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Influence of Feeding Intensity on Blood Concentrations of Glucose, Ketone Bodies and Free Fatty Acids in Nutritionally Adapted Dairy Cows Fed 24 Times Daily*

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Nielsen, T. K. and K. Jensen: Influence of feeding intensity on blood concentrations of glucose, ketone bodies and free fatty acids in nutritionally adapted dairy cows fed 24 times daily. Acta vet. scand. 1991, 32, 301–306. – Concentrations of acetoacetate, D-3-hydroxybutyrate, glucose and free fatty acids were determined in blood from 3 Jersey cows during a 5-months period. The cows were fed an identical complete feed every hour, from ad libitum intake to maintenance level, for 28 days at each of the following levels of intake: 170, 138, 102, 53 and 37 g organic matter per kg bodyweight$^{0.75}$ (metabolic bodyweight). Throughout the experimental period, the cows were in negative energy balance, which was reflected in an average weight loss of 640 g per day. None of the metabolites differed from reference values for healthy lactating cows. The concentration of D-3-hydroxybutyrate decreased linearly with the level of feeding and reflected a decreased ruminal production of butyrate.
The high feeding frequency to lactating cows may be considered an essential factor in the prevention of ketosis.

energy balance; feeding frequency; FFA.

Introduction

Changes in the concentrations of e.g. glucose, ketone bodies, and free fatty acids in the blood are commonly observed in ruminant ketosis. The use of these blood metabolites as indicators of the energy balance (*Coggins & Field* 1978, *Dale et al.* 1978, *Andersson & Lundström* 1984, *Vik-Mo* 1984a) seems to open up interesting possibilities for control and prevention of ketosis, as well as for optimizing the energy supply in animal production.

Most studies of indicators for carbohydrate and fat metabolism have been performed in ruminants under heavy physiological stress, i.e. multiparous sheep or dairy cattle with excessive milk production, in both instances with significant negative energy balance.

The aim of the present study was to elucidate the effects of feeding intensity on the concentrations of glucose, ketone bodies,

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and free fatty acids in the blood of dairy cows, from maximum energy intake to maintenance level, when fed frequently to ensure minimum metabolic variation.

Materials and methods

Three Jersey cows (Nos. 15, 16, and 17) were fed automatically every hour during 5 months from the second month of the second lactation period. The cows were dried off when fed at maintenance level. The daily rations consisted of complete fed of identical composition throughout the experimental period, in the form of feed pellets and long straw (90/10). The chemical composition of the ration was:

Dry matter	89.2 %
Crude protein	16.7 %
Crude fat	4.3 %
Nitrogen-free extract	43.1 %
Crude fibre	16.2 %
Crude ash	8.9 %

The diet was supplemented with vitamins and minerals to Danish standards.

The sequence of feeding intensities were scheduled to be 200, 150, 100, 50, and 35 g organic matter per kg bodyweight$^{0.75}$ daily. The cows were fed at each level for 28 days, starting with the highest level. The daily feed intake was recorded, and the cows were weighed twice a week. After 25 days' adaptation to each feeding level, blood samples were collected from the vena jugularis through a 14-gauge needle at 2 p.m. The blood samples were stabilized with heparin sodium and stored in liquid nitrogen (*Työppönen & Kauppinen* 1980) until analyzed. The concentrations of acetoacetate and D-3-hydroxybutyrate in full blood were determined according to the enzymatic method by *Williamson et al.* (1962), and by the use of reference standards and D-3-hydroxybutyrate dehydrogenase from BOEHRINGER, Mannheim, FRG. The contents of plasma-free fatty acids were determined by spectrophotometry after liquid-liquid extraction (*Falholt et al.* 1973). The glucose content in full blood was analysed by the hexokinase/glucose-6-phosphate dehydrogenase principle with a Sigma test combination.

Results

Energy balance

The average body weight of the cows decreased from 340 to 269 kg during the 5-month period, reflecting a mean daily loss of 640 g body weight or a total weight loss of 24 % (Table 1).

Blood parameters

The concentrations of glucose, ketone bodies and free fatty acids are shown in Table 2.

Table 1. Average bodyweight and intake of organic matter (OM) of three dairy cows during a five-month period of the second lactation.

	Scheduled feed intake, g OM/kg BW$^{0.75}$/day				
	200	150	100	50	35
Bodyweight (BW), kg:	340.1	319.1	302.1	286.2	268.2
Feed intake:					
kg OM/day	13.4	10.4	7.4	3.7	2.4
g OM/kg BW$^{0.75}$/day	170.3	137.7	101.5	52.9	37.0
Loss of BW between levels, g/day		269	568	607	750

Table 2. The concentrations (mmol) of glucose (GLU), acetoacetate (ACAC:, D-3-hydroxybutyrate (HBU) and free fatty acids (FFA) in blood from 3 dairy cows fed different amounts of a complete feed during a 5-month period of the second lactation.

Cow No.	Metabolite	Feed intake, g OM/kg $BW^{0.75}$/day				
		170	138	102	53	37
15	GLU	2.28	3.78	2.38	2.81	3.39
	ACAC	0.105	0.076	0.031	0.073	0.057
	HBU	0.525	0.509	0.516	0.262	0.231
	FFA	–	0.361	0.321	0.255	0.252
16	GLU	2.73	3.31	2.48	2.21	2.21
	ACAC	0.061	0.054	0.158	0.144	0.143
	HBU	0.566	0.412	0.281	0.190	0.126
	FFA	–	0.532	0.364	0.296	0.291
17	GLU	3.81	3.51	2.83	1.67	1.72
	ACAC	0.117	0.169	.0032	0.147	0.153
	HBU	0.549	0.534	0.297	0.203	0.148
	FFA	–	0.386	0.363	0.157	0.451
Average	GLU	2.94	3.53	2.87	2.22	2.44
	ACAC	0.083	0.100	0.073	0.121	0.118
	HBU	0.546	0.485	0.364	0.218	0.168
	FFA	–	0.426	0.649	0.236	0.331

The levels of glucose, acetoacetate, and free fatty acids were not affected by the feeding intensity, although the concentrations of glucose of cow No. 17 decreased with decreasing feed intake. The content of D-3-hydroxybutyrate declined linearly from 0.55 to 0.17 mmol/l full blood from ad libitum feed intake to maintenance level. The difference was statistically highly significant (Table 3).

Discussion

The concentrations of acetoacetate, D-3-hydroxybutyrate and free fatty acids found in the blood were low, whereas the glucose level was close to average when compared with the reference values for these blood metabolites in healthy animals in their first month of lactation (*Baird* 1977, *Qvesel* 1983, *Andersson* 1984, *Vik-Mo* 1984a). Thus, at no time has there been any uncompensated stress on the carbohydrate or fat metabolism.

An increase in the concentrations of ketone bodies and free fatty acids in ruminants is

Table 3. Analysis of variance (F-values) of main effect on blood content of glucose (GLU), acetoacetate (ACAC), D.3.hydroxybutyrate (HBU), and free fatty acids (FFA).

	GLU	ACAC	HBU	FFA
Feeding level	1.98^{ns}	0.44^{ns}	21.23^{***}	2.76^{ns}
Cows (animals)	1.10^{ns}	1.67^{ns}	3.02^{ns}	0.49^{ns}

usually correlated with a negative energy balance, especially at the beginning of the lactation period, because of increased fat mobilization and increased ketogenesis in the liver. A concurrent hypoglucemia is presumably due to that fact that the gluconeogenesis has limited possibilities of supplying the tissues, including foetus and milk glands, with glucose (*Bergman* 1973, *Hibbett* 1979, *Baird* 1982). The biochemical changes are even more pronounced in ruminants with clinical ketosis.

The animals in the present experiment lost considerable weight, i.e. equivalent to 150 kg of a 600 kg dairy cow. Despite the reduced energy intake, the cows were able to produce milk to the level of 35 g organic matter per kg bodyweight$^{0.75}$, when they were dried off. Nevertheless, mobilization of energy reserves never resulted in non-physiological increases in the blood level of ketone bodies or in decreases in blood glucose concentrations even at the highest feeding level 2 months postpartum as has been found by others in cases of negative energy balance of the same magnitude (*Jönsson & Pehrson* 1972, *Dale et al.* 1978, *Parker & Lewis* 1978, *Halse et al.* 1983, *Andersson & Lundström* 1984). Neither were the concentrations of free fatty acids in plasma found to correlate with the energy balance as observed by many other investigators (e.g. *Bowden* 1971, *Erfle et al.* Parker & Lewis 1978), but not by *Jönsson & Persson* (1972). The lack of correlation between the blood metabolites and the energy balance found in the present study, especially 2 and 3 months postpartum, may be explained partly by the animals being fed 24 times a day, which results in an ideal fermentation in the rumen and hence constant absorption of all nutrients (*Jensen & Wolstrup* 1977) including glucose precursors and energy metabolites, partly by the long time of adaption. Thus, the gluconeogenesis was not stressed to such an extent that an accumulation of fatty acids and increased ketogenesis occurred (*Bergman* 1973, *Baird* 1982).

The almost linear correlation between feeding intensity and the D-3-hydroxybutyrate concentration in the blood is probably a result of an increased contribution from the conversion of butyric acid by the ruminal mucous membrane during the increased ruminal metabolism (*Leng & West* 1969, *Bergman* 1971, *Coggins & Field* 1978, *Wik-Mo* 1984b). The content of D-3-hydroxybutyrate in the blood at maintenance level must be assumed to be a result of an interaction with endogenous acetoacetate owing to the given metabolic conditions (*Bergman* 1971).

It is worth noting that the ratio between D-3-hydroxybutyrate and acetoacetate was less than 10 in all cases, a situation which is usually observed in animals with ketosis, in which the endogenous acetoacetate production is greatly increased (*Baird* 1977). It might be considered whether the glucose precursors in the liver was limited after all, resulting in a relatively high acetoacetate concentration, but that the constant supply of nutrients in fact counteracted increased ketogenesis.

In conclusion, high feeding frequency to lactating cows may be considered an essential factor in the prevention of ketosis.

References

Andersson L: Concentrations of blood and milk ketone bodies, blood isopropanol and plasma glucose in dairy cows in relation to the degree of hyperketonaemia and clinical signs. Zbl. Vet. Med. A. 1984, *31*, 683–693.

Andersson L, Lundström K: Effects of energy balance on plasma glucose and ketone bodies in blood and milk and influence of hyperketonemia on milk production in post-parturient

dairy cows. Zbl. Vet. Med. A. 1984, *31,* 539–547.

Baird GD: Aspects of ruminant intermediary metabolism in relation to ketosis. Biochem. Soc. Trans. 1977, *5,* 819–827.

Baird GD: Primary ketosis in the high-producing dairy cow: Clinical and subclinical disorders, treatment, prevention, and outlook. J. Dairy Sci. 1982, *65,* 1–10.

Bergman EN: Hyperketonemia-ketogenesis and ketone body metabolism. J. Dairy Sci. 1971, *54,* 936–948.

Bergman EN: Glucose metabolism in ruminants as related to hypoglycemia and ketosis. Cornell Vet. (Suppl.), 1973, *63,* 341–382.

Bowden DM: Non-esterified fatty acids and ketone bodies in blood as indicators of nutritional status in ruminants. Can. J. Anim. Sci. 1971, *51,* 1–13.

Coggins CRE, Field AC: Changes in plasma concentrations of glucose, free fatty acids, ketone bodies, thyroxine and insulin of lactating beef cows in relation to time of feeding and energy status. In: Blood Profiles in Animal Production. Occasional Publication No. 1, ed. D. Lister. Brit. Soc. Anim. Prod. 1978, p. 41–47.

Dale H, Vik-Mo L, Halse K: Ei feltgranskning over ketose hjå mjølkekyr sett i høve til energidekninga i tidleg laktasjon. (A field trial of ketosis in dairy coes in relation to energy supply in early lactation). Norsk Vettidsskr. 1978, *90,* 3–10.

Erfle JD, Fisher LJ, Sauer FD: Interrelationships between blood metabolites and an evaluation of their use as criteria of energy status of cows in early lactation. Can. J. Anim. Sci. 1974, *54,* 293–303.

Falholt K, Lund B, Falholt W: An easy colorimetric micromethod for routine determination of free fatty acids in plasma. Clin. Chem. Acta 1973, *46,* 105–111.

Halse K, Standal N, Syrstad O: Fatty acid composition of milk fat related to the concentration of acetoacetate and glucose in blood plasma of cows. Acta Agric. Scand. 1983, *33,* 361–368.

Hibbett KG: Bovine ketosis and its prevention. Vet. Rec. 1979, *105,* 13–15.

Jensen K, Wolstrup J: Effect of feeding frequency on fermentation pattern and microbial activity in the bovine rumen. Acta Vet. Scand. 1977, *18,* 108–121.

Jönsson GB, Pehrson BG: Some blood parameters in dairy cows at different feeding intensities. Proc. 7th Int. Meeting on Disease of Cattle. London 1972, p. 250–257.

Leng RA, West CE: Contribution of acetate, butyrate, palmitate, stearate and oleate to ketone body synthesis in sheep. Res. Vet. Sci. 1969, *10,* 57–63.

Parker BNJ, Lewis G: The effect of energy level on body condition and some blood compounds in dairy cows. In: Blood Profiles in Animal Production. Occasional Publication No. 1. Ed. D Lister. British Society of Animal Production. 1978, p. 121–132.

Qvesel J: Epidemiologisk undersøgelse over bovin ketose i en vestjysk kvægpraksis. (An epidemiological investigation of bovine ketosis in western Jutland). Dansk Vettidskr. 1983, *66,* 378–386.

Työppönen J, Kauppinen K: The stability and automatic determination of ketone bodies in blood sample taken in field conditions. Acta Vet. Scand. 1980, *21,* 55–61.

Vik-Mo L: Effects of feeding on blood concentrations of acetoacetate, glucose and free fatty acids in dairy cows at substandard energy level during early lactation. Dept. Anim. Nutr., Agric. Univ., Norway. 1984a, Rep. No. 221.

Vik-Mo L: Rumen fermentation pattern and its effect on ketonemia in dairy cows during early lactation. Dept. Anim. Nutr., Agric. Univ., Norway. 1984b, Rep. No. 222.

Williamson DH, Mellanby J, Krebs HA: Enzymic determination of D-(-)-3-hydroxybutyric acid in blood. Biochem. J. 1962, *82,* 90–96.

Sammendrag

Indflydelse af fodringsintensiteten på blodkoncentrationen af glukose, ketonstoffer og frie fedtsyrer hos ernæringsmæssigt veladapterede malkekøer ved høj fodringsfrekvens.

Koncentrationen af acetoacetat, D-hydroxybutyrat, glucose og frie fedtsyrer blev analyseret i blod

fra tre lakterende jerseykøer, som gennem en 5 måneders periode blev fodret 24 gange i døgnet med samme foderblanding. Hver 28. dag blev foderrationen reduceret fra ad libitum gradvist ned til vedligeholdelsesniveau, hvor køerne blev goldet. Fodringsintensiteter var 170, 138, 102, 53 og 37 g organisk stof pr. kg metabolisk legemsvægt (kg legemsvægt^{0,75}).

Under forsøget var køerne i stor negativ energibalance, idet det totale vægttab udgjorde 24 % svarende til et gennemsnitligt dagligt tab på 640 g.

Ingen af de undersøgte metabolitter fandtes under disse omstændigheder afvigende fra referenceværdier hos tilsyneladende sunde og raske malkekøer. D-3-hydroxybutyratkoncentrationen faldt lineært med aftagende ruminal produktion af smørsyre.

Undersøgelsen understreger betydningen af høj fodringsfrekvens hos højtydende malkekøer med vægt på forebyggelse af ketotiske tilstande.

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