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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATION

The Three Hundred and Thirty-fifth Meeting of the Nutrition Society (One Hundred and Thirty-first of the Scottish Group) was held at the Freedom Inn, Aviemore on Tuesday and Wednesday 2 and 3 October, 1979 when the following papers were read:

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Effect of diet upon serum ribonuclease concentration in the rat. By A. SHENKIN, *Department of Biochemistry, Royal Infirmary, Glasgow* and S. BIELECKA, *Vitrum Institute for Human Nutrition, Stockholm, Sweden*

Serum ribonuclease (RNase) concentration has been shown to be high in a number of clinical situations associated with a negative nitrogen (N) balance (Albanese *et al.* 1972; Sigulem *et al.* 1973). However, in man, the presence of renal or hepatic disease can also lead to elevation in serum RNase, and the effect of nutritional status alone on serum RNase may be difficult to assess (Shenkin *et al.* 1976).

Male rats (mean weight 72 g) were given either a high quality diet including casein+methionine, an isoenergetic diet with gluten as N source, or an isoenergetic protein-free diet. Animals were pair fed to the protein-free group. After 5 d the diet including casein+methionine was given to all animals. Five animals in each group were killed and serum RNase measured after 2 and 5 d on test diet and after 2, 5 and 7 d refeeding.

The control animals taking the complete diet were in positive N balance and gained weight throughout the study. Serum RNase in these animals rose from a mean basal level of 0·067 to 0·107 mg/l at the end of the experiment. The animals given the gluten diet remained in N balance and maintained their body-weight for the 5 d period. However, mean serum RNase decreased to 0·054 mg/l after 2 d and then increased slightly to 0·061 mg/l after 5 d. On refeeding, body-weight increased rapidly and serum RNase also rose. The animals on protein-free diet and hence in negative N balance, lost weight progressively for 5 d and the mean serum RNase fell to 0·041 mg/l. When the complete diet was introduced and growth recommenced, serum RNase again showed a striking increase.

In a subsequent experiment using six diets of different net protein utilization (NPU) a marked correlation (r 0·84) was observed between NPU and serum RNase after 9 d diet, the better the NPU, the higher the serum RNase.

It is concluded that serum RNase does not correlate inversely with N balance in the rat. The serum concentration of RNase is probably dependent both on the weight of the animal and the rate of change in weight.

Albanese, A. A., Lorenze, E. J., Orto, L. A., Wein, E. H., Zavattaro, D. N. & de Carlo, R. (1972). *N.Y. St. J. Med.* **72**, 1595.
Shenkin, A., Citrin, D. L. & Rowan, R. M. (1976). *Clin. Chim. Acta* **72**, 223.
Sigulem, D. M., Brasel, J. A., Velasio, E. G., Rosso, P. & Wirick, M. (1973). *Am. J. clin. Nutr.* **26**, 793.

0029–6651/79/3803–335A $00.35

Maternal and foetal glucose metabolism in the exercising pregnant ewe.

By K. D. CHANDLER, SHAIO-LIM MAU and A. W. BELL, *School of Agriculture, La Trobe University, Bundoora, Victoria* 3083, *Australia*

Despite recent major advances in the study of foetal energy metabolism (Battaglia & Meschia, 1978) little is known about the responsiveness of foetal substrate supply and utilization to factors which may affect maternal metabolism, such as exercise and climatic changes. The present results arise from a preliminary study of substrate utilization by the gravid uterus in resting and exercising ewes during late pregnancy.

Polyvinyl catheters were surgically implanted in the maternal carotid artery, the uterine vein draining the pregnant horn of the uterus and the foetal aorta in four single-pregnant Merino ewes at 116–128 d gestation. At least 6 d after surgery, when the ewes' appetites had returned to pre-surgery levels, several sets of blood samples were drawn from the catheters while the ewe was standing at rest. This sampling procedure was then repeated while the ewe was walking on a moving belt treadmill at 33 m/min on a 10° slope. Results presented in the Table are means for four animals.

Concentrations of glucose and lactate in arterial blood and uterine A-V differences (mmol/l)

		Arterial concentration				Uterine A-V difference			
		Glucose	SE of difference	Lactate	SE of difference	Glucose	SE of difference	Net CHO (glucose+ lactate)	SE of difference
Ewe:	Rest	2·54	0·39	0·72	0·43	0·22	0·03	0·10	0·016
	Exercise	5·69	($P<0·01$)	2·45	($P<0·05$)	0·48	($P<0·01$)	0·45	($P<0·05$)
Foetus:	Rest	0·81	0·17	1·58	0·10				
	Exercise	1·81	($P<0·01$)	2·64	($P<0·01$)				

These changes in blood metabolite concentrations were accompanied by significant increases in maternal and foetal arterial blood pH ($P<0·05$) and significant decreases in arterial Pco_2 in ewes and foetuses during exercise ($P<0·01$), while foetal arterial Po_2 also decreased ($P<0·05$). Uterine blood flow (measured by a dye dilution technique in one ewe) decreased from 1322 to 700 ml/min during exercise.

Similar exercise-induced changes in maternal and foetal blood gases and uterine blood flow have been recently reported from another laboratory (Longo *et al.* 1978). Our results suggest that despite decreased uterine blood flow, umbilical uptake of glucose and possibly, lactate is quickly increased during maternal exercise as a consequence of maternal hyperglycaemia.

Battaglia, F. C. & Meschia, G. (1978). *Physiol. Rev.* **58**, 499.
Longo, L. D., Hewitt, C. W., Lorijn, R. H. W. & Gilbert, R. D. (1978). *Fedn Proc. Fedn Am. Socs exp. Biol.* **37**, 905.

0029–6651/79/3803–335A $00.35

Alternative uses for the liquid scintillation counter in general laboratory techniques. By R. C. NOBLE and J. H. SHAND, *The Hannah Research Institute, Ayr KA6 5HL*

Most biological research establishments possess a sophisticated liquid scintillation counter. The cost effectiveness of these expensive instruments can be maximized by altering their role in general laboratory analysis and by considering alternative uses for their highly sensitive light detecting abilities in addition to their normal role in the measurement of radioactivity. The introduction of miniature vials to liquid scintillation counting has required the simple construction of sealed glass miniature standards, volume 5·0 ml, which could be screwed into the normal 20 ml glass scintillation vial (Noble & Drummond, 1977). Using the annular space between the two vials, it is possible through the attenuation of the photons emitted from the inserted miniature standard, to provide quantitative techniques not normally associated with liquid scintillation counting. Thus, by the introduction of developed coloured products into the annular space, analysis may be readily achieved for many commonly used colorimetric techniques. Amongst the many other general laboratory methods to which the technique has been applied are measurements involving ultraviolet absorption, particle sedimentation rates and the monitoring of enzymic and other reactions which rely upon changes in the colour or opacity of a medium. In the case of colorimetric analyses the range of substrate concentrations that can be determined accurately using the liquid scintillation counter is extensive and may considerably exceed the range that can be accommodated within the useable range of many spectrophotometers. It is clear from the results that the simple construction of matched miniature radioactive standards, adds a new dimension in the use of a liquid scintillation counter.

Noble, R. C. & Drummond, J. T. (1977). *Lab. Pract.* **26**, 23.

0029-6651/79/3803-335A $00.35

Effect of dilution rate on distribution and composition of bacterial matter in Rusitec, using soluble food in presence of undigested hay residues.

By J. W. CZERKAWSKI and GRACE BRECKENRIDGE, *The Hannah Research Institute, Ayr KA6 5HL*

Rumen fermentation can be successfully simulated in vitro using normal solid food (Czerkawski & Breckenridge, 1977) or using soluble food in presence of an inert matrix of undigested residues of hay (Czerkawski & Breckenridge, 1979).

In the experiment reported here, soluble food (a mixture of carbohydrates, casein and minerals) was infused continuously into four vessels, each of which contained four nylon gauze bags with extracted hay. Every 7 d one bag was removed from each vessel and replaced by a bag of new hay. The nominal dilution rates varied from 0·3 to 1·8/d and the concentrations of food in the infusates were adjusted to give approximately similar food inputs in all vessels.

The production of acetic and butyric acids and methane declined with increasing dilution rate and then increased. These decreases were compensated for by increased production of propionic and C_5 acids. The output of particulate dry matter in the effluent did not change significantly, but the concentration of diaminopimelic acid (DAP) in the particulate matter declined with increasing dilution rate and the concentration of protein increased slightly. Consequently, the DAP:protein value declined rapidly with increasing dilution rate in the effluent and in the free liquid reaction mixture (29 to 14 mg/g). This value decreased slowly in the liquid that is associated with solid digesta (washings, 13 to 10 mg/g) and remained practically unchanged in the washed residues (7–8 mg/g). The total bacterial counts/unit volume were linearly related to DAP concentrations in both liquid fractions, but the line did not pass through the origin. Although protozoa were found in the liquid that was associated with the solid ($5·7\times10^4$/ml), no protozoa could be detected in the effluent or the liquid reaction mixture.

The output of ATP did not change with dilution and therefore the efficiency of microbial synthesis calculated from the output of particulate DM did not change ($Y_{ATP} = 9·5\pm0·2$ g/mol for four vessels). If the DAP output is used to calculate the net synthesis of bacterial matter, Y_{ATP} would change markedly with dilution rate. These results show that although DAP is a convenient and valuable bacterial marker, its use requires utmost caution, even in a simplified model system.

Czerkawski, J. W. & Breckenridge, Grace (1977). *Br. J. Nutr.* **38**, 371.
Czerkawski, J. W. & Breckenridge, Grace (1979). *Br. J. Nutr.* **42**, 219.

0029–6651/79/3803–335A $00.35

The effect of diet on the citric acid and soluble calcium content of cow's milk. By I. H. L. ORMROD* and P. C. THOMAS, *The Hannah Research Institute, Ayr KA6 5KL* and J. V. WHEELOCK, *University of Bradford, Bradford BD7 1DP*

As part of an investigation of factors influencing the composition and processing characteristics of cow's milk, two 4×4 Latin Square experiments were conducted with Ayrshire cows in mid-lactation, to describe the effect of diet on milk citric acid and soluble calcium contents; these constituents influence the heat stability of milks concentrated to approximately 45% solids (Banks & Muir, 1978). In the first experiment four cows and four diets were used and the experimental periods were 4 weeks. The diets were two mixtures of grass silage and a dairy concentrate containing barley (60:40 and 22:78 respectively) and two similar mixtures in which the concentrate contained barley and flaked maize. The second experiment was of similar design to the first but the diets consisted of grass silage, dairy concentrates and ground-pelleted dried grass (50:50:0, 30:50:20, 20:50:30 and 0:50:50). In both experiments diets leading to a reduction in milk fat content i.e. both high concentrate diets in the first experiment and the 0:50:50 diet in the second experiment, led to consistent reductions in milk citric acid content and milk soluble calcium content, and there were significant correlations between soluble calcium and citric acid (r 0·89, $P<0·001$, and r 0·86, $P<0·001$ for the first and second experiments respectively). However, the effects of diet were not sufficiently large to achieve $P<0·05$ levels of statistical significance with the number of animals used.

To examine the relationships between fat, citric acid and soluble calcium further, two cows were given in consecutive 1 week periods over a total period of 5 weeks, a series of diets containing chopped hay and dairy concentrates in varying proportions, and designed to produce extreme variations in milk fat content. The results from this experiment confirmed fully the relationships between the contents of fat, citric acid and soluble calcium in milk suggested by the earlier experiments. Milk fat contents ranged from 1·70 to 3·62%; corresponding ranges for citric acid and soluble calcium were 93·2–180·6 and 33·5–47·2 mg/100 g respectively. There was significant correlation between milk citric acid and fat contents (r 0·81, $P<0·001$) and between milk soluble calcium and fat content (r 0·79, $P<0·001$).

An interpretation of the relationships is that the secretion of soluble calcium in milk is linked with the secretion of citric acid which is in turn dependent on the synthesis of fat in the mammary gland, and thus on the composition of the diet.

Banks, W. & Muir, D. D. (1978). *A. Rep. Hannah Res. Inst.* p. 118.

*I.H.L.O. is a CASE postgraduate scholar.

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The nutritive value of feed proteins which escape degradation in the rumen. By J. C. MATHERS, R. J. THOMAS, N. A. M. GRAY and I. L. JOHNSON*, *Department of Applied Biology, University of Cambridge, Pembroke Street, Cambridge CB2 3DX*

Ruminant animals obtain their amino acids largely from microbial protein synthesized in the rumen and from feed proteins which escape rumen degradation. In the absence of direct information on the nutritive value of undegraded feed proteins (Smith & Mohamed, 1977), Roy *et al.* (1977) assumed that all feed proteins entering the small intestine are of equal quality.

Feed proteins that escape degradation in the rumen were obtained as follows: sheep were maintained on semi-purified diets in which all the nitrogen was supplied by either fish meal (FM)+urea or sunflower-seed meal (SFM). FM or SFM in polyester bags were incubated in the rumens of sheep (given the corresponding protein source) for 6 h (Mathers *et al.* 1977) after which time, the bags were washed and the feed residues freeze-dried.

The quality of protein in the original FM and SFM and in their partially digested residues (BR) was investigated in a rat growth trial. Semi-purified diets containing 100 g crude protein and 50 g fibre/kg were formulated in which all the N was derived from FM, SFM, FM-BR or SFM-BR. A casein+methionine control diet was included. Four weanling Wistar rats were offered each diet *ad lib.* for 14 d. Results are given in the Table.

Protein source	Growth rate (g/d)	Protein efficiency ratio	Apparent N digestibility
FM	5·59	2·91	0·911
FM-BR	5·05	2·93	0·895
SFM	4·25	2·41	0·809
SFM-BR	3·06	2·06	0·831
Casein+methionine	6·15	3·11	0·930
LSD (P=0·05)	1·12	0·33	0·047

Partial degradation within the rumen did not affect the protein quality of FM but the protein efficiency ratio of SFM-BR was significantly lower than that of SFM. The reduced protein quality of SFM-BR was not due to impaired N digestibility suggesting that the pattern of absorbed amino acids was poorer for growth in the rat than that from the original SFM. These observations indicate that not only is the nutritional quality of feed protein escaping degradation in the rumen likely to differ for different feeds but that the quality of the original feed protein may be an unreliable guide to that of the protein reaching the small intestine.

Mathers, J. C., Horton, C. M. & Miller, E. L. (1977). *Proc. Nutr. Soc.* **36**, 37A.
Roy, J. B., Balch, C. C., Miller, E. L., Ørskov, E. R. & Smith, R. H. (1977). In *Protein Metabolism and Nutrition*, p. 126. [S. Tamminga, editor]. Wageninger.
Smith, R. H. & Mohamed, O. E. (1977). *Proc. Nutr. Soc.* **36**, 153A.

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Retention time and digestibility of milled hay in sheep and red deer (*Cervus elaphus*). By M. SANCHEZ-HERMOSILLO and R. N. B. KAY, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Previous experiments comparing digestion in sheep and red deer indicated that deer digested coarse fodders rather less well and that this was associated with a shorter mean retention time (MRT) (Kay & Goodall, 1976; Milne *et al.* 1978). An experiment was carried out to see if differences in MRT were associated with food particle size. Since small particles generally pass rapidly through the gut, better maceration by deer might lead to shorter MRT than in sheep, but the difference would diminish if the food were finely milled.

Two young ewes and two stag calves, about 35 and 50 kg respectively were cannulated in the rumen and in the ascending duodenum. They were given a hay diet which was milled through either a 40 mm screen (modulus of fineness, MF 3·9) or a 5 mm screen (MF 2·1) (ASAE, 1967). The diets were given at near maintenance level, 55 g hay/kg body-weight$^{0.75}$ daily, during two 15 d periods; sheep 1 and deer 1 received the fine hay first, sheep 2 and deer 2 the coarse hay first. The MRT of stained hay particles to duodenum and to rectum was measured in samples taken from these sites (Castle, 1956). Apparent digestibility of dry matter (DDM) was determined in pooled rectal samples using Cr_2O_3 as a reference substance. The MF was calculated for hexane-washed dried samples of duodenal contents and faeces.

	Duodenum		Faeces		DDM (%)
	MRT (h)	MF	MRT (h)	MF	
Fine hay:					
Sheep 1	53·1	1·55	66·4	1·17	55
Sheep 2	49·4	1·59	63·1	1·24	53
Deer 1	60·2	1·43	72·1	1·27	49
Deer 2	66·2	1·37	74·2	1·28	51
Coarse Hay:					
Sheep 1	52·1	1·17	71·0	1·01	58
Sheep 2	55·2	1·14	73·9	1·07	65
Deer 1	70·2	1·18	97·2	1·10	52
Deer 2	75·2	1·10	80·0	1·20	51

Unexpectedly, the MRT of these milled diets was longer in the deer than in the sheep, yet digestibility was lower in the deer. In both species the fine hay tended to have shorter MRT and lower digestibility than the coarse. Duodenal particles were similar in size in the two species, as were faecal particles. Curiously, particle size was almost always larger for the fine diet than for the coarse.

American Society of Agricultural Engineers (1967). *Agricultural Engineers Yearbook*, 1967, p. 301.
Castle, E. J. (1956). *Br. J. Nutr.* **10**, 15.
Kay, R. N. B. & Goodall, E. D. (1976). *Proc. Nutr. Soc.* **35**, 98A.
Milne, J. A., MacRae, J. C., Spence, A. M. & Wilson, S. (1978). *Br. J. Nutr.* **40**, 347.

0029 6651/79/3803–335A $00.35

Possible use of urinary nitrate as a measure of total nitrate intake. By BARBARA BARTHOLOMEW, CHRISTINE CAYGILL, RANJNA DARBAR and M. J. HILL, *Bacterial Metabolism Research Laboratory, Central Public Health Laboratory, London NW9 5HT*

Several workers have suggested an involvement of ingested nitrate in gastric cancer (e.g. Hill *et al.* 1973; Zaldivar & Robinson, 1973; Cuello *et al.* 1976). In order to assess whether urinary nitrate can be used as a measure of nitrate ingestion it is important to clarify whether nitrate can be synthesized endogenously as suggested by Tannenbaum *et al.* (1978).

Six healthy subjects consumed a nitrate-free liquid diet (formulated by Dr. Howard, West Middlesex Hospital) and distilled water for 7 d. The concentration of urinary nitrate of one subject was monitored over this period and found to drop from 83 to 23 μg/ml in 3 d and to reach a basal value of 18 μg/ml on the 4th day. A dose of either 25, 50, 100 or 170 mg nitrate was administered to each subject after 3 d. A different dose was then given to each after a further 48 h on the nitrate-free diet. Nitrate and nitrite concentrations were measured in saliva and urine collected at 30 and 60 min intervals respectively.

The urinary nitrate concentration reached a maximum 3–6 h (depending on dose) after challenge and slowly returned to basal levels after about 13 h. In saliva some of the nitrate is reduced to nitrite, and the total nitrate+nitrite concentration reached a maximum 1 h after challenge, returning to basal levels within 5 h. At all dose levels approximately 65–70% of the nitrate consumed was recovered in the urine, a further 20% in saliva, the remainder being possibly lost in other body secretions.

These results do not show any evidence of endogenous nitrate synthesis as reported by Tannenbaum *et al.* (1978) and it would appear that a 24 h urine collection may be used as an estimate of total nitrate intake. Further studies to clarify this are currently in progress.

This work is supported by the Department of the Environment.

Cuello, C., Correa, P., Haenszel, W., Gordillo, G., Brown, C., Archer, M. & Tannenbaum, S. (1976). *J. natn. Cancer Inst.* **57**, 1015.
Hill, M. J., Hawksworth, G. & Tattersall, G. (1973). *Br. J. Cancer* **28**, 562.
Tannenbaum, S., Tett, D., Young, V., Land, P. & Bruce, W. (1978). *Science*, N.Y. **200**, 1487.
Zaldivar, R. & Robinson, H. (1973). *Z. Krebsforsch.* **80**, 289.

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Whole-body protein synthesis and oxidation rates in streptozotocin diabetic rats. By E. C. ALBERTSE, P. J. GARLICK and V. M. PAIN, *Department of Human Nutrition, London School of Hygiene and Tropical Medicine, London WC1*

A study of protein metabolism in adult diabetic patients did not show any difference between rates of whole-body protein synthesis before and after short periods of insulin treatment (Albertse *et al.* 1979). This contrasts with the large decrease in muscle-protein synthesis found in streptozotocin diabetic rats (Pain & Garlick, 1974). Whole-body protein synthesis and oxidation rates were therefore determined in diabetic and insulin treated rats. 1-^{14}C-leucine was infused intravenously over 4 h, $^{14}CO_2$ collected and the specific activity of leucine in the plasma measured (Waterlow *et al.* 1978).

In Expt 1 (see Table), 4 d after diabetes was induced by injection of streptozotocin food intake of the diabetic rats were similar to that of control rats. Despite a highly significant ($P<0.001$) increase in oxidation rate, synthesis dropped only slightly. In Expt 2 the rats had been diabetic for 8 d and were also hyperphagic compared with their controls. Rates of oxidation were even more elevated and there was a statistically significant decrease in the rate of the whole-body protein synthesis, as expected from in vivo and in vitro studies on individual tissues. In Expt 3 4 d diabetic rats were treated with insulin P.Z.I. (4 units/d) for a further 4 d. This normalized blood glucose values and caused a similar hyperphagic response as in 8 d diabetic rats. Growth rates and body-weights were comparable to those of control rats within this time period. Insulin treatment also brought the protein synthesis rates back to near normal values, but oxidation rates were still significantly increased. Protein breakdown rates can be calculated but in practise were difficult to interpret.

Changes in protein (*μmol leu/min per 100 g body-wt*)

	Experiment 1		Experiment 2		Experiment 3	
	Control	Diabetic (4 d)	Control	Diabetic (8 d)	Control	Insulin treated diabetic
Oxidation	0·222	0·428	0·242	0·671	0·230	0·314
SEM	0·018**	0·035**	0·017**	0·056**	0·021*	0·028*
Synthesis	1·455	1·358	1·671	1·385	1·441	1·384
SEM	0·064	0·074	0·077*	0·057*	0·129	0·091

*$P<0.05$, **$P<0.001$.

We conclude that in rats made diabetic with streptozotocin, unlike in adult human diabetics, there is a fall in the rate of whole-body protein synthesis which can only be corrected by insulin treatment.

Albertse, E. C., Garlick, P. J., Pain, V. M., Reeds, P. J., Watkins, P. J. & Waterlow, J. C. (1979). *Proc. Nutr. Soc.* (In the Press).
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Food intake in relation to dietary energy and nitrogen concentrations in 'lean' and 'fatty' Zucker rats. By RUTH B. S. HARRIS*, G. R. HERVEY and G. TOBIN, *Department of Physiology, The University of Leeds, Leeds LS2 9JT*

It has been suggested that the primary defect in congenitally obese Zucker rats is inefficiency of protein deposition; that their food intake is regulated in relation to protein storage and not to energy and hence individuals with the defect eat more and become obese (Radcliffe & Webster, 1976). To test this, 'lean' and 'fatty' Zucker rats have been given, in separate experiments, diets designed (1) to vary energy concentration while keeping protein concentration constant and (2) to vary protein concentration while remaining isoenergetic. Treatment groups were of three to five individually caged female rats aged 4 to 5 weeks at the start. The experimental diets were as similar as possible and based on normal 'chow'. Treatment periods were preceded by a period on control diet for all rats. Results are reported for control periods and 10 d periods beginning 10 d after changes of diet.

In the first experiment the absorbable (i.e. 'digestible') energy concentrations of the three diets, measured by bomb calorimetry of diets and faeces, were 9·2, 13·4 and 17·4 kJ/g diet for both 'lean' and 'fatty'. Concentrations of absorbable nitrogen were always in the range 30–34 mgN/g diet.

Mean daily food intakes of rats given diets of different energy concentrations

	Energy concentration of diet					
	Low		Normal		High	
	g	kJ	g	kJ	g	kJ
'Lean' rats: Control	15·0	215	15·5	222	16·2	232
Experiment	20·8	191	16·2	217	13·8	237
'Fatty' rats: Control	22·5	322	22·4	320	21·9	313
Experiment	29·9	275	23·8	319	19·6	337

The 'lean' rats, when given diluted and concentrated diets, compensated to an extent which left energy intake changed by −11 and +2% respectively; the 'fatty' rats by −15 and +8%. N intakes changed by +39 and −15% in the 'lean' rats by +33 and −11% in the 'fatty' rats.

In the second experiment six diets provided a range of absorbable N of 4·5–53·2 mg N/g (energy of 13·5–15·1 kJ/g). 'Lean' and 'fatty' rats showed similar energy intakes on all diets; differences were unrelated to N concentration. The amounts of N absorbed were almost proportional to the absorbable N in the diet. Carcass analysis showed no changes other than failure to increase body N content on the diet with the lowest N content, in both 'lean' and 'fatty' rats.

Young 'fatty' Zucker rats, therefore, appear to regulate food intake in relation to dietary energy concentration only slightly less well than do 'lean' rats. The experiments lend no support to Radcliffe & Webster's hypothesis.

Radcliffe, J. D. & Webster, A. J. F. (1976). *Br. J. Nutr.* **36**, 457.

*SRC postgraduate student.

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Voluntary food intake in Zucker rats in relation to protein intake fixed by tube-feeding. By NELIDA A. GARCIA, RUTH B. S. HARRIS*, G. R. HERVEY and G. TOBIN, *Department of Physiology, University of Leeds, Leeds LS2 9JT*

If the need for protein determines the voluntary food intake, it would be expected that decreasing an externally controlled protein intake would lead to increased voluntary intake of energy, and vice versa.

'Lean' and 'fatty' Zucker rats have been given protein by stomach tube feeds, which provided about a third of the normal energy intake; they were also offered a protein-free diet *ad lib.*

The six treatment groups, three of 'lean' and three of 'fatty' rats, were composed of six individually caged male rats aged 10 weeks at the start of the experiment. During the first phase of the experiment, to establish a baseline all groups were offered *ad lib.* a powdered diet containing approximately 17% protein. From this the 'lean' and 'fatty' rats obtained approximately 0·54 and 0·74 g N/d respectively. In the second phase, which accustomed the rats to tube-feeding and allowed selection of matched groups, a liquid diet was given by stomach tube, which gave the 'lean' and 'fatty' rats 0·50 and 0·69 g absorbed N and 100 and 140 kJ/d respectively (the reduction in absorbed N by a factor of approximately 0·75 was not intended). In the third phase the three groups of 'lean' rats received by tube 0·28, 0·57 and 0·68 g absorbed N/d and 110 kJ; and the 'fatty' rats received 0·38, 0·79 and 1·05 g N/d and 150 kJ. Protein-free diet was available *ad lib.* during the second and third phases.

Total energy intakes (kJ/d)

	Phase 1	Phase 2	Phase 3	Protein in Phase 3
'Lean' rats:	343	317	296	(Low)
	364	312	304	(Normal)
	386	343	299	(High)
'Fatty' rats:	526	398	303	(Low)
	506	409	330	(Normal)
	548	411	338	(High)

In the 'fatty' groups the differences in energy intake in the final phase were in the expected direction, but, at +2 and −8% relative to the 'normal-N' group, they were small compared with the −53 and +34% differences in N intake; and much below the level which could have been shown to be significant (least significant difference for $P<0.05 \sim 25\%$ of control intake). The reasons for the general decreases in total energy intake during tube-feeding are not clear.

The results do not suggest that availability of protein was a major factor in determining the food intake of these rats.

*SRC postgraduate student.

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Predicted and measured performance of Friesian cows fed on rations calculated to differ in rumen degradable protein and undegraded protein. By J. D. OLDHAM, W. H. BROSTER, D. J. NAPPER and T. SMITH, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

The acid test of the proposals for a new system for rationing protein to ruminants (Roy *et al.* 1977) will be its ability to predict animal performance accurately. The aim of the present work was to give some background to the effectiveness of the initial proposals by a comparison of predicted (Roy *et al.* 1977) with measured performance of cows.

Sixteen mature Friesian cows were given a standard ration (UF_1) for the first 2 weeks post-partum. From 2–12 weeks post-partum groups of four cows were offered one of four rations with different values for rumen degradable protein:undegraded protein. All cows were offered 20 kg maize silage, 2 kg alkali-treated straw cubes and 11·5 kg concentrates/d. The concentrates contained urea:fish-meal (g/kg); 25:0(U), 16:40(UF_1), 8:80(UF_2) or 0:122(F). All rations contained 139–145 g crude protein/kg DM. Digestibility of the rations was measured in a Latin Square experiment with four heifers fed at maintenance.

		Yield			DM		PP output	
Treatment	Milk yield (kg/d)	Milk protein (g/d)	Milk fat (g/d)	Live-weight gain (g/d)	Digest-ibility	Calculated RDP:UDP	Measured	Calculated*
U	26·1	785 b	669 b	370 ab	0·71 c	3·6	841	693
UF_1	29·4	894 a	642 b	530 a	0·76 b	2·5	974	834
UF_2	28·4	888 a	664 b	180 ab	0·81 a	1·8	915	936
F	28·1	848 ab	797 a	130 b	0·78 ab	1·4	868	904
SE of difference between means	1·95	31·4	44·3	165	0·012	—	—	—

RDP, rumen degradable protein.
UDP, undegraded protein.
PP, productive protein above maintenance.
a, b. Values which do not share a common superscript differ significantly ($P<0·05$).
*Roy *et al.* (1977).

Substitution of urea-N by fish-meal-N significantly increased DM digestibility at all but the highest level of fish-meal inclusion and consequently digestible energy intakes differed between treatments. Milk and protein yields differed beween U and UF_1, UF_2 and F, significantly so for protein. Live weight change showed a significant reverse pattern. The highest milk and protein yields were obtained with values for RDP:UDP of the order 1·8–2·5 (see Table).

Calculations based on digestible OM intakes, underestimated the productive protein above maintenance (PP) output with U and UF_1 and overestimated it with UF_2 and F compared with 'measured' PP. The progressive changes in measured PP output were not in accord with the predicted pattern of change. In particular only the first level of substitution of urea-N by fish-meal-N resulted in an increase in measured PP output.

Roy, J. H. B., Balch, C. C., Miller, E. L., Ørskov, E. R. & Smith R. H. (1977). In *Protein Metabolism and Nutrition* p. 126. Pudoc: Wageningen.

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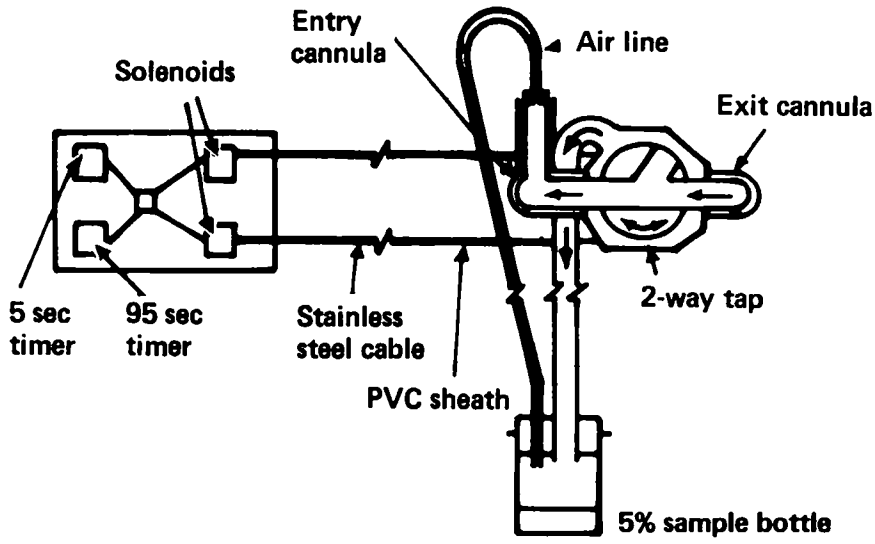
A new automatic method for measuring dry matter and nitrogen flow through re-entrant cannulas in the duodenum of growing pigs. By A. G. Low, *National Institute for Research in Dairying, Shinfield, Reading, Berks. RG2 9AT*

Two of the current methods for measuring dry matter and nitrogen flow through re-entrant cannulas in the duodenum of pigs have been shown to give markedly different results (Low & Zebrowska, 1977). Because these methods interfere with the flow of digesta, a novel sampler has been made (from acetal co-polymer plastic); this fits into the centre of a re-entrant cannula and interrupts the normal flow of digesta from exit to entry cannulas for 5 s in every 100 s. During the 5 s periods, digesta pass to a sample bottle maintained at gut lumen pressure. The sampler is controlled through two 2 m cables by two timers and solenoids standing in a box behind the cage in which the pig is housed.

EQUIPMENT FOR AUTOMATIC SAMPLING OF PIG DIGESTA



In preliminary tests with two 40 kg pigs the equipment was used for 24 h collections. The pigs received diets based on barley, weatings and soya-bean meal. Intakes and duodenal flows respectively in 24 h were: DM, 1488 and 1577 g; N, 41 and 52 g; chromic oxide 10·0 and 10·2 g (flow calculated as amount in sample bottle ×20).

I am grateful to Mr R. T. Budd and his staff for helpful discussions and for constructing the equipment.

Low, A. G. & Zebrowska, T. (1977). *Br. J. Nutr.* **38**, 145.

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Effect of type of carbohydrate on the production of microbial nitrogen in the rumen. By F. J. HART* and E. R. ØRSKOV, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The effects of different sources of dietary carbohydrate on the net production of microbial protein were measured in sheep. Three sheep with re-entrant cannulas in the proximal duodenum were given three diets in successive periods of three weeks according to a Latin Square design. The duodenal digesta were collected over 48 h periods. Daily production of microbial protein was estimated to consist of the daily flow of N×6·25 less N present as ammonia and as undigested dietary N. Each diet contained 25 g/kg of N in a degradable form (85% as urea and 15% as lactic casein). Alkali-treated barley straw, starch and sucrose were the major sources of carbohydrate in the diets; 9% cotton linters were added to the starch and sucrose diets to maintain normal ruminal function. The ratio degradable N to S was 8:1 in each diet. The results are summarized in the Table and show that the fluid flow through the duodenum was greater and the organic matter disappearing anterior to the duodenum smaller for the diet containing alkali-treated straw than for the others. This could have arisen from greater salivary flow with the more fibrous diet. The estimated production of microbial N was marginally greater for the diet containing the alkali-treated straw, a trend which agrees with that noted by Harrison *et al.* (1976). The fermented organic matter (FOM) was estimated by assuming that microbial organic matter contains 10·5% N.

Organic matter intake (OMI), water intake, rumen fluid outflow, fermentable organic matter (FOM) intake and microbial nitrogen (N) production (g of microbial N per 100 g of actually fermented OM)

Diet	OM intake (g/d)	Water intake (l/d)	Rumen fluid outflow (g/g OMI)	FOM intake (g/d)	Microbial N production (g/100 g FOM)
Alkali-treated straw	459	4·4	17·8	304	3·3
Starch	582	3·9	10·4	457	2·4
Sucrose	457	3·8	8·0	432	2·3
SE of means		0·7	0·4	60	0·2

Harrison, D. G., Beever, D. E., Thompson, D. J. & Osbourne, D. F. (1976). *J. Sci. Fd Agric.* **27**, 617.

*Present address: 18 Allan Street, Curtan A.C.T. 2505, Australia.

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The effect of proportion of concentrate and method of cereal processing on type of rumen fermentation and milk quality in Friesian cows. By E. R. ØRSKOV and G. W. REID, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The low-fat content in milk from dairy cows fed on high quantities of grain in their diet is associated with a high molar ratio propionic:acetic acids in rumen fluid. We have shown that in sheep the ratio propionic:acetic acid in rumen fluid correlated with the degree of physical processing of barley, whole grain giving a high proportion of acetic acid (Ørskov *et al.* 1974). However, the digestibility of whole grain fed to dairy cows is low. It can be increased to that of rolled barley by spraying with caustic soda (Ørskov & Greenhalgh, 1977).

Sixteen Friesian cows in mid lactation were fed on a complete diet of 30:70 or 20:80 hay and concentrates to appetite. The concentrates contained 60 or 70% of either rolled barley or barley treated with caustic soda (35 g NaOH/kg). At both levels of barley feeding the cows eating alkali-treated barley had a greater proportion of acetic acid in the rumen and a higher percentage of fat in milk compared with the cows on rolled barley.

The effect of the proportion of concentrate and method of grain processing on rumen VFA proportions (mmol/mol) butter fat content and yield of fat corrected milk (FCM) in dairy cows

(Each value is the mean of 8 observations)

		Molar proportions of:				
Hay:Concentrate	Processing method of grain	Acetic acid	Propionic acid	Butyric acid	Butter fat (g/kg)	FCM (kg/d)
30:70	Rolled	56·0	31·0	10·8	33·0	14·7
30:70	NaOH	64·0	18·0	15·6	39·0	15·5
20:80	Rolled	49·5	37·8	9·5	28·4	13·0
20:80	NaOH	61·4	19·0	16·7	38·5	14·6
SE of treatment differences		1·7	2·3	1·7	2·7	1·8

The results indicate that the method of processing of grain can greatly influence the amount of concentrate that can be included in diets for dairy cows before a depression in milk quality occurs.

Ørskov, E. R., Fraser, C. & Gordon, J. G. (1974). *Br. J. Nutr.* **32**, 59.
Ørskov, E. R. & Greenhalgh, J. F. D. (1977). *J. agric. Sci., Camb.* **89**, 253.

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Some aspects of nitrogen metabolism during simulated saturation dives of up to 43 bar (420 metres of sea water). By R. F. CARLYLE, SHIRLEY-ANN COLLIS and M. P. GARRARD, *PL(AMTE), Fort Road, Alverstoke, Gosport, Hampshire*

During two dives of 420 metres of sea water (msw; 43 bar), divers were given a fixed diet enabling investigations into nitrogen, mineral and energy metabolism to be carried out. The four subjects showed an increased 24 h urinary-N (with constant faecal-N) on reaching maximum depth, the bulk of which was increased urea excretion; creatinine and uric acid remained constant. Collagen metabolism was apparently normal as urinary levels of hydroxyproline, and circulatory levels of proline and proline-iminopeptidase remained unchanged throughout the dives. Confinement and lack of exercise that might have caused muscle wasting appear not to have contributed as the N excretion had returned to normal by 300 msw during the decompression and 3-methylhistidine in plasma was also unchanged. However, of the twenty-one amino acids surveyed in plasma only glycine, valine and lycine were raised at 420 msw with only the glycine levels being raised significantly; normal levels were recorded at the start of compression and during the decompression.

Circulatory concentrations of free fatty acids (FFA), triiodothyronine (T_3), T_3 resin uptake, and anti-diuretic hormone did not significantly change on either of the 420 msw dives. During a subsequent 300 msw dive (31 bar) a similar pattern was seen with no change in the circulatory levels of T_3, T_3 resin uptake, and growth hormone. Thyroxine (T_4) levels, however, were raised during both the 420 msw dives and the 300 msw dives in all seven subjects, reaching a maximum at varying times during the decompression, decreasing to normal post-dive. Thyroid stimulating hormone (TSH) levels followed the T_4 result closely. Serum cortisol and plasma noradrenaline were also significantly increased at 420 msw, returning to normal half-way through the decompression.

The increases in urea excretion, circulatory levels of T_4, cortisol, noradrenaline, glycine, valine, lysine and the decreases in serum insulin levels, correspond closely with the maximal severity of the High Pressure Neurological Syndrome (HPNS) in each dive. It would therefore appear that the effects of pressure and decompression on these dives have been to stimulate catabolism of endogenous protein stores via T_4, mediated centrally through the pituitary-thyroid axis.

Nevertheless certain anomalies were observed: (a) The increase in circulatory noradrenaline was not accompanied by increased FFA levels; (b) T_4 appears to act alone as T_3 levels were unaltered; (c) The proposed stimulation of protein catabolism could have been specific, as only glycine, valine and lysine were mobilized into the circulation in significantly increased amounts.

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Rates of protein synthesis in rat liver and small intestine in protein deprivation and diabetes. By MARGARET A. MCNURLAN and P. J. GARLICK, *Department of Human Nutrition, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1*

We have previously shown that in starvation the rate of protein synthesis is reduced in both liver and small intestine (McNurlan *et al.* 1979). The effect of changes in amino acid supply on rates of protein synthesis in the intact animal was further investigated by studying rats which were: (a) given a protein-free diet for 8 d and (b) hyperphagic, 10 d after induction of diabetes with streptozotocin. Rates of protein synthesis were measured by assessing incorporation of isotope 10 min after injecting [1-^{14}C]leucine (100 μmol/100 g body-weight).

	Control rats		Protein-deprived rats		Diabetic rats	
Tissue	Mean	SEM	Mean	SEM	Mean	SEM
Liver	97	2	68	2*	52	4*
Jejunal mucosa	143	3	112	4*	140	10
Jejunal serosa	69	3	44	2*	69	4

*Significantly different from control, $P<0.0001$.

In the liver the rate of total protein synthesis (hepatic + secreted) was markedly reduced by both protein deprivation and diabetes (see Table). This contrasts with results obtained earlier by constant infusion of [^{14}C]tyrosine. This method measures the rate of synthesis of non-secreted liver proteins only; this rate was elevated in protein deprivation (Garlick *et al.* 1975) and was unchanged in diabetes (Pain & Garlick, 1974). The discrepancy cannot be explained simply by a change in the rate of synthesis of secreted protein. Although depression of albumin synthesis has been reported in protein deprivation (Morgan & Peters, 1971) and diabetes (Pain *et al.* 1978) these changes are far too small to account for the change in total protein synthesis. Consequently, it seems more likely that results from constant infusion were influenced by failure to define accurately the specific radioactivity of the free amino acid at the site of protein synthesis, which the large dose method was designed to overcome.

In intestine protein deprivation resulted in a 20% decrease in the rate of synthesis in jejunal mucosa and a 40% decrease in the serosa. Interestingly, in diabetic rats the rate of protein synthesis in mucosa and serosa was unchanged despite hyperphagia, when increases in cell mass and cell proliferation rate have been shown to occur (Miller *et al.* 1977).

Garlick, P. J., Millward, D. J., James, W. P. T. & Waterlow, J. C. (1975). *Biochim. biophys. Acta* **44**, 71.
McNurlan, M. A., Tomkins, A. M. & Garlick, P. J. (1979). *Biochem J.* **178**, 373.
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Morgan, E. H. & Peters, T. (1971). *J. biol. Chem.* **246**, 3500.
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The effect of a protein extraction process on some apparent digestibility coefficients of fresh pasture. By T. E. TRIGG and A. M. BRYANT, *Ruakura Animal Research Station, Private Bag, Hamilton, New Zealand*

It has been estimated that approximately 5·5 million hectares of New Zealand is potentially suitable for simultaneous production of protein from both pasture plants and grazed ruminants (Hutton, 1972). An important determinant of the profitability of a system which combines these enterprises is the extent that the partial extraction of protein from pasture modifies the performance of animals grazed on the processed herbage (pressed pasture, PP).

During the last 2 years, five experiments involving 206 lactating cows at Ruakura Animal Research Station have shown that cows offered fresh PP produce less milk (⩽15%) of lower fat (⩽0·28% units) and protein (⩽0·17% units) content than their contemporaries offered fresh pasture. Reasons for this decreased production are being sought. During the course of these experiments lactating identical twin cattle have been used to derive estimates of some apparent digestibility coefficients. Each estimate (Table 1) was based on the quantitative 10 d collection of faeces and urine from animals offered pasture or PP *ad lib.*

Mean voluntary intake and mean digestibility coefficients of organic matter (OMI, OMD), gross energy (GE) and nitrogen (N) in pasture (P) or pressed pasture (PP) in early or late spring, using lactating dairy cows

Ration	*n*	Month	OMI (kg/d)	OMD	GEI (MJ/d)	GED	NI (g/d)	ND
P	10	Sept–Oct	10·7	0·80	223	0·76	396	0·76
PP	10		9·3	0·77	188	0·72	305	0·72
SE (a)			0·3	0·003	6·7	0·005	11·3	0·009
Statistical significance of difference			**	***	***	***	***	**
P	7	Nov–Dec	13·8	0·73	278	0·69	430	0·69
PP	7		11·7	0·71	236	0·67	330	0·67
SE (a)			0·8	0·003	16	0·005	23	0·012
Statistical significance of difference			**	***	**	***	***	**

NS, not significant.
*$P<0·05$, **$P<0·01$, ***$P<0·001$.

The results indicate the decreased production of cows offered PP may be explicable in part by a reduction in both intake and apparent digestibility of nutrients.

Hutton, J. B. (1972). *Proc. N.Z. Soc. Anim. Prod.* **32**, 160.

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Treatment of bovine hypocuprosis by the oral administration of cupric oxide needles. By N. F. SUTTLE, *Moredun Research Institute, 408 Gilmerton Road, Edinburgh EH17 7JH*

Cupric oxide needles (CuO_N) given orally to sheep, are retained in the alimentary tract and have produced large increases in liver Cu concentrations (Dewey, 1977) but they are regarded as a poor source of available Cu for the bovine (Chapman & Bell, 1963). The object of the experiment was to study further the properties of CuO_N given to cattle. Nine calves, weighing approximately 250 kg, were given a semi-purified diet of low Cu content (Suttle & Angus, 1976) until they were hypocupraemic (plasma Cu<0·5 mg/l) and some showed clinical signs of Cu deficiency (chiefly loss of coat condition and colour). Five were then given 50 g CuO_N needles, providing 40 g Cu in a filter paper capsule, by means of a balling gun and four were left untreated. After 41 d, the calves were slaughtered and their livers removed for Cu analysis. The effects of CuO_N on Cu status are illustrated by the results in the Table.

Mean Cu concentrations in the plasma (mg/l), liver, hair and faeces (mg/kg DM*) of initially hypocuprotic cattle, 41 d after the oral administration of cupric oxide needles compared with those of an undosed group*

(Mean values with their standard errors; number of determinations in parentheses)

	Cu concentration							
	Plasma		Liver		Hair		Faeces	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Dosed	0·72	0·03 (5)	342	54 (5)	6·2	0·9 (3)	79·5	24·2 (3)
Undosed	0·16	0·03 (4)	5	0·3 (4)	2·6	0·7 (2)	3·0	0·5 (2)

CuO_N alleviated all clinical symptoms of deficiency and significantly increased Cu concentrations in liver, plasma and hair. The presence of elevated faecal Cu concentrations after 41 d confirmed that CuO_N were well retained in the bovine alimentary tract and were probably still releasing absorbable Cu. Although the increase in liver Cu stores was large it represented only 1% of the Cu dose. It is concluded that CuO_N provides an attractive alternative to repeated parenteral Cu injections for the prevention of bovine hypocuprosis. The reason for the successful use of CuO_N lies in the large amount of Cu which can be administered in a slow release form rather than an intrinsically high biological availability of the Cu which they contain.

Chapman, H. L. & Bell, M. C. (1963). *J. anim. Sci.* **22**, 82.
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The importance of non-skeletal muscle sources of urinary 3-methylhistidine in the rat. By P. C. BATES, G. K. GRIMBLE and D. J. MILLWARD. *Clinical Nutrition and Metabolism Unit, Department of Human Nutrition, London School of Hygiene and Tropical Medicine, 4 St. Pancras Way, London NW1 2PE*

The excretion rate of 3-methylhistidine (3MH) is widely used as an index of skeletal muscle protein degradation in several species. Although at least 75% of protein-bound 3MH occurs in skeletal muscle it should be recognized that turnover rate as well as pool size determines production rate. Rapidly turning over pools in skin and intestinal muscle could make substantial contributions to 3MH excretion (Nishizawa *et al.* 1977). We have investigated this in two experiments. We have measured turnover rates of 3MH in skeletal muscle, skin and intestine in rats by measuring incorporation rates of radioactivity from S-adenosyl methionine labelled with [^{14}C]methionine (methyl). Assuming that in non-growing rats protein synthesis and degradation rates are equal, the degradation rates of 3MH in skeletal muscle, skin and intestine were 1·0, 3·2 and 9·9%/d respectively. Furthermore, if the degradation rate of the 3MH in the rest of the body is the mean value of these three tissues, then taking account of their respective pool sizes, skeletal muscle may account for as little as 25% of urinary excretion.

In a second experiment we plotted the time course of the change in labelling of urinary 3MH in rats over 1 month after a single injection of [^{14}C]methionine (methyl). This curve resolved into two exponentials and from their slopes and intercepts it appeared that turnover of whole body 3MH could be represented by two pools contributing 88 and 12% of total excretion with degradation rates of 53 and 3%/d respectively. Although there are several reasons why the interpretation of the urinary decay curve is equivocal (e.g. non-random degradation, Ward *et al.* 1976, and prolonged incorporation of isotope) nevertheless these results together with those of the first experiment indicate that the common assumption that skeletal muscle accounts for most of the urinary 3-methylhistidine excretion is erroneous, at least in the rat.

Nishizawa, N., Noguchi, T. & Hayeyama, S. (1977). *Br. J. Nutr.* **38**, 149.
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Cathepsin D and acid autolytic activity in skeletal muscle of protein deficient, severely protein-energy restricted and refed rats. By S. ROSOCHACKI and D. J. MILLWARD, *Clinical Nutrition and Metabolism Unit, Department of Human Nutrition, London School of Hygiene and Tropical Medicine, 4 St. Pancras Way, London NW1 2PE*

Previous measurements indicated that proteolytic activity was increased in skeletal muscle, only in protein-deficient rats in a terminal state, while during the first 7 d of rehabilitation the activity fell (Millward, 1971). We now report on the effects of prolonged severe protein–energy deficiency, moderate protein deficiency, and more extended rehabilitation on rat muscle proteinases.

Male rats (130 g) were given either a protein-free diet (LE) at 5 g/d or a diet containing 40 g protein/kg *ad lib.* (LP); all rats were given a 200 g protein/kg diet after 21 d. The LE diet induced a 33% loss of muscle whereas the LP diet slowed growth to near-total suppression. Proteinases were assayed with haemoglobin as cathepsin D (at pH 3·42, triton×100) and by autolytic activity (pH 3·5, triton×100, 1 mM Mg^{++}). Pepstatin the specific inhibitor of cathepsin D suppressed 70% of the activity towards haemoglobin and 58% of the autolytic activity (see Table).

Effect of malnutrition and rehabilitation on proteolytic activity in rat skeletal muscle (μg tyrosine equivalents/mg protein per h)

Treatment (d)	Well fed rats		Protein–energy deficient (LE)		Protein deficient (LP)	
	Cathepsin D	Autolysis	Cathepsin D	Autolysis	Cathepsin D	Autolysis
0	34·7	18·1	—	—	—	—
3	—	—	37·5*	20·1	33·4	18·9
7	34·0	17·6	38·6*	23·2*	31·5	21·0*
14	31·9	18·9	34·3	21·6*	33·5	19·4
21	30·4	18·1	33·6	22·3*	32·5	19·1
Refed rats						
1	—	—	31·8	21·1*	24·7*	16·1
3	—	—	33·1	17·3	28·5	14·9*
7	29·4	16·8	42·3*	20·0*	33·6*	16·9
14	26·4	15·5	44·0*	24·0*	37·9*	19·7*

Statistical significance of difference from control (Student's *t* test) *$P<0.001$.

The results show that the marked muscle wasting in the LE fed rats was accompanied by increased proteinase activity whereas with one exception the growth suppressed rats exhibited little change. On refeeding both activities initially fell but then increased to higher levels than previously observed. The results are consistent with increased muscle protein degradation occurring in severe energy restriction as well as during rapid growth of nutritional rehabilitation (Millward & Waterlow, 1978).

Millward, D. J. (1971). *Proc. Nutr. Soc.* **31**, 3A.
Millward, D. J. & Waterlow, J. C. (1978). *Fedn Proc. Fedn Am. Socs exp. Biol.* **37**, 2283.

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Prospective laboratory methods for estimating the susceptibility of feed proteins to microbial breakdown in the rumen. By D. G. CHAMBERLAIN and P. C. THOMAS, *The Hannah Research Institute, Ayr KA6 5HL*

New systems for protein rationing have highlighted the need for laboratory tests to assess the susceptibility of feed proteins to microbial breakdown in the rumen. We have examined three tests as a preliminary to investigating their correlation with ruminal degradability measured with duodenal-cannulated animals. The tests were applied to four protein feeds differing in type and in reported in vivo degradability values (see Table). In the first test feeds (2·0 g) were incubated with 100 ml of artificial saliva at pH 7·0 and 39° for 6·5 h to determine soluble nitrogen (N) as a proportion of total-N. The second test was similar to the first but the saliva contained a mixed protease (from *Streptomyces griseus*) and the breakdown was measured by the proportion of total-N not precipitated with 1·2 M-trichloroacetic acid. In the third test breakdown was estimated from the net production of ammonia-N from 1·0 g of feed incubated anaerobically with 100 ml of strained rumen fluid at 39° for 6·5 h.

Results of measuring the extent of protein breakdown of various feeds using three tests

(Mean values with their standard errors; number of determinations in parentheses)

Feed	Reported in vivo degradability	Artificial saliva test (7)		Protease test (7)		Rumen fluid incubation test (9)	
		Mean	SE	Mean	SE	Mean	SE
Casein	0·90	0·87	0·01	0·89	0·02	0·57	0·01
Groundnut meal	0·71–0·90	0·65	0·01	0·40	0·01	0·30	0·01
Soya-bean meal	0·51–0·71	0·26	0·01	0·38	0·01	0·06	0·002
White fish meal	0·51–0·71	0·29	0·01	0·64	0·02	0·27	0·01

All tests were reproducible. The solubility and protease tests were simple to conduct but, except for casein, gave different values for corresponding foods. The rumen fluid test was potentially the best but breakdown of groundnut and soya-bean meal relative to casein, were underestimated because of fixation of ammonia due to fermentation of carbohydrate in the feeds. Incubations of ammonium sulphate and glucose showed that ammonia fixation increased linearly (r 0·995) with carbohydrate supply and was 2·27±0·051 mg ammonia-N/100 mg glucose (mean±SE, n 8). Other carbohydrates have yet to be examined but correction of breakdown values for ammonia fixation using factors derived from the amount and type of carbohydrate in the feed appears feasible.

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Catabolism of branched chain amino acids by ruminant muscle. By B. JANE COWARD and P. J. BUTTERY, *Department of Applied Biochemistry and Nutrition, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, Leics. LE12 5RD*

Branched chain amino acids are extensively oxidized by rat muscle (see for example Goldberg & Odessey, 1972). It has been suggested that this may not be the case in sheep (Ballard *et al.* 1976). In order to investigate this we used a perfused sheep diaphragm preparation (Coward & Buttery, 1978).

A comparison of the proportions of the branched chain amino acids to tyrosine for sheep diaphragm muscle protein with the corresponding proportions for the efflux of these amino acids by the preparation, suggested that valine, leucine and isoleucine are only metabolized to a limited extent.

Perfusions were made in which a synthetic amino acid mixture was used, to give concentrations twice those found in plasma (Tao *et al.* 1974) and containing either L-[U-^{14}C]valine, L-[U-^{14}C]isoleucine or L-[U-^{14}C]leucine to give a specific activity at the start of the perfusion of 0·65, 0·8 and 1·0 μCi/μmol respectively. The initial concentrations of valine, isoleucine and leucine at the start of the perfusion were 0·46, 0·28 and 0·29 μmol/ml respectively. $^{14}CO_2$ leaving the perfusion apparatus was determined, as was the $^{14}CO_2$ content of the perfusate at the end of the perfusion (routinely 3 h). The rate of catabolism was calculated from the extracellular specific activity of the amino acids, and assuming that only the carboxyl group gave rise to $^{14}CO_2$. Little or no oxidation of valine was detected <0·07 μmol/h per 30 g wet wt. (three perfusions).

For isoleucine values of 0·33 and 0·21 were recorded while leucine gave a value of 0·21. Corresponding results calculated from the results of Goldberg & Odessey (1972) for the incubated rat diaphragm with [1-^{14}C]valine, [1-^{14}C]isoleucine and [1-^{14}C]leucine were 1·36, 2·30 and 3·32 μmol/h per 30 g wet wt. The concentration of each branched chain amino acid was 0·1 μmol/ml.

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The effect of energy restriction on nitrogen balances in obese subjects on varying protein intakes. By P. S. SHETTY, R. T. JUNG and W. P. T. JAMES, *MRC Dunn Clinical Nutrition Centre, Addenbrookes Hospital, Cambridge*

Energy intake is an important factor that alters nitrogen balances when protein intake is maintained constant. A group of sixteen obese females on varying levels of protein intake were studied to assess the effect of energy restriction on their N balances. They were divided into groups of four; each group receiving either 80, 60, 40 or 20 g milk protein/65 kg ideal body-weight (IBW) per d. After an initial 10 d equilibration on a high-energy diet (HED) providing 167 kJ (40 kcal)/kg IBW per d they were given a low-energy diet (LED) of 38·5 kJ (9·2 kcal)/kg IBW per d for a period of 3 weeks. The reduction in energy was achieved by decreasing carbohydrate intake only. During the entire study N balances were computed from total urinary, faecal and menstrual collections. Faecal N losses were corrected by use of the continuous marker technique; N lost in blood samples was also accounted for.

On energy restriction urinary creatinine excretion decreased significantly in all four groups and did not depend on the protein intake. Urea excretion increased significantly during the first week of semi-starvation and returned to pre-energy restricted levels during the second week. However, in the low-protein (20 g) group the urea excretion in urine continued to fall. During the period on the HED diet faecal N excretion correlated positively (r+0·74) with urinary N on high-protein intakes (80 g/65 kg IBW per d) and negatively (r −0·69) on low-protein intakes (20 g/65 kg IBW per d). A reduction in faecal weight and N excretion occurred on energy restriction despite varying protein intakes.

	N balance (g/d)			
		LED		
Protein intake (g/65 kg IBW per d)	HED	Week 1	Week 2	Week 3
80	+0·01	−0·91	+0·56	+0·34
60	−1·93	−3·24	−1·53	−0·67
40	−1·01	−1·62	−1·83	−0·93
20	−2·28	−2·44	−2·45	−2·06

N balance on the HED was negative on all but the highest protein intake. With 'adequate' protein intakes, (60–80 g/65 kg IBW per d), increased N losses occurred on energy restriction. On the lowest protein intakes energy restriction did not alter the N balance (Calloway & Spector, 1954). Despite varying levels of protein intake, after 3 weeks of semi-starvation the obese subjects were beginning to reduce their N excretion. This suggests that obese subjects conserve N efficiently during energy restriction and adapt progressively to retain much of the dietary protein when they are provided with low-energy diets (Van Itallie & Yang, 1977). An intake of 80 g protein/65 kg IBW per d seems necessary if N loss is to be minimized.

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The influence of dietary fibre on faecal nitrogen excretion in man. By ALISON M. STEPHEN and J. H. CUMMINGS, *MRC Dunn Clinical Nutrition Centre, Addenbrookes Hospital, Trumpington Street, Cambridge*

Faecal nitrogen is an important component of total N metabolism, and should be measured in all N balance studies. N in faeces is thought to be derived from gut secretions and sloughed epithelial cells, from the intestinal microflora, or to be unabsorbed dietary-N. It has been observed in controlled diet studies of healthy individuals that changing protein intake alters faecal-N very little but increasing dietary fibre intake leads to large increases in N excretion.

We have investigated the way in which dietary fibre increases faecal N by measuring faecal composition in two groups of six subjects given a metabolically controlled British type diet for a 3 week period and then given the same diet for a further 3 weeks with either 18·3 g or 18·0 g dietary fibre from cabbage or bran respectively. Faeces collected during the third week of each period were weighed, pooled and analysed for total nitrogen, for neutral detergent fibre (Goering & Van Soest, 1970) and for total bacterial solids (Stephen & Cummings, 1979).

	Diet			
	Control	Control +cabbage	Control	Control +bran
Faecal weight (g/d)	88·2	142·5	95·5	197·0
Faecal solids (g/d)	26·1	34·6	27·0	46·0
Neutral detergent fibre (g/d)	4·1	5·7	4·6	16·2
Bacterial solids (g/d)	14·5	19·3	15·0	17·3
Total faecal nitrogen (g/d)	1·4*	2·1	1·4†	2·0
Bacterial nitrogen (g/d)	0·8*	1·3	0·9†	1·1
Faecal nitrogen in bacteria (%)	58·9	59·7	59·7	54·3

*Three determinations.
†Four determinations.

Both cabbage and bran fibre increased faecal weight, faecal-N excretion and the excretion of bacteria. The N content of the bacterial fraction for all dietary periods was approximately 6%. An increased output of bacteria therefore accounts for the greater N excretion associated with the intake of fibre. Bacteria make up 55% of the solids and since they contain 80% water, larger bacterial dry weights imply increases in the over-all water content of the stool. Hence dietary fibre appears to increase faecal weight and faecal-N through the same mechanism, namely increased microbial growth.

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Ruminal and post-ruminal digestion of pasture herbage by grazing lambs.

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Weaned lambs 4 months old (Expt 1) and 2 months old (Expt 2) with cannulas into the rumen and the abomasum, grazed three pastures: *Phalaris aquatica* (high (PH) and low (PL) herbage availabilities) and lucerne. Individual intakes of organic matter (OM) and nitrogen were determined from faeces output during two 4 d (Expt 1) and three 5 d (Expt 2) periods. The digestibility in vitro and N content, adjusted for salivary-N, of extrusa from companion sheep with oesophageal fistulae was also determined. In the same 4–5 d periods, flows of OM and components from the stomach were calculated by reference to the ^{51}Cr-EDTA and ^{103}Ru-P markers that were infused into the rumen (Corbett *et al.* 1976*b*) and had their concentrations measured in digesta. Adjustments were made as described by Corbett *et al.* (1979). The ^{51}Cr-EDTA counts in ruminal fluid after infusions had been stopped and 6 h later were used to calculate fluid volumes and dilution rates.

(Mean values with their standard errors; number of lambs in parentheses)

	Expt 1 (Feb–April)						Expt 2 (Oct–Dec)					
Sets of results	Lucerne 7(4)		PH 4(2)		PL 3(2)		Lucerne 12(4)		PH 10(4)		PL 12(4)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Live weight (kg)	21·2	1·2	20·5	0·8	19·5	1·5	16·8	2·4	15·3	0·5	15·3	1·8
OM digestibility	0·68		0·66		0·57		0·74		0·62		0·67	
Digestible OM (g/d)	416	20	406	12	251	37	353	25	182	20	165	5·4
Fraction of DOM digested in the rumen	0·57	0·03	0·55	0·08	0·48	0·04	0·61	0·04	0·60	0·03	0·62	0·02
N intake (g/d)	22·1	1·0	23·5	1·4	12·2	1·3	21·7	1·4	9·3	0·8	11·8	0·4
N leaving stomach (g/d)	22·0	1·4	22·2	0·7	15·1	2·8	19·1	1·2	9·8	1·2	10·3	0·5
Non ammonia-N leaving stomach (g/d)	19·7	1·4	19·3	1·1	13·4	2·0	18·4	1·1	9·7	1·2	9·8	0·4
Digestibility of non ammonia-N in intestines	0·70	0·01	0·71	0·02	0·65	0·02	0·69	0·01	0·62	0·04	0·62	0·02
Rumen fluid volumes (l)	1·5	0·1	2·5	0·9	1·4	0·07	2·5	0·2	2·2	0·2	1·6	0·1
Dilution rate/h	0·28	0·01	0·27	0·07	0·27	0·02	0·22	0·02	0·15	0·01	0·16	0·01

The fraction of digestible OM that was digested in the rumen did not differ ($P>0·05$) between treatments. More N left the stomach than was consumed when the feed provided about 5 g N/100 g DOM (PL (Expt 1) and PH (Expt 2)) but less when the feed provided 5·3–7·2 g N/100 g DOM. The digestibility of non ammonia-N and dilution rate both differed between pastures ($P<0·05$); they were generally greatest for lucerne, the pasture that gave most rapid lamb growth (Corbett *et al.* 1976*a*). All dilution rates were greater than commonly reported for animals given dry feeds.

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Nitrogen intake, digestion and retention by grazing lambs. By J. L. CORBETT, *CSIRO Division of Animal Production, Armidale, NSW, Australia,* and C. J. PEREZ, *INIA, Apartado 22, Badajoz, Spain*

For dried forages, Hogan & Weston (1974) calculated the relation between crude protein (g) leaving the stomach (CPLS):crude protein intake (CPI;g), and digestible organic matter (DOM;g):(CPI;g) and reported; CPLS:CPI=0·18 DOM:CPI+0·33. The coefficients in the equation from our own results with grazed herbage (Corbett *et al.* 1979) were respectively 0·22 (±0·05) and 0·32 (±0·14); the residual SD was ±0·14 (r 0·52). The relationships between non ammonia-nitrogen disappearing from the intestines (NAN_{di};g/d) and N intake (NI;g/d) did not differ between pastures or experiments and was; NAN_{di}=0·543 (±0·053) NI+0·822 (RSD±2·31; r 0·84).

Non-cannulated lambs grazed with the similar cannulated lambs on all pastures in both experiments and their intakes of OM and N were determined concurrently. The non-cannulated lambs were slaughtered after about 8 weeks and their N retention in carcass plus wool (NR;g/d), as well as energy retention (Perez *et al.* 1976), were determined by comparing with a group slaughtered at the start.

(Mean values with their standard errors; number of lambs in parentheses)

	Expt 1 (Feb–April)						Expt 2 (Oct–Dec)					
	Lucerne		PH		PL		Lucerne		PH		PL	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Live weight (kg)	26·1	2·5(6)	25·2	1·4(6)	24·2	3·8(5)	19·8	0·9(6)	18·6	1·4(6)	17·7	1·5(6)
Digestible OM (g/d)	616	39	530	43	364	47	430	25	313	19	290	16
N intake (g/d)	33·0	2·1	30·3	2·4	17·8	1·0	26·1	1·5	15·6	0·9	20·8	1·1
NAN_{di} (g/d)*	18·7		17·3		10·5		15·0		9·3		12·1	
N retention (g/d)	4·3	0·4	2·8	0·3	1·1	0·1	2·9	0·2	2·0	0·2	1·0	0·2
Wool N (g/d)	1·3	0·3	0·6	0·2	0·5	0·1	0·7	0·1	0·7	0·1	0·5	0·09

*Calculated from NI using the equation given in the text.

The relation between N retention (NR) and NAN_{di} (NR=0·24 (±0·04) NAN_{di}−1·04; RSD±0·57) indicated low efficiency (0·24) of use of apparently absorbed NAN and this may reflect a relative shortage of energy (see Weston (1971)) and low efficiency of use of NAN_{di} for wool, estimated by Hogan *et al.* (1979) as about 0·12. The remaining fraction (0·88) might lack sulphur amino acids, because of sequestration in wool, and so be of low biological value for growth. The regression of (NR-Wool N) on (NAN_{di}−(Wool N×8·3)) indicated that the remaining NAN_{di} was used with an efficiency of 0·82 (P≃0·1) for body tissue.

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Phosphatidyl choline as a marker of duodenal flow of rumen protozoa in sheep. By A. JOHN and M. J. ULYATT, *Applied Biochemistry Division, D.S.I.R., New Zealand*

Studies of microbial growth in the rumen have been handicapped by the lack of a satisfactory marker of protozoa. Phosphatidyl choline (PC) could fill this role because it is widely found in protozoa (Thomson & Nozawa, 1972) including rumen protozoa (Dawson & Kemp, 1967). It occurs only rarely in bacteria (Shaw, 1974), and it is not secreted into the gastro-intestinal tract of sheep anterior to the entrance of the bile duct in significant amounts.

Four Romney Marsh wether sheep each with a rumen cannula and a duodenal T-piece cannula, were fed on chaffed lucerne hay at two intake levels (700 (L) and 1050 (H) g DM/d) and at two feeding frequencies within each intake level (once a day (×1) and once an hour (×24)). These treatments were replicated three times with sheep changing treatment between replicates. Each sheep received a continuous intra-ruminal infusion of ^{32}P-labelled sodium dihydrogen phosphate along with Ru-phenanthroline and Cr-EDTA as markers of duodenal digesta flow.

The extent of degradation of the PC present in the diet (<1 μg PC-P/g DM) was tested by comparing the specific radio-activities of ^{32}P-labelled PC in duodenal digesta and mixed rumen protozoa and demonstrating that essentially all dietary PC-P was degraded in the rumen and that that reaching the duodenum was of protozoal origin. Further, PC could not be detected in mixed rumen bacteria prepared from sheep fed on lucerne chaff and therefore it was concluded that PC can be used a protozoal marker.

Total N:PC-P in mixed rumen protozoa showed little variation with respect to intake level or feeding frequency, the mean (±SD) being 185±7 (*n* 19).

The effect of feeding level and frequency on protozoal-N reaching the duodenum

Feeding regimen	Protozoal-N:microbial-N at the duodenum	Protozoal-N entering the duodenum (g/kg OM truly fermented)
L× 1	0·23	4·91
L×24	0·22	4·47
H× 1	0·20	3·37
H×24	0·12	2·30

The mean total microbial-N entering the duodenum (estimated using DNA) was 19·9 g/kg OM truly digested in the stomach. Approximately 19% of this microbial-N was of protozoal origin (see Table). Differences in the efficiency of protozoal growth with intake level are also apparent in the Table.

Dawson, R. M. C. & Kemp, P. (1967). *Biochem. J.* **105**, 837.
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The use of lactating ewes in evaluating protein sources for ruminants. By J. S. GONZALEZ, J. J. ROBINSON, I. MCHATTIE and A. Z. MEHREZ, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Finnish Landrace×Dorset Horn ewes, each suckling two lambs, were used to evaluate urea (U), groundnut meal (GNM), soya-bean meal (SBM), meat-and-bone meal (MBM), linseed meal (LM), fish meal (FM) and blood meal (BM) as nitrogen sources for milk production. Each source was added to a basal diet (C) which had a metabolizable energy (ME) and crude protein (CP) content of 10 MJ and 94 g/kg dry matter (DM) respectively and supplied 0·9 MJ of ME/kg $W^{0.75}$ per d. Apart from urea the supplements were each tested at three levels, low (L), medium (M) and high (H) using a 3×3 Latin Square design. A measure of the rumen degradability of each supplement was obtained using the Dacron bag technique (Mehrez & Ørskov, 1977) in combination with an estimate of rumen retention time (Ørskov & McDonald, 1979).

Protein supplement	N intake (g/d) (percentage contributed by supplement) Level of supplementation L	M	H	Proportion of protein supplement degraded in the rumen	Milk yield (kg/d) Level of supplementation L	M	H	True protein N in milk (g/d) Level of supplementation L	M	H
C		26				1·93			11·9	
U		41		1·00		2·08			12·5	
GNM	38 (32)	47 (51)	56 (61)	0·47	2·31	2·22	2·26	16·0	15·1	16·3
SBM	38 (32)	47 (51)	57 (61)	0·47	2·29	2·47	2·45	14·4	17·0	16·8
MBM	38 (37)	47 (51)	57 (63)	0·37	2·35	2·39	2·49	14·9	15·7	16·4
LM	37 (35)	46 (54)	54 (67)	0·31	2·60	2·66	2·68	16·4	17·8	17·5
FM	38 (32)	47 (49)	57 (58)	0·26	2·59	2·83	2·84	17·1	19·6	21·2
BM	37 (32)	47 (47)	56 (59)	0·01	2·57	2·75	2·91	16·5	18·7	19·5
SE of means				0·013		0·201			1·04	

The small increases in milk yield when urea was added suggest an increase in microbial protein synthesis. The magnitude of the further increases for each protein supplement imply that LM, FM and BM were less degraded in the rumen than GNM, SBM and MBM. This is supported by the direct estimates for degradation. The correlation coefficient between milk yield and the degradability of the protein supplement was −0·89 ($P<0·01$). On the basis of degradability BM gave a lower than expected milk yield. Part of the response in yield to this protein source arose from recycling to the rumen of a proportion of the undegraded N fraction.

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Efficiency of microbial protein synthesis in cattle. By P. A. MEGGISON*, N. P. MCMENIMAN† and D. G. ARMSTRONG, *Department of Agricultural Biochemistry, University of Newcastle upon Tyne NE1 7RU*

Six mature Jersey cattle, equipped with rumen fistula and re-entrant intestinal cannulas, were used to measure efficiency of microbial protein synthesis (g microbial N entering the small intestine/kg organic matter (OM) actually digested in the rumen when fed on diets (a) cold-rolled barley–hay (7:3; w/w), (b) steam-flaked barley–hay (7:3; w/w), (c) ground maize–hay (3:1; w/w) and (d) ground barley–chopped alkali-treated straw (1:1; w/w). These diets were fed alone or supplemented with urea or biuret or isobutylidene diurea (IBDU) in two or three feeds/d; in certain experiments the urea was given 'continuously' by slow infusion in a dilute solution of molasses into the feed troughs. Microbial protein was measured in the digesta entering the small intestine (SI) using the technique of Elliott (1975). The OM actually digested in the rumen was determined as the difference between OM ingested and entering the SI less that of the microbial biomass entering the SI.

Efficiencies of microbial protein synthesis ranged from a value of $17{\cdot}4\pm1{\cdot}00$ when the diet comprised rolled barley–hay, through $24{\cdot}6\pm0{\cdot}73$ on the flaked barley–hay diet (neither of these diets was supplemented with non protein-nitrogen) to a value of $28{\cdot}8\pm2{\cdot}25$ for rolled barley–hay plus IBDU. On the alkali-treated straw–barley diets values were $21{\cdot}0\pm1{\cdot}24$ when urea was fed twice/d and $27{\cdot}8\pm1{\cdot}73$ when urea was given continuously; when IBDU was given twice/d the value was $29{\cdot}6\pm1{\cdot}80$. The mean value (sixteen experiments) was $24{\cdot}3\pm0{\cdot}92$ g microbial N/kg OM actually digested in the rumen which is in agreement with results obtained from sheep fed on high cereal, low roughage diets viz $22{\cdot}0\pm0{\cdot}20$ (McMeniman *et al.* 1976). This mean value and that of $0{\cdot}972\pm0{\cdot}22$ for OM actually digested in the rumen:digestible OM ingested gives a yield of $23{\cdot}6\pm1{\cdot}24$ g microbial-N/kg digestible OM ingested.

For predicting protein requirements of cattle under the proposed ARC Metabolizable Protein System (Roy *et al.* 1977) a yield of 30 g microbial-N/kg OM apparently digested in the rumen and a ratio of this to digestible OM intake equal to 0·65 have been assumed, giving 19·5 microbial-N/kg digestible OM intake. This value is appreciably lower than that of 23·6 g obtained in the studies reported herein.

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Efficiency of utilization of non protein-nitrogen in cattle. By P. A. MEGGISON*, N. P. MCMENIMAN** and D. G. ARMSTRONG, *Department of Agricultural Biochemistry, University of Newcastle upon Tyne NE1 7RU*

The ARC Protein Sub-group (Roy *et al.* 1977) have suggested that in situations where there is a requirement for rumen-degradable protein (RDP) urea-nitrogen should be ascribed a value of 0·80 for its efficiency of conversion to microbial N.

Meggison *et al.* (1979) fed Jersey cattle, equipped with a rumen cannula and re-entrant intestinal cannulas, basal diets comprising various cereals given individually with hay, and one comprising ground barley and alkali-treated straw. Each diet was fed either two or three times/d with and without the addition of individual non protein-N (NPN) sources. Amounts of microbial N (g/d) entering the small intestine were measured. Efficiency of NPN use is defined as the increase in microbial-N (g) at the duodenum over that observed when no NPN supplement was given/kg N in the NPN supplement.

Values for efficiency of NPN use approached 800, the value suggested by Roy *et al.* (1977), only when NPN was supplied either as urea given 'continuously' or in the form of a sustained release source of NPN fed infrequently. Efficiency of microbial protein synthesis was high when flaked barley was fed, even when no NPN supplement was fed.

Basal diet	NPN source	Frequency of feeding NPN (per d)	Efficiency of NPN utilization
Rolled barley–hay (7:3; w/w)	Urea	3	46
	IBDU	3	875
	biuret	3	583
Flaked barley–hay (7:3; w/w)	urea	3	−83
	IBDU	3	−9
	biuret	3	357
Ground maize–hay (3:1; w/w)*	urea	2	147
	urea	2	174
Ground barley–alkali-treated straw (1:1; w/w)	urea	2	−139
	urea	continuously†	786
	IBDU	2	746

*Mineral salts included at 70 g/kg feed dry matter.

†Urea dripped into feed trough at constant rate in a very dilute solution of molasses.

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An attempted evaluation of the proposed ARC protein system with reference to the lactating cow. By P. A. BRETT, M. ALMOND, D. G. HARRISON, P. ROWLINSON, J. ROOKE and D. G. ARMSTRONG, *School of Agriculture, University of Newcastle upon Tyne NE1 7RU*

When four heifers fitted with a rumen fistula and a re-entrant cannula at the proximal duodenum were fed at a level just above maintenance with a diet of (g/kg) 270 barley, 270 dairy cake and 460 wilted grass silage (DM basis), the mean values for the proportion of digestible organic matter (DOM) apparently digested in the stomachs and for g microbial N synthesized/kg OM apparently digested in the stomachs were 0·78 and 27·72 respectively. The corresponding values for a diet containing (g/kg) 60 barley, 270 cake, 120 soya and 450 silage were 0·75 and 34·75. The rumen degradability of silage N, cake plus barley N and soya N were 0·69, 0·95 and 0·74 respectively.

Twenty Friesian cows were fed to milk production (MAFF bulletin 33) in a subsequent experiment using a cross-over design. Two diets containing the same barley, cake, soya and a virtually identical silage were fed. Diet A (151·5 g crude protein (CP)/kg DM) contained these components in the respective proportion of 0·277, 0·275, 0·005 and 0·443. In diet B (196·4 g CP/kg DM) part of the barley was isoenergetically replaced with soya giving the proportions of 0·170, 0·275, 0·116 and 0·439 respectively. The dietary addition of soya had no significant effect upon milk yield and composition.

The quantities of nitrogen (g/d) theoretically available to the animals for milk production using (1) the suggested ARC rumen values and (2) our measured values were calculated together with (3) the quantities of nitrogen actually produced in the milk. The values for 1, 2 and 3 obtained with diet A were 126·1, 122·2 and 123·9 respectively and those for diet B were 153·0, 153·9 and 128·0 g/d. Although the suggested ARC values (Roy *et al.*) and our measured rumen values were markedly different, their use gave remarkably similar predictions for N available for milk production. The correlation between N available for milk production and found in the milk was excellent with diet A, but, due to the lack of productive response to the dietary soya, was poor with diet B.

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Maternal weight, reproduction, calf mortality and calf growth in farmed red deer (*Cervus elaphus*). By K. L. BLAXTER, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB* and W. J. HAMILTON, *Hill Farming Research Organisation, Glensaugh, Laurencekirk, Kincardineshire AB3 1HB*

The joint investigations by the Hill Farming Research Organisation and the Rowett Research Institute to find ways of farming red deer were started in 1970 at Glensaugh in Kincardine. Six cohorts of hinds born in successive years have been reared and bred to stags under hill farming conditions. The reproductive performance of this base herd has been examined up to the calving of June 1978 in relation to the weight of the hinds measured in late September immediately before the rut.

Whether or not a hind became pregnant depended on her weight at the time of the rut, and this was independent of her age. No hind weighing less than 52 kg proved pregnant and fertility increased to a maximum close to 100% at 95 kg. Birth weight of calf was also positively related to the weight of the hind. Stag calves were heavier at birth than hind calves. Additionally, heavier hinds calved earlier in the season than lighter ones. Subsequently, the daily gain in weight of stag calves was greater than that of hind calves and for both sexes there was a significant effect of hind weight on calf growth rate. Mortality of calves was 100% if birth weight was less than 4 kg, fell to 5% for weights of 7–8 kg and increased at heavier weights.

Even if a small hind calves, its calf is disadvantaged in several ways. It is small at birth, subject to greater mortality, has a shorter summer season in which to grow and its growth rate is less than that of a calf born to a heavier hind. Comparing hinds weighing 60 and 90 kg, fertility was 49% in the former, 96% in the latter, survival rate was 83% and 88%; the number of calves weaned/100 hinds was thus 41 and 84. The mean weaning weight of male and female calves born to hinds weighing 60 kg was 32 kg and to those weighing 90 kg, 41 kg. Annual production in terms kg live weight of weaned calves was 13·2 kg/hind for 60 kg hinds and 34·5 kg/hind for those weighing 90 kg.

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Voluntary intake of feed and equilibrium body-weight in sheep. By K. L. BLAXTER and J. C. GILL, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Lambs (Suffolk×(Finnish Landrace×Polled Dorset)), weighing 25–30 kg and aged 4–5 months, were given *ad lib.* access to two pelleted diets, Diet A (Wainman *et al.* 1970) or Diet AA6 (Wainman *et al.* 1975), for periods of up to 4·5 years. They were shorn every 6 months, weighed at these times and also fortnightly. Daily intake of feed was measured throughout. Animals were killed at intervals to determine body composition and eight were killed when aged more than 4 years.

Mean daily feed intake was 13% higher in the 8 weeks before shearing in July than in the 8 weeks before shearing in January. Mean intakes averaged over 6 month periods, however, were invariant with time. The sheep consumed the same amount in the first 6 months as they did in the same period 3 years later. There were, however, large differences between individuals, the range for Diet A being 1·87 to 2·41 kg/d and for Diet AA6 1·61 to 2·10 kg/d (SE±0·076 kg/d).

The time course of shorn body-weight (W) was described by the equation $W=A-B\ (\exp(-kt))$ for each individual, t being measured in months. The mean asymptotal weights (A) for nine sheep given Diet A was 130 kg (113–144) and 95% of this was reached at 41 months from the start. For Diet AA6 the corresponding values were 126 kg (102–147) and 95% of asymptote was reached in 36 months.

Asymptotal weight was correlated with feed intake. Daily metabolizable energy intake (in the first 2 years) was 459 kJ/kg asymptotal weight raised to the power 0·75 for sheep given Diet A and 499 kJ for sheep given Diet AA6. The efficiencies of utilization of energy of these diets (k_m) were 0·66 and 0·68 respectively (Blaxter & Boyne, 1978). Estimated fasting metabolism was thus 306 kJ and 341 kJ/kg $W(A)^{0·75}$ for the two diets. Directly determined fasting metabolism measurements made on seventeen of the sheep were 322±5 kJ/kg $W^{0·75}$.

The results imply that some of the variation between laboratories in estimates of voluntary intake could be due to failure to take account of the effect of season. Additionally, they show that voluntary intake is established early in life and this determines the ultimate body size sheep attain on a particular diet.

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