Effects of previous diet and duration of soybean oil supplementation on conjugated linoleic acid and octadecenoic isomers in lamb meat.

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Abstract:

Forty Merino Branco lambs were housed and randomly assigned to four groups. The trial duration was 42 days divided in 3 periods of 14 days. The diets used in the trial were commercial concentrate (C), dehydrated lucerne pellets (L), and pellets consisting in 90% of lucerne meal and 10% of soybean oil (O). Experimental groups were named as CCO, COO, LLO and LOO according to diet fed in each period (i.e. the CCO group were fed C diet in the first 2 periods and O in the last). After 42 days the lambs were slaughtered and *Longissimus thoracis* muscle was sampled. Lipids were extracted and fatty acid methyl esters were prepared and analyzed by gas chromatography and expressed as % of total fatty acids. ANOVA was conducted using a model considering the effect of initial diet (C vs. L), duration of lipid supplementation (14 days vs. 24 days) and their interaction. The C lambs (CCO and COO) had higher 18:1 *trans*-10 and less 18:2 *cis*-9, *trans*-11 (0.98 vs. 1.38) than L lambs (LLO and LOO). The duration of lipid supplementation increased ($p<0.05$) 18:1 *cis*-12, 18:2 n-6, 18:3 n-3 only in C lambs whereas the concentration of 18:1 *trans*-11, 18:1 *trans*-12 and 18:2 *cis*-9, *trans*-11 (1.02 *vs.* 1.34) increased in both C and L lambs.

Introduction:

The nutritional modulation of fatty acid profile of ruminant edible fats is currently an important research topic (Demeyer and Doreau, 1999). The inclusion of dietary lipid supplements in order to decrease saturated fatty acid and promote the enrichment in potential health beneficial unsaturated fatty acid including a conjugated isomer of linoleic acid, rumenic acid (18:2 *cis*-9, *trans*-11) and n-3 polyunsaturated fatty acids are particularly effective (Raes et al., 2004).

Lamb meat production is frequently based on concentrate feeding of weaned lambs, however the inclusion of high levels of polyunsaturated oil in diets is more effective using forage based diets, particularly dehydrated lucerne (Bessa, 2001; Santos-Silva et al., 2004). The potential negative digestive interactions associated with feeding high levels of polyunsaturated oils to ruminant are attenuated by high fiber diets (Palmquist, 1988). Moreover, isomeric pattern of octadecenoic fatty acid derived from ruminal

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biohydrogenation are clearly distinct between high concentrate and high forage diets leading to failure to increase the rumenic acid in animal fed high concentrate diets (Beaulieu et al., 2002).
This trial intends to evaluate if increased rumenic acid concentration in lamb meat can be achieved with short terminal periods of high oil forage based diet administration, and if previous diet type of lambs affects the response to high oil forage based diet.

Material and methods:

Forty Merino Branco lambs were housed and randomly assigned to four groups. The trial duration was 42 days divided in 3 periods of 14 days. The diets used in the trial were commercial concentrate (C), dehydrated lucerne pellets (L), and pellets consisting in 90% of lucerne meal and 10% of soybean oil (O). Chemical composition of diets is presented in Table 1. Experimental groups were named as CCO, COO, LLO and LOO according to diet fed in each period (Figure 1). After 42 days the lambs were slaughtered at EZN experimental abattoir. Soon after slaughter *Longissimus thoracis* muscle was sampled at 9th thoracic vertebra and kept at -80ºC until further analysis.

Table 1 – Chemical composition of experimental diets

	Dehydrated lucerne	Dehydrated lucerne with soybean oil	Commercial concentrate
Crude protein (%DM)	13.4	12.7	17.0
Ether extract (% DM)	3.9	12.1	3.2
NDF (% DM)	52.9	48.6	21.3
Ash (%DM)	13.8	10.6	6.8
Fatty acid composition			
10:0	0.38	0.31	1.34
14:0	0.38	0.23	0.00
16:0	13.2	11.3	12.3
18:0	2.99	2.99	1.04
18:1 *cis*-9	17.2	18.0	13.1
18:2 n-6	44.9	47.9	35.0
18:3 n-3	9.23	6.99	3.29

Muscle lipids were extracted by the method of Folch et al. (1957) and esterified fatty acids methyl esters were prepared by base catalysed transesterification using sodium methoxide according to Christie (1993). The fatty acid methyl esters were analysed using a HP5890A series II chromatograph (Hewlett-Packard, Avondale, PA, USA), equipped with a flame ionisation detector and fused silica capillary column (CP-Sil 88 100 m x 0.25 mm x 0.20 µm of Chrompack CP 7489, 1990-1998). Carrier gas was helium and the split ratio was 1:50. The column temperature was programmed to a constant temperature of 170 ºC. The injector and detector temperature was 250 ºC.

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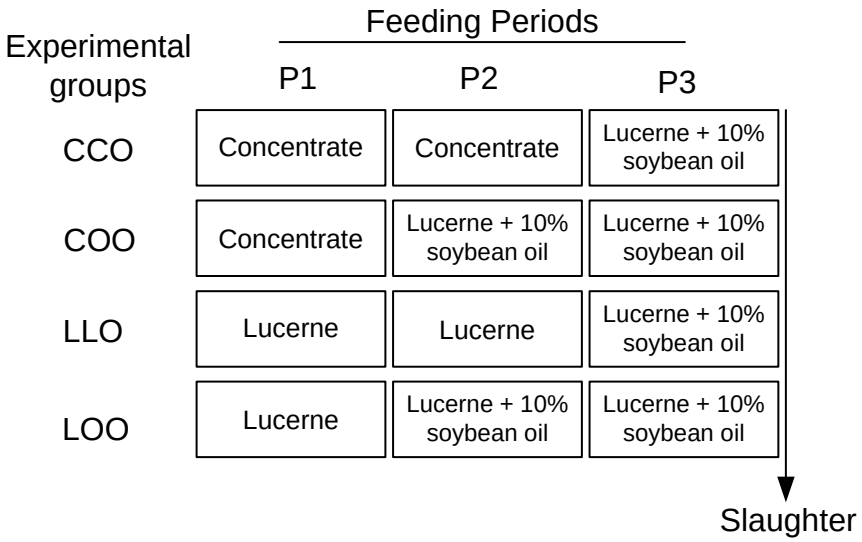

Figure 1 – Experimental groups

Peak identification was based on co-chromatography with known standards of FAME (Sigma, St. Louis, MO, USA). The chromatographic resolution of 18:1 isomers was not complete with coelution of *trans*-6, *trans*-7 and *trans*-8 isomers (that are presented as 18:1 *trans*-6+8), of *trans*-13, *trans*-14, *cis*-6, *cis*-7 and *cis*-8 isomers (presented as 18:1 ct), of 18:1 *trans*-15 and *cis*-10 isomers and of 18:1 *trans*-16 and cis-14 isomers.

Data were analysed using the GLM procedure of SAS (1989). Analysis of variance was conducted including in the model the effects of initial diet (I) (lucerne or concentrate), duration of soybean oil supplementation (O) (14 days or 28 days) and their interaction (I*O).

Results and Discussion

The lipid content and composition of fatty acid of lamb Longissimus thoracis muscle are presented in table 2. Lambs fed initially concentrate (CCO and COO) had higher lipid extract than LLO and LOO. The fatty acid concentration determined by GC was not significantly different.

Rumenic acid (18:2 cis-9, trans-11) were higher for lambs fed initially lucerne but increase with duration of soybean oil-lucern diet administration in lambs from both previous diet (non significant I*O interaction). These results suggest that lower concentration of rumenic acid in lambs initially fed concentrate are due to a lower starting point rather than to some lack of response to oil enriched diet. In our laboratory, experiments with this type of lambs, similar animal management and same analytical methodology show that lambs fed concentrate in all experimental period had rumenic acid concentration of 0.55 % of total fatty acids (Bessa et al., 2003), whereas for those fed dehydrated lucerne rumenic acid concentration ranged from 0.64 (Santos-Silva et al., 2004) and 0.85 (Bessa et al., 2003).

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Table 2 – Effect of initial diet (I)[1] and time on finishing diet (O)[2] on total lipids, fatty acid composition of Longissimus thoracis muscle of lambs.

	CCO	COO	LLO	LOO	*SEM*	I	O	I*O
mg/g DM								
Total lipids	83.2	86.3	70.7	75.3	*5.32*	*	ns	ns
Total fatty acids	47.1	47.4	39.3	44.5	*4.97*	ns	ns	ns
% of total fatty acids								
12:0	0.18	0.18	0.17	0.16	*0.020*	ns	ns	ns
iso-15:0	0.09	0.14	0.10	0.07	*0.018*	ns	ns	ns
anteiso-15:0	0.09^{ab}	0.06^{ab}	0.05^{a}	0.10^{b}	*0.013*	ns	ns	*
14:0	2.35	2.14	2.01	1.98	*0.161*	ns	ns	ns
15:0	0.32	0.30	0.28	0.23	*0.023*	*	ns	ns
iso-16:0	0.10	0.11	0.10	0.08	*0.009*	ns	ns	ns
16:0	20.5^{b}	17.7^{a}	17.2^{a}	17.9^{a}	*0.441*	***	*	***
16:1 *cis*-9	1.39^{b}	0.89^{a}	0.79^{a}	0.84^{a}	*0.075*	***	***	***
iso-17:0	0.31	0.30	0.21	0.25	*0.023*	**	ns	ns
anteiso-17:0	0.04^{a}	0.10^{b}	0.13^{b}	0.11^{b}	*0.011*	**	ns	**
17:0	1.07	0.81	0.70	0.63	*0.059*	***	**	ns
17:1 *cis*-9	0.61^{b}	0.32^{a}	0.26^{a}	0.26^{a}	*0.031*	***	***	***
18:0	13.0	13.4	14.3	13.3	*0.411*	ns	ns	ns
18:1 *trans*-6-8	0.33	0.44	0.41	0.36	*0.044*	ns	ns	ns
18:1 *trans*-9	0.40	0.45	0.46	0.47	*0.070*	ns	ns	ns
18:1 *trans*-10	3.14	2.66	0.64	1.97	*0.606*	*	ns	ns
18:1 *trans*-11	2.06	4.62	4.09	4.52	*0.569*	ns	*	0.07
18:1 *trans*-12	0.33	0.81	0.76	0.93	*0.144*	ns	*	ns
18:1 *tc**	0.28^{a}	0.61^{b}	0.69^{b}	0.66^{b}	*0.085*	*	ns	*
18:1 *cis*-9	31.1^{b}	25.0^{a}	24.3^{a}	23.7^{a}	*0.868*	***	***	***
18:1 *trans*-15 + *cis*-10	0.36	0.45	0.48	0.40	*0.061*	ns	ns	ns
18:1 *cis*-11	1.46	1.29	1.23	1.31	*0.069*	ns	ns	ns
18:1 *cis*-12	0.66^{a}	1.96^{b}	2.27^{b}	2.30^{b}	*0.169*	***	***	***
18:1 *cis*-13	0.13^{a}	0.20^{b}	0.16^{ab}	0.15^{a}	*0.012*	ns	*	**
18:1 trans-16 + *cis*-14	0.16^{a}	0.30^{bc}	0.34^{c}	0.27^{b}	*0.021*	**	ns	***
18:1 *cis*-15	0.07^{a}	0.10^{b}	0.09^{b}	0.09^{ab}	*0.006*	ns	ns	*
18:2 n-6	9.44^{a}	13.3^{b}	13.9^{b}	14.8^{b}	*0.699*	***	**	*
18:3 n-3	0.82^{a}	1.01^{b}	1.17^{c}	1.14^{c}	*0.0435*	***	ns	*
18:2 *cis*-9,*trans*-11	0.75	1.21	1.28	1.47	*0.116*	**	**	ns
20:0	0.11^{a}	0.13^{a}	0.15^{b}	0.12^{a}	*0.009*	*	ns	*
20:4 n-6	2.58	2.96	3.92	3.60	*0.302*	**	ns	ns
20:5 n-3	0.36	0.36	0.61	0.52	*0.049*	***	ns	ns
22:5 n-3	0.66	0.69	1.03	0.90	*0.072*	***	ns	ns
22:6 n-3	0.24	0.21	0.29	0.26	*0.025*	0.06	ns	ns
Other	3.11	2.71	3.37	1.97	*0.335*	ns	*	ns

1 – concentrate vs Lucerne ; 2 – finishing diet - lucern with 10% of soybean oil

* - 18:1 tc = sum of *trans*-13, *trans*-14, *cis*-6, *cis*-7 and *cis*-8.

ns—not significant ($p>0.05$); *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$); SEM – Standard error of means

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The 18:1 *trans*-11 also increases with duration of soybean oil-lucern diet administration for both initial diets. The 18:1 *trans*-10 characteristic of concentrate fed ruminants (Bessa et al., 2003) was higher in CCO and COO lambs. 18:1 *cis*-9 was higher in CCO lambs and decreasing in COO lambs to levels similar to LLO and LOO lambs. Higher concentration of 18:1 *cis*-9 are also characteristic of concentrate fed lambs (Daniel et al., 2004).
Lambs from CCO and COO had lower concentrations of 18:3 n-3, most of n-3 polyunsaturated fatty acids (see also table 3) and also of for 20:4 n-6. Some of nutritionally relevant sums and ratios of fatty acids are presented in the table 3. CCO lambs had lower concentration of n-6 polyunsaturated and hypocholesterolaemic fatty acids and higher hypercholesterolaemic fatty acids. Duration of soybean oil-lucern diet administration affected negatively the n-6/n-3 ratio.

Table 3 - Effect of initial diet (I)[1] and time on finishing diet (O)[2] on nutritional relevant ratios and sums of fatty acids.

	CCO	COO	LLO	LOO	*SEM*	I	O	I*O
∑ n-6	12.6[a]	17.0[b]	18.5[b]	19.1[b]	*0.92*	***	**	*
∑ n-3	2.06	2.28	3.08	2.82	*0.155*	***	ns	ns
∑ *hypercholesterolaemic*[3]	23.0[b]	20.1[a]	19.4[a]	20.0[a]	*0.53*	**	*	***
∑ *hypocholesterolaemic*[4]	15.4[a]	20.5[b]	22.9[b]	23.4[b]	*1.05*	***	*	*
Ratio n-6/n-3	6.25	7.54	6.03	6.83	*0.23*	*	***	ns
Ratio hH [5]	0.67[a]	1.03[b]	1.19[b]	1.21[b]	*0.074*	***	*	*

1 – effect of initial diet (concentrate vs Lucerne) ;
2 – effect of duration of finishing period (animals fed lucern and soybean oil) ;
3 – 12:0 + 14:0 + 16:0
4 – 18:1 cis-9 + n-6 PUFA + n-3 PUFA
ns—not significant (p>0.05); *p < 0.05; **p < 0.01; ***p < 0.001); SEM – Standard error of means.
5 – Ratio hH = ∑ hypocholesterolaemic /∑ hypercholesterolaemic fatty acids

Conclusions:

Concentrations of rumenic acid and 18:1 *trans*-11 in muscle of lambs fed concentrate respond positively to a finishing period with diet rich in soybean oil and dehydrated lucerne although 18:1 *trans*-10 remains higher and n-3 polyunsaturated fatty acids lower than lambs fed dehydrated lucern. However, in the concentrate fed lambs, 14 days of finishing with CLA promoting diet is not enough to the levels of CLA and 18:1 *trans*-11 reach close of those fed lucern and finished 14 days with CLA promoting diet.
Duration of oil supplementation period and residual effect of previous diets are factors to be considered when designing nutritional approaches for modification of fatty acid profile in ruminant meat.

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