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The influence of slowly and rapidly degradable concentrate protein on a number of blood parameters in dairy cattle

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Abstract

In three feeding trials with high-yielding fresh cows and heifers (102, 72 and 92 animals respectively), concentrates with slowly and rapidly degradable protein were compared. The roughage consisted of grass silage and maize silage. The cows were fed according to the standard or slightly above the standard for digestible crude protein and net energy for milk production. A sample of peripheral blood was taken immediately before the morning feed and then again 3 to 4.5 hours after the concentrate was fed. On a ration with slowly degradable protein the concentration of urea in the blood plasma was higher than in the case of rapidly degradable protein, both before and after the morning feed of concentrates. In one trial, in which the concentration of amino acids in the blood was also measured, a significantly lower concentration of the glucogenic amino acids as a whole was also recorded. There was a positive correlation between the urea concentration in the blood and milk production. These observations indicate that a ration containing slowly degradable protein promotes gluconeogenesis from amino acids. Moreover, insulin which inhibits gluconeogenesis showed a smaller increase in concentration in the blood after feeding when the ration contained slowly degradable protein. On a ration with slowly degradable concentrate protein the concentration of the non-esterified fatty acids tended to be lower directly before the morning feed. The difference in the degradability of the concentrate protein had no significant effect on the concentration of ketone bodies in the blood before the morning feed and 3.5 hours later.

Introduction

A previous publication (Veen, 1986) deals with the effect of the degree of degradability of concentrate protein on several rumen parameters in lactating dairy cows. Each trial involved a small number of rumen-fistulated cows, and in the majority of the trials discussed, roughage was not fed until after samples of rumen fluid had been taken at intervals during a period of three hours. During several feeding trials

– the results of which have been published elsewhere (Bakker et al., intern rep. 1982, 1983, 1984) – blood samples were taken from a number of cows, and various blood parameters were subsequently determined. The cows were housed and fed under normal practical conditions. The choice of blood parameters was determined by the data available in the literature and the results of previous trials, so that the same parameters were not used in all of the trials. The present paper contains the most important results from the 1980/81 trial onward.

As a ration with rapidly degradable protein causes the concentration of ammonia in the rumen to rise more rapidly, it was to be expected that the urea concentration in the blood would likewise increase more rapidly. On the other hand, the presence of urea in the blood may also be due to the conversion of amino acids into carbohydrates. For this reason we examined the concentration of the individual amino acids as well as that of urea in the blood. Differences in the concentration of the volatile fatty acids in the rumen fluid under the influence of the degradability of concentrate protein may be reflected in a difference in the concentration of insulin in the blood plasma, although there is no concensus concerning the influence of volatile fatty acids on insulin production (Stern et al., 1970; Bines & Hart, 1984). Differences in the concentration of non-esterified fatty acids and ketone bodies may point to differences in the mobilization of body energy (Bowden, 1971).

Materials and methods

The cows were Holstein-Friesians or crossbreeds of Holstein-Friesians and Dutch Friesians. They were kept in a cubicle house and were fed individually by means of Calan Broadbent selective feed gates. All the cows entered the trial 7-10 days after calving. Where a preliminary phase was introduced, all cows received the same concentrate, with a protein degradability which was the average of that of the trial feeds. In addition to the concentrates administered via the feed gates, the cows were given 0.5 kg concentrate at each milking, of a type which was also fed during the preliminary period. Both concentrate and roughage were administered in 2 daily portions, the concentrate at 7:00 and 16:00, the roughage at 8:30 and 17:00.

The trial designs are given schematically in Table 1. With the exception of trial RM-B22, concentrates were fed according to the requirement for milk production. In trial RM-B22 the cows received a fixed daily quantity of 10 kg concentrates and the heifers 7 kg. The standard for protein supply was met by preparing mixtures of concentrates with a high and a low protein content. The composition of the concentrates is given in Table 2. The protein degradability was determined by incubating the concentrates in nylon bags in the rumen of fistulated cows for 1, 6, 18 and 24 hours. On the basis of the course of the degradability curve, the degradability of the crude protein after 12 hours was estimated, and this value was used as the criterion.

In all the trials, peripheral blood was taken from all the animals; in trial RM-B20, samples were taken from the vena jugularis and in the other two trials from the vena coccygea. The blood was taken with vacutainers with heparin or EDTA as anti-clotting agents.

Table 1. Experimental design.

	Trial		
	RM-B20 1980/81	RM-B21 1981/82	RM-B22 1982/83
Number of animals	78 cows 24 heifers	52 cows 20 heifers	60 cows 32 heifers
Preperiod	none	week 2-5	none
Experimental period[1]	week 2-8	week 6-17	week 2-13
Type of concentrate[2]	b or B	b or B	bL + bH, or BL + BH
Roughage	prewilted grass silage and corn silage, ca 2:1 2 times a day	idem	morning: corn silage; afternoon: prewilted grass silage
Roughage supply	1.5 kg dry matter per 100 kg body weight	idem	corn silage: 5 kg dry matter for cows, 4 kg for heifers; grass silage ad lib.
Protein and energy supply	dcp[3]: norm NEL[4]: norm	dcp: norm NEL: 750 VEM above norm	dcp: norm or 15 % above
Sampling time[1]	week 7, before morning feeding	week 9, before morning feeding and 3.5 h after concentrate supply	week 6, before morning feeding and 4.5 h after concentrate supply

[1] Weeks of lactation.
[2] b = rapidly degradable protein; B = slowly degradable protein; L = low protein content; H = high protein content.
[3] dcp = digestible crude protein.
[4] NEL = net energy for lactation (van Es, 1978).

Chemical determinations

In trials RM-B20 and B21 the non-esterified fatty acids were determined according to the method of Regouw et al. (1971). Later a titration method was used. 2 ml of blood was extracted with 10 ml of a mixture of iso-propylalcohol, n-heptane and 1 M H_2SO_4 (40:10:1). After addition of 4 ml heptane, 3 ml of the upper layer was titrated with 0.02 M NaOH. Palmitic acid was used as a standard.

Ketone bodies were determined in heparinized blood. The ketone bodies were converted into acetone, which was isolated with the aid of a microdiffusion method. After reaction with salicyl-aldehyde the extinction was measured at 265 nm.

Table 2. Composition of the concentrates[1] (%).

	RMB-20/21		RMB-22			
	b	B	bL	bH	BL	BH
Maize	–	8.00	–	–	9.60	–
Lupin	–	–	20.00	25.00	–	–
Horsebeans	10.00	–	–	–	–	–
Maize gluten meal	–	12.00	–	–	8.67	11.67
Maize gluten feed	13.00	–	11.53	3.00	–	4.33
Beet pulp, dried	20.00	20.00	15.00	10.00	20.00	10.00
Molasses, cane	5.00	5.00	6.00	6.00	6.00	6.00
Breweries grains, dried	–	16.70	–	–	10.00	27.53
Malt sprouts	8.70	–	–	–	–	–
Citrus pulp, dried	20.00	20.00	25.00	30.00	20.00	30.00
Tapioca meal	4.10	13.80	15.00	0.67	20.00	–
Soya beans	2.40	–	–	–	–	
Soya beans, heat treated	–	2.00	–	–	3.00	2.50
Groundnut meal, solv. extr.	–	–	–	10.00	–	–
Rape seed meal, solv. extr.	–	–	5.00	13.33	–	–
Linseed expeller	15.00	–	–	–	–	–
Soya bean meal, solv. extr.	–	–	–	–	–	6.00
$CaHPO_4$	0.50	1.20	1.17	0.70	1.43	0.67
Salt	0.80	0.80	0.80	0.80	0.80	0.80
Limestone	–	–	–	–	–	–
Vitamins/minerals concentrate	0.50	0.50	0.50	0.50	0.50	0.50
VEM[2], calculated	939	940	937	939	939	939
dcp, calculated, g/kg	124	124	100	170	99	170
Protein solubility[3], %	42	12	48	49	13	11
Protein degradability[4], %	54	15	70	73	33	35
cp, determined, g/kg	160	165	138	200	140	216

[1] b = rapidly degradable protein; B = slowly degradable protein; L = low protein content; H = high protein content.
[2] Dutch feed unit net energy lactation (van Es, 1978).
[3] In mineral buffer solution according to Crooker et al. (1978).
[4] After 12 h incubation in nylon bag in rumen.

The urea in the blood plasma was determined according to the enzymatic colorimetric method of Boehringer Mannheim GmbH Diagnostica.

To determine the urea content in milk, the sample was warmed to 40 °C and thoroughly homogenized, after which 10 ml was centrifuged at 7000 rpm. Then 1 ml of the homogenized liquid was deproteinized with 10 ml URAC solution (Boehringer Mannheim) and 0.2 ml of the deproteinized liquid used for the urea determination, in the same manner as in the blood plasma.

Insulin was determined with the aid of a radio-immuno assay.*

* We are indebted to Dr G. J. Garssen of the Research Institute for Animal Husbandry, Zeist, Netherlands, for carrying out this determination.

Amino acids were determined in deproteinized plasma with the aid of HPLC equipment (Waters). The separation of the amino acids was carried out on an ion-exchange column. After separation the amino acids were derivatized with o-phtalic aldehyde and detected with a fluorescence detector. Norleucine was used as external standard.

During the statistical evaluation of the results, only the effect of the degradability of the concentrate protein was taken into account (B vs. b). In trial RM-B22 it had been our intention to create a difference in dcp (digestible crude protein) supply of 100 vs. 115 %, but the actual difference in dcp supply in the 6th week of lactation proved to be smaller at 107 vs. 116 %. This difference did not significantly affect any of the above-mentioned parameters, and there was no interaction with the degradability of the concentrate protein.

Results

Non-esterified fatty acids

The concentration of the non-esterified fatty acids (NEFA) was determined in blood which was taken immediately before the morning feed. Table 3 shows the results. There was wide variation among the individual animals. With the exception of trial RM-B22 the concentration of NEFA tended to be lower on treatment B. In trial RM-B22 the energy supply at the time of blood sampling, which was subsequently calculated, proved to be 5 % lower on treatment B, namely 99 % of standard vs. 104 % of standard for treatment b. This difference was highly significant. In the trials RM-B20 and RM-B21 the differences were 96 vs. 99 % and 100 vs. 102 % respectively. These differences were not significant.

Insulin

Table 4 shows the average values of the insulin concentration immediately before the morning feed and 3.5 hours later (trial RM-B21). Before the morning feed the concentration was somewhat higher on treatment B; 3.5 hours later, treatment b showed a slightly higher level. After a feed the insulin concentration increased on both treatments, but on treatment b the increase was 50 % higher. Due to the large range of individual values, none of these differences was statistically significant. The negative correlation between insulin and NEFA concentration just before a feed was negligible.

Ketone bodies

The concentration of ketone bodies is given in Table 5. The results of trial RM-B20 show that the ketone bodies in the blood immediately before the morning feed consisted largely of 3-hydroxybutyric acid. In trial RM-B21, in which ketone bodies were determined after a feed, the concentration was clearly higher than in trial RM-B20, where measurements were carried out in blood taken directly before the feed. As the production of volatile fatty acids in the rumen increases after a feed, it is to be expected that the concentration of 3-hydroxybutyric acid in the blood will also show an increase.

Table 3. Concentration of NEFA in blood plasma (μmol/l) shortly before the morning feeding. b: rapidly degradable protein; B: slowly degradable protein.

Trial	Lactation period (wk)	Concentration		Significance of difference
		b	B	
RM-B20	7	440	311	$P < 0.01$
RM-B21	9	338	262	$P < 0.07$
RM-B22	6	670	670	n.s.

Table 4. Concentration of insulin in blood plasma (in mU/l) before and after the morning feeding (Trial RM-B21). b: rapidly degradable protein; B: slowly degradable protein.

Time of sampling	Concentration		Significance of difference
	b ($n = 36$)	B ($n = 36$)	
Before feeding	9.71	11.24	n.s.
3.5 h after feeding	16.40	15.51	n.s.
Difference	6.69	4.27	n.s.

Table 5. Concentration of total ketone bodies and 3-hydroxybutyric acid in blood before and after the morning feeding (expressed in mmol acetone/l blood). b: rapidly degradable protein; B: slowly degradable protein.

Trial	Lactation period (wk)	Time of sampling	Concentration		Significance of difference
			b	B	
RM-B20					
Total ketone bodies	7	before feeding	0.28	0.31	n.s.
3-hydroxybutyric acid			0.24	0.27	n.s.
RM-B21					
Total ketone bodies	9	3.5 h after feeding	1.08	0.92	n.s.

Urea

In trial RM-B20 the urea concentration was only determined in blood taken before the morning feed. On treatment b the concentration was 4.02 mmol/l and on treatment B 5.00 mmol/l. The difference was significant ($P < 0.01$). In the subsequent trials the urea concentration was measured in blood samples taken before and after the feed. The results of these observations are given in Fig. 1. The urea concentration 3 or 3.5 hours after the feed was markedly higher than before the feed. In trial RM-B22 the difference 4.5 hours after the feed was much smaller. In all trials the average urea concentration was significantly higher on treatment B than on treatment b. In trial RM-B20 more urea was found in the milk on treatment B than on treatment b, namely 133 vs. 117 mg urea per 100 ml. This difference was highly significant. The urea content in the milk showed a strong positive correlation with that in the blood ($r = 0.718$).

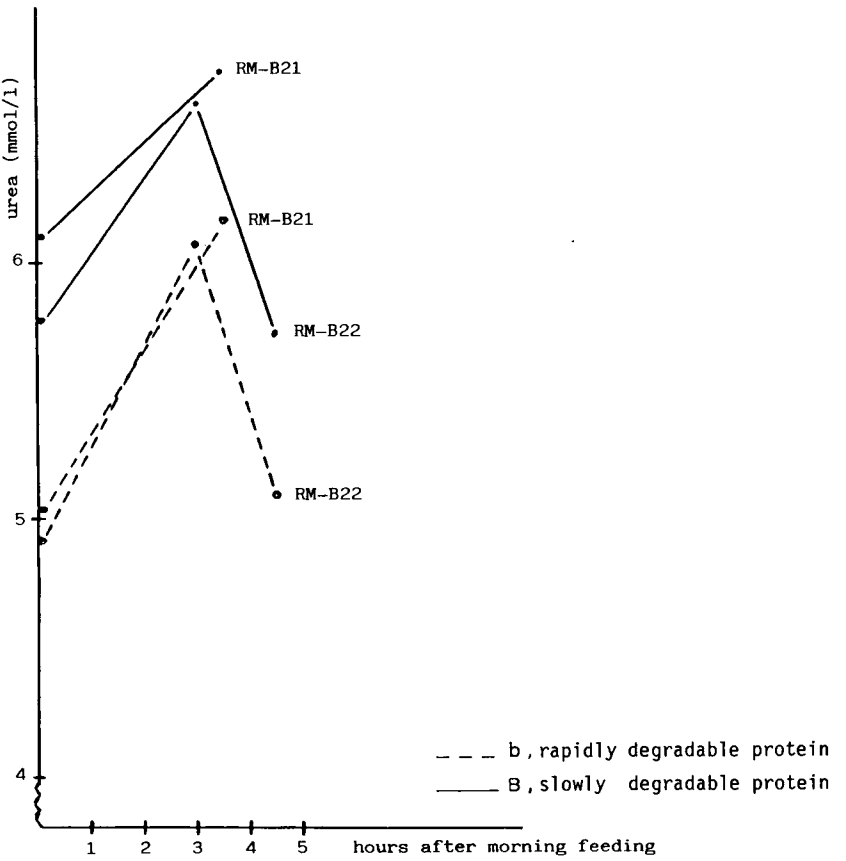

Fig. 1. Concentration of urea in blood plasma (2 trials).

Amino acids

The results are given in Table 6. The determination of proline and histidine presented a number of problems, and the content of these amino acids has not been included here. Only for the concentration of leucine the overall difference between treatment B and b was statistically significant (B > b; $P < 0.05$). The concentration of the non-essential amino acids together was significantly higher on treatment b as compared to B ($P < 0.05$). The differences in the concentrations of the individual amino acids before and after feeding were extremely small. The concentration of

Table 6. Concentration of amino acids (μmol/l) in blood plasma before and 4.5 h after the morning feeding (Trial RM-B22). b: rapidly degradable protein; B: slowly degradable protein.

Amino acids	Before feeding			After feeding		
	Concentration		Significance of difference	Concentration		Significance of difference
	b (mean ± s.d.)	B (mean ± s.d.)		b (mean ± s.d.)	B (mean ± s.d.)	
Threonine	242 ± 76	240 ± 73		252 ± 58	246 ± 82	
Valine	245 ± 64	250 ± 54		265 ± 63	271 ± 76	
Methionine	18 ± 5	20 ± 7		20 ± 6	18 ± 6	
Isoleucine	109 ± 26	112 ± 25		143 ± 37	126 ± 28	$P < 0.05$
Leucine	134 ± 30	194 ± 44	$P < 0.05$	162 ± 43	216 ± 48	$P < 0.05$
Phenylalanine	44 ± 10	49 ± 10		50 ± 11	51 ± 10	
Tryptophan	75 ± 31	80 ± 37		79 ± 34	75 ± 32	
Lysine	83 ± 20	77 ± 21		94 ± 28	78 ± 25	$P < 0.05$
Arginine	82 ± 28	75 ± 21		97 ± 30	76 ± 30	$P < 0.05$
Sum of essential amino acids	974 ± 162	1056 ± 164		1123 ± 224	1089 ± 203	
Aspartic acid	11 ± 5	12 ± 6		14 ± 6	13 ± 6	
Serine	117 ± 32	110 ± 32		113 ± 27	101 ± 28	
Glutamic acid	73 ± 22	68 ± 21		85 ± 23	75 ± 20	
Glycine	375 ± 108	283 ± 70	$P < 0.05$	315 ± 95	245 ± 55	$P < 0.05$
Alanine	261 ± 87	233 ± 67		222 ± 48	214 ± 64	
Tyrosine	48 ± 14	54 ± 14		61 ± 15	63 ± 15	
Sum of non-essential amino-acids	851 ± 183	751 ± 143	$P < 0.05$	811 ± 152	717 ± 146	$P < 0.05$

the essential amino acids after a feed increased slightly more on treatment b than on treatment B. For the essential amino acids as a whole this difference was significant ($P < 0.05$). As regards the non-essential amino acids, both treatments produced a slight decrease in the concentration of serine, and a more marked decrease in the concentration of glycine and alanine. The concentration of the latter amino acids declined more sharply on treatment b.

Discussion

The choice of blood parameters and of the time of sampling in each trial was based on the results of the preceding trial(s) and data from the literature available at that moment. This caused differences in the determinations of blood parameters between the trials. These differences limited the possibilities of interpretation of the results. The results gave rise to the design of another trial with only a few cows in which the post-prandial time-course of concentration was studied (Veen et al., 1988).

All the blood parameters measured showed a wide range of individual values, so that it is not possible to indicate more than a number of tendencies. The concentration of NEFA showed a particularly wide range of values. The difference in the level registered during trials RM-B20 and B21 on the one hand, and RM-B22 on the other hand was probably due to differing methods of determination. The concentration of NEFA in the blood just before the morning feed tended to be lower on a ration with slowly degradable concentrate protein. This difference was not observed during trial RM-B22, but there the energy supply was significantly lower on treatment B. In another experiment (Veen et al., 1988) we noted a lower NEFA concentration on treatment B at 2, 3 and 4 hours after the feed, which may point to a relatively better energy supply. The higher milk fat content which we observed on treatment B is presumably not due to a higher utilization of adipose fat. In that case we could expect a higher concentration of NEFA on the blood on treatment B.

The insulin concentration was higher 3.5 hours after a feed than directly before. At the time of blood sampling the insulin concentration was probably at or near its highest value (Veen et al., 1988), although other researchers found that this concentration peak occurred somewhat later (Evans et al., 1975; Trenkle, 1972). The higher insulin concentration on a ration with rapidly degradable concentrate protein is probably connected with the peak in the concentration of volatile fatty acids in the rumen which occurs approximately 2 hours after a feed (Evans et al., 1975; Veen, 1986). When the absorption of amino acids commences, this may also increase the concentration of insulin.

The treatments had no significant effect on the concentration of ketone bodies, either directly before the feed (trial RM-B20) or 3.5 hours afterwards (trial RM-B21). On both treatments the concentration of ketone bodies is probably determined both by the production of butyric acid in the rumen and by oxidation of fat in the liver. This may explain why in the two trials in which the concentrations of NEFA and ketone bodies in the blood were determined there was no correlation between these parameters.

Contrary to expectations, the concentrate with slowly degradable protein resulted in the highest urea concentration in the blood, both directly before the morning feed and several hours later (Fig. 1). Nevertheless, the larger increases in plasma urea concentration after feeding for treatment b than for treatment B suggested that plasma urea concentration was affected by rumen ammonia concentrations. In trial RM-B20 the concentration of urea increased in the first 3.5 hours with 0.65 mmol/l on a ration with slowly degradable concentrate protein, and with 1.1. mmol/l when the ration contained rapidly degradable protein. In trial RM-B22 the increase after 3 hours was 0.23 and 0.57 mmol/l respectively. In trial RM-B22, all the cows were given 11 kg of concentrates and all the heifers 8 kg, including 1 kg in the milking parlour. The treatments displayed no significant differences in the intake of total dry matter, so that there was no significant difference in the intake of grass silage. Thus the intake of grass silage was not a determining factor in the differences in urea concentration in the blood.

Correlations were calculated between urea concentration and average milk production in the week during which blood samples were taken. At $t = 0$, the following correlation coefficients were observed: on treatment b, $r = 0.325$ and on treatment B, $r = 0.50$. After feeding ($t = 4.5$ h), these values were $r = 0.323$ and $r = 0.417$ respectively. All correlations were statistically significant ($P < 0.05$). Thus the closest correlation with the urea content in the blood was recorded when slowly degradable concentrate protein was fed, in particular before the morning feed. When the cows and heifers were ranked according to the degree to which the dcp requirement was met, there proved to be no correlation with the urea content of the blood. Blauwiekel & Kincaid (1986) did record an increase in the urea concentration in the blood when the protein requirement was raised above the standard. From our data it can be concluded that the urea concentration in the blood is determined more by protein catabolism than by the ammonia production in the rumen. However, from the results of a later experiment we concluded that the higher concentration of urea in the blood did not result from catabolism of tissue amino acids (Veen et al., 1988).

In trial RM-B22 the urea concentration after 4.5 hours was almost the same as that directly before the morning feed. Therefore, we may assume that at that moment the influence of gluconeogenesis from amino acids was more of a determining factor for the urea concentration than the ammonia production in the rumen. We determined the amino acid concentration in the blood at that time. The amino acids alanine, serine, glycine, aspartic acid and glutamic acid sometimes act as precursors of glucose synthesis (Kaneko, 1980). For the sum total of these amino acids, we registered a significantly lower concentration on the treatment with slowly degradable protein, both before and after the feed. Champredon et al. (1977) found a connection between gluconeogenesis and a reduced concentration of alanine, serine and aspartic acid, in combination with an increase in urea concentration. Lindsay (1982) found only alanine, glutamine and glutamic acid to be of any importance in glucogenesis from amino acids. Glucose is the most important precursor of lactose (Bickerstaffe et al., 1974). Thus, higher milk production on treatments with slowly degradable protein may be linked to more gluconeogenesis from amino acids. Insulin inhibits gluconeogenesis and stimulates the uptake of glucose by liver, muscles

and fat. The insulin level has no influence on the uptake of glucose by the udder (Brockman & Laarveld, 1986). In trial RM-B21, insulin concentration in the blood 3.5 hours after feeding was slightly lower on treatment B.

When a barley ration was supplemented with more slowly degradable protein as compared to a rapidly degradable protein, Mercer & Miller (1982) found a higher concentration of essential amino acids in the plasma of sheep. The concentration of branched amino acids in particular was found to increase under these circumstances (Kung et al., 1984). We found that only leucine appeared in a significantly higher concentration on treatment B, both before and after a feed (Table 6). However, in an experiment in which the course of the concentration of metabolites in the blood was determined during several hours, we found that in general the individual concentration of the essential amino acids was lower on treatment B than on treatment b (Veen et al., 1988).

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