Metabolic Disorders in Farm Animals

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DISORDERS OF THIAMINE METABOLISM IN YOUNG RUMINANTS

E. E. Edwin and R. Jackman
Central Veterinary Laboratory, Ministry of Agriculture, Fisheries & Food, Weybridge

ABSTRACT

Thiamine is an obligatory requirement in energy metabolism and thus for growth and maintenance of live. In ruminants a large part of the thiamine supply will come from microbial synthesis in the rumen. However it is now recognised that under certain conditions significant amounts of thiamine destroying enzymes, presumably of microbial origin, may be found in the rumen and alimentary tract. Evidence ist accumulating that this may occur periodically under different types of management and if this situation lasts for any length of time, the host animal must be regarded as being in subclinical states of thiamine deficiency. In extreme cases death will result. The significance of this can be important in production as it can be assumed that intensively fed animals would require larger amounts of thiamine.

INTRODUCTION

It ist now widely recognised that the precept that ruminants do not require an extraneous supply of thiamine must be reconsidered as studies on the nature, aetiology and distribution of cerebrocortical necrosis (CCN) have revealed that it is a disease in which thiamine-deficiency plays an important role. The disease may be of increasing importance under conditions of intensive feeding for higher rates of weight gain.

MATERIALS AND METHODS

Thiamine was estimated by a modified thiochrome procedure (9), thiaminase by a rapid radioactive procedure (8) and transketolase by a modification (10) of the Brin method.

RESULTS AND DISCUSSION

Much biochemical evidence has accumulated to show that animals affected with CCN also suffer from thiamine deficiency. This ist shown by elevation in blood pyruvate, fall in tissue thiamine levels and lower transketolase activity in erythrocytes (5, 7, 25). Table I shows data obtained in our laboratory from field cases of CCN in sheep and cattle. For comparison, data from apparently healthy animals is also given.

Biochemical findings in CCN

Tissue Thiamine Concentration (µg/g wet wt)		
Sheep	CCN	Normal
Liver	0.421 ±0.060 (27)	2.07 ±0.474 (17)
Heart	0.581 ±0.093 (31)	3.10 ±0.432 (17)
Brain	0.592 ±0.111 (25)	1.21 ±0.101 (13)
Cattle		
Liver	0.613 ±0.102 (10)	2.81 ±0.515 (9)
Heart	0.549 ±0.118 (10)	2.81 ±0.46 (8)
Brain	0.301 ±0.061 (7)	1.40 ±0.284 (8)
Mean Erythrocyte Transketolase (% TPP effect)		
Sheep	122 (33)	23 (17)
Cattle	172 (8)	15 (5)
Ruminal or Faecal Thiaminase Activity		
Sheep, Cattle	Mostly Positive	Mostly Negative

The primary cause of the disease is the continuous production of thiaminase by bacteria in the rumen and intestines of affected animals; several species of thiaminase-producing bacteria have now been isolated from the rumen contents of such animals. The intensity of the activities and pH optima of the thiaminases vary with the bacterial source and it cannot be said with certainty that CCN will occur only at certain ruminal pH ranges. The thiaminases have been found to be of both types I and II. The rumen contents, consisting of semi-digested food, microbiota, and metabolic intermediates will also contain cosubstrates for the thiaminase I reaction and it is possible that the products of thiamine breakdown could further aggravate the thiamine deficiency that must result from the presence of ruminal thiaminase activity.

Many estimates have been made of the intake of thiamine, consisting of the dietary content and the contribution from ruminal synthesis. These estimates are likely to be erroneous when the effect of ruminal thiaminase activity has not been taken into consideration.

The presence of ruminal thiaminase activity can be correlated with faecal excretion of the enzyme and also with the thiamine status of the host animal (measured as the TPP effect of erythrocyte transketolase). Surveys on farms and fattening units using these parameters have led us to believe that at least 25 % of young cattle must be regarded as bein in a thiamine-deficient state (10, 26). Information is lacking on whether or not these animals would reach their full growth potential or attain their optimum rate of food conversion, since thiamine must be supplied at an adequate rate for carbohydrate utilisation.

Thiaminase-producing bacteria are not listed among the normal population of ruminal microorganisms (17). The conditions under which they proliferate in the rumen of affected animals are not understood; CCN has been reported to occur under many different types of management, grazing as well as housed and concentrate-fed animals succumb. Experiments to induce CCN by delibera-

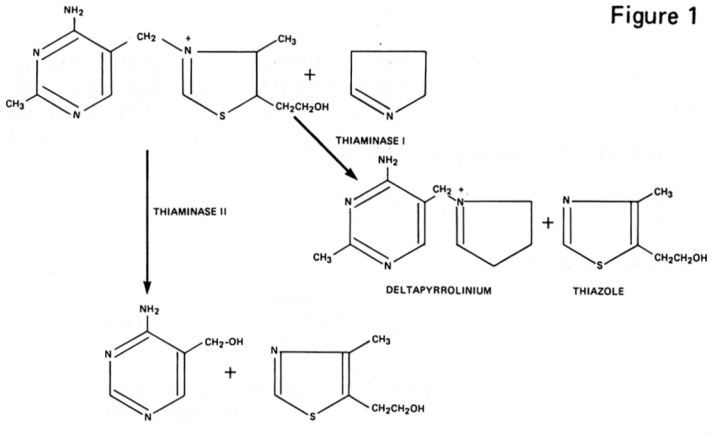
tely introducing live cultures of thiaminase-producing microorganisms have been uniformly unsuccessful (6, 22, 29, and our own unpublished observations), although an encephalopathy indistinguishable from CCN was produced by feeding sheep with bracken rhizomes which were known to contain large amounts of thiaminase I (13). The inference from these experiments is that control mechanisms must prevail in the rumen to maintain the harmonious symbiotic operation of ruminal fermentation whereby proliferation of undesirable bacteria or the elaboration of enzymes with undesirable consequences by normally quiescent organisms will be prevented. If these control mechanisms break down, conditions for the proliferation of thiaminase-producing microorganisms and their synthesis of thiaminase, may become favourable. Outbreaks of CCN are often preceded by some change in management or treatment such as movement to a new pasture, abrupt introduction of a new diet, or anthelmintic or antibiotic treatment.

The role of the natural cosubstrates for the thiaminase I reaction must also be considered. Several have been found in rumen liquor and identified (11). They have been shown to be formed during the metabolism of substances such as putrescine and cadaverine or amino acids such as ornithine, proline, hydroxyproline and lysine by plant and bacterial enzymes (21). Of the many studied, Δ^1-pyrroline, which has only a transient existence in the rumen before being further metabolised, appears to be the most potent, having the lowest Km value for the thiaminase I reaction. The reaction sequence is shown in Fig. 1.

The resultant pyrimidinyl derivative of Δ^1-pyrroline (deltapyrrolinium) is thought to aggravate the concurrent thiamine deficiency of CCN. This compound has been consistently isolated from the brain of animals affected with CCN (11). It has also been shown to reduce oxygen uptake by brain slices and accelerate pyruvate decarboxylation, in the same way as the thiamine antagonist amprolium (1). It is interesting that an encephalopathy similar to CCN can be induced by feeding amprolium to calves and lambs.

It has been suggested that other compounds, such as thiabendazole (27) or histamine (3) may act as cosubstrates for the thimainase I reaction, and that these might be the important precipitating factors. In our hands these compounds have not shown any potential for cosubstrate activity, and we have been unable to synthesise the relevant pyrimidinyl compounds by techniques that would normally yield such derivatives.

Figure 1



Encephalic lesions indistinguishable from those seen in CCN have also been described in molasses toxicity, a disease prevalent on feedlots employing a liquid molasses-urea feeding system with limited access to forage. Thiamine does not appear to play a part in the aetiology of this disease, as it can be prevented by increasing the fibre content of the diet. It has been stated that the chemical composition, physical form and in some circumstances the dietary intake would influence the pattern of microbial population (18). The proportion of ciliate protozoa to rod types would alter and the relative molar composition of the volatile fatty acids produced in the rumen would be changed. This appears to be the situation in molasses toxicity (12, 19). It has not been unequivocally established whether or not thiaminase-producing microorganisms also become establismed in this feeding regimen.

The incidence of CCN is said to be higher in intensively fed beef-units (2, 14, 15, 30), or on cobalt-deficient diets (20), or urea containing liquid dietary systems (23). Because of this, it has been said that the thiamine requirement of animals on these systems must be higher. However an alternative explanation, that on such exotic diets the control mechanisms may not function effectively and that the proliferation of thiarninase-producing organisms may be favoured, has not been properly explored.

Various estimates have been made on the thiamine intake of ruminants and divided into the amount from food and the contribution from ruminal synthesis. *Phillipson & Reid* (24) have shown that the rate of thiamine synthesis is greater when the grain content of the ration is increased. *Höller et al* (16) found that the prolonged administration of a protein-free diet to sheep reduced the thiamine concentration of their ruminal contents. *Breves et al* (4) have calculated that in sheep the daily flow of thiamine to the duodenum exceeded the daily intake by a factor of 18 or 24. *Steinberg et al* (28) found in 8 experiments that dairy cows synthesised 32 mg of thiamine per day.

In the presence of thiaminases the amount of thiamine available to the animal would be extremely low. It can be readily calculated that in 20 kg of rumen content levels of thiaminase activity usually found in CCN or in the prodromal stage of the disease would account for the destruction of up to 20 mg of thiamine per minute.

It seems reasonable to assume also that the presence of thiaminases would not interfere with the rate of thiamine synthesis. However the formed thiamine would be broken down rapidly and compounds such as deltapyrrolinium would now be synthesised at nearly the same rate, provided there were adequate supplies of cosubstrates.

In summary it is necessary to appreciate that thiamine requirement of ruminants may increase with faster growth, and that the ruminal synthesis of the vitamin would proceed at an adequate rate. However under certain circumstances, if the proliferation of thiaminase producing bacteria was not controlled, ruminal and intestinal thiaminase activity could destroy the thiamine formed. As long as the rate of synthesis of thiamine is greater than the rate of breakdown the host animal will receive some thiamine. If the rate of thiamine breakdown exceeded the rate of synthesis, the host will go into negative thiamine balance and tissue levels of the vitamin will fall. The situtation may be exacerbated by the concomitant synthesis of thiamine breakdown products, leading ultimately to the development of CCN. It is difficult to interpret the results of experiments to measure the rate of ruminal thiamine synthesis or dietary intake if these studies do not allow for the persistent or sporadic presence of thiaminase activity.

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GENETIC INFLUENCES ON THE VALUES OF METABOLITES AND ENZYMES IN THE BLOOD OF DAIRY COWS

F. Graf, H. Tenhumberg and H. Kräußlich
Institut für Tierzucht und Tierhygiene der Universität München

ABSTRACT

During a period of two years blood samples were taken regularly from 19 progeny-groups of AI-bulls, each consisting of about 15 Simmental cows, all being in the first lactation.
The blood samples were analysed for hematocrit, the serum for LDH, AST, CK, SDH, γ-GT, ICDH, GLDH, glucose, cholesterol and total protein values.

We found significant differences between the progeny-groups in the values of some of the blood parameters mentioned above. We also found progeny-groups with high values in most of the investigated enzymes-reacting strongly to the load as we presume- and other groups with the low values in these enzymes.

INTRODUCTION

Our Hypothesis: a) The serum levels are not only changed by clinical diseases, in clinical healthy cows they also change, though less as a consequence of load of performance.
b) there are genetic differences in the serum levels due to different reactions to this load.

The first part of the hypothesis is confirmed in an earlier paper (2,3). The second part is the object of this paper.

MATERIALS AND METHODS

All cows were kept under equal housing conditions. Each cow was bled 2 weeks prepartum, 2, 5, 10 weeks postpartum and afterwards in intervalls of 8 weeks. The blood samples were drawn from jugular vein and analysed on the same day.
The influence of time of day was eleminated by sampling blood always at the same time (7-8 o'clock a.m.).

The influence of diseases was eleminated by analysing only data of cows which showed no signs of disease. The influence of month was accounted for by adjustment-factors.
Stage of lactation has a significant influence (GRAF et al., 1979), but blood samples were taken in the same stages of lactation from all animals, as mentioned above.

RESULTS

Repeatability from day to day, 25 cows were bled on two successive days. Correlations between the blood values of these two days were calculated and the differences were tested for significance (table 1).

Table 1: Repeatability from day to day. Correlations and t-test. n = 25

		r	t
SDH,	Sorbit dehydrogenase, EC 2.7.3.2	0.93	0.23
GIDH,	Glutamate dehydrogenase EC 1.1.1.27	0.90	0.67
γ-GT,	Glutamyltransferase EC 2.3.2.2	0.85	0.22
ICDH,	Isocitrate dehydrogenase EC 1.1.1.42	0.82	0.20
AST (GOT),	Aspartate aminotransferase EC 2.6.1.1	0.88	0.14
LDH,	Lactate dehydrogenase EC 1.1.1.27	0.80	0.03
CK,	Creatine phosphokinase EC 2.7.3.2	0.95	0.01
Glucose		0.45	1.63
Total protein		0.53	1.88
Cholesterol		0.74	0.23

Differences between progeny-groups, Table 2 shows the analysis of variance for progeny-groups and the lowest and highest group-means.

Ranking of progeny-groups, Table 3 shows the ranking of the progeny-groups for liver specific enzymes and for glucose. Ranking number 1 is given for the lowest enzyme value but for the highest glucose value.

Table 2. Lowest and highest group-means, F-values and intra-class correlations

	Total mean	Lowest mean	Highest value	F FG:18/290	Intra-class correlations
Hemat. (%)	36,7 ± 1,31	35,9 ± 0,92	38.0 ± 1,17	$2,58^{++}$	0,094 ± 0,051
LDH (U/l)	1141 ± 118	1068 ± 82	1219 ± 127	1,62	
AST (U/l)	30,9 ± 4,3	28,7 ± 4,2	34,2 ± 3,6	$2,78^{++}$	0,104 ± 0,054
γ-GT (U/l)	11,1 ± 3,2	8,6 ± 1,0	14,4 ± 4,1	$4,70^{++}$	0,195 ± 0,084
CK (U/l)	32,8 ±12,8	26,8 ± 4,8	40,2 ±14,2	1,26	
SDH (U/l)	2,5 ± 0,6	2,2 ± 0,3	3,1 ± 0,6	$2,61^{++}$	0,095 ± 0,052
ICDH (U/l)	14,7 ± 1,9	12,5 ± 1,9	18,3 ± 2,8	$6,11^{++}$	0,248 ± 0,102
GLDH (U/l)	4,9 ± 2,3	3,0 ± 1,2	7,4 ± 2,9	$5,31^{++}$	0,220 ± 0,092
T. prot. (g/100 ml)	7.9 ± 0,6	7,6 ± 0,3	8,3 ± 0,8	$2,28^{++}$	0,077 ± 0,046
Glucose (mmol/l)	3,32 ± 0,28	3,15 ± 0,20	3,51 ± 0,53	$2,23^{++}$	0,075 ± 0,045
Cholest. (mmol/l)	2,97 ± 0,54	2,54 ± 0,40	3,31 ± 0,39	$1,71^{++}$	0,044 ± 0,035

++ $p < 0.01$

Table 3. Ranking of progeny groups 1-19

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
AST	19	11	2	7	18	1	6	5	10	13	14	12	4	3	17	16	8	15	9
γ-GT	6	5	2	12	7	1	3	11	17	10	13	14	15	4	16	18	9	19	8
SDH	8	5	1	14	3	2	7	4	11	16	6	15	9	13	18	17	10	19	12
GLDH	13	14	5	7	1	3	4	2	9	16	8	11	10	6	17	18	12	19	15
ICDH	19	18	1	15	9	8	2	17	3	11	12	6	7	4	14	13	5	16	10
Gluc.	14	17	5	11	13	4	6	16	8	10	19	7	9	2	15	3	1	18	12
Average	13,2	13,6	2,7	9,7	8	3,2	4,7	9,2	9,7	12,7	10,3	10,8	9,0	5,3	16,2	11,2	7,5	17,7	11,0

DISCUSSION

Liver specific enzymes SDH, GLDH, γ-GT, ICDH, AST (FRAHM et al., 1978) show significant differences between progeny-groups. For hematocrit, total protein, glucose and chollesterol the differences between groups are lower, but still significant. There are progeny-groups with high values in most of liver specific enzymes – reacting strongly to the load as we presume – and other groups with low values in these enzymes. Good ranking numbers – i. e. low enzyme values and high glucose value – are found for the groups 3, 6, 7, 14, poor ranking numbers for the groups 18 and 15. The intraclass correlation correspond to the repeatabilities from lactation to lactation (GRAF et al. 1979). They allow the assumption of genetic influences on these blood values. For a reliable estimation of heritabilities the number of animals is too low.
The relationship between these genetic differences and the load of performance is being investigated.

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USE OF THE COMPUTER IN THE STUDY OF METABOLIC DISORDERS OF DAIRY COWS

I. Beseda, K. Boda and J. Vál'ka
Institute of Animal Physiology of the Slovak Academy of Sciences, Košice, Laboratory Zvolen

ABSTRACT

A system was worked out for a computer evaluation of metabolic profile test. The computer calculated and plotted the results of biochemical analysis in the pictorial print-out form-histogram and percentual graph which show the average levels of metabolic parameters with regard to the reference limits. The input datalevels of metabolic profile have been used for the estimation of their inner linkage using multidimensional statistical analysis. As an example the metabolic profile data of cows suffering of subclinical ketosis are used and the results are compared with the control group.

INTRODUCTION

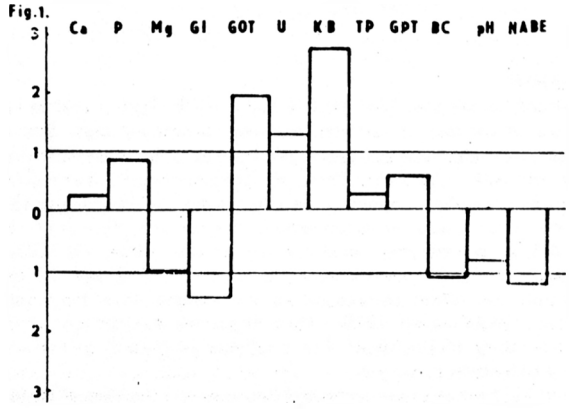
For the evalution of the results of biochemical analysis a computer can be used very effectively. In presented paper the computer elaborates the metabolic profile data in easily interpretable form of histograms and percentual graphs. Furthermore the system of the evaluation of the input data is completed using multidimensional statistical analysis of principal components which should help us on the example of ketotic cows to determine the inner linkage among individual varieties of the profile. With the aid of this method it is possible to evaluate and classify the diagnostic importance of used parameters of profile simultaneously.

MATERIAL AND METHODS

Using the computer the results of metabolic profile on 46 subclinical ketotic cows were elaborated in pictorial form of histogram and percentual graph according to method (2). Furthermore the input data were evaluated usung multidimensional stat. analysis (3,4). For comparison the data of 50 cows with normal profile were carried out using the same method. The blood serum samples were analysed for Ca, inorg.P, Mg, glucose (GI), urea (U), ketobody (KB), total protein (TP), GOT, GPT, buffer capacity (BC) and the samples of urine were analysed for pH and net acid-base excretion (NABE). For the computer the programme language FORTRAN IV and the analytical methods as in our previous work (2) were used.

RESULTS

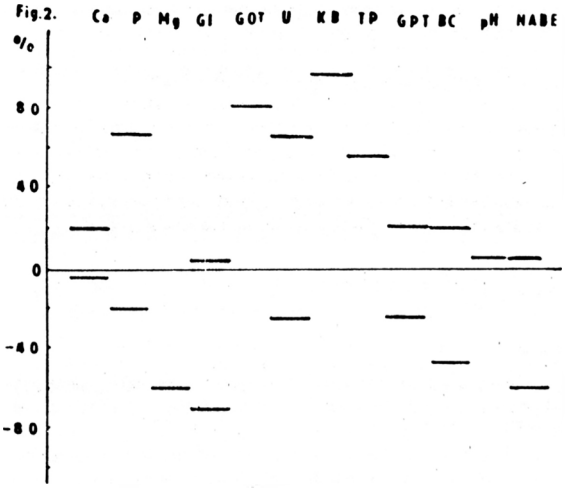
Figure 1 shwos the histogram of 46 subclinical ketotic cows.



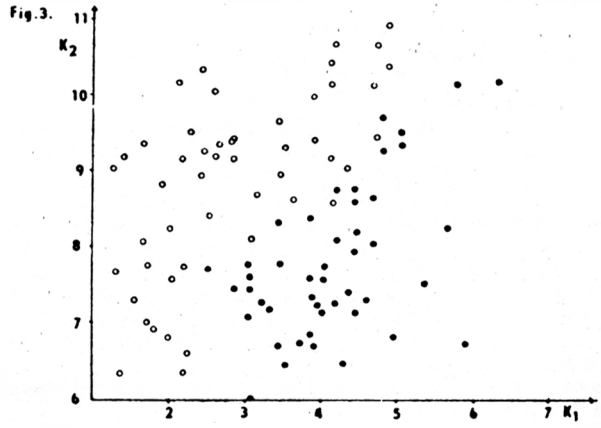
DISCUSSION

Data of subclinical ketotic cows using a computer were elaborated as an example. The histogram (Fig. 1) and the percentual graph (Fig. 2) give a distribution of the levels of the profile variates. Their inner linkage is demonstrated in the Fig. 3. The positions of the cows using the first two principal components K_1 and K_2 illustrate the differentiation of healthy cows from cows with increased level of ketobodies in blood serum. Cows which are close to each other have also a very similar profile. The latent

Distribution of decreased and increased levels of profile tested cows with subclinical ketosis ist shown in the Fig. 2.



According to calculation of multidimensional analysis of principal components the positions of each individual is shown in the Fig. 3 between the first two principal coordinates. Every point represents a cow.



The participation of individual variates of metabolic proflie on the variance of the first, second and the third latent roots (λ_i) are shown in the Table 1.

Table 1.

Variates	Latent vectors 1	2	3
Ca	0,277	0,368	-0,328
P	-0,355	0,223	-0,032
MG	0,301	-0,079	0,216
GI	0,282	-0,355	0,109
U	0,165	0,110	-0,568
KB	-0,200	0,413	0,328
TP	0,340	-0,290	-0,211
GOT	0,223	0,445	-0,223
GPT	0,126	0,222	-0,127
BC	0,050	0,343	0,307
pH	0,430	0,221	0,219
NABE	0,440	0,058	0,388
λi	2,569	1,865	1,585
% variance	21,41	15,54	13,21
Σ %	21,41	36,95	50,16

roots show participation of each component on the total variance. The latent vectors determine which of the variates are bearing the main part of the variability. On the first component the highest loading had NABE and pH of urine, TP and inorganic P in the blood serum. On the second one the GOT, KB, GI and BC of blood serum took a main part.

According to our findings it seems that 8 variates would be sufficient for the biological interpretation of the metabolic state of the cows. The method bears the possibility for the correct choice of the suitable variates to test the cows. Using this way the proposed number of variates could be narrowed for the new structure, which is derived from the analysis of the principal components. Our work points only at some possibilities of the computer data elaboration using multidimensional statistical analysis.

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LIVER METABOLISM IN THE DAIRY COW: PROBLEMS INVOLVED IN MEETING THE DEMANDS OF HIGH PRODUCTIVITY

G. D. Baird

A. R. C. Institute for Research on Animal Diseases, Compton

LIVER METABOLISM

Metabolism in ruminant liver differs somewhat from that in non-ruminant liver. This is because the nutrients absorbed from the gut and presented to the liver are different in the two cases. The major difference lies in the nature of the nutrients derived from ingested carbohydrate. In monogastric animals the products of carbohydrate digestion are monosaccharides, chiefly glucose, while in ruminants they are the short chain volatile fatty acids (VFA's), i. e. acetate, propionate and butyrate. Little glucose is normally absorbed from the ruminant gut. Glucose is still required, however, and must be synthesised from non-carbohydrate precursors by the process of gluconeogenesis, some 85 % of which takes place in the liver. By contrast with the non-ruminant liver, therefore, there is a net output of glucose from ruminant liver at all times. The main precursors for this glucose are propionate, glucogenic amino acids, lactate and glycerol (7). There is little net uptake of glucose, and it is questionable whether glycolysis proceeds at any significant rate.

Acetate is the main lipogenic compound in the ruminant, and is that VFA which is absorbed in greatest quantity from the gut. The ready availability of acetate, taken together with the fact that glucose is not lipogenic, may explain another peculiar feature of ruminant liver, which ist the apparent absence of an active pathway of lipogenesis (6). Other features of hepatic lipid metabolism are similar to those of non-ruminant liver, however, and include either esterification of assimilated non-esterified fatty acids (NEFA) to triacylglycerol, or oxidation of the NEFA to ketone bodies or to carbon dioxide. Acetate itself is not metabolised by the liver to any major extent (1), and passes through to peripheral organs. Virtually all the butyrate that reaches the liver is assimilated, and for the most part tranformed into hydroxybutyrate. In the fed state the liver secretes hydroxybutyrate at a similar rate to the gut, and much of this hydroxybutyrate is probably derived from butyrate. The remainder is derived from NEFA. An interesting feature of ruminant liver is that in the fed state there is a small constant net uptake of acetoacetate. This switches to a net output in situations of marked energy deficiency (1).

Other features of ruminant liver metabolism, including protein synthesis, urea synthesis, triacylglycerol export, cholesterol and phospholipid synthesis, detoxification processes, bilirubin assimilation, and bile formation and secretion, are probably similar to the corresponding processes in non-ruminant liver. However, it should be pointed out that these features have been investigated to a much more limited extent in ruminant liver than have the pathways of glucose and fat metabolism.

CONSTRAINTS ASSOCIATED WITH HIGH PRODUCTIVITY

During early lactation, the dairy cow has to contend with a variety of special factors that potentially can lead to "bottle-necks" in, or outright impairment of, hepatic function. These factors include the following:

(a) Overall metabolic demand is greatly increased, and the rates of metabolic processes within the liver have to be increased in proportion.

(b) Priority appears to be accorded to the nutrient demand of the lactating mammary gland, particularly as regards the requirement of the gland for glucose. One way in which the glucose requirement may be accorded priority is by the maintenance of a low blood insulin concentration, since glucose transport into the gland may be insulin-independent (14).

(c) Voluntary feed intake is frequently insufficient to meet the combined energy needs of maintenance and productivity, so that the animal is in a state of negative energy balance.

(d) The hormonal environment is such as to encourage lipid mobilisation.

Similar factors may also pertain in late pregnancy. Blood insulin concentration is also low at this time and, furthermore, there is evidence that the pregnant uterus, like the lactating mammary gland, may not require the mediation of insulin for glucose uptake (18).

ADVERSE EFFECTS OF HIGH PRODUCTIVITY ON LIVER METABOLISM DIMINISHED CARBOHYDRATE STATUS.

The increase in hepatic glucose output that occurs with the onset of lactation appears to be determined by the hormonal environment as well as by substrate supply, since some increase in output will occur even in the absence of any increase in feed intake (17). In fact, there seems to be a fairly close coordination between the magnitude of the increase in hepatic gluconeogenesis and the level of milk output. This coordination is not perfect, however. Thus, when comparison is made with fed non-lactating cows, fed cows in early lactation routinely show signs of diminished carbohydrate status. These signs include a decrease in the arterial concentration of glucose, decreases in the intrahepatic concentration of glycogen and some glucogenic intermediates, including pyruvate, citrate and 2-oxoglutarate, and, finally, decreases in the arterial concentration of glucose precursors such as lactate, pyruvate and alanine (2, 5). The signs probably arise because glucose demand tends to outstrip the ability of the liver to synthesise glucose. That this is so is indicated by the fact that qualitatively similar, but much more marked, changes are produced when lactating cows are subjected to fasting for 6 days (4). Other significant changes that occur in the fasted animals include decreases in the hepatic content of ATP and total NAD^+ (4).

The factor that is likely to be most important in limiting hepatic gluconeogenesis in the fed cow is the availability of endogenously-derived glucogenic precursors. The function of these precursors is to make good any deficit in the supply of exogenously-derived precursors that arises as a result of energy imbalance. It is unlikely that the ability of the liver to assimilate the percursors is an important limiting factor, since hepatic extraction of glucogenic amino acids and lactate increases in response to energy deficiency, while the level of hepatic extraction of propionate and glycerol is consistently high under all circumstances (1, 5, 9 and G. D. Baird and M. A. Lomax, unpublished work). Furthermore, in *vitro* measurements suggest that the activities of key gluconeogenic enzymes are likely to be adequate (1, 3, 4).

The percentage extraction, and rate of uptake, of lactate by the liver is routinely observed to be higher in fed lactating cows than in fed non-lactating cows (5). This difference may be another indication of the fact that lactating cows are suffering some degree of carbohydrate insufficiency. Recent findings *(J. G. v. d. Walt, E. N. Bergmann & G. D. Baird,* unpublished work) suggest that only a portion of the lactate taken up by the liver during lactation is used for glucose synthesis. The remainder may either be oxidised, or else be used to help replace the loss of carbon skeletons that is occuring within the gluconeogenic pathway. In the same study, parameters of lactate metabolism were compared in sheep and cows during late pregnancy and early lactation. The results suggested that the metabolic situations were similar in the two species in pregnancy but differed in lactation. Specifically, it was found that in early lactation the circulating concentration of lactate, and lactate turnover related to metabolic body weight, were lower in the cows than in the sheep, while the percentage of lactate turnover that was converted to glucose was higher. The findings indicated, therefore, that in early lactation the cows were more deficient in energy than the sheep, even

though all animals were fed to recommended standards (26). This type of study helps to explain why dairy cows, but not sheep, are susceptible to spontaneous lactational ketosis. This disorder probably arises because of the development of a serious imbalance between glucose demand and the rate at which the liver can supply glucose.

LIMITATION TO GLUCOSE OUTPUT.

The control mechanisms that are involved in coordinating hepatic glucose output and milk output also appear to ensure that glucose entry rate, and hence hepatic gluconeogenesis, do not rise above the minimum that is necessary to meet the total demand for glucose (15). The reasons for this limitation may be twofold. Firstly, there is the need to conserve energy, and, secondly, there is the desirability of maintaining a low blood insulin concentration.

RAPID FAT MOBILIZATION.

The tendency towards diminished carbohydrate status, and the prevailing hormonal environment, both encourage adipose tissue mobilization in early lactation. If mobilization is sufficiently rapid, the rate of uptake of NEFA by the liver may be too great for the NEFA to be processed normally. The two main consequences of this event are usually an increase in the rate of hepatic ketogenesis, and an increase in fat deposition within the liver. Increases in liver fat content and in blood ketone-body concentrations are frequently seen in dairy cows in early lactation, suggesting that a high rate of fat mobilization is commonplace at this time (21, 23). The mobilization may in fact begin in late pregnancy, since fat deposition in the liver can frequently be observed at one to two weeks prepartum (21). It must be remembered, however, that some increase in hepatic ketogenesis will occur postpartum, even in the absence of any increase in fat mobilization, as the result of an increase in butyrate supply. Hyperketonaemia and fatty infiltration of the liver become particularly pronounced during subclinical and clinical ketosis, and when cows in early lactation are fasted (1). Fatty liver also occurs in cows which have been grossly overfed prepartum. Rapid fat mobilization in association with diminished appetite is important here (16).

There are probably two reasons for the increase in hepatic ketogenesis that occurs in response to increased fat mobilization. The first is that more precursor (i. e. NEFA) is being made available. The second is that the mobilization is usually accompanied by a decrease in carbohydrate sufficiency that in turn results in an increase in the proportion of NEFA assimilated by the liver that is transformed into ketone bodies. One possible link between increased hepatic ketogenesis and drecreased carbohydrate sufficiency is the intrahepatic concentration of oxaloacetate (1). Other links have been proposed, however (27). The main lipid component that ist deposited in the liver of lactating dairy cows in the fed or fasted state is triacylglycerol (11, 19). This suggests that there could be some defect in the ability of the liver either to oxidise activated NEFA or to re-export the triacylglycerol in the form of very-low-density lipoprotein (VLDL). One possibility is simply that the capacity of these processes is being exceeded. Support for the view that impairment of the ability to export VLDL could be an explanation for the accumulation of triacylglycerol is provided by the observation that triacylglycerol output from the liver ceases when cows are fasted (20). Such an impairment could arise as a consequence of a shortage in one or more of the other substances involved in the formation of the VLDL such as choline, inositol, methionine, protein and cholesterol.

IMPAIRED CLEARANCE.

Several workers have reported that in situations of marked energy deficiency, such as fasting and spontaneous ketosis, the liver of the cow is less able to clear bromosulphthalein (a liver-function dye) from the blood (e. g. 10,12). Parallel studies have suggested that in these same situations the ability of the liver to clear bilirubin is also diminished. Decrease in bilirubin clearance in cattle does not appear to have been measured directly, however, but has simply been deduced from an increase in bilirubin concentration in the blood. One explanation for the decrease in clearance is that during energy deficiency there is increased competition between bromosulphthalein and bilirubin on the one hand, and NEFA on the other, for liver binding sites; another is that turnover within the enterohepatic cycle is decreased (13). Alternatively, the ability of the liver to conjugate bromosulphthalein and bilirubin and excrete them into the bile could be impaired. A strong positive correlation can also be demonstrated between hepatic fat content and blood bilirubin concentration in healthy fed cows during pregnancy and lactation (21). This suggests that the signs of energy deficiency that occur in late pregnancy and early lactation in normal animals may be associated with some decrease in bilirubin clearance. There do not seem to have been any direct demonstrations of an effect of energy deficiency on the ability of the liver to clear other substances of physiological importance.

IMPAIRED PROTEIN SYNTHESIS.

Although this area has not been well-researched, there is circumstantial evidence in cattel that energy deficiency may be associated with a decrease in the ability of the liver to synthesize protein. This evidence includes the following observations: 1. The circulating concentration of plasma albumin, which is synthesized exclusively in the liver, often decreases in dairy cows postpartum (24). 2. In the livers of fasted animals there is a decrease in mitochondrial number and in the content of ATP and rough endoplasmic reticulum (4, 19). Oxidation within the mitochondria is necessary to produce energy for protein synthesis in the form of ATP, while the rough endoplasmic reticulum is the intracellular organelle on which the protein synthesis takes place. Failure to synthesize the protein moiety of lipoprotein could be one explanation for fatty infiltration of the liver (see above). Another important factor that could limit hepatic protein synthesis is the availability of amino acids. During heavy lactation there will be a substantial drain of amino acids into the mammary gland, and amino acids will also be used for glucose synthesis and for oxidative purposes.

THE LIVER AND METABOLIC DISORDER.

There have been numerous suggestions that impaired hepatic function is implicated in the aetiology of the various periparturient disorders to which dairy cows are susceptible, including, besides ketosis, diminished fertility, increased susceptibility to infection, endometritis, retained placenta, and others (e. g. 16, 22, 25). However, in many instances there is a noticeable dearth of hard evidence to indicate which particular aspect or aspects of liver function are involved. In some cases it would be of value if the defective biochemical and physiological mechanisms that were immediately responsible for the disorder were better understood. Identification of the precise involvement of the liver would then be facilitated. Although it is widely assumed that the various changes that occur in the liver in conjunction with energy deficiency, i. e. decrease in carbohydrate status, fatty infiltration and ultrastructural changes, are detrimental to liver function, there is in many cases still no evidence to indicate which particular hepatic processes are deranged, and to what degree. Increase in serum enzyme concentration in early lactation has also been correlated with impairment of liver function (e. g. 16). Again, however, the particular hepatic processes that could be affected have not been elucidated. The use of changes in serum enzyme content to diagnose liver dysfunction is complicated by the fact that the hepatic content of these enzymes may itself change during energy deficiency (8). Furthermore, some of the enzymes under consideration, for example serum aspartate aminotransferase, may originate from other tissues besiders liver (12).

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FATTY LIVER IN DAIRY COWS – INCIDENCE, SEVERITY, PATHOLOGY AND FUNCTIONAL CONSEQUENCES

I. M. Reid
ARC, Institute for Research on Animal Diseases, Compton

ABSTRACT

Fatty liver is common in high-yielding dairy cows immediately after calving with 2/3 of cows suffering from moderate or severe fatty liver. The fatty liver is related to the negative energy balance and loss of condition which commonly occurs after calving. Severe fatty liver is accompanied by enlargement of liver cells with compression of hepatic sinusoids, and damage to mitochondria and rough endoplasmic reticulum.

This evidence of liver damage is accompanied by a number of blood metabolite changes including elevated free fatty acid levels, raised serum enzyme and lowered serum albumin concentrations. One important clinical consequence of fatty liver appears to be an adverse effect on fertility. Cows with moderate or severe fatty liver have significantly longer calving intervals when compared to cows with mild fatty liver.

INTRODUCTION

Fat accumulates in the livers of cows around the time of parturition. However, the incidence, severity and clinical significance of this post parturient fatty liver is only now beginning to be appreciated. In this paper I will review recent work on the incidence and severity of fatty liver in high-yielding dairy cows together with comments on the pathology and clinical significance of the condition.

MATERIALS AND METHODS

A total of 155 Friesians and 43 Guernseys were sampled from 5 herds calving between September 1978 and April 1979. The average cow milk yield of the Guernsey herd was 4040 kg and of the 4 Friesian herds 7400, 6500, 5850 and 6100 kg respectively. Samples of liver were taken by percutaneous neddle biopsy from each animal at 1 week (range 7-13 days) and 8 weeks (range 56-62 days) after calving. The amount of fat in the liver cell was estimated from ORO-stained sections of biopsy samples by point counting methods (4), and is expressed as % of the liver cell volume occupied by fat.

RESULTS AND DISCUSSION

Incidence, Severity and Duration. On average, fat occupied between 20-30% of the liver cell of cows at 1 week after calving (Table 1). In heifers, the figure is considerably lower. The fatty liver has essentially disappeared by 8 weeks after calving.

Table 1. Mean % Fat in Liver Cell of Friesians by week after calving and lactation number

	1	2	3	4	5	6 and >
1 week	10.3 (21)	31.9 (14)	20.8 (39)	29.1 (24)	21.2 (18)	28.2 (14)
8 week	0.7 (18)	1.3 (13)	1.0 (28)	1.2 (21)	0.8 (17)	2.2 (13)

( ) = number of cows

The mean values for hepatic fat were classified on the basis of % fat in the liver at 1 week after calving into 3 groups, mild (0-20% fat), moderate (20-40% fat) and severe fatty liver (> 40% fat in liver parenchyma). Using this scheme, 15% of the cows sampled had severe fatty liver and 48% had a moderate fatty liver. It is apparent that fatty liver is common in high-yielding dairy cows but not heifers 1 week after calving and is moderate or severe in some 2/3 of cows.

Pathology. Fatty liver in the cow is caused by an accumulation of triglyceride (1) in the liver cell which has a number of harmful consequences (4). The accumulation of fat causes the liver cell to increase in size and cosequently to compress hepatic sinusoids. In some cases, the liver cells become so engorged with fat that they rupture and stimulate a local inflammatory response. There is also damage to the metabolic machinery of the hepatic parenchymal cells, particularly to the organelles associated with protein synthesis and energy metabolism.

Pathogenesis. Early in lactation, dairy cows are subjected to energy deficit and mobilise body reserves for milk production. This mobilisation involving adipose tissue and muscle results in the accumulation of fat in liver and other organs e.g., muscle and kidney (3). In the present survey, the % fat in the liver cell at 1 week was significantly related to the loss of condition score between 1 and 8 weeks after calving, and to plasma NEFA concentrations confirming the relationship between severity of fatty liver and extent of body tissue mobilisation.

Clinical Significance. Blood glucose is significantly reduced in cows with moderate or severe fatty liver (Table 2). This reduced energy status results in fat mobilisation which is reflected in the elevated NEFA levels (Table 2). The low albumin levels in the blood probably indicate reduced synthesis in the liver and the high ASAT levels indicate liver cell damage.

Table 2. Blood changes in cows with fatty liver

	Fatty liver		
	mild	moderate	severe
Glucose (mmol/l)	2.25	1.94***	2.02**
Mg (mmol/l)	0.95	0.87**	0.84*
Albumin (g/l)	27.4	26.8	24.0***
NEFA (μmol/l)	562	837***	901**
AST (IU/l)	52.8	61.3**	65.0**

* significantly different from mild $P < 0.05$
** significantly different from mild $P < 0.01$
*** significantly different from mild $P < 0.001$

The reason for the low Mg concentrations in cows with severe fatty liver is not immediately apparent.

An important clinical consequence of fatty liver may be an adverse affect on fertility. In a recent study (5), we provided direct evidence for a link between the liver and infertility by showing that cows with severe fatty liver had a significantly longer calving interval compared to cows with mild fatty liver. Preliminary evidence from a further 68 cows from two herds (Tabel 3) confirms our original finding that fatty liver, when moderate or severe, is associated with a lengthened calving interval.

The reason for this relationship is not at present understood but our observations are cosistent with those of *Haresign* (2) showing that fertility is impaired by loss of body weight and condition after calving.

Table 3. Fatty liver and reduced fertility.

	Farm*	Fatty liver mild	Fatty liver mod.-severe
Calving	1	355	386
Interval (days)	2	368	409

* 1) 40 cows
2) 28 cows

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THE LOW MILKFAT SYNDROME: SOME METABOLIC CONSEQUENCES OF HIGH GRAINRESTRICTED ROUGHAGE RATIONS

P. van Beukelen, Th. Wensing and H. J. Breukink
Kliniek voor Inwendige Ziekten, Rijksuniv. Utrecht

ABSTRACT

In order to study the etiology of the low milkfat syndrome, a milkfat depression was induced with a ration including expanded corn. $NaHCO_3$ and a supplement of fibrous content to the diet were used as a therapy. All cows showed a milkfat depression in the first induction period, during a second induction period a milkfat depression did not always occur. The milkfat depression was accompanied by changes in the ruminal fluid and in the blood. The abolition of the milkfat depression by way of $NaHCO_3$ was different than by supplement of fibrous content to the diet.

INTRODUCTION

The low milkfat syndrome in high yielding daily cows is regularly established in Holland in the past decade. A reduction in the amount of fibrous content is an important predisposition for the low milkfat syndrome. This can be caused by high gifts of concentrate or by grinding and pelleting the roughages. There are several theories in explaining the low milkfat syndrome. On high concentrate-restricted roughage rations a decreased proportion of acetate in the ruminal fluid could bring on a decreased supply of acetate to the udder (1) . Acetate is the principal precursor for the *"de novo"* synthesis of fatty acids in the udder. Another theory about the etiology of the low milkfat syndrome starts from the increased proportion of propionate on these rations, which results in an increased activity of insulin, probably with glucose as intermediate. The antilipolytic effect of insulin will decrease the supply of plasma triglycerides, main precursors of fatty acids in the milk, to the udder (1, 3). In a third theory, a decreased hydrogenation of diet-fat in the ruminal fluid results in an increased proportion of unsaturated longchain fatty acids in the blood (6). Unsaturated fatty acids are used less effectively in the udder and may work inhibitory on esterification of other fatty acids (2).

The etiology of the low milkfat syndrome has been studied in this experiment and some methods to evoke the milkfat depression were tested.

MATERIAL AND METHODS

Four high producing dairy cows, fitted with large ruminal fistulas, were involved in the experiment, which was divided in serval periods. In a pre-experimental period of 20 days a control ration consisting of 10 kg hay, 8 kg of a commercial concentrate mixture and 2 kg pulp was given. A milkfat depression was induced in the first induction period of 30 days with a ration consisting of 5 kg hay, 12 kg concentrate and 3 kg expanded corn. In the first recovery of 30 days two cows were fed the control ration and two cows remained on the induction ration with addition of 200 g $NaHCO_3$ twice daily during feeding, given trough the ruminal fistula. In the second induction period of 30 days alls cows received the induction ration, while in the second recovery period, 30 days, the two cows, who were treated with $NaHCO_3$ in the first recovery period, were given the control ration, while the other two cows received the $NaHCO_3$-treatment.

In all cows samples of ruminal fluid and blood were collected the last 4 days of the pre-experimental period and the last 8 days of the other periods. Samples of the morning milk were collected every day. Methods used for analyzing are presented elsewhere. In this paper only a part of the results is discussed.

RESULTS

The mean milkfat percentages and the daily milkfat productions are given in Fig. 1. In all cows a milkfat depression was achived in the first induction period. No complete recovery of the milkfat percentages was found in the first recovery period. In the second induction period no milkfat depression was seen in 3 cows on the induction ration, only 1 cow reacted with a renewed milkfat depression. In the second recovery period the milkfat depression of the last cow was evoked completely.

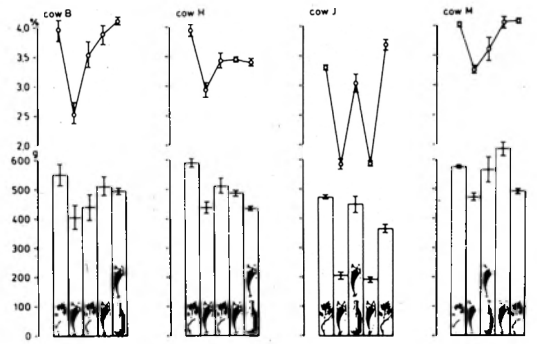

Figure 1. The mean milkfat percantages and the milkfat productions of the morning milk in the five periods, together with the S. E. M.

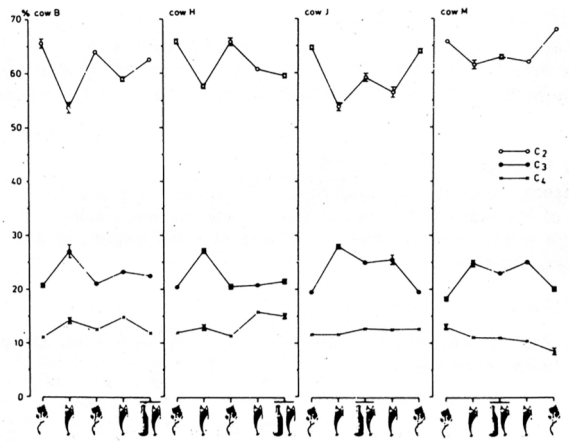

Figure 2. The percentages of C_2, C_3 and C_4 in the ruminal fluid in the five periods, together with the S. E. M.

The influence of the various rations on the volatile fatty pattern of the ruminal fluid is given in Fig. 2. The mean figures are values of the ruminal fluidsamples taken two hours after feeding. In the first induction period in all cows the milkfat depression was associated with an increase in the proportion of propionate and a decrease in the proportion of acetate. On the control ration in the recovery periods values of the pre-experimental period were obtained, while a different individual reaction was seen on the addition of $NaHCO_3$ in both recovery periods.

The distribution of the fatty acids in the milkfat and the fraction of the free fatty acids in the blood is given in fig. 3 for one of the cows, as an example of the changes in the four cows. In both induction periods

in the milkfat a large increase in the percentage of C 18 : 1 and a marked decrease in the percentage of C 18 : 0 was established. Small differences were found in the other fatty acids. In both recovery periods the percentages of C 18 : 0 and C 18 : 1 returned to the values of the pre-experimental period. In the fraction of the free fatty acids in the blood a large increase in the percentage of C 18 : 2 was established in the first induction period, on the cost of several other fatty acids. In the first recovery period a further increase in the percentage of C 18 : 2 was found, while pre-experimental values were achieved in the second recovery period.

The activities of the LDH, the SDH and the γGT in the four cows are given in fig. 4. In the first induction period nearly all enzyme activities were elevated. In the first recovery period a decrease in the liver enzyme activities was seen, although no normal values were reached.

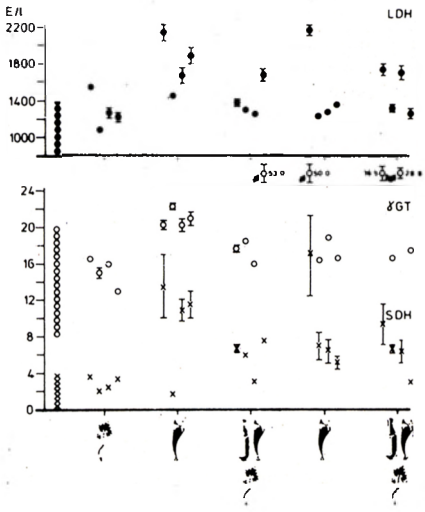

Figure 3. The distribution in 1 of the cows of the fatty acids in the milkfat and the F.F.A. in the blood in the 5 periods, with the S. E. M.

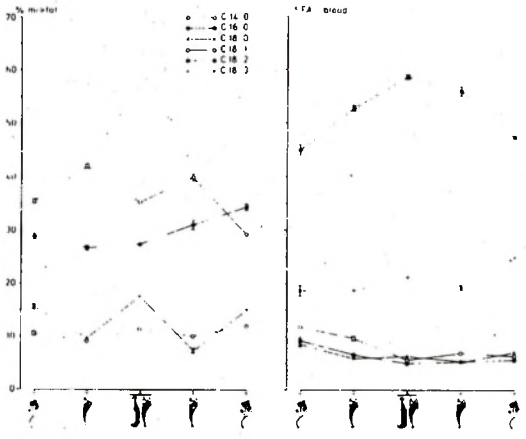

Figure 4. The mean activities of the LDH, the SDH and the γ-GT in the 5 periods, together with the S. E. M. if possible.

DISCUSSION

The increased proportion of propionate in the ruminal fluid in the milkfat depressed cows fits with results from literature (1). In the cows who did not experienced a milkfat depression in the second induction period an increased proportion of butyrate instead of an increased proportion of propionate was established. This phenomenon is also found by others (8) and in recently executed own experiments (4), maybe explained by an adaptation of the ruminal microbial population.

The increased percentage of unsaturated fatty acids in the milkfat and the blood in the milkfat depressed cows corresponds with results from others (2, 6). From the difference in reaction in the blood and the milkfat upon the addition of $NaHCO_3$ can be concluded that abolition of a milkfat depression by addition of $NaHCO_3$ to the ration follows a different pathway than through a supplement of fibrous content to the diet. From these figures an influence of $NaHCO_3$ on the metabolism of the udder can be assumed. This doesn't fit with figures in literature (5) in which an influence of $NaHCO_3$ on the composition of the volatile fatty acids in the rumen is presumed. On one and the same ration changes in the fatty acid pattern of the milk are found during lactation (7). Some indications for this statement are found in this experiment, not only in the milkfat, but also in the blood.

The increased activities of the liver enzymes in the milkfat depressed cows can not be easily explained. A different supply of fatty acids to the liver in cows with a milkfat depression can possibly result in a different way of triglycerides synthesis. In recent experiments liver biopsies are obtained for further investigation.

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METABOLIC DISORDERS ASSOCIATED WITH HYPOMAGNESEMIA AND HYPOCALCEMIA IN GRASS TETANY IN RUMINANTS

Y. Rayssiguier

Laboratoire des Maladies Métaboliques – Institut National de la Recherche Agronomique
Theix

ABSTRACT

Some metabolic disturbances associated with grass tetany have been studied in dairy cows after outdoor grazing. Hypomagesemia was accompanied by significant hypocalcemia. The increase in free fatty acids in spontaneous hypomagnesemia cows suggests that increased lipolysis is a feature of grass tetany. Hypomagnesemic cows developped hyperglycemia with no corresponding increase in plasma insulin concentration and high levels of plasma aldosterone. These results are discussed in relation with magnesium metabolism.

INTRODUCTION

Decreased magnesium absorption does not entirely account for the hypomagnesemia leading to grass tetany in dairy cows and it seems necessary to look for other contributing factors (2). Previous studies in ruminants showed that stimulation of lipolysis was accompanied by a decrease in plasma magnesium (4). It was therefore interesting to examine whether spontaneous hypomagnesemia in dairy cows put out to grass was also accompanied by increased lipolysis. Hypomagnesemia animals showed change in intermediary carbohydrate metabolism, and therefore plasma insulin was measured. Since the disease has been associated with high potassium and low sodium contents in grass, blood plasma aldosterone levels were also determined.

MATERIAL AND METHODS

The study was carried out in a veterinary practice in northern France were cases of grass tetany arise frequently in spring. Blood was collected before the animals were put outdoors to grass or 3 or 4 weeks after. Each time that an animal showed the first clinical signs of hypomagnesemia, a blood sample was taken from the animal and from a random control animal from the same herd.

Magnesium (Mg) and calcium (Ca) were estimated by atomic absorption spectrophotometry, sodium and potassium by flame photometry, phosphorus by colorimetry. The following metabolites were determined by enzymic methods: glucose, lactate, urea and non esterified fatty acids (FFA). Insulin

concentration in the plasma samples was determined by using the radioimmunoassay kit supplied by C.E.A. France. Aldosterone levels were determined by radioimmunoassay (5). Hydroxyproline was dermined according to the Bergman-Löxley method (6).

RESULTS AND DISCUSSION

Table 1. Plasma values in cows before and after outdoor grazing

	BEFORE	AFTER	
		Controls	Hypomagnesemic animals
Mg (mM)	0.92 ± 0.06	0.92 ± 0.06	0.26 ± 0.05[a]
Ca (mM)	2.52 ± 0.10	2.67 ± 0.07	1.99 ± 0.22[a]
P (mM)	1.77 ± 0.44	1.97 ± 0.15	1.62 ± 0.32
Na (mM)	131.5 ± 1.6	134.1 ± 1.9	133.3 ± 2.1
K (mM)	4.47 ± 0.22	4.65 ± 0.20	4.73 ± 0.35
Free fatty acids (mM)	0.49 ± 0.09	0.31 ± 0.03	0.84 ± 0.15[a]
Glucose (g/l)	0.64 ± 0.02	0.66 ± 0.02	0.83 ± 0.09[a]
Lactate (mM)	0.72 ± 0.13	0.87 ± 0.08	1.85 ± 0.32[a]
3-hydroxybutyrate (mM)	0.47 ± 0.09	0.32 ± 0.5	0.44 ± 0.14
Urea (mM)	2.75 ± 0.02	4.96 ± 0.40	4.81 ± 0.39[b]
Insulin (mU/l)	8.25 ± 0.82	12.81 ± 2.78	8.88 ± 1.35
Aldosterone (ng/100ml)	29.8 ± 14.4	44.9 ± 11	72.4 ± 11[b]
Hydroxyproline (ng/ml)	2.01 ± 0.21	1.38 ± 0.11	0.62 ± 0.12[a]

Mean ± SEM of 10-12 determinations except for aldosterone. In this case, each value is the mean of 5-6 determinations. Each obtained by pooling plasma from 2 animals.
[a] Statistically different from controls
[b] Statistically different from value before outdoor grazing.

Severity of hypocalcemia in grass tetany probably depends on a double mechanism : the lack of response of the parathyroid glands to a fall in plasma Ca and the lack of response of target organs to PTH and vitamin D metabolites. Mg depletion inhibits the normal process of collagen resorption in various experimental conditions and it has been suggested that the lack of sensitivity of bone might be related to the effect of Mg deficiency on proteic bone matrix (6).

Previous studies in the ruminant showed that stimulation of lipolysis was accompanied by a decrease in plasma Mg (4). For example, adrenalin or theophyllin infusion and different experimental stimulants such as cold exposure, or fasting bring about a decrease in plasma Mg with the increased lipolysis. The administration of propanolol (β blocking agent) or sodium nicotinate (antilipolytic agent) inhibits both the increase in FFA and hypomagnesemia. Same results have been obtained in other species (4). The increase in FFA in spontaneous hypomagnesemic cows suggests that increased lipolysis is a feature of grass tetany. Thus the different factors known to be implicated in the disease such as cold stress and undernutrition may bring about their effects via stimulation of lipolysis. Pratical implications concerning grass tetany can be obtained from this experiment. Apart from clinical methods consisting of large Mg supplements given to animals, interventions aimed at controlling lipolysis appear to be worthy of consideration. Readily fermentable carbohydrates added to the diet might be beneficial by increasing Mg absorption and by provinding energy (7).

It has been suggested that elevated levels of insulin stimulated by the high potassium content of tetany prone grass may contribute to hypomagnesemia by transfering Mg from extracellular fluids to intracellular spaces. Our results are not in agreement with this hypothesis (3). Hypomagnesemic cows developed marked hyperglycemia with no corresponding increase in mean insulin concentration in the plasma. Ca ions are necessary for a proper release of insulin from cells of the pancreatic gland and hypocalcemia might have interfered with release of insulin. The increase in FFA, glucose, lactate, suggest the possibility of increased sympathetic activity which can also abolish the release of insulin to various stimuli.

The grass associated with outbreaks of grass tetany is usually high in potassium and low in sodium but the increase in plasma aldosterone level seems rather related to the magnesium status of the animals. The increase in aldosterone secretion in hypomagnesemic cows agrees with previous results in laboratory animals during experimental Mg deficiency (1). The increase in aldosterone secretion seems to be related to increased renin secretion. In ruminants, this endocrine modification appears to act against Mg conservation. Martens has shown that the low Na/K ratio in the rumen contents reduces the absorption of Mg (4) and the possibility exists that a high level of aldosterone causes a fall in salivary sodium concentration along with an increase in the concentration of salivary potassium. This in turn would affect the Na/k ratio in the rumen.

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THE EFFECT OF DIFFERENT SELENIUM SUPPLEMENTATIONS TO CATTLE

G. Jönsson, S. Johnsson and B. Pehrson
The Veterinary Institute, Skara

ABSTRACT

Thirty mg of Se given twice during last month of pregnancy to beef cows on a Se-deficient diet gave satisfactory Se-status in the calves at birth, estimated by the activity of glutathione peroxidase in erythrocytes. The activity decreased gradually and reached the risk margin of nutritional myodegeneration after 2 - 3 months. Parenteral Se treatment at birth did not prolong the period of satisfactory Se-status. Se supplementation to the dams two months after calving gave no response in the calves. For further protection the calves themselves have to be Se supplemented. Results of GSH-Px and Se determinations of different organs from bulls on different dietary Se levels indicate that the accepted optimal Se level of 0.1 p.p.m. is too low, and should be somewhere between 0.1 and 0.5 p.p.m.

INTRODUCTION

Almost all Sweden has a pronounced selenium-deficiency. Homegrown feeds for cattle are inadequate in this element (1). Cattle management where imported and often Se-rich protein feed supplements are excluded, has become more common in the last decade. Concurrently increased risk for nutritional myodegeneration in calves has been observed. At the prospect of an expected allowance of Se supplementation to cattle feed in Sweden studies on the effect of different Se supplementations were urgent.

MATERIAL AND METHODS

Analyses. *Glutathione peroxidase* (GSH-Px) according to (3). *Selenium* fluorimetrically according to (2).

Experiment 1. *Animals:* 74 cow-calf pairs. *Se supplementation:* Cows were given 30 mg Se orally as Na_2SeO_3 twice at four weeks interval during the last month of pregnancy. The basal diet consisted of home-grown, Se-deficient but not analyzed feeds. At birth 3 mg Se was given intramuscularly to every second calf. The others were untreated controls. *Sampling:* Blood samples before supplementation and then once a month.

Experiment 2. *Animals:* 32 cow-calf pairs, randomly divided into four equal groups. *Se supplementation:* All cows treated during pregnancy as in Experiment 1. Approximately two months after calving every second cow was given 60 mg Se and every second calf 12 mg Se orally as Na_2SeO_3 according to the following scheme (+ treated, – untreated):

Group	1	2	3	4
Cow	+	+	–	–
Calf	+	–	+	–

Sampling: Blood samples were taken on day of Se supplementation and four weeks later.

Experiment 3. *Animals:* 36 bull calves, randomly divided into four groups. *Se supplementation:* 0, 0.1, 0.5 and 1.0 p.p.m. to a "barley-beef" diet, the Se content of which was approx. 0.1 p.p.m., during four months before slaughter. *Sampling:* Pieces of liver, kidney, cardiac and skeletal muscles, taken as

soon as possible after slaughter, immediately frozen in liquid nitrogen and kept below -20° C until analyzed.

RESULTS AND CONCLUSIONS

The results are presented in figures 1 - 3.

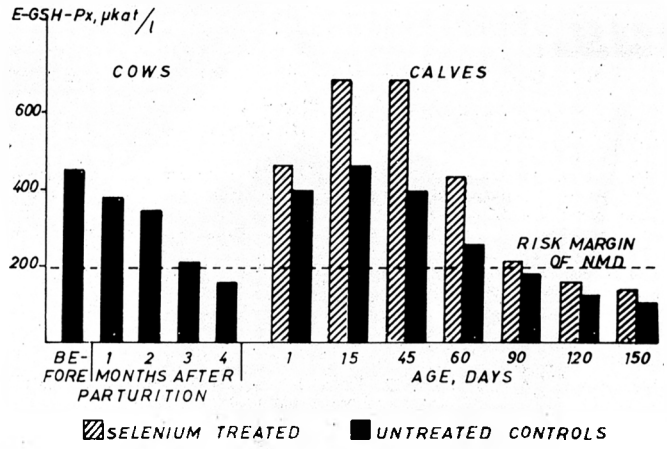

Figure 1. Experiment no 1. The effect of 30 mg Se given perorally twice to cows during last month of pregnancy, and of 3 mg Se intramuscularly to new-born calves on the erythrocyte glutathione peroxidase levels (E-GSH-Px).

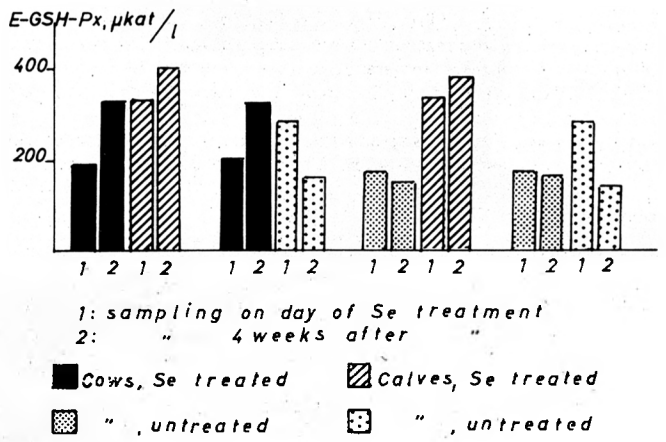

Figure 2. Experiment no 2. The effect of 60 mg Se given perorally once to dams, and of 12 mg Se given perorally to their calves two months after calving on the E-GSH-Px levels.

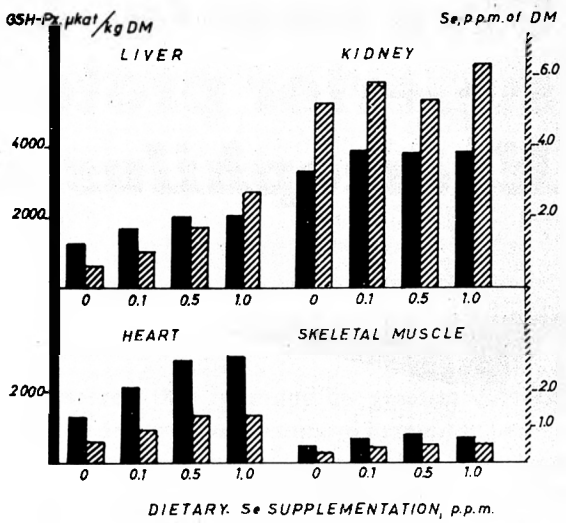

Figure 3. Experiment no 3. GSH-Px activity and Se content of liver, kidney, cardiac and skeletal muscles of bulls given four different Se supplementations during four months before slaughter.

A. Treatment of pregnant cows with 30 mg of Se twice during the last month of pregnancy gave glutathione-peroxidase levels in erythrocytes (E-GSH-Px) well above the empirically set risk margin of nutritional myodegeneration (NMD). The levels were decreasing successively and reached the risk margin at approx. 60 days after birth (Experiment no. 1).

B. A single treatment of the newborn calf with 3 mg of Se gave a considerably higher E-GSH-Px level but did not seem to proling the period of protection against NMD (Experiment no. 1).

C. Se treatment of the dams increased their own E-GSH-Px levels but did not influence the levels of their suckling calves, indicating that no or negligible amounts of Se are transferred to milk. For further protection against NMD, Se treatment has to be given directly to the calves (Experiment no. 2).

D. The GSH-Px activities of liver, cardiac and skeletal muscles increased when increasing the Se supplementation from 0.1 to 0.5 p.p.m. but levelled off when increasing to 1.0 p.p.m. The Se content of cardiac and skeletal muscles behaved in a similar way, while the Se content of the liver increased further with the highest supplementation. The results from the kidney were not quite consistent with those from the other organs, possibly due to high initial values. The results may indicate that a dietary Se content of somewhere between 0.1 and 0.5 p.p.m. would be more appropriate than the commonly recommended 0.1 p.p.m. (Experiment no. 3).

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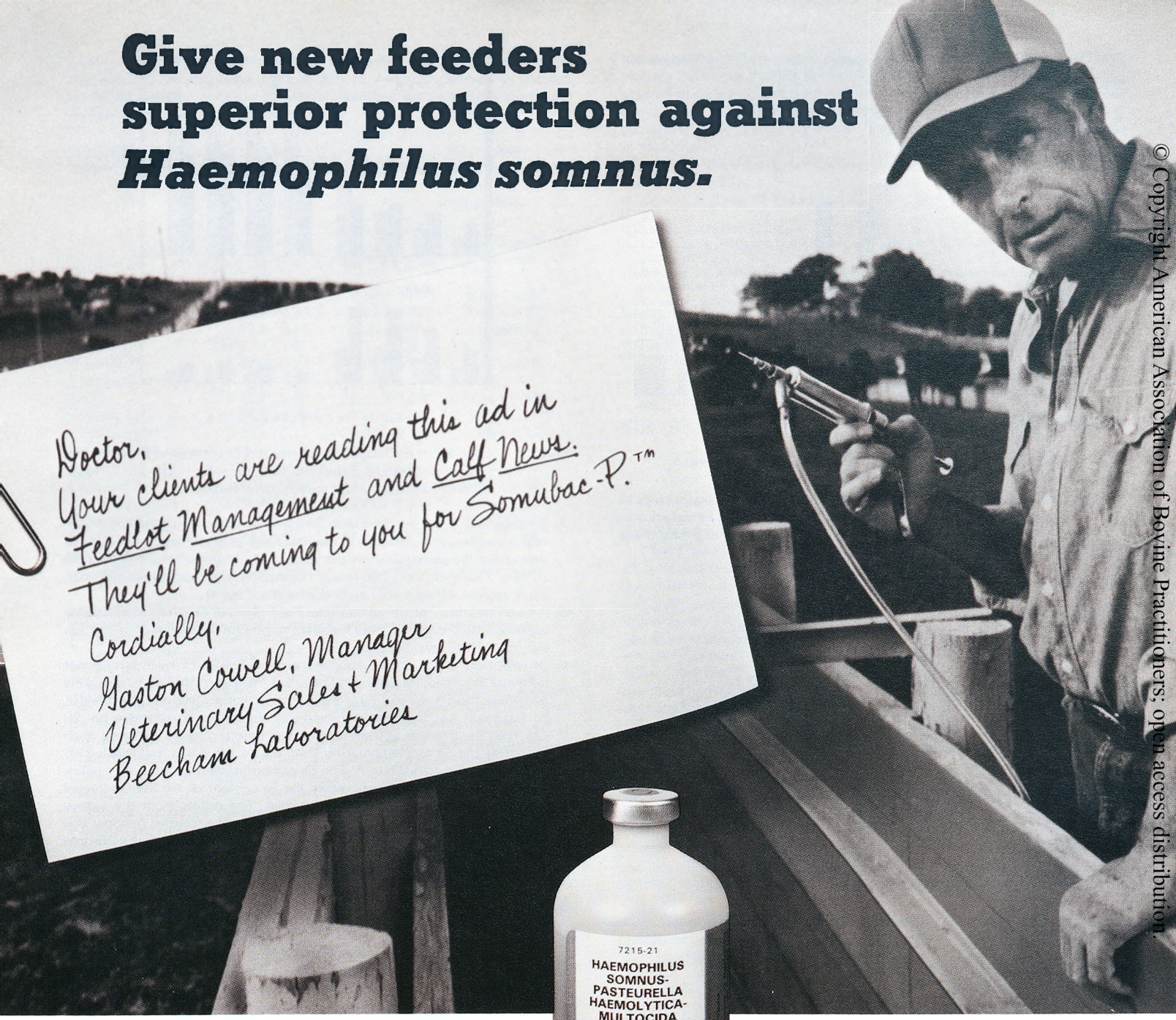

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