual methane production is a promising candidate trait; however, the best way to calculate residual methane has not yet been determined. This research compared six methods of calculating residual methane production. Methane and DMI records were obtained from 331 cows measured over a 5-day period from 12 batches across 5 years using the SF_6 tracer method and an electronic feed recording system, respectively. The six methods of calculating residual methane included regression of methane production on DMI adjusted for DIM, parity and experimental batch using linear and quadratic models that were unfixed and fixed at an intercept of zero, as well as models based on genetic and phenotypic regressions. The R^2 of models ranged from 0.48 to 0.98 ($P < 0.001$) with DMI accounting for 22% - 67% of the explained variation. Heritabilities for RMP (0.20 - 0.24) were estimated using univariate models correcting for fixed effects and fitting a genomic relationship model. The phenotypic and genetic correlation between definitions of residual methane estimated using bivariate models were high (> 0.9). Our results indicate that these methods of estimating residual methane production are similar.

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O 45

Transition dairy cows less-resilient to metabolic stress have increased markers of subacute inflammation and oxidative stress during climatic heat stress

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Cows that are less resilient to metabolic stress exhibit increased adipose tissue (AT) lipolysis and oxidative stress postpartum (PP). We hypothesized that increased AT lipolysis will be related to elevated subacute inflammation, oxidative stress and higher release of endotoxins (such as lipopolysaccharide, LPS) from AT to blood PP, and that seasonal heat stress may exacerbate these effects.

Methods: The study included 24 multiparous dairy cows calving during winter (W, n = 12) or summer heat stress (S, n = 12) at the Volcani research farm (Israel). Cows were categorized retrospectively to those with low (LWL) or high weight loss (HWL) during the first month PP, indicating on metabolic resilience. Blood samples were obtained twice a week during the transition period for tumor necrotizing factor alpha (TNF-α, by ELISA), oxidative stress marker (malondialdehyde, MDA, by TBARS assay) and LPS-binding protein (LBP, by ELISA). Subcutaneous AT biopsies were collected at 7 d PP during S for immunoblots of LBP and TNF-α. Data were analyzed by PROC MIXED (SAS).

Results: Blood TNF-α was 6.7-folds higher in S vs. W ($P < 0.0009$), and was 1.8-folds higher in HWL than in LWL cows during S ($P < 0.05$), but not between HWL and LWL cows at W. Blood MDA was 5-folds higher in S than in W ($P < 0.0001$), and was 2-folds higher in HWL than in LWL ($P < 0.05$) during S, and tended to be higher in HWL vs. LWL at W ($P < 0.1$). Across seasons, blood LBP was 1.7-folds higher in HWL than in LWL at 7 d PP ($P < 0.05$). In AT of S cows, the abundances of LBP ($P < 0.05$) and TNF-α ($P < 0.001$) were higher in HWL than in LWL.

Discussion: Together, cows that were less-resilient to metabolic stress had a higher inflammatory response and increased signs of endo-toxemia in blood and AT specifically during heat stress. Seasonal heat stress has a dramatic effect on the degree of oxidative stress, subacute inflammation and immune function in transition cows.

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O 46

Serum biochemical parameters, neutrophil phagocytic activity,and monocyte subsets during the transition period in ewes

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The transition period (TP) is characterized by many physiological changes which contribute to immunodysregulation. This could affect myeloid cell functions such as neutrophil phagocytosis or the capabilities of monocytes which differentiate into functionally diverse tissue macrophages and dendritic cells. The aim of this study was to assess physiological changes occurring in the ewe´s metabolic profile and to establish basic values regarding the peripheral blood neutrophil phagocytic activity and monocyte subsets during TP.

Blood samples were collected from nine healthy pregnant German Blackhead ewes (2nd and 3rd parity) 1 month and 2 weeks ante partum (a.p.), 1 day post partum (p.p.), 2 weeks and 1 month (p.p.) to cover the TP. Serum total protein, albumin, globulin, glucose, beta-hydroxybutyrate (BHB), magnesium, calcium, and phosphate levels were determined using an autoanalyzer/spectrophotometer. Total leukocytes counts (TLC) were counted by using light microscopy. Whole blood neutrophil phagocytic activity (uptake of bacteria *in vitro*) and monocyte subsets were evaluated by flow cytometry. Monocyte subsets were determined based on their differential expression of CD14 and CD16 and they were divided into classical (cM, CD14+/CD16−), intermediate (intM, CD14+/CD16+) and non-classical monocytes (ncM, CD14−/CD16+). One way RM ANOVA (GraphPad Prism 8) was used for comparison between the different time points.

TP had significant effects on calcium, phosphate, glucose, total protein, albumin, and globulin serum concentrations in ewes ($p < 0.05$-0.001). One day p.p. we observed a significant increase in TLC and neutrophil counts ($p < 0.05$-0.001). Total numbers of monocytes and the three monocyte subsets (cM, intM and ncM) displayed the highest values one month p.p. ($p < 0.05$-0.001).

Flow cytometric analysis revealed that neutrophil phagocytic activity is lowest 1 month a.p. and 1 day p.p. ($p < 0.05$-0.001). Interestingly, a negative correlation was observed between neutrophil phagocytic activity and serum Ca ($r = - 0.56$) 1 months a.p., whereas a positive correlation was observed between serum BHB and neutrophil phagocytic activity 1 day p.p. ($r = 0.72$).

Taken together we were able to show that the composition and function of myeloid cells display significant changes during the transition period. Regarding the neutrophil phagocytic activity, our findings are in line with previous studies carried out in cows; with respect to the monocyte subsets, the ewe´s pattern seems to differ. Since this is the first study addressing the ovine monocyte subsets composition around TP, further research is required to cover their functional properties.

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O 47

Correlation of gene expression of FGF21 in the liver of cows with genes of stress signaling pathways and lipid metabolism

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Fibroblast growth factor (FGF21) has been identified as a stress hormone which plays an important role in the adaptive response to various stress factors. Recent studies in dairy cows have shown that the expression of FGF21 in the liver is dramatically increased during the transition period. The present study aimed to investigate potential metabolic stimuli of the induction of FGF21 in the liver of dairy cows during the transition period. 50 Holstein cows (19 primi- and 31 multiparous) were included in the study. Milk yield, feed intake and body weight were recorded. Blood samples and liver biopsies were taken at the time points -2, +1, +4, +7 wks relative to calving. Blood was analyzed for NEFA and BHBA using commercial kits. Relative mRNA concentrations of FGF21 and other genes were measured by qPCR. Pearson's correlation coefficients between various parameters were calculated. For the cluster analysis, the 10 highest or lowest animals in milk yield, NEB, NEFA, BHBA and FGF21 concentrations were selected. Differences in means were considered significant at $P < 0.05$. Relative mRNA concentrations of FGF21 in the liver were strongly increasing from -2 wk to +1 wk (13-fold in primiparous, 24-fold in multiparous cows). Cows with high or low milk yield in average from wk 1 to wk 14 (46 ± 3 vs. 28 ± 4 kg/day), high or low NEB (-86 ± 17 vs. -34 ± 10 MJ NEL/d in wk 1), high or low plasma BHBA concentrations (1519 ± 409 vs. 401 ± 70 µmol/L in wk 1), high or low NEFA concentrations (872 ± 209 vs. 185 ± 49 µmol/L in wk 1) did not differ in hepatic mRNA concentration of FGF21 on wk +1. On wk +1, there were significant positive correlations between hepatic mRNA concentration of FGF21 and mRNA concentrations of genes involved in endoplasmic reticulum (ER) stress genes (ATF4, HSPA5), and inflammation (ceruloplasmin, haptoglobin). Cows with high mRNA concentrations of FGF21 had higher mRNA concentrations of genes of ER stress (ATF4, DDIT3, HSPA5, spliced XBP1) and inflammation (haptoglobin) than cows with low hepatic mRNA concentrations of FGF21. Relative mRNA concentrations of lipid metabolism (β-oxidation, lipogenesis) did not correlate with mRNA concentration of FGF21. The data of this study suggest that FGF21 induction in the liver of dairy cows might be induced by ER stress and inflammation. The data moreover shows that milk yield, energy balance, and concentrations of NEFA and BHBA are unrelated to hepatic FGF21 expression.

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High-resolution Immunophenotyping in bovine blood and milk

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Bovine mastitis is one of the most prevalent and costly diseases in the dairy cattle industry and significantly impairs animal welfare. Furthermore, the use of antibiotics, which is currently the main strategy for therapy and prophylaxis of mastitis, could lead to increased bacterial resistance to antimicrobials. Therefore new biomarkers are needed to enable early and reliable detection of mastitis. In addition, it is desirable that the health management of other infectious or non-infectious cattle diseases will also change from curative to preventive.

The estimation of the total somatic cell count (SCC) of a raw milk sample is one of the most common procedures to monitor the udder health status. Since the percentages of the different somatic cell types vary depending on the immunological state of the udder, it makes sense to consider more than just the absolute cell count number. The determination of a differential cell count (DCC) in milk is a promising instrument for the identification of infected mammary glands, even in an early or subclinical stage. Moreover, the determination of the DCC has the potential to provide information on the systemic health status of the cow.

As a method for immunophenotyping flow cytometry offers clear advantages in terms of practicability and efficiency. We established a high-resolution DCC to detect 13 populations and subpopulations of the major immune cells parallel in blood and milk by using fluorochrome conjugated antibodies that bind to the cell-specific surface molecules (cluster of differentiation, CD). For epithelial cells we use a fluorochrome conjugated pan-cytokeratin antibody. This enables us to reliably detect 10 subpopulations in addition to the 3 main populations of immune cells (granulocytes, monocytes/ macrophages and lymphocytes). Furthermore, we determine the degree of cell death using a viability dye. Within our long-term immunophenotyping project we monitor initially eight cows throughout their entire lactation (ca. 305 days). In the early lactation phase (ca. 100 days) we take blood and milk samples twice a week, during the remaining lactation both samples are taken once a week. If one of the other cows at our experimental farm falls ill, it will be sampled more closely and for additional cows approved vaccines were used as an immune stimulus. The reference values, obtained with our long-term immunomonitoring project, can serve as an indicator of the health status of a single animal or can be used as an early warning system in dairy herd management.

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Effect of Phyto Ax'Cell plant based additive on the metabolic status of peri-partum dairy cows

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The time around calving is the key transition period for farmers to improve milk production, health and reproduction. In addition to good nutritional strategies, the use of plant bioactives offers a new approach to improve metabolic health. The objective was to evaluate the activity of a commercial blend of plants and plant extracts (Phyto Ax'Cell, Phytosynthese) on the inflammation and health of dairy-cows around peripartum. Phyto Ax'Cell is a standardized plant additive with anti-inflammatory and antioxidant properties (such as curcumins, turmerone or flavonoids and artepillin C from propolis). The trial was carried out on a commercial farm of 600 Holsteins in Europe from June to September 2018. 2x23 cows were placed in a control group (CON) and in an experimental group (EXP) according the calving date (=D0). The EXP group received 25 g/cow/day of Phyto Ax'Cell, top dressed on TMR, from approximately 15 days pre-calving to 7 days post-calving. Blood analyses were done weekly from D-7 to D+14 to evaluate energy metabolism and inflammatory status; rectal temperature was measured daily during 14 days from D0; 2 ultrasound examinations were realized at an average of D+14 and D+26; daily milk production was recorded until 60 days of lactation. Results were analyzed with XLStat, Student or Mann-Whitney tests. The EXP group showed a lower haptoglobin level at D+7 (0.55 vs 0.79; $p<0.05$) and D+14 (0.44 vs. 0.66; $p<0.05$); the anti-oxidant capacity (TAC) was numerically improved. Fewer cows from EXP showed hyperthermia during the first 2 weeks (-7%, $p<0.05$). Energy metabolism represented by NEFA (mmol/L)/cholesterol (mmol/L) ratio was significantly improved (0.21 vs. 0.36 at D0; $p<0.1$; and 0.19, 0.15 vs 0.36, 0.32 respectively at D+7, D+14; $p<0.05$), and glucose concentrations tended to be higher at calving (3.93 vs 3.43 mmol/L; $p<0.1$). Ultrasound examination indicated a trend for better uterine health through fewer cows in EXP showing the presence of uterine content (26% vs. 48%) and a better uterus involution in the pelvis (80% vs. 55% at D14 and 100% vs. 90% at D26). Milk production was numerically improved (1878 vs. 1785 kg). The addition of Phyto Ax'Cell at peri-partum helped to improve the energy and inflammatory status of dairy cows. These results might explain the better uterine health and milk performance trend.

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A postbiotic from *Aspergillus oryzae* improves productive and inflammatory responses to heat stress in lactating Holstein cows

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The objective of this study was to evaluate the effect of a postbiotic from *Aspergillus oryzae* (AO) on productive and inflammatory responses in lactating dairy cows exposed to heat stress. Forty-eight Holstein cows (105 ± 27 days in milk, 704 ± 23 kg body weight) were used in a completely randomized design for 36 d and randomly assigned to 1 of 4 treatments: 0 (control; CTL), 3 (low), 6 (medium), and 18 g/d (high) of AO (Biozyme Inc., St. Joseph, MO). A total mixed ration (18.1% CP, 33.0% NDF, 1.61 Mcal/kg NE_L) was individually fed twice daily with 41% forage and 59% concentrate and AO was top-dressed at each feeding. Cows were exposed to warm climate and provided with heat abatement in period 1 (d 1-10) and without it in period 2 from 0900 to 2200 h (d 11-36). Milk yield and composition, body temperature, and plasma acute phase protein concentrations were measured. On d 36, whole blood was collected from cows in CTL, low, and medium treatment groups for an ex-vivo challenge with 5 µg/µL lipopolysaccharide (LPS). The expression of genes encoding cytokines was analyzed as 1) no-LPS stimulation, 2) LPS stimulation, and 3) ratio of LPS to no-LPS stimulation. Mild heat stress was attained in period 1, whereas heat stress intensity was increased in period 2 [temperature-humidity index = 74.6 ± 2.4 and 77.3 ± 4.2, respectively]. In both periods, DMI was similar among treatments. In period 1, the AO postbiotic tended to quadratically increase (P = 0.06) milk and energy-corrected milk (ECM) compared with CTL (+3.2 and 3.6 kg/d, respectively). Additionally, AO quadratically decreased (P = 0.03) plasma concentrations of LPS binding protein by 25.7%. In period 2, AO quadratically increased (P < 0.01) ECM, protein, and fat yields compared with CTL (+3.8, 0.08, and 0.16 kg/d, respectively). Furthermore, AO linearly decreased (P = 0.02) SCC and morning vaginal temperature quadratically decreased (P = 0.01) plasma concentrations of serum amyloid A (< 65.6%) and tended to quadratically decrease (P ≤ 0.10) concentrations of haptoglobin and LPS binding protein (< 35.4 and 23.3%, respectively). In the ex vivo experiment, blood from AO-fed cows registered a linear decrease (P = 0.02) in the LPS to no-LPS stimulation ratio of IL-6 expression (< 65.6%). In summary, the AO postbiotic increased milk yield and decreased vaginal temperature and markers of inflammation in heat-stressed dairy cows.

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Dynamic of circulating leukocyte subsets in lactating dairy cows in response to an acute systemic endotoxin challenge

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The innate immunity is a highly specialized and complex part of the immune system in all metazoan. It serves as first line of defense, plays a fundamental role in preventing infections and enables adaptive immunity. Insufficient or excessive activation of the cellular innate immune response might be associated with an increased susceptibility to diseases. Therefore, this study focused on main cellular components involved in the innate immune response. We aimed to characterize the kinetic responses of circulating leukocyte subsets in lactating dairy cows subjected to an endotoxin-induced, acute systemic inflammation to elucidate crucial factors of individual responsiveness. For this purpose, three healthy, lactating Holstein cows received a single intravenous endotoxin bolus (0.5µg/kg body weight, *E.coli* O111:B4 lipopolysaccharide) and were subsequently examined for 96h. Blood was collected via venous catheter before LPS-exposure (0h) and in high frequent intervals post LPS-administration (0.5h, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 9, 12, 24, 48, 72, and 96h). White blood cell Count (WBC) was determined using automatic cell analyzer and leukocyte subsets were phenotyped by expression of surface receptors by flow cytometry. LPS-induced changes in leukocyte functions such as phagocytosis and signaling were evaluated by flow cytometry.

Total count of circulating WBC showed a biphasic change, with an initial decrease peaking at 2.5h (0.8 G/L) and a subsequent recovery to basal value (7.4 G/L) after 96h. Further, LPS-administration resulted in an activation of neutrophils as shown by a temporal increase of basal intracellular radical production within the first 4h after LPS-exposure. Percentages of phagocytic neutrophils decreased significantly within 2h (52.4%) after LPS-treatment recovering after 4h (79.6%) and remained on this level until the end of the trial, whereas the phagocytic capacity of these cells is unchanged in the beginning, increased by 16% after 24h and remained constant at that higher level during the next three days.

In conclusion, LPS-induced profound dynamic changes in all analyzed leukocyte subsets and induced an acute and transient response characterized by a biphasic time course. Initially, LPS-induced responses on cellular level were similar in all tested animals, which resulted mainly from the redistribution of these cells into the tissues. However, the following recovery phase exhibited strong differences. These individual differences on long-lasting response to LPS should be further characterized as variations in susceptibility to diseases could arise from immune response individuality.

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Cryptosporidium parvum infection alters glucose transport in infected enterocytes

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Introduction: The protozoan *Cryptosporidium parvum* causes enteropathies in humans and animals all over the world. In calves, it leads to severe diarrhoea that is often associated with bacterial or viral infections (Göhring et al. 2014) threatening animal welfare and causing economic losses (Lendner et al. 2011). Although a lot of work has been conducted on cryptosporidiosis in the last decades, the parasite-host interactions are still not completely unraveled. The parasite destructs parts of the intestinal brush-border membrane which is crucial for the uptake of nutrients, e.g. glucose and galactose, the main energy source for suckling ruminants. In this study, we investigated whether glucose transport mechanisms of the host cells are influenced by infection with *C. parvum.*

Material & Methods: IPEC-J2 cells (an intestinal porcine epithelial cell line) were infected with *C. parvum* oocysts and the success of infection was ensured by quantification of the hsp70 gene expression using qPCR and immunofluorescent staining of *C. parvum*. The gene expression levels of glucose transporter (GLUT) 1 and 2 and Na^+-coupled glucose transporter (SGLT) 1 were compared in infected and uninfected cells 24, 48, 72 and 96 h p. i. by RT-qPCR. Furthermore, the protein expression of SGLT 1 was quantified in Western blot studies.

Results: While the protein expression of SGLT 1 was not altered in infected cells, gene expression of SGLT 1 and GLUT 1 was significantly increased 24 h p. i. The gene expression of GLUT 2 was significantly decreased at nearly all time points measured and this decrease correlated significantly with the infection dose.

Conclusion: To our knowledge, this is the first *in vitro* study about the influence of the parasite *C. parvum* on glucose transport of host cells. IPEC-J2 cells proved to be a suitable model for investigating the effects of *C. parvum* infection in enterocytes. Our results point to an adaptation of the host cells taking place in the acute phase of the infection. Thus, an early onset of therapeutic oral administration of high glucose solutions is of imminent importance. The mechanism mediating the adaptation of glucose transport could be a promising therapeutic onset and should thus be further investigated.

Göhring, F; Möller-Holtkamp, P; Daugschies, A; Lendner, M. (2014): Untersuchungen zur Häufigkeit von *Cryptosporidium parvum* bei Durchfallkälbern und der Einfluss von Koinfektionen auf das Krankheitsgeschehen. In: Tierärztliche Umschau 69 (4), S. 112-120.
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P 137

Oral administration of lipopolysaccharide from Escherichia coli does not induce an effective systemic immune response in milk-fed Holstein calves

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Escherichia coli, a gram-negative bacteria, is one of the major pathogens causing diarrhoea in pre-weaned calves. Lipopolysaccharides (LPS) are cell wall components from gram-negative bacteria that are commonly used to mimic immune responses caused by these microorganisms. To our knowledge, studies based on the oral administration of LPS to calves do not exist in literature. Hence, the main objective of this study was to induce a systemic immune response in milk-fed calves through oral administration of LPS derived from *E. coli* (strain 0111:B4).

Twenty Holstein calves with a birth BW ranging from 35 to 50 kg and plasma brix ≥ 8.7 % at second day of life were used. Calves were housed in individual pens and fed with 4 L of cow milk (40 ± 2 ºC) twice a day. In addition, calves had free access to calf starter, hay and water. On day 35 ± 1, the LPS group (n = 10) received 4 L of milk containing LPS (12 µg/ kg of BW) in the morning feeding. The control group (n = 10) received 4 L of milk without LPS. On that day, blood samples were collected from the jugular vein right before morning feeding and then at 3, 6, 24, 48, 72 and 168 hours after feeding. Simultaneously, rectal temperature and heart rate were recorded. Blood plasma concentrations of C-reactive protein (CRP) and immunoglobulins (IgG, IgM and IgA) were analysed by ELISA, fibrinogen concentration by immune reaction and turbidimetry and, total protein, albumin and haptoglobin (Hp) by spectrophotometric method. The PROC MIXED procedure of SAS was used for statistical analysis and the model included treatments, hours, and their interaction and animal as a repeated effect. Values were significant when $P < 0.05$.

Body temperature (38.8 ± 0.04 °C) and heart rate (113.1 ± 5.3 beats/min) were not different between the LPS vs control calves ($P > 0.05$). Plasma concentrations of acute phase proteins (Hp: 0.26 ± 0.04 mg/mL, CRP: 3.0 ± 0.1 mg/L, fibrinogen: 3.6 ± 0.4 g/L) were not affected by the oral LPS challenge ($P > 0.05$). Similarly, plasma concentrations of total proteins (62.2 ± 1.0 g/L), albumin (38.1 ± 0.5 g/L), immunoglobulins (IgG: 12.5 ± 1.1 mg/mL, IgM: 422.9 ± 27.7 µg/mL, IgA: 37.9 ± 3.5 µg/mL) did not differ between LPS and control calves ($P > 0.05$). In conclusion, the oral administration of LPS (12 µg/ kg of BW) was not able to induce an effective systemic immune response in milk-fed calves.

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Changes in metabolic and immune parameters of young veal calves following different pre-transport diets, transport durations and transport conditions

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The experiment had a 2 × 2 × 2 factorial design with 3 factors: 1) provision of breeding milk or electrolytes prior to transport; 2) transport duration (6 or 18 h) and 3) transport conditions (open truck or conditioned truck). The aim of the current study was to investigate effects of these factors on metabolic and immunological variables of calves upon arrival at the veal farm. A total of 368 Holstein-Friesian and cross-bred calves (18 ± 4 days; 45.3 ± 3.3 kg body weight (BW)) were randomly assigned to the different treatments and transported in two consecutive batches from a collection center to a veal farm. Blood samples were collected from calves before transport, immediately post-transport (0 h) and 4 h, 24 h, 48 h, wk 1, wk 3 and wk 5 post-transport. Blood was analyzed for cortisol, bilirubin, haptoglobin, IgG and IgM. The blood profile, including hematocrit, hemoglobin, neutrophils, lymphocytes, monocytes, basophils and eosinophils was measured in blood samples taken before and after transport. Flow cytometry analysis (FACS) was conducted on samples before and after transport to characterize different lymphocyte populations, including CD8+, NK+, CD4+, δγ+, CD14+ and CD21+ cells. Cells were also stimulated with perforin in order to quantify effects of transport on cell activity. Body weight, rectal temperature (RT) and skin elasticity were determined before and after transport. Calves fed with either milk or electrolytes and transported for 18 h had an increase in plasma bilirubin concentrations upon arrival at the veal farm (Δ = 1.83 µmol/l and Δ = 1.50 µmol/l, respectively). The increase in bilirubin concentrations was also found in calves fed with electrolytes after 6 h transport (Δ = 3.64 µmol/l), whereas calves fed with milk and transported for 6 h showed a decrease in bilirubin concentration immediately post-transport (Δ = -3.75 µmol/l) (P = 0.03). Upon arrival at the veal farm, calves transported in the open truck had a lower number of white blood cells (WBC) (8.12 × 10^9/l) than calves in the conditioned truck (9.51 × 10^9/l) (P = 0.01). Results showed that feeding milk at the collection center and 6 h transport prevented a rise in bilirubin. The type of truck affected the total number of WBC and specific cell populations.

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Effects of *Spartina alterniflora* extract on serum biochemical indices and immune function of lactating dairy cows

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Introduction: Spartina alterniflora (S.A) is nutritious, especially the 14 essential trace elements, as well as bioactive materials that are beneficial to animal health. S. A in the diet of dairy cattle has no drastic effect on milk production and composition (Wang et al., 2008), and contains abundant bioactive materials, such as total flavonoids. Given these valuable findings, S. A extract should be evaluated as a potential ruminant feed resource.

Material and Methods: This experiment was conducted to determine the effects of different levels of S. A extract supplementation on blood parameters in lactating dairy cows. Sixty lactating Holstein dairy cows with similar body condition, parity, lactation stage and milk yield were blocked and randomly allocated into 6 groups with different supplementation levels of S. A extract [0, 5, 10, 25, 50, 100 g/d per cow]. The experiment was continued up 90d, including 10d of adaptation period and 80d of experiment period. Blood sample was collected at the end of experiment. Serum biochemical indices were analyzed using fully automatic chemistry analyzer and imunoglobulin concentrations were analyzed using ELISA kit. Data were analyzed by ANOVA using SPSS22.0. $P < 0.05$ was used as the significant threshold.

Results: The serum albumin (ALB) concentration, uric acid (UA) concentration and creatinine (CREA) concentration was numerically increased with the S.A extract levels increased, while adding 100 g showed a negative effect on these parameters ($P < 0.05$). Serum alkaline phosphatase (ALP) concentration in 50 g group was lower than other groups ($P < 0.05$). Adding S.A extract reduced the concentration of urea nitrogen (BUN, $P < 0.05$). There is no significant difference in other serum biochemical indices, such as aminotransferase (ALT) concentration, aspartate aminotransferase (AST) concentration and glucose (GLU) concentration. At the end of the experiment, up to 50g level of S.A extract significantly increased the blood IgA, IgG and IgM concentration compared with the control group ($P < 0.05$).

Conclusion: The spartina alterniflora extract addition at 50g increased ALB, UA, CREA and improved IgA, IgG and IgM concentration. This also indirectly reflects S. A extract can enhance the immunity of dairy cows.

Wang G, Qin P, Wan SW, Zhou WZ, Zai XM, Yan DL 2008. Ecological control and integral utilization of Spartina alterniflora. Ecological Engineering 32, 249–255.

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Effect of soluble carbohydrate diets on reticuloruminal pH, motility, hematological and biochemical health indicator in cattle.

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The study involved six adult, not lactating, rumen fistulated Jersey cows. The study design was a 3 × 3 Latin square that lasted 9 weeks. During the first 2 weeks of each experimental period cows were fed with a maintenance diet and in the last week of each experimental period the animals were allocated to three diets: a control diet (CON), continuation of the maintenance diet, and 2 different diets designed to induce a subacute acidosis challenge. The 2 challenge diets were isoenergetic and isonitrogenous, the former high in starch (HSt) the latter high in sugar (HSu). Reticular pH and motility were recorded throughout the study period, blood and ruminal samples were taken the 1st, the 2nd and the last day of each challenge week for a total number of 9 sampling times during the entire study. Cows' health was monitored daily using 4 parameters: diarrhea, inappetence, depression and ruminal tympany. The data comprised 56160 pH, 23883 motility, 1134 hematology and biochemical and 90 health-related observations. The effects of treatment, hour of day and sequence day after treatment on clinical parameters monitored, were analyzed using linear mixed effect (LME) models. The challenge diets resulted in a decline in pH, an increase in the absolute pH residuals and an increase in the number of minutes per day under pH 5.8 with an effect size of 147 and 144 min/d for HSu and HSt respectively. pH values achieved with challenge diets were consistent with conventional definitions of subacute rumen acidosis (SARA). Systemic inflammation, characterized by increased plasmatic concentration of Serum amyloid A (SAA) neutrophils and decrease in plasmatic concentration of lymphocytes, was only detected with HSt diet despite a similar extent of variance registered in pH values with HSu diet. Amplitude of motility recordings was significantly increased by starch but not by sugar. The period between movements was decreased by both HSt and HSu diets. The sugar treatment strongly reduced the probability of an animal consuming its complete allocation, while starch treatment tended to reduce the proportion consumed. Both challenge diets were associated with an increased probability of having diarrhea. In conclusion this study indicated that similar values in ruminal pH can reflect differently on clinical and subclinical health parameters.

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P 141

Peripartal changes in immunoglobulin concentrations in comparison between cattle and goats

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During the peripartal period high yielding cows are highly susceptible for different diseases such as ketosis, different reproductive disorders, disturbances of mineral homeostasis and mastitis and metritis as dominating infectious diseases. A link between energy balance and immune functions in early lactation has been suggested which might result in impaired immune reactions of both, cellular and humoral immunity in response to the negative energy balance. In a recent study, a substantial decrease in plasma immunoglobulin G1 (IgG1) concentrations could be demonstrated around parturition which reached prepartal concentrations approximately 6 to 8 weeks after parturition. The IgG2 concentrations were not affected while IgM exhibited a sharp decrease 3 days before parturition with a rapid return to prepartal values after parturition. Therefore, it was the aim of the present study to measure peripartal immunoglobulin concentrations in low producing cattle (White Park cattle) and to compare these data with respective values as obtained from 2 different breeds of goats (White German goat and Thuringian goat). Blood samples were taken two to seven weeks before parturition, at parturition and up to six weeks after parturition. Enzyme linked immunosorbent assays (ELISAs) using isotype-specific coating and detection antibodies were used to determine plasma concentrations of IgG1, IgG2 and IgM.

In White Park cattle immunoglobulin concentrations were basically in a similar range as in German Holstein cattle. However, in contrast to German Holstein cattle IgG1, IgG2 and IgM concentrations in White Park cattle did not exhibit any changes around parturition.

In goats, mean IgG concentrations were lowest at parturition and one day after parturition and tended to increase until the sixth week after parturition irrespective of both breeds. However, these differences were not significantly different.

From these studies it may be concluded that the genetic background and/or the level of productivity may affect the peripartal immunoglobulin profile in cattle. White German goats are characterized by higher productivity for milk yield in comparison with the Thuringian goat. This, however, did not result in any differences in peripartal immunoglobulin profiles. Thus, species differences have also to be discussed between cattle and goats.

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P 142

The effects of in-feed resin acid composition on the colostrum composition and immunity of dairy cows

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Dairy cows prevalently undergo a subacute systemic inflammation during the transition period. A slow resolution of postpartum inflammation may predispose dairy cows to metabolic disorders and divert the nutrients that could be used for production and normal physiological functions. In-feed coniferous resin acids in sow diets have been reported to increase immunoglobulin G (IgG) concentration in colostrum and to improve piglet performance. The objective of this study was to investigate the effects of in-feed resin acids on colostrum composition of dairy cows during early and mid-lactation.

Thirty-six Nordic Red cows were assigned to a basal diet based on grass silage and concentrate (CON) and two treatment diets supplemented with the same level of resin acids, from 3 wk prior to the predicted parturition to 10 wk postpartum. The two treatment diets were the basal diet supplemented either with tall oil fatty acids (TOFA; Progres®, Hankkija Ltd, Finland; 9 % resin acids) at 7 g/cow/day or with resin acid concentrate (RAC; Forchem Ltd; 37.5% resin acids) at 1.68 g/cow/day. The first colostrum was measured with Brix refractometer and representative samples were collected for the analysis of fat, protein, lactose, urea, somatic cell count, and IgG.

The RAC group had a higher colostrum urea concentration (8.6 mg/ml) compared with the CON (6.2 mg/ml; $P < 0.05$) and TOFA group (6.3 mg/ml; $P < 0.05$), which may reflect the modification of rumen microbiota or ruminal nitrogen metabolism by the resin acids supplement. Colostrum lactose content of the RAC group (3.0 g/kg) was lower compared with the TOFA group (3.4 g/kg; $P < 0.05$) and tended to be lower compared with the CON group (3.3 g/kg; $P < 0.10$). The decreased colostrum lactose content may reflect the decrease of blood glucose level in the RAC group, which may have possibly resulted from changes in ruminal fermentation. In contrast, the diet had no effect on colostrum fat, protein, and dry matter contents, the weight of first milking colostrum, and somatic cell count in colostrum. Moreover, colostrum IgG concentration (56.1 mg/ml for CON, 54.2 mg/ml for TOFA, and 57.6 mg/ml for RAC) and Brix value were not affected by the resin acids supplement.

In conclusion, the changes in colostrum composition reflect the potential influence of resin acids supplement on rumen microbial fermentation. Further studies on rumen microbiota will provide clearer insight into the effect of resin acids.

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P 143

The effects of administration of aqueous extracts of *Rhizopus oryzae* on the postpartum hepatic injury and dysfunction in dairy cows

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Objective: In dairy cows, most production diseases occur during early lactation, immediately after calving. Therefore, the prevention of these diseases to improve health of dairy cows by metabolic control is a most important challenge. In our previous study, just after calving, some hepatic gene expression levels were temporarily downregulated, and liver injury markers gradually increased from −1 to 1 weeks relative to parturition (Haga et al., 2018). On the other hand, it has been reported that aqueous extracts from Rhizopus oryzae (RU) had protective effect on liver injury induced by carbon tetrachloride in rats (Suzuki et al., 2015). Therefore, the aim of this study was to evaluate the preventive effects of oral administration (OA) of aqueous extracts of RU on the puerperal hepatic injury and suppression of genes expression in dairy cows.

Methodology: Six multiparous Holstein cows were used (from –3 to 3 weeks postpartum). Treatments (each n = 3) were as follows: forced OA (10 g/day) of (ⅰ) RU (RU group) and (ⅱ) placebo (P group) from –3 weeks to 0 day postpartum. Blood samples were taken from the cows by jugular venipuncture at −3, −1, 0, 0.5, 1, 2 and 3 weeks relative to parturition. Liver tissue was obtained by biopsy at −3, 0.5, 1 and 3 weeks relative to parturition, and at 0 week, which was conducted within 14 h from parturition. Liver injury was assessed by the activities of serum aspartate transaminase (AST) and alanine aminotransferase (ALT). The hepatic mRNA expression levels of albumin, insulin-like growth factor-1 (IGF-1), catalase, vitamin E-related proteins, haptoglobin (Hp) and endoplasmic reticulum stress-induced unfolded protein response marker (XBP1s) were measured by RT-qPCR. All statistical analyses were conducted with the MIXED model procedure (SAS).

Results: After calving, in RU group, serum AST activity was significantly lower ($P < 0.05$) and ALT activity also tended to be lower ($P = 0.09$) than those in P group, respectively. The hepatic mRNA expression levels of albumin, IGF-1, catalase and vitamin E-related proteins were temporarily downregulated after calving, and the hepatic mRNA expression levels of Hp and XBP1s increased at calving. However, we could not confirm the significant difference between these data in two groups. These results indicate that the OA of RU (10 g/day for 3weeks prepartum) may prevent the liver injury after calving, while, could not show clearly the preventive effects on the puerperal acute hepatic dysfunction (suppression of genes expression) in dairy cows.

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P 144

Evaluation of d-ROMs test for oxidative stress among periparturient cows

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Extent and nature of oxidative stress in periparturient cows has been investigated measuring both antioxidants and peroxidation products such as thiobarbituric acid test ("TBARS"). The use of TBARS is criticized for its low specificity as it may react with several compounds other than malondialdehyde (MDA), a peroxidation product of polyunsaturated fatty acids (del Rio et al, 2005). The measurement of F2 - isoprostanes is considered to be superior to MDA (Roberts et al, 2002), but until recently, this was not measured among dairy cattle. This in contrast to the d-ROMs test, that stands for derivatives of Reactive Oxygen Metabolites. This test measures primarily hydroperoxides (Vincent et al, 2007). Plasma TBARS was found to increase shortly before calving and increased further after calving, but plasma d-ROM remained relatively steady except a dramatic drop just before calving (Bernabucci et al, 2005). Serum d-ROM did not correlate with serum MDA in periparturient heifers (Dobbelaar et al, 2010). This suggest that TBARS and d-ROMs reflects other peroxidative pathways. Indeed, although d-ROMs tests was measured in many human and animal studies, it is also been criticised by Harma et al (2006) stating that the chromogen used in this method is also a substrate for the ceruloplasmin (ferroxidase) enzyme. Therefor we calculated the correlation between d-ROMs and ceruloplasmin in serum of dairy cows.

We collected data from 250 cows on five commercial dairy herds in the Netherlands. These herds participated in a project to evaluate the effect of two levels of vitamin E supplementation on the mastitis incidence (Bouwstra, 2010). MDA and d-ROMs and several antioxidants were measured in serum. Cows were sampled on average 54, 26 and 12 days before calving, with 24 h after calving and on average 19 days after calving. Serum d-ROMs was measured colorimetrically with the chromogenic substrate N,N,-diethyl-p-phenylenediamine on a LX-20 auto-analyzer, using a kit from Diacron (Grosseto, Italy). Ceruloplasmin in serum (3,3',5,5'-tetramethylbenzidine oxidase activity) was measured on an auto-analyzer (LX-20 of Beckman-Coulter, Woerden, The Netherlands). Cows with mastitis were excluded form analysis. Log serum MDA was weakly and negatively correlated with squared d-ROMs ($r = -0.084$, $P = 0.004$). Squared d-ROMs was positively correlated with serum CP concentration ($r = 0.419$, $P < 0.001$). We concluded that d-ROMs is probably a proxy for ceruloplasmin.

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Effects of raspberry feeding on peripheral blood immune cells populations in calves

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Introduction: Weanling calves are immature in immunity for many reasons, which can result in various diseases. Like as inter alia diarrhea. Vitamin E deficiency is known as one of the factors that lowers the calf immunity, and calves tend to be deficient in vitamin E because vitamin E is difficult to pass through the placenta. In recent years, several studies have been reported to feed calves with vitamin E. Therefore, we focused on the vitamin E-rich raspberry and the aim of this study was to investigate the fluctuation of peripheral immune cells by the raspberry feeding to calves.

Materials and Methods: The experimental design was approved by the Animal Care and Use Committee of Akita Prefectural University. Three Japanese Shorthorn cattle (4 month old, 131.3 ± 9.22 kg BW) and three Japanese Black cattle (4 month old, 116.3 ± 3.84 kg BW) were bred with two pens each. The animals were fed hay and formula supplement twice a day at 9:00 and 16:00 each day, with free access to water and mineral salts ad libitum. Raspberries ('Heritage') were lyophilized and then ground and mixed with formula feed for feeding to calves. The amount of raspberry fed was 2g per kg of body weight each day, and the feeding period of the raspberry was set to 2 weeks. Blood samples were collected three times at 2-week intervals from the start of the feeding experiment. The leukocyte subsets (granulocytes, T cells, B cells and monocytes) in whole blood and T cell subsets ($CD4^+$, $CD8^+$ and γδ T cells) in peripheral blood mononuclear cells were analyzed using flow cytometry. The results were considered significantly different when $P<0.05$, and a “tendency” was defined when $P<0.10$. The paired *t*-test was used to identify significant differences from the values before raspberry feeding.

Results and conclusion: There was no significant change in the leukocyte subsets and $CD4^+$ and $CD8^+$ T cells populations by feeding with raspberry. On the other hand, feeding of the raspberry increased the percentage of γδ T cells, and two weeks after raspberry feeding ended, it tended to increase compared to the value before raspberry feeding. These results were similar for both cattle breeds.The results of this study suggested that feeding a raspberry may increase γδT cells. So, the next task is to identify the active ingredients contained in raspberries and to examine their effects on calf diseases.

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Tolerance and rapid recovery following exposure to lipopolysaccharide in isolated ruminal epithelial cells

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Ruminal acidosis may increase potential for interaction of microbial-associated molecular patterns (MAMP) such as lipopolysaccharide (LPS) with ruminal epithelial cells (REC) thereby inducing a local pro-inflammatory response. MAMP concentration, duration, and repeated exposure to MAMP may influence the pro-inflammatory response. However, the nature of the pro-inflammatory response initiated by REC is poorly understood. The objective of this study was to investigate the local pro-inflammatory response in REC when differentially exposed to LPS, in vitro. Primary REC were isolated from ruminal papillae from 4-mo old bull calves (n=5) and yearling beef heifers (n=4) and grown in culture until confluence. Cells were then exposed to either low (100 ng/mL) or high (5000 ng/mL) LPS concentrations with: 1) no LPS exposure (control); 2) 12 h of LPS exposure; 3) 24 h of LPS exposure; 4) 36 h of LPS exposure; 5) 12 h of LPS exposure followed by 36 h of no exposure (recovery); 6) 12 h of LPS exposure followed by 24 h without LPS followed by an additional 12 h of exposure (repeated exposure). Total RNA was extracted from REC and real-time qPCR was used to determine relative expression of TLR4, TNFα, IL-1β, CXCL2, and CXCL8 normalized to the geometric mean of three reference genes. Statistical analysis was conducted using LPS dose, exposure, and their interaction as fixed effects in a 2 × 6 factorial arrangement. The LPS × exposure interaction was not significant and was removed from the model. All genes were up-regulated compared to control; however, there were no differences in expression between cells that were exposed to 12, 24, or 36 h of LPS. Greater expression (fold change) was observed after 12 h of LPS compared to repeated exposure for TLR4 (2.0 vs 1.1; $P < 0.01$), TNFα (12.8 vs 8.0; $P = 0.02$), CXCL2 (15.0 vs 9.3; $P = 0.02$) and CXCL8 (54.0 vs 26.7; $P = 0.03$). Expression of these genes was similar to control for the recovery treatment. Expression of IL-1β increased 262-fold at 12 h compared to 17-fold ($P < 0.01$) following recovery. Expression of CXCL2 was greater in cells exposed to high LPS concentration compared to low (10.6 vs 9.0; $P = 0.04$). These data are interpreted to indicate that LPS dose and timing relative to exposure influence the nature of the pro-inflammatory response, and when LPS is removed, REC recovered rapidly. They also indicate that the REC may develop tolerance to repeated LPS exposure.

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The effect of bovine Lactoferrin and probiotic on blood parameters and health status in Ghezel lambs during the pre-weaning phase

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Due to increase mortality in ruminant neonate in initial days of life and positive effects of lactoferrin (LF) and probiotic in immune system; We hypothesis that bovine lactoferrin (bLF) and probiotic could promote immune system and health status in pre-waning phase of ruminant neonates. Thirty six Ghezel suckling male lambs (3.9±0.65 kg body weight (BW)) were selected for the experiment from the 3rd day of age and housed in individual pens. Lambs were assigned randomly to 6 Groups included: 1) control (without bLF and probiotic), 2) 1g/d probiotic, 3) 0.25 g/d bLF, 4) 0.25 g/d bLF and 1 g/d probiotic, 5) 0.5 g/d bLF, 6) 0.5 g/d bLF and 1 g/d probiotic. Bovine lactoferrin (Shangqiu Kangmeida Bio-Technology Co. Ltd) and probiotic (Primalac™) were given orally every day (09:00) in 56 days. Suckling lambs were fed fresh milk from ewes by nipple bottle three times per day (06:00, 14:00 and 22:00). Starter (Alfalfa Hay (17.54%), Soybean Meal (17.54%), Barley Grain (29.24%), Wheat bran (31.11%), Sodium Bicarbonate (1.00%), Mineral and vitamin premix (1.50%), Calcium carbonate (1.17%), Salt (0.90%)) and water were available ad libitum from day 14th. Blood samples was collected from jugular veins prior to feeding for blood's metabolites and Complete blood cell count (CBC) on days 3, 31 and 59. Feces were scored 3 days in week on a scale of 1 through 7(1 = separate hard lumps and 7 = liquid consistency without solid space diarrhea Rectal temperatures (≤37.50 and 39.50≤) were determined in lambs that appeared languid, listless to eat and had diarrhea. Days medicated were recorded as each days that a lamb received drug. Statistical analysis was performed as repeated measures data using the Mixed Proc model of SAS software 9.2. No significant differences was found among health status indices except medicated days ($P<0.05$). Moreover, no differences were observed in erythrocyte, MCV, MCH and MCHC. Conversely hemoglobin, white blood cells (WBC), segmented neutrophil and lymphocyte concentrations were significantly affected by the treatments ($P<0.05$). Additionally experimental treatments significantly changed plasma concentrations of Fe, non-esterified fatty acids (NEFA) and glucose ($P<0.05$). According to results of present experiment, it seems that bLF plus probiotic can have synergic effect on performance of Ghezel lamb breed in pre-weaning phase.

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Blood profiles in fattening bulls of different horn status under consideration of feed intake and growth

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Whereas multiple studies have reported short-term effects of disbudding including pain, changes in behavior and physiological parameters, possible long-term effects of disbudding are not yet exhaustively studied. The aim of this study was to investigate the effect of horn status on blood parameters under consideration of feed intake and growth in fattening bulls.

Therefore, two runs of crossbred (Limousin x dairy breed) bulls (n=41 and n=40) were raised and fattened under similar conditions (housing, feeding, interventions) in three rearing groups: horned (H+), disbudded (H-) and mixed (M; half H+, half H-).

During fattening, dry matter intake (DMI) was recorded daily by automatic weighing troughs. Bulls were weighed regularly in intervals of five weeks. Blood samples were taken at three time points (hereinafter: periods; before disbudding (P1), two months after disbudding (P2) and in the end of the fattening period (11 months after disbudding; P3) and were analyzed for concentrations of minerals, hematological and metabolic parameters.

For analysis, DMI was averaged per week. Average daily gain (ADG) and ratio of total DMI (kg)/ weight gain (kg) (FCR) were calculated for each interval between two consecutive weigh-ins.

We computed different linear mixed models with the fixed factors rearing group, period, their interaction (only for blood parameters), body weight at grouping (except for blood parameters), and the random factors animal and run.

Rearing group had no effect on DMI ($P=0.81$), ADG ($P=0.34$) or FCR ($P=0.064$). Blood parameter concentrations were mostly situated within physiological reference ranges. Period had an effect ($P<0.01$) on all blood parameters. No rearing group effect was present at P1. Compared to the other rearing groups, H- bulls had throughout higher phosphor ($P<0.05$), at P2 a higher white blood cell count than H+ bulls and at P3 higher β-hydroxybutyrate ($P<0.01$) and urea ($P<0.01$) concentrations. These results could indicate an inflammatory process in H- bulls at P2, potentially due to disbudding, and differences in energy or protein metabolism in adult cattle of different horn status. Compared to H+ and H- bulls, M bulls had a lower concentration of plasma total protein at P3 ($P<0.01$).

No differences between rearing groups were found in red blood cell counts, hemoglobin, albumin, creatinine, urea, glutamate-dehydrogenase, gamma-glutamyl-transferase, glucose, non-esterified fatty acid, magnesium, sodium, calcium and potassium concentrations.

These data suggest no consistent differences between fattening bulls of different horn status. Only within specific periods we observed minor differences, which may be further investigated.

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P 149

Effect of ergovaline exposure on serotonin receptor 5HT2A in bovine lateral saphenous vein

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Cattle can be exposed to ergot alkaloids through fungal contamination of feed and forage that can result in severe reductions in productivity. Previous work has demonstrated that exposure to ergot alkaloids like ergovaline cause vasoconstriction in peripheral and visceral vasculature primarily through serotonin receptor 5HT2A. Effects ergot alkaloids have on receptor signaling are not fully understood and ergopeptide interaction with vascular receptors that regulate smooth muscle contraction could alter serotonin receptors leading to observed decreases in vascular reactivity. Objectives of this study were to determine if exposure of bovine lateral saphenous veins to ergovaline, 5HT2A antagonist, inhibitors of phospholipase C and protein kinase C will alter the vasoactivity to serotonin and the concentration of 5HT2A receptors. Lateral saphenous veins were collected from Holstein steers (n=8) at a local abattoir. Blood vessels were cleaned of external adipose and connective tissues and sliced in 2-mm cross sections. Cross-sections were incubated for either 2 hr or 24 hr in oxygenated Krebs-Henseleit buffer (95%O_2/5%CO_2; pH=7.4; 37°C) containing 1x10^{-6} M of either a vehicle (Control), ketanserin (KET; $5HT_{2A}$ receptor antagonist), 1-[6-[[(17β)-3-Methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1*H*-pyrrole-2,5-dione (PLC-; phospholipase C inhibitor), 2-[1-(3-Dimethylaminopropyl)indol-3-yl]-3-(indol-3-yl) maleimide (PKC-; protein kinase C inhibitor), or ergovaline (ergopeptide alkaloid). Following the incubation, blood vessel cross-sections were either mounted in a multi-myograph or ground in liquid N, homogenized in a protein extraction buffer, and frozen for later 5HT2A receptor protein quantification with a 5HT2A ELISA. Cross-sections in the myograph were exposed to increasing concentrations of serotonin. Contractility data were normalized as percent contractile response induced by a reference addition of 1x10^{-4} M norepinephrine and both the contractile response and the protein expression data were analyzed as a completely randomized design using SAS for effects of incubation treatment and incubation time. Veins that were incubated for 2 hr and 24 hr with KET, PLC-, and PKC- all had similar response curves that did not differ from the Control vein responses ($P>0.05$). Veins exposed to ERV for 2 hr and 24 hr did not respond to increasing concentrations of serotonin and differed from Control veins and all compounds evaluated for both incubation intervals ($P<0.05$). There were no effects of incubation treatment or time on the quantity of 5HT2A receptor protein. These results indicate ergovaline-derived effects on 5HT2A receptor function occur by means other than receptor number or interrupting signaling pathways associated with smooth muscle contraction.

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Influence of body weight at slaughter and dietary energy concentration on carcass tissue composition of Fleckvieh bulls

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The objective of this study was to specify the effect of body weight at slaughter and dietary energy concentration on carcass composition of growing Fleckvieh bulls of modern type.

Methods: 74 Fleckvieh bulls (age: 44 d, body weight (BW) 81 kg) were fed on restricted milk replacer and a concentrates/hay-based total mixed ration (TMR) until weaning at an average BW of 123 kg and subsequently on a TMR based on maize silage and concentrates for ad libitum intake. At a BW of about 220 kg the bulls were divided in two feeding groups “energy norm” and “energy high” with 11.2 and 12.0 MJ ME/kg DM, respectively. Individual feed intake was recorded daily and BW was determined every four weeks. The bulls were slaughtered in five weight groups: 120, 200, 400, 600, and 780 with average final weights of 121 (n=8), 200 (n=10), 400 (n=18), 597 (n=16) and 781 (n=18) kg, respectively. After slaughtering, the dressed carcasses were chilled for 20 hours at 4 °C and dissected to muscle, fat, tendons and bone. Statistical analysis was performed using Proc GLM of SAS (Version 9.3). Results are shown in LSMEANS.

Results: There were only minor effects of dietary energy concentration on carcass tissue composition in feeding groups energy norm and energy high. During growth the percentage of bone in the chilled carcasses decreased from 23.1% in 120 kg bulls to 13.2% in 780 kg bulls ($p<0.05$). Comparing the lowest and highest weight groups with 120 and 780 kg, muscle percentage decreased from 67.5% to 63.5% ($p<0.05$) while percentage of fat tissue increased from 2.7% to 16.3% ($p<0.05$). However, percentage of tendons did not vary between weight groups 120 and 780 kg with 6.0% and 5.9%, respectively.

Conclusions: Carcass compositions of bulls in lower weight classes corresponded widely to literature data from past decades while contemporary high end weights of 750 kg and above showed considerably more whole body fat at the expense of muscle tissue. Variations in dietary energy concentrations within margins found under practical conditions do not alter body composition to a relevant extent.

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Effects of cow genotype on production, viability of calves and reproductive related traits

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Demand for high biological value dairy products is ever increasing. Using CSN3*BB (κ-casein) genotyped sires has led to an increased frequency of the BB genotype and B allele in cow's herds in order to provide milk with increased protein content, thus significantly influencing the cheese yields but also the both dams and calves metabolic state.

The aim of the current research was to assess the effects of cows' genotypes on production and reproductive indices, and also on subsequent calves' viability. The study was carried out on 148 Romanian Simmental cows and 70 calves separated from dams in first hour after birth. Three production traits were considered: milk production, fat and protein percentage in order to evaluate the effect of genotype on cows' performances. Two reproductive indices were considered: days open and the number of inseminations in order to assess the cows' metabolic rebalacing capacity. Suckling behaviour in first day of life was considered in order to assess the calves' viability according to dams' genotype. Data analysis was performed using the one-way ANOVA protocol, with the categorical factor being "the cows' genotype". No significant difference was recorded for milk production ($P>0.17$) according to cows' genotype (6011±93 vs. 5913±111 kg for AA and BB genotypes). The genotype of cows significantly influenced the fat (4.29±0.02 vs. 4.23±0.02%, $P\leq0.017$ for AA and BB genotypes) and protein percentage (3.31±0.01 vs. 3.43±0.02%, $P\leq0.039$ for AA and BB genotypes). The BB genotype did not influence the days open (131±9.6 vs. 137±4.2 days, $P>0.26$) or the number of inseminations (1.9±0.2 vs. 2.2±0.1, $P>0.19$) compared to AA genotyped cows. The genotype had no effect on calves' viability. The prevalence of viable calves was comparable according to AA and BB genotypes (82.3% vs. 81.8%, $P>0.06$). The assessment of calves' viability based on the difference between required intervals for consumption of the first and the second colostrum bout associated to the first day of life revealed close values (15.3±0.2 vs. 16.1±0.2 min, / boat, $P>0.063$). Knowledge of these factors could encourage the use of new traits in order to assess the efficiency and welfare in herds. Current results suggest that applying breeding programs to cows in order to improve their genetic structure and provide high biological products could be done without negative effects on rearing efficiency. Overall, results have shown that cows' genotype did not influence the calves' welfare.

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Effect of milk replacer including innovatively treated zinc oxide on calf performance

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Calf raising focuses on reduced health events/treatments and especially for male calves on maximized weight gain. This study investigated the effects of milk replacer supplemented with 100 ppm zinc, on calf weight gain and therapeutic treatments. Zinc was supplemented as zinc oxide (ZnO) or activated ZnO (aZnO). The aZnO is produced using a vibrating eccentric mill and has shown comparable higher efficiency against E. coli development in vitro.

Methods: Fourty male HF calves (20.6±3 days of age, 58.0±5.9 kg live weight) were allocated to two treatments groups (n = 20) with similar average live weight. Calves were fed 7 weeks a milk replacer (MR, 50% skim milk, concentration 140g/L, approx. 1 kg/head/day) supplemented with 135 ppm ZnO or aZnO using an automated system. Calves had access to fresh water, hay ad lib. and concentrate feed max. 1, 1.5 and 2 kg in weeks 1-5, week 6 and 7, respectively. Cow TMR was fed in week 8. Live weight was recorded weekly, MR intake was recorded individually. Concentrate, hay and TMR consumption was recorded at group level. Treated health events were recorded individually including diarrhea, colic, fever and pneumonia. Weight data are means ± SD. Analysis of variance was performed using the procedure for linear mixed models (PROC MIXED) of SAS.

Results: Two animals died in the ZnO and one in the aZnO group and were excluded from the data analysis. MR, hay and TMR intake was similar. The aZnO treatment had on average 12 kg higher concentrate intake, resulting in a higher energy intake overall. Final average group weight (kg, 108.6±8.5 vs. 113.5±10.5), average weight gain (kg, 50.1±5.9 vs. 55.6±8.4) and daily weight gain (g, 895±106 vs. 993±150) were significantly higher for the aZnO treatment compared to ZnO ($P < 0.05$). Treated health events were eight compared to five in the ZnO and aZnO treatment, respectively.

Conclusion: Comparing the effects of the supplementation of 135 ppm ZnO or aZnO in MR, aZnO showed beneficial effects on weight development and reduced health treatments. It is speculated that the activated ZnO might be more effective, due to used production technique (activation and enlarged surface - higher reactivity) which is proven in vitro and as well in piglets. Further studies will be done to examine the mode of action and optimize the use of this new modified ZnO as an effective source to support efficient calves rearing.

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Productive and physiological responses of lactating dairy cows supplemented with phytogenic feed ingredients

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This experiment compared milk production, milk composition, and physiological responses in lactating dairy cows supplemented or not with a mixture of condensed tannins, encapsulated cinnamaldehyde, curcumin, capsaicin, and piperine. Thirty-six lactating multiparous, pregnant ¾ Holstein x ¼ Gyr cows were maintained in a single dry-lot pen with *ad libitum* access to water and a total-mixed ration, and milked twice daily (d -7 to 84). On d 0, cows were ranked by days in milk (86 ± 3 d), milk yield (27,8 ±1.0 kg), body weight (BW; 584 ± 10 kg) and body condition score (BCS; 3.04 ± 0.06) and assigned to receive (SUPP; n = 18) or not (CON; n = 18) 30 g/cow daily (as-fed basis) of Actifor Pro (Delacon Biotechnik GmbH; Steyregg, Austria). From d 0 to 84, SUPP cows individually receive (as-fed basis) 15 g of Actifor Pro mixed with 85 g of finely-ground corn through self-locking head gates prior to each milking of the day. Each CON cows concurrently received 85 g (As-fed basis) of finely-ground corn through self-locking head gates. Throughout the experiment period (d -7 to 84), cows from both treatments were administered 500 mg of sometribove zinc at 14-d intervals. Individual milk production was recorded daily, whereas milk samples were collected weekly for analysis of milk composition. Cow BW, BCS and blood samples were also collected weekly. Cows receiving SUPP gained more BCS ($P = 0.05$) and had greater ($P = 0.04$) milk yield during the experiment compared with CON cows (0.22 vs. 0.07 of BCS, SEM = 0.05; 29.5 vs. 27.9 kg/d, SEM = 0.5). Milk composition did not differ ($P \geq 0.15$) between SUPP and CON cows; hence, SUPP cows also had greater ($P \leq 0.02$) production of fat-corrected and energy-corrected milk. No treatment differences were also detected ($P \geq 0.21$) for serum concentrations of glucose and serum urea N. Mean serum haptoglobin concentration during the experiment was greater ($P = 0.05$) in CON vs. SUPP cows. Cows receiving SUPP had less ($P \leq 0.04$) serum cortisol concentrations on d 21 and 42, and greater ($P \leq 0.05$) serum concentrations of insulin-like growth factor-1 on d 7, 35, and 63 compared with CON cows (treatment x day interactions; $P \leq 0.02$). Therefore, supplementing phytogenic feed ingredients improved nutritional status, milk production, and welfare of lactating ¾ Holstein x ¼ Gyr cows.

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Milk fatty acids as possible predictors of energy balance in dairy cows

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Postpartum energy balance (EB) is critical for lactation success, health, reproduction and lifespan of dairy cows. Different methods have been suggested for indirect EB estimation with milk fat to protein ratio (FPR) being the most widely used. The origin of milk fatty acids (MFA) from two sources – plasma lipids from feed, rumen or fat mobilization and de novo synthesis in mammary gland - makes them also good candidates for possible biomarkers. The aim of the study was to compare the following markers or their combinations to predict the EB of the dairy cow: body condition score (BCS) at calving, milk FPR, selected MFA ratios and days in milk (DIM). The experiment with 30 multiparous Estonian Holstein cows with different BCS (range 2.25 - 4.0) at calving was conducted at the Eerika Experimental Farm of the Estonian University of Life Sciences within 150 days postpartum. Cows were fed a total mixed ration ad libitum and milked twice daily. They were weighed daily automatically on average from the 6th to the 153rd day after calving; the average number of daily weighings per cow was 120. Two trained persons registered BCS from calving to 12th week with an average number of observations of 7.3 per cow. Milk samples were collected from the first commercial milking postpartum twice weekly until confirmed pregnancy of the cow, followed by once a week sampling, and were analysed for fatty acid composition by gas-chromatography. The daily EB of cows was estimated based on body weight and BCS according to Thorup et al (2012). Variance partitioning analysis was used to study the relative importance of selected MFA ratios, BCS at calving, milk FPR and DIM to predict the EB. Results showed that BCS at calving, milk FPR, selected MFA ratios, and DIM together account for 63.1% of EB variance. Selected MFA ratios alone explained 62.9%, DIM alone 28.2%, milk FPR alone 26.0% and BCS at calving alone 4.8% of EB variance. The MFA ratios' effect almost totally covers the effects of BCS at calving, milk FPR and DIM and adds an extra 21.4% of EB variance. MFA ratios can therefore be used as a more reliable indicator of EB than current use of FPR.

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O 48

Effect of weaning strategy on calf milk replacer and starter feed intake and on growth of ad libitum fed calves

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The effect of weaning strategy on intake of calf milk replacer (CMR) and starter feed (SF) and growth of calves from birth to 16 weeks of age was determined. The trial was conducted at a farm in Eastern Germany with 80 calves in 4 groups. Calves were fed CMR and SF with automated feeders. Calves were fed CMR *ad libitum* in the first 5 weeks. Weaning started in week 6 and calves were linearly weaned from 12 L/d to 2 L/d in 5 weeks at 10 weeks of age (control=W10) or in 9 weeks at 14 weeks of age (delayed=W14). Starter feed intake was available from the start of the trial, but restricted to 2.5 kg/d. Hay was offered ad libitum and when the youngest calf was 8 weeks old a total mixed ration (TMR) was offered ad libitum. Individual intake of CMR and SF was measured daily, hay and TMR were not measured. Body weight was measured twice a week and average daily gain (ADG) was determined per week of each calf. Data were analysed with weaning strategy as fixed factor, week as repeated measurement, interaction of strategy x week, and calf within week as random factor. Differences were tested with a t-test.

Weaning calves at a later age (W14) resulted in a higher ($P<0.001$) body weight of 10.7 kg (W10= 137.4, W14= 148.1 kg) at 16 weeks of age and a higher ($P<0.01$) ADG of 90 g/d (W10= 860 g/d, W14= 950 g/d). There were no significant differences in the first 5 weeks, from week 8 onwards the body weight and ADG differed between weaning strategies. Total intake of CMR was higher ($P<0.01$) with weaning at W14 with 100.8 kg DM/calf compared to W10 with 69.3 kg DM/calf, whereas the total SF intake was lower at W14 with 79.1 compared to W10 with 98.5 kg DM/calf. Intake of SF was higher ($P<0.05$) on W10 from week 9 until week 13 of age than on W14. This means a slower increase in SF intake with delayed weaning and more time for rumen and intestinal development. During weaning, the decrease in calculated daily energy intake was lower with delayed weaning. Weaning ad libitum CMR-fed calves over a longer period reduces the drop in energy intake at the start of weaning and improves growth of calves during and after weaning.

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O 49

Oral vitamin A supplementation during late-pregnancy and birth stage enhances growth, pre-adipocyte and muscle development in Korean native calves

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This study investigated the effect of oral supplementation of vitamin A in the diet on growth, pre-adipocyte and muscle development of Korean native calves, from late pregnancy of the heifers to postnatal birth stage. Twenty-seven heifers (initial average BW = 320 kg [SD 31.2]) were selected for the current study. Heifers were fed high levels of vitamin A (100,000 IU/day) during the late pregnancy stage. Newborn calves (initial average BW = 31.6 kg [SD 3.98]) were then randomly divided into two groups with similar heads of bulls and heifers (control: 9 bulls and 4 heifers; treatment: 10 bulls and 4 heifers). All calves were treated with or without vitamin A supplementation (20,000 IU vs. 45,000 IU vitamin A/day, retinyl acetate) for two months until weaning. BW, feed intake and blood sampling were conducted during the experimental period. Meanwhile, longissimus dorsi muscle samples were obtained by biopsy from two-months aged bulls. Vitamin A supplementation resulted in both higher BW and higher average daily gain (ADG) at day 45 ($P < 0.05$ and $P < 0.05$, respectively), as well as an upward tendency of BW and ADG toward day 60 in the treatment group ($P = 0.079$ and $P = 0.058$, respectively). Serum vitamin A showed an increasing trend on day 45 and 60 ($P = 0.098$ and $P = 0.060$, respectively) with vitamin A supplementation. However, we did not find a sex effect in this study. Complete blood counts showed no significant changes in all calves. The gene expression found in the *longissimus dorsi* muscle sample revealed that vitamin A supplementation promoted preadipocyte, muscle development, and related signaling pathways by upregulating the gene expression of CTNNB1, ERK1, ERK2 (ERK and β-catenin pathway), Pref-1, Zfp423 and Myf6 ($P < 0.05$), without disturbing the mature adipocyte marker (PPARγ and FABP4). Additionally, the tissue section for *longissimus dorsi* muscle proved to have a more massive amount of muscle fiber based on the same area. In conclusion, high vitamin A supplementation during late pregnancy in pregnant heifers (100,000 IU/day) and in the postnatal period in newborn calves (45,000 IU/day) enhanced calf growth performance without an adverse effect on health status. In addition, according to the gene expression in two-months aged calves, positive impacts were observed on preadipocyte and muscle development with vitamin A supplementation. Collectively, vitamin A supplementation to calves would be beneficial for future production property, including marbling development and carcass traits.

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O 50

Maternal-Fetal Hepatic Mineral Interactions: Liver Mineral Ratios

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Transfer of minerals from maternal blood to the gravid uterus is recognized as a critical physiologic process for development and survival of the fetus and neonate. The study objective was to characterize relationships between paired maternal and fetal hepatic mineral concentrations to better understand the dynamics in mineral homeostasis during pregnancy. Paired maternal and fetal livers (n=185), including 11 sets of twins, were collected. Cattle breeds were identified as dairy (Holstein) or beef (Angus or Hereford). Fetal crown-to-rump length was measured and used to estimate gestational age (GA). Maternal and fetal liver mineral concentrations were determined by inductively coupled plasma mass spectroscopy (ICP/MS; Perkin Elmer Elan 6100). Mineral concentrations (µg/g dry weight [DW]) were determined by mass-to-charge based on respective internal standards used for differing minerals of interest. Measured minerals included calcium (Ca), cobalt (Co), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), selenium (Se) and zinc (Zn). Paired maternal-fetal liver mineral concentrations (DW) were used to calculate the fetal-maternal mineral concentration ratio (FMR) or maternal-fetal mineral concentration ratio (MFR). Mineral data were assessed for normality and transformed as necessary. Population statistics and correlation were determined. Analysis of variance (ANOVA) models evaluated main effects of breed (Dairy or Beef), GA, gender, and sampling period on FMR. Mean (± SD) fetal age was 6.4±1.5 mo (range: 3.7-9.4 mo). An overall association between maternal and fetal liver mineral concentrations were only found with Cu ($r=0.29$; $P<0.0001$) and Se ($r=0.64$; $P<0.0001$). Mean (± SD) FMR values were >1 indicating fetal concentrating ability for Ca (1.69±0.64), Cu (3.01±6.91), Fe (4.93±4.50), Mg (1.33±0.23), Se (1.71±1.25) and Zn (4.50±2.72), whereas FMR was < 1 for Mn (0.76±0.30), Mo (0.21±0.12) and Co (0.38±0.33). Mean (±SD) MFR values were >1 for Cu (1.19±0.87), Mn (1.61±0.96), Co (4.13±6.15) and Mo (6.69±3.98), but highly variable. Age influenced FMR and MFR for Mg ($P<0.0001$), Ca ($P<0.004$), Mn ($P<0.0001$), Fe ($P<0.0002$) and Mo ($P<0.0001$) adjusting for breed and sampling period. Generally, FMR declined and MFR increased with GA, though these were not always linear relationships. In contrast, both Mn and Mo showed increasing FMR and declining MFR with GA. These data provide a different perspective on maternal-fetal hepatic mineral interactions by pairing samples and show a concentrating ability of the fetal liver and associations between maternal and fetal concentrations that can change over gestational time.

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Maternal-Fetal Hepatic Mineral Interactions: Mineral Association Relationships

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The study objective was to further characterize relationships between paired maternal and fetal hepatic mineral concentrations to better understand the dynamics in mineral homeostasis during pregnancy. A population of abattoir collected paired maternal and fetal livers (n=185) were used to evaluate liver mineral relationships. Maternal and fetal liver mineral concentrations were determined by inductively coupled plasma mass spectroscopy (ICP/MS; Perkin Elmer Elan 6100). Mineral concentrations (µg/g dry weight [DW]) were determined by comparison of mass-to-charge ratio with internal standards for respective minerals of interest. Measured minerals included calcium (Ca), cobalt (Co), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), selenium (Se) and zinc (Zn). Paired maternal-fetal liver mineral DW concentrations were used to calculate the fetal-maternal ratio (FMR) or maternal-fetal ratio (MFR) for all minerals. Mineral data were assessed for normality and transformed as necessary. Linear and nonlinear regression modeling was used to determine relationships between fetal or maternal liver mineral concentrations and FMR or MFR. Refer to companion abstract for further methods and study population statistics. Relationship between FMR and maternal liver mineral concentration was fitted with a power function where at low maternal mineral concentrations there was a high FMR and low FMR with high maternal liver mineral. Minerals Cu (r^2=0.94, P<0.0001), Co (r^2=0.89, P<0.0001), Se (r^2=0.42, P<0.0001), Fe (r^2=0.24, P<0.0001), Zn (r^2=0.57, P<0.0001) and Ca (r^2=0.53, P<0.0001) all showed this response. A strong linear relationship between MFR and maternal Cu (r^2=0.76, P<0.0001) or Co (r^2=0.89, P<0.0001) liver concentration. There was a quadratic relationship between maternal (r^2=0.39, P<0.0001) and fetal (r^2=0.56, P<0.0001) liver Mg concentrations and Mg-FMR. Maternal-fetal Mg ratio was linearly associated with maternal (r^2=0.37, P<0.0001) and quadratically with fetal (r^2=0.60, P<0.0001) liver Mg concentration. Both Mn and Mo showed similar relationships between fetal liver mineral concentration and FMR and MFR. Fetal Mo (r^2=0.83, P<0.0001; r^2=0.77, P<0.0001) and Mn (r^2=0.51, P<0.0001; r^2=0.69, P<0.0001) concentrations were associated linearly with FMR and nonlinearly with MFR, respectively. Additionally, Fe (r^2=0.74, P<0.0001), Zn (r^2=0.60, P<0.0001) and Ca (r^2=0.53, P<0.0001) showed a lower fetal liver mineral concentration with higher MFR, but this relationship was not observed with Cu, Co or Se. The power response observed with many of the minerals suggests a high maternal to fetal transfer at low maternal mineral concentration but a more limited transfer as maternal mineral content increase; possibly a protective mechanism. Further research is needed to explore these maternal-fetal mineral relationships.

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Effect of maternal supplementation with essential fatty acids and conjugated linoleic acid on the endocrine growth regulation in neonatal calves

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Due to the replacement of pasture by corn silage based rations in modern dairy nutrition, the availabilities of α-linolenic acid and conjugated linoleic acid for cows are reduced. First results of the present study indicate that an altered maternal supply with these fatty acids can change the fatty acid status and energy metabolism of neonatal calves (1), which might be accompanied by modulations of their somatotrophic axis and growth. Thirty-eight Holstein-Friesian calves from dams abomasally infused during the last 9 wk of gestation and the subsequent lactation either with linseed and safflower oil (EFA; n = 9), Lutalin® (CLA; n = 9), EFA and CLA (EFA+CLA; n = 11) or coconut oil to counterbalance energy intake from the EFA infusion (CNTR; n = 9) were studied in their first 5 d of life. The study was performed in 5 blocks of 8 (or 7) calves with random assignment of each treatment in each block, respectively. Blood samples for analysis of growth hormone, insulin-like growth factor-I (IGF-I) and IGF-binding proteins (IGFBP) -2 and -3 in calf plasma were collected daily before feeding of colostrum from the own dam. After slaughter, kidney, kidney fat, liver, pancreas, spleen and thyme were weighed and duodenum, mid jejunum and ileum sampled for morphometric measurements. Data were analysed by repeated measures ANOVA of SAS using a mixed model approach. The model included the fixed factors treatment (EFA, CLA), time or gut segment, respective interactions, block and sex. The durations of supplementation and gestation were included as covariates. During the 5 d, plasma IGF-I concentration was lower in CLA groups than in calves, whose dams received no CLA ($P<0.05$). The IGF-I concentration was higher in EFA compared to EFA+CLA calves directly after birth ($P<0.01$). The ratio of IGFBP-3 to IGFBP-2 in blood plasma was higher in EFA than EFA+CLA calves on d 2 ($P<0.05$). Neither birth weight nor average daily gain or the body weight corrected organ masses were affected by maternal supplementations. The intestinal development was only modulated in the ileum, where maternal CLA supplementation reduced the crypt depth ($P<0.05$) and increased the ratio of villus height to crypt depth ($P<0.05$). Despite a modification of the IGF status, only minor effects on the intestinal development of the neonatal calf were induced by maternal fatty acid supplementation. However, CLA seems to affect ileal mucosa growth.

1) Uken et al. (2018) Proc. Soc. Nutr. Physiol., 27, 183.

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P 157

Growth and health of lambs artificially reared with casein- or whey-based milk replacer

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High cost of milk proteins has driven development of cheaper whey-based milk replacer (WBMR) as an alternative to skim-milk (casein)-based milk-replacer (CBMR) for artificial-rearing of young ruminants. Lower weight gains are associated with WBMR in calves while disease incidence and mortality were similar. Similar data is not available for lambs. This study compared growth, antibiotic use and mortality in lambs reared on either WBMR or CBMR in the first three weeks of rearing at commercial scale. East Friesian cross-bred lambs born to naturally-mated ewes lambed outdoors were enrolled in the study at 2 days of age to enable consumption of natural colostrum from their dam. Lambs were reared in a well-ventilated indoor facility in 3 x 3 metre group pens (16-20 mixed sex lambs/pen with 2 teats/pen) with 8 replicated pens per treatment. Lambs were randomly-allocated to either WBMR (n=138) or CBMR (n=151) groups balanced for sex, birth-rank and date of birth. Lambs were offered *ad libitum* MR using automatic-feeders dispensing warm reconstituted CBMR (25.2% protein, 26.4% fat, 40.5% carbohydrate and 35.4% lactose and 8.9% ash) or WBMR (22.2% protein, 22.9% fat, 43.3% carbohydrate, 19.2% lactose and 8.9% ash) mixed at 230 g/L, with lamb starter and fresh water freely available. Lambs were weighed on entry and exit and health/mortality data collected daily. There was a diet x rank interaction (P=0.001) whereby CBMR-fed single/twin and triplet/quad lambs had the highest average daily gain (ADG) independent of birth-rank (362 *vs.* 358 respectively, SED=16.7) compared to WBMR-fed lambs where higher ADG was observed in triplet/quad compared to single/twin lambs (275 *vs.*213 g/d respectively, SED=16.7). Males tended to have higher ADG than females (311 *vs.* 294, SED=9.7 g/d, P=0.064). Mortality (10% *vs.* 4%), and overall antibiotic treatments (18% *vs.* 4%) were greater in WBMR- than CBMR-fed lambs. These results highlight that CBMR under an *ad libitum* milk feeding regime supports greater ADG independent of birth-rank and reduces the incidence of disease in the first 3 weeks of life compared to WBMR. Furthermore, WBMR under an *ad libitum* milk feeding regime results in lower ADG of single/twin-born lambs compared to their triplet/quad counterparts. Longer-term implications on growth, health and wellbeing, and interaction with birth-rank, warrant further investigation. Selection of MR for rearing should consider not just feed cost, but economic and welfare implications of slower growth, increased antibiotic treatment and increased mortality.

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P 158

Influence of maternal conjugated linoleic acid and essential fatty acid supply on the intestinal immune system of neonatal calves

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Fatty acids (FA) play a key role in gastrointestinal growth and immunomodulation and their pattern in colostrum/ milk depends on maternal supplementation (MS). The present study investigated the impact of colostral FA by MS with conjugated linoleic acid (CLA) and essential FA (EFA) on the intestinal immune system of neonatal calves.

Nine German Holstein cows were abomasally supplied with coconut oil (control; 76 g/d), CLA (Lutalin®; 38 g/d) or CLA+EFA (Lutalin®+ Linseed oil+ safflower oil; 78+ 4+ 38 g/d) from nine weeks antepartum until early lactation (n = 3/ group). Their calves (4 male, 5 female) were fed with colostrum/ milk of respective dams and slaughtered five days postnatal. Leucocytes isolated from jejunal and ileal epithelium and *lamina propria* (LP) were stained with CD2-, CD4-, CD8- and CD21-antibodies. Cell subsets were measured by flow cytometry. First colostrum was analyzed according to dry matter (DM) content, fat content and FA pattern. Data were analyzed by single and multifactorial variance analyses of SAS 9.4 including in case of milk traits MS as fixed factor and in case of cell subsets the MS and sex of calves.

MS influenced colostral FA composition, ω-3 FA, ω-6 FA and DM content ($p < 0.05$). Maternal CLA supplementation decreased $CD2^+$ subsets in jejunal LP ($p < 0.05$), tended to decrease $CD8^+$ subsets in jejunal epithelium ($p < 0.1$) and increased $CD21^+$ subsets in jejunal and ileal LP compared to control calves. In case of $CD21^+$ cells a MS-by-sex interaction was detected ($p < 0.001$). $CD4^+$ subsets in the jejunal epithelium and $CD2^+$, $CD4^+$ and $CD8^+$ subsets in the jejunal LP were affected by sex ($p < 0.05$). Interestingly, T and B cell subsets in considered localizations of CLA+EFA calves equaled subsets in control calves.

$CD4^+$ subsets in ileal epithelium correlated with polyunsaturated FA, ω-3 and ω-6 FA proportions ($r > 0.70$; $p < 0.05$). DM content in colostrum correlated positively with $CD2^+$ subsets in jejunal and ileal epithelium, $CD8^+$ subsets in the jejunal epithelium ($r > 0.77$; $p < 0.05$) and negatively with $CD21^+$ subsets in jejunal epithelium and ileal LP ($r < -0.75$; $p < 0.05$). Total fat content in colostrum correlated with $CD4^+$ subsets in jejunal epithelium ($r = 0.76$; $p = 0.03$). Summarizing, present results revealed strong relationships between alterations in colostral FA composition due to CLA and EFA MS and modifications in T and B cell subsets in the neonatal bovine intestine.

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Effect of abrupt or step-down weaning at different starter intakes on growth performance of Holstein female calves.

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Feeding schemes with elevated quantities of milk are adopted more frequently in commercial dairy farming. This makes it more challenging to reach sufficient starter intake before weaning with potential negative effects on growth performance after weaning. The objective of this study was to evaluate the effect of abrupt or step-down weaning on female calf growth performance. The start of weaning was determined by the amount of starter intake over the previous week. Sixty Holstein Friesian calves were randomly assigned to one of three weaning strategies at birth. Feeding scheme before weaning was the same for all treatments: calves were fed 2x 2.5L of milk replacer/d at a concentration of 125 g/L, starter and hay was provided ad libitum until weaning. Weaning strategies combined age and starter intake (daily average over the last week). The first group (G1) was abruptly weaning at d 56 and a starter intake of 0,75kg/d. Group 2 (G2) was weaned in 2 steps starting at d 56 and a starter intake of 0,5kg/d and only completely weaned at 1 kg/d starter intake. Weaning of group 3 (G3) started earlier at d 42 and minimum starter intake of 0,5 kg/d, was done in 3 steps and was only completed at a starter intake of 1kg/day. Milk intake was measured daily, starter and hay intake was measured weekly. Body weight was measured every 2 weeks till 12 weeks of age, then every 2 months till 12 months of age. Calves were housed individually till 12 weeks of age. Data was analyzed using the linear mixed model that accounted for the fixed effects of treatment, time and their interaction. Calf was considered a random effect.

Bodyweight and starter intake did not differ between treatments before weaning. A treatment x time interaction was observed for starter intake in week 8 and 9, the G3 had higher starter intake compared to G1 and G2 ($P<0.05$). The G1 had a temporarily reduced post-weaning growth but this effect disappeared later on. Overall weaning strategy had no significant effect on feed conversion and growth performance. In this study was shown that a reduced post-weaning growth can be prevented by using a step-down weaning strategy in case of a restricted milkregime. The long term growth performance were not affected by starter intake at weaning or weaning strategy in case a minimum starter intake of 0.75 kg/day at weaning was required.

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P 160

Effects of incremental amounts of supplemental leucine to milk-fed neonatal Holstein bull calves on pancreatic and intestinal enzyme activity

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Leucine is an essential amino acid and is a regulator of metabolic pathways for maintenance and growth. Supplementation of leucine has been shown to affect pancreatic α-amylase activity and protein accretion; however, effects of supplemental leucine on intestinal enzymes are not well known. Twenty-three Holstein bull calves were sorted into four groups and fed milk replacer twice daily for 28 days with or without supplemental leucine (0, 0.4, 0.6, or 0.8 g/kg initial body weight). On d 29, calves were euthanized for tissue collection. A sample of pancreas and duodenum was taken for determination of protein concentration and enzyme activity. Data were analyzed as a completely randomized block design with arrival date as block. Enzyme activity and protein concentration were analyzed using the GLM procedure of SAS, with calf as the experimental unit and effects of block and treatment. Linear and quadratic effects of treatment were determined by orthogonal contrasts. Pancreatic α-amylase and trypsin were not affected ($P > 0.1$) by leucine supplementation. Pancreatic protein concentration and content relative to body weight was quadratically affected by leucine supplementation ($P \leq 0.01$) as was mg protein per kg of calf body weight ($P < 0.001$) where calves on 0 or 0.4 g/kg body weight leucine showed a decrease in both pancreatic protein concentration and content relative to body weight compared to all other treatments. Intestinal protein concentration and glucoamylase activity was not affected ($P > 0.2$) by treatment. Intestinal isomaltase activity (U/g intestine, and U/g protein) linearly decreased with increasing leucine supplementation ($P \leq 0.03$). Intestinal maltase activity (U/g intestine, and U/g protein) linearly decreased with increasing leucine supplementation. Intestinal lactase activity (U/g intestine) showed quadratic effects of leucine supplementation ($P = 0.02$) where calves receiving 0.4 g/kg body weight leucine were lower than all other treatments. These results indicate that supplemental leucine at low levels negatively affected intestinal carbohydrate enzymes; however, long-term effects of supplemental leucine on carbohydrate digesting enzymes are unclear.

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Effects of collection time and colostrum quality on calf rumen and faecal bacterial communities

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Management and feeding regimes implemented at birth may influence the establishment of gastrointestinal microbial communities in calves. In New Zealand's seasonal dairy systems management of newborn calves is challenging because farmers only collect calves once or twice daily from the calving paddocks, and colostrum feeding practices vary. We investigated the effects of calf collection time and quality of colostrum fed during the first 2 days of life on the calf gastrointestinal microbiota. Forty Friesian × Jersey crossbred calves were removed from their dams and collected from the calving paddock either EARLY (within 12 h after birth) or LATE (between 18-24 h after birth). Calves were then fed either high-quality colostrum (HQC; first milking colostrum, Brix% = 22.7 ± 1.8) or low-quality colostrum (LQC; pooled from the first 8 milkings, Brix% = 12.3 ± 1.0) at 7.5% (vol/wt) of bodyweight within the first two days of life (4 feeds to EARLY, and 3 feeds to LATE calves). Thereafter, all calves were group-housed and fed milk replacer (28% CP, 20% fat, mixed at 125 g/L, at 20% of initial bodyweight) and a pelleted calf starter (20% CP, ad libitum) using automatic feeders. Calves were slaughtered at 35 ± 2 days and rumen and rectum content samples were collected. Short-chain fatty acid (SCFA) concentrations of the rumen and faecal samples were determined by gas chromatography. Faecal acetate and propionate concentrations tended to be greater in the LQC (mean 47.1 mM acetate, and 25.6 mM propionate), than the HQC fed calves (mean 38.9 mM acetate and 21.3 mM propionate; $P < 0.1$, two-factor analysis of variance). Individual SCFA concentrations did not otherwise differ significantly ($P < 0.05$) by colostrum quality or collection time. Bacterial communities were assessed by sequencing 16S rRNA gene (V3-V4 hypervariable region) amplicons prepared from the rumen and faecal samples. The communities in each treatment group had overlapping distributions by nonmetric multidimensional scaling using Bray-Curtis dissimilarities. However, differences were observed using partial least squares discriminant analysis, and calf collection time appeared to have a greater influence on the rumen and faecal bacterial communities than colostrum quality, which segregated communities in the LATE, but not the EARLY, collected calves. These data indicate that time spent in the calving paddock with the dam, and the quality of the colostrum received thereafter, can influence the composition of the rumen and faecal microbial communities of artificially-reared calves over one month of age.

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O 51

Dietary cation and anion difference: Effects on feed intake, ruminal function and plasma leptin in dairy goats under tropical condition

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Dairy goat is small ruminant raised in various climatic conditions. Under high ambient temperature (HTa) in tropical areas, milk synthesis and evaporative heat dissipation are both considered homeorhetic controls. Hence, it is important by to find the appropriated means to improve the living quality and optimize production. We have shown that high dietary cation and anion difference (DCAD) increased water intake (WI) and total body water which may improve heat dissipation. Supplementation with high DCAD also increased dry matter intake (DMI). Moreover, plasma leptin has been shown previously to mediate eating behavior in crossbred dairy goat. This study was carried out to determine the effect of high DCAD on DMI, ruminal function and plasma leptin in lactating dairy goats under tropical condition. Ten dairy goats were divided into two groups with five animals each. The treatment diets were formulated at different DCAD level; either control (22.81 mEq/100 g DM) or high DCAD (39.08 mEq/100 g DM) diet. After parturition, DMI and WI were recorded daily. At the 4th and the 8th weeks post-partum (PP-4 and/or PP-8), ruminal fluid, blood and urine samples were collected and the nutrient digestibility measurement was evaluated. Dry matter intake/body weight (DMI/BW) from high DCAD group (38.1±1.2 g/kg BW) was significantly higher ($P<0.05$) than control group (32.8±1.9 g/kg BW) at PP-8. Animals fed with high DCAD diet consumed more water than control, particularly at night time ($P<0.05$). Ruminal pH, acetate concentration and urinary allantoin excretion increased in high DCAD diet, whereas ruminal butyrate concentration was lower. Other ruminal parameters such as total VFA concentration, propionate molar proportion and average ratio of acetate/propionate were not affected by high DCAD supplementation. The apparent digestibility was improved and the plasma leptin concentration was elevated by high DCAD supplementation. The results from this study suggest that, under HTa condition, high DCAD diet may increase DMI especially at PP-8 in relation to improving nutrients apparent digestibility, ruminal fermentation and microbial protein synthesis. Since leptin is known as anorectic hormone, an increase in plasma leptin concentration was not involved in the DMI. We speculate that the improvement of ruminal function after high DCAD supplementation facilitated eating behavior.

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O 52

Modelling forage intake in new world camelids

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The literature between South and North America has contrasting recommendations for dietary nutrient densities for llamas and alpacas in-spite-of use of the same nutrient requirement models due to differences in intake expectations. The study objective was to summarize and model published research describing dry matter intake (DMI) of llamas and alpacas to determine if dietary neutral detergent fiber (NDF) influences intake capacity. A retrospective study was performed on summarized data from 9 published studies characterizing individual DMI of forage diets by llamas and alpacas. Daily DMI was quantified and characterization of the diet relative to crude protein (CP) and NDF was provided. In these 9 studies data were derived from a total of 27 llamas and 38 alpacas. Intake comparisons were determined for 23 forage combinations. Intake was calculated on g/kg body weight (BW) and metabolic BW (g/kg.75, MBW). In the experimental diets, forage CP and NDF ranged from 22 to 205 and 452 to 746 g/kg dry matter, respectively. Analysis of variance (ANOVA) and multiple regression modelling were used to define effect of species, dietary CP and NDF content, study and interactions on intake metrics. Study was confounded by diet composition thus regression modelling used CP and NDF to model intake. Across studies DMI (1485 vs 721 g/d, P<.0001) and MBW intake (46.4 vs 35.2 g/kg MBW, P=.0006) were higher for llamas compared to alpacas. Observed intakes within species were highly variable given the wide range of forage quality fed. Least squared mean DMI (g/kg BW) for llamas (14.7 ± 0.6) and alpacas (13.0 ± 0.7) was not different. Least squared mean dietary NDF intake (g/kg BW) tended (p=0.08) to be higher for llamas (8.68 ± 0.31) compared to alpacas (7.61 ± 0.37). Modelling DMI and NDF intake across species did not provide robust predictive models. Multiple regression modelling including dietary CP and NDF content (1st, 2nd and 3rd order variables) and an interaction term between dietary CP and NDF content provided the best predictive models for DMI (g/kg BW) for alpacas (r^2=.69) and llamas (r^2=.92). These findings suggest dietary NDF and CP content control DMI capacity of camelids like other ruminants, but due to a slower fiber rate of passage have a lower NDF capacity (8-9 g/kg BW). Llamas seem more sensitive to dietary NDF as an intake limiter compared to alpacas given the stronger relationship observed between NDF, CP and DMI capacity.

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Feed intake pattern did not explain individual variation of feed efficiency in dairy goats fed a Total Mixed Ration

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Feeding behavior is a parameter involved in feed efficiency in ruminants. It shows variation among individuals. Feed intake pattern, a major aspect of feeding behavior, is a repeatable trait. This study aimed to evaluate feed efficiency in dairy goats with a set of 35 animals (13 Alpine and 22 Saanen) at three life stage periods (middle of lactation (P1), end of first lactation and beginning of second gestation (P2), second lactation (P3)). Goats were fed twice a day a Total Mixed Ration adapted to requirements (20 % concentrate, 20% meadow hay, 30 % chopped dried alfalfa, 30 % sugar beet pulp silage). Feed efficiency varied from 0.453 to 1.69 kg FCMY (fat corrected milk yield) per kg dry matter intake (DMI) with a mean value of 1.16 and a standard deviation of 0.299. Feed efficiency was different between periods: 1.37 ± 0.108 (P1), 0.79 ± 0.144 (P2) and 1.32 ± 0.161 (P3) and significantly lower for P2 compared to P1 and P3 ($P < 0.05$). This can be explained by the physiological status of the animals as during P2, goats were in gestation and rebuilt their reserves for the next lactation. During P1 and P3, animals were at an earlier stage of lactation and mobilized more their reserves as observed by the Non-Esterified-Fatty-Acid levels in plasma (P1: 152.7; P2: 107.9; P3: 232.2 (µmol/l)). Feed efficiency was significantly ($P < 0.01$) driven by milk yield on the whole data set ($r = 0.92$, $n = 105$) and at each period (P1, $r = 0.50$; P2, $r = 0.92$; P3, $r = 0.81$). Several aggregate measures of feed intake patterns were calculated for each goat with three days of recording per goat and per period: DMI during the 90 or 180 minutes following afternoon feed allowance, proportion of feed allowance eaten during these first 90 or 180 min, asymptote, initial rate of intake and residual mean square error of the adjustment of the curve describing DMI evolution with an exponential model, NDF sorting (ratio between NDF content of intake and NDF content of offered diet). Except for the asymptote of the curve describing DMI evolution, none of these aggregate measures evaluated within period or across periods could explain individual variation of feed efficiency. Thus, with this total mixed ration, individual variation of feed efficiency in dairy goats was mainly explained by milk yield and did not depend on feed intake pattern.

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O 54

Feeding behaviour of Nellore cattle fed increasing levels of dry corn gluten feed

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Introduction: A higher proportion of rumen degradable starch can cause ruminal acidosis and change feeding behaviour. Little is known about how replacing corn by Dry Corn Gluten Feed (DCGF), a high-fiber and low-palatability by-product, could affect this trait. The aim was to evaluate the effects of increasing levels of DCGF on cattle feeding behaviour.

Materials and Methods: A hundred-twenty Nellore bulls with initial body weight of 360 ±15 were allocated in a completely randomized blocks design in pens with 5 animals in each and 6 pens per treatment. Animals were fed for 113 days. There were four treatments with DCGF (T1 – Control, T2 – 180 g/kg, T3 - 360 g/kg and T4 - 540 g/kg of DCGF). The diets contained 135, 135, 139 and 166 g/kg of crude protein and 557, 486, 408 and 306 g/kg of non-fiber carbohydrates, respectively. All diets contained 112 g/kg of roughage NDF. The 24-hour visual observations were performed three times over the trial. The following information was collected: time spent eating (EAT), ruminating (RUM) and resting (RES), expressed in minutes; and number of meals per day. Dry matter intake (DMI) was measured everyday. Meal length (minutes) was calculated by dividing EAT by number of meals/day. Dry matter intake per meal (kg) was calculated by dividing DMI by the average number of meals per pen in each day. EAT and RUM data were used to calculate DM consumption rate (DMCR; EAT/DMI) and rumination rate (DMRR; RUM/DMI) expressed in minutes per kg of DM. Data were analyzed using the MIXED procedure of SAS with contrasts.

Results: DMI was quadratically affected by the treatments (10.42; 11.72; 11.53; and 11.28 kg/day; $P<0.02$). The inclusion of DCGF caused linear increases in EAT (167; 179; 197 and 207 minutes/day; $P<0.01$). However, RUM was not affected ($P>0.05$). Resting time (RES) also decreased linearly (1014; 976; 963 and 955 minutes/day; $P<0.01$). The number of meals (10.1; 12.3; 11.8 and 15.1) and DMI per meal (1.2; 1.03; 1.04 and 0.78 kg/meal) increased linearly ($P<0.01$). Meal length was linearly reduced according to the treatments (16.7; 14.7; 16.8 and 13.8 minutes/meal; $P<0.01$). The DMCR increased linearly (16.3; 15.5; 17.3 and 18.5 minutes/kg of DM; $P<0.01$) and DMRR was not affected by the treatments ($P>0.05$).

Conclusion: The replacement of corn by DCGF do not affect rumination but cause changes in eating behaviour.

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O 55

Renal control of feed intake during adaptation in ruminants grazing low dry matter forages

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Fodder beet (*Beta vulgaris*) grazing systems for cattle and sheep have been pioneered in New Zealand over the past decade. The DM% of the crop is low (<15%), but intakes are high (>2.2% LWT/d). This study sought to establish intake and water balance in sheep fed beet dominant diets.

Eight Coopworth hogget rams, individually pen fed, were used in sequence. The three diet treatments were: Diet A (control) - FB 0%: grass silage (GS) 100%; Diet B (transition) - FB 30%: GS 70%; and Diet C (beet dominant)- FB 90%: GS 10%. Each diet treatment had a 7 d collection period of faeces and urine. LWT gain and water balance were also calculated.

Intake maximums ranged from 3.4% of LWT (Diet A) to 3.1% (Diet C). The mean urine output rose from 29 mL (/ kg LWT daily) in diet A to 162 mL in diet C, with mean LWT gain at <50g/ d at diet A but 160g/ d for diet C.

Urine volumes followed water consumed in feed rather than imbibed, with recorded water drinking intakes effectively zero at Diet C (<100ml/ d), resulting in periods of approximately 30 days with effectively no water drinking, but urine volumes above 100mls/ kg LWT daily. The highest recorded daily urine outputs were >200 mL/ kg LWT, fourfold above reference range maximums. The strong LWT gain for Diet C amply demonstrated there is no diuresis effect for this diet, simply a high water loading.

Adapting from Diet A to Diet B took 5 d, but Diet B to Diet C took 12 d to maximum beet intake, and intakes lifted approximately every 3 d then remained stable again for this period. As sheep had been on the FB diet for c.30 d by initiation of Diet C, we suggest it was renal adaptation to the high water loads eaten, not rumen adaptation, that controlled intake. Previous studies have reported very low rumen ammonia despite high microbial protein production on identical FB rations in steers. The absence of an ammonia 'brake' on rumen epithelial blood flow means rumen water absorption can be maximal, and we suggest it then requires direct intake control to avoid subsequent haemolysis, unless renal water excretion can be up-modulated to cope. This may explain why typical transition to *ad libitum* FB diets are at least 14 d, while similar energy density concentrate diets can be 7 d.

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O 56

Prediction of dry matter intake using milk fatty acid composition for dairy cows during early lactation

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It is important to perform proper feeding management during the perinatal period to increase the productivity of dairy cows, and many studies have attempted to improve the negative energy balance during the early lactation period. Although it is essential to understand dry matter intake (DMI) to improve the energy balance status, it is difficult to measure DMI on a farm. Hence, a simple and practical method is required that can be used in the field. In recent years, there have been reports that the fatty acid composition of milk fat can be used as an index for health management of dairy cows, and mid-infrared spectroscopic analysis will allow rapid and non-invasive analysis of fatty acids in a large number of milk sample. Therefore, we evaluated the DMI estimation method using milk fatty acid composition during early lactation.

A total of 307 pairs of morning and evening milk samples were collected from 58 Holstein lactating cows (9 first lactation cows, 20 second lactation cows, and 29 third lactation cows) during 1-8 weeks after parturition. Fat, protein, and lactose content in milk samples were analyzed using infrared analysis. The composition of fatty acids was analyzed using gas chromatography. DMI and milk yield (MY) were measured daily. DMI prediction equation was developed by multiple regression analysis using variable specification method or stepwise method.

The results obtained were as follows:

1) DMI was 19.7 kg (4.6-41.4 kg), and MY was 35.0 kg (12.4-58.0 kg). The proportion of major fatty acids in milk included $C_{14:0}$ 9.8 % (2.7-14.8 %), $C_{16:0}$ 34.6 % (28.0-49.3 %), $C_{18:0}$ 12.3 % (3.7-18.3 %), $C_{18:1}$ 26.6 % (13.0-46.0 %).

2) The following DMI (kg/day) prediction equations were obtained by multiple regression analysis using the parameters such as lactation numbers, week after parturition (WK), MY (kg/day), milk components, and milk fatty acids (%).

$DMI = 3.33 + 0.469 \times MY$ (Adjusted $R^2 = 0.775$, RMSE = 4.06)

$DMI = -5.06 + 0.371 \times MY + 1.208 \times C_{14:0}$ (Adjusted $R^2 = 0.848$, RMSE = 2.56)

$DMI = -2.38 + 0.655 \times WK + 0.321 \times MY + 0.837 \times C_{14:0}$ (Adjusted $R^2 = 0.852$, RMSE = 2.33)

It has been shown that it is possible to improve the accuracy of DMI prediction during early lactation using the information on milk fatty acid composition. As $C_{4:0}$ ~ $C_{14:0}$ is derived from the rumen fermentation products, it is presumed that the ratio of $C_{14:0}$ was used in the equations to reflect the status of the rumen fermentation, i.e., the feed intake status.

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Meal pattern, dry matter intake and digestibility in goats supplemented with oil palm frond and oil palm meal product

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Para-grass (*Brachiaria mutica*) is easily grown in Thailand and has been used by the farmer as a basic feed stuff for goats. However, oil palm frond (OPF) and oil palm meal (OPM) from bio-diesel plant are being used for roughage substitution since it is a low-cost by-product available throughout the year as one of eco-feeds. The effects of OPF and OPM added to basal para-grass on body weight, meal pattern, nutrient utilization in relation to rumen fermentation were evaluated in six female crossbred goats aged 6-8 months old. By using randomized 3x3 Latin square design, each goat was fed for 28 days of 3 diet treatments; 100 % para-grass (T1); 50 % para-grass + 50 % oil palm frond (T2), and 30 % para-grass + 50 % OPF + 20 % oil palm meal (T3). The nutrient composition of 3 diets were estimated as %DM basis for ADF (35.4, 36.4, 38.9), NDF (58.4, 52.9, 54.4), CP (11.4, 11.5, 11.3) and fat (1.7, 2.5, 3.6), ash (11.9, 10.6, 9.8), respectively. Body weight, feed intake and dry matter intakes were recorded weekly while meal pattern was recorded at week 1 and 3 after treatment. The nutrient digestibility was recorded on the last 5 consecutive days whereas rumen pH, protozoa and urinary allantoin and creatinine ratio were measured at the end of each treatment. The results showed that body weight was not different among treatment groups. Feed intake was slightly lower in T3 but DMI was significantly higher in T2 and T3 compared with T1 ($P<0.05$) starting from week 1 throughout the 4 week periods. The meal patterns (duration, size and frequency of meal) were not different among groups. The percent digestibility of protein was lower in both T2 and T3 while fat digestibility was lower only in T2. The rumen C2/C3 and urinary allantoin/creatinine were higher in T2 and T3 compared with T1. It is concluded that OPF with and without OPM can be used for roughage substitution. Other sources of feed with high protein content should be supplemented to address low protein digestibility.

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Influence of 150 vs 250g concentrate/kg ECM on milk performance, energy balance in a long-term experiment with Simmental cows.

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Long-term studies on the input of concentrates (C) in the feeding of dairy cows are lacking. Therefore the objective of the present trial was to verify the response of Simmental cows to a long-term reduction of C.

Materials and Methods: Over two lactations, 48 dairy cows in 2 groups (n=24) H and L were fed differently with C (H: 250 g C/kg ECM, L: 150 g C/kg ECM). Roughage consisting of grass silage, maize silage and straw/hay amounted to 6.6 MJ NEL/kg DM energy during lactation. The components of C were wheat, barley, field beans rapeseed meal and minerals. The rate of C (in DM) in the total mixed ration was 35 % in H until 165th day of lactation (R1), then 22 % (R2), so in average 250 gC/kg ECM were allocated during lactation. L was fed with 150 g C/kg ECM on average. Until the 165th day of lactation, L received R2, then R3 (14% C). During the dry period, both groups were fed equally. Mixed models were used to carry out separate statistical evaluations for lactation and dry period.

Results: Between H and L, signifcant differences in daily intake of energy, roughage and C were observed. H ingested significantly more energy and C, but significantly less roughage. The substitution of roughage averaged 0.70 MJ NEL/MJ NEL C. Until the 165th day of lactation, substitution of roughage was lower (0.51 MJ NEL/MJ NEL C) than in the second half (0.86 MJ NEL/MJ NEL/C).

The ECM performance per day of lactation amounts 27.8 kg ECM in both groups. H produced 51 % of the ECM from roughage while L produced 73 %. Fat and protein contents of the milk did not differ. The average daily energy balance and body condition score was significantly higher in H than in L during lactation. Body weight did not differ. H achieved a balanced energy supply on the 37th lactation day and L on the 72th lactation day. In L energy surplus increased steadily but slower after having reached a balanced supply. H changed earlier from a catabolic to an anabolic metabolic state after calving.

During the dry period, L ingested significantly more DM per day. Hence, at the end of gestation L had compensated the difference of the BCS (3.7; H 3.8).

Conclusion: Roughage of high enery content is the prerequisite for saving C without reducing performance.

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Feed intake and liveweight gain of Bali Bulls fed low quality forage supplemented with increasing levels of cassava and gliricidia

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Fattening of bulls is commonly practised by smallholder farmers in villages in Indonesia.This study was carried out to evaluate the effect of increasing the intake of a supplement made of cassava (*Manihot esculenta*) and gliricidia (*Gliricidia sepium*) on intake, liveweight gain, faecal pH and water intake of Bali bulls fed elephant grass (*Pennisteum purpureum*). Cassava is low in crude protein (CP) but high in metabolisable energy (ME) content with variable levels of hydrogen cyanide (HCN) and a combination with a readily available tree legume, gliricidia, would make a good supplement mix. Fifty Bali bulls (*Bos javanicus)*(initial body weight (W) 157±3.73 kg) were used in a completely randomised block design, with a combination of ground whole cassava tubers and gliricidia (1:1 on estimated DM basis) offered at 0, 4, 8, 12 and 16 g DM/kgW/day (d), fed with elephant grass *ad libitum*. The experimental site was Malonas village, Central Sulawesi.There were 10 animal/replications per dietary treatment. Drinking water was offered *ad libitum* daily, and measured during weeks 3, 9 and 15 together with measurement of faecal pH. The experiment lasted for 18 weeks including a two week adaptation period. The nutrient content of elephant grass was 63 g CP, 661 g NDF/kg DM, cassava was 19 g CP,300 g NDF and 17.9 mg HCN/kg DM and gliricidia was 223 g CP, 384 g NDF/kg DM.

Increasing supplement intake caused substitution and a decrease in intake of the elephant grass ($P<0.05$), but significantly ($P<0.05$) increased total feed DM intake. At supplement levels of 0, 4, 8, 12 and 16 g DM/kg W/d, elephant grass DM intake was 22.9, 21.5, 20.0, 17.8, 16.1 g DM/kgW/d, respectively, while total DM intake was 22.9, 25.4, 27.8, 29.6 and 31.8 g DM/kg W/d, respectively and liveweight gain was 0.20, 0.27, 0.35, 0.39 and 0.46 kg/d, respectively. Water intake and faecal pH were not affected significantly ($P>0.05$) by increasing supplement intake, with a mean value of 90.1 g water/kg W/d and a faecal pH of 6.50. It was concluded that inclusion of cassava and gliricidia intake up to 16 g DM/kgW/d resulted in high total feed intake and liveweight gain with no negative effect of cassava inclusion.

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Effects of rumen-protected folic acid supplementation on amino acid compositions of *longissimus dorsi* muscles in lambs

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Folic acid, as the acceptor and donor of one-carbon units, participates in the synthesis of purines and pyrimidines, methionine cycle and the mutual conversion of Ser and Gly, as well as the conversion of His and Glu. To evaluate the effects of rumen-protected folic acid (RPFA) supplementation on nutrition composition of longissimus dorsi muscles in lambs, twenty-two Hu lambs (49 ± 4.3 days of age and 16.0 ± 2.23 kg of body weight [BW], mean ± SD) were blocked by BW and assigned into two groups of 11 each in a randomized experimental design. All lambs were fed a total mixed ration with roughage to concentrate ratio of 50:50 on a DM basis. One group was not supplemented RPFA, while another group was offered with 4.0 mg FA from RPFA per kg dietary DM. The feeding experiment lasted for 130 days with 10 days of adaption period and followed by 120 days of collection period. Then lambs were slauthered the end of feeding experiment. There was no significant difference in final body weight (43.63 vs 43.80 kg for control and RPFA addition, respectively). Dressing percentage and meat percentage of supplementary RPFA group were 4.2% and 3.5% higher than control. Moisture, crude protein, ether extract and ash of longissimus dorsi muscles in lambs with RPFA supplementation were 74.01%, 20.52%, 4.49% and 1.03%, whereas those of in control were 74.33%, 20.45%, 4.23% and 1.06%, respectively. Expressed as mg/g crude protein, essential amino acid (EAA) content of longissimus dorsi muscles had no significant difference between RPFA supplementation and control (489.6 vs 488.1 mg/g). Contents of Ser, Leu, Lys, Asp and Glu tended to be higher for RPFA supplementation. These results indicated that supplementation of RPFA in lamb diets increased dressing percentage and meat percentage, but had no significant influence on nutrition composition of longissimus dorsi muscles.

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Effect of wood kraft pulp feed on growth performance, feed digestibility, and rumen fermentation in japanese black fattening steers

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Japanese Black fattening cattle are generally fed large quantities of concentrated feed over a period of 20 months. The oversupply of concentrate feed during fattening may lead to a decrease in the daily pH of ruminal fluid, causing ruminal acidosis and thereby increasing the risk of metabolic disorders such as anorexia and diarrhea. Nutritional management of the Japanese Black steer during fattening is necessary to control rumen fermentation without reducing the energy content of the feed, thereby improving the health and productivity of the animals.

Recently, wood kraft pulp (KP), which is high in total digestible nutrients and neutral detergent fiber, has been developed as a feed. Kraft pulp is a feed primarily composed of cellulose. Providing KP to the Japanese Black fattening steers is expected to both stabilize the ruminal environment and improve productivity; however, to our knowledge, no reports are available on the effect of feeding KP to Japanese black fattening steers. Therefore, this study, examined the effects of KP feed on growth performance and rumen fermentation in Japanese Black fattening steers.

Ten Japanese Black fattening steers (26 months old) were randomly divided into control and KP groups. The control group (n = 5) was fed concentrate feed without KP, and the KP group (n = 5) was fed concentrate feed containing 10% KP. Both groups were provided rice straw as roughage. Animals were allocated individual pens. The experiment was conducted over a period of 12 weeks. Feed digestibility was measured through the collection of total feces. The ruminal fluid samples (collected −4, 0, 4, 8, and 12 weeks after the onset of the experiment) were aspirated using an oral tube and analyzed for volatile fatty acids (VFAs). The pH of the ruminal fluid was measured using pH sensors orally administered to each animal. There were no significant differences in dry matter intake, daily body weight gain, nutrient digestibility, and the ruminal concentrations of VFAs between the groups. At 8 and 12 weeks after the onset of the experiment, the acetate to propionate ratio in the ruminal fluid of the KP group was significantly higher than that in of the control group. The average daily pH of ruminal fluid and activity of ruminal lipopolysaccharide did not differ between the groups. Our results suggest that growth performance and feed digestibility in the Japanese Black fattening steers was not influenced by replacing concentrate feed with KP.

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Calcium-magnesium ratio in the serum of newborn calves correlates with the level of their vitality

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The study of calcium and magnesium homeostasis in newborn mammals is of scientific interest because of the possible connection between the health status of newborns and changes in the metabolism of these chemical elements in their bodies due to premature birth or hypoxia during parturition. Calves experienced difficult calving are more acidotic, have less motivation to get up, reduced sucking reflex and vitality in general. The study purpose was to test the content of calcium, magnesium and their ratio in the serum of newborn calves with different levels of vitality. The calves' vitality was assessed shortly after birth by VIGOR score (VS), similar to Apgar score in humans. 100 Holstein calves, weighting 29-45 kg, at 268-284 days gestational age, separated into 5 groups with n = 20 each: (1) excellent vitality, 26-27 VS; (2) very good vitality, 23-25 VS; (3) good vitality, 21-22 VS; (4) marginal vitality, 17-20 VS; (5) poor vitality, 17-20 VS. Blood samples were obtained from the jugular vein of calves, 12-24 hours after birth, in the morning before feeding; the content of calcium and magnesium in serum was determined on Shimadzu AA6300 atomic adsorption spectrophotometer. All data were expressed as mean ± standard deviation and median. The differences between groups were determined by independent-samples Kruskal-Wallis test, and the correlation analysis was performed by non-parametric two-dimensional Spearman significance criterion in IBM SPSS Statistics 20.0. The null hypothesis was rejected when $P<0.05$. Groups of calves did not differ in body weight and gestational age at birth. The serum content of calcium in calves of groups 1-5 was: (1) 2.86 ± 0.17, (2) 3.22 ± 0.24, (3) 2.82 ± 0.20, (4) 2.91 ± 0.05 and (5) 2.84 ± 0.09 mmol/L, respectively. The lowest serum magnesium content was found in group 1 calves - 0.77 ± 0.10 mmol/L, in animals of groups 2-5 it was higher by 27.3, 24.7, 28.6 and 45.6%, respectively ($P<0.001$). The serum calcium-magnesium ratio in group 1 calves was 3.75 ± 0.27: 1, and in calves of groups 2-5 - lower by 14.1, 20.0, 21.6 and 31.5% respectively ($P<0.001$). A direct correlation was found between the serum calcium-magnesium ratio and the assessment of calves according to VS reflected their vitality level ($r = 0.89$, $P<0.001$). In conclusion, calcium-magnesium ratio and magnesium levels in sera seem to be associated with calves' vitality at birth. Future follow-up studies of hypermagnesemia nature in calves with low vitality are recommended.

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Effect of wood kraft pulp feed on digestibility, ruminal characteristics, and milk production performance in lactating dairy cows

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Approximately 67% of the land in Japan is forested. Although several studies on the utilization of wood-based feed for cows have been conducted, such materials contain lignin, which interferes with digestion. Wood kraft pulp (KP) is a cellulosic pulp that is produced by removing lignin from wood chips via a kraft process treatment. Because the total digestible nutrient content of KP is equivalent to that of corn grains, KP may be used as a replacement for concentrated feed. In this study, the effects of feeding KP to lactating dairy cows were evaluated with regard to digestibility, rumen fluid pH, rumen fermentation characteristics, and milk production. Four lactating dairy cows were used for this crossover design feeding experiment, which included a control group and a KP-fed group. The control group was a fed total mixed ration (TMR; 40% roughage and 60% concentrate) and the KP group was fed a TMR in which half of the rolled corn was replaced with 12% KP. The dry matter intake, digestibility of the feed components, and milk yield did not differ significantly between the control and KP groups. The number of times that the ruminal fluid pH dropped below 6.1 was lower in the KP group than that in the control group ($P<0.10$). The acetic acid ratio in the ruminal fluid of the KP group was higher than that in the control group ($P<0.05$), and the propionic acid ratio in the ruminal fluid of the KP group was lower than that in the control group ($P<0.05$). The acetate:propionate acid ratio was higher in the KP group than in the control group ($P<0.05$). Lipopolysaccharide (LPS) levels in the ruminal fluid of the KP group was lower than those in the control group ($P<0.10$). The milk yield, milk protein content, milk lactose content, and solid-non-fat content were not affected by the diets. The milk fat content tended to increase in the KP group compared with that in the control group ($P=0.07$). These results indicate that the use of KP feed in lactating dairy cows induced the same rumen fermentation characteristics as those in cows fed a large amount of roughage without decreasing milk productivity. KP could be a valuable feed resource substitute for grains, as it maintained ruminal fluid pH and decreased ruminal fluid LPS level.

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Acceptance of phytogenic prototypes fed to rearing calves from d 12 to d 23 of age

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The present study is a part of a transversal project, aiming to develop phytogenic feed additives conferring livestock animals a higher resilience to *Eimeria spp.* infections. In the current trial the palatability of 6 different phytogenic prototypes (PT) which reduced *Eimeria* sporozoite invasion in an *in vitro* assay efficiently, supplemented into milk replacer of rearing calves was tested. In contrast to PT 5 and 6, the immunomodulating core of combinations 1 to 4 did not contain humic acid. All PTs contained various essential oil mixes from myrtaceae,- laminaceae,-zingiberaceae- and lauraceae plants. The animal trial was conducted with 75 rearing bull calves (Deutsches Fleckvieh) assigned to 15 groups of 5 calves to which two different PT were offered for choice. In the choice test, each calf had time limited access (15 min per meal) to 2 buckets of milk replacer containing different PT, offered at 7:00 h and 16:00 h and containing 5.0 l milk replacer. During the 12-d evaluation period calves received in addition to the milk replacer hay and pre-weighed starter feed. Intake from individual buckets was calculated by weighing residues after withdrawal of the buckets. For determination of palatability ranking, preference indices based on milk replacer intake from buckets 1 and 2 was calculated using a 50% difference as discrimination criterion. Individual body weight gain, overall feed intake as well as scoring of fecal consistency and health status was recorded daily. Average body weight gain of bull calves was 5.4 kg during the trial, corresponding to 447 g per calf and day. Statistically relevant synergistic effects between feeding the different additives and weight gain did not exist. Average cumulative feed conversion ratio based on overall daily dry matter intake from milk replacer plus starter feed plus hay was 2.574 and without differences between the additives. Feacal scoring varied within the normal physiological range. In contrast, clear preferences for the phytogenic combinations could be observed. PTs 5 and 6, containing humic acid were clearly omitted by the calves. PTs 1 to 4 without humic acid and with various essential oil combinations were comparably accepted by the calves. It can be concluded, that successful product development should include palatability testing in sensitive animal species. In the next step, the *in vivo* efficacy of the 4 preferred PTs will be tested on oocyst shedding in Eimeria infected calves.

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The effects of the preservation method of grass forages on feed intake, chewing behaviour, and milk yield of dairy cows.

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The preservation method of grass forages (i.e., grass hay vs. silage) affects both the DM content and its physical effectiveness. Yet, these effects remain unclear, despite being of considerable interest, especially in forage-based dairy production systems (e.g. organic production). Therefore, the aim of this study was to evaluate the effects of the preservation method of grass forages with a similar botanical composition on the DM intake, chewing behavior, and milk yield of organic dairy cows.

A wilted grass sward (*Dactylis glomerata, Lolium multiflorum, Poa pratensis, Alopecurus pratensis, Anthoxanthum odoratum*; DM, 55.2%; CP, 11.3%; NDF, 46.6%; on DM basis) was harvested with a loading wagon (theoretical length of cut 8cm), but windrows were collected alternately, either for barn-drying or ensiling in a horizontal silo. Nine months later, the differently conserved forages were fed *ad libitum* (10% refusals, as-fed) over a period of 34 d to one of two animal groups, each comprising 9 lactating Holstein cows. Each cow ingested additionally a fixed amount of concentrate per day (3.6 kg; CP, 28.8%; on DM basis). Assignments of cows to groups were based on previous milk yield (30.0 ±5.9 kg) and body weight (717 ±53 kg) as well as DIM (171 ±104 d) and parity (3.8 ±2.1). Data collection of cows' individual feed intake (20 d; CALAN gate system), chewing behaviour (7 d; RumiWatch halters), and milk yield (20 d) started after an adaptation period of 14 d. ANOVA (SAS 9.4) included fixed effects (day and treatment), covariables for the respective dependent variable, and random effects (cow nested within the group). Covariables of cows were collected (DMI, 9 d; chewing behaviour, 3 d; and milk yield, 9 d) when they were being fed a covariate TMR before being switched to experimental feeding.

Total DM intake (21.9 vs. 21.3 kg/d) and ECM yield (30.1 vs. 28.5 kg/d) were higher ($P < 0.10$) in the hay than the silage feeding group. However, preservation method did not affect ($P > 0.10$) daily eating time (339 vs. 318 min) or rumination time (531 vs. 545 min), indicating no impact of the preservation method on the physical effectiveness. In conclusion, our data seem to show an advantage of hay feeding in terms of forage DM intake and energy output via milk, while the physical effectiveness of the diet was not impacted under current feeding conditions (*ad libitum*, low concentrate levels).

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The effect of energy and protein supplementation on feed Intake, digestibility and liveweight gain of Donggala bulls fed corn stover

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Donggala (*Bos indicus*) bulls is the local cattle breed raised by smallholder farmers in Central Sulawesi, Indonesia for cow- calf systems and fattening purposes. In fattening practice, farmers usually feed Donggala bulls with low quality roughages such as corn stover as the only feed stuff. Therefore supplementation with energy and protein is required to increase their liveweight gain. This study aimed to examine the effect of adding rice bran (source of energy) and palm kernel meal (source of protein) or their combination on feed intake, digestibility and weight gain of Donggala bulls fed corn stover. The experiment was done at an experimental farm located at Potoya village, Sigi district, Central Sulawesi province, Indonesia. Donggala bulls (n=28) average initial body weight (BW) 181±4.35 (SE) kg were stratified on BW and randomly allocated to 4 dietary treatments in a completely randomised block design (n=7 bulls/treatment). The dietary treatments were corn stover *ad libitum* (CS), CS plus rice bran (RB) at 1% of BW dry matter (DM)/day (d), CS plus palm kernel meal (PKM), 1% BW DM/d, and CS plus mixture of RB+PKM (1:1), 1% BW DM/d. Water was available *ad libitum* during experimental period. The experiment lasted for 16 weeks, in which 2 and 14 weeks were allowed for adaptation and collection periods respectively. The crude protein content of CS, RB and PKM was 74, 127 and 172 g/kg DM respectively, while neutral detergent fibre contents was 674, 452 and 634 g/kg DM, respectively. Data were analysed using MInitab statistical package.

Donggala bulls receiving RB, PKM and RBPKM consumed 0.92, 0.74 and 0.84 of their total diet allocation, respectively. Supplementation with energy and protein decreased ($P<0.05$) basal diet DM intake but increased ($P<0.05$) total DM intake, DM digestibility and average daily gain (ADG) of Donggala bulls fed CS. Basal diet intake of bulls fed CS, CS plus RB, CS plus PKM and CS plus RBPKM was 2.62; 2.14; 2.04 and 2.30% W/d, respectively. Bulls fed CS plus RBPKM had the highest ($P<0.05$) total DM intake, DM digestibility and ADG with value 3.34%BW/d; 69% and 0.63 kg/d, respectively. Bulls given CS only had lowest total DM intake, DM digestibility and ADG with value of 2.62 %BW/d; 57% and 0.34 kg/d, respectively. The results from this study clearly indicated that addition of combination of energy and protein can boost liveweight gain of Donggala bulls fed low quality roughage.

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External marker administration through an automated head-chamber system provides analogous estimates of fecal output compared to traditional hand feeding

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The objective of this experiment was to determine if titanium dioxide (TiO_2) dosed through an automated head-chamber system (AHCS; GreenFeed; C-Lock Inc., Rapid City, South Dakota, USA) is an acceptable way to administer a fecal output marker to grazing cattle. The AHCS used has a 2-bin bait feeding system. Bin 1 contained unmarked alfalfa pellets (UMP) and Bin 2 contained alfalfa pellets marked with 1% TiO2 on a dry matter basis (MP). Twelve heifers (body weight = 402 ± 5 kg) were stratified by body weight and then randomized to either 1) dosed with TiO2 by hand (HFD) or 2) dosed with TiO2 through the AHCS (AHCSD) for 19 days. During the morning (0800) all heifers were offered UMP at 0.25% of BW in individual feeding stanchions. The AHCSD heifers received a single dose (32 ± 1.6 g) of MP at their first visit each day to the AHCS system and all subsequent feeding was UMP. The HFD received only UMP during AHCS system visits but received their 32 g of MP during the morning feeding in the stanchions. On day 15, all heifers were dosed with a bolus containing 2.5 g of ytterbium chloride (Yb). Fecal samples were collected via rectal grab every 4 h for the first 12 h and then every 12 h till the end of day 19; concentrations of Yb in feces were fitted to an one-compartment, age-dependent model. There was statistical agreement (P = 0.04) and little difference (P = 0.43) between the HFD and AHCSD methods for the fecal output estimates. However, the among-animal variation differed between the 2 dosing methods (standard deviations of 0.1 and 0.7 kg/day, respectively). Increased variability associated with the AHCSD system probably resulted from greater variability in the dose timing because MP were consumed at appetency. There was a lack of agreement between the pulse dosed Yb and Ti fecal output estimates (P = 0.15), but they were also not statistically different (P = 0.30). Bland-Altman analysis showed an acceptable bias (-0.5 kg/day) between Ti and Yb fecal outputs, however, there were wide Limits of Agreement with large 95% confidence intervals, further indicating the lack of agreement between the two dosing methods. Labor savings associated with dosing cattle with indigestible markers through an AHCS may allow more use of the technique; however, caution is recommended due to the increased variability noted in fecal output estimates.

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Differential gene expression in three regions of the hypothalamus of steers with different protein and energy intake

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The hypothalamus controls feed intake in response to various physical, nutrient and hormone signals that are integrated in the arcuate nucleus (ARC). The ARC contains both orexigenic and anorexigenic neurons which communicate with the theoretical hunger (lateral hypothalamic area; LHA) and satiety (ventromedial hypothalamus; VMH) centres to control feed intake. Cattle in northern Australia have a 35 to 60% reduction in voluntary feed intake during the annual dry season (April to November) due to a very low crude protein [CP; 25 to 40g/kg dry matter (DM)] content of available pasture. This reduction in intake is likely regulated by metabolic mechanisms. It was hypothesised that gene pathways within the ARC, LHA and VMH of steers would be differentially expressed in response to the CP content of the diet and metabolisable energy (ME) intake.

Bos indicus steers (n=15; 194 ± 10kg liveweight, mean ± S.D.) were fed a high CP-high dry matter digestibility (DMD) diet *ad libitum* to provide unrestricted ME intake (HCP-HDMD-U), a low CP-low DMD diet *ad libitum* to provide unrestricted ME intake (LCP-LDMD-U) or a HCP-HDMD diet restricted to an equivalent ME intake of the LCP-LDMD diet (HCP-HDMD-R) for 98 days. Intake of steers was significantly different between all treatments ($P<0.001$; 28.5, 17.0 and 9.7 gDM/kg LW.day for HCP-HDMD-U, LCP-LDMD-U and HCP-HDMD-R respectively). ME intake was significantly higher ($P<0.001$) for steers fed the HCP-HDMD-U (0.24 MJ/kg LW.day) but was similar for steers fed the LCP-HDMD-U and HCP-HDMD-R (0.07 MJ/kg LW.day) diets.

Steers were euthanised and RNA was extracted from the ARC, VMH and LHA of these steers followed by preparation of cDNA libraries and subsequent sequencing on Illumina NovaSeq 6000 (producing 100 bp single end reads). FASTQ files were trimmed using 'trimmomatic' and mapped against *Bos taurus* reference genome release 9 using HISAT2 (average 56% reads mapped within genes). Within the ARC, 179 and 142 genes were differentially expressed between the HCP-HDMD-U and the LCP-LDMD-U and HCP-HDMD-R treatments, respectively. The VMH and LAT had minimal differentially expressed genes in response to dietary treatments. These data indicate the importance of the ARC in integrating signals in steers with different levels of ME intake.

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Estimation of nitrogen and phosphorus flows in dairy production with by-product feeding in Eastern China

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Dairy farms utilized many by-product feeds such as soybean hull, tea leave, ginkgo leave and molasses, in the intensive dairy production in Jiangsu Province in Eastern China. The objectives of this study were to estimate nitrogen (N), and phosphorus (P) budgets and nutrient flows in the by-products based dairy system in Eastern China. Face-to-face interviews and feed samples were conducted in the Tailaishen dairy company, where the average milk yield was 32 kg per day per head. The supplies of N and P from both local and external feeds and the retentions, products and excretions of dairy cattle were calculated on an individual animal level. The estimations of N and P flows on the farm scale were predicted by multiplying the individual N and P budgets by the number of dairy cattle. The results showed that the N and P intakes of each dairy cattle were 63.7 and 3.2 kg/year from local feeds, and 250.0 and 10.6 kg/year from external feeds, respectively. The productions of N and P were 53.5 and 9.6 kg/year, and the excretions of N and P were 260.2 and 4.1 kg/year, respectively. Thus, we found that local feeds provided less N and P (20.3 and 24.4% in total feeds, respectively) than external feeds on farm scale. The use efficiencies of N and P from feeds to products were 17.1 and 69.6%, respectively. Excretions of N and P in the form of manure were 134.7 and 30.6 t/year on farm scale. This study indicated that large amounts of N output through manure into the cropland and the N load from manure should be reduced for the development of sustainable agricultural production system in the study area.

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Effects of tannins in banana stalks on in situ digestion in dairy cows

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Banana stalks, the by-products of banana (*Musa sp*.) cultivation, are promising feed resources for ruminants. They contain tannins, which in suitable concentrations may be beneficial for ruminant protein utilization. The objective of this study was to compare the *in situ* digestive characteristics of alfalfa and banana stalks as well as to assess the impact of tannins in banana leaves and stems on the ruminal digestion in dairy cows. Banana stalks were combined with alfalfa in ratios of 0:100, 50:50 and 100:0. Polyethylene glycol (PEG) was added in each group to evaluate the effect of tannins. *In situ* dry matter (DM), neutral detergent fibre (NDF) and crude protein (CP) degradability were determined by incubating the conserved forages in 3 rumen cannulated Holstein cows for 0, 2, 4, 8, 12, 24, 48 and 72 h. Compared to alfalfa, banana stalks had lower soluble (*a*), potential degradable (*b*) fractions, fractional disappearance rate (*c*) and effective degradability (ED, $P<0.001$) of DM and CP. However, the ED of NDF was similar between alfalfa and banana stalks. Both banana stalks and the mixture forage with PEG had greater *a* fractions and ED of DM, NDF and CP than two groups without PEG ($P<0.001$). The results indicated that tannins in banana stalks could inhibit the ruminal degradation of forages and thus their level of inclusion level has to be controlled when fed to ruminants.

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O 57

Is there any evidence of glyphosate effects on rumen metaproteome and rumen microbial metabolism in vitro?

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Glyphosate is an organophosphorus compound which inhibits the enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) in the shikimate pathway leading to plant death. The fact that EPSPS is also present in microorganisms and fungi raises the question whether the rumen microbial ecosystem of cattle can be affected by residues of glyphosate-based herbicides (GBH) in feed. The rumen simulation technique (RUSITEC) with twelve fermentation vessels served as an appropriate *in vitro* investigation method. Nylon bags were filled with 15 g of a mixture of dried grass silage (49.5%), dried maize silage (39.7%), cracked wheat cracked (5.0%), dried soya cake (5.0%) and mineral feed (0.8%). The experiment was divided into an equilibration (6 days) followed by an experimental period (9 days). During the experimental period, three dosages (0.1 mg/l, 1 mg/l, 10 mg/l) of glyphosate (monoisopropylamine salt solution; 400 mg/ml**)** and of two GBH (Roundup LB plus and Durano TF; 486 mg/ml) were applied directly into the fermentation vessels daily. The substrate was free of glyphosate and the treatment solutions contained the expected amounts of glyphosate. Thus, per run three fermentation vessels served as controls and nine vessels received one of the above mentioned treatment. Four RUSITEC runs were conducted with randomly assigned treatments to the fermentation vessels. Totally, the runs represented the replicates (n = 4). Effects of treatment, glyphosate concentration, time and their interaction on metabolic parameters as well as on microbial protein synthesis and metaproteomics were statistically analyzed using the Mixed Models Analysis (SAS Enterprise Guide). Values were regarded as significant at $p < 0.05$. All measured values of redox potentials, pH, NH_3-N concentrations, total SCFA production, molar SCFA proportions, degradation of crude nutrients and microbial protein synthesis were in the physiological range known for RUSITEC experiments. None of these parameters were significantly affected by the treatment or the glyphosate concentration. Significant effects of the factor time were found for pH, redox potentials, NH_3-N concentrations, total SCFA production and the molar proportions of propionate, isobutyrate, butyrate, isovalerate and valerate. The functions and taxonomic composition of the rumen microbiota, as separately analyzed on the protein level by metaproteomics for liquid and solid associated microorganisms, did not change. This is in accordance with the results of the metabolic parameters. In conclusion, no negative effects of the GBH or glyphosate alone on rumen microbial metabolism, the microbial protein synthesis or the metaproteome could be identified *in vitro*.

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O 58

Daily shifts in composition and function of the rumen metaproteome in liquid and solid rumen fractions

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The assembly of the rumen microbiome and its metabolic activity varies between individuals, rumen fractions and animal diets. The microbiome also changes with increasing age and varies substantially during the course of each day. Examination of diurnal fluctuations in arrangement and function of bacteria and archaea is of relevance for future studies and may allow an improved assessment of sampling times while providing further insights into the relationship of ruminants and their microbial communities.

At 7.30 each day, three rumen cannulated, lactating Jersey cows were fed ad libitum with 48% grass silage and 52% concentrate including permanent access to water. After 20 days of adaptation, rumen matter was taken from five positions at six time points within 24 hours and squeezed using disposable gloves to obtain the liquid fraction while the remains constituted the solid rumen fraction. Equal parts of the five liquid and solid fractions were pooled. Subsequent to enrichment of prokaryotic cells, extracted peptides were analyzed by high-resolution mass spectrometry (LC-MS/MS). Identification and quantification of proteins was optimized by a two-step search approach. Additionally, raw data were searched against a recently published collection of ruminal metagenome-assembled genomes. Functional assignments were performed using GhostKOALA, eggNOG-mapper and dbCAN metaserver.

Average daily feed intake ranged from 21 to 32 kg. Significant differences in phylogeny and metabolic functions of the active bacteria were detected between time points. Diurnal fluctuations were more evident in the liquid fraction. Tremendous taxonomical shifts occurred in protein abundance ratio of the major bacterial phyla, Bacteroidetes and Firmicutes. Fiber-degraders associated with the solid fraction appeared resilient to diurnal changes. Regarding feeding time, ribosomal proteins increased in abundance whereas proteins belonging to the starch and sucrose metabolism as well as ABC transporters decreased. The abundance of CAZymes exhibited a diurnal pattern further emphasizing differences between rumen fractions. Proteins involved in acetate production increased in abundance after feeding while butyrate producing enzymes decreased. Archaeal proteins were less affected by diurnal changes. Independent bioinformatic search databases resulted in various but complementary protein identifications.

The current study accentuates the importance of daily fluctuations for appropriate rumen sampling. Our findings reveal that the time point of feeding is the most determinant factor regarding the diurnal shifts. An increased availability of substrates from ingested feed leads bacteria to focus rather on growth instead of further intensifying nutrient uptake. Separate bioinformatic data processing procedures highlight the importance of sample-specific search databases for metaproteomics.

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O 59

MitoCow - Longitudinal characterization of ruminal and duodenal microbiota and metabolites in LPS stimulated transition dairy cows.

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Introduction: The dairy cow is exposed to multiple environmental and physiological stressors, such as the metabolically challenging transition phase and microbial infections impacting health and performance. Besides the animal itself, the dynamics of the microbial community of the gastrointestinal tract under stress situations is less studied so far. The microbiota plays a crucial and indispensable role for the cow and it is of importance to shed more light in the interaction between both. This study investigated the ruminal and duodenal microbiota during calving and an LPS induced inflammation as two different types of stressor in the same individuals. The intensive sampling enabled the detection of changes in the microbiota revealing the power of these stress situations.

Methods: This work is part of the MitoCow project, which aims to elucidate the functionality of the cow's mitochondria during individual and standardized stress situations, interlinking a broad variety of metabolic, physiologic and genetic data. Further information about the animal trial can be taken from Meyer et al. (Conference proceedings ISRP Leipzig 2019). Out of this project, eight rumen and duodenum fistulated Holstein cows were sampled at day 42, 14 a.p.,14, 100, 118 and 126 p.p. and 12, 24, 72 hours after calving and LPS injection (day 111, using 0.5 µg/BW). The cows were divided into a control and carnitine supplemented group (n=4 each). Bacterial DNA was extracted from all samples. The 16S rDNA gene was sequenced by Illumina amplicon sequencing. Sequences were clustered into operational taxonomic units (OTUs). Nuclear magnetic resonance technique (NMR) was used for metabolomic analyses.

Results: Sequence data leads to 17 bacterial phyla, 22 families and 1,990 OTUs. The microbiota in the rumen and duodenal fluid changed remarkably during the transition ($p=<0.001$), but the microbial diversity was not affected during the LPS challenge. Carnitine supplementation did not reveal an impact on the microbial composition. Clear animal individual shifts were noted during the complete trial period. Clear differences were seen between different time points as well as for single OTUs such as *Fibrobacter succinogenes* spp.. Forty metabolites, such as fatty and amino acids, amines and urea, which were identified by NMR showed clear shifts along the trial period.

Conclusions: Applying Illumina sequencing and NMR, we could demonstrate that microbial communities and metabolites changed differentially at the two stress situations. It is the first long-term challenge study comparing microbial and ruminal metabolic interaction during the critical transition time of the dairy cow.

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O 60

Metabolism of linolenic, linoleic and vaccenic acid by pure cultures of rumen bacteria

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To meet the high energy requirements of our high yielding dairy cows, grains and fats have been increasingly incorporated in ruminant diets. Moreover, lipid supplements have also been included in ruminant diets to increase concentrations of human-health promoting n-3 fatty acids and bioactive CLA in milk and meat. However, such feeding practices might result in alterations in the rumen biohydrogenation of poly-unsaturated fatty acids resulting in the build-up of *trans*-10 intermediates at the expense of *trans*-11 intermediates. This so-called *trans*-11 to *trans*-10 shift is often associated with milk fat depression in dairy cattle. Nevertheless, the microbial etiology of this condition is not well understood. Therefore, the aim of this study was i/ to identify rumen bacteria that produce trans-10 intermediates from 18:3n-3, 18:2n-6 or trans-11 18:1, and ii/ to elucidate the influence of lactate on 18:2n-6 metabolism. Based on previous studies, it was hypothesized that at least some of the investigated strains produce trans-10 intermediates. Furthermore, it was hypothesized that lactate-utilizing bacteria would grow better under lactate-enriched conditions and would alter their metabolism and convert 18:2n-6 to trans-10 intermediates. Pure cultures of 30 rumen bacterial species were incubated individually in the presence of 40 µg/mL 18:3n-3, 18:2n-6 or *trans*-11 18:1 under control or lactate-enriched (200 mM Na lactate) growth conditions. If available, bacterial strains isolated from the rumen were used, if not, strains originated from the gut or feces from other animals or from human tissue. The incubation was stopped after 24 h, after which subsamples were analyzed for long-chain fatty acid composition by gas chromatography. Of the 30 studied strains, *Propionibacterium acnes* was the only bacterium found to produce *trans*-10 intermediates from 18:3n-3 (i.e. *trans*-10, *cis*-12, *cis*-15 CLnA) and 18:2n-6 (i.e. *trans*-10, *cis*-12 CLA), irrespective of the growth condition. None of the other bacteria produced *trans*-10 intermediates from 18:3n-3, 18:2n-6 or biohydrogenation intermediates, irrespective of lactate supplementation. Other bacteria were confirmed or found to produce either *trans*-11 intermediates (i.e. *Butyrivibrio fibrisolvens*, *Butyrivibrio proteoclasticus* and *Sharpea azabuensis*) or hydroxy FA (i.e. *Bifidobacterium adolescentis*, *Bifidobacterium pseudolongum*, *Streptococcus equinus*, *Streptococcus gallolyticus* and *Megasphaera elsdenii* 2602A and 5052B) from 18:3n-3 and 18:2n-6, which were not further metabolized to *trans*-10 intermediates by mixed rumen inoculum.

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O 61

Effect of a fodder beet versus ryegrass-dominant diet on placental and fetal arginine metabolism in sheep

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Arginine has multiple metabolic fates and is, therefore, one of the most versatile amino acids serving as a precursor for the synthesis of protein, nitric oxide, creatine, poly amines, and urea. During pregnancy, arginine directly supports fetal growth and is used by the placenta to produce nitric oxide, a key promoter of angiogenesis and blood flow, and thus placental nutrient/oxygen exchange. The objective of this study was to determine whether lower birth-weight of lambs born to ewes grazed on fodder beet (low protein diet) compared to ryegrass-based pasture from mid-late pregnancy to term was associated with changes in arginine metabolism using a metabolomics approach. Twin-bearing ewes were grazed on either ryegrass-dominant pasture plus supplementary ryegrass/clover hay (RG) or 100% fodder beet plus supplementary ryegrass/clover hay (FB; from 100 days of gestation to term (n=100/group). At 135 days gestation, a subset of 10 ewes per treatment was euthanised and blood samples collected immediately post-mortem from the placental artery and vein, and the fetus via cardiac puncture. Plasma samples were subjected to metabolomics analysis using the AbsoluteIDQ p180 kit of Biocrates Life Science AG. Fetuses from FB ewes had 1.9-fold ($P<0.001$), 1.3-fold ($P<0.05$), and 1.3-fold ($P=0.08$) lower plasma arginine, ornithine and citrulline concentrations than their RG counterparts. Symmetric dimethylarginine (SDMA), an indirect inhibitor of nitric oxide synthesis that reduces arginine availability to nitric oxide synthase (NOS), was 1.6-fold higher ($P<0.001$) in fetuses from FB- than RG-fed ewes. Plasma concentrations of arginine, ornithine and citrulline were lower in the placental artery (1.8-, 2.4-, and 2.0-fold, respectively, $P<0.001$) and placental vein (1.8-, 1.8-, and 2.1-fold, respectively, $P<0.001$) in FB than RG-fed ewes. Asymmetric dimethylarginine (ADMA), a direct inhibitor of NOS, was 1.3- and 1.2-fold lower in placental artery and vein plasma respectively in FB compared to RG-fed ewes ($P<0.05$), while SDMA was 1.3-fold greater ($P<0.05$) in placental vein plasma with a tendency for 1.3-fold higher ($P=0.08$) concentrations in placental artery samples from FB- compared to RG-fed ewes. Collectively, these results indicate that the previously observed reduction in birth-weight of lambs from ewes grazed a FB diet in mid-late pregnancy may be driven by reduced placental transfer of arginine, citrulline, and ornithine to the fetus. Elevated concentrations of SDMA in both the fetus and placental artery/vein plasma indicate that restricted fetal growth in FB-fed ewes may also be mediated by inhibition of arginine transport, blood flow, oxidative stress and/or inflammatory responses.

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The bacterial community dynamics in a subacute ruminal acidosis evaluated by Rumen Simulation Technique (RUSITEC)

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The rumen simulation technique (RUSITEC) is a well-established semi continuous *in-vitro* model for investigating ruminal fermentation and microbial dynamics. Here we investigated the dynamics in the bacterial community during a subacute ruminal acidosis (SARA) model in the RUSITEC. To get a better taxonomic resolution of the most abundant phylotypes present in the RUSITEC fermenters, for the first time, we combined Illumina 16S rRNA amplicon sequencing (variable regions 3/4) to cover the full bacterial diversity and PacBio 16S rRNA full-length sequencing.

Eight fermenters were used to evaluate combinations of two different SARA buffers, which were reduced in buffer capacity, and one standard buffer with a low concentrate diet, a high concentrate diet or a switch from a low concentrate diet in the control phases to a high concentrate diet in the SARA challenge. To do so, three fermenters were infused with SARA buffer 1, three fermenters were infused with SARA buffer 2, and two fermenters were infused with the standard buffer during the SARA challenge. After seven days of equilibration, samples were collected on days 12 (after the first 5-day control phase), 17 (after the 5-day SARA challenge), and 22 (after the second 5-day control phase). The experiment was repeated four times and pH was measured on a daily base.

The pH remained stable within the physiological range (about 6.7) during both control phases for all treatment groups as well as during the SARA phase for the two groups infused with standard buffer. In groups infused with the SARA buffers, pH decreased during SARA challenge and stabilized below the SARA threshold of pH 5.8 from day 15 to 17. Initial values were regained within 2 days after SARA challenge. Species richness and diversity were lower during the SARA challenge compared to the control phases. The weighted UniFrac distances also revealed a separate clustering of the samples collected from SARA buffer treated groups during the SARA challenge compared with samples from both control phases. There was no difference between SARA buffers; neither did the concentrate-forage ratio affect the overall community composition.

We conclude that the shifts in species richness and diversity were mainly caused by the lower pH in the fermenters infused with SARA buffer 1 and 2.

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The effects of concentrate feeding during the close-up period on the rumen function and metabolic adaptation of dairy cows

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We tested the hypothesis that concentrate supplementation of grass silage-based close-up diet primes rumen for high concentrate feeding after calving and improves adaptation to lactation.

During the 3 wk close-up period, 16 multiparous cows received ad libitum feeding of grass silage (11.4 MJ ME/kg DM) either as sole feed (GRASS) or supplemented with increasing amount (1 to 4 kg/d) of concentrate (CONC). After parturition, both groups were offered grass silage ad libitum and similar amount of concentrate. Feed intake and rumination time were recorded daily. Reticular pH was monitored with wireless sensors. Blood was sampled via tail vessel puncture at d -21, -7, +1, +10 and +21 relative to parturition. Oral and faecal microbiota samples were collected at d -21, -3, and +10 relative to calving. Composition of oral and faecal microbiota, analysed with 16S rRNA amplicon sequencing, were used as proxies for rumen and hindgut microbiota. The bacterial alpha diversity was determined using Shannon diversity index and composition of microbiota was analysed using linear models. Feed intake, rumination time, rumen pH and blood composition were analysed by ANOVA.

Silage dry matter intake (DMI) did not differ between the treatments prepartum, while total DMI was higher in CONC (12.4 vs. 10.8 kg/d). Daily rumination time was shorter in CONC than GRASS (395 vs. 465 min) prepartum. No differences in total DMI (21.6 kg/d) and rumination time (439 min/d) were observed postpartum. Average reticular pH was similar between treatments pre- and post-partum, with no indications of subclinical rumen acidosis. Oral or faecal microbial diversity did not differ between the treatments. However, potentially important differences were observed in the abundances of specific bacterial taxons. In the oral microbiota 3 d prior to calving, streptococci and Gemella, and in faeces 10 d after calving, Ruminococcaceae, Prevotellaceae and Rikenellaceae were more abundant in CONC than GRASS.

Plasma insulin (37.4 vs. 16.7 µIU/ml) and BHBA (0.59 vs. 0.36 µIU/ml) were increased in CONC relative to GRASS, and NEFA decreased (0.16 vs. 0.23 mmol/l) at d 7 prior parturition. No differences were observed in blood composition postpartum.

In conclusion, concentrate supplementation prepartum induced transient changes in rumination time, composition of gastro-intestinal microbiota, and concentrations of insulin and energy metabolites. Reticular pH and feed intake during early lactation were not affected. The results suggest no substantial benefit of concentrate supplementation of high-digestibility grass silage during the close-up period.

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Effectiveness of rumen community DNA extraction methodologies and the analysis of differing sampling points as indicators of the rumen microbiome

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Throughout the entire length of the gastrointestinal tract of ruminants a complex environment of dynamic microorganisms can be found as part of the host microbiome. Previous studies have observed correlations between the diversity of the rumen microbiome and a variety of effects, from feed efficiency to host health. In recent years, metagenomics has become the primary methodology to examine and analyse the microbiome. Here we investigate some of the challenges involved in sampling of the ruminant GI tract, using Faecal, Oral and Rumen sampling. The effectiveness of several available methodologies has been examined and the extracted community DNA has been examined for quality, yield and the presence of inhibitors that could affect downstream processes such as sequencing. Further to this, DNA samples were then utilised to see if it was possible to draw conclusions from less invasive sampling methods. Due to the invasiveness of direct rumen sampling methodologies, the use of alternative sampling sites could offer a useful indication of the overriding makeup of the rumen microbiome, what organisms are present, and their relative abundance. Most importantly, it is imperative that samples be processed in a consistent manner in order to reduce variability, and the use of universal standards is imperative if meaningful comparisons are to be made.

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Resin acid composition increases propionate and butyrate production in a Rumen Simulation Technique (RUSITEC) model

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Tall oil fatty acid -based resin acid composition (TOFA) with 9% coniferous resin acids is a known microbial modulator and performance enhancer for chickens and pigs, but the effects of resin acids on rumen fermentation are incompletely understood. Here we studied the effects of TOFA on the ruminal fermentation of dairy cows in a 16-day Rumen Simulation Technique (RUSITEC) experiment.

Six 750-ml RUSITEC fermentation vessels were loaded with substrate bags containing 6 g hay and 4 g concentrate feed, and seeded with fresh bovine rumen fluid. After an equilibration phase of eight days, either TOFA (330 µl/day) or ethanol (550 µl/day) was added to the fluid of the fermenters during the daily exchange of the substrate bags. Ethanol was used as a solvent for another treatment not included here. Earlier studies suggest no effects on the used level of ethanol. On days 8-16, the vessels were measured for redox potential, pH, NH_3-N and short chain fatty acid (SCFA) concentrations, gas volume and percentages of CH_4 and CO_2. Organic matter degradation was analysed from substrate bags of days 9 - 11, and 13-15. On days 8, 12 and 16, the vessels were sampled for 30 ml of fluid and the substrate bags for liquid- and solid-associated microbes, respectively. Microbial DNA was extracted and subjected to microbial population analysis by Illumina MiSeq 16S rRNA amplicon sequencing.

The pH and the redox values stayed at the physiological range of 6.6 to 6.8 and -270 mV to -290 mV, respectively, with no treatment effects. The total SCFA production was similar in both treatments, but TOFA significantly increased the production rates and the molar proportions of propionate and butyrate, and decreased those of isovalerate. The molar proportion of acetate decreased significantly by TOFA. Degradation of organic matter and the concentrations of NH_3-N, gas volume and percentages of CH_4 and CO_2 were not affected by TOFA. The treatments showed no differences in relative abundances of microbial phyla at the individual time-points of either the liquid or solid phase. Analysis for the operational taxonomic units (OTUs) revealed only a few transient changes as a response to TOFA.

In conclusion, TOFA shifted the fermentation towards propionate and butyrate production but did not have other major impacts on fermentation in this model. Increased ruminal propionate production has been linked to increased capacity of milk production. Studies with lactating dairy cows are needed to verify such an effect for TOFA.

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Supply of methionine during late-pregnancy alters fecal microbiome and metabolome in neonatal dairy calves without changes in daily feed intake

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Maternal methionine supplementation in pregnant sows induced distinctive changes in the hindgut microbiome in piglets which likely supports better growth performance during the preweaning period. However, the influence of maternal methionine in dairy cows on hindgut microbiome of neonatal calves is unknown. Therefore, the objective of the current study was to evaluate the impact of maternal methionine supply during late-pregnancy in dairy cows on fecal microbiome and metabolome in neonatal calves, and its association with body development and growth performance during preweaning period. To achieve this, 26 heifer calves, i.e. neonatal female offspring, born to Holstein cows receiving either a basal control (CON) close-up diet (1.47 Mcal/kg DM and 15.3% CP) with no added methionine or CON plus methionine added in the form of ethyl-cellulose rumen-protected methionine (MET, Mepron®, Evonik Nutrition & Care GmbH, Germany) at a rate of 0.09% of dry matter intake during the last 28 d of pregnancy were selected for the current study. That rate ensured lysine:methionine ratio in metabolizable protein was close to 2.8:1 which is based on recent experiments demonstrating a benefit of this ratio in terms of production performance and health. We collected fecal samples from heifers from birth until 6 weeks of age to study changes in fecal microbiome and metabolome during preweaning period. Fecal microbiome was analyzed with QIIME 2 whereas fecal metabolites were measured using LC-MS untargeted approach. At birth, MET heifers had greater ($P \leq 0.05$) body weight (BW), hip height (HH) and wither height (WH) than in CON group. During the preweaning period, no differences between groups were detected for starter dry matter intake ($P = 0.77$). However, MET heifers maintained greater ($P \leq 0.05$) BW, HH and tended ($P = 0.06$) to have greater WH and average daily gain (ADG) ($P = 0.10$). Fecal microbiome and metabolome profiles through 42 days of age in MET heifers indicated greater capacity for hindgut production of endogenous antibiotics. The positive effect of maternal methionine on the enrichment of Nocardioidaceae and Amycolatopsis in the hindgut, well-known bacteria for producing antibiotics, underscores the protective effect of maternal methionine against pathogens in newborn hindgut. Furthermore, maternal methionine induced greater energy production in MET heifers. For example, MET heifers had greater butyrate-producing bacteria such as Ruminococcus and Fusobacterium, suggesting a better capacity for generating this important volatile fatty acid in the hindgut. Enhancing maternal methionine supply during late-gestation in dairy cows has a positive effect on hindgut functionality and health in their offspring through alterations in the fecal microbiome and metabolome without affecting feed intake. Those alterations could limit pathogen colonization of the hind-gut while providing essential nutrients to the neonate. Together, such responses contribute to the ability of young calves to achieve better rates of nutrient utilization for growth.

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Effects of $CoCO_3$ on rumen fermentation and hematopoietic function in lactating dairy cows

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Introduction: Cobalt is an essential nutrient for the synthesis of vitamin B12. Deficiency of vitamin B12 reduced average feed intake and daily gain (Wang et al., 2007). Supplementing with 0.5 mg/kg cobalt sulfate ($CoSO_4$) increased plasma vitamin B12 and glucose of lambs (Bishehsari et al., 2010). However, effects of different dietary cobalt carbonate ($CoCO_3$) levels on blood metabolites and rumen bacterial composition in lactating dairy cows are unclear.

Material and Methods: This experiment was conducted to determine the effects of different levels of $CoCO_3$ supplementation on blood parameters and ruminal bacterial community in lactating dairy cows. Sixty lactating Holstein dairy cows with similar body condition, parity, lactation stage and milk yield were blocked and randomly allocated into 5 groups with different supplementation levels of $CoCO_3$ [0, 6, 12, 30, 60 mg/d per cow]. The ratio of dietary concentrate to roughage was 40:60, the average DMI was ≈18 kg. The experiment was kept up to 56 days, including 14d of adaptation period. Data were analyzed using SPSS 22.0. $P<0.05$ was used as the significant threshold.

Results: Vitamin B12 content in blood and milk was numerically increased, and red blood cells (RBC), haematocrit (HCT) and haemoglobin (HGB) in blood samples increased by adding $CoCO_3$ ($P < 0.05$). $CoCO_3$ addition significantly increased microbial crude protein (MCP) yield ($P < 0.05$). In contrast, $CoCO_3$ levels ranged to 60 mg/d did not change chao1 and Shannon indices, the relative abundance of phylum. Furthermore, 30 mg $CoCO_3$ increased Ruminiclostridium_5 and Defluviitaleaceae_UCG-011 genera relative abundance, however, $CoCO_3$ levels increased to 60 mg, their abundance dropped ($P < 0.05$).

Conclusion: The $CoCO_3$ addition enhanced hematopoietic function, did not dramatically change rumen bacterial community. The optimal supplement level of $CoCO_3$ in the dietary is 30 mg/d.

Wang RL, Kong XH, Zhang YZ, Zhu XP, Narenbatu, ZH Jia 2007. Influence of dietary cobalt on performance, nutrient digestibility and plasma metabolites in lambs. Animal Feed Science and Technology 135, 346–352.
Bishehsari S, Tabatabaei MM, Aliarabi H, Alipour D, Zamani P, Ahmadi A 2010. Effect of dietary cobalt supplementation on plasma and rumen metabolites in Mehraban lambs. Small Ruminant Research, 89(1), 170-173.

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P 188

Using high throughput sequencing to describe protozoal communities in RUSITEC fermenters fed two different diets

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Novel techniques of sequencing have given researchers a useful tool to assess the composition of microbial populations. In the case of ruminant's gastrointestinal tract, these techniques are widely used to study bacteria and archaeal populations but the rest of microbial are less studied. Olive cake is a by-product of oil extraction that contains bioactive compounds with antimicrobial and antioxidant activities that could be beneficial for the animals, but might also affect the ruminal microbial communities. The aim of this study was to assess protozoal populations in Rusitec fermenters when they received either a conventional diet for dairy sheep (50:50 forage:concentrate) or a diet including 16.7% of olive cake (OC) in replacement of corn silage. Diets were incubated in four Rusitec fermenters in a cross-over design with two 14-day incubation periods. Fermenters were given daily 30 g of diet, and in each period half of them received the diet with no olive cake (CON) and the other half received the OC diet. Samples of solid and liquid digesta were collected from four donor sheep and from the fermenters at the end of the incubation period, DNA was extracted, and protozoal profiling was performed using 18S rRNA gene amplicon sequencing. The reads generated were processed using the FROGS pipeline. Only reads that correspond to protozoa were kept for further analysis using R and the Phyloseq package. Protozoa were detected only in sheep inocula and liquid samples from CON fermenters. In the inocula, number of reads was more than 20 times higher in liquid samples comparing to the solid ones and more than 99% of reads in the solid inocula were unidentified protozoa. Six genera (*Ophyroscolex, Entodinium, Polyplastron, Isotricha, Dasytricha* and *Enoploplastron*) were detected in the liquid inocula, and only 4 were present in the CON-fermenters liquid samples. *Ophyroscolex* was the most abundant (55.40% of reads, median values) genus in liquid inocula samples followed by *Polyplastron* (10.70%). In CON-fermenters *Entodinium* was the most abundant genus (57.44%) followed by *Ophyroscolex* whose abundance was lower than in the inocula (30.49%). In summary, a loss in protozoal diversity was detected in Rusitec fermenters over the incubation period and a defaunation effect was observed when OC was included in the diet. High throughput sequencing seems to be an efficient tool to characterise protozoal populations in ruminal samples.

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Using the oro-ruminal FLORA® device, volatile fatty acid proportions but not concentrations and pH were valid in rumen fluid samples

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Five rumen cannulated lactating Holstein cows were used to validate the intra-ruminal sampling site of the oro-ruminal FLORA device (Geishauser et al., 2012). Rumen pH, volatile fatty acid (VFA) concentration, and VFA proportion were determined in obtained samples. All cows were fed a diet (on dry matter basis 31.4, 23.9, and 44.8 % of maize silage, grass-clover silage, and concentrates, respectively) ad libitum once daily. Dry matter intake averaged (± SD) 22.1 ± 2.1 kg/day during a seven-day sampling period. Rumen fluid samples were obtained using the FLORA device and concurrently from the ventral ruminal sac via the rumen cannula using a suction strainer mounted with a syringe. Three sets of samples were obtained with two to three days interval. The FLORA device was rinsed and functionality secured between each sampling. Saliva contamination of the FLORA sample was scored visually (0-100 %) based on viscosity, mucus, and colour. When determining the intra-ruminal location of the FLORA sampling cup, firmness of rumen content was scored from soft to firm depending on the force needed to introduce the hand into the rumen mat. Data was analysed using a model containing sampling method, sampling day, and their interaction as fixed effects, cow as random effect, and sampling day as repeated measure.

Firmness of rumen content was assessed as soft in one cow and as normal to firm in the other four cows. The FLORA sampling cup was only located in the ventral sac in the cow having soft rumen contents, whereas it was located in the atrium and middle to dorsal sac in the other cows. Compared to the FLORA device, pH was 0.51 units lower ($P < 0.01$) and concentration of total VFAs was 27.3 mM higher ($P < 0.01$) when using the strainer. Sampling method did not affect the proportion of individual VFAs. In FLORA samples, degree of saliva contamination was positively correlated to pH ($r = 0.52$, $P < 0.05$) which probably caused a dilution of VFAs in the samples taken.

In conclusion, rumen fluid taken in the ventral rumen sac had comparable VFA proportions to samples taken with the FLORA device, but had lower pH and higher absolute concentrations of VFAs, likely due to saliva contamination. The intra-ruminal site was not consistent for the FLORA device.

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P 190

Influence of different sources of copper and zinc on *in vitro* rumen fermentation characteristics at optimal and suboptimal pH level.

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Introduction: For high milk producing dairy cows, optimal fermentation in the rumen is required. Rumen fermentation can be influenced by pH and trace elements like copper (Cu) and zinc (Zn). In this study, the effects of different Cu and Zn additives, on gas production (GP) and fermentation end products, under optimal and suboptimal pH conditions, were evaluated *in vitro*.

Materials and methods: Five different sources of inorganic (sulphate and hydroxychloride) and organic (glycinate, amino acid complex and proteinate) Cu and Zn were tested. Two buffers were used to compare *in vitro* fermentation at optimal rumen pH of 6.6 (CONE bicarbonate-phosphate buffered rumen fluid) and in a suboptimal situation with pH level of 5.8 (SARA buffered rumen fluid). Supplementations of 40 mg/g and 100 mg/g Cu and Zn, respectively, were added to a substrate of dried grass silage (500 mg) and incubated in buffered rumen fluid in triplicate for 72 h at 39°C. Real time gas production was measured, using an automated gas production system, and controls without added trace elements were included. At the end of the experiment, pH and volatile fatty acid (VFA) concentrations were measured.

Results: Cu and Zn hydroxychlorides inhibited GP ($P=0.012$) and total VFA ($P<0.001$) only under SARA conditions (reduction in GP of 17%), whilst sulphates and organic sources all strongly depressed GP and total VFA under either optimal (CONE, $P<0.001$) or suboptimal (SARA, $P<0.001$) pH conditions, compared to the control (reductions in GP all exceeding 82%). Additionally, control and hydroxychlorides were the only two treatments that showed a multiphasic response in GP, whereas all other treatments stopped fermentation within 1.6 h and only revealed GP data with a monophasic response, indicating affected microbial activity.

As intended, pH at the end of fermentation differed between buffers (mean pH of 6.59 and 5.95 in CONE and SARA, respectively). Also differences in relative proportions of individual VFA could be observed, depending on used buffer and on trace element source.

In conclusion, this study demonstrated that the extent of *in vitro* GP was depressed at suboptimal pH levels. Of the 5 different sources of trace elements tested, hydroxychlorides clearly distinguished themselves by their low influence on rumen fermentation and this only under suboptimal pH condition, whereas Cu and Zn sulphate, glycinate, amino acid complex and proteinate all strongly inhibited fermentation and altered fermentation characteristics both under optimal and suboptimal pH conditions.

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Residual feed intake and rumen bacterial diversity in lactating sheep: A preliminary study of their potential link

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Improvements in sustainability include the enhancement of feed efficiency, which in ruminant livestock might be associated with the diversity of the rumen microbiome. Results in the literature are inconsistent; efficient animals have been related with both high and low microbial diversity. However, no studies are available in dairy ewes. Therefore, we aimed at examining the relationship between feed efficiency, measured as residual feed intake (RFI), and rumen bacterial diversity in lactating sheep. The latter was estimated through the richness, Shannon and evenness indices, using T-RFLP and 3 enzymes: *HhaI, MspI* and *HaeIII*. The RFI is defined as the difference between the actual and expected feed intake, which was determined from feed requirements for maintenance and lactation according to the AFRC system. Animals with lower RFI eat less than predicted, being more efficient. Data from two studies performed by our team (with 20+15 animals fed a 50:50 F:C diet) were employed. Regression analyses were conducted to investigate the link between RFI and diversity measures. RFI averaged 0.29±0.062 (range: -0.75 to 1.01) and showed no significant relationship with most diversity indices. However, trends to significance ($P<0.10$) were observed in the quadratic regression using all data and the evenness index with *HhaI* ($R^2=0.15$) and in the linear regression using data from one experiment and the Shannon index with *MspI* ($R^2=0.15$). Furthermore, a significant quadratic relationship ($P<0.01$) was found for data from one experiment and richness with *MspI* ($R^2=0.43$). In the linear regression, RFI decreased (efficiency increased) as diversity augmented. In the two quadratic correlations, however, the most efficient animals were those with the greatest and least diverse bacterial communities, which might be associated with apparent inconsistencies in the literature. In this regard, one hypothesis postulates that a greater microbial diversity might also imply a larger diversity in microbial metabolic networks that would favor a better use of metabolites by the host. Conversely, another hypothesis suggests that a less diverse microbiome may use less substrate and energy for microbial proliferation and direct more nutrients to the host. In this study, constraints related to the number of experiments and individual animals, the qualitative methodology to measure diversity, and the use of only taxonomic information may have hindered a clearer outcome. Nonetheless, our results might support a non-linear association between feed efficiency and rumen bacterial diversity in dairy ewes, suggesting a more complex scenario than initially expected. Further studies are warranted.

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Changes in the ruminal fermentative activity by including urea as a protein replacement in fattening lamb diets

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Rumen contents from twelve lambs fed three isonitrogenous diets (n=4) were used as inocula for in vitro incubations to study fermentation of different substrates. Diets consisted of a standard fattening diet (CON), a diet with 0.6 % urea (URE1) and a diet with 0.95% urea (URE2) partially replacing soybean meal of CON. Substrates of varying composition (CON diet, starch, cellulose or pectin) were incubated in vitro (300 mg of substrate) in batch cultures inoculated with rumen fluid from lambs fed one of the experimental diets. Gas production, volatile fatty acid (VFA) production and ammonia concentration were measured after 24 h of incubation. Data were subjected to one-way ANOVA using a mixed model with diet as the fixed effect and animal as random effect. No differences (P>0.05) were observed in ammonia or VFA concentrations, VFA molar proportions or pH in the ruminal fluid used as inocula. When the CON diet was incubated no differences (P>0.05) were observed among the three inocula in the fermentation parameters. Gas production ranged from 2.1 to 2.2 mol/300 mg substrate, ammonia concentrations from 352 to 391 mg/L and total VFA production from 2.63 to 2.80 mol. Accumulation of fermentation end-products in the cultures was not affected by the source of inoculum when either pectin or starch were incubated. Conversely, when cellulose was incubated, the amount of gas produced decreased linearly (P<0.01) from 2.1 mol/300 mg substrate with the CON inoculum to 1.9 (URE1) and 1.2 mol/300 mg substrate (URE2) in cultures inoculated with rumen liquid from lambs fed diets in which soybean was partially replaced with urea. Concurrently, VFA production was also linearly decreased (P<0.01) as urea was increased in the diet of rumen donor lambs (2.51 mol/300 mg substrate for CON, 2.26 mol/300 mg substrate for URE1 and 1.39 mol/300 mg substrate for URE2). Only the molar proportion of butyrate was affected by the source of inoculum when cellulose was incubated, with no differences in the molar proportions of other VFA or in the acetate to propionate ratio. The results suggest a similar fermentative activity in the rumen contents of lambs fed the three diets, except for an apparent decreased degradation of cellulose when soybean meal was substituted for urea as a replacement for a part of the protein in fattening lamb diets.

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Effects of replacing barley maize by citrus pulp in a dairy sheep diet on microbial populations in RUSITEC fermenters

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Citrus pulp is a by-product highly available in the Mediterranean area. It is rich in rapidly degradable carbohydrates and it has been used in ruminant diets replacing partially the cereals with no negative effects on animal performance, but there is little information of its effects on microbial populations in the rumen. Two 50:50 forage:concentrate diets were used, with the concentrate containing either maize (20% fresh matter basis) or being totally replaced by citrus pulp. Four Rusitec fermenters were used in a cross-over design with two 14-day incubation periods, and three rumen-cannulated Merino sheep were used as ruminal content donors for inoculating the system. Samples of liquid and solid digesta were collected from the fermenters at the end of each incubation run. DNA was extracted and the abundance of bacteria and protozoa, as well as the relative abundance of fungi and archaea, were assessed by qPCR. Bacterial diversity was analysed using Automated Ribosomal Intergenic Spacer Analysis (ARISA). Data were processed using R with the vegan package. The number of peaks detected in the ARISA electropherograms and the Shannon's index were not affected by the inclusion of citrus pulp in the diet ($P>0.05$). When representing the principal coordinates analysis based on Bray-Curtis distance, samples clearly grouped according to diet, suggesting different bacterial community composition. Abundance of bacterial and protozoa populations were similar in both diets in the solid digesta ($P>0.05$), but the presence of citrus pulp in the diet tended to increase the abundance of bacteria ($P=0.099$) and increased protozoa abundance ($P=0.041$) in the liquid digesta. Relative abundance of archaea increased in the liquid digesta of the citrus pulp diet ($P=0.024$), but there were no differences between diets ($P>0.05$) in the solid digesta. Finally, fungi populations were affected by diet, with higher relative abundance in both solid and liquid digesta ($P=0.001$ and $P=0.022$) for the citrus pulp diet compared to the one containing maize. In conclusion, replacing maize by citrus pulp in a dairy sheep diet promoted changes in microbial populations and a greater abundance of microorganisms, especially in the liquid digesta of Rusitec fermenters, which might be related to the high sugar content of citrus pulp.

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Effects of replacing barley strw and corn silage by olive cake in the diet on microbioal populations in RUSITEC fermenters

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By-products are an important waste in agro-food industry in many countries, and they can represent an environmental problem, as they are difficult to eliminate. Their use in ruminant feeding could alleviate this problem and contribute to a more sustainable livestock production. Olive cake is a by-product of olive oil extraction that contains bioactive compounds with antimicrobial and antioxidant activities that could also affect the ruminal microbiota, but this aspect has not yet been investigated. Four Rusitec fermenters were used in a cross-over design in two 14-day incubation runs to study the effects of replacing part of the forage by olive cake in a dairy sheep diet on microbial populations. Three rumen-cannulated Merino sheep were used as ruminal content donors for inoculation of the system. Experimental diets were a conventional diet for dairy sheep (50:50 forage:concentrate) with corn silage and barley straw as forage, and a diet in which corn silage and barley straw were partially replaced by olive cake (16.7%). Samples of liquid and solid digesta were collected from the fermenters at the end of each incubation run. DNA was extracted and the abundance of bacteria and protozoa, as well as the relative abundance of fungi and archaea, were assessed by qPCR. Bacterial diversity was analysed using Automated Ribosomal Intergenic Spacer Analysis (ARISA). Data were processed using R with the vegan package and the PROC MIXED from SAS. The number of peaks detected in the ARISA electropherograms and the Shannon's index were not affected (P>0.05) by the inclusion of olive cake in the diet, but they were higher (P=0.006 and P=0.005, respectively) in liquid than in solid digesta. Principal coordinates analysis based on Bray-Curtis distance showed that samples were not clearly grouped by diet, due to the variability of samples from the same diet and the strong effect of the digesta phase. Bacterial and protozoal abundance in the liquid digesta and the relative abundance of fungi and archaea in both digesta phases were not affected (P>0.05) by the inclusion of olive cake in the diet. In the solid digesta, diet affected (P=0.013) the bacterial abundance and tended to affect (P=0.06) the protozoal abundance, both being higher for the diet containing olive cake than for the conventional diet. In conclusion, the addition of olive cake in substitution of corn silage and barley straw in the diet promoted greater bacterial and protozoal growth in the solid digesta of Rusitec fermenters.

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The new anaerobic rumen fungus producing zoospores containing both flagellum and cilia from Hanwoo, Korean Native Cattle

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The anaerobic fungi isolated from the digestive tract and feces of herbivores belong to phylum *Neocallimastigomycota*, class *Neocallimastigomycetes*, order *Neocallimastigales* and family *Neocallimastigaceae* in taxonomy and eleven genera of anaerobic fungi have been described, currently. In this study, anaerobic fungus was isolated from the rumen of Hanwoo, Korean Native Cattle and identified based upon morphological and molecular characteristics. Rumen fluid was collected from three cannulated Hanwoo cows and anaerobic fungi were isolated according to Hungate's roll tube method. The selected fungal culture (J18) was examined with light microscope (LM) and scattering electron microscope (SEM) and fluorescence microscope for morphological characterization. Fungal DNAs were extracted and analyzed for molecular characterization using internal transcribed spacer 1(ITS1) region ribosomal DNA and D1/D2 domain of large subunit (LSU) ribosomal DNA for identification markers. For phylogenetic analyses, MEGA7 software was used for sequence alignment and Maximum likelihood method was used for phylogenetic tree construction. The phylogenies were tested based on 2,000 bootstrap replications. From the results of LM and SEM examination, the shapes of J 18 sporangia were either ovoid or spherical with monocentric thallus and the size was ranged from 2.5 μm to 30 μm. Zoospores of J18 were spherical with the range from 0.2 to 10.0 μm and the length of flagella ranged from 4.7 to 3.0 μm. Unlike other described anaerobic fungi, J18 produced zoospore containing both flagellum and cilia. Isolated J18 colony secreted mucilage like substance on the surface of glassware or substrate, however appressorium was not observed. The ITS1 sequence of J18 was highly similar to that of uncultured *Cyllamyces* clone (GQ850330.1) with 100% identity and LSU sequence was highly similar to that of uncultured *Neocallimastigomycota* clone (MF872501.1) with 99.09% identity. According to phylogenetic analyses, ITS1 of J18 was placed in the clade of uncultured *Cyllamyces* clones and close to the clades of bulbous fungi including *Caecomyces* and *Cyllamyces* rather than filamentous, however LSU of J18 was placed in the clade of uncultured *Neocallimastigomycota* and close to *Piromyces* super clade. The morphological characteristics of J18 were filamentous fungi with monocentric thallus and uniflagellated zoospore, however the average size of sporangia were smaller than other isolated fungi. The genetic characteristics of J18 were close to those of anaerobic bulbous fungi *Caecomyces* and *Cyllamyces*. The isolated anaerobic fungi from Hanwoo was suggested to be as *Hanwoomyces koreanojoo*, gen. nov., sp. nov. Detailed genome analysis is under progress.

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Can early life interventions leave an imprint in the rumen microbiome in calves?

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In ruminants, the microbiota is shaped mainly by the diet offered at the time. To date, attempts to permanently change the microbiota in adults have been unsuccessful. The developing microbiota of young animals could be more malleable to an imprint.

In a series of experiments, we attempted to alter the rumen microbiome using different dietary strategies in calves. In the first experiment the effects of a fibre vs a concentrate-based pre-weaning diet on the rumen microbiome were compared in 16 calves up to 9 months of age. In the second experiment a methane inhibitor was given to twelve pre-weaned calves to evaluate its effect on methane emission and microbiota at different ages pre and post-weaning compared to a control group (n=12). A third approach was to inoculate eight calves during the first two weeks of life with rumen fluid from an adult cow, and to follow the microbiome and compare their microbiota to a control group of eight calves until one year of age.

Roughage fed calves pre-weaning had a microbiota and metabolite composition similar to a pasture-fed heifer, and different from the concentrate fed calves while the different diets were fed. These differences however were not observed when the diet of the two groups was similar. In the second study methane emissions were decreased by more than 50% using chloroform and 9,10-anthraquinone without negative effects on growth, intake, or rumen function. The rumen microbiome responded with increased molecular hydrogen, propionate, and butyrate, and decreased acetate. However, the differences in the microbiota composition were small, indicating that the fermentation effects were driven by changes in the metabolic pathways of the rumen microbiota rather than a change in microbial makeup. An inoculation within the first 10 days of life led to a differential bacterial community after 2 weeks. This effect was not persistent after 4 and 10 weeks, but the microbiota of the two groups separated again after 50 weeks. Rumen metabolites were not affected by the inoculation at any stage. Our results are in agreement to most of the current literature on the ability to imprint the rumen microbiota past the intervention itself.

In conclusion, like adult animals, a young animal's rumen microbiota is mainly driven by diet, and the possibilities to imprint the rumen appear to be limited. This is not surprising given the metabolic flexibility of the rumen microbiota under methane inhibition, as shown in experiment two.

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Source and level of zinc supplementation can alter fermentation by rumen microbes

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Zinc (Zn) is an essential mineral required for fiber digestion (Martinez and Church, 1970). Rumen microbes use soluble Zn to degrade cellulose to meet energy requirement. Solubility can vary with source and level of dietary Zn. The objective of this study was to compare ruminal effects of level and source of dietary Zn. We compared inorganic sources, zinc sulfate ($ZnSO_4$) and zinc oxide (ZnO) with a potentiated zinc oxide (HiZox®; Animine, Sillingy, France), at two supplemental levels.

Rumen content of one non lactating, ruminally cannulated Holstein cow (4 years of age, 650kg, predominantly forage diet) was fermented in 7 dual-flow continuous rumen cultures. Experimental treatments included 1) control (no supplemental Zn), 2) HiZox®, 30 mg of Zn/kg of DM, 3) HiZox®, 120 mg of Zn/kg of DM, 4) ZnO, 30 mg Zn/kg of DM, 5) ZnO, 120 mg Zn/kg of DM, 6) $ZnSO_4$, 30 mg Zn/kg of DM, 7) $ZnSO_4$, 120 mg Zn/kg of DM. Diets consisted of a TMR blend with corn silage, corn, soybean meal, salt and mineral vitamin premix and formulated to meet lactating cow requirements (NRC 2001). An experiment consisted of 8 days with 4 days for stabilization followed by 4 days for data collection. Each experiment was repeated three times. Data were analysed according to a randomized complete block design with repeated measures using the PROC MIXED procedures of SAS (SAS Inst., Inc., Cary, NC). Preplanned orthogonal contrasts were used to compare means.

As expected, level of Zn increased ruminal Zn compared to control. At 120 ppm, HiZox® resulted in greatest ($p<0.005$) ruminal Zn compared to ZnO and $ZnSO_4$. Culture pH was higher ($p<0.01$) with supplemental Zn; HiZox® resulted in higher ($p<0.01$) pH compared to ZnO and $ZnSO_4$. Methane was similar between control, $ZnSO_4$ and HiZox® but reduced with ZnO. Cultures receiving 120 ppm of ZnO had a lower acetate (A) to propionate (P) ratio and reduced isoacids concentration when compared with 120 ppm $ZnSO_4$ and HiZox®.

Supplemental Zn affected ruminal fermentation. Zinc oxide seemed to favour non-cellulolytic microbes whereas $ZnSO_4$ and HiZox® seemed to promote fiber digestion.

Martinez, A. and D. C. Church. 1970. Effect of Various Mineral Elements on In Vitro Rumen Cellulose Digestion. J Anim Sci. 31:982-990.

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Differences in fermentation kinetics by age of donor animals

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Fermentation kinetics are investigated using *in-vitro* gas curves. During research concerning early fermentation kinetics, differences by age of the donor of rumen fluid were seen during incubation of the same feeds. Total accumulated gas production was detected by pressure sensors during incubation, after which residues were filtered to determine dry matter degradation. Despite similar endpoint gas production (ml gas produced at STP per gram DM) and dry matter degradation after 24 or 48 hours, significant differences were seen in the fermentation kinetics of ruminant concentrate feeds. The same 10 concentrate samples were tested in duplicates, triplicates or quadruplicates in each of 2 trials with rumen fluid from 6 calves 5-6 weeks old, 4 calves 10-12 weeks old and 2 heifers between 1 and 1.5 years old. The samples were heat treated rapeseed meal, soybean meal, faba beans (toasted and untoasted), rapeseed cake, 2 different calf starter types, organic alfalfa pellets, grass pellets and soybean cake. One and half year old heifers almost always had a fermentation curve which could best be described by the equation:

(1): $Y_t = b / (1+(H^c / t^c))$

while the fermentation kinetics of the 6 week old calves was always best described by a simple exponential:

(2): $Y_t = b\,(1-e^{-ct})$

where Y_t is the total gas produced at time t (ml/g DM incubated), b is the asymptotic gas production (ml/g DM), and c is the constant determining the steepness of the curve. H (equation 1) is the time at which half the asymptotic amount of gas was produced. The fermentation kinetics of 10-12 week old calves was intermediate. The 10-12 week old calves had the greatest gas production per gram DM (asymptote) for all concentrates. Toasted and untoasted faba beans were not significantly different in DM degradation or gas production within each of the three age groups after 24 hours. However, while the untoasted faba beans were significantly more rapidly fermented in the 10-12 week old calves, the toasted faba beans were slightly more rapidly fermented in the youngest and oldest age groups, thereby changing the ranking of the feeds from 5-10 hours after start of fermentation. We conclude that early fermentation kinetics is affected by the age of the donor animal, and this may affect the ranking of feeds. Research regarding composition of the gas produced and fermentation products can indicate age dependent differences in microbial populations.

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Rumen fermentation characteristics, microbial population and meat fatty acids profile of West African dwarf rams fed water-washed neem fruit diets

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This study was conducted to determine the effect of diets containing water-washed neem (Azadirachta indica A. Juss) fruit on rumen fermentation characteristics, microbial population and meat fatty acid profile of fattened West African dwarf rams. Twenty-five yearling rams (12.3±2.0 kg) were assigned to one of five dietary groups with five animals per group in a completely randomised design. Each group received a total mixed ration formulated with 0% (T1), 2.5% (T2), 5.0% (T3), 7.5% (T4), and 10.0% (T5) water-washed neem fruit (WNF) inclusion for 90 days. Rumen liquor was collected for volatile fatty acids and microbial assay. At the end of the feeding trial, animals were slaughtered and Longissimus dorsi muscle from the loin meat cut was taken for the determination of fatty acids using standard procedures. Water-washed neem fruit initially increased but subsequently reduced ($P<0.05$) the population of bacteria and fungi with increased inclusion. However, the population of protozoa was consistently reduced with increased inclusion of WNF. Methane production (mL) and acetic-propionic acid ratio reduced ($P<0.05$) from 15.00-6.00 and 5.51-4.02 respectively with WNF inclusion. Palmitic and stearic acids constituted a greater portion of the saturated fatty acids (SFA) but were not significantly ($P>0.05$) affected by the different treatments. There was a significant ($P<0.05$) and consistent increase in the concentration of lauric, oleic, vaccenic, rumenic, linolenic and arachidonic fatty acids in T5 compared to T1. Dietary inclusion of WNF did not affect linoleic acid, SFA, and DI-Rumenic acid but significantly increased ($P<0.05$) the concentration of unsaturated fatty acids (UFA), polyunsaturated fatty acids (PUFA), UFA/SFA, PUFA/SFA, and total DI-rumenic acid in T5 compared to other treatments. It was concluded that increased incorporation of water-washed neem fruit reduced methane production, rumen protozoa population, acetic-propionic acid ratio and increased unsaturated fatty acids including rumenic acid, a conjugated linoleic acid of mutton. Inclusion of water-washed neem fruit in the diet affected rumen fermentation, rumen microbiota and intermediary metabolism especially biohydrogenation and methane production.

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About the organisers

The University of Veterinary Medicine Hannover, Foundation (TiHo)
The University of Veterinary Medicine Hannover, Foundation (TiHo) is a nationally and internationally recognized institution of higher education known for its excellence in veterinary science and its interdisciplinary focus. We are a leader in research, teaching and services in veterinary medicine and train young scientists in all areas of veterinary science. Our overall goal is the continual development of the science of veterinary medicine.

The TiHo stands for long-standing competence in the field of veterinary medicine. It is an eminent scientific institution connecting modern science with university tradition. Since its founding in 1778 as the Roß-Arzney-Schule it has kept its independent status up until today thereby assuming an exceptional position in Germany. At the beginning of 2003 the TiHo was transformed to a university foundation – the State of Lower Saxony granting the university a greater personal responsibility and thereby more flexibility for legal arrangements.

The TiHo has built up six specialist centres and integrates six clinics, 18 institutes and three special units. Additionally, in Ruthe, south of Hannover, and in Bakum near Vechta the University of Veterinary Medicine Hannover runs two field offices training students, and conducting research projects.

University of Leipzig, Institute of Veterinary Physiology (VMF)
The VMF is one of five establishments for veterinary education in Germany and the only one located in Central Germany. The faculty was originally located in Dresden and moved from Dresden to Leipzig in 1923. This was mainly motivated to get a closer contact of veterinary and human medicine already following the “One World One Health One Medicine”-concept.

Today, the VMF is an educational and service providing institution embedded in a national and international research network. The faculty consists of 16 institutes and clinics, organized in 5 centres and a research farm south of the campus. Teaching and research is provided by 38 professors and assistant professors, 125 researches and 140 members of the technical staff.

The VMF and its interdisciplinary and partially modularized curriculum were repeatedly positively evaluated by E.A.E.V.E. Last evaluation was done in 2018. Each winter semester 130–140 students join the faculty. To date 816 students are learning at the faculty. 92 percent of the students finish their studies within the standard period of study of 11 semesters. Besides the education of veterinary students, the faculty confers the grade “Dr. med. vet.” to about 60 scientists per year.

Leipziger Messe GmbH
Leipziger Messe is one of the ten leading German trade fair companies and one of the top 50 worldwide. It organises events in Leipzig and at various locations in Germany and abroad. With its five subsidiaries and the Congress Center Leipzig (CCL), Leipziger Messe is a comprehensive service provider covering the entire chain of the events business. As a result, customers and visitors voted Leipziger Messe – for the fifth time in a row – the trade fair industry service champion, in Germany’s largest service ranking in 2018. The Leipzig fairgrounds comprise an exhibition area of 111,900 m² and an open-air exhibition area of 70,000 m². More than 263 events – trade fairs, exhibitions, congresses and events – take place annually with 15,214 exhibitors and over 1.2 million visitors from all over the world. Leipzig was the first German trade fair company to be certified according to the Green Globe standards. Sustainability is a recurring theme in the Leipziger Messe’s corporate activities.

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