Indicators of undernutrition in cattle

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Abstract

Potential biochemical indicators of long-term undernutrition in cattle, which could be used objectively, reliably and routinely, were investigated by evaluating frequently analysed metabolites in cattle. In an initial study, a meta-regression of literature data for glucose, urea, non-esterified fatty acids (NEFA) and β–hydroxybutyrate (BHB) against body condition score (BCS), body weight (BW) and its change (BWC) was conducted. The credible intervals of the gradients included zero for all regressions, showing that there were no significant relationships between any of the blood metabolites and BCS, BW or BWC across the 13 included studies. In a second study, fresh field samples from nine herds of adequately-nourished suckler cows and stored samples from two herds of suckler cows, which had experienced severe undernutrition, were analysed for serum albumin, total protein, urea, BHB, NEFA, creatinine, fructosamine and globulin. Positive regressions with BCS were obtained for albumin and creatinine, and a negative regression with fructosamine/albumin ratio. With the threshold for detecting undernutrition set at ≥ 10.75 μmol g^{-1}, the fructosamine/albumin ratio gave sensitivity and specificity of 100%. Therefore, it is probably necessary to combine several blood measures to obtain a valid assessment of the nutritional state of ruminants, and we advise against the use of a single plasma metabolite concentration in assessing the nutritional state and welfare of individual cows.

Keywords: albumin, animal welfare, cattle, fructosamine, plasma metabolites, undernutrition

Introduction

Adequate nutrition, when nutrient intake meets nutrient requirements, is a fundamental requirement for the welfare of all livestock. Inadequate nutrition is either caused by malnutrition — an improper balance of nutrients in the diet — or undernutrition — an insufficient total nutrient intake. Problems related to malnutrition are well researched within the field of cattle nutrition whereas there is less information available on the effects of under-nutrition on animal welfare. Undernutrition, defined as a prolonged low nutrient intake, is particularly likely in cattle when the standard of husbandry is inadequate, when the profitability in cattle farming is low, when there are structural changes in the industry or when there are movement restrictions imposed during epidemics. The effects of undernutrition on animal welfare are determined by the degree and length of undernutrition. However, there is limited information available on different stages of undernutrition in ruminants and the opportunities to collect such data are limited for ethical and legislative reasons. Objective measures of undernutrition are required to assist in the determination of possible infringements of legislation by livestock farmers.

Evidence of undernutrition in farm animals is currently a matter of subjective veterinary assessment. Attempts have been made to standardise the subjective visual assessment of the nutritional status of cattle by developing systems for scoring body condition (Lowman *et al* 1976; Edmonson *et al* 1989). People experienced in body condition scoring may be capable of achieving a high degree of repeatability between different occasions, but the risk of inter-person variation remains (Calavas *et al* 1998; Veerkamp *et al* 2002).

The research reported here was undertaken to investigate potential biochemical indicators of long-term undernutrition in cattle, which could be used objectively, reliably and routinely by veterinary practitioners. The objectives were to determine the value of frequently analysed metabolites in cattle, to establish definitive reference ranges for these metabolites and to suggest possible indicators of undernutri-tion. The objectives were addressed in two studies: (1) a literature study that included a well-defined literature search to identify literature sources of metabolites in cattle plasma, and a meta-regression analysis on these data; and (2) a field sample study that included analysis of metabolites from fresh samples obtained from adequately nourished beef

Table 1   Search phrase used in the initial phase of the literature searches.

Animal terms		Nutritional state terms
Cattle		Undernutrition
Bovine		Malnutrition
Dairy		Starvation
Beef		Feed-intake
Ruminant	Ruminants	Feed intake
Cow	Cows	Dry matter intake
Heifer	Heifers	Feed-deprivation
Bull	Bulls	Feed deprivation
Bullock	Bullocks	Energy deficiency
Steer	Steers	Protein deficiency
		Low intake
		Low feed-intake
		Low feed intake
		Negative energy balance

suckler cows, and from stored samples from beef suckler cows that experienced severe undernutrition.

Materials and methods

Study 1: literature survey and analysis

Criteria for the literature survey

Literature reports were only included if they contained data on body condition scores (BCS), and/or body weights (BW), and/or changes in body weight (BWC) of beef or dairy cattle that were at least two years old and had received the same diet for 21 days or longer, in order to avoid confounding effects on BCS, BW and BWC attributable to variation, such as changes in rumen fill. The time period of 21 days represents the maximum time taken for rumen conditions, and the corresponding effects on BW and production responses to stabilise in response to varying nutrition levels, and was chosen because it is the shortest possible period after which steady state nutrition can be assumed in ruminants. The 21 day period for the rumen to adjust to dietary regimes is well established, for example in research that studied the transition from winter housing to pasture (eg Taylor 1954; Balch & Line 1957; Storry & Sutton 1969; Wilkinson & Cumberland 1970). Reports containing information on dairy cows were only included in the study if the cows were non-lactating or were believed to have passed peak production. Therefore, we aimed to include only animals that were in a steady metabolic state, without rapid changes in metabolism or negative nutrient balance driven by high milk yield rather than limited nutrient intake. In addition, only reports in English or Scandinavian languages were considered.

Literature searches

The online literature databases ISI Web of Science and PubMed, and the CD–Rom literature database Vet CD

1973–1988 (CABI Silverplatter Information) were searched. The initial searches were conducted in September 2002 and included all combinations of at least one of the animal terms and at least one of the nutritional state terms presented in Table 1. The identified references were downloaded into the EndNote reference software (EndNote 7.0, Thomson ISI, Philadelphia, USA) and were then searched for the words 'blood' or 'plasma' in any field of the reference, with the objective of identifying reports relating to plasma parameters.

The databases of the references retained were combined into one, discarding duplicate references. All references were then assessed for suitability, on the basis of the information given in title, keywords and abstract, for inclusion in the meta-analysis.

The remaining studies were examined for the reporting of data relating changes in body weight and body condition to changes in the concentrations of suitable plasma metabolites. Data was extracted from either tables or graphs, and in the case of the latter, graph digitalising software was used (Ungraph, Biosoft, Cambridge, UK).

Meta-regression — model description

In determining whether there was a relationship between the nutritional state of cows and plasma constituents, a meta-regression analysis was performed on the literature data set. We designated the nutritional state measures BCS, BW and BWC as true measures, and the plasma measures as surrogate measures. The simplest situation was data comprising one true and one surrogate measure for each individual cow in a group. The surrogate was regressed against the true measure and the gradient coefficient estimated. If the gradient coefficient was significantly different from zero it implied a relationship.

Data was suitable from 13 studies. Each of these studies consisted of one or more different experimental (treatment) groups, each on a different diet or of a different breed. For each treatment group, true and surrogate outcomes collected over time for each cow, the mean and standard error of the surrogate outcomes, the mean of the true outcomes, aggregated at each time point for the treatment group were available for the meta-regression. The order of the time points was considered unimportant for the current study, and replication over time was a mechanism for generating true/surrogate data pairs.

There were three potential complications. First, there were different numbers of cows in each study; therefore, the analysis was weighted accordingly. At each time point observation, the mean surrogate measure for a group of cows was assumed to be sampled from a normal distribution with standard deviation equal to the standard error of the treatment group mean.

Second, repeated measures from the same treatment group are expected to be more similar than observations from different treatment groups. This intra-group correlation was accounted for by regression of the observed mean at each time against the true observation, with a different intercept

Box 1

$$s_{ijk} \sim N(\alpha_{ij} + \beta_{ij}t_{ijk}, e_{ijk})$$

$$\alpha_{ij} \sim N(\alpha_i, \sigma_\alpha^2)$$

$$\beta_{ij} \sim N(\beta_i, \sigma_\beta^2)$$

$$\alpha_i \sim N(\alpha_0, \sigma_\alpha^2)$$

$$\beta_i \sim N(\beta_0, \sigma_\beta^2)$$

Let i denote the study, j the treatment group within a study and k the observations over time. s_{ijk} denotes the surrogate measure and t_{ijk} the true measure at time k, in treatment group j, in study i. e_{ijk} is the (known) variance of s_{ijk}.

Figure 1

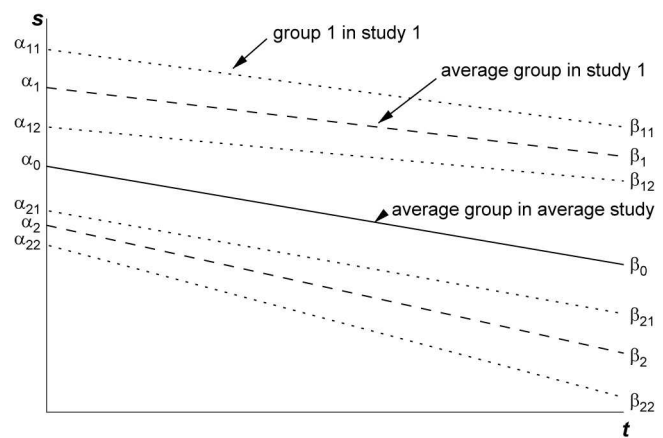Graphical explanation of the model used for meta-analysis of the literature data.

and gradient for each treatment group in each study. These intercepts and gradients were each sampled from random-effects normal distributions (level 2 random effects). Therefore, observations over time on cows are assumed to be independent conditional on their treatment group, while allowing observations from the same group to be more similar than observations from a different one.

Third, measures from different treatment groups in the same study were assumed to be related. This was accounted for by allowing the means of the level 2 random effect to be different for each study, but to have common variance across studies. Therefore, treatment groups are assumed to be independent conditional on the study.

The intercept and gradient means for each study were each assumed to come from random-effect normal distributions with common means and variances. These estimates of the means were used as estimates of intercept and gradient for the overall relationship between the surrogate and true outcomes. The essential information in the output of this kind of analysis is the overall gradient of the relationship between the surrogate and true outcome. This has to be significantly different from zero for the surrogate outcome candidate to be a useful surrogate. To be a useful surrogate the estimates of the gradients between studies also need to be reasonably homogeneous because wide variability between studies would imply that the surrogates were unreliable. Furthermore, if the study gradients were homogeneous, the individual treatment group means would also be required to be homogeneous. Visual inspection of gradient homogeneity was used to draw conclusions regarding the strength of the relationships.

The algebraic definition of the model is given in Box 1: i denotes the study, j the treatment group within a study and k the observations over time. s_{ijk} denotes the surrogate measure and t_{ijk} the true measure at time k, in treatment group j, in study i. e_{ijk} is the (known) variance of s_{ijk}. β_0 is the overall gradient of the relationship between the surrogate and true outcome. The model is described graphically in Figure 1. The dotted line shows the surrogate/true relationship for each treatment group; these corresponded to

α_{ij} and β_{ij}. The dashed lines give the relationship for each study, α_i and β_{il}; the solid line represents the overall relationship, corresponding to α_0 and β_0.

The model fitting was achieved using Bayesian inference Using Gibbs Sampling (Gilks *et al* 1994). This uses Bayesian Markov Chain Monte Carlo (MCMC) techniques to estimate parameters (Gilks *et al* 1996) and requires prior distributions. Means of normal distributions, α_0 and β_0 were given $N(0, 10^6)$ prior distributions. Log standard deviations of normal distributions were given uniform distributions. These prior distributions were locally almost uniform for mean parameters, were uniform for log variance parameters, and were close to 'non-informative'. MCMC runs with a burn-in of 5000 iterations followed by monitoring over 20 000 iterations, which indicated satisfactory convergence. Means and 95% credible intervals of the posterior distributions were used to summarise the results.

Study 2 — field sample study

Collection and analysis of blood samples from adequately-nourished animals

Blood samples were collected by veterinary practitioners from nine herds of beef cattle in England from the counties of Derbyshire, Devon, Norfolk and Yorkshire. These herds were of adequately-nourished cattle (ie considered by the attending veterinary surgeon to be receiving adequate nutrition). The herds were routinely sampled by the veterinary practitioners in February and March 2003, in connection with a Brucellosis Eradication Scheme (Defra 1999). The practitioners were asked to provide aliquots of blood from mature beef suckler cows, and to record their body condition score at the time of sampling. All practitioners had participated in further education in body condition scoring and they all used the same scoring technique (Defra 2002). The practitioners also reported whether the herd was housed

indoors or kept outdoors, and the principal season of calving. The aliquots of blood samples, collected into plain tubes, were forwarded on ice to the Department of Clinical Veterinary Medicine, University of Cambridge, UK, where the serum was separated and frozen for subsequent dispatch for analysis at the Veterinary Laboratories Agency (VLA), Shrewsbury, UK.

Analysis of serum constituents

Seven analytes were determined at VLA, and two further values were calculated. The reasons for selection of analytes and the methods used at VLA are as follows:

(1) Albumin, total protein, urea — reduced concentrations of albumin (bromocresol green: Randox Laboratories Ltd, Crumlin, UK), total protein (biuret reaction: Sigma, Gillingham, UK) and urea (urease reaction: Bayer Diagnostics, Newbury, UK) are considered to indicate short-term negative protein balance (Payne *et al* 1970).

(2) β–hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA) — elevated concentrations of BHB (enzymic oxidation: Randox Laboratories Ltd, Crumlin, UK) and NEFA (ACS–ACOD method: Wako, Saitama, Japan) are considered to indicate short-term negative energy balance and adipose tissue catabolism (Reist *et al* 2002).

(3) Creatinine — reduced concentrations of creatinine (Jaffe reaction: Thermotrace Ltd, Rawdon, UK) indicate reduced muscle bulk which may result from prolonged active tissue protein catabolism (Istasse *et al* 1990).

(4) Fructosamine, fructosamine/albumin ratio — reduced concentrations of glucose are considered to indicate short-term negative energy balance (Reist *et al* 2002), but glucose determinations can only be performed on fresh samples (analysed or frozen within approximately 1 h of sampling), which were not available in the present study. Therefore we chose to measure serum fructosamine (NBT reduction: ABX, Shefford, UK), which reflects average glucose concentration over the preceding two to three weeks (Armbruster 1987). Changes in fructosamine might be masked if there are concurrent changes in concentrations of serum protein, particularly albumin. The fructosamine/albumin ratio allows for such protein changes (McCance *et al* 1989) and was investigated here in view of the low albumin values expected in undernourished cattle. Although the use of such a correction to fructosamine was not considered important in assessing diabetics (Brown & Grunberger 1991; Jensen 1993) and was not found helpful in studies on healthy calves (Coppo 2001), it has been suggested as a useful approach in cases were albumin is likely to be low (McCance *et al* 1989; Kawamoto *et al* 1992).

(5) Globulin — elevated globulin (total protein minus albumin) concentrations may be a sign of chronic inflammation or immune-mediated disease. As total protein is a linear combination of albumin and globulin, only the latter analytes were subjected to statistical analysis.

(6) Statistical analysis of data from adequately-nourished animals — multiple linear regression analysis was used with the objective of defining relationships between serum analyte values and BCS, with one analyte as a dependent variable and BCS as an independent variable, with Herds 1–8 each entered as independent dichotomous variables. An alternative, non-parametric approach was also investigated in which, for each analyte, the median value for animals with BCS 3 in each herd was calculated and subtracted from all the values for the herd, so that the trends in each herd were aligned. Trends within this 'difference data' were then assessed by Spearman rank correlation. Furthermore, a reference range (2.5–97.5 percentile) was determined for each analyte from the 389 adequately-nourished animals sampled. Analyses were carried out using Excel 98 (Microsoft Corporation) and SPSS version 6.1 (SPSS Inc: Chicago, USA) software.

(7) Collection and analysis of blood samples from undernourished animals — within the time frame of this investigation, it proved impossible to obtain fresh blood samples from cattle that had been subjected to severe undernutrition sufficient to cause emaciation, but frozen serum samples from two herds (Herds A and B) were made available to us by the Royal Society for the Protection of Cruelty to Animals. These samples were collected in the spring of 1998 in connection with suspected severe undernutrition in two herds of beef suckler cows. The herds comprised mature animals, some with calves at foot, that had been offered a restricted allocation of a total mixed ration and were emaciated with a BCS of 1.0 on a 1–5 scale (Defra 2002), according to the attendant veterinarian. The frozen serum samples from both herds were dispatched for analysis at VLA, Shrewsbury, UK. A total of 27 samples were analysed in 2003, after five years of storage at –20°C. For 20 animals (Herd B) the samples had also been analysed when fresh in 1998 at a regional VLA for albumin, BHB, total protein (allowing globulin to be calculated) and urea.

Statistical analysis of data from under-nourished animals

The first objective was to determine whether any of the analytes had significantly different average values in either of the under-nourished herds compared with adequately-nourished animals (taken as a whole). The two under-nourished herds were each compared with the combined adequately-nourished animals using the Mann-Whitney U test. The 1998 values for Herd B were also compared with the adequately-nourished animals using the Mann-Whitney U test. The 1998 and 2003 analytical results were compared by paired *t*-tests.

The second objective was to determine whether any of the analytes provided a useful diagnostic test for undernutrition, on the basis of the reference ranges determined above for the adequately-nourished animals. The sensitivity and specificity of each analyte as a test for undernutrition was assessed. Relative Operating Characteristic (ROC) analysis was carried out to optimise the test parameters.

Table 2   Schematic overview of literature searches.

Step	ISI Web of Science	PubMed	Vet CD 1973	Other sources
Initial search phrase	2976	12 958	148	–
Blood or plasma	531	5326	32	–
Duplicates	Combined into one database and 2030 duplicates deleted			–
	3859			
Identifying papers for full text revision	Titles, abstracts and keywords reviewed			–
	126 references chosen for full text revision			
Identifying papers for inclusion in meta-analysis	10			3
Data extraction	Data extracted from 13 papers			–

Table 3   Studies included in the meta-regression and the body weight, body condition score and metabolite data used.

ID study number	Number of animals	Groups	Duration (days)	Number of samples per cow	BW	BCS	BWC	Data availability BHB	Glucose	NEFA	Urea
1	12	2	28	5	yes	no	yes	×	×	×	×
2	16	2	77	4	no	yes	yes	×	×	×	×
3	18	2	42–70	4–6	no	yes	no			×	
4	21	2	56	9	yes	yes	yes			×	×
5	19	2	49	1	no	no	yes		×		×
6	17	3	70	4	yes	yes	yes		×		×
7	11	2	115	4	yes	yes	yes	×		×	
8	8	2	21	1	no	no	*		×	×	
9	18	1	133	11	yes	yes	yes	×	×	×	×
10	18	3	28	1	yes	no	yes	×	×	×	
11	43	3	98	1	yes	yes	no	×	×	×	×
12	48	4	91	1	yes	yes	no	×	×	×	
13	24	3	70	1	yes	yes	no	×	×	×	×

* estimated from data on metabolisable energy.

Results

Literature data

Literature searches

The initial searches resulted in 2976 references in ISI Web of Science, 12 958 references in PubMed and 148 references in Vet CD 1973–1988 (Table 2). Requiring the words 'blood' or 'plasma' in any of the fields reduced this to 531 references in the ISI Web of Science file, 5326 references in the PubMed file and 32 in the Vet CD file. After the deletion of duplicate references, a total of 126 references were found suitable for full text review. The most common reasons for not being included in the full text review were language, and the species and age of the animals. From the 126 references, 10 studies were identified as suitable for inclusion in the meta-analysis. The most common reasons for omitting papers were experimental designs with short experimental periods or lack of data on BCS and BW. In addition to the 10 papers chosen from the searches, 3 papers that fulfilled

the criteria were already known to the authors. The references used in the meta-analysis are listed in Appendix 1.

There was sufficient data for meta-regression for glucose, urea, NEFA and BHB (Table 3). The authors of study number 1 provided additional data. Urea values for studies number 11 and number 12 were corrected by a constant of 0.214, after discussions with the authors.

The number of data points extracted differed between the studies because of differences in experimental design. The number of groups of cows that were possible to include from each study ranged from 1 to 4. The study sub-groups were usually different treatment groups but in one case (study number 3) the sub-groups consisted of different dairy breeds. The studies reported data from experimental periods ranging from 21 to 133 days in length and the number of samples obtained during the experimental periods ranged from 1 to 11. The numbers of animals included in the studies ranged from 8 to 48 and in total the meta-analysis included data from 273 animals. Most of the cows were of dairy breed

Table 4   Mean gradient and credible intervals of meta-regression on metabolic data across body condition scores (BCS), body weight (BW) and rate of body weight change (BWC).

Metabolite	Versus	Mean gradient	Credible interval of gradient		Unit
			2.5%	97.5%	
BHB	BCS	−0.17	−1.55	1.23	mmol l^{-1} unit^{-1}
	BW	0.00	−1.30	1.34	mmol l^{-1} kg^{-1}
	BWC	−0.05	−1.90	1.77	mmol l^{-1} kg^{-1} day^{-1}
Glucose	BCS	−0.02	−1.51	1.53	mmol l^{-1} unit^{-1}
	BW	0.00	−1.31	1.34	mmol l^{-1} kg^{-1}
	BWC	0.12	−1.12	1.33	mmol l^{-1} kg^{-1} day^{-1}
NEFA	BCS	0.13	−1.71	1.35	mmol l^{-1} unit^{-1}
	BW	0.00	−1.41	1.40	mmol l^{-1} kg^{-1}
	BWC	−0.03	−1.17	1.09	mmol l^{-1} kg^{-1} day^{-1}
Urea	BCS	0.24	−1.24	1.80	mmol l^{-1} unit^{-1}
	BW	0.00	−1.14	1.13	mmol l^{-1} kg^{-1}
	BWC	−0.50	−2.06	1.06	mmol l^{-1} kg^{-1} day^{-1}

Table 5   Distribution of body condition scores (BCS) within herds of adequately-nourished cattle; values are numbers of animals.

Herd	Body condition score							
	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0
1	1	3	4	10	–	15	2	2
2	–	3	–	33	–	28	1	1
3	–	2	–	13	–	6	–	–
4	–	–	–	1	–	12	–	1
5	8	37	9	5	–	–	–	–
6	1	–	–	3	2	9	1	7
7	–	–	–	13	–	1	–	–
8	–	–	3	3	–	2	–	–
9	–	–	7	49	33	7	–	–

but three of the studies were performed on beef cows (numbers 4, 6 and 7). One study (number 7) included metabolic data from three time-points during the day; a mean value was calculated and used for the meta-regression. Metabolic data was presented either as group mean values representing one time-point or as means over a time-period if several samples were taken. In one study (number 8), BWC was estimated from data on energy balance. These estimates were based on a conversion factor of 16 MJ kg^{-1} BW (Alderman & Cottrill 1993). All papers that reported BCS used a scale of 1–5, emaciated–obese, respectively.

Meta-regression

The credible intervals of the gradients included zero for all regressions, showing that there were no significant relationships between any of the plasma metabolites and BCS, BW or BWC across the 13 included studies (Table 4; Figure 2). Despite this, the regressions against BWC (Figure 2) showed the most promising patterns, with all studies showing a negative slope for NEFA and urea, and positive slope for glucose.

Adequately-nourished cattle

The herds sampled ranged in size from 8 to 147 animals (median 23). All herds were housed indoors and all but one was predominantly spring-calving. A total of 389 animals were sampled and BCS were available for 338 of these; BCS was recorded to a precision of 0.5. The range of BCS values differed between herds (Table 5).

BCS and serum analyte

Multiple linear regression analysis revealed no significant association with BCS for BHB, fructosamine, globulin,

Figure 2
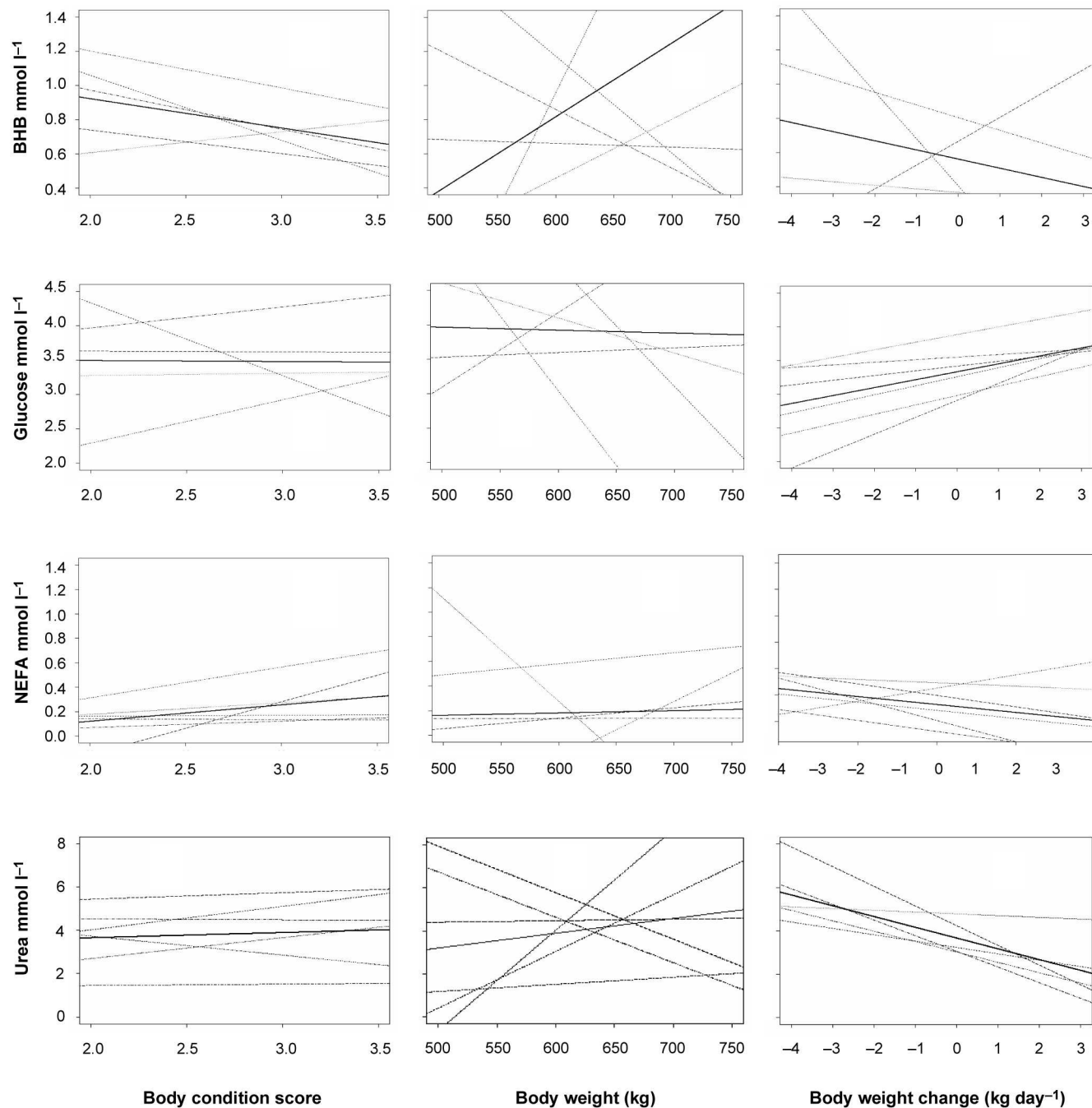
Graphs showing results from meta-regression of literature data on plasma β–hydroxybutyrate (BHB), glucose, non-esterified fatty acids (NEFA) and urea against body condition score, body weight and body weight change. The solid line in each graph represent regressions over 13 studies and dotted lines represent individual studies.

NEFA or urea ($P > 0.05$). As NEFA values were not normally distributed in four out of the nine herds (Shapiro-Wilks and Lillefors tests), this variable was also analysed as $\log_{10}$ (NEFA), but it still had no significant regression with BCS. Significant regressions were only found for albumin (regression coefficient: 1.11 ± 0.35, $P = 0.001$), for creatinine (6.65 ± 2.25, $P = 0.003$) and for fructosamine/albumin ratio (-0.27 ± 0.09, $P = 0.004$).

Trends were also investigated using a non-parametric method. Difference data was subjected to Spearman rank correlation against BCS. The strongest correlation was a positive correlation of BCS with creatinine ($r^2 = 0.21$), the next strongest was a positive correlation with albumin ($r^2 = 0.14$). For fructosamine/albumin ratio, globulin, NEFA and urea, $r^2 < 0.1$. There was no significant correlation with BHB or fructosamine.

Table 6 Reference ranges for serum constituent concentrations in adequately-nourished beef cattle.

Analyte	Units	Reference range
Albumin	g l⁻¹	25.00–44.40
β–hydroxybutyrate	mmol l⁻¹	0.12–0.61
Creatinine	µmol l⁻¹	110.00–225.00
Fructosamine	µmol l⁻¹	183.00–365.00
Fructosamine/albumin	µmol g⁻¹	5.63–9.70
Globulin	g l⁻¹	27.20–49.20
NEFA	µmol l⁻¹	176.00–1317.00
Total protein	g l⁻¹	59.20–87.50
Urea	mmol l⁻¹	1.88–7.00

Table 7 Median values for analytes in adequately-nourished animals and under-nourished Herds A and B.

Analyte	Units	Adequately-nourished	Herd A	Herd B 2003	Herd B 1998
Albumin	g l⁻¹	36.10	27.10***	26.70***	31.00***
β–hydroxybutyrate	mmol l⁻¹	0.31	0.29	0.27	0.23*
Creatinine	µmol l⁻¹	157.00	81.00***	134.00***	–
Fructosamine	µmol l⁻¹	248.00	367.00***	337.00***	–
Fructosamine/albumin	µmol g⁻¹	7.00	15.53***	12.53***	–
Globulin	g l⁻¹	36.60	34.90	45.60***	47.50***
NEFA	µmol l⁻¹	467.00	343.00	346.00*	–
Urea	mmol l⁻¹	4.30	6.50***	2.10***	1.70***

*, *** = significantly different from adequately-nourished animals at $P < 0.05$ and $P < 0.001$, respectively.

Reference range

For each analyte a reference range was determined as 2.5 percentile to 97.5 percentile of values for all adequately-nourished cattle (389 animals) (Table 6).

Under-nourished cattle

Comparison of 1998 and 2003 values for Herd B

Some analytes had been measured for Herd B at a regional VLA when the samples were fresh. Following five years of storage at –20°C, there was a significant loss of protein (albumin: –16%, $P < 0.001$; calculated globulin: –5%, $P < 0.001$). The metabolites increased significantly (BHB: +13%, $P < 0.001$; urea: +19%, $P < 0.001$). These changes were just beyond what we would assume to be the expected limits of analytical variation, although information on the actual limits is not available from VLA. The largest proportional difference was seen in urea, but the absolute values for urea were small; therefore a small change appeared as a large percentage.

Comparison of under-nourished herds and adequately-nourished cattle

The two under-nourished herds, A and B, were each compared with the combined adequately-nourished animals (Table 7). The 1998 data on Herd B was also compared, and although there are differences in detail this data leads to the same

conclusions as the later data. For BHB the under-nourished herds are the same ($P \geq 0.05$), or lower (Herd B: 1998, $P = 0.002$) than the adequately-nourished group. For NEFA the under-nourished herds are the same ($P \geq 0.05$), or lower (Herd B: $P = 0.019$) than the adequately-nourished group. For albumin and creatinine both herds had values significantly lower than the adequately-nourished animals ($P < 0.001$), whereas both were significantly higher for fructosamine ($P < 0.001$) and for fructosamine/albumin ratio ($P < 0.001$). Herd B was higher for globulin ($P < 0.001$). Although Herd A was significantly higher for urea ($P < 0.001$), Herd B was significantly lower for this analyte ($P < 0.001$).

Using the reference ranges to detect under-nourished cattle

The reference ranges derived above provide diagnostic tests for undernutrition. These indicate undernutrition when an animal has an analyte value that is higher or lower (depending on analyte) than the reference range. The specificity of each test (the proportion of true negatives, ie normal animals, found negative by the test) is set at 97%, by the nature of the reference range. The sensitivity of the procedure as a 'diagnostic' test for undernutrition (the proportion of true positives, ie under-nourished animals, found positive by the test) can be calculated from our data for each of the under-nourished herds (A and B) and for the two herds combined (ie A + B).

Only two analytes had a sensitivity value ≥ 90%. Creatinine below the reference range had a sensitivity of 100% in Herd A, but of only 30% for combined Herds A + B. The fructosamine/albumin ratio above the reference range had a sensitivity of 100% for both Herd A and Herd B.

ROC analysis, in which the threshold value of the test is adjusted to optimise specificity and sensitivity, gave the following results for the fructosamine/albumin ratio with the condition for undernutrition set at ≥ 10.75 µmol g⁻¹:

Sensitivity = 100%;

Specificity = 100% (95% confidence interval: 99–100%);

Likelihood ratio for condition with a positive test = 389 (95% confidence interval: 55–2755);

Likelihood ratio for condition with a negative test = 0.

Discussion

Literature

Suitability of literature

Despite the fact that ruminant nutrition is an extensively studied area, few studies were found that included information about both BCS and BW, and plasma parameters, and many of those that did had been performed on well-nourished animals (McGuire *et al* 1995; Ndibualonji *et al* 1997; Agenäs *et al* 2003). Most dairy cows responded by adjusting production levels to meet nutrient intake (Phillips & Kitwood 2003). However, those in early lactation maintained production and increased mobilisation of their body tissues with dramatic increases in NEFA and BHB (Hartmann & Lascelles 1965; Baird *et al* 1972; McGuire *et al* 1995; Agenäs *et al* 2003), and reductions in insulin and glucose (Baird *et al* 1972; McGuire *et al* 1995; Ndibualonji *et al* 1997). Many feed-deprivation studies restricted nutrient intake to 0–50% of the requirement for 1–7 days, which was considered too short in the criteria set for this study. In the 13 studies that we identified as matching the criteria of long-term treatment periods (≥ 21 days), adult animals (≥ 2 years) and reports of the BCS or BW of the animals and metabolic data, the cattle had long-term diminished body tissue reserves because these had already been mobilised and tissue mobilisation metabolites, for example NEFA and BHB, were not elevated.

Outcome of the meta-analysis

No plasma constituents with any value as indicators of undernutrition in the long-term were found. Several studies reported strong correlations between changes in feed intake and changes in plasma metabolite concentrations, but these correlations were not consistent across studies, implying variation between animals and situations. No generic relationship could be derived from the existing data because all of the credible intervals spanned zero. However, the regressions against BWC (Figure 2) showed the most promising pattern and suggest that with additional data it may be possible to find a correlation across studies. It is likely that a combination of BWC and BCS would improve the strength of the regression and give significant gradients. The data available in this study was not adequate to attempt such an analysis. It would therefore be valuable to repeat this analysis when more data is available, to investigate whether a model that includes BCS as well as BWC could establish a correlation with any plasma metabolite.

Field data

Field data problems: five-year storage

The re-analysed data on albumin, BHB, total protein and urea from Herd B revealed that clinically important changes occurred during storage, just beyond the limits of analytical variation. The stability of two other analytes — creatinine and fructosamine — was reviewed in published data: creatinine concentration of 10 samples of canine serum stored at –20°C for 8 months increased by 8.3% (Thoresen *et al* 1995); therefore the reduction observed in under-nourished cattle was unlikely to be due to storage.

The literature on fructosamine stability in frozen serum is inconsistent. The best study (Koskinen & Irjala 1988) found a mean change in 27 samples of human serum stored at –20°C for 16 months of 4.8%; marked variability between samples was noted. Thoresen *et al* (1995) found a change of –17.9% in 8 months in 10 dog sera. It is possible that fructosamine would also have increased in samples stored for five years, so our deductions on the basis of these measurements are tentative.

Metabolic interpretation

Adequately-nourished animals

Regression analysis revealed little relationship between BHB, fructosamine, globulin, NEFA or urea with BCS in adequately-nourished animals. This was likely to be because there was no overriding metabolic process affecting these analytes in such stock. The fructosamine/albumin ratio had a negative coefficient, reflecting increasing albumin with BCS, whereas fructosamine was constant across body condition scores.

Under-nourished animals

The lower or equal values for BHB and NEFA, compared with adequately-nourished animals, probably indicate that acute fat mobilisation had been completed and tissue protein catabolism was the dominant energy supply. The low creatinine values in under-nourished animals, particularly in Herd A, were taken to represent low muscle mass, suggesting mobilisation of muscle protein for a considerable period. Therefore, the amino acids produced must be deaminated for conversion to glucose, generating urea. This mobilisation may have been more recent in Herd B than in Herd A, as urea in the animals in Herd B was mostly low, suggesting attempts to control negative nitrogen balance, and creatinine nearer normal, suggesting muscle bulk was not so severely diminished. Increases in fructosamine were seen in both herds and the fructosamine/albumin ratio (FAR) was markedly elevated, providing a test that sensitively detected the shifts to higher fructosamine and lower albumin. Neither of these underlying shifts was large enough alone to provide a sensitive test.

Increased fructosamine/albumin ratio

An increase in this ratio, the most marked feature of the serum chemistry of the under-nourished herds must reflect reduced albumin, increased fructosamine, or both. There was evidence of reduced albumin (both herds had significantly lower albumin than the adequately-nourished animals, $P < 0.0001$). A reduced level of serum albumin is an expected effect of negative nitrogen balance (Payne *et al* 1970). The elevation of fructosamine was also significant for both herds ($P < 0.0001$). Three factors could lead to increases in fructosamine: an artefact of storage, increased average serum or equivalently plasma, and decreased serum protein turnover.

First, there may be an artifactual elevation in fructosamine arising from five years' storage of the frozen serum (see discussion above). Increases of up to 20% have been reported for quite short storage periods, but the careful study of Koskinen and Irjala (1988) found the average increase to plateau after a few months at approximately 5%. The fructosamine increase in Herd A, compared with the mean of the nine adequately-nourished herds, averaged 50%, and in Herd B nearer 30%.

Second, the elevation of fructosamine could be the result of increased average serum (plasma) glucose. This might indicate a move toward glucose-based metabolism, resembling that of mono-gastrics, with plasma glucose higher than in a fed ruminant. The fructosamine increases observed here could reflect increases in plasma glucose of up to 50% higher than normal. Such glucose-related changes in bovine serum fructosamine have been observed in calves, which are undergoing rumen development, with fructosamine 35% lower at six months than at two months (Coppo 2001). It may be estimated from data in Coppo (2001) that the average plasma glucose falls by 35–40% in the same period.

The third possible cause of elevated serum fructosamine is reduced turnover of serum protein. This is typically seen in hypothyroidism. Hypothyroid dogs have fructosamine that is 37% higher than normal (Reusch *et al* 2002), and in hypothyroid humans the elevation is 20% (Sako *et al* 1989). Reduced turnover of serum albumin would also be expected in animals with severely limited nitrogen intake (Weigand 1977; Smith *et al* 1994). Cattle on restricted diets show reduced circulating levels of thyroid hormones (Heitzman & Mallinson 1972; Vicini *et al* 1988; Capuco *et al* 2001) and this may be the mechanism of the reduced turnover.

General discussion

A suitable indicator of undernutrition would be a measure that determines the degree of undernutrition across the different stages of severity. However, because of homeostatic and other physiological mechanisms, this may be difficult to find. In 1978 Lee *et al* (1978) commented that "concentrations of metabolites are of almost no practical use for individual cows because of extreme variations in diet required to generate abnormal concentrations of blood metabolites". In a more recent study on estimates of energy balance in high-yielding dairy cows in early lactation, NEFA and T4 were found to be the most informative traits but the authors pointed out that the precision of energy balance in individual cows was low (Reist *et al* 2002). Plasma metabolite concentrations are a result of many factors and it will therefore always be difficult to prove the cause of an abnormal metabolite concentration. Therefore, we strongly advise against the use of a single plasma metabolite concentration in investigations and discussions regarding the nutritional state and welfare of individual cows. Future work in this field should be targeted at identifying a combination of plasma parameters that will serve as a clinical test of undernutrition.

The apparently satisfactory sensitivity of the fructosamine/albumin ratio to detect significant differences between undernourished and adequately nourished cattle offers some promise for the determination of critical levels, but further confirmatory work is required because of the uncertainty of the effects of storage on fructosamine concentrations.

Conclusions and animal welfare implications

Adequate nutrition is a requirement for the welfare of cattle, but establishing whether cattle are undernourished is difficult. Two studies, a literature study and a field sample study, were conducted to identify objective indicators of undernutrition. The literature study did not identify any suitable indicators of undernutrition in cattle. The field sample study generated reference ranges for adequately nourished cattle for a number of metabolites. It was shown that low creatinine may be a possible indicator of impending undernutrition and it is suggested that the ratio between fructosamine and albumin could serve as an indicator to identify undernourished animals. However, it is probably necessary to combine several blood measures to obtain a valid assessment of the nutritional state of an animal and we strongly advise against the use of a single plasma metabolite concentration in assessing the nutritional state and welfare of individual cows.

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References

Agenäs S, Dahlborn K and Holtenius K 2003 Changes in metabolism and milk production during and after feed deprivation in primiparous cows selected for different milk fat content. *Livestock Production Science 83*: 153-164

Alderman G and Cottrill BR 1993 *Energy and Protein Requirements of Ruminants: An Advisory Manual Prepared by the AFRC Technical Committee on Responses to Nutrients.* CAB International: Wallingford, UK

Armbruster DA 1987 Fructosamine: structure analysis and clinical usefulness. *Clinical Chemistry 33*: 2153-2163

Baird GD, Heitzman RJ and Hibbitt KG 1972 Effects of starvation on intermediary metabolism in the lactating cow: a comparison with metabolic changes occurring during bovine ketosis. *Biochemical Journal 128*: 1311-1318

Balch CC and Line C 1957 Weight changes in grazing cows. *Journal of Dairy Research 24*: 11-20

Brown EM and Grunberger G 1991 Usefulness of albumin-based and total protein-based fructosamine correction formulas in diabetes practice. *Diabetes Care 14*: 353-354

Calavas D, Sulpice P, Lepetitcolin E and Bugnard F 1998 Assessing the accuracy of a body condition scoring in ewes under field conditions. *Veterinary Research 29*: 129-138

Capuco AV, Wood DL, Elsasser TH, Kahl S, Erdman RA, Van Tassell CP, Lefcourt A and Piperova LS 2001 Effect of somatotropin on thyroid hormones and cytokines in lactating dairy cows during *ad libitum* and restricted feed intake. *Journal of Dairy Science 84*: 2430-2439

Coppo JA 2001 Evolution of fructosaminaemia and glucaemia during the growth of unweaned and early weaned half-bred zebu calves. *Veterinary Research Communications 25*: 449-459

Department for Environment, Food and Rural Affairs (Defra) 1999 Prevention and Control of Animal Diseases. http://www.defra.gov.uk/corporate/publications/pubcat/cvo/1999/chapter1.pdf (accessed 10 April 2006)

Department for Environment, Food and Rural Affairs (Defra) 2002 *Condition Scoring of Beef Suckler Cows and Heifers.* Department for Environment, Food and Rural Affairs: London, UK

Edmonson AJ, Lean IJ, Weaver LD, Farver T and Webster G 1989 A body condition scoring chart for Holstein dairy cows. *Journal of Dairy Science 72*: 68-78

Gilks WR, Richardson S and Spiegelhalter DJ 1996 *Markov Chain Monte Carlo in Practice.* Chapman & Hall: London, UK

Gilks WR, Thomas A and Spiegelhalter DJ 1994 A language and program for complex Bayesian modelling. *The Statistician 43*: 169-177

Hartmann PE and Lascelles AK 1965 The effect of starvation on the uptake of the precursors of milk fat by the bovine mammary gland. *Australian Journal of Biological Sciences 18*: 1025-1034

Heitzman RJ and Mallinson CB 1972 A comparison of the thyroxine levels in the plasma of healthy starved and acetonaemic dairy cows. *Research in Veterinary Science 13*: 591-593

Istasse L, Vaneenaeme C, Gabriel A, Clinquart A, Maghuinrogister G and Bienfait JM 1990 The relationship between carcass characteristics, plasma hormones and metabolites in young fattening bulls. *Veterinary Research Communications 14*: 19-26

Jensen AL 1993 Various protein and albumin corrections of the serum fructosamine concentration in the diagnosis of canine diabetes mellitus. *Veterinary Research Communications 17*: 13-23

Kawamoto M, Kaneko JJ, Heusner AA, Feldman EC and Koizumi I 1992 Relation of fructosamine to serum-protein albumin and glucose-concentrations in healthy and diabetic dogs. *American Journal of Veterinary Research 53*: 851-855

Koskinen P and Irjala K 1988 Stability of serum fructosamine during storage. *Clinical Chemistry 34*: 2545-2546

Lee AJ, Twardock AR, Bubar RH, Hall JE and Davis CL 1978 Blood metabolic profiles: their use and relation to nutritional status of dairy cows. *Journal of Dairy Science 61*: 1652-1670

Lowman BG, Scott NA and Sommerville SH 1976 Condition Scoring for Cattle. East of Scotland College of Agriculture. *Bulletin Number 6*, 31 pp. Edinburgh School of Agriculture: Edinburgh, UK

McCance DR, Clarke KC and Kennedy L 1989 Serum fructosamine in uremia myeloma and acute inflammatory disorders — relationship to serum glucose and albumin levels. *Annals of Clinical Biochemistry 26*: 63-68

McGuire MA, Bauman DE, Dwyer DA and Cohick WS 1995 Nutritional modulation of the somatotropin/insulin-like growth factor system: response to feed deprivation in lactating cows. *The Journal of Nutrition 125*: 493-502

Ndibualonji BB, Dehareng D and Godeau JM 1997 Effects of starvation on plasma amino acids urea and glucose in dairy cows. *Annales de Zootechnie 46*: 163-174

Payne JM, Dew SM, Manston R and Faulks M 1970 The use of a metabolic profile test in dairy herds. *The Veterinary Record 87*: 150-158

Phillips CJC and Kitwood SE 2003 The effects of feed supplements on the production, metabolism and behaviour of dairy cows fed low levels of concentrates during lactation. *Journal of Applied Research in Veterinary Medicine 1*: 187-195

Reist M, Erdin D, von Euw D, Tschuemperlin K, Leuenberger H, Chilliard Y, Hammon HM, Morel C, Philipona C, Zbinden Y, Kuenzi N and Blum JW 2002 Estimation of energy balance at the individual and herd level using blood and milk traits in high-yielding dairy cows. *Journal of Dairy Science 85*: 3314-3327

Reusch CE, Gerber B and Boretti FS 2002 Serum fructosamine concentrations in dogs with hypothyroidism. *Veterinary Research Communications 26*: 531-536

Sako Y, Umeda F, Hashimoto T, Haji M and Nawata H 1989 Serum fructosamine in assessment of diabetic control and relation to thyroid function. *Hormone and Metabolic Research 21*: 669-672

Smith G, Weidel SE and Fleck A 1994 Albumin catabolic rate and protein-energy depletion. *Nutrition 10*: 335-341; Discussion 353

Storry JE and Sutton JD 1969 The effects of change from low-roughage to high-roughage diets on rumen fermentation, blood composition and milk fat secretion in the cow. *British Journal of Nutrition 23*: 511-521

Taylor JC 1954 Techniques of weighing the grazing animal. *Proceedings of the British Society of Animal Production 1954*: 3-16

Thoresen SI, Tverdal A, Havre G and Morberg H 1995 Effects of storage time and freezing temperature on clinical chemical parameters from canine serum and heparinized plasma. *Veterinary Clinical Pathology 24*: 129-133

Veerkamp RF, Gerritsen CL, Koenen EP, Hamoen A and De Jong G 2002 Evaluation of classifiers that score linear type traits and body condition score using common sires. *Journal of Dairy Science 85*: 976-983

Vicini JL, Cohick WS, Clark JH, McCutcheon SN and Bauman DE 1988 Effects of feed-intake and sodium-bicarbonate on milk-production and concentrations of hormones and metabolites in plasma of cows. *Journal of Dairy Science 71*: 1232-1238

Weigand K 1977 [The regulation of serum albumin in physiological and pathological conditions (Author's translation)]. *Klinische Wochenschrift 55*: 295-305

Wilkinson JM and Cumberland PH 1970 Grazing behaviour and weight changes in calves turned out to pasture. *Journal of the British Grassland Society 25*: 214-219

Appendix 1. Studies included in the meta-analysis.

Agenäs S, Burstedt E and Holtenius K 2003 Effects of feeding intensity during the dry period 1. Feed intake body weight and milk production. *Journal of Dairy Science 86*: 870-882

Berry NR, Sutter F, Bruckmaier RM, Blum JW and Kreuzer M 2001 Limitations of high alpine grazing conditions for early-lactation cows: effects of energy and protein supplementation. *Animal Science 73*: 149-162

Bruckmaier RM, Gregoretti L, Jans F, Faissler D and Blum JW 1998 Longissimus dorsi muscle diameter backfat thickness body condition scores and skinfold values related to metabolic and endocrine traits in lactating dairy cows fed crystalline fat or free fatty acids. *Zentralblatt für Veterinarmedizin Reihe A45*: 397-410

Fisher LJ, Donnelly PE, Hutton JB and Duganzich DM 1975 Relationships between levels of feeding and certain blood metabolites in dairy cows in mid lactation. *Journal of Agricultural Science (Cambridge) 84*: 29-37

Knaus W, Luger K, Zollitsch W, Gufler H, Gruber L, Murauer C and Lettner F 1999 Effects of grass clover-pellets and whole plant maize-pellets on the feed intake and performance of dairy cows. *Animal Feed Science and Technology 81*: 265-277

Moorby JM, Dewhurst RJ, Evans RT and Fisher WJ 2002 Effects of level of concentrate feeding during the second gestation of Holstein-Friesian dairy cows 2. Nitrogen balance and plasma metabolites. *Journal of Dairy Science 85*: 178-189

Moorby JM, Dewhurst RJ, Tweed JKS, Dhanoa MS and Beck NFG 2000 Effects of altering the energy and protein supply to dairy cows during the dry period 2. Metabolic and hormonal responses. *Journal of Dairy Science 83*: 1795-1805

Ortigues I, Petit M, Agabriel J and Vermorel M 1993 Maintenance requirements in metabolizable energy of adult non-pregnant nonlactating Charolais cows. *Journal of Animal Science 71*: 1947-1956

Ponter AA, Douar C, Mialot JP, Benoit-Valiergue H and Grimard B 2000 Effect of underfeeding post-partum Charolais beef cows on composition of plasma non-esterified fatty acids. *Animal Science 71*: 243-252

Rabiee AR, Macmillan KL and Schwarzenberger F 2001 The effect of level of feed intake on progesterone clearance rate by measuring faecal progesterone metabolites in grazing dairy cows. *Animal Reproduction Science 67*: 205-214

Rastani RR, Andrew SM, Zinn SA and Sniffen CJ 2001 Body composition and estimated tissue energy balance in Jersey and Holstein cows during early lactation. *Journal of Dairy Science 84*: 1201-1209

Strickland JR, Gulino-Klein LF, Ross TT, Slate S, Peterson MK, May T and Taylor JB 1998 Effects of nutrient supplementation in beef cows of poor body condition fed snakeweed (*Gutierrezia* spp). *Veterinary and Human Toxicology 40*: 278-284

Vicini JL, Cohick WS, Clark JH, McCutcheon SN and Bauman DE 1988 Effects of feed-intake and sodium-bicarbonate on milk-production and concentrations of hormones and metabolites in plasma of cows. *Journal of Dairy Science 71*: 1232-1238