Influence of the degree of dietary fatty acid unsaturation on rumen fermentation parameters

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SUMMARY – The influence of the degree of dietary fatty acid unsaturation on rumen fermentative activity was tested by comparing two extruded oilseeds (rapeseed or R and linseed or L) incorporated at a level of 8% dry matter (DM) in ruminant diets. With the R diet (rich in $C_{18:1}$), the ruminal pH of fistulated goats was statistically lower and the number of protozoa was higher than with the L diet (rich in $C_{18:3}$). However, the molar composition of the volatile fatty acid (VFA) did not differ between diets at the different sampling times. The *in sacco* dry matter degradability of the diets was measured on fistulated cows adapted to the experimental diets. It was higher with the R diet. In conclusion, the increase in the degree of dietary fatty acid unsaturation seems to decrease the ruminal fermentative activity.

Key words: Dietary fatty acids, unsaturation, oilseeds, rumen, ruminants.

RESUME – *"Influence du degré d'insaturation des acides gras sur les paramètres de fermentation ruminale". Afin de mesurer l'effet du degré d'insaturation des acides gras alimentaires sur l'activité ruminale, nous avons comparé deux graines extrudées (colza ou C et lin ou L) incorporées à un niveau de 8% de matière sèche (MS) dans des régimes destinés à des ruminants. Le régime C (riche en $C_{18:1}$) a induit un pH ruminal des chèvres fistulées statistiquement plus faible que le régime L (riche en $C_{18:3}$). Le nombre des protozoaires a été plus élevé avec le régime C qu'avec le régime L, mais la composition molaire en acides gras volatils (AGV) n'a pas été influencée par le changement de régime. La dégradabilité* in sacco *de la matière sèche des régimes a été mesurée sur des vaches recevant les deux régimes expérimentaux. Elle a été plus élevée pour le régime C. En conclusion, l'augmentation du degré d'insaturation des matières grasses alimentaires semble décroître l'activité fermentaire du rumen.*

Mots-clés : *Matière grasse alimentaire, insaturation, graines oléagineuses, rumen, ruminants.*

Introduction

Fat is often given in early lactation to ruminants because its high energy level is of particular importance at a physiological stage when energy intake is a limiting factor for milk production. Several authors pointed out that fat might alter the ruminal microbial ecosystem, because it often has a negative effect on feed intake and fibre digestibility (Doreau and Chilliard, 1997). This effect is more noticeable with polyunsaturated fatty acids (Czerkawski, 1973; Palmquist and Jenkins, 1980; Sutton *et al*., 1983).

The objective of this study was to test the influence of the degree of dietary fatty acid unsaturation of extruded oilseeds on rumen fermentative activity. The extrusion process is of interest because it partially protects protein from ruminal degradation (Clinquart *et al*., 1993). Therefore, in the literature, an extruded oilseed has been usually compared to a control seed (McGuffey and Schingoethe, 1982; Ferlay *et al.*, 1992) rather than to another extruded oilseed. In this paper, we compared two extruded oilseeds [rapeseed (R) and linseed (L)] differing in the degree of their dietary fatty acids unsaturation: they were respectively rich in $C_{18:1}$ and $C_{18:3}$. This kind of oilseeds can modify significantly milk fatty acids composition, and especially the C_{18} fractions (Duvaux-Ponter *et al.*, 2001; Giger-Reverdin *et al.*, 2001). We used two complementary approaches: the *in vivo* kinetics of rumen fermentation parameters and the *in sacco* dry matter degradability of the experimental diets with fistulated animals receiving these diets.

Material and methods

Diets

The oilseeds were incorporated at a level of 8% DM in the diet which also included corn silage (50% DM) and dehydrated alfalfa (10% DM). The rest of the diet was made up of concentrate (32% DM) in order to meet the requirements of dairy goats fed in early lactation. Diets were offered *ad libitum* after morning milking (around 08:00) which is considered as T0.

In vivo kinetics of rumen parameters

Four fistulated goats in mid-lactation received the 2 diets (2 goats for each diet). After 4 weeks of adaptation, ruminal fluid was collected before the morning feed (time = 0) and 2, 4, 6 and 8 hours after feeding. Kinetics were performed in duplicate on each goat on 2 separate days. The pH was immediately recorded. Samples were quickly deep-frozen at –20°C with 5% orthophosphoric acid until their analysis for volatile fatty acid (VFA) content by capillary chromatography (Broudiscou and Lassalas, 2000). The number of protozoa was also estimated 6 hours after the morning meal by the method described by Broudiscou *et al.* (1997).

In sacco dry matter degradability

A modification of the nylon bag technique proposed by Michalet-Doreau *et al.* (1987) was used to measure the disappearance of dry matter (DM) of the two diets. Bags were removed after 2, 4, 8, 16, 24 and 48 hours of incubation. Each of the three fistulated cows received 8 kg DM of the two experimental diets at two different periods instead of the standard diet used for determining the protein dispersibility index (PDI) value of feedstuffs (Michalet-Doreau *et al.*, 1987). These daily allowances met the requirements of the animals. Within each period, 2 kinetics of measurements were performed.

Statistical analysis

In vivo *kinetics of rumen parameters*

The statistical design was a double-repeated one (duplicate day and several samplings on the same animal at a given day). We used a split-plot analysis with the "AR(1) proc mixed" procedure of SAS/STAT (1987). We took into account the following effects: diet, day, hour of sampling and their interactions. This model was used since the same goats were sampled at different times (Littell *et al*., 1998).

In sacco *dry matter degradability*

These results were also analysed with a proc mixed procedure. Following effects were tested: diet received by the cow, feedstuff, duration of incubation and their interactions.

In all the statistical procedures, we removed from the model non-significant interactions or effects.

Results and discussion

The rapeseed meal induced more fermentation than the linseed one, especially during the first 2 hours of fermentation. The pH decreased first very quickly and significantly. It reached a plateau value of 6.03 after 4 h and then increased significantly after 6 h, just before the second meal of the day (Fig. 1). Linseed diet had a pH significantly higher than the rapeseed one, but there was no interaction diet-hour of sampling.

The quantity and the different percentages of VFA were studied at T0, T4 and T8. The dietary effect was only significant for total VFA in agreement with pH variations (Table 1).

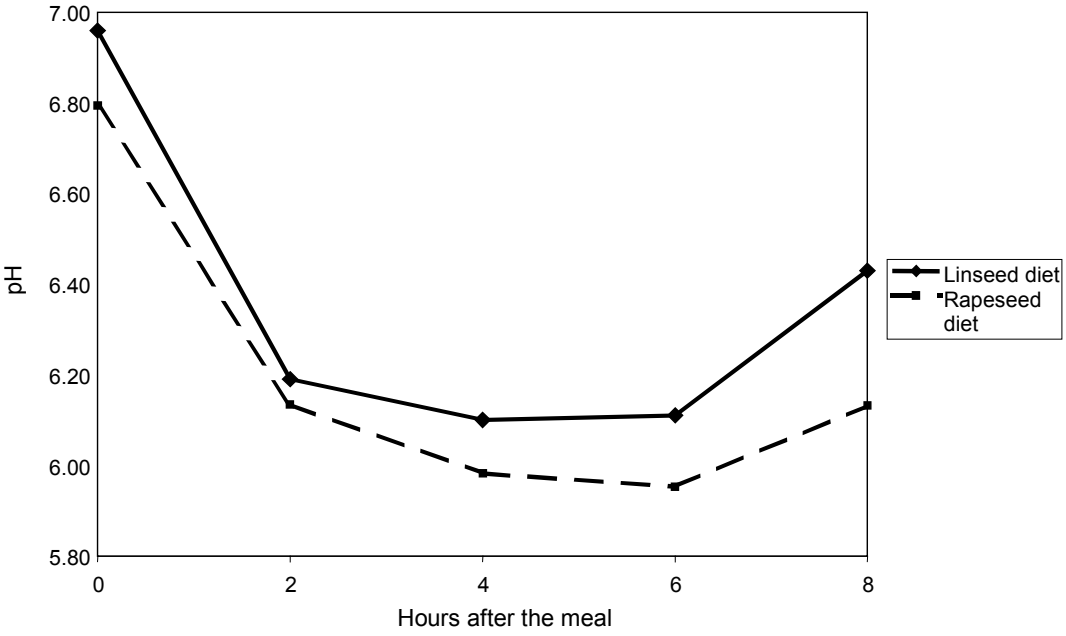

Fig. 1. Evolution of pH after feeding in four fistulated goats receiving either a linseed diet or a rapeseed one.

Table 1. Dietary effect on the ruminal kinetics for the concentration of volatile fatty acids (VFA) and percentages of the different VFA

Diet	Sampling time					
	0 h		4 h		8 h	
	Rapeseed	Linseed	Rapeseed	Linseed	Rapeseed	Linseed
VFA (mmoles/l)	95[a]	61[b]	165[c]	152[c,d]	146[d]	118[e]
% C2	65.5[a]	63.6[a]	62.0[b,c]	60.2[b]	61.8[c]	62.4[c]
% C3	16.6[a]	16.5[a]	22.0[b]	22.4[b]	22.3[b]	20.6[b]
% iC4	1.6[a]	2.5[b]	1.0[c]	0.7[c]	1.0[c]	0.9[c]
% C4	12.6	12.2	12.3	13.2	12.5	13.2
% iC5	2.2[a]	3.2[b]	0.9[c]	0.8[c]	0.9[c]	0.9[c]
% C5	1.1[a]	1.0[a]	1.4[b]	1.2[a,b]	1.3[b,c]	1.1[a,c]
% C6	0.1	0.1	0.3	0.4	0.2	0.4

[a,b,c,d,e]Within a row, values with the same letter do not differ significantly ($P > 0.05$).

The VFA composition was modified by dietary intake since the acetic acid percentage decreased very quickly while the percentage of propionic acid increased in the first 2 hours after the morning feeding. This is in agreement with the specific effect of corn silage or the addition of fat, which enhance propionate production (Broudiscou *et al.*, 1990). The non-significant dietary effect is in agreement with the results of Sutter *et al.* (2000) who compared rapeseeds and linseeds and with the review of Sauvant and Bas (2001) who pointed out that the degree of unsaturation of fatty acids did not significantly affect the C_2/C_3 ratio.

After 6 hours of fermentation, the number of total protozoa was significantly higher for the rapeseed meal compared to the linseed one ($1.36/10^6$ ml vs $1.05/10^6$ ml). This was due only to the holotrich protozoa. This agrees with Sauvant and Bas (2001) who stressed that the increase in dietary fatty unsaturation decreased the ruminal density of protozoa. Sutter *et al.* (2000) found a non-

significant decrease when replacing rapeseed by linseed. These high values point out that extruded seeds do not have a defaunating effect on the contrary to oils (Sutton *et al.*, 1983; Broudiscou *et al.*, 1990).

Whatever the duration of the incubation, DM degradability of the samples (diets R or L offered to goats) was higher when incubated in cows receiving the rapeseed diet (dotted lines, see Fig. 2). This could be related with a linearly decrease in apparent ruminal neutral detergent fibre (NDF) digestibility when esterification of fat decreased (Elliott *et al*., 1997).

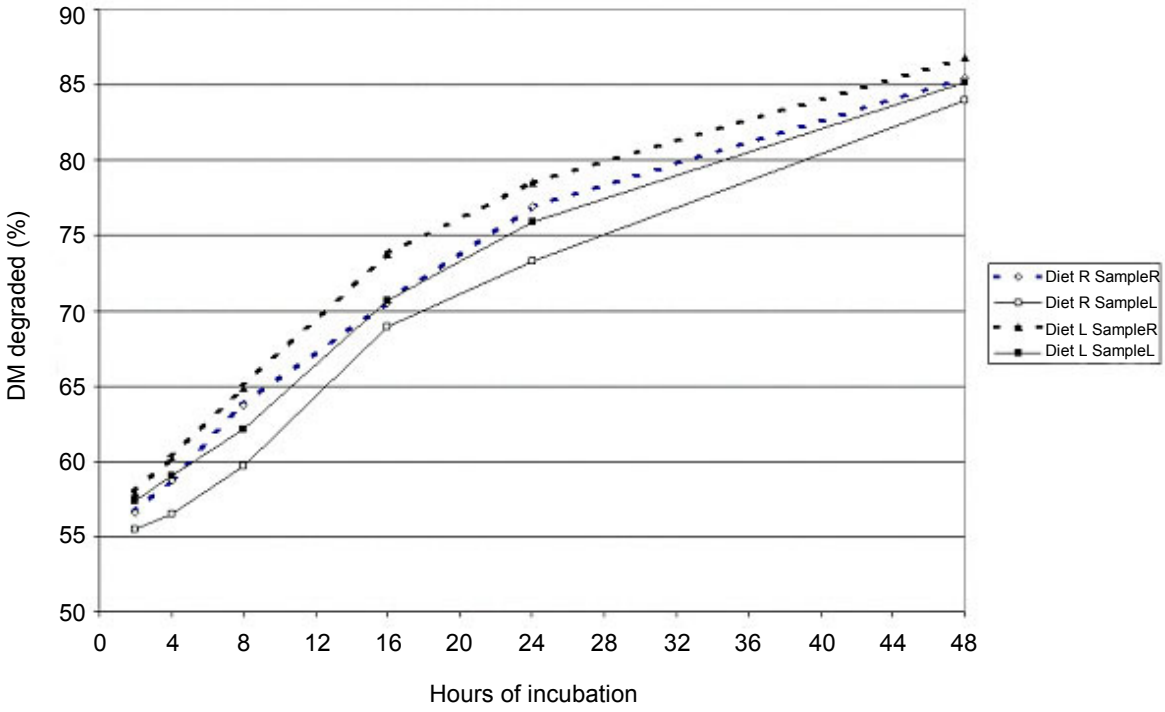

Fig. 2. Effect of the diets given to fistulated cows on *in sacco* dry matter degradability of diets samples (R: rapeseed, L: linseed).

The samples of the rapeseed diet (Sample R) were significantly less degraded than those of the linseed one (Sample L), whatever the kind of diets received by the cows. The differences were statistically significant between 4 and 24 hours of incubation. There was no significant interaction between samples and diets. This means that the rapeseed diet which was less degraded than the linseed one, seemed to induce less fermentation in cows, on the contrary to goats.

Conclusion

These two experiments bring new information about extruded oilseeds that had been seldom studied on the contrary to oils. They indicated that the degree of dietary fatty acid unsaturation had an influence on rumen fermentation. When it was increased, fermentation seemed to be less efficient, but the proportion of the different VFA in the rumen remained the same.

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