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A note on the effect of rape seed oil in concentrate/hay ration on microbial protein synthesis in sheep

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ABSTRACT

The effect of rape seed oil supplementation on efficiency of microbial protein synthesis was estimated on three rams fitted with rumen cannulae in 3x3 Latin square design consisting of three experimental diets differing in the percentage of rape seed oil supplement. The basic diet for all animals was composed of 60% concentrate and 40% meadow hay which was supplied with 0. 3 or 6% of rape seed oil. Supplementation of the diet with 3 or 6% of rape seed oil did not have a significant effect on rumen pH and total volatile fatty acid concentrations ($P < 0.05$). There was a statistically significant difference in the level of valeric acid among groups. Also the level of ammonia-N was altered significantly. Daily output of microbial protein-N, determined from allantoin content in the urine, decreased with the amount of fat added to the ration from 15 to 13 and 12 g, respectively, but these differences were not statistically significant.

KEY WORDS: rumen, fat, microbial protein, allantoin, sheep

INTRODUCTION

Supplementation of diets for ruminants with fat is used to increase their energy concentration. Fat is commonly added in the amount of 2-8% DM (Palmquist and Jenkins, 1980; Sklan et al., 1991; Grant and Weidner, 1992). However, higher levels of fat can reduce fibre digestibility (van der Honing and Tamminga, 1986; Tackett et al., 1996). Kowalczyk et al. (1977), suggested that

lipids can inhibit the growth of certain bacteria species, especially cellulolytic ones. In the case of reducing fibre digestibility in the rumen, the amount of energy available for synthesis of microbial protein in the rumen may decrease as it depends on the quantity of digestible organic matter (Khorasani et al., 1991). According to Ørskov (1992) in sheep 10-55 g N can be converted into bacterial protein when 1 kg organic matter is digested in the rumen. Sutton et al. (1983) and Murphy et al. (1987) maintain that the yield of microbial protein synthesis is not reduced but rather increased as the result of diet supplementation with fat. The effect of fat on ruminal processes depends not only on its type and amount but, first of all, on the fatty acid spectrum. Rumen microflora is particularly affected by fats containing unsaturated fatty acids (van der Honing and Tamminga, 1986).

The aim of this study was to determine the rate of microbial protein synthesis in sheep fed a diet supplemented with rape seed oil, containing a high concentration of unsaturated fatty acids.

MATERIAL AND METHODS

The experiment was carried out on 3 rams of mean body weight of 40 ± 3kg equipped with rumen cannulae in 3x3 Latin square design. All animals were fed a diet consisting of 60% concentrate and 40% meadow hay. The energy value of the ration was 5.87 MJ NE, whereas the crude protein content was 125 g per 1 kg, which complies with standard requirements of adult sheep (Ryś et al., 1993). Experimental treatments were: diet without supplement, control-treatment 1; diet supplemented with 3% – treatment 2 or with 6% of rape seed oil in DM – treatment 3. The daily ration (1200 g) was divided into two equal portions and fed at 8.00 and 15.00 h for 15 days in each period. Water was available *ad libitum*. On the last day of each period rumen liquor samples were withdrawn before the morning feeding and 3 and 6 h after feeding (Grummer et al., 1993). Urine was collected for 2 days into containers with sulphuric acid using a urine collection device according Kowalczyk et al. (1996).

Rumen liquor samples were analysed for ammonia-N (Conway, 1962), VFA with GLC method (Ziołecki and Kwiatkowska, 1973) and pH potentiometrically. Allantoin in urine was determined according to Ballcels et al. (1992). The amount of microbial nitrogen (N_M) was calculated according to the equation: $N_M = (N_N - N_{AF}) \times 4 \times (110/18)$, where N_M is microbial N (g d^{-1}), N_A is allantoin N excreted during 24 h and N_{AF} is allantoin N excreted during 24 h of fasting (Puchala and Kulasek, 1992).

The results were subjected to analysis of variance using SAS (6.03) software (SAS 1990).

RESULTS

The composition of diet is shown in Table 1. The pH value of rumen liquor was similar in all groups and ranged from 5.5 to 5.8 (Table 2). No differences were found either in the concentration of fatty acids (Table 3) which ranged: for acetic acid, from 47-48 mmol/l; propionic acid, from 29-33 mmol/l; and butyric acid, from 12.5-12.9 mmol/l. Only the valeric acid concentration changed under the influence of rape seed oil supplementation from 1.6 to 2.7 mmol/l. Also the level of ammonia-N was altered significantly ($P < 0.05$) with treatment; at lower rape seed oil supplementation its level was lower (11.0 mmol/l) than in the control group (14.8 mmol/l) and at the higher supplementation level, it reached the lowest value, 8.53 mmol/l; differences were statistically significant (Table 2). Daily output of microbial protein-N decreased with the amount of fat added to the ration from 15 to 13 and 12 g, respectively, but these differences were not statistically significant (Table 2).

TABLE 1

Feed composition, DM %

Feeds	Group I	Group II	Group III
Rape seed oil meal	6.0	5.5	5.0
Wheat, ground	9.0	8.5	8.5
Rye, ground	17.0	17.0	16.5
Barley, ground	28.0	27.0	26.0
Meadow hay	38.0	37.0	36.0
Rape seed oil	0.0	3.0	6.0
Mineral-vitamin mix	1.5	1.5	1.5
Trace mineral salt*	0.5	0.5	0.5

* contains: NaCl, 95.8%

TABLE 2

Daily production of microbial nitrogen (N_M), ammonia nitrogen (NH_3-N) and pH in rumen fluid $\pm$SD

	Group I	Group II	Group III
N_M, g/day	15.0 $\pm$5.69	13.3 $\pm$6.58	12.6 $\pm$3.38
NH_3-N, mmol/l	$14.8^a \pm 8.83$	$11.0^{ab} \pm 6.05$	$8.5^b \pm 8.59$
pH	5.6 $\pm$0.48	5.8 $\pm$0.56	5.8 $\pm$0.68

a, b – $P \leqslant 0.05$

TABLE 3

Concentration of volatile fatty acids in rumen fluid, ±SD, mmol/l

Acid	Group		
	I	II	III
Acetic	47.1 ±9.43	47.2 ±5.65	48.2 ±5.75
Propionic	29.8 ±6.12	33.0 ±7.08	30.0 ±6.89
Butyric	12.9 ±0.78	12.5 ±2.11	12.5 ±1.46
Isobutyric	5.7 ±1.51	6.2 ±1.86	5.3 ±1.08
Valeric	1.6^{a} ±1.02	1.9^{ab} ±0.95	2.7^{b} ±1.38
Isovaleric	4.1 ±2.14	3.8 ±1.83	3.4 ±1.66
Total VFA	101.2	104.6	102.1

a,b – $P \leqslant 0.05$

DISCUSSION

According to Chen et al. (1991) the yield of protein synthesis expressed as microbial nitrogen (N_M) ranges from 14-19g N_M per kilogram of organic matter digested in the rumen depending on the diet. Ørskov (1992) reported that N_M yield determined with the use of DAPA and 35S as markers ranges from 10–55 g N_M per kilogram of organic matter digested in the rumen of sheep. The rate of microbial protein synthesis (MCP) expressed as grams of MCP per MJ of fermentable metabolizable energy (FME) depends on the level of feeding and the rate of digesta outflow from the rumen: for sheep and cattle fed at maintenance level – 9 g MCP/MJ FME; for growing sheep and cattle – 10 g, while for lactating cows and sheep – 11 g (Alderman and Cottrill, 1995). In the present study, addition of rape seed oil resulted in a slight, but not significant, decline in synthesis of microbial protein (Table 2) amounting to 15.0, 13.3 and 12.6 g N_M/d^{-1}, in the control and experimental groups, respectively. Puchala and Kulasek (1992), Czauderna and Kowalczyk (1995) suggested that, in order to estimate the extent of N_M synthesis correctly, it would be recommendable to determine not only the amount of excreted allantoin but also other purine derivatives excreted in the urine. In practice, however, the method of measuring excreted allantoin alone is used frequently as a marker for the evaluation of N_M synthesis.

In studies on the effect of fat on protein synthesis, both positive and negative results have been obtained. Hussein et al. (1996) found a linear increase of the yield of microbial protein biosynthesis when cows were supplemented with 10% (in dietary DM) whole canola seeds (CS) treated with alkaline H_2O_2 or as crushed CS (partially protected and unprotected fat). Supplementing diets with other sources of fat also increases the output of ruminal N_M synthesis. Murphy et al. (1987) found a positive correlation between the amount of added fat and yield of

N_M synthesis when effects of full-fat crushed rape seed (0, 1 or 2 kg/d) on rumen microbial protein synthesis were studied in lactating cows. Nevertheless, inclusion of fat into the diet, especially unprotected, may provoke a negative effect on ruminal processes. Refraining from feeding high amounts of fat prevents the negative effect on microorganisms development and averts the decrease in fibre digestion in the rumen (Palmquist and Jenkins, 1980; Palmquist, 1994). In our experiment, inclusion of dietary fat in the form of rape seed oil led to a slight, but statistically non-significant, decline in the synthesis of microbial protein. Also Khorasani et al. (1991) reported that high concentrations of fat containing polyunsaturated fatty acids inhibits growth of microorganisms and fibre digestibility. Tackett et al. (1996) also found that primarily unprotected fat may cause disturbances in ruminal fermentation which consequently lead to reduced fibre digestibility. However, Wiseman (1984) claims that supplementation with fat inhibits fibre digestibility in sheep, while a similar effect is not always observed in dairy cattle. A negative influence of dietary lipids above 5% in the diet for sheep on the development of microorganisms, especially of cellulolytic bacteria, was also reported by Kowalczyk et al. (1977). Devendra and Levis (1974), Ben Salem et al. (1993), found that the negative effect of fat on ruminal processes can be attributed to physical coating of the fibre with fat preventing microbial attack, to modification of the rumen microbial population as the result of possible toxic effects of fat on certain microorganisms, to reduced cation availability from formation of insoluble complexes with long-chain fatty acids, and also to inhibition of microbial activity due to surface-active effects of fatty acids on cell membranes. Palmquist (1984) suggests that the negative influence of fat on digestibility is less conspicuous if the dietary fibre intake is high.

In our experiment a considerable drop in NH_3-N concentration was observed when the level of ration supplementation was 6% (Table 2), which is in agreement with the finding of Kowalczyk et al. (1977) who noted a linear decline in NH_3-N concentration in the rumen liquor with the increase of tallow in diet. Tsefa et al. (1993) also reported that 0.5 kg rape seed oil in rations for Fresian bulls reduces NH_3-N level and inhibits development of protozoa in the rumen.

No statistically significant differences in the ruminal pH level were observed in our experiment (Table 2). The concentration of volatile fatty acids (VFA) in the rumen fluid was similar and depended, primarily, on the type of feed. Significant differences ($P < 0.05$) in the level in valeric acid were found between groups I and III (Table 3) which is in contradiction with Kowalczyk et al. (1977) who reported a reduced proportion of all volatile fatty acids, particularly of acetic, propionic and isobutyric acids, with an increasing level of tallow in the diet for sheep.

In conclusion, the results presented indicate that addition of rape seed oil to the diet may depress microbial protein synthesis in the rumen but, on the other hand, may favourably influence other rumen parameters. However, having in

mind the conflicting results by authors of different experiments, more work is needed to find the best way of feeding ruminants rations with fat as a source of energy and desired fatty acids without harm for microorganism development and fibre digestion.

REFERENCES

Alderman G., Cottrill B.R., 1995. Energy and Protein Requirements of Ruminants. University Press, Cambridge

Balcells J., Guada J.A., Peiro J.M., 1992. Simultaneous determination of allantoin and oxypurines in biological fluids by high-performance liquid chromatography. J. Chromatogr. 575, 153-157

Ben Salem H., Krzeminski R., Ferlay A., Doreau M., 1993. Effect of lipid supply on in vivo digestion in cows: comparison of hay and silage diets. Can. J. Anim. Sci. 73, 547-557

Chen X.B., Chen Y.K., Franklin E.R., Ørskov E.R., Shand W.J., 1991. The effect of feed intake and body weight on purine derivative excretion and microbial protein supply in sheep. J. Anim. Sci. 70, 1534-1542

Chen X.B., Gomes M.J., 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives – an overview of the technical details. International Feed Resources Unit, Rowett Research Institute, Bucksburn, Aberdeen (UK). Occasional Publication, 1992

Conway E.J., 1962. Microdiffusion Analysis and Volumetric Error. Crosby Lockwood, London

Czauderna M., Kowalczyk J., 1995. Determination of allantoin in blood by high-performance liquid chromatography with pre-column derivatization. J. Anim. Feed Sci. 4, 351-358

Devendra C., Lewis D., 1974. Fat in the ruminant diet : review. Indian J. Anim. Sci. 44, 917-938

Grant R.J., Weidner S.J., 1992. Effect of fat from whole soybeans on performance of dairy cows fed rations differing in fiber level and particle size. J. Dairy Sci. 75, 2742-2751

Grummer R.R., Melissa L.L., Barmore J.A., 1993. Rumen fermentation and lactation performance of cows fed roasted soybeans and tallow. J. Dairy Sci. 76, 2674-2681

Honing van der Y., Tamminga S., 1986. Effect of fat on rumen fermentation and gastrointestinal absorption. A seminar in the EC programme of coordination of agricultural research, organized by the National Institute of Animal Science, Forsogsanlag Foulum (Denmark), 25-27 June 1985, pp. 55-69

Hussein H.S., Merchen N.R., Fahrey G.C., 1996. Effects of forage percentage and canola seed on ruminal protein metabolism and duodenal flows of amino acids in steers. J. Dairy Sci. 79, 98-104

Khorasani G.R., Robinson P.H., de Boer G., Kennelly, 1991. Influence of canola fat on yield, fat percentage, fatty acid profile and nitrogen fractions in Holstein milk. J. Dairy Sci. 74, 1904-1911

Kowalczyk J., Ørskov E.R., Robinson J.J., Stewart C.S., 1977. Effect of fat supplementation on voluntary food intake and rumen metabolism in sheep. Brit. J. Nutr. 37, 251

Kowalczyk J., Skiba B., Buczkowski Z., Kowalik B., 1996. A device for quantitative urine collection from male sheep in balance trials. J. Anim. Feed Sci. 5, 297-301

Murphy M., Uden P., Palmquist D.L., Wiktorsson H., 1987. Rumen and total diet digestibility in lactating cows fed diets containing full-fat rapeseed. J. Dairy Sci. 70, 1572-1582

Ørskov E.R., 1992. Protein Nutrition in Ruminants. Academic Press Ltd., London

Ørskov E.R., 1994. Recent advances in understanding of microbial transformation in ruminants. Livest. Prod. Sci. 39, 53-60

Palmquist D.L., Jenkins T.C., 1980. Fat in lactation rations : review. J. Dairy Sci. 63, 1-14
Palmquist D.L., 1984. Use of fats in diets for lactating dairy cows. In: J. Wiseman (Editor). Fats in animal nutrition. Butterworths, London (UK), pp. 357-381
Palmquist D.L., 1994. The role of dietary fats in efficiency of ruminants. American Institute of Nutrition, pp. 1377-1382
Puchala R., Kulasek G.W., 1992. Estimation of microbial protein flow from the rumen of sheep using microbial nucleic acid and urinary excretion of purine derivatives. Can. J. Anim. Sci. 72, 821-830
Ryś R., 1993. Nutrient Requirements for Cattle and Sheep. Tables of Nutritional Value of Feedstuffs (in Polish). Institute of Zootechnic, Kraków
SAS, 1990. SAS/STAT Users guide (Release 6.03). SAS Institute Inc., Cary, NC, USA
Sklan D., Moallem U., Folman Y., 1991. Effect of feeding calcium soaps of fatty acids on production and reproductive responses in high producing lactating cows. J. Dairy Sci. 74, 510-517
Sutton J.D., Knight R., McAllan A.B., Smith R.H., 1983. Digestion and synthesis in the rumen of sheep given diets supplemented with free and protected oils. Brit. J. Nutr. 49, 419-432
Tackett V.L., Bertrand T.C., Jenkins T.C. Pardue F.E., Grimes L.W., 1996. Interaction of dietary fat and acid detergent fiber diets of lactating dairy cows. J. Dairy Sci. 79:270-275
Tsefa A.T., 1993. Effects of rape-seed oil supplementation on digestion, microbial amino acid composition in ruminants. Anim. Feed Sci. Technol. 41, 313-328
Wiseman J., 1984. (Editor). Fats in Animal Nutrition. Butterworths. London (UK)
Ziołecki A., Kwiatkowska E., 1973. Gas chromatography of C_1 to C_5 fatty acids in rumen and fermentation media. J. Chromatogr. 80, 250-254

STRESZCZENIE

Wpływ dodatku oleju rzepakowego na syntezę białka mikroorganizmów u owiec żywionych dawką składającą się z paszy treściwej i siana

Materiał doświadczalny stanowiły trzy tryki kaniulami żwaczowymi. Dawka pokarmowa składała się z paszy treściwej i siana, w stosunku wagowym 60:40%. Dawkę dzielono na dwie równe części i skarmiano w dwóch odpasach o godzinie 08:00 i 15:00. Doświadczenie przeprowadzono w układzie kwadratu łacińskiego 3x3 stosując trzy dawki:

I – kontrolna bez dodatku tłuszczu
II – z dodatkiem 3% oleju rzepakowego w sm dawki
III – z dodatkiem 6% oleju rzepakowego w sm dawki.

Próby treści żwacza pobierano przed odpasem oraz w 3 i 6 godzin po odpasie. W próbach oznaczano poziom lotnych kwasów tłuszczowych, azotu amonowego oraz pH treści żwacza. Rozmiar biosyntezy białka mikroorganizmów określano na podstawie ilości wydzielonej alantoiny w moczu. Dodatek 3 i 6% oleju rzepakowego nie miał wpływu na poziom pH treści żwacza. Dodatek tłuszczu nie miał wpływu na całkowity poziom lotnych kwasów tłuszczowych. Stwierdzono jedynie statystycznie istotny wzrost ilości kwasu walerianowego przy dodatku oleju rzepakowego. Wyraźnie zmieniał się także poziom azotu amonowego, który przy mniejszym dodatku oleju obniżał się z 14,8 do 11,0 mmol/l, a przy większym do 8,53; różnice te były statystycznie istotne. Dobowa produkcja białka mikroorganizmów zmniejszała się wraz z dodatkiem tłuszczu z 15 g do 13 g i 12 g N, odpowiednio w grupie II i III, ale różnice te nie były statystycznie istotne.