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Effects of Heat Processing of Soybeans and Linseed on Ruminal Fatty Acid Biohydrogenation *in situ*

Research Article

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ABSTRACT

The aim of this study was to determine and compare *in situ* biohydrogenation (BH) fatty acids in three forms of soybeans and linseed (raw, extruded and roasted). Nylon bags (5×10 cm) containing 4 g of raw, extruded or roasted soybeans or raw, extruded or roasted linseed were incubated in the rumen of fistulated ewes for 4, 8, 12 and 24 hours. Results for linoleic acid (C18:2) showed that the extent of BH only in 4h of incubation was significantly higher than other times of incubation in raw linseed ($P<0.05$). Proportions of linoleic acid (C18:2) were significantly higher for raw compared to extruded and roasted soybeans for 8 and 12 hours of incubation ($P<0.05$). Amounts of trans-C18:1 (octadecadienoic acid) in all 4 times of ruminal incubation were similar between each three forms of linseed ($P>0.05$). But proportions of this fatty acid in roasted soybeans were significantly lower than raw and extruded soybeans in 12 h of incubation ($P<0.05$). No significant difference was observed for extent of linolenic acid (C18:3) BH between three different forms of linseed and soybeans ($P>0.05$). Differences were not significant between produced cis-9, trans-11 conjugated linoleic acid (CLA) in three forms of linseeds in all times of incubation ($P>0.05$), but amounts of CLA in 12 and 24 hours of incubation were significantly higher for extruded soybeans ($P<0.05$). Proportions of stearic acid (C18:0) for the three forms of linseed (raw, extruded and roasted) in all hours of incubation showed no significant difference ($P>0.05$). The amount of C18:0 after 24 h of incubation was significantly higher for raw soybeans ($P<0.05$). According to the results, heat processing had better effects on the preservation of linoleic acid compared to linolenic acid in soybeans and linseeds against rumen BH.

KEY WORDS biohydrogenation, heat processing, *in situ*, linseed, soybeans.

INTRODUCTION

In recent years, the levels and sources of supplemental fat in the ruminants' diet has been recognized, especially in lactating dairy ruminants. Oilseeds are the most commonly used supplemental sources of lipids in the diet of ruminants. Most naturally occurring fats are present in plants as triglycerides, phospholipids or glycolipids, and these esters can be hydrolyzed by ruminal bacteria (Kaleem *et al.* 2013b) releasing fatty acids and glycerol.

If the released fatty acids are unsaturated BH is likely to occur (Reiser, 1951) (Figures 1 and 2), as unsaturated fatty acids are potentially toxic to bacteria, so many ruminal bacteria use BH as a detoxification reaction (e.g., *B. fibrisolvens*) (Harfoot *et al.* 1973a). Oilseeds (such as linseed) have fatty acids with 3 double bonds (α or γ linoleic), so these acids are hydrogenated in the rumen via conjugated intermediates. Awareness and control of the BH rate of unsaturated fatty acids is very important in the ruminant, as the absorption of unsaturated fatty acids by ruminants can

influence reproduction (e.g. establishment of puberty (Smith *et al.* 1989), semen production (Castellano *et al.* 2010), and follicle development and quality of oocytes (Staples and Thatcher, 2005; Bilby *et al.* 2006c), and production e.g. milk (Rego *et al.* 2004; Bernal *et al.* 2010) or meat (Wood *et al.* 2003) quality.

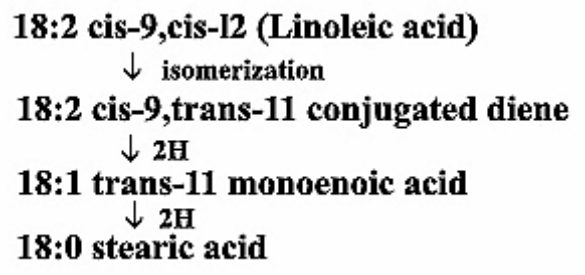

Figure 1 Sequence of reactions in the BH of linoleic acid in the rumen

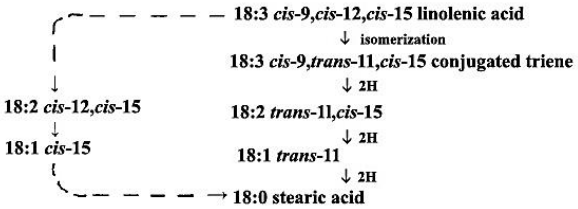

Figure 2 Metabolic pathways in the BH of linolenic acid in the rumen

The process of creating ruminant feeds often requires the oilseeds to be processed using heat; for example by extrusion, roasting or flaking (Table 2) (Troegeler-Meynadier *et al.* 2014). Treatments such as extrusion reduce the moisture level of feeds (Kaleem *et al.* 2013b). However the effect of heat processing on the extent of BH is not clear, with variable results obtained from in *in situ* (Agazzi *et al.* 2004; Akarim *et al.* 2006; Chouinard *et al.* 1997; Enjalbert *et al.* 2003; Perrier *et al.* 1992), *in vivo* (Gonthier *et al.* 2005) and *in vitro* (Akarim *et al.* 2006; Enjalbert *et al.* 2003; Harfoot *et al.* 1973a; Jenkins and Adams, 2002) studies. Differences in rumen function, particularly pH, may be responsible for the variability in the *in vivo* studies (Van and and Demeyer 1996) whereas the presence/absence of food particles may be a significant factor producing the *in vitro* variability as 80% of the BH occurs in association with the fine food particles (Harfoot *et al.* 1973a; Harfoot *et al.* 1973b). Understanding the impact of heat on the BH of unsaturated fatty acids from oilseeds is important as soybeans and linseed are the most common sources of linoleic acid (C18:2) and linolenic acid (C18:3), respectively, in the ruminant's diet. The aim of this study was to determine the effects of type of heat processing of soybeans and linseed on BH using an *in situ* study.

MATERIALS AND METHODS

In situ experiment

Three fistulated, 3-year-old, Zel ewes with an average weight of 40 kg were used in this study. Prior to the start of the study, all three ewes were fed an adaptation diet based on corn silage, wheat straw and a mix of soybeans and linseed (5% dry matter/diet) for 2 weeks (Table 1). All ewes were kept in individual stalls.

Table 1 Components of ewe's adaptations diet for two weeks prior to the rumen incubation

Diet ingredients (%)	Amounts in diet (% DM)
Maize silage	27.00
Wheat straw	19.30
Barley grain	18.65
Corn grain	13.60
Beet pulp	6.15
Wheat bran	4.10
Soybean meal (44%)	2.10
Molasses	3.40
Dicalcium phosphate	0.40
Supplement mixture fats (linseed and soybeans)	5.00
Salt	0.30
Diet nutrients	
Protein	9.04
Fat	6.8
NDF	26.4

NDF: neutral detergent fiber and DM: dry matter.

Nylon bags (5×10 cm, with pore size 45 µm) containing 4 g of test material and 1 g of straw were placed into the rumen through the fistula and incubated for 4, 8, 12 or 24 hours (3 bags per ewe per incubation time). The straw was added to facilitate distribution of fatty acids, prevent fat agglomeration and limit leaching of fatty acids into the rumen (Agazzi *et al.* 2004).

Samples were ground using a ball mill for 1 min (0.5 mm diameter screen). After incubation, the bags were withdrawn and washed with cold water until the water was clear in order to remove any feed particles and prevent further fermentation.

The washed bags were then dried at 65 ˚C for 48 h or until they reached a constant weight, whichever was longer. The bags were then frozen at -18 ˚C for at least 24 h. Before testing the bags were thawed and washed as described earlier. The 0 h samples used the same test volumes and the same nylon bags but were not incubated. After the samples were placed in the bag was washed for 5 min as described above, except that the water was at 25 ˚C.

Chemical analyses

Fatty acid extraction was undertaken using the same method as Park and Goins (1994).

Table 2 The fatty acid composition of the three forms (raw, extruded or roasted) of soybeans and linseed used in experiment

Fat source	Fatty acid profiles (%)				
	C16:0 Palmitic acid	C18:0 Stearic acid	C18:1 Oleic acid	C18:2 Linoleic acid	C18:3 Linolenic acid
Raw linseed	5.05	4.65	20.38	14.58	51.36
Extruded linseed	7.64	5.22	20.05	14.81	51.28
Roasted linseed	8.41	5.75	19.31	14.11	49.96
Raw soybeans	11.84	3.81	21.10	53.31	4.65
Extruded soybeans	9.65	4.43	21.64	50.21	4.90
Roasted soybeans	9.80	4.38	20.81	50.48	4.82

The concentrations of fatty acids methyl esters were measured using gas chromatography, with borontrifluoride being replaced by a solution of methanol-acetylchloride in ratio of 10:1 (Enjalbert *et al.* 2003). The proportions of linoleic acid (C18:2), trans-octadecenoic acid (trans-C18:1), linolenic acid (C18:3), CLA and stearic acid (C18:0) were then calculated. The extent of BH in the rumen, was calculated using the model developed by Enjalbert *et al.* (2003) model based on the equation recommended by Ørskov and McDonald (1979).

$P = P_0 e^{-c(t-l)}$.

P= percentage disappearance at time t.

P_0= initial proportion of unsaturated fatty acids

C= extent of BH.

l= lag time before BH begins.

Statistical analysis

The data were analyzed with the MIXED procedure of SAS (2009), with individual means compared using Duncan's range test the model used was follows:

$$Y_{ij} = \mu + L_i + C_j + P_k + e_{ijk}$$

Where:

Y_{ij}: dependent variable.

μ: overall mean.

L_j: effect of oilseed's forms.

C_j: effect of ewes.

P_k: effect of incubation times.

e_{ij}: error term.

RESULTS AND DISCUSSION

Figures 3 to 12 show the changes in each of the 18-carbon unsaturated fatty acid, for all of the three processing procedures (raw, extruded and roasted) for both linseed and soybean for 4, 8, 12 and 24 h of incubation, respectively. No differences in ruminal BH of linoleic acid in 8, 12 and 24 h of incubation were seen between extruded, roasted and raw linseed (P>0.05), but at 4 h of incubation BH of linoleic acid was significantly higher in the raw linseed sample than the extruded and roasted linseeds (P<0.05) (Figure 3). No significant difference between three forms of linseed was found during ruminal incubation for the proportion of trans-octadecenoic acid (trans-C18:1) (Figure 4), CLA (Figure 5), or BH of linolenic acid (P>0.05) (Figure 6).

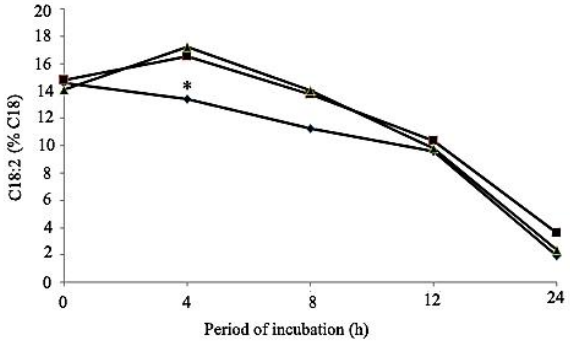

Figure 3 Evolution of linoleic acid (C18:2) percentages reported to the C18 quantities according to the duration of incubation and the heat treatment of linseed: raw linseed (♦), extruded linseed (■) and roasted linseed (▲)

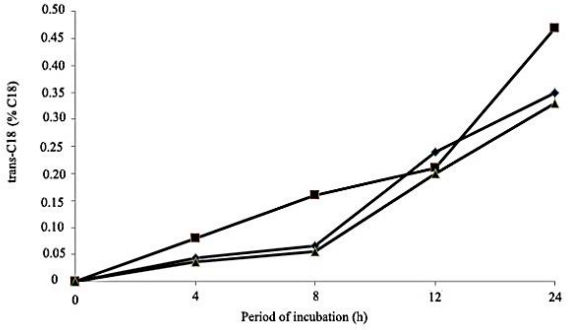

Figure 4 Evolution of *trans*-octadecenoic acid (*trans*-C18:1) percentages reported to the C18 quantities according to the duration of incubation and the heat treatment of linseed: raw linseed (♦), extruded linseed (■) and roasted linseed (▲)

Although the amount of CLA produced from raw linseed tended to be lower at 12 h of incubation (P=0.108) (Figure 5). The process of stearic acid (C18:0) production in nylon bags through BH was similar for all forms of linseed during incubation (P>0.05) (Figure 7).

The trans-octadecenoic acid (trans-C18:1) amount (Figure 9) was significantly lower in the roasted soybeans sample than the raw and extruded soybeans in 12 h of incubation (P<0.05).

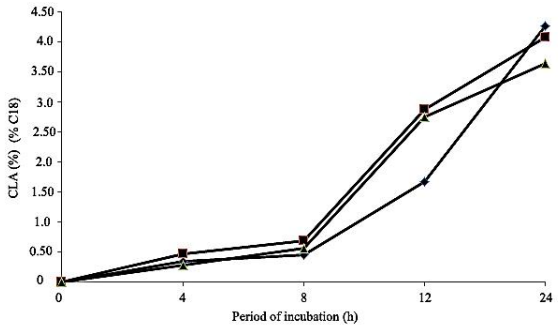

Figure 5 Evolution of conjugated linoleic acids (CLA) percentages reported to the C18 quantities according to the duration of incubation and the heat treatment of linseed: raw linseed (♦), extruded linseed (■) and roasted linseed (▲)

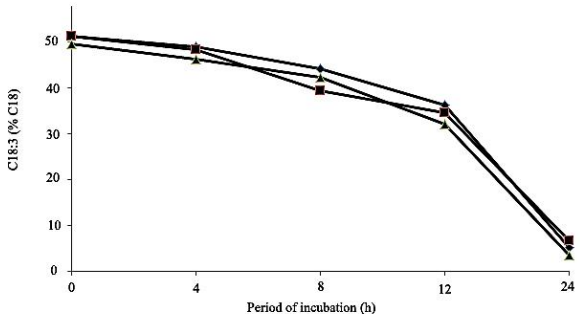

Figure 6 Evolution of linolenic acid (C18:3) percentages reported to the C18 quantities according to the duration of incubation and the heat treatment of linseed: raw linseed (♦), roasted linseed (■) and extruded linseed (▲)

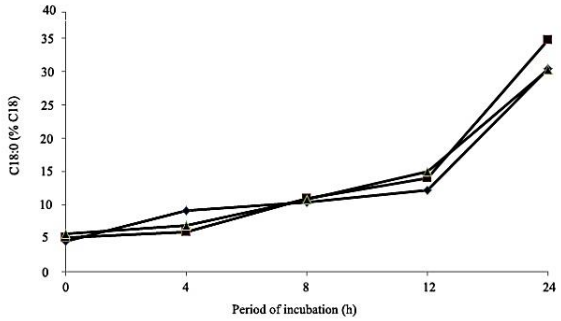

Figure 7 Evolution of stearic acid (C18:0) percentages reported to the C18 quantities according to the duration of incubation and the heat treatment of linseed: raw linseed (♦), extruded linseed (■) and roasted linseed (▲)

The disappearance of linoleic acid was greater for raw soybeans than extruded and roasted soybeans after 8 and 12 h of incubation (P<0.05) (Figure 8). No effect of heat treatment on the linolenic acid (C18:3) BH in the three forms of soybeans was seen (P>0.05) (Figure 11). The proportion of CLA in 24 h of incubation was about 2 and 5 times higher with roasted and extruded soybeans than raw soybeans, respectively (P<0.05).

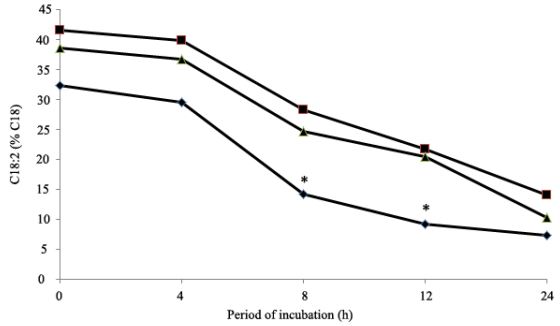

Figure 8 Evolution of linoleic acid (C18:2) percentages reported to the C18 quantities according to the duration of incubation and the heat treatment of soybeans: raw soybeans (♦), extruded soybeans (■) and roasted soybeans (▲)

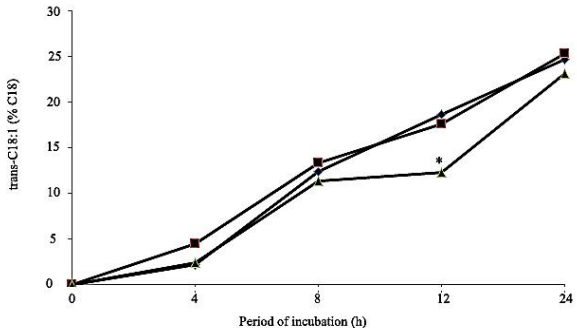

Figure 9 Evolution of *trans*-octadecenoic acid (*trans*-C18:1) percentages reported to the C18 quantities according to the duration of incubation and the heat treatment of soybeans: raw soybeans (♦), extruded soybeans (■) and roasted soybeans (▲)

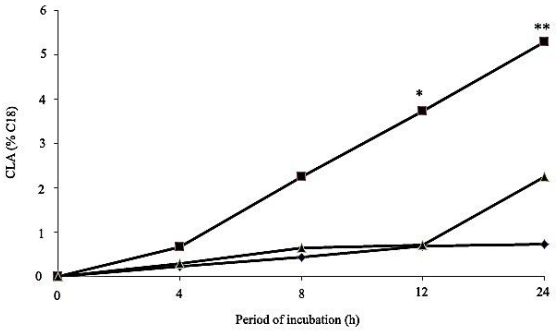

Figure 10 Evolution of conjugated linoleic acids (CLA) percentages reported to the C18 quantities according to the duration of incubation and the heat treatment of soybeans: raw soybeans (♦), extruded soybeans (■) and roasted soybeans (▲)

Moreover, the amount produced of CLA in 12 h of incubation was significantly higher in extruded than raw and roasted soybeans (P<0.05) (Figure 10).

Stearic acid (C18:0) production (Figure 12) from raw soybeans was about 2 times higher than that for extruded and roasted soybeans, but only in 24 h of incubation (P<0.05).

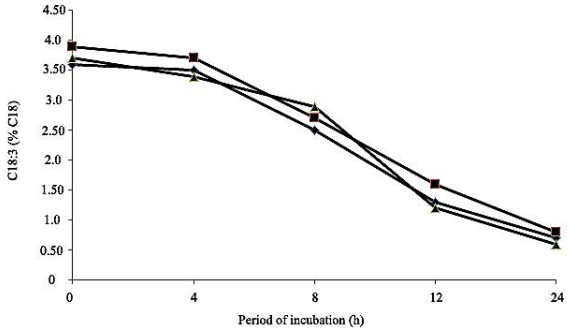

Figure 11 Evolution of linolenic acid (C18:3) percentages reported to the C18 quantities according to the duration of incubation and the heat treatment of soybeans: raw soybeans (♦), extruded soybeans (■) and roasted soybeans (▲)

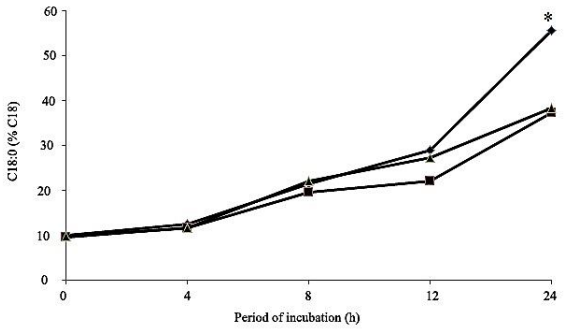

Figure 12 Evolution of stearic acid (C18:0) percentages reported to the C18 quantities according to the duration of incubation and the heat treatment of soybeans: raw soybeans (♦), extruded soybeans (■) and roasted soybeans (▲)

Through Figures 3 to 12, asterisks indicate a significant statistical difference between the treatments in similar time (P<0.05).

These results are consistent with those reported by Akarim *et al.* (2006), showing no significant difference in the extent of BH of linoleic and linolenic acids between processing forms (preconditioning and extrusion) of linseeds. In the other *in vitro* experiments, similar rates of BH for linoleic acid (C18:2) in dairy cows fed soybean oil (5% DM in diet) have been reported (Beam *et al.* 2000). Perrier *et al.* (1992), using ground soybeans found that 40% of the linoleic acid disappeared after 8 h of incubation. In most of the experiments, the range of disappearance of linoleic acid was between 19 to 48% (Agazzi *et al.* 2004; Reddy *et al.* 1994).

In our experiment, heat processing had no significant effect on the extent of BH contents of linolenic acid in soybeans. *In situ* versus *in vitro* ruminal BH of unsaturated fatty acids from canola oils showed higher lag time (Enjalbert *et al.* 2003). They suggested that after 24 h of incubation, BH of linolenic and linoleic acids from *in situ* studies were not complete. *In situ* studies of raw and processed soybeans, canola and sunflower seeds showed that rate of disappearance is 2 to 4 times higher than disappearance rate of dry matter. Furthermore, rate of disappearance of polyunsaturated fatty acids (PUFA) were more than saturated fatty acid. This is due to more outflow of PUFA from nylon bags with feed particles and also higher rate of BH in PUFA compared to monounsaturated fatty acids (Agazzi *et al.* 2004; Troegeler-Meynadier *et al.* 2006b). In our study, the production of *trans*-octadecenoic acid (trans-C18:1) for extruded linseed was higher compared to raw and roasted linseeds. Similarly, Akarim *et al.* (2006) found that the extrusion of linseed increased proportion of trans-octadecenoic acid (trans-C18:1) in 16 h of ruminal incubation. They suggested that extrusion of oilseeds could be a suitable way for protection of unsaturated fatty acids against ruminal BH. Differences between studies could be due to the temperature of extrusion. For soybeans, amounts of trans-octadecenoic acid (trans-C18:1) decreased during 16 and 24 h of incubation (Agazzi *et al.* 2004). According to our results, amounts of this intermediate fatty acid during ruminal BH for roasted soybeans for 12 h of incubation were significantly lower (P<0.05). Higher levels of *trans*-octadecenoic acid (trans-C18:1) was reported in milk composition in lactating dairy cows fed extruded compared to roasted soybeans (Chouinard *et al.* 1997).

The CLA is an intermediate composition synthesis during BH. Previous studies showed that production of CLA for extruded linseed was lowest during 16 h in ruminal incubation (Agazzi *et al.* 2004; Akarim *et al.* 2006). Kaleem *et al.* (2013a) found no significant difference for ruminal CLA in dairy cows fed roasted soybeans. In other study, production of CLA for extruded soybeans during 12 and 24 h of incubation was higher than other incubation hours. Production of stearic acid (C18:0) is the final step of unsaturated fatty acids BH in the rumen. Once liberated as free fatty acids, any unsaturated fatty acids are subject to BH by rumen bacteria, the end-product of this hydrogenation being stearic acid (18:0). In previous experiments, minimum amounts of stearic acid production for raw incubated soybeans in 2 hours after incubation reported by Troegeler-Meynadier *et al.* (2014). While the highest amounts of stearic acid proportions in the rumen were reported after 16 h incubation of nylon bags (Reddy *et al.* 1994; Chouinard *et al.* 2001). In other study, Troegeler-Meynadier *et al.* (2006b) showed that heat treatments of soybeans (extruded and roasted) *in*

situ had no significant effects on production of stearic acid (C18:0) during 6, 12, 18 and 24 h of ruminal incubation. While, they reported that raw soybeans had more amounts of stearic acid (c18:0) in all times of ruminal incubation.

CONCLUSION

Results of the effect of heat processing on ruminal BH of oilseeds are variable. Temperature employed and methods of heat and pressure application for processing of linseeds could affect the rate of ruminal BH. However, application of heat and pressure could be favorable means for decreasing the extent of BH in the rumen. *In situ* BH of unsaturated fatty acids is slower than *in vitro*. So, comparing the extent of ruminal BH for both methods does not seem logical. As a result, extrusion and roasting of linseeds as for economic justification could add to ruminant's diet as an energy source and according to our results, we recommend that extruded oilseeds add to ruminant's diet as fat sources for their more resistance against ruminal BH.

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