



Growth, feed intake, carcass characteristics, and meat fatty acid profile of lambs fed soybean oil partially replaced by fish oil blend



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ABSTRACT

The objective of this study was to evaluate the effects of partial replacement of soybean oil by fish oil on dry matter intake (DMI), growth, carcass characteristics, and meat fatty acid profile of feedlot lambs. Fifty Santa Ines male lambs with 17.1 ± 2.8 of initial body weight (BW) were individually penned and used in a randomized complete block design with 10 blocks and 5 treatments. Dietary treatments, dry matter (DM) basis, consisted of: (1) control diet (CONT) with a 10:90 of forage to concentrate ratio, (2) control diet supplemented with 40 g/kg of soybean oil (OFO), (3) control diet supplemented with 2.5 g/kg of fish oil blend + 37.5 g/kg of soybean oil (25FO), (4) control diet supplemented with 5 g/kg of fish oil blend + 35 g/kg of soybean oil (50FO), and (5) control diet supplemented with 7.5 g/kg of fish oil blend + 32.5 g/kg of soybean oil (75FO). Diets were mixed once daily and fed *ad libitum*. At the end of the 84-day feeding trial, all animals were slaughtered for carcass characteristics evaluations and meat fat acid profile determination. Animals fed soybean oil had reduced DMI compared to control; however, the average daily gain (ADG), feed efficiency (FE) and final BW were not affected. The animals fed fish oil had similar DMI, ADG, FE and final BW to those receiving the control treatment. The DMI, ADG, FE and final BW were not affected by the increasing substitution of soybean oil for fish oil. Most carcass characteristics were not affected by treatments. The shrink after chilling was lower for the 50FO diet. Short, medium, and long-chain fatty acids were similar for all diets. Stearic acid concentration was higher for lambs fed the fat diets vs. control. However, stearic acid concentration decreased linearly when fish oil replaced soybean oil. Vaccenic acid concentration was higher for lambs fed fat diets vs. control. In addition, vaccenic acid increased linearly with fish oil inclusion. The conjugated linoleic acid (CLA) C18:2 *cis*-9, *trans*-11 showed higher concentration in

Abbreviations: OFO, control diet supplemented with 40 g/kg DM of soybean oil; 25FO, control diet supplemented with 2.5 g/kg DM of fish oil blend + 37.5 g/kg DM of soybean oil; 50FO, control diet supplemented with 5 g/kg DM of fish oil blend + 35 g/kg DM of soybean oil; 75FO, control diet supplemented with 7.5 g/kg DM of fish oil blend + 32.5 g/kg DM of soybean oil; ADF, acid detergent fiber; ADG, average daily gain; BW, body weight; BWS, body weight before slaughter; CCW, chilled carcass weight; CLA, conjugated linoleic acid; CONT, control diet; CP, crude protein; DE, digestible energy; DHA, docosahexaenoic acid; DM, dry matter; DMI, dry matter intake; EE, ether extract; EPA, eicosapentaenoic acid; FE, feed efficiency; HCY, hot carcass yield; HWC, hot carcass weight; LM, *Longissimus dorsi*; ME, metabolizable energy; N, nitrogen; n-3, omega-3; n-6, omega-6; NFC, non-fibrous carbohydrate; NDF, neutral detergent fiber; SC, shrink after chilling; SFT, subcutaneous fat thickness; TDN, total digestible nutrients.

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meat of animals fed diets containing fish oil compared to control, but it was not affected by soybean oil inclusion. Feeding small amounts of fish oil blend plus soybean oil does not exert an additional effect on the concentration of CLA C18:2 *cis*-9, *trans*-11 in relation to the exclusive use of soybean oil. However, the mixture of 7.5 g/kg DM of fish oil blend with 32.5 g/kg DM of soybean oil is recommended, because it improves the lipid profile of the meat by increasing the concentration of vaccenic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Additionally, supplementing 7.5 g/kg DM of fish oil blend mixed with 32.5 g/kg DM of soybean had no negative effect on the feed intake, ADG, FE and carcass characteristics of the lambs fed high concentrate diet.

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1. Introduction

The intake of omega-3 (*n*-3) fatty acids and conjugated linoleic acid (CLA) is beneficial to human health (Park, 2009). Fish oil is a rich source of *n*-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). When supplemented in ruminant feed, fish oil favors the accumulation of vaccenic acid in the rumen (Toral et al., 2010), which can then be used for endogenous CLA synthesis in various tissues (Piperova et al., 2002). Significant increases in the concentration of vaccenic acid and CLA in ruminant products have been obtained with the supply of fish oil as the sole source of supplemental fat (Donovan et al., 2000; Toral et al., 2010). However, Whitlock et al. (2002) demonstrated a synergistic effect of fish oil mixed with soybean oil with regard to the increase in the concentrations of these fatty acids in milk.

The use of fish oil (Donovan et al., 2000; Shingfield et al., 2003) or soybean oil separately (Bouattour et al., 2008) and the mixture of these sources in different ratios has been thoroughly evaluated for lactating dairy cows (Ramaswamy et al., 2001; Whitlock et al., 2002, 2006). A consistently observed response to the supplementation of fish oil in dairy cows is its effect on reducing dry matter (DM) intake, particularly when included in the diet at amounts above 10 g/kg DM (Donovan et al., 2000; AbuGhazaleh et al., 2002; Whitlock et al., 2002). Nevertheless, little information exists with respect to the performance of growing animals and the chemical composition and meat fatty acid profile from sheep fed both fat sources. This study aimed to evaluate the effects of providing increasing dietary levels of fish oil blend (<10 g/kg DM) mixed with soybean oil on the dry matter intake (DMI), average daily weight gain (ADG), carcass characteristics and meat fatty acid profile from sheep.

2. Material and methods

2.1. Location, animals and experimental facilities

The experiment was conducted at the sheep confinement facility of the Sheep and Goat Intensive Production System, Animal Science Department, "Luiz de Queiroz" College of Agriculture, São Paulo University, located in Piracicaba-SP (22°42'24" S and 47°37'53" W), Brazil.

A total of 50 male non-castrated Santa Inês lambs, with an initial average body weight (BW) of 17.1 ± 2.8 kg and at 66.0 ± 9.0 days old, were used in the study. The animals were housed in covered pens (one animal/pen) with concrete floor and dimensions of 1.3 m × 3.5 m. All animals were dewormed with 10 g/kg moxidectin (Cydectin, Fort Dodge Animal Health, Campinas, SP, Brazil) at a dose of 1 ml/50 kg of body weight and were given vitamin supplement (vitamins A, D and E) before the start of the experiment.

2.2. Experimental design, treatments and dietary management

The experimental design consisted of a randomized complete block, with 5 treatments and 10 blocks per treatment. The blocks were defined according to the weight and age of the animals at the beginning of the experiment. The experiment lasted 84 days.

The treatments were defined by the addition of increasing levels of fish oil blend (59 g/kg of DHA plus EPA and 4.2 *n*-6/*n*-3 ratio; Azevedo Indústria e Comércio de Óleos LTDA, São Paulo, SP, Brazil; Table 1) as a replacement for soybean oil (Campestre Indústria e Comércio de Óleos Vegetais LTDA, São Paulo, SP, Brazil; Table 1) in the diet, maintaining the content of supplemental fatty acid at 40 g/kg DM (Tables 2 and 3). The oils were added to a basal diet that contained 900 g/kg DM of concentrate and 100 g/kg DM of forage (fresh sugarcane bagasse). The treatments were as follows: (1) basal diet without added oil (CONT); (2) 40 g/kg DM of soybean oil (OFO); (3) 2.5 g/kg DM of fish oil blend + 37.5 g/kg DM of soybean oil (25FO); (4) 5 g/kg DM of fish oil blend + 35 g/kg DM of soybean oil (50FO); and (5) 7.5 g/kg DM of fish oil blend + 32.5 g/kg DM of soybean oil (75FO). The experimental diets were formulated according to National Research Council (NRC, 2007). The fatty acid composition of oils is shown in Table 1. The ingredient composition of the diets is shown in Table 2 and, the chemical composition and fatty acid profiles are shown in Table 3.

Corn and soybean hulls were coarsely ground using a grinder (Nogueira® DPM – 4, Itapira, Brazil) and mixed with soybean meal, urea, limestone, mineral premix and monensin sodium (Elanco do Brasil, São Paulo, SP, Brazil) using a horizontal mixer with a 500-kg capacity (Lucato®, Limeira, Brazil). The monensin sodium was added at a dosage of 25 mg/kg (natural matter

Table 1

Fatty acid composition of oils.

Fatty acid (g/100 g FAME) ^d	Soybean oil	Fish oil ^a	SEM ^b	P-Value ^c
C12:0	n.d. ⁱ	0.10	0.03	<0.01
C14:0	0.08	1.30	0.35	<0.01
C16:0	11.60	12.40	0.29	0.20
C16:1	n.d.	1.28	0.37	<0.01
C18:0	4.14	3.54	0.18	0.03
C18:1 n-9	23.24	21.50	0.54	0.07
C18:2 n-6	54.55	46.79	2.26	0.01
C18:3 n-3	4.98	5.09	0.06	0.51
C20:5 n-3 (EPA) ^e	n.d.	2.83	0.82	<0.01
C22:6 n-3 (DHA) ^f	n.d.	3.05	0.88	<0.01
Saturated total	16.0	18.03	0.63	0.08
PUFA ^g	60.76	58.89	0.55	0.02
MUFA ^h	23.24	23.09	0.14	0.69
PUFA n-6	55.82	47.54	2.39	<0.01
PUFA n-3	4.94	11.35	1.85	<0.01

^a A fish oil blend containing 59 g/kg of EPA plus DHA, with 4.2 n-6/n-3 ratio.^b SEM = standard error of the mean ($n=3$ samples for each oil).^c Probability value for comparing the oils sources by Tukey test.^d FAME = fatty acid methyl esters.^e EPA = eicosapentaenoic acid.^f DHA = docosahexaenoic acid.^g PUFA = polyunsaturated fatty acids.^h MUFA = monounsaturated fatty acids.ⁱ n.d. = non detected.

basis). The oil was added to the concentrate in the diet immediately before feeding. The concentrate and fresh sugarcane bagasse were separately weighed using an electronic scale (Marte®, LC 100, São Paulo, SP, Brazil), mixed and offered daily as complete diets. All animals had *ad libitum* access to feed and fresh water. Feed offered and refused were recorded daily to adjust feed offered for 0.10 refusal. Both were sampled weekly and frozen at -20°C for later analysis. Animals were weighed after a 14 h fast on days 0, 28, 56 and 84 of the experimental period to determine the average daily gain (ADG) and feed efficiency (FE; g of BW gain/kg of feed).

2.3. Animal slaughtering and carcass characteristics

At the end of the confinement period, all animals were slaughtered and hot carcass weight (HCW) obtained; after 24 h of cooling at 4°C , the carcasses were weighed again to determine the chilled carcass weight (CCW). The hot carcass yield (HCY), chilled carcass yield (CCY) and shrink after chilling (SC) were calculated by the following formulae: HCY = (HCW/BWS) \times 100; CCY = (CCW/BWS) \times 100 and SC = ([HCW – CCW]/HCW) \times 100, where BWS = the body weight of animals immediately before slaughter.

The subcutaneous fat thickness (SFT) was measured on the *Longissimus dorsi* (LM) muscle, between the 12th and 13th rib. After 24 h of cooling, the LM muscle was transversely cut, and the SFT was measured from both sides of the carcass using an

Table 2

Ingredient composition.

Item	Diets ^a				
	CONT	OFO	25FO	50FO	75FO
Ingredients (g/kg DM)^b					
Fresh sugarcane bagasse	100	100	100	100	100
Ground corn	563	514	514	514	514
Soybean meal	100	109	109	109	109
Soybean hulls	200	200	200	200	200
Urea	5	5	5	5	5
Ammonium chloride	5	5	5	5	5
Limestone	14	14	14	14	14
Mineral premix ^c	13	13	13	13	13
Fish oil ^d	–	–	2.5	5.0	7.5
Soybean oil	–	40	37.5	35.0	32.5

^a CONT = diet without added oil; OFO = 40 g/kg DM of soybean oil; 25FO = 2.5 g/kg DM of fish oil blend + 37.5 g/kg DM of soybean oil; 50FO = 5 g/kg DM of fish oil + 35 g/kg DM of soybean oil; 75FO = 7.5 g/kg DM of fish oil + 32.5 g/kg DM of soybean oil (DM basis).^b DM = dry matter (3 samples for each diet).^c Concentrations in the diets: 0.98 g/kg P, 1.74 g/kg Ca, 0.13 g/kg Mg, 0.91 g/kg S, 1.88 g/kg Na, 6.5 mg/kg Fe, 3.9 mg/kg Cu, 59.8 mg/kg Zn, and 0.2 mg/kg Se (DM basis).^d A fish oil blend containing 59 g/kg of EPA plus DHA, with 4.2 n-6/n-3 ratio.

Table 3

Chemical composition of experimental diets.

Item	Diets ^a					SEM ^b	P-Value ^c			
	CONT	OFO	25FO	50FO	75FO		CONT*OFO	CONT* fish oil	L	Q
Chemical composition (g/kg DM)^d										
DM, as-fed basis	835	836	837	833	838	0.70	0.80	0.64	0.73	0.31
CP	148	151	148	151	152	1.89	0.60	0.62	0.77	0.68
NFC	469	424	428	429	422	5.31	<0.01	<0.01	0.93	0.45
aNDF	301	313	314	310	314	1.66	0.02	<0.01	0.92	0.59
ADF	183	187	188	189	188	1.02	0.20	0.07	0.73	0.85
Ash	49	52	51	50	51	0.52	0.08	0.31	0.25	0.40
ME, MJ/kg DM ^e	12.2	12.8	12.6	12.9	13.0	0.08	0.02	0.01	0.25	0.18
Fatty acid composition (g/kg DM)										
C12:0	0.01	0.004	0.02	0.02	0.02	0.002	0.08	<0.01	<0.01	0.02
C14:0	0.04	0.07	0.10	0.14	0.16	0.01	<0.01	<0.001	<0.001	0.61
C16:0	4.55	8.68	8.45	8.86	8.59	0.34	<0.001	<0.001	0.83	0.88
C16:1	0.05	0.08	0.10	0.13	0.15	0.01	<0.01	<0.001	<0.001	0.86
C18:0	1.06	2.20	2.20	2.31	2.19	0.10	<0.001	<0.001	0.85	0.62
C18:1 n-9	9.33	16.48	15.50	16.20	15.50	0.56	<0.001	<0.001	0.38	0.81
C18:2 n-6	13.00	29.27	29.70	30.26	29.17	1.30	<0.001	<0.001	0.90	0.13
C18:3 n-3	0.60	1.85	1.94	2.02	1.98	0.12	<0.001	<0.001	0.35	0.59
C20:5 n-3 (EPA) ^f	0	0	0.06	0.12	0.17	0.01	1.00	<0.001	<0.001	0.64
C22:6 n-3 (DHA) ^g	0	0	0.06	0.11	0.17	0.01	1.00	<0.001	<0.001	0.59
Saturated total	5.80	11.30	10.92	11.49	11.11	0.45	<0.001	<0.001	0.99	0.98
PUFA ^h	13.78	31.35	31.99	32.76	31.72	1.50	<0.001	<0.001	0.49	0.18
MUFA ⁱ	0.33	0.87	0.59	0.55	0.67	0.07	0.01	0.10	0.35	0.20
PUFA n-6	13.18	29.49	29.93	30.50	29.40	1.37	<0.001	<0.001	0.89	0.14
PUFA n-3	0.60	1.85	2.06	2.25	2.32	0.14	<0.001	<0.001	0.01	0.54
Total fatty acids	29	60	59	61	59	3.48	<0.01	<0.01	0.98	0.96

^a CONT = diet without added oil; OFO = 40 g/kg DM of soybean oil; 25FO = 2.5 g/kg DM of fish oil blend + 37.5 g/kg DM of soybean oil; 50FO = 5 g/kg DM of fish oil + 35 g/kg DM of soybean oil; 75FO = 7.5 g/kg DM of fish oil + 32.5 g/kg DM of soybean oil (DM basis).

^b SEM = standard error of the mean ($n=3$ for each diet).

^c CONT*OFO = control diet vs. diet containing 40 g/kg DM of soybean oil; CONT*fish oil = control diet vs. diets containing fish oil (CONT vs. 25FO, 50FO, and 75FO); L = linear effect; Q = quadratic effect.

^d DM = dry matter; CP = crude protein; NFC = non-fiber carbohydrates; NDF = neutral detergent fiber; ADF = acid detergent fiber; ME = metabolizable energy.

^e NRC (2007).

^f EPA = eicosapentaenoic acid.

^g DHA = docosahexaenoic acid.

^h PUFA = polyunsaturated fatty acids.

ⁱ MUFA = monounsaturated fatty acids.

outside digital caliper. The exposed side of the LM muscle was drawn on tracing paper, and its area was then measured with a planimeter, to the nearest cm^2 , to obtain the LM area. The values obtained from the right and left sides of the carcass were used to calculate the arithmetic mean of the SFT and LM area per carcass. The perirenal fat was separated from the pelvic fat and removed, together with the kidneys, from the carcass. Then, perirenal fat was separated from the kidneys and weighed using an electronic scale (Marte®, LC 100, São Paulo, SP, Brazil). Approximately 15 cm sample of the LM muscle was removed from the right half of the carcass of each animal. This sample was divided into two sub-samples: one for the determination of the total fat and the other for determining the fatty acid profile. The samples were kept at -20°C until analysis.

2.4. Chemical analysis and calculations

After the end of the trial, samples of diets and orts were thawed and pooled by diets and experimental sub-period. Then, three samples of each diet and orts were ground through a 1-mm Wiley Mill screen (Marconi, Piracicaba, Brazil). The DM content of feed offered and orts was determined after oven-drying the samples at 105°C for 24 h according to the method of the Association of Official Analytical Chemists (AOAC, 1990; #934.01). Ash was determined by incinerating the samples in a muffle furnace at 550°C for 4 h (AOAC, 1990; #942.05). Total nitrogen (N) concentration was determined using a LECO® FP-528 Total Nitrogen Analyzer (LECO® Corporation, St. Joseph, MI, USA; AOAC, 1990; #968.06). Crude protein (CP) was obtained by multiplying the total N content by 6.25. Neutral detergent fiber (aNDF) was determined according to Van Soest et al. (1991), using heat-stable alpha-amylase and sodium sulfite, and acid detergent fiber (ADF) according to AOAC (1990; #954.01) with an Ankom 2000 Fiber Analyzer (Ankom Tech. Corp., Fairport, NY, USA). The ether extract (EE) was determined using a LECO® TFE-2000 Fat Analyzer (LECO® Corporation, St. Joseph, MI, USA).

Non-fibrous carbohydrates (NFC) were estimated according to the equation: $\text{NFC} = 100 - (\text{NDF} + \text{CP} + \text{EE} + \text{Ash})$. The total digestible nutrients (TDN) were calculated according to Weiss et al. (1992): $\text{TDN} = \text{CP}_{\text{digested}} + (\text{EE}_{\text{digested}} \times 2.25) + \text{NDF}_{\text{digested}} + \text{NFC}_{\text{digested}}$. The metabolizable energy (ME) values for each diet were

based on the assumption that 1 kg of TDN is equal to 4.409 Mcal of digestible energy (DE) and 1 Mcal of DE is equal to 0.82 Mcal of ME (NRC, 2007). The data used to calculate dietary ME were obtained from Ferreira (2011).

The total fatty acid concentration of the diets was obtained from 2 g composited samples (3 samples per diet), which were subjected to fatty acids extractions according to Folch et al. (1957). The total fat of the meat was determined using the LECO® TFE-2000 Fat Analyzer (LECO® Corporation, St. Joseph, MI, USA). Approximately 2 g of diets, orts or LM muscle were subjected to FA extraction by the protocol of Folch et al. (1957). The samples were homogenized in 20 ml chloroform:methanol (2:1). The homogenate was then centrifuged for 20 min at 2400 × g, and the supernatant transferred to a 50-ml Falcon tube using a glass syringe. An aliquot of 4.4 ml NaCl solution (15 ml/l) was added to the supernatant, and centrifuged again for 20 min at 2400 × g. The lower phase, which contained the lipid components diluted in chloroform, was collected using a glass syringe and transferred to another tube. To complete the extraction process, the contents of the tube were evaporated with gaseous nitrogen to completely remove the solvent.

Lipid extracted from the diets, orts, LM muscle, soybean oil and fish oil (3 samples per oil) were methylated by a two steps methylation procedure, using 2 ml of 0.5 M sodium methoxide (10 min at 50 °C) followed by the addition of methanoic HCl (10 min at 80 °C), according to Kramer et al. (1997), and stored at –20 °C in 1.5 ml amber vials containing nitrogen gas. The quantification and determination of fatty acids was performed using Agilent 7890A gas chromatograph equipped with flame ionization detector (7683B) and a fused-silica capillary column (J & W 112-88A7, Agilent Technologies, Santa Clara, CA, USA), 100 m in length and 250 µm internal diameter, containing 0.20 µm cyanopropyl polysiloxane. The data acquisition was performed using the ChemStation software (Agilent Technologies, Santa Clara, CA, USA). The total chromatographic run time was 87.5 min, divided into four heating cycles, as follows: 70 °C (1 min); 100 °C (5 °C/min for 2 min); 175 °C (10 °C/min for 40 min); 225 °C (5 °C/min); and 245 °C (20 °C/min for 20 min). Hydrogen was used as the carrier gas at a flow rate of 1.0 ml/min, and the temperature of the injector and the detector was 260 °C. Nitrogen gas was used as the make-up gas at a rate of 30 ml/min. The split option at a ratio of 50:1 was used. The sample fatty acid identification was based on the retention time of the methyl esters of the fatty acid standards; a 37-component standard was used (Supelco mix C4-C24/n 18919). In addition, individual standards (Nu-Chek Prep, Elysian, MN, USA) were used to identify C18:0, C18:2 *cis*-9, *trans*-11, and C18:2 *trans*-10, *cis*-12 fatty acid.

2.5. Statistical analysis

A completely randomized design was used to analyze the chemical composition and fatty acid profile of the diet. The analyses were performed using data from four samples taken over the entire trial. The PROC MIXED procedure of Statistical Analysis System (SAS Institute, 1999) was used. The means were obtained using the LSMEANS command.

The dry matter intake (DMI), ADG and FE were analyzed as repeated measures using the PROC MIXED procedure of SAS Institute (1999), according to the following statistical model: $Y = \mu + B_i + D_j + S_{ij} + T_k + (DT)_{jk} + E_{ijk}$, where μ = the overall mean; B_i = the random effect of block ($i = 1-10$); D_j = the fixed effect of diet ($j = 1-5$); S_{ij} = the residual error associated with the animal effect (block × diet); T_k = the fixed effect of time (days 28, 56 and 84 of the experimental period; $k = 1-3$); $(DT)_{jk}$ = the interaction of the diet × time; and E_{ijk} = the residual error. The covariance matrix that best fit the data set was "autoregressive" (AR 1). The treatment means were obtained using the LSMEANS command. There were two previously defined contrasts: (1) the control diet (CONT) vs. the diet containing 40 g/kg DM of soybean oil (0FO); and (2) CONT vs. the diets containing fish oil (25FO, 50FO and 75FO). The effects of the fish oil content (0FO, 25FO, 50FO or 75FO) included in the diets as the replacement for soybean oil were evaluated using linear and quadratic orthogonal contrasts. The effects of the time and the diet × time interaction were defined by an F test analysis of variance.

The carcass parameters and the meat fatty acid profile were analyzed using the PROC MIXED procedure of SAS Institute (1999), according to the model: $Y = \mu + B_i + D_j + E_{ij}$, where μ = the overall mean; B_i = the random effect of block ($i = 1-10$); D_j = the fixed effect of diet ($j = 1-5$); and E_{ij} = the residual error. The means were obtained using the LSMEANS command. The previously described contrasts (control diet vs. diet containing 40 g/kg DM of soybean oil and control vs. diets containing fish oil) were performed. The effects of the fish oil contents included in the diets as the replacement for soybean oil were evaluated using linear and quadratic orthogonal contrasts. Significance was declared at $P < 0.05$.

3. Results

3.1. Diets and animal performance

Dietary DM, CP and ADF content were similar among treatments. By contrast, NFC was lower ($P < 0.01$) in diets with oils compared to the control, and the oil diets showed a similar chemical composition (Table 3). Total fatty acids were similar (approximately 60 g/kg DM) among oil diets. For all fatty acids evaluated (Table 3), except C12:0, diets with supplemental oil had a higher ($P < 0.05$) concentration than the control diet. As intended, the replacement of the soybean oil with fish oil resulted in a linear increase ($P < 0.001$) in dietary EPA (C20:5 *n*-3) and DHA (C22:6 *n*-3) concentration (Table 3). The diets with oils had also a similar concentration of linoleic (C18:2 *n*-6) and linolenic (C18:3 *n*-3) acids.

Animals fed soybean oil had reduced ($P = 0.02$) DMI (kg/day, g/kg of BW^{0.75} and kg/100 kg BW) compared to the control diet. However, the ADG and final BW were not affected. Because the 14% reduction ($P = 0.02$) in the DMI was accompanied by an 11.3% decrease in the ADG (no significant effect, $P = 0.14$), there was no difference in the FE between OFO and CONT.

Table 4

Age, BW, and growth of feedlot lambs fed diets with soybean oil or soybean oil blended with fish oil (experimental period = 84 days).

Item	Diets ^a					SEM ^b	P-Value ^c			
	CONT	OFO	25FO	50FO	75FO		CONT*OFO	CONT*fish oil	L	Q
Initial age (days)	69	64	66	67	63	–	–	–	–	–
Initial BW (kg)	17.2	17.1	17.1	17.0	17.1	0.34	–	–	–	–
Final BW (kg)	37.3	35.0	34.8	38.5	36.7	0.82	0.19	0.69	0.17	0.55
DMI ^d										
kg/d	0.99	0.85	0.87	0.99	0.92	0.02	0.02	0.19	0.15	0.34
g/kg BW ^{0.75}	84.2	74.1	76.9	83.4	79.9	1.14	<0.01	0.18	0.08	0.29
kg/100 kg BW	3.7	3.3	3.5	3.7	3.6	0.04	<0.01	0.20	0.07	0.32
ADG ^e (g)	239	212	210	254	234	7.50	0.14	0.73	0.09	0.51
FE ^f (g/kg)	247	244	245	268	254	7.20	0.86	0.52	0.37	0.55

^a CONT = diet without added oil; OFO = 40 g/kg DM of soybean oil; 25FO = 2.5 g/kg DM of fish oil blend + 37.5 g/kg DM of soybean oil; 50FO = 5 g/kg DM of fish oil + 35 g/kg DM of soybean oil; 75FO = 7.5 g/kg DM of fish oil + 32.5 g/kg DM of soybean oil (DM basis).

^b SEM = standard error of the mean ($n = 10$ lambs for each diet).

^c CONT*OFO = control diet vs. diet containing 40 g/kg DM of soybean oil; CONT*fish oil = control diet vs. diets containing fish oil (CONT vs. 25FO, 50FO, and 75FO); L = linear effect; Q = quadratic effect.

^d DMI = dry matter intake.

^e ADG = average daily gain.

^f FE = feed efficiency (g of BW gain/kg of feed).

The animals fed diets containing fish oil had similar DMI, ADG, FE and final BW to those receiving the control treatment (Table 4). The DMI, ADG, FE and final BW were not affected by the increasing substitution of soybean oil for fish oil (Table 4). There was a time effect ($P < 0.05$) on final BW, DMI, ADG and FE; however, a diet \times time interaction was not observed (data not shown).

3.2. Carcass

HCW, CCW, HCY and CCY values did not differ among the experimental diets (Table 5). The SC was similar between the control diet and diets with supplemental fatty acids. However, the SC had a quadratic relationship ($P < 0.01$) as soybean oil was replaced by fish oil, with lower values observed for 50FO (Table 5).

There was no effect of fatty acid supplementation on the LM area, LM fat thickness, perirenal fat weight and LM fat concentration (Table 5).

3.3. Meat fatty acid composition

There was no effect of oil source on the total concentrations of short, medium and long-chain fatty acids of meat (Table 6). Also, meat unsaturated fatty acids concentration was not affected by the experimental diets (Table 6). However, the concentration of polyunsaturated fatty acids in the meat increased ($P < 0.01$) in the diets containing fish oil.

On the other hand, the monounsaturated fatty acids were lower ($P < 0.001$) in the meat of animals fed oil diets. The monounsaturated fatty acids identified were myristoleic acid (C14:1), palmitoleic acid (C16:1), oleic acid (18:1 *cis*-9) and

Table 5

Carcass characteristics of feedlot lambs fed diets with soybean oil or soybean oil blended with fish oil.

Item	Diets ^a					SEM ^b	P-Value ^c			
	CONT	OFO	25FO	50FO	75FO		CONT*OFO	CONT*fish oil	L	Q
Slaughter weight (kg)	39.2	37.2	37.2	41.0	39.0	0.90	0.29	0.97	0.16	0.49
HCW ^d (kg)	19.8	18.7	18.6	20.3	19.6	0.46	0.29	0.75	0.23	0.70
Chilled carcass weight (kg)	19.3	18.4	18.3	20.0	19.1	0.46	0.30	0.82	0.25	0.58
Hot carcass yield (kg/100 kg)	50.5	50.2	50.1	49.4	50.2	0.21	0.50	0.13	0.68	0.32
Chilled carcass yield (kg/100 kg)	49.5	49.1	49.2	48.8	48.9	0.20	0.54	0.29	0.63	0.94
Shrink after chilling (kg/100 kg)	2.1	2.1	1.7	1.4	2.4	0.12	0.99	0.30	0.53	<0.01
LM ^e area (cm ²)	12.3	12.8	13.0	14.5	12.8	0.24	0.48	0.75	0.42	0.08
Fat thickness (mm)	1.5	1.4	1.5	1.6	1.7	0.05	0.85	0.40	0.10	0.80
Perirenal fat (kg)	0.6	0.6	0.5	0.7	0.7	0.04	0.49	0.29	0.32	0.78
Fat (g/kg LM)	23	21	21	25	21	0.90	0.47	0.92	0.51	0.29

^a CONT = diet without added oil; OFO = 40 g/kg DM of soybean oil; 25FO = 2.5 g/kg DM of fish oil blend + 37.5 g/kg DM of soybean oil; 50FO = 5 g/kg DM of fish oil + 35 g/kg DM of soybean oil; 75FO = 7.5 g/kg DM of fish oil + 32.5 g/kg DM of soybean oil (DM basis).

^b SEM = standard error of the mean ($n = 10$ lamb carcasses for each diet).

^c CONT*OFO = control diet vs. diet containing 40 g/kg DM of soybean oil; CONT*fish oil = control diet vs. diets containing fish oil (CONT vs. 25FO, 50FO, and 75FO); L = linear effect; Q = quadratic effect.

^d HCW = hot carcass weight.

^e LM = *Longissimus dorsi* muscle.

Table 6Fatty acid composition of *Longissimus dorsi* muscle from lambs fed diets with soybean oil or soybean oil blended with fish oil.

Fatty acid (g/100 g FAME) ^d	Diets ^a					SEM ^b	P-Value ^c			
	CONT	OFO	25FO	50FO	75FO		CONT*OFO	CONT*fish oil	L	Q
C10:0	0.15	0.13	0.14	0.14	0.13	0.00	0.22	0.42	0.89	0.25
C12:0	0.08	0.11	0.09	0.10	0.09	0.01	0.17	0.51	0.31	0.98
C14:0	2.04	2.33	2.10	2.30	2.20	0.05	0.06	0.23	0.66	0.44
C14:1	0.05	0.08	0.06	0.10	0.06	0.01	0.29	0.38	0.70	0.62
C15:0	0.29	0.31	0.31	0.34	0.33	0.01	0.22	0.03	0.18	0.95
C16:0	24.10	23.71	23.36	24.18	24.24	0.18	0.44	0.67	0.16	0.58
C16:1	0.56	0.53	0.54	0.58	0.53	0.01	0.31	0.73	0.78	0.10
C18:0	13.57	14.75	15.12	14.53	13.68	0.21	0.03	0.05	0.04	0.14
C18:1 n-9	44.47	36.03	36.61	36.35	35.85	0.64	<0.001	<0.001	0.80	0.45
C18:1 trans-11	1.68	4.72	5.23	6.93	5.95	0.32	<0.001	<0.001	0.02	0.15
C18:2 cis-9, trans-11	0.78	1.11	1.07	1.22	1.13	0.07	0.11	0.04	0.75	0.90
C18:2 trans-10, cis-12	0.09	0.07	0.08	0.09	0.10	0.01	0.37	0.91	0.28	0.84
18:2 n-6 c9, c12	6.45	10.00	9.64	8.51	10.02	0.37	<0.001	<0.001	0.74	0.20
C18:3 n-6	0.10	0.17	0.20	0.15	0.16	0.01	0.04	0.02	0.45	0.82
C18:3 n-3	0.15	0.32	0.32	0.29	0.34	0.02	<0.001	<0.001	0.80	0.28
C20:5 n-3 (EPA)	n.d. ^g	n.d.	0.12	0.12	0.19	0.01	0.16	<0.001	<0.01	0.18
C22:6 n-3 (DHA)	n.d.	n.d.	0.12	0.14	0.23	0.01	0.95	<0.001	<0.001	0.20
Other	5.46	5.61	4.90	3.92	4.78	0.17	0.78	0.04	0.05	0.04
Short-chain (C4:0–C12:0)	0.22	0.24	0.23	0.25	0.21	0.01	0.53	0.82	0.47	0.65
Medium-chain (C14:0–C16:1)	27.12	27.00	26.40	27.46	27.36	0.22	0.80	0.91	0.28	0.60
Long-chain (C17:0–C22:6)	72.66	72.79	73.39	72.29	72.43	0.23	0.83	0.92	0.31	0.63
Saturated total	41.44	42.45	42.24	42.71	41.88	0.21	0.14	0.13	0.57	0.52
Unsaturated total	58.56	57.55	57.76	57.29	57.86	0.21	0.13	0.09	0.83	0.71
MUFA ^e	47.75	42.04	43.10	44.56	42.96	0.50	<0.001	<0.001	0.19	0.07
PUFA ^f	10.81	15.51	14.65	12.73	15.16	0.50	<0.001	<0.01	0.48	0.09
Total n-3	3.19	3.84	3.41	2.56	3.46	0.16	0.11	0.89	0.17	0.03

^a CONT = diet without added oil; OFO = 40 g/kg DM of soybean oil; 25FO = 2.5 g/kg DM of fish oil blend + 37.5 g/kg DM of soybean oil; 50FO = 5 g/kg DM of fish oil + 35 g/kg DM of soybean oil; 75FO = 7.5 g/kg DM of fish oil + 32.5 g/kg DM of soybean oil (DM basis).

^b SEM = standard error of the mean ($n=10$ samples for each diet).

^c CONT*OFO = control diet vs. diet containing 40 g/kg DM of soybean oil; CONT*fish oil = control diet vs. diets containing fish oil (CONT vs. 25FO, 50FO, and 75FO); L = linear effect; Q = quadratic effect.

^d FAME = fatty acid methyl esters.

^e MUFA = monounsaturated fatty acids.

^f PUFA = polyunsaturated fatty acids.

^g n.d. = non detected.

vaccenic acid (18:1 *trans*-11), whereas no treatment effect was found for the myristoleic and palmitoleic acids, the concentration of vaccenic acid was higher ($P<0.001$) in the meat of animals fed diets containing fish oil compared to the control. Oleic acid had 18.4% lower concentration ($P<0.001$) in the meat from the lambs fed fish oil diets compared to the control. Feeding soybean oil (40 g/kg DM) also reduced (19%) the concentration ($P<0.001$) of oleic acid. Compared to the control diet, the concentration of vaccenic acid was 181.0% ($P<0.001$) and 259.3% ($P<0.001$) higher in the meat from the lambs fed soybean and fish oil diets, respectively (Table 6). Furthermore, the replacement of the soybean with fish oil resulted in a linear increase ($P=0.02$) in the concentration of vaccenic acid.

Stearic acid concentration was higher in meat from lambs in OFO ($P=0.03$) and fish oil ($P=0.05$) treatments compared to the control. Further, the replacement of up to 7.5 g/kg soybean oil with fish oil linearly decreased ($P=0.04$) the concentration of stearic acid in meat. There was no treatments effect on the percentage of C18:2 *trans*-10, *cis*-12 CLA isomer in meat, whereas the concentration of the C18:2 *cis*-9, *trans*-11 isomer was 42.3% higher (no significant effect, $P=0.11$) for OFO when compared to CONT (Table 6). When the three diets containing fish oil were analyzed together, there was a 51.3% increase ($P=0.04$) in the concentration of CLA C18:2 *cis*-9, *trans*-11 in meat compared to the control; however, its concentration was not affected by the substitution of soybean oil for fish oil. The supply of oil-containing diets did not affect the concentration of n-3 fatty acids in meat. However, there was a quadratic effect ($P=0.03$) in response to the substitution levels of soybean by fish oil, with the lowest value observed in meat from animals fed the 50FO diet. This result was modulated by numerical reductions in the concentrations of C18:3 n-3 and C18:3 n-6 fatty acids, which, although not significant, promoted this response when combined. The concentration of EPA (C20:5 n-3) and DHA (C22:6 n-3) in meat increased linearly ($P<0.01$) with the increasing levels of fish oil.

4. Discussion

4.1. Performance

The DMI decrease is not a predictable response associated with the supply of soybean oil for ruminants (AbuGhazaleh et al., 2002; Whitlock et al., 2002; Loor and Herbein, 2003; Maia et al., 2006; Gómez-Cortés et al., 2009; Freitas et al., 2010).

The supply of 45 g/kg DM of soybean oil or sunflower oil to goats did not affect the DMI, which can be attributed to the similar content of ME of the diets (Roy et al., 2013). However, the lower DMI observed for the OFO treatment was a reflection of its higher energy density compared to the control (Table 3). The absence of effect of increasing substitution of soybean oil with fish oil on DMI and ADG explains the similar FE observed in response to the fish oil supply (Table 4). The similar DMI by the animals fed diets containing fish oil compared to those receiving the CONT treatment agrees with other reports in the literature, in which the supply of fish oil, when compared to diets without oil (Shingfield et al., 2003; Whitlock et al., 2006) did not affect DMI. However, in most cases, the use of fish oil as the sole source of supplemental fat (Donovan et al., 2000; AbuGhazaleh et al., 2002; Whitlock et al., 2002; Toral et al., 2010) or mixed with other fat sources (AbuGhazaleh et al., 2002; Whitlock et al., 2002; Toral et al., 2010) caused a decrease in intake.

4.2. Carcass

The absence of treatment effects on HCW, CCW and CCY (Table 5) could be attributed to similar final BW of the lambs among treatments (Table 4). The carcass yield data are within the range commonly observed for Santa Inês lambs. For example, Ferreira et al. (2011) reported values of 50.6 kg/100 kg and 49.2 kg/100 kg for the HCY and CCY, respectively.

Our data indicated that LM fat thickness is not a good modulator of SC, since LM fat thickness was similar among treatments and the SC was lower in treatment 50FO (Table 5). A possible explanation for this finding is that the SFT measured only on the LM muscle may not have been representative of the overall carcass fat cover (Osório et al., 2002). Cooper et al. (2004) also did not verify effect of feeding fish oil on the carcass fat levels of the lambs.

4.3. Meat fatty acid composition

The long-chain fatty acids concentration was higher in diets with fish oil blend as compared to the control diet (Table 3). However, total concentrations of short, medium and long-chain fatty acids in the meat were similar among treatments (Table 6), suggesting that the efficiency of absorption and/or transfer of long-chain fatty acids from the diets to muscle was reduced. In addition, the supplementation of long-chain fatty acids did not affect the *de novo* synthesis of fatty acids in fat tissue. To support this idea, the supply of long-chain fatty acids to lactating animals usually results in increased concentrations of these fatty acids in milk of dairy cows, with a consequent reduction in the synthesis of short-chain fatty acids in the mammary glands (Grummer, 1991; Whitlock et al., 2006). Also, growing animals may be less responsive to changes in tissue fatty acid profile because supplemental fat may be used as energy source instead of being stored.

The lack of effect of oils supply on meat unsaturated fatty acids concentration was a result of the lower concentration of monounsaturated fatty acids found in the meat of the animals fed diets with oils, because the concentration of polyunsaturated fatty acids increased ($P<0.001$) in response to soybean oil and fish oil supplementations. The decrease in the oleic acid concentration was responsible for the reduction in the concentration of the monounsaturated fatty acids in the meat (Table 6). Indeed, a decreased concentration of oleic acid (C18:1 *cis*-9) in the meat of sheep supplemented with linoleic acid-rich oils has often been reported (Mir et al., 2000; Bas et al., 2007; Lee et al., 2007). The intake of oleic acid was higher in animals fed the diets with added oil (9.4, 14.0, 13.4, 16.0 and 14.3 g/day for CONT, OFO, 25FO, 50FO, and 75FO, respectively). Therefore, the lower level of oleic acid observed for the treatments with supplemental fatty acids could be attributed to the lower transfer of this fatty acid from blood to muscle and/or a decrease in its synthesis from stearic acid by the stearoyl-CoA desaturase. In addition, Sessler et al. (1996) demonstrated that linoleic and linolenic acids exert an inhibitory effect on the expression of this enzyme. In the present experiment, the supply of linoleic and linolenic acids was largely increased for all treatments with supplemental fatty acids (Table 3).

The observed increase in the concentration of vaccenic acid and C18:2 *cis*-9, *trans*-11 CLA in the meat of the lambs supplemented with fatty acids (Table 6) probably resulted from the higher synthesis of this fatty acid in the rumen. Vaccenic acid and C18:2 *cis*-9, *trans*-11 CLA are synthesized in the rumen as a transient intermediate in the process of biohydrogenation of linoleic acid (Bauman et al., 1999). The DHA decreases the biohydrogenation of vaccenic acid and consequently increases their concentration in the rumen (AbuGhazaleh and Jenkins, 2004), which may have caused the linear increase in the concentration of vaccenic acid as fish oil increased in the diets (Table 6). Because C18:2 *cis*-9, *trans*-11 CLA can be synthesized from vaccenic acid through the stearoyl-CoA desaturase activity (Adlof et al., 2000; Griinari et al., 2000), endogenous synthesis may also have contributed to the increase in the concentration of C18:2 *cis*-9, *trans*-11 CLA in the meat of the lambs supplemented with fatty acids. The linear decrease in the stearic acid concentration in meat according to fish supply demonstrates that small amounts of EPA and DHA present in diets with fish oil blend (Table 3) were effective in reducing ruminal biohydrogenation of vaccenic acid to stearic acid. Other authors have demonstrated the efficiency with which fish oil favors the accumulation of vaccenic acid in ruminant products (Ramaswamy et al., 2001; AbuGhazaleh et al., 2002; Whitlock et al., 2002; Bharathan et al., 2008; Toral et al., 2010); however, these authors evaluated inclusions of fish oil in amounts equal to or greater than 10 g/kg DM, a value that may compromise animal performance (Donovan et al., 2000; Kitessa et al., 2001; Annett et al., 2009). The benefits of using small amounts of fish oil blend on lipid composition of meat, without compromising animal performance, create a new possibility for the use of this technology. Because the concentration of vaccenic acid increased linearly with the addition of fish oil, a similar increase was expected in the concentration of CLA C18:2 *cis*-9, *trans*-11. This result reinforces the idea that the activity of the stearoyl-CoA desaturase enzyme was negatively affected by

the supplemental fatty acids (Sessler et al., 1996), but also allows an exploration of the hypothesis that the flow of vaccenic acid exceeded the desaturation capacity of stearoyl-CoA desaturase in muscle tissue (Chilliard et al., 2001).

The EPA and DHA have high rates of ruminal biohydrogenation (Scollan et al., 2001; Duckett and Gillis, 2010), which explains their low concentration in the meat (Table 6). The observed increase in the meat fat concentrations of EPA and DHA according to fish oil supply is desirable due to beneficial effects of these fatty acids on human health (Ruxton et al., 2007). According Dohme et al. (2003), lipolysis and biohydrogenation rates decrease with increasing dietary concentrations of fish oil, increasing, therefore, the availability of EPA and DHA for absorption. This observation explains the linear increase in the concentrations of EPA and DHA in the meat fat with the increasing levels of fish.

5. Conclusions

Feeding small amounts of fish oil blend in combination with soybean oil does not exert an additional effect on the concentration of CLA C18:2 *cis*-9, *trans*-11 in relation to the exclusive use of soybean oil. However, the mixture of 7.5 g/kg DM of fish oil blend with 32.5 g/kg DM of soybean oil is recommended, because it improves the lipid profile of the meat by increasing the concentration of vaccenic acid, EPA and DHA. Additionally, supplementing 7.5 g/kg DM of fish oil blend mixed with 32.5 g/kg DM of soybean oil had no negative effect on the feed intake, ADG, feed efficiency and carcass characteristics of the lambs fed high concentrate diet.

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References

- AbuGhazaleh, A.A., Jenkins, T.C., 2004. Short communication: docosahexaenoic acid promotes vaccenic acid accumulation in mixed rumen cultures when incubated with linoleic acid. *J. Dairy Sci.* 87, 1047–1050.
- AbuGhazaleh, A.A., Schingoethe, D.J., Hippen, A.R., Kalscheur, K.F., Whitlock, L.A., 2002. Fatty acid profiles of milk and rumen digesta from cows fed fish oil, extruded soybeans or their blend. *J. Dairy Sci.* 85, 2266–2276.
- Adlof, R.O., Duval, S., Emken, E.A., 2000. Biosynthesis of conjugated linoleic acid in humans. *Lipids* 35, 131–135.
- Annett, R.W., Dawson, L.E.R., Edgar, H., Carson, A.F., 2009. Effects of source and level of fish oil supplementation in late pregnancy on feed intake colostrums production and lamb output of ewes. *Anim. Feed Sci. Technol.* 154, 169–182.
- AOAC, 1990. *Official Methods of Analysis*, 15th ed. Association of Official Analytical Chemists, Arlington, VA, USA.
- Bauman, D.E., Baumgard, L.H., Corl, B.A., Griniari, J.M., 1999. Biosynthesis of conjugated linoleic acid in ruminant. *Proc. Am. Soc. An. Sci.*, 15–77.
- Bas, P., Berthelot, V., Pattier, E., Normand, J., 2007. Effect of level of linseed on fatty acid composition of muscles and adipose tissues of lambs with emphasis on trans fatty acids. *Meat Sci.* 77, 678–688.
- Bharathan, M., Schingoethe, D.J., Hippen, A.R., Kalscheur, K.F., Gibson, M.L., Karges, K., 2008. Conjugated linoleic acid increases in milk from cows fed condensed corn distillers soluble and fish oil. *J. Dairy Sci.* 91, 2796–2807.
- Bouattour, R., Casals, R., Albanell, E., Such, X., Caja, G., 2008. Feeding soybean oil to dairy goats increases conjugated linoleic acid in milk. *J. Dairy Sci.* 91, 2399–2407.
- Chilliard, Y., Ferlay, A., Doreau, M., 2001. Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. *Livest. Prod. Sci.* 70, 31–48.
- Cooper, S.L., Sinclair, L.A., Wilkinson, R.G., Hallett, K.G., Enser, M., Wood, J.D., 2004. Manipulation of the n-3 polyunsaturated fatty acid content of muscle and adipose tissue in lambs. *J. Anim. Sci.* 82, 1461–1470.
- Dohme, F., Fievez, V., Raes, K., Demeyer, D.I., 2003. Increasing levels of two different fish oils lower ruminal biohydrogenation of eicosapentaenoic and docosahexaenoic acid in vitro. *Anim. Res.* 52, 309–320.
- Donovan, D.C., Schingoethe, D.J., Baer, R.J., Hippen, A.R., Frankling, S.T., 2000. Influence of dietary fish oil on conjugated linoleic acid and other fatty acids in milk fat from lactating dairy cows. *J. Dairy Sci.* 83, 2620–2628.
- Duckett, S.K., Gillis, M.H., 2010. Effects of oil source and fish oil addition on ruminal biohydrogenation of fatty acids and conjugated linoleic acid formation in beef steers fed finishing diets. *J. Anim. Sci.* 88, 2684–2691.
- Ferreira, E.M., Pires, A.V., Susin, I., Mendes, C.Q., Gentil, R.S., Araujo, R.C., Amaral, R.C., Loerch, S.C., 2011. Growth, feed intake, carcass characteristics, and eating behavior of feedlot lambs fed high-concentrate diets containing soybean hulls. *J. Anim. Sci.* 89, 4120–4126.
- Ferreira, E.M., 2011. Diets with Fish Oil in Partial Replacement of Soybean Oil for Sheep (Doutorado em Ciência Animal e Pastagens)–Escola Superior de Agricultura Luiz de Queiroz., Universidade de São Paulo, Piracicaba, SP, Brazil, pp. 156.
- Folch, J., Lees, M., Sloane Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226, 497–509.
- Freitas Jr.de, J.E., Rennó, F.P., Santos, M.V., Gandra, dos., Maturana Filho, J.R., Venturelli, M.B.C., 2010. Productive performance and composition of milk protein fraction in dairy cows supplemented with fat sources. *Rev. Bras. Zootec.* 39, 845–852.
- Gómez-Cortés, P., Bach, A., Luna, P., Juárez, M., Fuente, M.A.da la., 2009. Effects of extruded linseed supplementation on n-3 fatty acids and conjugated linoleic acid in milk and cheese from ewes. *J. Dairy Sci.* 92, 4122–4134.
- Griniari, J.M., Corl, B.A., Lacy, S.H., Chouinard, P.Y., Nurmiela, K.V.V., Bauman, D.E., 2000. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by delta9-desaturase. *J. Nutr.* 130, 2285–2291.
- Grummer, R.R., 1991. Effect of feed on the composition of milk fat. *J. Dairy Sci.* 74, 3244–3257.
- Kramer, J.K., Fellner, V., Dugan, M.E., Sauer, F.D., Mossoba, M.M., Yurawecz, M.P., 1997. Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. *Lipids* 32, 1219–1228.
- Kitessa, S.M., Gulati, S.K., Ashes, J.R., Fleck, E., Scott, T.W., Nichols, P.D., 2001. Utilisation of fish oil in ruminants I. Fish oil metabolism in sheep. *Anim. Feed Sci. Technol.* 89, 189–199.
- Lee, J.H., Waller, J.C., Yilmaz, Y., Melton, S.L., 2007. Effect of feeding rumen-protected dietary protein-oil supplements on fatty acid composition and α -tocopherol content of blood serum and muscle lipids of lambs. *Small Rumin. Res.* 72, 101–110.
- Loor, J.J., Herbein, J.H., 2003. Dietary canola or soybean oil with two levels of conjugated linoleic acids (CLA) alter profiles of 18:1 and 18:2 isomers in blood plasma and milk fat from dairy cows. *Anim. Feed Sci. Technol.* 103, 63–83.

- Maia, F.J., Branco, A.F., Mouro, G.F., Marcantonio, C., Santos, G.T. dos, Minella, T.F., Macedo, F.A.F., 2006. Feeding vegetable oil to lactating goats: nutrient digestibility and ruminal and blood metabolism. *Rev. Bras. Zootec.* 35, 1496–1503.
- Mir, Z., Rushfeldt, M.L., Mir, P.S., Paterson, L.J., Weselake, R.J., 2000. Effect of dietary supplementation with either conjugated linoleic acid (CLA) or linoleic acid rich oil on the CLA content of lamb tissues. *Small Rumin. Res* 36, 25–31.
- NRC, 2007. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids and New World Camelids. The National Academic Press, Washington, DC, USA.
- Osório, J.C.S., Oliveira, N.M., Osório, M.T.M., Jardim, R.D., Pimentel, M.A., 2002. Meat production in male lambs derived from the crossing between Border Leicester ram with Corriedale and Polwarth ewes. *Rev. Bras. Zootec.* 31, 1469–1480, Suplemento.
- Park, Y., 2009. Conjugated linoleic acid (CLA): good or bad trans fat? *J. Food. Compos. Anal.* 22S, S4–S12.
- Piperova, L.S., Sampugna, J., Teter, B.B., Kalscheur, K.F., Yurawecz, M.P., Ku, Y., Morehouse, K.M., Erdman, R.E., 2002. Duodenal and milk trans octadecenoic acid and conjugated linoleic acid (CLA) isomers indicate that post absorptive synthesis is the predominant source of cis-9 containing CLA in lactating cows. *J. Nutr.* 132, 1235–1241.
- Ramaswamy, N., Baer, R.J., Shingoethe, D.J., Hippen, A.R., Kasperton, K.M., Whitlock, L.A., 2001. Composition and flavor of milk and butter from cows fed fish oil, extruded soybeans, or their combination. *J. Dairy Sci.* 84, 2144–2151.
- Roy, A., Mandal, G.P., Patra, A.K., 2013. Evaluating the performance carcass traits and conjugated linoleic acid content in muscle and adipose tissues of black Bengal goats fed soybean oil and sunflower oil. *Anim. Feed Sci. Technol.* 185, 43–52.
- Ruxton, C.H.S., Reed, S.C., Simpson, M.J.A., Millington, K.J., Ruxton, C., 2007. The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. *Commentary J. Hum. Nutr. Diet.* 20, 275–287.
- SAS Institute, 1999. SAS Systems for Windows, Version 8. 2. SAS Institute Inc., Cary, NC, USA.
- Scollan, N.D., Dhanoa, M.S., Choi, N.J., Maeng, W.J., Enser, M., Wood, J.D., 2001. Biohydrogenation and digestion of long chain fatty acids in steers fed on different sources of lipid. *J. Agric. Sci.* 136, 345–355.
- Sessler, A.M., Kaur, N., Paltas, J.P., Ntambi, J.M., 1996. Regulation of stearoyl-CoA desaturase 1 mRNA stability by polyunsaturated fatty acids in 3T3 adipocytes. *J. Biol. Chem.* 271, 29854–29858.
- Shingfield, K.J., Ahvenjarvi, S., Toivonen, V., Arola, A., Numereka, K.V.V., Huhtanen, P., Griinari, J.M., 2003. Effect of dietary fish oil on biohydrogenation of fatty acids and milk fatty acid content in cows. *Anim. Sci.* 77, 165–179.
- Toral, P.G., Hervás, G., Gómez-Cortés, P., Frutos, P., Juárez, M., Fuente, M.A.de.la., 2010. Milk fatty acid profile and dairy sheep performance in response to diet supplementation with sunflower oil plus incremental levels of marine algae. *J. Dairy Sci.* 93, 1655–1667.
- Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74, 3583–3597.
- Weiss, W.P., Conrad, H.R., St Pierre, N.R., 1992. A theoretically-based model for predicting total digestible nutrient values of forages and concentrates. *Anim. Feed Sci. Technol.* 39, 95–110.
- Whitlock, L.A., Schingoethe, D.J., AbuGhazaleh, A.A., Hippen, A.R., Kalscheur, K.F., 2006. Milk production and composition from cows fed small amounts of fish oil with extruded soybeans. *J. Dairy Sci.* 89, 3972–3980.
- Whitlock, D.J., Schingoethe, D.J., Hippen, A.R., Kalscheur, K.F., Baer, R.J., Ramaswamy, N., Kasperton, K.M., 2002. Fish oil and extruded soybeans fed in combination increase conjugated linoleic acids in milk of dairy cows more than when fed separately. *J. Dairy Sci.* 85, 234–243.