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Further studies on the effect of fat supplementation of concentrates fed to lactating dairy cows. 3. Effect on rumen fermentation and site of digestion of dietary components

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Summary

Fat-supplemented concentrates were fed to dairy cows equipped with rumen and duodenal cannulae, and the effect on rumen fermentation and site of digestion of dietary components was studied. Treatments were feeding concentrates supplemented with 7 % of fat (treatment C7), supplemented with 12 % of fat (treatment C12) and supplemented with 12 % of fat adsorbed on a carrier (treatment C12C).

Small but significant changes in rumen fermentation characteristics such as pH, total VFA (volatile fatty acids) and NGR (non-glucogenic/glucogenic ratio) were observed with treatments C12 and C12C as compared with treatment C7. Numbers of protozoa were severely reduced with treatments C12 and C12C, but not with treatment C7.

Site of digestion of organic matter and energy was affected by treatments C12 and C12C but this could be attributed entirely to an increased postruminal fat digestion. Total digestion and site of digestion of carbohydrate fractions (crude fibre, neutral detergent fibre, starch, sugars) was not affected by fat supplementation. Recovery of hydrogen in CH_4 actually measured was close to 90 % of that estimated from the rumen fermentation balance. N flow to the small intestine was not affected by replacing part of the carbohydrates by fat, indicating an increased efficiency of microbial protein synthesis in the rumen when fat was increased.

Introduction

Supplementation of dairy diets with fat has still considerable interest. Because of its

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high energy density it might help to fill the gap between intake of energy and energy requirement in early lactation, when dry matter intake and energy intake are often inadequate. However the inclusion of fat in dairy diets may interfere with rumen fermentation (Devendra et al., 1974; Henderson et al., 1977; Kowalczyk et al., 1977; Knight et al., 1978; Rohr et al., 1978; Ikwuegbu & Sutton, 1982), site of digestion (Knight et al., 1978; Ikwuegbu and Sutton, 1982) and energy utilization (Kronfeld, 1976; Brumby et al., 1978).

The effect of fat supplementation was studied in dairy cows equipped with ruminal and duodenal cannulae. With these animals energy balance studies were performed, the results of which were reported in a previous paper (van der Honing et al., 1983). The effect on rumen fermentation and site of digestion of various dietary components is reported in this paper.

Materials and methods

The experiments were performed with 4 lactating dairy cows. All animals were fitted with a rumen cannula and two animals were also fitted with a re-entrant cannula at the beginning of the small intestine just beyond pancreatic and bile duct. Housing, feeding and maintaining the animals was as described in a previous paper (van der Honing et al., 1983). Experimental diets were one third of long meadow hay and two thirds of one of three concentrates supplemented with 7 % of fat (treatment C7), supplemented with 12 % of fat (treatment C12) or supplemented with 12 % of fat adsorbed on a carrier of Palabora vermiculite (treatment C12C). Hay was fed at 6h00 and 15h30; concentrates were offered one hour later. Composition of the concentrate mixtures was reported in a previous paper (van der Honing et al., 1983). Animals with only a rumen cannula received all three treatments, animals also equipped with duodenal cannulae received treatments C12 and C12C only.

Experimental periods were 6 weeks for animals with a rumen cannula only and 7 weeks for the animals with also a duodenal cannula. During weeks 4 and 5 digestion and energy balance studies were performed with all animals, the results of which were reported in a previous paper (van der Honing et al., 1983). In week 6 digestion and duodenal flow measurements were made continuously for 96 hours with the animals fitted with duodenal cannulae, according to the methods described earlier (Tamminga, 1981). In weeks 3 and 6 or 7, extensive measurements were made in the rumen during 1 day. Rumen sampling was at 0, 1, 2, 3, 5 and 7 hours after feeding concentrates in the morning for pH, buffering capacity, ammonia, volatile fatty acids (VFA) and at 4 hours after feeding for protozoa. Rumen samples for pH, NH_3 and VFA were treated and analysed as described by van Vuuren et al. (1983). Samples for counting protozoa were preserved with an equal volume of an aqueous solution containing 50 % of glycerin and 5 % of formalin. After an appropriate dilution varying from 50 to 200-fold with the same solution, protozoa were counted through a microscope in a McMaster counting chamber. In order to measure the rumen fluid dilution rate (D) the animals were dosed with 1 litre of a Cr-EDTA solution (Binnerts et al., 1968) containing 2.8 g of Cr in the rumen at 12h00 and rumen

fluid samples were taken after 3, 4, 5, 6, 9, 18, 20, 22 and 24 hours. Samples were centrifuged at high speed and the remaining clear supernatant was directly injected into the flame of an atomic absorption spectrophotometer for Cr analysis. Buffering capacity was measured by titrating 10 ml of fresh rumen fluid with 0.1 mol/l HCl to pH 4.0 and calculated as the first derivative of the titration curve (Counotte et al., 1979).

Sampling and analysis of feeds, duodenal digesta and faeces was done as described by Tamminga (1981). In addition to the normal fat analysis based on extraction with hexane, samples were also analysed with hexane extraction preceded by hydrolysis with HCl. In addition samples were analysed for carbohydrate fractions: starch and sugars according to the instructions of the Netherlands Normalization Institute (NEN 3574 for starch and NEN 3571 for sugars) and neutral detergent fibre (NDF) according to the methods of Van Soest & Wine (1967).

Results and discussion

Effect on rumen fermentation

Average values of samples taken in the period up to 7 hours post-feeding during 2 days (12 samples) of pH, total volatile fatty acids (VFA), non-glucogenic/glucogenic ratio (NGR) (Ørskov, 1975), NH_3 content, rumen liquor dilution rate (D) and rumen volume are given in Table 1. Differences between treatments were small and became significant only for measurements made during the period between 3 and 7 hours after feeding. Largest differences were found between treatments C7 and C12 in the animals with rumen cannulae only. VFA concentrations and NGR were significantly lower with treatment C12 during this period. Differences between

Table 1. Rumen fermentation characteristics.

	C7	$C12_A$[1]	$C12C_A$[1]	$C12_B$[2]	$C12C_B$[2]
N	24	24	24	24	24
pH	6.1	6.2	6.3	6.2	6.3
total-VFA (mmol/l)	137	121	116	108	110
Acetate (%)	63.8	63.4	63.4	65.5	66.8
Propionate (%)	21.0	22.6	22.2	22.1	21.6
Butyrate (%)	15.1	14.0	14.4	12.4	11.6
NGR[3]	4.2	3.7	3.9	3.6	3.7
$BC_{4.75}$[4] (mmol/l)	78	74	71	69	67
$BC_{6.25}$[5] (mmol/l)	13	9	16	18	15
NH_3 (mmol/l)	16.6	13.8	12.2	15.6	14.8
Liquid dilution rate (h^{-1})	0.164	0.149	0.158	0.090	0.097
Rumen volume (l)	77	93	80	85	75

[1] Cows with rumen cannula only.
[2] Cows with ruminal and duodenal cannulae.
[3] Non-glucogenic/glucogenic ratio.
[4] Buffering capacity: mmol HCl required to shift pH one unit.
[5] If pH $\leq$ 6.25, then BC was assumed zero.

treatments C7 and C12C in the same animals during this period were significant for VFA and pH only in that VFA was lower and pH higher with treatment C12C. No significant differences were found between treatments C12 and C12C for either group of animals. Changes in rumen volume and rumen liquor dilution rate (D) with different treatments were not statistically significant.

The 10 to 15 % decreased total VFA with treatments C12 and C12C as compared with treatment C7 reflects the relative decrease of the amount of substrate available for the microbes (carbohydrates in particular). The supplemented fat which replaced part of the carbohydrates cannot be converted into VFA under the anaerobic conditions in the rumen. The lower total VFA concentrations also explains the slightly higher pH with treatments C12 and C12C. Increasing the amount of added fat from 7 to 12 % shifted the VFA proportions to some more propionate and less butyrate. As a consequence the NGR was decreased, particularly during the period between 3 and 7 hours after feeding. Ruminal ammonia concentrations decreased slightly with increasing the amount of supplemented fat from 7 to 12 %.

Reduction in ruminal ammonia concentrations after supplementing the diet of sheep with fats or oils was also observed by Kowalczyk et al. (1978), Henderson et al. (1977) and Ikwuegbu & Sutton (1982). This reduction has been associated with reduced numbers of protozoa. In Table 2 the numbers of protozoa as found in our experiments are shown. As a comparison protozoal numbers of the same animals receiving the same amount of hay and concentrates without supplemented fat are also included in Table 2. The results show reduced numbers of protozoa with treatments C12 and C12C but not with treatment C7. Henderson et al. (1971) observed in sheep also a dramatic decrease in numbers of protozoa after the addition of 10 % tallow to the diet, but not after the addition of 5 %.

The shift in VFA pattern is also in agreement with observations reported in literature. However the changes observed in our experiments were much less dramatic than those observed in the experiments of Rohr et al. (1978). In their experiments increasing the daily amount of crude fat in the diet of dairy cows from 388 to 973 g by the inclusion of beef tallow in the concentrate part of the diet changed the acetate/propionate/butyrate ratio from 64.3/16.8/18.9 to 62.3/23.7/14.0. Comparing treatment C7 with C12 in our experiments meant an increase in the daily fat consumption from 1482 g to 1998 g and our results are therefore not directly compara-

Table 2. Numbers of protozoa ($\times 10^6$) per litre of rumen fluid

Diet[1]	Holotrichs	Entodinia	Epidinia	Total
Hay, unsupplemented concentrates	20	650	26	696
C7	17	650	46	713
$C12_A$	4	340	9	353
$C12C_A$	5	450	30	485
$C12_B$	7	205	6	218
$C12C_B$	6	270	11	287

[1] For explanation see footnotes to Table 1.

ble with those of Rohr et al. (1978). Palmquist & Conrad (1978) when feeding diets with a low proportion of long roughage (lucerne hay and maize silage) in the diet increased fat intake from 1215 to 2180 g/day. This caused a shift in acetate/propionate/butyrate ratio from 66.2/21.3/12.5 to 64.2/24.3/11.6 which is more in line with our observations.

Changes in buffering capacity between treatments could largely be attributed to changes in VFA concentrations. According to Counotte et al. (1979) VFA concentrations can be estimated from buffering capacity at pH 4.75 and at pH 6.25. Estimated values averaged 104 ± 1.8 % of the measured values in our experiments (n = 120). Individual variation was still rather high and the recovery (measured values as percentage of estimated values) showed a coefficient of variation of 20 %. Recovery was slightly lower but coefficient of variation considerably higher than in the experiments reported by Counotte et al. (1979).

From these results it must be concluded that the adsorption of fat on a carrier had only a marginal effect with respect to preventing the interference of fat with rumen fermentation. The latter effect was however small.

Digestion of dietary components

Figures on total digestion and site of digestion in animals fitted with duodenal cannulae, after receiving treatment C12 or C12C are given in Table 3. As a comparison

Table 3. Site of digestion of dietary components.

Component[1]		$C12_B$[2]	$C12C_B$	Mean	Unsuppl.
I_O	(kg)	12.2	10.7	11.2	11.5
d_O		74	76	75	80
$d_{O,S}$		45	49	48	56
I_E	(MJ)	264	229	241	227
d_E		70	72	72	76
$d_{E,S}$		25	29	28	43
I_{XF}	(kg)	1.8	1.7	1.7	1.9
d_{XF}		69	74	73	72
$d_{XF,S}$		92	90	91	80
I_{XX}	(kg)	6.0 (6.0)[3]	5.4 (5.4)	5.6 (5.6)	6.8
d_{XX}		74 (79)	77 (82)	76 (81)	86
$d_{XX,S}$		64 (70)	69 (72)	67 (72)	84
I_{XL}	(kg)	1.4 (1.4)	1.2 (1.3)	1.3 (1.3)	0.4
d_{XL}		87 (64)	88 (64)	88 (64)	63
$d_{XL,S}$		−8 (−59)	−1 (−36)	−3 (−43)	−218
I_{XP}	(kg)	2.9	2.4	2.6	2.4
d_{XP}		70	71	71	73
$d_{XP,S}$		7	1	3	−16

[1] I = intake (kg/day); d_X = apparent digestibility of fraction X (% of I); $d_{X,S}$ = apparent digestibility in stomachs (% of d_X). Suffixes: O = organic matter; XP = crude protein; XL = crude fat; XF = crude fibre; XX = N-free extractives; E = energy (MF).

[2] For explanation see footnotes to table 1.

[3] Figures in parentheses refer to results if fat analysis was based on extraction with hexane preceded by hydrolysis with HCl.

data from a previous experiment with 3 animals fed diets of comparable composition at the same level of intake except that no supplementary fat was given, are also included in Table 3. With respect to the digestion of crude fat two analytical procedures were followed. Figures refer to crude fat analysis based on extraction with hexane and the figures in brackets refer to extraction with hexane preceded by hydrolysis with HCl. As can be seen from the figures the effect of HCl hydrolysis is considerable.

The results on overall digestibility obtained in a 4-day collection period agreed reasonably well with those obtained in the same animals in the 10-day collection period during the energy balance studies (van der Honing et al., 1982) in the weeks prior to the duodenal flow measurements.

No differences could be observed with respect to total digestion and site of digestion between treatments C12 and C12C. When comparing the pooled results of fat supplemented diets (C12 and C12C) with the results of unsupplemented diets some striking differences become apparent. First of all a shift in the site of digestion of organic matter and an even larger shift in the site of energy digestion. Similar observations were made by Knight et al. (1978) and Ikwuegbu & Sutton (1982) in sheep. However in our experiments this shift could entirely be explained by the replacement of carbohydrates by fat, the latter of which cannot be degraded in the rumen other than hydrolysed to free fatty acids. Surprisingly enough no reduction in crude fibre digestion or site of digestion was found. Reduced crude fibre digestion after fat supplementation has been observed repeatedly, both in sheep (Buysse, 1962; Dijkstra, 1969; Sundstol, 1974) and cows (Rohr et al., 1978). No apparent reason can be found for the absence of an effect of fat supplementation on total digestion and partial digestion in the forestomachs in our experiments. The high digestibility of the crude fibre might be a reason for the absence of a fat effect. Total apparent digestion of fibre was high indeed in our experiments: 0.75 in sheep for fibre in both hay and concentrates without supplemented fat (van der Honing et al., 1983). This could however hardly explain the absence of an effect of fat. Fibre digestion in the experiments of Rohr et al. (1978) was considerably lower, but in the experiments of Palmquist & Conrad (1978) no effect was observed either and apparent cellulose digestion in their experiment was low and in the order of 0.50.

The apparent reduction and shift in the digestion of NfE (XX) could be explained as an artefact. Fat analysis based on hexane extraction only, severely underestimated duodenal and faecal fat content. If fat analysis was based on hexane extraction preceded by HCl hydrolysis the apparent differences disappeared. As a consequence of the latter analytical procedure apparent digestion of fat decreased dramatically from 0.88 to 0.64 with a concomitant rise in the digestion of NfE.

Digestion of carbohydrate fractions and fermentation balance

In Table 4 total apparent digestion (d_X) and partial digestion in the stomachs ($d_{X,S}$) of various carbohydrate fractions with treatments C12 and C12C is shown. These pooled results are very similar to those obtained in other experiments with dairy cows if no supplemental fat was fed (Tamminga et al., 1982). The residual fraction (R) increased between mouth and duodenum with an average of 165 g, resulting in

Table 4. Site of digestion of carbohydrate fractions.

Fraction	I_X[1] (kg/day)	d_X (% of I_X)	$d_{X,S}$ (% of d_X)
Carbohydrates[2]	7.3	79	75
NDF	4.0	76	87
Starch	0.8	97	82
Soluble sugars	1.2	99	100
Residue[3]	1.3	57	–23

[1] For explanation see footnote 1 to Table 3.
[2] Carbohydrates = OM – XP – XL; XL analysis based on extraction with hexane preceded by hydrolysis with HCl.
[3] Residue = carbohydrate – (NDF + starch + sugars).

a negative $d_{R,S}$. This fraction contains all analytical errors. Part of the dietary carbohydrates may become converted to microbial carbohydrates of which it is uncertain if they are included in one of the other fractions. It is also questionable if the crude protein entering the small intestine contains 16 % of N (the basis of crude protein equals N × 6.25), because of the presence of N in nucleic acids and some N in ammonia. So not too much emphasis should be placed on the increase of this residual fraction. Our observations in dairy cows are very much in line with observations made in sheep in similar experiments (Armstrong & Smithard, 1979).

By accepting a number of assumptions an attempt can be made to calculate a stoichiometric fermentation balance. In the following calculations it was assumed that:
- CH_4 originates exclusively from the fermentation of carbohydrates with a chemical composition of $(C_6H_{10}O_5)_n$;
- VFA proportions present in the rumen reflect the proportions in which they are produced.

Both assumptions are open to criticism as it is known that, for instance, the fermentation of protein may also yield CH_4 (Demeijer & van Nevel, 1979), that in microbial growth some surplus [H] resulting from rumen fermentation may become incorporated (Demeijer & van Nevel, 1975) and that the VFA proportions present in the rumen with concentrate rich diets not always accurately reflect the proportions in which they are produced (Sutton & Morant, 1978).

If it is now assumed that the carbohydrates fermented in the forestomachs are entirely converted to VFA in the proportions present in the rumen it becomes possible to estimate the amount of CH_4 produced from the equation (Tamminga, 1976):

$$CH_4 = \frac{(4a - 2b + 4c)}{162 \times 8(0.5a + 0.5b + c)} \times 22.4 \text{ l/g carbohydrates fermented, in which}$$

a, b and c represent the proportions in which acetate, propionate and butyrate are present in VFA. The results of such calculations can than be compared with actually measured quantities of CH_4 and recoveries estimated. The result of this exercise is

Table 5. Fermentation balance in the rumen of dairy cows fed with fat supplemented diets.

Cow	Treatment	Carbohydrates fermented (kg/day)	HAc:HP:HB (mol/100 mol)	CH_4 produced (l/day)		
				calc.	measured	fraction recovered
A	C12	4.47	65:22:13	367	360	0.98
A	C12C	4.62	68:21:14	395	360	0.91
B	C12C	3.84	66:22:12	299	278	0.93

shown in Table 5. Recoveries are surprisingly high. It must be stressed however that between 10 and 20 % of the structural carbohydrates (NDF) were digested postruminally. Besides some incorporation of non-structural carbohydrates in microbial mass may take place. Such microbial carbohydrates are mainly α-linked polymers which are believed to be hydrolysed in and absorbed from the small intestine and therefore not to contribute to VFA and CH_4 resulting from hindgut fermentation. However, CH_4 resulting from hindgut fermentation of structural carbohydrates would not be accounted for in the calculated CH_4 production, but would be part of the measured CH_4 production. NDF digested postruminally accounted for 9 % of the total carbohydrates fermented in the rumen, therefore recoveries would be overestimated by some 10 %. After correction for this the recoveries are very close to the recoveries obtained with incubations in vitro (Demeijer, 1976).

N flow to the small intestine

Extremely high values for the efficiency of microbial protein production after the inclusion of linseed oil (Knight et al., 1978; Ikwuegbu & Sutton, 1982) or beef tallow (Knight et al., 1978) in the rumen of sheep have been reported, resulting in an elevated N flow to the small intestine. In our experiments carbohydrates were replaced by rather than supplemented with fat, which would normally have to result in a decreased flow of microbial and total N to the small intestine. In Table 6 intestinal total N flow after feeding fat supplemented diets is related to a number of dietary components and compared with results of previous experiments with the same animals but fed diets without supplemented fat. The results show a quite 'normal' intestinal N flow if related to ingested digestible organic matter (DOM). If the intestinal N flow is however related to the amount of carbohydrates fermented in

Table 6. Total N flow to small intestine in dairy cows as related to organic matter apparently digested (DOM) in the total tract and carbohydrates fermented in the rumen (carb. F) and absorbed N as related to apparently digested energy (DE).

Diet	*n*	Duodenal flow		Absorbed N/DE (g N/MJ)
		g N/kg DOM	g N/kg $carb._F$	
No fat added	8	48 ± 1.5	76 ± 2.6	1.74 ± 0.058
12 % fat added	3	48 ± 2.1	94 ± 5.6	1.66 ± 0.058

the forestomachs rather than to DOM digested in the total tract duodenal N flow is over 20 % higher after feeding fat supplemented diets than after feeding unsupplemented diets. This observation seems to confirm the higher efficiency of microbial protein synthesis with high-fat diets, reported in literature (Knight et al., 1978; Ikwuegbu & Sutton, 1982), presumably due to a specific inhibiting effect of fat on protozoa (Demeijer, 1981).

Fat supplementation results in a higher energy density of the absorbed nutrients, which also may have a negative influence on the ratio absorbed protein to absorbed energy. However the decrease in this ratio after feeding fat supplemented diets was only marginal and by no means statistically significant.

Conclusions

Fat supplementation of dairy diets does interfere with rumen fermentation, but not always as severely as suggested in literature.

Numbers of protozoa become severely reduced when high levels of fat are included.

Interference by fat supplementation of total and partial digestion in the forestomachs of structural carbohydrates can be avoided, even with high levels of supplemented fat, but the reason why is not clear.

The expected decrease in the ratio absorbed protein to absorbed energy after feeding fat supplemented diets to dairy cows is largely compensated for by relatively more protein leaving the forestomachs presumably as result of a more efficient microbial protein synthesis.

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