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The influence of supplemental fat on rumen volatile fatty acid profile, ammonia and pH levels in sheep fed a standard diet*

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ABSTRACT

The effect of different types of fat supplementation on basic rumen parameters was estimated on three milking sheep fitted with rumen cannulae in a 3x3 Latin square design consisting of three experimental diets differing in the percentage of fat supplement. The basic ration for the control group consisted of meadow hay and concentrate (60:40) and was supplemented for experimental groups with rape seed oil, soyabean oil, linseed oil, tallow, Bergafat® or fish oil at a level of 0 (control), 4, and 6% in dry matter. Samples of rumen fluid were analyzed for volatile fatty acids, ammonia ($N-NH_3$) and pH. The acetic acid level increased significantly ($P<0.05$) when 4 and 6% of soyabean oil was added to the ration. Similarly, addition of linseed oil increased ($P<0.05$) the level of acetic acid in experimental groups. A significant ($P<0.05$) increase in the acetic acid level was observed when 6% Bergafat® was added, whereas the level of this acid was reduced when 6% fish oil was added to the diet. The butyric acid level decreased significantly ($P<0.05$) when the diet contained 6% linseed oil, and there was a decrease ($P<0.05$) in isobutyric acid in both groups receiving fish oil. A slight but statistically significant ($P<0.05$) increase in the isobutyric acid level when the diet was supplemented with 4% soyabean oil was also observed. The addition of rape seed oil and tallow

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had no influence on fatty acid levels in the experimental groups. There were no main treatment effects on daily mean pH and ammonia concentration in the rumen. With all fat additives, except for fish oil, the ruminal N-NH_3 concentration was lower in both experimental groups, but the differences were not significant. Only the addition of 4% fish oil to the ration was accompanied by a significant ($P<0.05$) increase in the ammonia level.

KEY WORDS: volatile fatty acids, ammonia, pH, rumen, fat, sheep

INTRODUCTION

Carbohydrates make up the major proportion of the diet for ruminants. The principal end products of their fermentation are volatile fatty acids, which are used by animals as a source of energy for life processes and bacterial protein production in the rumen. Unfortunately, in the case of high-producing ruminants it is difficult to reach the required energy level in the diet without adding fat. Consumption of lipids by herbivorous animals is low because most forages contain only limited amounts of fats. On the other hand, the rumen microbial population is intolerant to high dietary fat levels. When fat is added to the diet, it is fed normally at no more than 5 to 7% of the total diet DM. Higher levels are apt to result in abnormal rumen fermentation unless the fat is protected from microorganisms by coating lipid droplets with casein, and then treating the complex with formaldehyde (Pond et al., 1995) or the fat is administered as fatty acid calcium salts (Kowalski, 1997; Brzóska et al., 1999).

The effect of added fat on basic rumen parameters seems to be dependent on the fatty acid composition of the supplemented fat. Unsaturated fatty acids appear to be more toxic to ruminal microbes than saturated fatty acids (Sklan et al., 1984). This study was designed to determine whether diets supplemented with different types of plant and animal fats affect rumen metabolites in sheep.

MATERIAL AND METHODS

Animals and diets

The experiments were carried out in a 3 x 3 Latin square design on 3 milking ewes (50±5 kg) fitted with permanent rumen cannulas to determine the effects of fat source on basic rumen parameters. The basic ration for the control group consisted of meadow hay and concentrate (60:40), which was supplemented for the experimental groups with rape seed oil (RSO), linseed oil (LSO), soyabean oil (SBO), fish oil (FIO), tallow (TAL) or Bergafat® (BER) at a level of 0 (control), 4 and 6% in dry matter of the diet. The concentration of fatty acids in the used fats is presented in Table 1.

TABLE 1

Fatty acid concentration in fats used in experiments, %

Fatty acids	RSO[1]	SBO[2]	LSO[3]	TAL[4]	BER[*,5]	FIO[6]
C 14:0	-	-	-	7	3	9.23
C 15:0	-	-	-	-	-	0.89
C 16:0	5.11	10.84	5.03	29	47-53	25.23
C 17:0	-	-	-	-	-	1.66
C 18:0	1.84	3.77	3.43	21	39-44	3.75
C 18:1	60.23	25.75	16.45	41	6	32.27
C 18:2	20.55	50.47	15.14	2	-	6.54
C 18:3	10.35	8.18	59.66	-	-	5.22
C 20:0	0.95	0.51	0.29	-		7.02
C 20:1	-	0.41	-	-	1	-
C 20:2	0.42	0.07	-	-		8.19
C 20:3	0.55	-	-	-		-

[1] rape seed oil; [2] soyabean oil; [3] linseed oil; [4] tallow; [5] Bergafat®, fatty acid content depends on the part of Bergafat preparation; [6] fish oil

The energy value of the rations was, depending on fat level in the ration, from 0.89 to 1.00 UFL, whereas the crude protein content was from 97 to 90 PDIN and PDIE. The ingredients and nutritional value of the diets are presented in Table 2. The daily ration was divided into two equal portions and fed at 8.00 and 18.00 h. Water was continuously available.

TABLE 2

Diets composition and nutritive values of diets

Ingredients	Level of added fat, %		
	0	4	6
Meadow hay	59.0	57.0	55.5
Wheat meal	21.0	20.0	19.5
Rapeseed meal	3.0	3.0	3.5
Wheat bran	15.0	14.0	13.5
Mineral additive, Polfamix OK	2.0	2.0	2.0
Nutritive value			
UFL	0.89	0.96	1.00
PDIN	97	93	90
PDIE	97	93	90

Sampling and analysis

The experiments consisted of three 16-day trials, 14 days for adaptation to the diet and the last two for sample collection. Samples of rumen fluid were collected before the morning feeding and 3 and 6 h after feeding (Grummer et al., 1993). pH was measured potentiometrically. Quantitative analysis of ammonia was carried out by a modification of Nessler's method. After the colour complex of ammonia from rumen fluid and Nesslers's reagent was formed, sample absorption was monitored using a spectrophotometer (absorbency to 400 nm). The ammonium quantity was calculated by the use of the following equation:

$$C = A \text{ mean} \times k \text{ mean} \times 14.70 \text{ (mmol/l)}$$

where:
A - mean absorption of samples
k - mean factor
14.70 - value including dilution and converted into moles.

Individual fatty acids were detected using the Tangerman and Negengast (1996) method.

Statistical analysis

All data were analyzed using SAS procedures (User's Guide, 1990).

RESULTS

The pattern of volatile fatty acids (VFAs) in the rumen fluid from all experimental groups tended towards more acetic acid. When soyabean, linseed, fish oils and also Bergafat® were added to the sheep ration, the ruminal level of acetic acid was significantly higher ($P<0.05$) than in control groups except of the group fed diet with rape seed oil (Table 3) where the increase was not significant ($P>0.05$).

TABLE 3
Effect of rape seed oil on concentration of individual VFA in the rumen of sheep, mmol/L

Acid	Fat addition to the diet, %					
	0		4		6	
	mean	CV	mean	CV	mean	CV
Acetic	70.25	20.04	73.43	20.92	73.76	20.84
Propionic	20.35	31.24	21.98	28.19	22.65	27.47
Butyric	11.34	41.04	12.34	37.21	12.40	29.80
Isobutyric	1.23	21.72	1.57	19.98	1.75	23.81
Valeric	2.15	49.04	1.45	47.25	1.68	42.91
Isovaleric	1.98	20.34	1.56	27.19	1.03	32.64

means in rows with the same letter are not significantly different [a,b]-$P<0.05$; [A,B]-$P<0.01$

Increasing the fat supplements to the diet was accompanied by an increase in the acetic acid level. The acetic acid level increased significantly (P<0.05) from 52.25 mmol/L in the control group to 68.70 and 61.03 mmol/L, respectively when 4 and 6% of soyabean oil was added to the ration (Table 4). Similarly, linseed oil increased (P<0.05) the level of acetic acid from 58.89 mmol/L in the control group to 68.19 and 67.53, respectively in the experimental groups (Table 5). Changes

TABLE 4

Effect of soyabean oil on concentration of individual VFA in the rumen of sheep, mmol/L

Acid	Fat addition to the diet, %					
	0		4		6	
	mean	CV	mean	CV	mean	CV
Acetic	52.25[a]	21.48	68.70[b]	20.19	61.03[b]	20.29
Propionic	19.44	32.72	21.26	23.84	19.89	30.40
Butyric	11.02[a]	38.09	11.65[a]	42.20	9.08[b]	27.18
Isobutyric	1.97[ab]	13.75	2.35[a]	17.23	1.51[b]	19.07
Valeric	1.62	39.78	2.49	41.02	1.52	38.38
Isovaleric	1.67	19.42	1.66	20.23	1.48	24.02

means in rows with the same letter are not significantly different [a,b]-P<0.05

TABLE 5

Effect of linseed oil on concentration of individual VFA in the rumen of sheep, mmol/L

Acid	Fat addition to the diet, %					
	0		4		6	
	mean	CV	mean	CV	mean	CV
Acetic	58.89[a]	21.72	68.19[b]	20.38	67.53[b]	20.51
Propionic	16.92	27.78	22.21	35.07	20.59	29.11
Butyric	10.06	30.39	10.88	28.72	13.84	34.90
Isobutyric	1.82	17.28	1.45	20.04	1.89	25.29
Valeric	1.33	42.27	1.80	49.78	1.07	42.28
Isovaleric	1.28	17.87	1.54	21.21	1.19	29.00

means in rows with the same letter are not significantly different [a,b]-P<0.05

in individual fatty acids in the rumen of sheep after feeding the ration with tallow (Table 6) were not significant (P>0.05) but a significant (P<0.05) increase in the acetic acid level was observed when 6% Bergafat® was added to the ration (Table 7). The level of this acid declined when 6% fish oil was fed with the diet (Table 8). Supplementation of the diets with fish oil modified the level of butyric and isobutyric acids. The butyric acid concentration decreased significantly (P<0.05) from 15.94 in the control group to 10.43 mmol/L in the rumen of animals fed the diet supplemented with 6% linseed oil, whereas a decrease (P<0.05) in isobutyric acid in both groups receiving fish oil and soyabean oil was found. A slight but significant (P<0.05) increase of isobutyric acid was observed when the diet contained 4% soyabean oil.

TABLE 6

Effect of tallow on concentration of individual VFA in the rumen of sheep, mmol/L

Acid	Fat addition to the diet, %					
	0		4		6	
	mean	CV	mean	CV	mean	CV
Acetic	75.56	19.12	74.00	19.87	77.02	19.04
Propionic	26.71	24.17	28.39	31.83	27.77	30.91
Butyric	10.57	19.89	14.29	23.71	13.15	29.87
Isobutyric	0.98	20.04	1.87	25.79	1.31	19.34
Valeric	1.85	49.17	1.37	51.07	1.32	50.38
Isovaleric	1.94	19.14	1.17	22.72	2.13	20.52

TABLE 7

Effect of Bergafat® on concentration of individual VFA in the rumen of sheep, mmol/L

Acid	Fat addition to the diet, %					
	0		4		6	
	mean	CV	mean	CV	mean	CV
Acetic	60.82[a]	19.82	64.86[a]	21.45	68.32[b]	21.82
Propionic	21.79	28.34	25.00	30.92	22.62	35.70
Butyric	10.07	29.02	12.00	37.43	13.01	31.98
Isobutyric	1.23	20.87	1.37	24.38	1.93	22.45
Valeric	1.31	49.38	1.52	57.08	0.79	55.19
Isovaleric	1.34	29.01	1.21	24.35	1.23	27.34

means in rows with the same letter are not significantly different [a,b]-P<0.05

TABLE 8

Effect of fish oil on concentration of individual VFA in the rumen of sheep, mmol/L

Acid	Fat addition to the diet, %					
	0		4		6	
	mean	CV	mean	CV	mean	CV
Acetic	72.93[a]	19.23	73.31[a]	19.78	65.88[b]	20.34
Propionic	19.07	23.42	18.32	29.54	17.76	31.19
Butyric	15.94[a]	17.34	16.43[a]	38.23	10.43[b]	30.32
Isobutyric	1.53[a]	18.19	0.85[b]	21.93	0.60[b]	24.72
Valeric	1.88	48.32	2.07	42.73	1.70	47.04
Isovaleric	1.79	19.72	1.20	21.23	1.08	27.04

means in rows with the same letter are not significantly different [a,b]-P<0.05

Addition of rape seed oil, a source of C 18:1, or tallow, a source of saturated fatty acids and also of about 40% C 18:1, had no influence on the fatty acid level in experimental groups (Tables 3 and 6). Soyabean and linseed oils, potential supporters of C 18:2 and C 18:3 in ruminal biohydrogenation slightly affected fermentation in the rumen (Tables 4 and 5). No significant changes in fatty acids were observed when Bergafat® (a source of saturated fatty acids) was added to the diet, except an increase in acetic acid when the diet containing 6% Bergafat® was fed.

There were no main treatment effects on daily mean pH and concentration of ammonia in the rumen (Table 9). With all fat additives, except fish oil, the N-NH_3 concentration in the rumen was lower in both experimental groups, but the differences were not significant. Only the addition of 4% fish oil to the sheep ration was accompanied by a significant ($P<0.05$) increase in the ammonia level, from 21.32 mmol/L in the control group to 27.25 mmol/L in the experimental group.

TABLE 9

Concentration of rumen ammonia (mmol/L) and mean rumen pH in sheep fed diets with different source of fat supplementation

Source of fat[1]	Rumen parameters					
	N-NH_3			pH		
	Level of added fat, %					
	0	4	6	0	4	6
RSO	10.12	9.78	7.03	6.90	6.72	6.53
SBO	29.07	22.54	16.05	6.54	6.16	6.48
LSO	12.95	12.03	11.25	7.52	6.42	6.65
TAL	13.11	9.16	10.83	7.71	7.04	6.88
BER	12.32	10.51	11.27	7.99	7.18	7.78
FIO	21.32[a]	27.25[b]	22.53[a]	6.60	6.41	6.54

means in rows with the same letter are not significantly different [a,b]-$P<0.05$

[1] explanation see Table 1

DISCUSSION

The effect of fat supplementation of ruminants' diets on rumen metabolism has been tested extensively for years. Devendra and Lewis (1974) outlined some principles of formulating ruminants' diets to avoid the negative influence of added fat on rumen parameters, mostly on microbial synthesis. Kowalczyk et al. (1977) found that the addition of more than 5% of tallow to diets for ruminants alters the nitrogen metabolism in the rumen, decreases feed intake and structural carbohydrate digestion. Schauff et al. (1992) reported that ruminal fermentation is not altered greatly when the diet consists of a proper amount of fibre in the ration and if fat does not exceed 4% of dietary dry matter. Palmquist and Jenkins (1980) and Palmquist (1994) also reported a negative influence of dietary lipids at a level above 5% in the diet for ruminants. The effect of fat supplementation on rumen metabolism depends on the fatty acid composition of added fat. However, fats rich in saturated fatty acids have less deleterious effect on rumen parameters, whereas the unsaturated fatty acids present in vegetable oils are metabolized in the rumen (lipolysis, isomerization, biohydrogenation) and influence the composition and quality of animal products.

In our study, the diets consisted of 60% meadow hay, but fat was added up to 6% in dry matter. Feeding animals diets with higher levels of fat modified mostly the proportion of acetic acid in the rumen liquid. When soyabean, linseed and fish oils as well as Bergafat® were added to the sheep ration, the ruminal level of acetic acid was higher than in the control groups, while the acetic acid level was reduced when the diet contained 6% fish oil. In our experiments there was a slight but statistically significant ($P \leq 0.05$) increase in the isobutyric acid level when the diet was supplemented with 4% soyabean oil. Fish oil in the experimental diets also modified the level of butyric and isobutyric acids in the rumen (Table 8). In the experiments of Avila et al. (2000) the molar proportion of acetate tended to increase ($P<0.10$) and the molar proportion of butyrate to decrease with supplemental dietary fat (tallow, yellow grease or both). According to Garnsworthy (1997) if added fats interfere with normal fibre digestion in the rumen, acetate and butyrate production would be reduced. In our studies similar results were obtained for butyric and isobutyric but not for acetic acid. The reason for the discrepancy in regards to acetic acid could be the different concentration of fat in the diets. The type of fermentation in the rumen depends not only on the level and type of fat supplementing the diet but also on the primary treatment of fat, as Tackett et al. (1996) found that primarily unprotected fat containing polyunsaturated fatty acids might cause disturbances in ruminal fermentation. In our previous experiments (Szumacher-Strabel et al., 2001), ruminal VFA profiles did not indicate modification except for the level of isovaleric acid when rape seed oil and hydrogenated rape seed oil were added to sheep rations.Whereas in experiments on sheep fed diets supplemented with vegetable oils and tallow, the concentration of acetic and propionic acids was depressed (Szumacher-Strabel, 1998). Onetti et al. (2002) in their work on the effect of supplemental tallow on the performance of dairy cows found that tallow tended to decrease the volatile fatty acid concentration in the rumen. Neither rape seed oil nor tallow supplementation affected the fatty acid composition of rumen fluid in these experiments. Both of these fats are sources of C 18:1, which in this case did not alter rumen fermentation, whereas the addition of tallow to rations for Holstein cows increased concentration of propionate and decreased concentrations of acetate and valerate in the rumen (Lewis et al., 1999).

There were no meaningful treatment effects on the ammonia concentration or pH in the rumen in our experiments. Only the addition of 4% fish oil to the ration was accompanied by a significant ($P<0.05$) increase in the ammonia level. Similarly

Avila et al. (2000) observed that rumen fluid pH values were not significantly different across diets supplemented with tallow, yellow grease or both. Rumen ammonia-N concentrations were in his experiments high for all diets. Khorasani and Kennely (1998) reported that the overall mean concentration of rumen N-NH_3 and mean rumen pH were not affected by diet. The rumen N-NH_3 concentration in the experiment of Avila et al. (2000) on cows was not altered by adding additional fat to diets containing fat from whole cotton seed. Ohajuruka et al. (1991) also obtained similar results with feeding ruminant animal-vegetable blended fat. Although Pantoja et al. (1994) measured no difference due to fat supplementation at 5% of DM, ruminal ammonia-N concentrations decreased linearly with increasing proportions of unsaturated fatty acids in the fat source. In our previous work the concentration of N-NH_3 was not affected by different types of fat added to the diet for sheep (Szumacher-Strabel, 1998).

CONCLUSIONS

In summary, the presented results show that adding moderate amounts of different types of fat to diets for ruminants does not meaningfully alter the concentrations of acetic acid, ammonia or the pH in the rumen, suggesting that fibre digestion remains on a normal level, however, feeding diets with higher levels of fat leads to a decrease in the acetate concentration in the rumen. Differentiation in the type of supplemented fat containing different proportions of fatty acids and their degree of saturation affects not only acetic acid but also other volatile fatty acids produced in the rumen.

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STRESZCZENIE

Wpływ dodanego tłuszczu do dawek dla owiec na poziom lotnych kwasów tłuszczowych, azotu amonowego i pH treści żwacza

Celem przeprowadzonych doświadczeń było określenie wpływu rodzaju dodanego tłuszczu do dawek składających się z siana i mieszanki treściwej (60:40%) na poziom podstawowych parametrów żwaczowych. Doświadczenia przeprowadzono na trzech owcach mlecznych z trwałymi kaniulami żwaczowymi, w układzie kwadratu łacińskiego 3x3. Do dawki kontrolnej dodano tłuszcze pochodzenia roślinnego i zwierzęcego, różniące się poziomem długołańcuchowych kwasów tłuszczowych: olej rzepakowy, olej sojowy, olej lniany, łój, Bergafat® lub olej rybny w ilości 0 (grupa kontrolna), 4 lub 6% suchej masy paszy. W pobranych próbach treści żwacza określono poziom lotnych kwasów tłuszczowych, amoniaku oraz pH. Stwierdzono statystycznie istotne ($P \leq 0.05$) różnice w stężeniu kwasu octowego pomiędzy grupami zwierząt otrzymujących dodatek oleju sojowego, lnianego, rybnego bądź Bergafatu®. Dodatek oleju sojowego i łoju do dawek dla owiec spowodował statystycznie istotne ($P<0.05$) zmiany stężenia kwasu masłowgo i izomasłowego, szczególnie w grupach otrzymujących 6% dodatek tłuszczu. Nie stwierdzono wpływu dodanego tłuszczu na pH płynu żwaczowego i poziom azotu amonowego z wyjątkiem dodatku 4% oleju rybnego, który spowodował statystycznie istotny ($P<0.05$) wzrost poziomu azotu amonowego w płynie żwacza.