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Performance, meat quality, intramuscular fatty acid profile, rumen characteristics and serum parameters of lambs fed microencapsulated or conventional linseed oil

Chang Gao[1,2,3], Dong Gao[1], Qiuxu Zhang[1], Yuan Wang[1,2,3], Aiqin Gao[1]**

[1]*College of Animal Science, Inner Mongolia Agricultural University, Hohhot, P.R. China*
[2]*Inner Mongolia Herbivorous Livestock Feed Engineering Technology Research Center, Hohhot, P.R. China*
[3]*Key Laboratory of Smart Animal Husbandry at Universities of Inner Mongolia Automomous Region, Hohhot, P.R. China*

**Corresponding authors: wangyuan@imau.edu.cn; gaoaiqin999@163.com*

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Abstract: Microencapsulated linseed oil (MLO) has been used in diets to enhance the omega-3 fatty acid concentration of meat in beef cattle without negatively affecting ruminal microbials and digestion; however, the growth performance, meat quality, intramuscular fatty acid profile, and rumen characteristics in response to MLO have not been examined in sheep. Thus, the aim of the present study was to evaluate the effects of microencapsulated or conventional linseed oil supplement on growth performance, meat quality, intramuscular fatty acid profile, rumen characteristics and serum parameters in Small-tailed Han sheep. Eighteen 5-months-old male sheep (25.31 ± 1.31 kg) were allocated to three groups. After seven days of adaptation to a basal diet, fed for 80 days, the treatments allocated were (1) basal diet (CON); (2) basal diet with added 2% linseed oil (LO); (3) basal diet with added 4% MLO. The results showed that treatments had no effects on growth performance ($P \geq 0.10$). Compared to CON and MLO group, the $pH_{24\,h}$ of the *longissimus dorsi* (LD) muscle in LO group was lower ($P = 0.07$), while the shear force was higher ($P = 0.01$). Compared to CON group, the addition of linseed oil increased proportions of C17:1, C18:2 n-6c, total polyunsaturated fatty acids (PUFA), PUFA/saturated fatty acids and total n-6, while it decreased the proportion of C17:0, C16:1 and C18:1 c-9 in LD muscle ($P < 0.10$). The lambs in LO and MLO group had higher proportions of C20:1, C18:3 n-3 and total n-3 in LD muscle than those in CON group ($P < 0.10$). Compared to CON group, the ruminal pH value of MLO group and the ruminal NH_3–N content of LO group were lower ($P < 0.10$). The total volatile fatty acid, proportion of acetate, and acetate/propionate were deceased in LO and MLO groups ($P < 0.05$). Furthermore, the two supplements significantly increased the proportions of propionate, butyrate and isovalerate ($P < 0.05$). Circulating cholesterol and high-density lipoprotein cholesterol concentrations were increased by linseed oil supplementation ($P = 0.04$). In conclusion, these results indicate that microencapsulated linseed oil did not exhibit any superior effects on muscle fatty acid composition and rumen fermentation of lambs.

Keywords: microencapsulated linseed oil; growth performance; meat characteristics; omega-3 fatty acids; rumen fermentation

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A recent meta-analysis of clinical trials concluded that the consumption of n-3 polyunsaturated fatty acids (PUFA) lowers the risk of myocardial infarction, total cardiovascular disease (CVD) and CVD death (Hu et al. 2019). Therefore, the enrichment with n-3 PUFA to balance the n-6/n-3 in mutton has been the important objective of study on lamb production. The nutritional strategies for increasing the content of n-3 PUFA in mutton include the use of vegetable oils or seeds (Miltko et al. 2019). Among these, linseed oil, rich in α-linolenic acid (ALA, C18:3 n-3), has been shown to effectively modify the proportion of n-3 PUFA in meat of lambs (Jeronimo et al. 2009). Wang et al. (2019a) also demonstrated that linseed oil effectively increased ALA and total n-3 contents in the subcutaneous adipose tissue of cashmere kids. However, it is reported that dietary addition of linseed oil decreased the count of protozoa and cellulolytic bacteria and the activity of cellulolytic enzyme (Majewska et al. 2017). Moreover, a loss of dietary n-3 PUFA was found due to extensive biohydrogenation in rumen (Wang et al. 2019a), so oil supplements should be protected to prevent these problems. Sun et al. (2021) described that the use of a rumen-protected microencapsulated supplement from linseed oil improved the growth performance, meat quality and intramuscular fatty acid composition of Korean native steers, without negatively affecting ruminal microbials and digestion. However, the effects of microencapsulated linseed oil in lambs require further research. Thus, the objective of this study is to evaluate the effects of the microencapsulated or conventional linseed oil supplementation on performance, meat quality, intramuscular fatty acid profile, rumen characteristics and serum parameters of lambs.

MATERIAL AND METHODS

This study was conducted on the experimental sheep farm of Inner Mongolia Agricultural University situated in Hohhot, Inner Mongolia, China (latitude 40.6° and longitude 111.4°). The experimental protocol ([2020]071) was approved by the Animal Care and Use Committee of the Inner Mongolia Agricultural University (Hohhot, China).

Experimental design, animals and diets

A single-factor completely randomized trial design was used. Eighteen 5-months-old male Small-tailed Han sheep (25.31 ± 1.31 kg) were selected and randomly assigned to one of the three groups, with each treatment six lambs. After 10 days of adaptation, lambs were allowed one of the following dietary treatments for 80 days: a basal diet with no supplementation (CON); an experimental diet containing 2% linseed oil (LO) and an experimental diet containing 4% microencapsulated linseed oil (MLO). The LO was purchased from a local market (Hohhot, China) and contained 98% of fatty acids (FA) with 5.60% palmitic acid (C16:0), 3.90% stearic acid (C18:0), 22.22% oleic acid (C18:1 c-9), 17.10% linoleic acid (C18:2 n-6) and 49.60% ALA (C18:3 n-3). The microencapsulated linseed oil used in our study was purchased from INNOBIO (Dalian, China). According to their instruction, the microencapsulated linseed oil was prepared via spray drying process using flaxseed gum, soy protein and maltodextrin as the wall materials and it was composed of 49% FA including 5.27% palmitic acid (C16:0), 2.66% stearic acid (C18:0), 16.71% oleic acid (C18:1 c-9), 15.63% linoleic acid (C18:2 n-6) and 58.99% ALA (C18:3 n-3). All diets were prepared as isoenergetic and isonitrogenous and in the form of a total mixed ration (TMR). The ingredient composition and nutrient levels of the experimental diets are shown in Table 1. Lambs were kept in individual pens (1.0 m × 1.2 m) with facilities for feeding and watering. Feed was offered twice daily at 07:00 am and 19:00 pm at a rate of 10% feed refusal.

Growth performance

The body weight (BW) of each lamb was recorded before morning feeding on day 1 (initial BW) and day 80 (final BW) to calculate the average daily gain (ADG). The feed offered and refused was collected and weighed daily to calculate the dry matter intake (DMI). The feed conversion ratio (FCR) was calculated as the ratio of DMI to ADG.

Carcass characteristics

All lambs were slaughtered by exsanguination at a commercial abattoir. Immediately after slaughter, carcasses were weighed to obtain hot carcass weight (HCW), and then the dressing percentage (HCW/final BW) was calculated.

https://doi.org/10.17221/77/2022-CJAS

Table 1. The ingredient composition and nutrient levels of experimental diets (air-dry basis)

Items	Treatment		
	CON	LO	MLO
Ingredients (%)			
Corn	30.00	25.00	25.00
Corn gluten meal 54%	3.00	3.00	3.00
Soybean meal 43%	5.00	5.00	5.00
Cottonseed meal	3.00	3.00	3.00
Rapeseed meal 36%	3.50	3.50	3.50
Sunflower skin	20.00	20.00	20.00
Peanut shell	15.00	18.00	16.00
Wheat straw	15.00	15.00	15.00
Linseed oil	–	2.00	–
Linseed oil microcapsules	–	–	4.00
Slow-release urea	1.50	1.50	1.50
$CaCO_3$	0.50	0.50	0.50
NaCl	0.50	0.50	0.50
$NaHCO_3$	0.50	0.50	0.50
$CaHPO_4$	0.50	0.50	0.50
Premix[1]	2.00	2.00	2.00
Total	100.00	100.00	100.00
Nutrient levels[2]			
Metabolic energy (MJ/kg)	7.91	8.18	8.05
Crude protein (%)	11.67	11.32	11.26
Ether extract (%)	2.35	4.18	4.15
Neutral detergent fiber (%)	43.16	45.12	43.50
Acid detergent fiber (%)	33.65	35.25	34.09
Total fatty acids (%)	1.18	2.75	2.75
Calcium (%)	0.62	0.63	0.61
Phosphorus (%)	0.32	0.34	0.33
Fatty acid (g/kg)			
C16:0	1.30	2.10	2.00
C18:0	0.20	0.90	0.70
C18:1 c-9	3.20	6.70	5.70
C18:2 n-6	6.50	8.60	8.30
C18:3 n-3	0.20	8.70	10.40
Calculated n-6/n-3	32.5	0.99	0.80

CON = basal diet; LO = linseed oil; MLO = microencapsulated linseed oil

[1]Provided the following per kilogram of premix: Ca 130 g, P 65 g, Fe 1300 mg, Cu 200 mg, Zn 1 200 mg, Mn 1 000 mg, I 9 mg, Se 7 mg, Co 12 mg, tertiary-butylhydroquinone 0.15 g, vitamin A 140 000 IU, vitamin D 37 500 IU, vitamin E 375 mg, vitamin K 25 mg, vitamin B_1 25 mg, vitamin B_6 25 mg, riboflavin 75 mg, vitamin B_{12} 0.28 mg, nicotinic acid 300 mg, pantothenic acid 200 mg, folic acid 15 mg, biotin 1.5 mg

[2]Metabolic energy was calculated based on the ingredients of the diet and their metabolic energy content, not based on the actual dry matter intake

Meat quality and intramuscular fatty acid profile

The pH, drip loss, cooking loss and shear force of the *longissimus dorsi* (LD) muscle were measured as described by Jiang et al. (2015). Lipids from the LD muscle were extracted with a chloroform/methanol (2 : 1 v/v) mixture, the saponification of extracted fats was done using NaOH and methyl esterification was done using a boron trifluoride-methanol solution. The FA profile was detected with a gas chromatograph (GC-2014; Shimadzu International Trading Co., Ltd, Kyoto, Japan) with a flame-ionisation detector and a CNW CD-2560 column (100 m in length with 0.25 mm inside diameter and 0.2 μm film thickness) as described by Wang et al. (2019b). The FA profile was expressed as g/100 g of total FA.

Rumen characteristics

Immediately after slaughter, ruminal fluid was collected from each lamb and strained through four layers of cheesecloth to detect the pH, NH_3–N and volatile fatty acids (VFA). Briefly, pH was measured immediately after collection via pH meter (Starter 2100; Ohaus Corp., Parsippany, NY, USA). The NH_3–N concentrations were measured by the colorimetric method using NH_4Cl as the standard according to Chen et al. (2017). A sample of ruminal fluid was mixed with 0.2 mol/l HCl (1 : 4) and stored at –20 °C for ruminal NH_3–N measurement. Samples were thawed and centrifuged at 4 °C at 4 000 × *g* for 15 min, 500 μl of sodium nitroprusside (0.2 g sodium nitroprusside dissolved in 250 ml of 14% natrium salicylicum) and 500 μl of prepared solutions (5 ml sodium hypochlorite solution mixed with 250 ml 0.3 mol/l sodium hydroxide solution) were homogenised with 100 ml of the supernatant solution ten minutes after incubation at room temperature. The absorbance was measured on a microplate reader (BioTek Instruments Inc., Winooski, VT, USA) at a wavelength of 700 nm. To determine VFA concentrations, 5 ml sample was centrifuged at 10 000 × *g* at 4 °C for 15 min, then 1.5 ml of the supernatant was taken and homogenized with 0.15 ml of metaphosphoric acid. The mixed solution was again centrifuged at 10 000 × *g* at 4 °C for 15 min, and the supernatant was used to determine VFA content with a gas chromatograph

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(Clarus 680; PerkinElmer, Inc., Waltham, MA, USA) with an Elite-FFAP column (30 m in length with a 0.25 mm inside diameter).

Serum parameters

Before morning feeding on day 80, blood samples were collected from all lambs from the jugular vein into a 5 ml vacuum tube without anticoagulant. The serum was extracted by centrifuging at 3 000 × *g* for 10 min and used to determine glucose (GLU), triglyceride (TG), cholesterol (CHO), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C), insulin and leptin content using commercial ELISA kits (Wuhan ColorfulGene Biological Technology Co., Ltd, Wuhan, China) in accordance with the manufacturer's instructions.

Statistical analysis

Each lamb was considered as the experimental unit. Data on performance, meat quality, intramuscular fatty acid profile, rumen characteristics and serum parameters were analyzed using the general linear model (GLM) procedure of SAS v9.2 (SAS Institute, Cary, NC, USA), and multiple comparison was conducted by Duncan's multiple range tests. Significance was considered when $P < 0.05$ and tendencies were discussed at $0.05 \leq P < 0.10$.

RESULTS

FA profiles of experimental diets

FA profiles of the experimental diets are presented in Table 1. LO diet had higher levels of C16:0, C18:0, C18:1 c-9 and C18:2 n-6 than MLO and CON diets, but the C18:3 n-3 levels were lower than in MLO diet. In addition, the CON diet had lower C18:3 n-3, thus the n-6/n-3 ratios of CON diet were higher than in LO and MLO diets.

Growth performance and carcass characteristics

The results for growth performance are presented in Table 2. The lambs in three groups had similar initial body weight ($P = 0.73$). Treatments did not influence final BW, ADG, DMI and FCR ($P \geq 0.10$). Hot carcass weight and dressing percentage results were not affected by treatments ($P \geq 0.10$).

Table 2. Effects of conventional and microencapsulated linseed oil on the growth performance and carcass characteristics of lambs

Items	Treatments			SEM	*P*-value
	CON	LO	MLO		
Initial weight (kg)	26.83	23.97	25.13	1.311	0.73
Final weight (kg)	45.70	45.17	46.83	1.326	0.96
Growth performance					
ADG (g/day)	235	265	271	9.408	0.29
DMI (kg/day)	1.66	1.65	1.64	0.025	0.96
FCR	7.17	6.25	6.05	0.291	0.37
Carcass characteristics					
Hot carcass weight (kg)	21.20	21.23	22.00	0.973	0.98
Dressing percentage (%)	46.21	46.84	47.00	1.204	0.97

ADG = average daily gain; CON = basal diet; DMI = dry matter intake; FCR = feed conversion ratio; LO = linseed oil; MLO = microencapsulated linseed oil

Meat quality

The $pH_{45\ min}$ of LD muscle was not affected by treatments (Table 3), but a decreasing trend in $pH_{24\ h}$ in LO group compared to CON and MLO groups was found ($P = 0.07$). Compared with

Table 3. Effects of conventional and microencapsulated linseed oil on the meat quality of the *longissimus dorsi* muscle in lambs

Items	Treatments			SEM	*P*-value
	CON	LO	MLO		
$pH_{45\ min}$	6.73	6.66	6.61	0.041	0.41
$pH_{24\ h}$	5.65^{x}	5.56^{y}	5.82^{x}	0.047	0.07
Drip loss (%)	23.21	20.58	26.59	2.157	0.71
Cooking loss (%)	56.34	54.38	57.70	0.790	0.39
Shear force (N)	66.92^{ab}	86.97^{a}	53.87^{b}	4.187	0.01

CON = basal diet; LO = linseed oil; MLO = microencapsulated linseed oil

^{a,b}Different upper-case letters present significant differences in the same index ($P < 0.05$)

^{x,y}Different upper-case letters tend to be different ($0.05 \leq P < 0.10$)

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LO group, the shear force was remarkably lower in MLO group ($P = 0.01$), but it did not differ from CON group. The drip loss and cooking loss were not affected by treatments ($P \geq 0.10$).

Meat fatty acid composition

Concerning the FA profile of LD muscle, several statistical differences were observed (Table 4). Compared to CON group, the addition of linseed oil significantly increased proportions of C17:1, C18:2 n-6c, total PUFA, PUFA/SFA and total n-6, while it decreased the proportion of C17:0, C16:1 and C18:1 c-9 in LD muscle ($P < 0.10$). The lambs in LO group and MLO group had higher proportions of C20:1, C18:3 n-3 and total n-3 in LD muscle than CON group ($P < 0.01$). Moreover, a decrease in the n-6/n-3 ratio of LD muscle was observed in LOM group compared to CON group ($P = 0.08$).

Table 4. Effects of conventional and microencapsulated linseed oil on the fatty acid profile in the *longissimus dorsi* muscle of lambs (%)

Items	Treatments			SEM	*P*-value
	CON	LO	MLO		
Fatty acid composition					
C14:0	1.85	1.08	1.29	0.165	0.14
C16:0	20.21	16.5	17.89	0.625	0.15
C16:1	1.45^{x}	1.04^{y}	1.19^{xy}	0.069	0.07
C17:0	0.69^{a}	0.56^{b}	0.66^{a}	0.024	0.01
C17:1	0.63^{b}	2.49^{a}	0.87^{b}	0.351	0.03
C18:0	15.22	15.30	16.05	0.313	0.55
C18:1 t-9	3.36	4.20	4.51	0.238	0.11
C18:1 c-9	32.38^{x}	24.73^{y}	29.87^{xy}	1.476	0.07
C20:1	0.33^{c}	1.51^{a}	1.17^{b}	0.180	< 0.01
C18:2 n-6c	10.43^{y}	15.03^{x}	12.42^{xy}	0.901	0.09
C18:3 n-3	0.72^{b}	1.73^{a}	1.75^{a}	0.175	< 0.01
C20:3 n-6	0.24	0.32	0.30	0.285	0.39
Fatty acid partial sums					
Total SFA	37.97	33.43	35.89	0.947	0.15
Total MUFA	38.14	33.97	37.6	1.105	0.17
Total PUFA	11.39^{b}	17.92^{a}	14.47^{ab}	1.127	0.03
PUFA/SFA	0.30^{b}	0.54^{a}	0.41^{ab}	0.040	0.03
Total n-6	10.67^{y}	16.20^{x}	12.72^{xy}	1.018	0.05
Total n-3	0.72^{b}	1.73^{a}	1.75^{a}	0.175	< 0.01
n-6/n-3	15.11^{x}	8.75^{xy}	7.08^{y}	1.649	0.08

CON = basal diet; LO = linseed oil; MLO = microencapsulated linseed oil; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids

[a,b]Different upper-case letters present significant differences in the same index ($P < 0.05$)

[x,y]Different upper-case letters tend to be different ($0.05 \leq P < 0.10$)

Rumen fermentation

Data for rumen characteristics are presented in Table 5. The pH tended to be lower in MLO group than in CON group ($P = 0.06$). The NH_3–N content of ruminal fluid in LO group was lower than that in CON group ($P < 0.01$). The total VFA, proportion of acetate, and acetate/propionate were deceased in LO and MLO groups ($P < 0.05$). Furthermore, the two supplements significantly increased the proportions of propionate, butyrate and isovalerate ($P < 0.05$).

Serum biochemical analysis

The serum biochemical analysis showed that CHO and HDL-C concentrations were increased by LO supplementation ($P = 0.04$). However, there were no significant differences in GLU, TG,

Table 5. Effects of conventional and microencapsulated linseed oil on the rumen characteristics of lambs

Items	Treatments			SEM	*P*-value
	CON	LO	MLO		
pH	6.49^{x}	6.27^{xy}	6.15^{y}	0.061	0.06
NH_3–N (mg/100 ml)	15.30^{a}	14.22^{b}	15.78^{a}	0.140	< 0.01
Total VFA (mmol/l)	86.90^{a}	60.82^{b}	63.03^{b}	4.589	0.01
Acetate (A, %)	64.40^{a}	59.24^{b}	55.99^{b}	1.427	0.02
Propionate (P, %)	16.11^{c}	21.42^{b}	26.31^{a}	1.530	< 0.01
Butyrate (%)	16.76	15.81	14.06	0.618	0.22
Isobutyrate (%)	0.57^{c}	0.80^{a}	0.74^{b}	0.034	< 0.01
Valerate (%)	1.21^{b}	1.70^{a}	1.83^{a}	0.106	0.01
Isovalerate (%)	1.02	1.06	1.09	0.029	0.67
A/P	4.08^{a}	2.77^{b}	2.13^{b}	0.321	0.01

CON = basal diet; LO = linseed oil; MLO = microencapsulated linseed oil; VFA = volatile fatty acid

[a,b]Different upper-case letters present significant differences in the same index ($P < 0.05$)

[x,y]Different upper-case letters tend to be different ($0.05 \leq P < 0.10$)

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LDL-C, insulin, and leptin content between treatment groups ($P \geq 0.10$).

DISCUSSION

Growth performance and carcass characteristics

Feed intake and growth of lambs were not affected by conventional and microencapsulated linseed oil supplementation. It was demonstrated that microorganisms in rumen could endure unsaturated FA (Nguyen et al. 2018), and thus they did not influence the DMI of lambs. For example, Kitessa et al. (2009) did not report any differences in dry matter intake in lambs supplemented with 3% linseed oil. The lack of effects of dietary treatments on DMI in this study was confirmed by other studies using linseed oil (Jeronimo et al. 2009; Kitessa et al. 2009). The absence of differences in final weight and ADG of lambs in LO and MLO groups compared to CON group was observed, which is consistent with previous results for lambs fed linseed oil (Urrutia et al. 2015; Malau-Aduli et al. 2019). Sun et al. (2021) also reported no differences in final weight and feed intake of steers fed microencapsulated linseed oil. It is suggested that the similarity of growth performance among treatment groups was generated by the similar protein and energy content of diets and DMI of lambs in this study.

Additionally, the hot carcass weight and dressing percentage of lambs were not affected by the dietary treatments as a consequence of the similar final weight of lambs. This result is in agreement with that published by Jeronimo et al. (2009), who reported no differences in these parameters of lambs fed vegetable oils.

Meat quality

The $pH_{45\ min}$ of LD muscle was unaffected by dietary linseed oil supplementation and the values of all treatments were comparable with previously reported values in lambs (Malva et al. 2017). The observed $pH_{24\ h}$ values of applied treatments and CON ranged from 5.56 to 5.82 within an acceptable range (5.4–5.8) for 24 h post slaughter. However, a decreasing trend in $pH_{24\ h}$ in LO group was found. This result may be attributed to unprotected linseed oil with the high levels of PUFA, which can be oxidized, then the lipid peroxidation leads to a decrease in $pH_{24\ h}$ Inconsistence with the present study, Najafi et al. (2012) found that diets containing different lipid sources did not affect the ultimate pH at 24 h of lamb meat. The similarity in pH value was reflected in drip loss and cooking loss observed in all treatments. It is consistent with others that did not find any differences in cooking loss by dietary lipid source supplementation, not only in Malpura lambs (Bhatt et al. 2020) but also in Nellore bulls (Fiorentini et al. 2018). Sanudo et al. (2000) clarified that lower shear force values were related to the higher fat diets fed to lambs. Nevertheless, compared with CON group, dietary linseed oil supplementation did not change the shear force of LD muscle in this study. Even though a previous study found that dietary microencapsulated linseed oil supplementation reduced the shear force values of steers (Sun et al. 2021), other research testing oils either protected or unprotected from rumen degradation to Nellore young bulls had no effect on the shear force (Oliveira et al. 2012). However, it is not easy to compare different feeding studies because of differences in the composition of basal diet as well as age, breed and muscle type of animals (Wang et al. 2021).

Meat fatty acid composition

The dietary inclusion of conventional and microencapsulated linseed oil decreased the proportion of C17:0. To some degree, this decrease is desirable because high values of SFA can lead to hyperlipoidaemia and increase the risk of cardiovascular disease (Chikwanha et al. 2018). This finding was consistent with previous research in dietary unsaturated oil supplementation. Guerrero et al. (2018) reported a greater decrease in C17:0 in the intramuscular fat of cull ewes that received linseed supplementation. According to Miltko et al. (2019), biosynthesis of acetyl-CoA carboxylase and fatty acid synthase, the main fat producing enzymes of fat formation, was inhibited by linseed oil supplementation. In addition, the decrease of the C17:0 proportion in lamb muscle may be related to incomplete hydrogenation induced by high consumption of unsaturated oil. Bessa et al. (2008) reported that linseed oil decreased the C18:1 c-9 concentration, the main MUFA, in the *longissimus thoracis* muscle of lambs. In accordance with previous evi-

https://doi.org/10.17221/77/2022-CJAS

dence, lambs of LO group in this study had a decreasing tendency in the C18:1 c-9 proportion. It is suggested by Bessa et al. (2007) that high dietary PUFA contents resulted in inhibition of enzyme activity and absence of C18:0 desaturation in rumen. Thus, the decrease in the C18:1 c-9 proportion of LD muscle in this study may be caused by higher levels of C18:1 c-9, C18:2 n-6 and C18:3 n-3 in LO diet. As expected, supplementing conventional and microencapsulated linseed oil to the lamb increased the proportion of C18:3 n-3 and total n-3 PUFA in LD muscle. Similar results were reported by Jeronimo et al. (2009) that linseed and its oil supplementation effectively increased the C18:3 n-3 content in the muscle of lambs. These increases indicate that part of dietary C18:3 n-3 could escape from biohydrogenation in the rumen and be absorbed by the tissue. Furthermore, the proportion of C18:2 n-6 tended to be higher in LO group, which could be attributable to higher total n-6 content in the LD muscle of LO group. These parameters were found to be controversial in previous studies, in which the C18:2 n-6 proportion in the muscle of lambs was decreased by linseed oil (Jeronimo et al. 2009). They suggested that the C18:2 n-6 proportion was decreased when the C18:3 n-3 proportion was increased, as the degree of unsaturation of C18 FA in phospholipids is maintained constant.

The PUFA/SFA and n-6/n-3 are indicators used to assess the nutritional value of fat for human consumption. Usually, lamb meat has higher SFA content and low PUFA/SFA. Increasing the PUFA concentration in the ration by including a fat source such as linseed and its oil generally improves the PUFA/SFA of meat (Guerrero et al. 2018). In the present study, the total PUFA and PUFA/SFA in LD muscle was increased by linseed oil supplementation, which is favourable in terms of human health. The excessive quantities of n-6 FA and the elevated n-6/n-3 ratio were found to prevent cardiovascular diseases, cancer, inflammatory diseases, and autoimmune disorders. The lower n-6/n-3 in LD muscle, although not significantly, was achieved in lambs fed conventional and microencapsulated linseed oil, an effect that can be explained by enhanced C18:3 n-3. Similar results of lambs fed protected linseed oil were obtained by Gravador et al. (2020). However, all the above results indicate that linseed oil is more effective in modifying the FA composition of LD muscle compared to the microencapsulated form.

Rumen fermentation

Generally, the addition of unsaturated fatty acids (UFA) has a toxic effect by inhibiting microbial metabolism in the rumen. A decreased ruminal pH value was observed in goats fed unsaturated oils (Kholif and Olafadehan 2021). A reduction in NH_3–N concentration was also found and it was likely due to a decrease in protease activity and rumen bacterial and protozoal populations caused by linseed oil supplementation (Benchaar et al. 2015; Majewska et al. 2017). In the present study, all ruminal pH values were within the normal range (Genis et al. 2021), while the pH values were lower in MLO group compared with CON group. The NH_3–N concentration was reduced by dietary linseed oil supplementation in this study. Moreover, the total VFA concentration was decreased by LO and MLO supplementation and the fermentation pattern shifted to propionate. In agreement with the present study, a reduction in the A/P ratio in goats with vegetable oils (Kholif and Olafadehan 2021) was found although it is reported that the rumen-protected UFA had no effect on the fermentation pattern including the pH, NH_3–N and VFA content (Sun et al. 2021). Considering these findings, we concluded that the microencapsulated linseed oil still negatively affected the ruminal fermentation of lambs.

Serum biochemical analysis

No change was observed in serum glucose, insulin and leptin contents in lambs supplemented with conventional and microencapsulated linseed oil (Table 6). Zhang et al. (2016) reported that there was no difference in the blood insulin concentration in cattle fed conjugated linoleic acid oil. Finding no change in these serum indices among treatment groups may be explained in great part by the isoenergetic and isoproteinic diets used in the present study. Previous study has demonstrated that both hormones, insulin and leptin, are related to the feeding level of sheep and consequently correspond to their energy balance and homeostasis (Tsiplakou et al. 2012). In the present study, the serum contents of cholesterol and HDL-C were significantly higher in LO group. It has been reported that feeding linseed oil increased the serum cholesterol concentration of cows (Oliveira et al. 2021). The serum cholesterol content was increased in lambs supplemented linseed oil probably due to the stimulating

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Table 6. Effects of conventional and microencapsulated linseed oil on the serum parameters of lambs

Items	Treatments			SEM	*P*-value
	CON	LO	MLO		
Glucose (mg/dl)	81.26	88.12	82.65	1.734	0.24
Triglyceride (mmol/l)	0.45	0.47	0.48	0.002	0.92
Cholesterol (mmol/l)	1.56^{b}	2.04^{a}	1.64^{b}	0.089	0.04
LDL-C (mmol/l)	0.49	0.29	0.30	0.059	0.33
HDL-C (mmol/l)	1.44^{b}	2.06^{a}	1.86^{ab}	0.106	0.04
Insulin (μIU/ml)	12.10	8.37	9.64	1.060	0.44
Leptin (pg/ml)	72.70	67.91	61.53	6.919	0.88

CON = basal diet; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; LO = linseed oil; MLO = microencapsulated linseed oil

^{a,b}Different upper-case letters present significant differences in the same index ($P < 0.05$)

effect of fat for HDL-C, which has previously been explained in Albas Cashmere Goats (Liu et al. 2021). However, in this study, no change in serum metabolites was detected in lambs fed microencapsulated linseed oil.

CONCLUSION

Our results indicated that feeding conventional and microencapsulated linseed oil can enhance C18:3 n-3, total n-3 and n-6/n-3, while only the conventional form impacted the proportion of C17:0, C18:1 c-9, C18:2 n-6c, PUFA, PUFA/SFA, n-6 and n-3. Both forms negatively affected rumen fermentation parameters. In summary, the present study indicated that microencapsulated linseed oil did not exhibit any superior effects on muscle FA composition and rumen fermentation of lambs.

Conflict of interest

The authors declare no conflict of interest.

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