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The effect of olive, sunflower or linseed oils on the fermentation pattern and methane production in the rumen simulating technique

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Abstract. The objective of this study was to investigate the effects of the addition of different vegetable oils to the diet on the fermentation pattern and methane production in Rusitec fermenters. For this purpose four treatments were randomly allocated to sixteen fermenters in a completely random design. Inoculum was obtained from four ruminally fistulated Merino sheep fed lucerne hay and concentrate. Treatments were defined by the diet supplied to the fermenters, so that the control treatment (C) diet was a total mixed ration for ewes on lactation. In the other experimental treatments the control diet was supplemented with olive oil (OO), sunflower oil (SO) or linseed oil (LO) to reach a final concentration of 50 g oil/kg diet. After 7 days of adaptation, samples of effluent, fermenters' fluid content and digesta of each fermenter were collected during 10 days. There were no significant differences (P>0.05) between treatments in pH values in the fermenters' fluid content. The LO diet showed (P<0.05) the highest dry matter, organic matter and NDF disappearance. The lowest values were observed with the SO diet (P<0.05). However, there were no differences for ADF disappearance (P>0.05). All three oils reduced methane production compared with control cultures (P<0.05), but there were no differences in the daily gas production. There were no differences in acetate production. The LO diet gave rise to the highest and the control diet the lowest propionate production (P<0.05), whereas butyrate production decreased as a result of any oil addition. The acetate to propionate ratio was also decreased by any oil addition. There were no significant effects (P>0.05) of oil supplementation on L-lactate concentration. Ammonia N concentration was higher (P<0.05) in the control treatment, but there were no significant differences between treatments in microbial protein output. In conclusion, these results indicate that fermentation pattern is affected by oil supplementation mainly by decreasing the acetate to propionate ratio, as well as methane production.

Keywords. Vegetable oils – Volatile fatty acids – Methane – Rusitec.

Effet de l'huile d'olive, de tournesol ou de graines de lin sur le patron de fermentation et la production de méthane dans le système de simulation ruminale RUSITEC

Résumé. *L'objectif de ce travail a été d'étudier les effets de 3 huiles végétales sur le patron de fermentation et la production de méthane dans le système RUSITEC. Nous avons évalué 4 traitements, appliqués à 16 fermenteurs selon un dispositif complètement aléatoire. L'inoculum a été obtenu de 3 brebis Mérinos alimentées à base de foin de luzerne et de concentré. Le traitement témoin (C) correspond à une ration complète pour des brebis en lactation. Concernant les autres traitements, le régime témoin a été supplémenté avec 50 g/kg d'huile d'olive (AO), d'huile de tournesol (AG) ou d'huile de lin (AL). Après 7 jours d'adaptation, des échantillons d'effluents, de liquide des fermenteurs et de digesta ont été prélevés tout au long d'une période de 10 jours. Aucune différence significative (P>0.05) du pH du contenu liquide des fermenteurs n'a été constatée entre les différents traitements. Le régime AL a montré (P<0.05) une plus importante disparition de matière sèche, matière organique et FDN. Les plus faibles taux de disparition ont été obtenues avec le régime AG (P<0.05). Néanmoins, il n'y a pas eu des différences au niveau de la disparition de FDA (P>0.05). En comparaison avec le traitement témoin, les 3 huiles ont réduit la production de méthane (P<0.05), mais il n'y a pas eu des différences dans la production de gaz et de l'acide acétique. La plus importante et la plus faible production de propionate ont été obtenues, respectivement, avec les traitements témoin et AL (P<0.05). La production de butyrate et le rapport acétate : propionate ont chuté avec l'addition des trois huiles. On n'a pas trou-*

vé des effets significatifs (P>0.05) de la complémentation avec des huiles sur la concentration de lactate. La concentration d'azote ammoniacal la plus élevée a été obtenue avec le régime témoin (P<0.05). Cependant, Les différents traitements ont abouti à la même synthèse des protéines microbiennes. En conclusion, ces résultats indiquent que la fermentation est affecté avec l'incorporation des huiles dans la ration en réduisant le rapport acétate : propionate et la production de méthane.

Mots-clés. *Huiles végétales – Acides gras volatils – Méthane – RUSITEC.*

I – Introduction

Additions of fat to feeds for ruminants have been a recurrent nutritional issue, aiming to increase milk fat content, to make the most of considerable quantities of inedible fats available to the feed industry and to match the energy requirements of high producing animals (Palmquist and Jenkins, 1980). More recently, driven by concerns on human health (Harris, 2008) and environmental sustainability, feeding lipids has received attention as a mechanism to increase conjugated linoleic acid in ruminant products (Chilliard *et al.*, 2000) and to reduce methane emission (Beauchemin *et al.*, 2007).

It is known that C:18 polyunsaturated fatty acids (sunflower oil and linseed oil) are among those fat sources with potential to both reduce methane production (Martin *et al.*, 2008) and increase conjugated linoleic acid or vaccenic acid in the rumen (AbuGhazaleh and Jacobson, 2007; Luna *et al.*, 2008). Potential adverse effects of fat addition, however, still deserve attention, depending on fat characteristics (chain length, saturation, esterification), basal diet composition (forage *vs* concentrate), and level of inclusion (Chalupa *et al.*, 1984; Doreau and Ferlay, 1994; Dewhurst *et al.*, 2006). Therefore, effects of specific oil sources on digestibility, rumen fermentation and microbial population need to be assessed. The Rumen Simulation Technique (Rusitec) is an experimental procedure that allows precise control of conditions and factors affecting fermentation and measurement of fermentation end-products formation during relatively long periods of time (Czerkawski and Breckenridge, 1977; Czerkawski and Breckenridge, 1979).

The objective of this study was to investigate the effects of the addition of different vegetable oils (olive, sunflower and linseed oils) to the diet on the fermentation pattern and methane production in Rusitec fermenters.

II – Materials and methods

1. Apparatus and experimental treatments

The study was carried out using sixteen fermentation vessels of the Rusitec system (Czerkawski and Breckenridge, 1977). Inoculum was obtained from four ruminally fistulated Merino sheep fed a diet consisting (per kg) of 700 g grass hay and 300 g concentrate. On the first day of the experiment, vessels were inoculated with 400 ml of strained rumen fluid diluted with 250 ml of artificial saliva and 80 g of squeezed rumen contents. Particulate rumen contents were placed inside a nylon bag (100 µm pore size) and then into the feed container together with a bag of fermentation substrate (15 g DM). After 24 h of incubation, bags with rumen contents were replaced by others with feed. Afterwards, bags with 48 h incubation residues were replaced with new bags containing feed. A continuous infusion of McDougall artificial saliva (pH 8.4) at a rate of 556 ml/day (dilution rate of 3.6%/h) was maintained through each vessel.

Four experimental treatments were defined by the diet used as fermentation substrate. Control treatment diet (C) consisted of a total mixed ration for ewes on lactation. In the other three treat-

ments the diet used in the control was supplemented by the addition of olive oil (OO), sunflower oil (SO) or linseed oil (LO) to reach a concentration of 50 g/kg. Ingredients and chemical composition are given in Table 1. Experimental diets to be fermented in Rusitec were prepared every three days and stored at 4ºC until use.

Table 1. Ingredients and chemical composition of the experimental diets

Treatment	C	OO	SO	LO
Ingredients (g/kg DM)				
Cracked corn grain	250	237.5	237.5	237.5
Barley grain	150	142.5	142.5	142.5
Soybean meal	200	190	190	190
Lucerne hay	200	190	190	190
Beet pulp	90	85.5	85.5	85.5
Molasses	60	57	57	57
Bicarbonate	15	14.3	14.3	14.3
Mineral vitamin premix	35	33.3	33.3	33.3
Olive oil	0	50	0	0
Sunflower oil	0	0	50	0
Linseed oil	0	0	0	50
Composition (g/kg DM)				
Organic matter	925	927	930	929
Crude protein	152	150	157	162
Neutral detergent fibre	236	240	237	233
Ether extract	21.7	73.4	72.3	70.7

The incubation trial consisted of a 7-day adaptation period followed by a 14-day collection period. On collection days 1, 2, 3, 8, 9 and 10, gas and liquid effluent were collected and measured. Samples of gas were taken to measure CH_4 concentration, and VFA, L-lactate and ammonia concentrations were determined in effluent. Bags withdrawn from the vessels after 48 h of incubation were used to determine DM disappearance, as a measure of diet digestibility. Chemical composition of dried incubation residues was determined to calculate digestibility of feed components.

On day 6 of the collection period, effluent and feed residues in bag were mixed (considered representative of whole digesta) with an electric blender for 1 min and processed to determine microbial protein synthesis by ^{15}N method as described by Carro and Miller (1999) and Giraldo *et al.* (2007), using a labelled microbial pellet obtained from differential centrifugation of digesta.

2. Chemical analysis

Experimental diets and incubation residues were analyzed to determine DM, ash and N according to the Association of Official Analytical Chemists (AOAC, 1999). NDF and ADF and lignin analyses were carried out according to Van Soest *et al.* (1991). Ether extract was determined by the filter bag technology, using an Ankom XT15 extraction system (Ankom®, Macedon, USA).

The volume of gas produced was measured with a drum-type gas meter (model TG1; Ritter Apparatebau GmbH, Germany) and CH_4 was analyzed with a gas chromatograph as described by García-González *et al.* (2008). VFA concentrations were determined by gas chromatography (López and Newbold, 2007). Ammonia concentration was measured by a colorimetric method (Weatherburn, 1967) and lactate by an enzymatic-colorimetric method (Taylor, 1996). Analyses of ^{15}N enrichment were performed by isotope ratio mass spectrometry (Carro and Miller, 1999).

3. Statistical analysis

Data were subjected to ANOVA using the GLM procedure of SAS (2002) for a complete randomized design with four replicates. The statistical model was $y_{ij} = \mu + O_i + e_{ij}$, where y_{ij} is the observation; μ the overall mean; O_i the supplemented oil and e_{ij} is the residual error. The random effect was fermentation vessel within treatment. Multiple comparisons of means among oil sources were performed using Tukey's test.

III – Results and discussion

There were not differences (P>0.05) between treatments in medium pH (Table 2). LO supplementation gave rise to higher OM, CP and NDF disappearance than SO treatment (P<0.05), with intermediate values in C and OO treatments (Table 2). Fat disappearance was higher in all supplemented treatments compared to control vessels (P<0.05). Although feeding lipids can inhibit fibre digestion in the rumen (Jenkins, 1993), our results are in line with those obtained in a Rusitec system by Jalc *et al.* (2007), who did not found differences in DM or NDF degradation when the diet was supplemented with oleic, linoleic or linolenic acids.

Table 2. Effects of oil addition on gas (total and methane), volatile fatty acids and L-lactate production and acetate to propionate ratio in the RUSITEC system

	C	OO	SO	LO	RSE	*P* ≤
pH	6.73	6.72	6.66	6.67	0.023	0.126
Disappearance of:						
Organic matter (fat free)	0.674^{ab}	0.708^{ab}	0.661^{b}	0.721^{a}	0.0254	0.022
Neutral detergent fibre	0.329^{ab}	0.347^{a}	0.290^{b}	0.354^{a}	0.0261	0.044
Crude protein	0.495^{ab}	0.549^{a}	0.476^{b}	0.547^{a}	0.0366	0.036
Fat	0.518^{c}	0.705^{b}	0.738^{b}	0.809^{a}	0.0286	<0.001
Gas production:						
Total (l/day)	3.01	2.75	2.81	2.98	0.131	0.470
CH_4 (mmol/g of OM fermented)	1.02^{a}	0.81^{b}	0.78^{b}	0.73^{b}	0.090	0.005
VFA (mmol/day)						
Acetate	24.9	23.8	22.0	24.1	0.91	0.206
Propionate	8.39^{b}	10.30^{ab}	9.87^{ab}	11.88^{a}	0.684	0.003
Butyrate	7.38^{a}	5.64^{b}	5.50^{b}	5.51^{b}	0.332	0.005
Total VFA	51.2	48.5	46.2	50.7	1.43	0.109
Acetate : propionate ratio	2.99^{a}	2.32^{b}	2.27^{b}	2.03^{b}	0.092	<0.001
L-lactate (mg/day)	5.56	6.67	7.27	6.48	0.708	0.431
Nitrogen metabolism						
Ammonia (mg/day)	89.1^{a}	72.4^{b}	65.0^{b}	65.0^{b}	3.17	0.001
Microbial protein (g/day)	1.06	1.02	0.90	1.14	0.091	0.353

As for volatile fatty acid production (Table 2), there were no differences in total VFA and acetate production (P>0.05). However, all supplemented treatments showed higher propionate and lower butyrate productions compared to the control. As a result, acetate to propionate ratio was noticeably decreased by oil addition in comparison with control. There were no differences on L-lactate concentration. Similar effects on VFA production and molar proportions have been observed by Getachew *et al.* (2001), Jalc *et al.* (2006, 2007) and Toral *et al.* (2009).

Effects of fat on ruminal fibre digestibility and fermentation parameters are known to vary depending on basal diet, esterification, saturation and level of fat. In the present work, fat was in form of triglycerides that are known to have lesser adverse effects on rumen fermentation (Chalupa *et al.*, 1984).

Compared to control all three oils reduced methane production (P<0.05), although there were no differences on total gas production (Table 2), in agreement with Gómez-Cortés *et al.* (2008) and Hervás *et al.* (2008) who observed (using the *in vitro* gas production technique) a lack of differences between control treatment (a high-concentrate diet) and the same diet supplemented with 6% of either soybean or sunflower oil, respectively. In general terms, it is known that lipid supplementation of diets reduces methane emissions by inhibiting organic matter fermented in the rumen, activity of methanogenic archae or ruminal protozoa, and through the disposal of hydrogen during the fatty acid biohydrogenation process (Johnson and Johnson, 1995). In the present work the reduction on methane production was not due to lower organic matter fermentation (Czerkawski *et al.*, 1966; Zhang *et al.*, 2008), and it seems likely that methanogenic archae could be affected. The lack of differences among the different oils used as supplements is in agreement with Jalc *et al.* (2007) and Beauchemin *et al.* (2007).

Ammonia concentration was higher in the control treatment (P<0.05) than in the treatments including oil. Beauchemin *et al.* (2007) observed that the only major effect of lipid feeding on ruminal fermentation was a reduction in ruminal ammonia, without differences among lipid sources. However there were not significant differences among treatments in microbial protein synthesis (Table 2). In line with our results, addition of oleic, linoleic or linolenic acid to a basal diet of high quality pasture in a continuous fermenter did not affect microbial synthesis (Kolver *et al.*, 2002).

IV – Conclusion

In conclusion, these results indicate that a 5% inclusion level of olive, sunflower or linseed oil to a diet for dairy ewes affects the fermentation pattern mainly by decreasing the acetate to propionate ratio and methane production.

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