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Changes in fermentation processes as the effect of vegetable oil supplementation in *in vitro* studies

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ABSTRACT

The effect of 5% rapeseed, sunflower or linseed oil supplementation to a high-concentrate diet on basic rumen parameters, methane emission, rumen bacteria and protozoa counts was estimated *in vitro* in batch culture studies. The inclusion oils differing in fatty acid composition did not affect the pH or ammonia level of rumen contents but decreased VFA and butyric acid levels ($P \leq 0.05$), whereas the level of particular VFAs and the acetate-to-propionate ratio were not influenced by different oils. Protozoa number was significantly decreased by all supplemented oils, whereas no effect of fat on bacteria count was noted. As the number of unsaturated bonds in the supplemented oils increased, the protozoa counts decreased. Each oil only slightly decreased *in vitro* dry matter disappearance ($P > 0.05$). Methane emission was reduced ($P \leq 0.01$) when oils were added to the incubates.

KEY WORDS: rumen parameters, methane, bacteria, protozoa, vegetable oils, rumen

INTRODUCTION

Fat supplementation to ruminant diets can result in abnormal rumen fermentation, including a decreased level of volatile fatty acids and ammonia. The effect of added fat seems to be dependent on its fatty acid composition. According to McAllister et al. (1996) C18 polyunsaturated fatty acids are usually

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characterized as the most toxic to rumen microflora and have a negative impact on ruminal processes.

There is relatively little information available detailing the effect of fat supplemented to high-concentrate diets, usually fed to young growing animals,on rumen metabolites and methane emission, therefore the objective of this study was to examine the effects of three vegetable commercial oils supplemented to a high-concentrate diet on methane emission, basic rumen metabolites and number of microorganisms grown in a batch culture system.

MATERIAL AND METHODS

Rumen fluid was collected 2 h after feeding from Polish Merino ewes maintained on standard concentrate-hay. Rumen fluid was diluted with buffer (2:3) solution (292 mg K_2HPO_4, 240 mg KH_2PO_4, 480 mg $(NH_4)_2SO_4$, 480 mg NaCl, 100 mg $MgSO_4 \cdot 7H_2O$, 64 mg $CaCl_2$ $2H_2O$, 4 mg Na_2CO_3 and 600 mg cysteine hydrochloride per liter) and 40 ml transferred to incubation vessels containing 0.4 g substrate and incubated in anaerobic conditions at pH 6.5 and 39^0C in duplicate samples. The substrate was composed of, g: hay 0.156, wheat 0.125, barley 0.078, rapeseed meal 0.031, mineral mixture 0.09 for control group but supplemented with 0.019 g rapeseed (source of C18:1), sunflower (source of C18:2) or linseed (source of C18:3) oil.

After 24 h incubation, samples of gas to measure methane content (GC, Hewlett Packard) and liquid were taken for analysis of pH and bacteria; protozoa classified into *Entodiniomorphs* and *Holotrichs* were counted. The remaining fluid was centrifuged (10 000 × g, 4ºC, 15 min) and the cell-free supernatant was stored at -20ºC until analysis for VFA (GC, Varian, Star 3400 CX) according to Tangerman and Negengast (1996), and ammonia spectrometrically (Nessler method, absorbance 400 nm). *In vitro* dry matter disappearance (IVDMD) was calculated from the difference of the original dry sample and dry residue weights.

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

RESULTS

The inclusion of 5% of vegetable oils differing in fatty acid concentrations (Table 1) did not affect the rumen fluid pH or ammonia level. Each of the fat supplements reduced ($P \leq 0.05$) the level of volatile fatty acids from 102.1 mmol/L in the control group to 91.5, 81.4 and 78.9 mmol/L in incubates with rapeseed, sunflower or linseed, respectively. Linseed oil, rich in linolenic acid, was found to have the highest detrimental effect on VFA level. All vegetable oils decreased ($P \leq 0.05$) the level of butyric acid and, similarly as in the case of VFAs, the highest effect was obtained when linseed oil was added. Oils did not influenceparticular VFAs or the

acetate-to-propionate ratio, but the butyrate level was significantly depressed in incubates containing oils.

Table 1. Effect of vegetable oils on 24 h *in vitro* fermentation pattern of high-concentrate diets studied in batch culture (mean values)

Item	Treatment			
	control	rapeseed oil	sunflower oil	linseed oil
	n			
Rumen fluid properties				
pH	7.0	6.6	6.3	6.4
ammonia, mmol/L	13.6	14.5	13.0	12.5
volatile fatty acids, mmol/L	102. 1^{AA}	91.5^{AB}	81.4^{BB}	78.9^{BB}
acetate (A)	56.9	52.8	45.9	45.7
propionate (P)	25.4	22.5	20.4	19.4
butyrate	12.8^{AA}	10.7^{AB}	10.3^{AB}	9.1^{BB}
A/P	2.2	2.3	2.2	2.3
Rumen fluid microbial counts				
bacteria, 10^8 ml^{-1}	14.4	13.6	14.0	11.7
protozoa, 10^3 ml^{-1}	9.3^{AA}	7.5^{AB}	6.4^{BB}	6.0^{BB}
IVDMD[1], %	42.3	41.6	41.7	38.0
methane, mM	7.4^{aa}	5.4^{bb}	4.8^{bb}	3.7^{cc}

means with the same letter are not significantly different, $^{A, B}$- $P<0.05$; $^{a, b, c}$ - $P<0.01$
[1] *in vitro* dry matter disappearance

The number of protozoa significantly decreased as the number of unsaturated bonds in the supplemented oils increased, but the bacteria count was not depressed by oils.

IVDMD decreased slightly but not significantly in incubates supplemented with oils. Nevertheless, the type of oil supplemented reduced ($P<0.01$) methane emission, as production of methane decreased from 7.48 mM in the control incubate to 5.4, 4.8 and 3.7 mM in incubates with rapeseed, sunflower or linseed oils, respectively.

DISCUSSION

In the presented experiments, when the substrate in the rumen liquid was mostly concentrate, a detrimental effect of supplementing 5% fat as a vegetable oil on rumen processes was not demonstrated. Oils did not alter pH or ammonia levels, bacteria counts were similar in all groups. A high concentrate diet with different supplemented oils did not change the IVDMD. Only slight but significant decreases in the total VFAs, butyrate level, and protozoa count were observed. Similar results were reported by Dong et al. (1997) who did not find a direct effect of rapeseed oil in concentrate diets on pH and ammonia levels *in vitro*, but fat addition decreased the VFA level in

rumen fluid. However, incorporation of sunflower oil *in vitro* in experiments carried out by Ivan et al. (2001) decreased acetate-propionate ratio in oil-supplemented rumen liquid in comparison with controls. A stronger effect of fat on feed digestibility and carbohydrateand nitrogen metabolic pattern could probably be achieved at a higher level of fat supplementation, as was demonstrated in experiments *in vivo* on sheep by Kowalczyk et al. (1977), Jenkins (1993) and Doreau and Chilliard (1997). In summarizing factors affecting the level and proportion of fatty acids in rumen fluid Hvelplund (1991) included the type of diet fed and fat supplements.

As an indirect effect of limited protozoa counts, we observed the suppressing effect of unsaturated fatty acids from oils on methane emission. According to Machmüller et al. (1998) the reason for the methane-suppressing effects of fats either rich in saturated fatty acids or unsaturated ones was a direct effect against the rumen microbes involved in methane production.

CONCLUSIONS

Applied up to 5% in a diet composed mostly of concentrate, vegetable oils differing in fatty acid composition did not have a detrimental effect on the rumen fermentation pattern. However, oils can decrease the butyrate level and protozoa counts in the rumen liquid resulting in a decrease of methane emission.

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