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

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ABSTRACT

Three experiments in a 4 x 4 Latin square design were carried out on four rams fitted with rumen cannulas to investigate the effect of varying sources and levels of fat addition on microbial protein net synthesis (MN) in sheep fed a concentrate-based diet (60% of concentrate and 40% of meadow hay). In the 1st experiment addition of rape seed oil (RSO) was tested, linseed oil (LSO) was added in the 2nd, and tallow (TAL) in the in 3rd experiment. Treatments in all experiments were similar: a control group without fat addition and experimental groups with 4, 8 and 10% of fat in DM of the diet. Microbial production in the rumen, purine derivatives in urine, ruminal ammonia, pH, fatty acids in the rumen were measured. In all cases fat addition resulted in decreased MN, however, 8% fat supplementation had the least deleterious effect on MN production. Fat addition significantly affected purine derivatives excretion. LSO as the supplemental fat led to a decrease in N ammonia concentration from 7.8 mmol/L in the control group to 6.39 mmol/L in the group that received 8% LSO, whereas a significant increase to 9.00 mmol/L was observed when 10% LSO in DM was fed. Ruminal fluid pH was not altered by treatments. In all of the experiments, the increased fat content in the diets decreased the molar percentage of acetic acid. Supplementation of the diet with LSO and TAL resulted in an increase of the propionic acid level ($P<0.05$).

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

INTRODUCTION

High producing animals usually have a negative energy balance. Dietary energy density in the diet can be increased by incorporation of additional concentrate,

supplemental fat or both (Grum et al., 1996). Supplementation with fat helps meet the energy requirement of ruminants without causing the metabolic disorders often observed when large amounts of grain are fed (Wu and Huber, 1994). Diets for ruminants are also supplemented with high-starch grains to increase energy density, however, the amount of grain that can be fed is limited because ruminants require fibre in the ration for adequate chewing activity and rumen function (Hoffman et al., 1991). Another discouragement to the use of high-starch concentrate diets in ruminant rations is their rapid fermentation, which causes a decrease in the pH in the rumen (Cone, 1991). The most efficient way to increase the energy concentration of a diet is to include fat. However, fat has an adverse effect on digestion of fibre in the rumen, often resulting in reduced feed intake (Houtert and Leng, 1993). Various effects of added fat depend on the level, source and type of fat, dietary carbohydrate source and feed intake (Garnsworthy, 1997).

The objective of this study was to investigate the effects of a high concentrate diet supplemented with different levels of fat on microbial net synthesis and other rumen parameters.

MATERIAL AND METHODS

Animals and diets

Four rams (average BW = 60±3 kg) with ruminal cannulas were assigned to three experiments. In all of the 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 and the last 2 for sample collection. Sheep received diets consisting of 40% meadow hay, chopped into 5 cm and 60% concentrate, supplemented with 4, 8 and 10% of fat on dry matter basis. The current study is a continuation of previous research based on hay diets (Szumacher-Strabel, 1998). Three experiments were carried out: the 1st experiment with added rape seed oil (RSO), the 2nd with linseed oil (LSO), and the 3rd with tallow (TAL). Water was available *ad libitum*. The daily experimental ration, 1500 g, was divided into two equal portions and fed at 08.00 and 15.00 h. The energy value of the experimental rations was 5.31 MJ NE/kg, whereas the crude protein content was 144 g per kg. Sheep were fed at the maintenance level plus 15%.

Sample collection and analysis

Samples of rumen fluid and urine were collected and analyzed as described in a previous paper (Szumacher-Strabel, 1998).

Statistical analysis

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

RESULTS AND DISCUSSION

Feed costs account for some 70-75% of the total cost of ruminant livestock production and therefore it is imperative that the adopted principles of feeding practice are those which ensure the most efficient production of livestock products compatible with maximum economic return (Armstrong, 1980). In the case of high producing ruminants the first factor that has played a part in this strategy is covering energy and protein requirements. Starch is commonly added mainly to increase the energy density of feeds for ruminants, although, as mentioned in the introduction, high amounts of starch lead to rumen fermentation disturbances. In this case, the best way to decrease starch feeding is introducing fat into the rations. Increasing use of fat in feeding programmes has provided documented, consistent production responses when dietary fats were included at up to 5 to 6% of dietary DM (Pantoja et al., 1996). Fat supplementation (about 3% of dietary DM) has often positively influenced the reproductive status of ruminants (Staples et al., 1998). Lipids added to ruminant diets can greatly disrupt fermentation in the rumen, causing reduced digestibility of nonlipid energy sources. Compared with fibre, dietary fat is less detrimental to digestibility of nonstructural carbohydrates (NSC) (Jenkins, 1993). Also Tackett et al. (1996) suggested that the type of fibre influenced the degree of negative effects caused by ruminally active fats. Elmeddah et al. (1991) did not demonstrate a significant interaction between the nature of the diet and fat. According to Elliot et al. (1995) replacement of NSC with supplemental fat decreases the amount of energy that is available for growth of ruminal microorganisms and may decrease microbial protein synthesis.

In the present experiment, in which diets containing 60% of concentrate were tested, in all cases fat addition resulted in decreased MN (Figure 1). Compared with previous experiments (Szumacher-Strabel, 1998), where sheep were fed hay diets, NM production in sheep fed concentrate-based diets tended to be slightly higher, but the results of the previous study, when hay diets were fed, were more uniform. Similarly, in the experiment of Perez et al. (1997) when sheep were fed diets consisting of rations with two forage:concentrate ratios (low concentrate-LC and high concentrate-HC) urinary excretion of purine derivatives and microbial yield were higher in animals offered HC than in those that were offered LC. In our studies the results were similar for all kinds of fat and statistically significant differences were not observed ($P>0.05$). Neither source and type of fat nor dietary carbohydrate source had an effect on net MN level when 8% of fat was added.

However, when the level of added fat was below or above this level, a high variability in MN production was observed, particularly with tallow, which is difficult to explain.

Inclusion of linseed oil increased efficiencies of ruminal microbial protein synthesis, but this was accompanied by decreased ammonia concentration and increased N flow into the duodenum. According to Jenkins (1993) these increased efficiencies have been attributed to an increased dilution rate of solids in the rumen because of the added fat. Similarly, the efficiency of bacterial protein synthesis was increased when whole canola seeds were supplemented to diets with two forage levels (70 vs. 30% in DM) in the experiment of Hussein et al. (1996).

The level of total purine derivatives (PD) in urine was estimated and microbial protein net synthesis was calculated, similarly as in previous studies, using allantoin as the indicator. There were no statistically significant differences between the groups and experiments (Figures 2 to 5). Surra et al. (1997) conclude that urinary excretion of PD may be affected by variations in the flow of undigested fibre along the small intestine. Figure 6 presents the changes in the amount of total purine derivatives for all of the added fats. Addition of fat to concentrate-based diets, in contrast with hay-based diets, caused a larger variation in the level of purine derivatives excreted. In the experiment of Vagnoni and Broderic (1997), protein synthesis was poorer in cows that consumed lucerne conserved as hay than as lucerne silage. Source, amount, and physical characteristics of dietary forage can interact with nonforage fibre sources and influence ruminal fibre digestion, passage, and performance of dairy cows fed diets containing substantial amounts of nonforage fibre in place of forage (Grant, 1997). According to Allen (1997) ruminal fermentation of both nonfibre carbohydrate and fibre is extremely variable, and this variability is not related to the nonfibre carbohydrate content of the diet. In spite of such large variability, neither carbohydrate source nor fat addition affected PD excretion in sheep fed concentrate-based diets in a statistically significant ($P>0.05$) manner.

Supplementary tallow had no effect on the ruminal ammonia N level. When LSO was the supplementary fat, a decrease in N ammonia concentration was observed in the group receiving 8% LSO (from 7.8 mmol/L in the control group to 6.39 mmol/L), whereas a statistically significant increase was observed when 10% LSO in DM was fed (Figure 7). The concentration of NH_3-N in ruminal fluid increased slightly ($P<0.07$) when supplemental fat as tallow was fed (Weigel et al., 1997). Satter and Slyter (Ivan et al., 1996) suggested that concentrations of 5 to 8 mg of ammonia N/100mL of ruminal fluid were optimal for maximizing microbial mass yield and that concentrations < 2 mg/100 mL were potentially limiting for microbial growth. However, other studies have indicated that chemical or structural characteristics of the degradable substrate might influence estimates of optimal ruminal ammonia N for microbial growth. Diets in the present experiments were

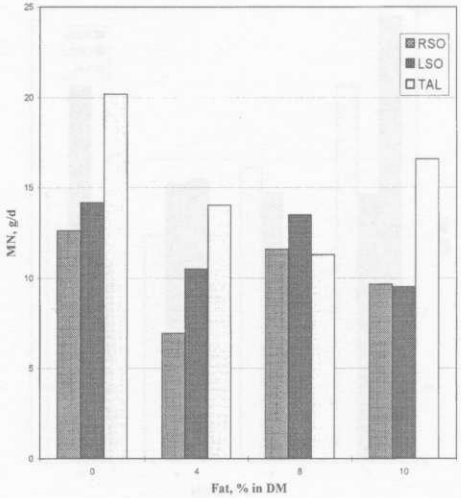

Figure 1. Effect of RSO, LSO and TAL on microbial protein (MN) production

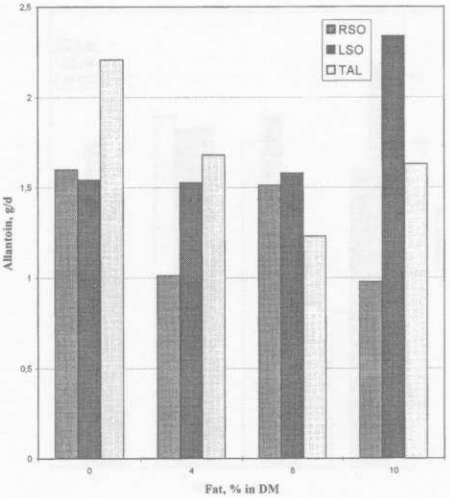

Figure 2. Effect of RSO, LSO and TAL on allantoin in urine of sheep

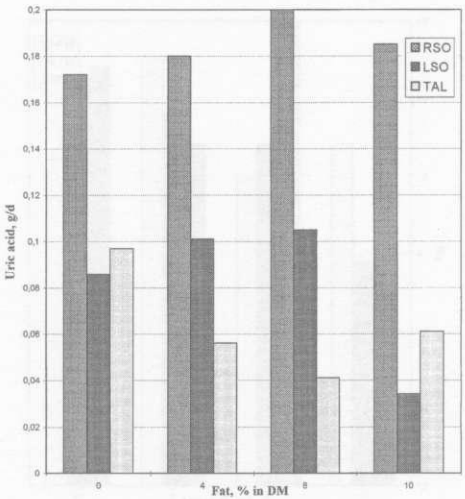

Figure 3. Effect of RSO, LSO and TAL on uric acid in urine of sheep

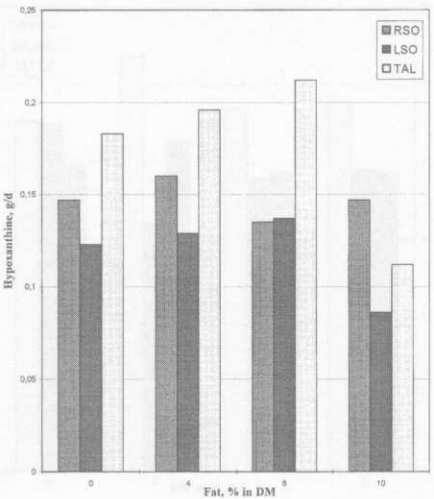

Figure 4. Effect of RSO, LSO and TAL on hypoxanthine in urine of sheep

sufficient for maximal microbial growth yield and also to meet the energy requirements of animals.

Ruminal fluid pH was not altered by treatments ($P>0.05$) (Figure 8) although it seems that concentrate-based diets tended to decrease the pH in comparison with hay-based diets. Madison-Anderson et al. (1997) reported that pH and ammonia

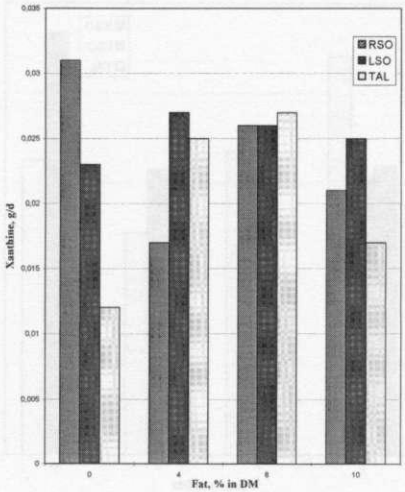

Figure 5. Effect of RSO, LSO and TAL on xanthine in urine of sheep

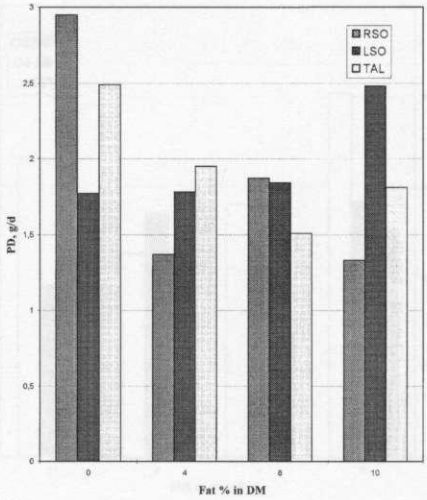

Figure 6. Effect of RSO, LSO and TAL on total purine derivatives (PD) content in urine

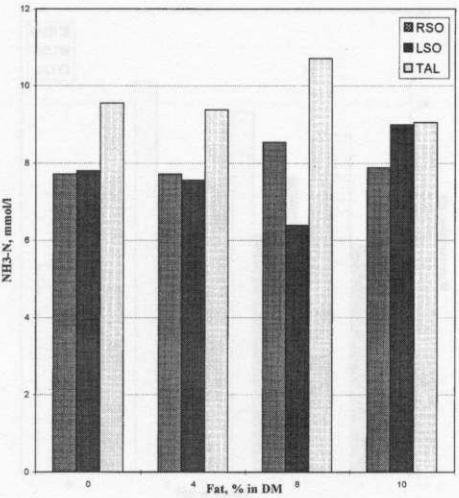

Figure 7. Effect of RSO, LSO and TAL on NH_3-N concentration in rumen fluid

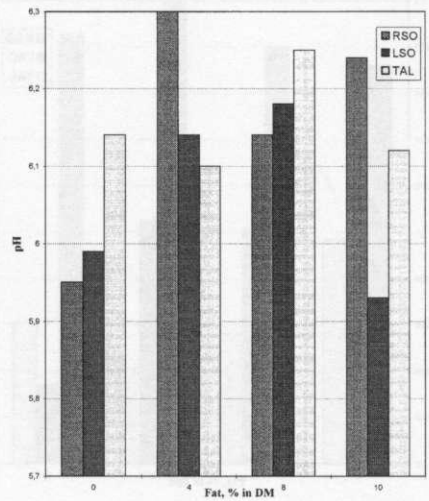

Figure 8. Effect of RSO, LSO and TAL on rumen fluid pH

were unaffected by supplemental fat (3% of DM extruded soyabeans). Low ruminal pH may decrease DMI, fibre digestibility, and microbial yield and increase feed costs (Allen, 1997).

Acid production in the rumen is due primarily to fermentation of carbohydrates, which represent over 65% of the DM in diets of ruminants (Allen, 1997). In all of

TABLE 1

Effect of rapeseed 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	35.03^{b}	6.55	42.30^{a}	9.05	31.30^{b}	7.23	25.37^{c}	6.21
Propionic	29.90	4.83	32.09	5.56	31.18	6.82	34.36	7.37
Butyric	10.30	3.44	10.29	2.00	10.60	2.68	12.48	2.47
Isobutyric	3.33	0.91	3.47	0.71	2.76	1.07	3.02	1.22
Valeric	3.59^{a}	0.93	3.00^{ab}	1.12	2.27^{b}	0.81	3.63^{a}	0.84
Isovaleric	3.05^{a}	0.84	3.58^{a}	0.90	1.55^{b}	0.76	1.67^{b}	0.80
Total VFA	85.22^{AB}	12.78	94.75^{A}	15.32	79.66^{B}	13.96	80.56^{B}	13.12

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	36.95^{a}	7.23	33.54^{ab}	5.48	33.03^{ab}	4.11	30.64^{b}	3.09
Propionic	24.30^{c}	4.27	26.54^{bc}	3.67	30.20^{b}	4.19	37.59^{a}	4.43
Butyric	8.54	3.46	8.66	2.90	8.55	2.59	9.50	3.14
Isobutyric	2.58	0.90	2.83	0.91	3.37	1.13	2.80	0.54
Valeric	2.54	0.90	2.41	0.59	2.57	0.52	2.42	0.55
Isovaleric	2.21	0.64	2.18	0.70	2.63	0.65	2.41	0.58
Total VFA	77.15	13.30	76.18	10.05	80.37	10.66	85.36	7.25

a, b, c – $P<0.05$

the described experiments the increased fat content in the diets decreased the molar percentage of acetic acid. In experiments with LSO and TAL elevated levels of propionic acid were observed ($P<0.05$) (Tables 1 to 3). Increasing the LSO and TAL levels also caused a rise in propionic acid levels. Similarly, the molar proportions of acetate tended to decrease and molar proportions of propionate tended to increase in the ruminal fluid when fat was fed in Schauff's et al. (1992) experiment.

The theory that the ratio of acetate to propionate produced in the rumen determines the energetic efficiency of growth has been tested extensively (Houtert and Leng, 1993). According to Schauff et al. (1992) ruminal fermentation is not altered greatly when ruminants ingest large amounts of feed and animal fats do not exceed 4% of dietary DM.

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	36.28[a]	4.64	34.39[a]	4.02	32.58[a]	5.43	26.65[b]	4.07
Propionic	26.29[b]	4.22	25.44b	4.91	28.34[b]	4.49	36.12[a]	5.24
Butyric	10.42	2.74	8.42	2.83	8.57	2.74	10.84	1.76
Isobutyric	2.91	0.84	3.32	2.28	2.36	0.69	2.90	1.20
Valeric	3.19[ab]	0.94	3.14[ab]	0.88	2.91[b]	0.91	3.88[a]	0.50
Isovaleric	2.63[a]	0.61	2.60[a]	0.50	2.61[a]	0.46	2.02[b]	0.51
Total VFA	81.74	10.25	77.33	10.48	77.40	10.83	82.44	9.83

a, b, c – $P<0.05$

Garnsworthy (1997) suggested that if added dietary fats interfere with normal fibre digestion in the rumen, acetate and butyrate production will be reduced. In the present experiment, the molar percentage of butyrate was not altered by fat addition, whereas supplementing fat in Weigel's et al. (1997) experiment slightly decreased ($P<0.08$) the butyrate level. In the experiment of Grummer et al. (1993), butyrate responded quadratically as tallow supplementation was increased. In the present experiment, an increased RSO and TAL ratio generally decreased ($P<0.05$) the molar percentage of isovalerate and valerate.

A less deleterious effect of fat on the molar proportion of fatty acids and moderate changes in other VFA patterns were observed when linseed oil was fed. Ruminal VFA patterns of sheep were not altered when 10% canola fatty acids were added to a diet containing ground lucerne hay (Tackett et al., 1996). Volatile fatty acid concentrations in the rumen were similar for diets consisting of 60% meadow hay as for diets composed of 65% maize silage supplemented with 7% RSO on a DM basis (Ben Salem, 1993).

As expressed in the previous study, the composition of the basal diet also may influence how a fat source affects ruminal fermentation (Szumacher-Strabel, 1998). According to Jenkins (1993) fats that normally inhibit fermentation and digestion often cause less inhibition when the hay content of the basal diet is high.

The obtained results are similar when compared with previous paper (Szumacher-Strabel, 1998). The results of these two studies indicate that addition of fat to sheep rations does not influence MN production to a significant degree. Additional research is needed to investigate the proper forage:concentrate level when fat is added to sheep rations so as not to greatly alter ruminal fermentation.

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STRESZCZENIE

Synteza białka mikroorganizmów u owiec żywionych dawkami złożonymi siana i paszy treściwej z dodatkiem różnego rodzaju i ilości tłuszczu

Przeprowadzono trzy doświadczenia na czterech trykach z trwałymi kaniulami żwaczowymi, w których badano wpływ diety składającej się z paszy treściwej i objętościowej (60:40%) z dodatkiem tłuszczu w postaci oleju rzepakowego (RSO) – doświadczenie 1, oleju lnianego (LSO) – doświadczenie 2 oraz 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 skarmiano w dwóch odpasach. Doświadczenia przeprowadzano w układzie kwadratu łacińskiego 4 x 4 testując w czterech grupach różne poziomy dodanego tłuszczu: 1 – grupa kontrolna bez dodatku tłuszczu, 2 – dodatek 4% tłuszczu (RSO, LSO lub TAL) w sm, 3 – dodatek 8% tłuszczu (RSO, LSO lub TAL) w sm, 4 – dodatek 10% tłuszczu (RSO, LSO lub TAL) w sm. Dodatek tłuszczu zmniejszał produkcję białka mikroorganizmów, lecz różnice te nie były statystycznie istotne. Źródło tłuszczu oraz jego poziom w dawce nie miały wpływu na poziom pochodnych purynowych w moczu owiec oraz na pH płynu żwaczowego. Dodatek 8% oleju lnianego do diety istotnie ($P<0,05$) obniżył, natomiast 10% dodatek istotnie ($P<0,05$) zwiększył poziom azotu amonowego. We wszystkich doświadczeniach dodatek tłuszczu obniżył ($P<0,05$) poziom kwasu octowego w płynie żwacza, natomiast olej lniany i łój zwiększyły ($P<0,05$) produkcję kwasu propionowego we wszystkich grupach.