Journal of Animal and Feed Sciences, 7, 1998, 385 - 394

Microbial protein net synthesis in sheep fed meadow hay supplemented with different source and level of fat

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(Received 29 June 1998; accepted 22 September 1998)

ABSTRACT

Three experiments were carried out on four rams fitted with rumen cannulas in a 4 x 4 Latin square design to investigate the effect of varying source and level of fat addition on microbial protein net synthesis (MN) in sheep fed meadow hay. The addition of rape seed oil (RSO) was tested in the 1st experiment, the addition of linseed oil (LSO) in the 2nd, and tallow (TAL) in the 3rd experiment. Treatments in all experiments were similar: a control group without added fat and experimental groups with 4, 8 and 10% of fat in DM of diet. The fat source did not affect microbial protein net synthesis, but addition of 8% of fat tended to increase MN. The highest level of all purine derivatives was observed when RSO was added to meadow hay, whereas tallow addition decreased level of all purine derivatives. The effect of fat was well demonstrated in the variation in pH, and the levels of NH_3-N and volatile fatty acids.

KEY WORDS: fat, meadow hay, purine derivatives, microbial protein net synthesis, sheep

INTRODUCTION

Ruminants in intensive production (growing lambs, high yielding cows) have a large requirement for energy, but the energy intake may be limited by dry matter intake (DMI). Inclusion of fat, such as oils and tallow, in the diets of ruminants may increase the energy density of the diet without depressing DMI and, on the other hand, it is not necessary to increase the intake of feeds. Ruminants have successfully utilized fats in their diets, but the optimal amounts of fat supplements in the rations have not yet been determined. Several researchers have studied the

effects of either oil or tallow supplementation to dairy cows and sheep diets (Schauff et al., 1992). They have observed either positive or negative effects of fat supplementation on microbial protein synthesis (MN), volatile fatty acids, their concentrations and proportions, as well as some other rumen parameters. These effects may be attributed to the type of added fat and diet composition. In the experiments of Ben Salem et al. (1993) the negative effects of lipids on rumen parameters were less important when fibre intake was high. Some other authors did not find a negative effect of fat on rumen parameters of ruminants fed concentrate rations. Generally, positive effects of adding unprotected fat on MN in the rumen were observed. Therefore, the objective of the present studies was to investigate the effects of feeding a hay diet supplemented with varying source and fat levels, rape seed oil (RSO), linseed oil (LSO) and tallow (TAL) on MN and some other rumen parameters in sheep.

MATERIAL AND METHODS

Animals and diets

Four rams (average BW = 60±3 kg) with ruminal cannulae, in 3 experiments, were assigned to three experiments. In all experiments the experimental design was a 4 x 4 Latin square with 16-d periods. The first 14 d were used for adaptation to the diet while the last 2 for sample collection. Sheep received meadow hay, chopped into pieces 5 cm, supplemented with 4, 8 and 10% of fat addition in dry matter. Rape seed oil was added in the 1st experiment, linseed oil in the 2nd, and tallow in the 3rd. Throughout the whole experiment water was available *ad libitum*. The daily ration (1200 g) consisting of meadow hay was divided into two equal portions and fed at 08.00 and 15.00 h. The energy value of the rations was 5.30 MJ NE per kg, whereas the crude protein content was 144 g per kg.

Sampling procedure

Samples of rumen fluid were collected via a rumen cannula using a simple device (Szumacher-Strabel et al., 1998). The levels of NH_3-N, VFA and value at pH were measured in ruminal samples, which were taken before the morning feeding and 3 and 6 h after feeding. pH was measured immediately after sampling, using an pH-Metr N517 apparatus. Ruminal samples (20 mL) were acidified with 10 mL of 0.5n H_2SO_4 and frozen for later analyses of NH_3-N. Next, 20 mL of rumen fluid were taken to analyse VFA concentrations. Urine was collected into containers with 50 mL 1M H_2SO_4, using a urine collection device according to Kowalczyk et al. (1996). Samples (about 30 mL) were frozen and then the purine derivatives were measured.

Chemical analyses

Purine concentrations in urine were determined by the modified HPLC method of Ballcels et al. (1992). Concentrations of NH_3-N in ruminal fluid samples were measured according to Conway (1962). The concentration of VFAs in the ruminal fluid was determined using a gas chromatography method with Chrom 5 according to Ziołecki and Kwiatkowska (1973).

Calculation

Allantoin excretion in the urine was used as a marker for estimation of microbial protein net synthesis (MN). MN was calculated according to the equation of Puchala and Kulasek (1992).

Statistical analysis

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

RESULTS AND DISCUSSION

To be utilized effectively in the diets of ruminants, fat must not alter ruminal fermentation (Weigel et al., 1997). Fat can be provided in various physical states, such as the whole seeds of oil plants, thermal-treated oil, soaps or coated fats, to increase energy intake. Fat supplements must be relatively inert in the rumen to reduce its detrimental effects on ruminal fermentation. Consequently, the rate of ruminal processes is related to the amount of lipids added and to their composition: polyunsaturated fatty acids have a more negative effect than saturated fatty acids (Ben Salem, 1993). High concentrations of fat, particularly fats containing polyunsaturated fatty acids, inhibit microbial growth and fibre digestion (Khorasani et al., 1991). In many experiments in which fat had been added to the diet of ruminants, rumen fermentation has been depressed, apparent digestibility of cell walls has been reduced, and its digestion has been shifted from the rumen to the hindgut (Tesfa, 1993).

There are few studies which have investigated the influence of fat on urinary excretion of purine derivatives and microbial protein synthesis. First, Topps and Elliott (1965) reported a correlation between urinary excretion of allantoin and the concentration of purines in the rumen of sheep, suggesting that allantoin excretion is an index of the ruminal flow of microbial protein (Vagnoni et al., 1997). Purine derivatives (PD) in the urine of ruminants originate mainly from the degradation of absorbed microbial nucleic acids and daily excretion is directly related to the daily amount of purine absorbed (Chen et al., 1992).

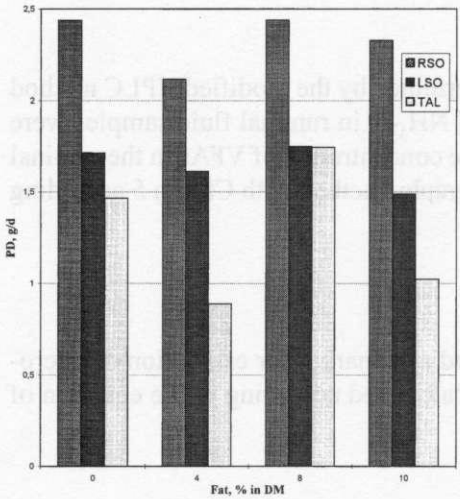

Figure 1. Effect of RSO, LSO and TAL on all purine derivatives (PD) in urine of sheep

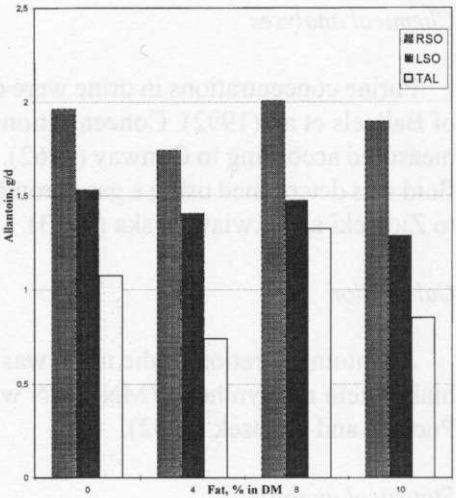

Figure 2. Effect of RSO, LSO and TAL on allantoin in urine of sheep

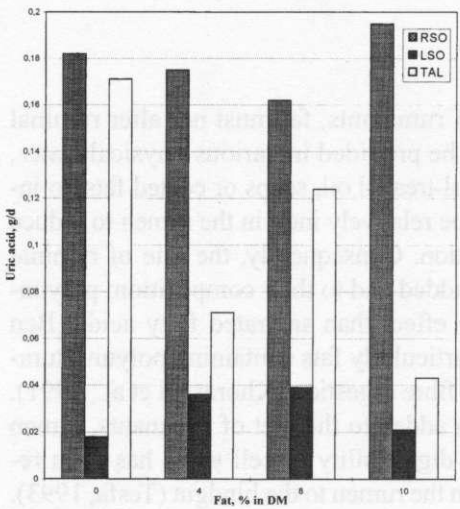

Figure 3. Effect of RSO, LSO and TAL on uric acid in urine of sheep

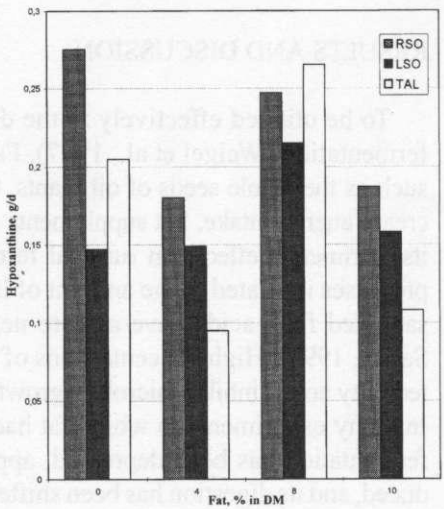

Figure 4. Effect of RSO, LSO and TAL on hypoxanthine in urine of sheep

Czauderna and Kowalczyk (1995) recommend determining all PD in urine of sheep, in order to estimate the extent of MN synthesis correctly. As total excretion increases, the proportion of allantoin increases. This is also a reason not to use allantoin excretion alone to calculate the microbial protein supply. The proportion of the individual components expressed as a percentage of the sum are as follows

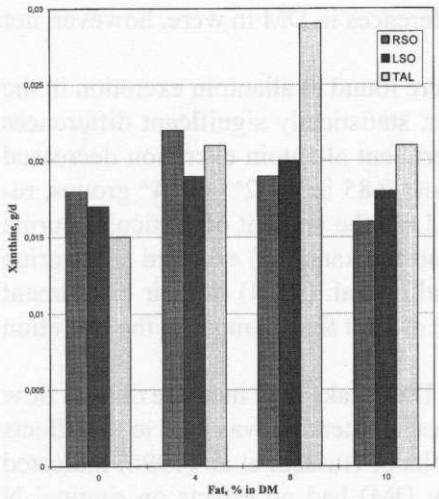

Figure 5. Effect of RSO, LSO and TAL on xanthine in urine of sheep

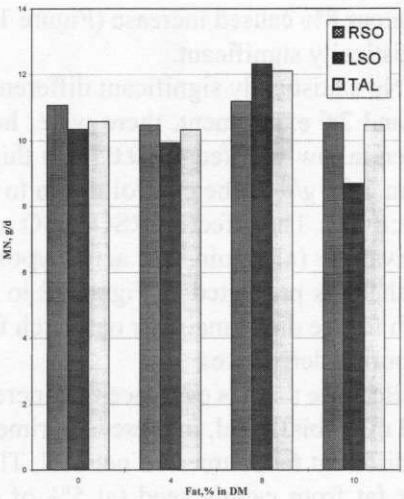

Figure 6. Effect of RSO, LSO and TAL on microbial protein (MN) production in rumen of sheep

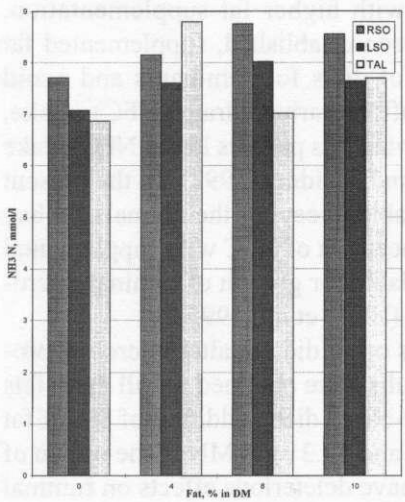

Figure 7. Effect of RSO, LSO and TAL on NH_3-N concentration in rumen fluid of sheep

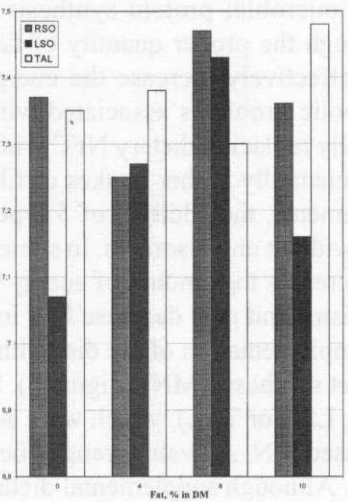

Figure 8. Effect of RSO, LSO and TAL on pH of rumen fluid in sheep

in sheep: allantoin 60-80%, uric acid 30-10%, xanthine plus hypoxanthine 10-5% (Chen and Gomes, 1992). Similar results were obtained in the present experiments.

The highest level of all purine derivatives was observed when RSO was added to the sheep ration, whereas tallow addition decreased their level in urine. Generally, 4 and 10% of RSO, LSO and TAL the ration decreased the level of PD,

whereas 8% caused increase (Figure 1). Differences in DM in were, however, not statistically significant.

No statistically significant differences were found in allantoin excretion in the 1st and 2nd experiment, there were, however, statistically significant differences when tallow was fed ($P<0.05$). In this experiment allantoin excretion decreased from 1.07 g/d in the control group to 0.74 and 0.85 in the 2nd and 4th groups, respectively. The effect of RSO, LSO and TAL on the amount of particular purine derivatives (allantoin, uric acid, hypoxanthine and xanthine) excreted in the urine of sheep is presented in Figures 2 to 5. Khalili et al. (1994) in their experiment with forage diets (maize or oat-vetch forage) did not find changes in the excretion of purine derivatives.

Because there is evidence that increasing DM intake may increase digesta flow and microbial yield, in these experiments special attention was paid to the effects of different fat sources on net MN. The results of Hussein et al. (1996) indicated that fat from canola seed (at 5% of dietary DM) had no effects on ruminal N metabolism; no relationship was found between forage and canola seed proportion. In the experiments of Sutton et al. (1983) in sheep fed linseed oil and in Murphy et al. (1987), when milking cows were fed full-fat rape seed, the efficiency of microbial protein synthesis increased with higher fat supplementation. Although the proper quantity of fat has not been established, supplemented fat may effectively increase the energy density of diets for ruminants and avoid metabolic problems associated with high nonfibre carbohydrate (NFC) intake. Partially replacing dietary NFC with supplemented fats permits lower NFC intake and potentially higher intakes of fibre (Grant and Weidner, 1992). In the present experiments, the addition of 3 types of fat enabled keeping the animals on hay diets without any disorders. In some cases replacement of NFC with supplemental fat decreases the amount of energy that is available for growth of ruminal microorganisms and may decrease MN in the rumen (Elliot et al., 1995).

Supplementation of the diet with three types of fat did not alter microbial protein net synthesis (MN) (Figure 6). Similar results were obtained for all three fats (RSO, LSO or TAL), which were added to hay-based diets: addition of 8% of fat increased MN. All values ranged between 8.69 and 12.31 g/d MN in the rumen of sheep. Although supplemental dietary fat can have deleterious effects on ruminal microbes and microbial activity, in these experiments, however, neither oils nor tallow affected MN.

There is a great interest in maximizing fat utilization for ruminants (particularly unprotected fats) because oil seeds, oils, and tallow are usually the cheapest sources of fat (Schauff et al., 1992). Tallow, which contains approximately 50% unsaturated fatty acids (Grummer et al., 1993), is the least expensive in comparison with other sources of fat and can be fed at 4 to 6% of the dietary DM without affecting ruminal fermentation and nutrient digestibility (Weigel et al., 1997). The present

experiments confirmed that addition of fat up to 8% in DM either as RSO, LSO or TAL, may be effectively used with hay-based diets in ruminant nutrition. The other results of our experiments seem to confirm this hypothesis.

Supplementary fat feeding has consistently been observed to depress rumen ammonia concentration (Hall, 1990) and is in agreement with observations of Kowalczyk et al. (1977). In our experiments the concentration of NH_3-N remained above 6.87 mmol/L and was not affected by feeding fat (Figure 7). The highest differences were noticed when sheep were fed the diet containing tallow and the results differed statistically ($P<0.05$). In this experiment, addition of tallow to hay diets increased the NH_3-N level from 6.87 mmol/L in the control group to 8.79, 7.01 and 7.69 mmol/L in the 2nd (addition of 4% fat), 3rd (addition of 8% fat) and 4th (addition of 10% fat) groups, respectively. Addition of RSO and LSO also increased the ammonia N level, but the differences were not statistically significant. In similar studies (Schauff et al., 1992) the concentration of ammonia N also was not altered by treatments, when tallow was given to cows fed lucerne hay, maize silage and concentrate (45:5:50, DM basis).

Acidity of rumen contents is one of the important factors affecting microbial production and its activity. The effect of added fat on pH value is given in Figure 8. The hay-based diet prevented a drop and maintained the rumen pH on a level above 7.07. The pH of the rumen fluid was not altered by fat addition, whereas in the experiment of Kowalczyk et al. (1977) rumen pH tended to increase with increasing amounts of tallow in the diet. Conflicting results were reported by Boila et al. (1993), where supplementary tallow had no effect on ruminal fermentation.

Increasing fat levels in the diets resulted, in all experiments, in substantial changes in ruminal concentrations of VFA and their proportions. Addition of RSO and TAL depressed the concentrations of acetic and propionic acids (Tables 1 and 2).

TABLE 1

Effect of rape seed oil on concentration of total and individual VFA

Acids mmol/L	Group I control	SD	II 4% fat	SD	III 8% fat	SD	IV 10% fat	SD
Acetic	47.29^{a}	10.11	47.13^{a}	5.77	37.80^{b}	5.36	35.56^{b}	10.35
Propionic	28.81^{a}	4.78	24.98^{ab}	2.70	22.97^{ab}	2.33	25.79^{b}	7.46
Butyric	12.64	3.82	12.50	1.33	11.93	2.34	11.02	4.57
Isobutyric	3.37^{ab}	0.45	2.66^{b}	0.91	3.68^{a}	1.35	2.82^{b}	0.82
Valeric	2.92^{ab}	0.66	3.43^{a}	0.71	2.62^{b}	0.56	2.71^{b}	1.10
Isovaleric	2.40^{b}	0.98	2.46^{b}	0.55	3.66^{a}	1.37	3.23^{a}	1.03
Total VFA	97.46^{a}	16.71	93.17^{ab}	9.24	82.70^{b}	5.90	81.17^{b}	19.52

a, b, c – $P<0.05$

TABLE 2

Effect of linseed oil on concentration of total and individual VFA

Acids mmol/L	Group I control	SD	II 4% fat	SD	III 8% fat	SD	IV 10% fat	SD
Acetic	45.48	8.52	42.83	6.72	42.19	6.73	39.00	7.08
Propionic	25.04^{b}	3.35	26.11^{b}	4.13	30.19^{a}	4.31	30.34^{a}	2.19
Butyric	10.32	3.08	10.99	1.89	10.55	1.54	10.10	1.96
Isobutyric	2.88^{a}	0.52	2.69^{ab}	0.58	2.67^{ab}	0.83	2.13^{b}	0.80
Valeric	2.71	0.81	2.69	0.81	2.53	0.59	3.01	0.87
Isovaleric	2.24	0.67	2.47	0.43	2.59	0.59	2.61	0.57
Total VFA	88.70	12.55	87.80	11.56	90.74	11.39	87.20	9.39

a, b, c – $P<0.05$

TABLE 3

Effect of tallow on concentration of total and individual VFA

Acids mmol/L	Group I control	SD	II 4% fat	SD	III 8% fat	SD	IV 10% fat	SD
Acetic	41.80^{a}	7.03	38.45^{ab}	6.43	35.07^{b}	6.55	32.65^{b}	6.84
Propionic	21.46^{c}	2.16	26.95^{b}	5.58	26.68^{b}	3.60	34.34^{a}	3.45
Butyric	10.83^{ab}	1.73	9.45^{b}	1.49	11.29^{a}	2.12	9.67^{b}	1.65
Isobutyric	2.86	0.83	3.03	0.84	2.96	0.81	2.47	0.58
Valeric	3.42	0.93	3.28	0.71	2.80	0.80	3.19	0.70
Isovaleric	2.15^{bc}	0.79	2.89^{a}	0.57	2.60^{ab}	0.94	1.85^{c}	0.81
Total VFA	82.55	10.10	84.08	11.29	81.20	10.65	84.18	10.16

a, b, c – $P<0.05$

Molar proportions of propionate tended to increase in ruminal fluid when LSO was fed (Table 3). In the experiment of Tackett et al. (1996) the pattern of VFA was not altered when 10% canola fatty acids were added to a diet containing ground lucerne hay, whereas the concentration of VFAs in ruminal fluid in Schauff's et al. (1992) experiment were decreased by feeding tallow.

Thus, the use of fats and oils to increase the energy density of ruminant diets has gained wide acceptance but more work is required to determine the type of diet and fat level to obtain satisfactory high-energy diets for ruminants. Further experiments with concentrate-based diets and types of fat addition should give more complete information.

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STRESZCZENIE

Synteza białka mikroorganizmów u owiec żywionych sianem łąkowym z dodatkiem różnej ilości i rodzaju tłuszczu

Przeprowadzono trzy doświadczenia na czterech trykach z trwałymi kaniułami żwaczowymi, w których testowano wpływ diety składającej się z siana z dodatkiem tłuszczu w postaci oleju rzepakowego (RSO) – doświadczenie 1, oleju lnianego (LSO) – doświadczenie 2 lub łoju (TAL) – doświadczenie 3, na syntezę białka mikroorganizmów w żwaczu, poziom pochodnych purynowych w moczu, poziom azotu amonowego i lotnych kwasów tłuszczowych w żwaczu oraz pH płynu żwaczowego. Dawkę pokarmową stanowiło natłuszczane siano łąkowe, które skarmiano w dwóch odpasach. Doświadczenia przeprowadzano w układzie kwadratu łacińskiego 4 x 4 testując w czterech grupach trzy poziomy dodanego tłuszczu (4, 8, 10% w sm). Rodzaj dodanego tłuszczu nie miał istotnego wpływu na rozmiar syntezy netto białka mikroorganizmów ($P>0,05$). Najwyższy poziom pochodnych purynowych (PD) w moczu stwierdzono przy dodatku oleju rzepakowego do siana, podczas gdy dodatek łoju obniżał poziom PD. Poziom azotu amonowego i pH płynu żwaczowego mieścił się w granicach norm fizjologicznych. Dodatek łoju spowodował statystycznie istotny wzrost poziomu azotu amonowego w grupach doświadczalnych w porównaniu z grupą kontrolną ($P<0,05$). W pozostałych doświadczeniach nie stwierdzono wpływu dodatku tłuszczu na poziom wymienionych wskaźników. We wszystkich doświadczeniach dodatek tłuszczu wpłynął istotnie ($P<0,05$) na stężenie lotnych kwasów tłuszczowych: spadek poziomu kwasu octowego oraz w doświadczeniu drugim i trzecim wzrost poziomu kwasu propionowego.