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Effects of Low Supplement Levels of Plant Oil and Type of Antioxidant on Meat Quality Parameters of Feedlot Lambs

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Abstract: Plant oil supplements have been used in ruminant feedlot diets as alternative high-density energy source. However, feeding ruminants with high levels of oil may adversely affect the rumen microbiome and function, negatively impacting performance and production. Plant oils high in polyunsaturated fatty acids (PUFA) may also affect animal products quality when PUFA escape or partially escape biohydrogenation. This study investigated the effects of a low inclusion level (1.62%) of a plant oil (commercial sunflower-soybean blend) on the physicochemical attributes, fatty acid profile, and shelf-display stability of *longissimus thoracis et lumborum* muscle of lambs fed high-energy starch-based diets. In addition, either a natural or synthetic antioxidant were included in the plant oil containing diets to evaluate their effects on maintenance of shelf-display stability with or without natural or synthetic antioxidants. Forty [$n = 40$; average weight (± standard error of mean) 28 (± 0.836 kg)] were blocked by weight and randomly allocated to 4 dietary treatment groups [(1) control (Grain); (2) grain plus plant oil only (Oil); (3) grain plus plant oil with synthetic antioxidant (OilCaps); and (4) grain plus plant oil with natural antioxidant (OilNat)]. Feeding lambs with grain-based diets supplemented with plant oil, with or without natural or synthetic antioxidants, had no effects on meat physicochemical attributes ($P > 0.050$) or color stability over 7 d of shelf display ($P > 0.050$). Regardless of antioxidant inclusion, oil supplementation increased conjugated linoleic acid (CLA) composition in lamb *longissimus* muscle ($P = 0.028$). Overall, results from this study suggest that grain-based feedlot diets supplemented with low inclusion levels of plant oil did not significantly affect shelf stability of lamb meat during display while both oil supplement and antioxidants increased the CLA content of *longissimus* muscle.

Key words: antioxidants, color, fatty acids, oxidation, plant oils
Meat and Muscle Biology 8(1): 17003, 1–12 (2024) **doi:10.22175/mmb.17003**
Submitted 17 October 2023 Accepted 18 January 2024

Introduction

The benefits of plant oil supplements in high-energy ruminant diets have been widely studied over the past decades. Plant oil is a source of high-density energy and thus can be used to reduce the levels of starch in feedlot diets, alleviating the risk of metabolic disorders such as acidosis caused by rapidly fermentable carbohydrates in grain-based diet as well as heat stress (McGrath et al., 2018). Furthermore, plant oils may improve physical characteristics of feeds by reducing dustiness and enhancing palatability, possibly increasing feed intake (Palmquist and Jenkins, 2017). Microbial populations may also be altered due to increased polyunsaturated fatty acid (PUFA) content in plant oils causing ruminal defaunation, thereby increasing bacterial population density and efficiency of bacterial protein synthesis, and reducing methane production (Qin et al., 2012; Ibrahim et al., 2021).

Despite the advantages of plant oils in ruminant diets, inclusion levels should be carefully managed.

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Total lipid concentration of more than 6% in ruminant diets reduces microbial growth (Santos-Silva et al., 2004), dry matter (DM) intake, and fiber digestion (Bessa et al., 2005; McDonald et al., 2010; Benchaar et al., 2012). Even though the complex microbial organization within the rumen utilizes processes such as biohydrogenation as a protective mechanism against the toxic effect of PUFA on microbes (Wood et al., 2003; Francisco et al., 2015; Enjalbert et al., 2017), high levels of PUFA have an inhibitory effect on the composition and population of rumen microorganisms, which reduces volatile fatty acid production and restrict growth performance (Yang et al., 2009). A maximum inclusion level of 4% oil in ruminant diets has been suggested (Ibrahim et al., 2021).

Apart from increasing energy concentration and palatability of ruminant diets, addition of plant oils may influence meat quality by increasing intramuscular fat (IMF) and PUFA content. There may also be a notable increase human health–benefiting fatty acids such as oleic acid, conjugated linoleic acid (CLA), and eicosapentaenoic acid (Santos-Silva et al., 2004; Bessa et al., 2005, 2008; Quiñones et al., 2019). Regrettably, increasing PUFA in meat may lower shelf stability, expressed as high lipid oxidation, color deterioration, and off-flavor development (Wood et al., 2003; Wanapat et al., 2011).

Addition of either synthetic or natural antioxidants in ruminant diets has shown positive effects on meat shelf stability through improved color stability and reduced fat oxidation (Francisco et al., 2015; Peng et al., 2016). Synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene (BHT), tertiary butylhydroquinone, and ethoxyquin are popular inexpensive and effective substances used to maintain oxidative stability of meat (Ribeiro et al., 2019; Estévez, 2021). However, these synthetic antioxidants have been linked with carcinogenic and cytotoxic effects (Estévez, 2021; Xu et al., 2021). Thus, due to increased consumer health concerns, there has been increasing interest in replacing synthetic antioxidants with natural antioxidants. Interestingly, addition of natural antioxidants in ruminant diets has been linked with beneficial effects such as improved immune function and rumen fermentation and protecting meat against discoloration and development of rancidity and off-flavors (Estévez, 2021; Kong et al., 2022).

When included in ruminant diets, plant oils are added at levels between 4% and 5% and limited to 8% (Francisco et al., 2015; Ibrahim et al., 2021). In this study, we investigated the effects on meat quality of a low inclusion level of a plant oil (sunflower-soybean blend) with additional inclusion of either a synthetic or natural antioxidant. The rationale behind the lower inclusion of oils was to mitigate the risk of metabolic disorders by reducing the level of rapidly fermentable carbohydrates from the maize component of a high-energy lamb feedlot diet without risking the negative effects of high fat inclusion in the diet. The hypotheses tested were that inclusion of plant oil in high-energy lamb feedlot diets would increase IMF and improve the PUFA profile, while color stability may be compromised during extended shelf display. In addition, it was expected that antioxidants would enhance color and oxidative stability of meat during shelf display.

Materials and Methods

Trial facilities, animal sourcing, processing, and ethical clearance

The trial was conducted at the research facilities of Chalmar Beef and Lamb commercial feedlot and slaughter plant (Farm Tweefontein 19 IR, Babsfontein, Gauteng Province, South Africa) under the regulations of South African National Standards for the Care and Use of Animals for Scientific Purpose (SANS 10386: 2021) with ethical clearance from the Stellenbosch University ethics committee (reference number ACU-2022-24893). In addition, the study was approved under Section 20 of the Animal Diseases Act, 1984 (Act No 35 of 1984) by the South African Department of Agriculture, Land Reform and Rural Development.

Forty ($n = 40$; average initial live weight of 28 ± 0.836 kg) Dohne Merino uncastrated lambs were sourced from a single farm. Upon arrival, lambs were weighed, ear tagged, drenched with a broad-spectrum anthelmintic (Trinex Plus 19.5%, Ascendis Animal Health, South Africa) and vaccinated against enterotoxaemia and *Pasteurella* using Multivax P Plus [MSD Animal Health, Intervet (Pty) Ltd., South Africa]. The lambs were allocated to single pens measuring 1 m × 2 m that were constructed in a well-ventilated shed with concrete flooring and wooden shavings for bedding. Each pen had a raised feeder and free access to fresh clean water. Pens and feeders were cleaned weekly and the automatic water troughs were cleaned daily. After 18 d of adaption to a standard feedlot grower diet (no trial additives), the lambs were weighed again, received a secondary anthelmintic drench [Startect; derquantel (10 mg/mL) and abamectin (1 mg/mL); Zoetis (Pty) Ltd., Australia], vaccination (Covexin 10; Cooper Veterinary Products (Pty) Ltd., South Africa) and

a hormone-free growth stimulant [{Zeranol 12 mg (73.8% m/m)}; Ralgro Sheep, MSD Animal Health, Intervet (Pty) Ltd., South Africa]. Weights at 18 d after arrival were used to allocate lambs to 4 different treatment groups.

Trial design and treatments

The lambs were randomly allocated to 4 dietary treatment groups (Table 1) according to weight so that the mean starting weights were similar for the 4 treatment groups (28 ± 0.836 kg). The diet of the control group (Diet 1: Grain) consisted of crushed yellow maize as main energy source 12.8 MJ/kg or 3.07 MJ/kg DM). The remaining diets were based on the control diet but were supplemented with either plant oil only [Diet 2: Oil, 1.62% DM basis; soybean-sunflower liquid oil blend, Southern Oil (Pty) Ltd., South Africa]; or plant oil and synthetic antioxidant [Diet 3: OilCaps; ethoxyquin (6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline, Capsoquin, ITPSA, Barcelona, Spain); or plant oil and natural antioxidant [Diet 4: OilNat; Biored, monomeric flavonoids and polymeric pro-anthocyanidins, Reg. nr. V20924, Act 36/1947; Biorem Biological Products, South Africa). The soybean-sunflower oil blend was in an unrefined, crude form as it is a by-product of the oil refinery industry. Across the treatments, the feed was formulated to be iso-energetic and iso-nitrogenous. The supplementation of oil was therefore not intended to increase energy levels but to provide a cost effective, safer form of energy than pure starch in a high-energy diet. The total lipid content of the supplemented diets (Oil, OilCaps, OilNat) was 5.5% compared to 4% for the control diet (Grain). Feed and water were available ad libitum through the duration of the trial.

Table 1. Ingredients of 4 treatment diets on a dry matter basis

Ingredients (%)	Grain	Oil	OilCaps	OilNat
Base mix				
Ammonium chloride	0.84	0.84	0.84	0.84
Yellow maize	77.0	75.4	75.4	75.4
Dried distillers grain	3.04	3.04	3.04	3.04
Maize plant roughage	2.49	2.49	2.49	2.49
Liquid molasses	2.60	2.60	2.60	2.60
Soya hulls	9.17	9.17	9.17	9.17
Soybean oilcake	1.62	1.62	1.62	1.62
Sheep premix	1.81	1.81	1.81	1.81
Urea	0.86	0.86	0.86	0.86
Salt	0.60	0.60	0.60	0.60
Supplements				
Soybean-sunflower oil	0.00	1.62	1.62	1.62
Capsoquin	0.00	0.00	0.015	0.00
Biored	0.00	0.00	0.00	0.02

Base mix: The standard base ingredients that were included in all the treatment diets.

Supplements: Ingredients that were subject to treatment differences.

Grain: Control, base mix feed supplemented with yellow maize; did not contain soybean-sunflower unrefined oil blend.

Oil: Treatment, base mix feed supplemented with soybean-sunflower unrefined oil blend only.

OilCaps: Treatment, supplemented with soybean-sunflower unrefined oil blend and the synthetic antioxidant, Capsoquin.

OilNat: Treatment, supplemented with soybean-sunflower unrefined oil blend and the natural antioxidant, Biored.

Table 2. Calculated nutritional value of 4 treatment diets on a dry matter basis

Nutrient	Grain	Oil	OilCaps	OilNat
TDN (g/kg)	816.4	802.6	802.6	802.6
Crude fiber (g/kg)	61.5	60.8	60.8	60.8
ME (Mcal/kg)	3.07	3.02	3.02	3.02
Ether extract (g/kg)	39.6	55.1	55.1	55.1
Crude protein (g/kg)	141	139	139	139

ME = metabolizable energy; TDN = total digestible nutrients.

Grain: Control, base mix feed supplemented with yellow maize, did not contain soybean-sunflower unrefined oil blend.

Oil: Treatment, base mix feed supplemented with soybean-sunflower unrefined oil blend only.

OilCaps: Treatment, supplemented with soybean-sunflower unrefined oil blend and the synthetic antioxidant, Capsoquin.

OilNat: Treatment, supplemented with soybean-sunflower unrefined oil blend and the natural antioxidant, Biored.

Animal slaughter, processing, and sampling

After 52 d on feed, the lambs were slaughtered at Chalmar Beef and Lamb abattoir according to commercial practice that included electrical stunning (1A peak current across the head, 2 s) followed by exsanguination, electrically stimulation with an ECS-1 Jarvis stimulator (Jarvis Products Corporation RSA (Pty) Ltd, Germiston, South Africa), with an output of rectangular DC wave, 150 V, maximum amplitude 17 Hz, 5 ms^{-1} pulse width, RMS-Voltage below 50 V for 20 s. Carcasses were chilled at 2°C ± 2.0°C for 18 h before sampling.

One hour and 24 h postmortem pH (pH_1, pH_{24}) and temperature values (T_1, T_{24}) of the *longissimus thoracis* (LT) were recorded between the 9th and the 10th rib with a handheld pH meter (Crison pH 25, Crison Instruments, S.A) fitted with a glass electrode and temperature probe. At both timepoints, the pH meter was calibrated with pH 7.00 and 4.01 buffers at room temperature of 12°C ± 2.0°C for pH_1 and 2.0°C ± 2.0°C for

pH_{24}, respectively. The loin section (bone-in) including the *M. longissimus thoracis et lumborum* (LTL of both sides were removed from the 9th rib to the 5th lumbar vertebrae [rack saddle (4928) and loin saddle (4883); UNECE Standard, 2006], vacuum-packed, labeled, and shipped to the meat laboratory at Stellenbosch University (Stellenbosch, Western Cape Province, South Africa) under refrigerated conditions, reaching the laboratory 3 d postmortem. Following the method described in Baldi et al. (2019), starting from the cranial toward the caudal end, the loin of the left carcass side was sectioned into one 5-cm portion (LT) for proximate analyses, one 3-cm portion (LT) for fatty acid composition, and three 2.5 cm portions (*m. longissimus lumborum*; LL) for shelf stability test (color and oxidative stability).

Meat proximate analyses

The LT samples for proximate analyses were deboned, trimmed of any visible subcutaneous fat (SCF) and connective tissue, and homogenized using a knife mill (Knifetec 1095, Höganäs, Sweden), vacuum-packed, and stored at −20°C. Moisture (method 950.46) and ash (method 942.05) were analyzed according to AOAC (2005) guidelines. Total meat lipids were extracted using chloroform:methanol (2:1 v/v) solution, and IMF was analyzed according to Lee et al. (1996). Nitrogen content was analyzed on the dried (60°C for 48 h), defatted meat using the Dumas technique (method 968.06; AOAC, 2005). A 0.15 g ground meat sample was encapsulated in a LECO foil sheet and analyzed utilizing a LECO Nitrogen/Protein Analyzer (FP-528, LECO Corporation, Miami, FL). Crude protein was calculated as the total nitrogen (percent) multiplied by 6.25.

Meat fatty acids

Extraction of lipids from 1 g of lyophilized LT muscle was done using chloroform and methanol mixture in a ratio of 2:1 (v/v; Folch, 1957). Thereafter, 10 mg of lipid extracts were dissolved in 1 mL of toluene containing 1 mg of *cis*10-heptadecenoic acid methyl ester (*c*10–17:1) as internal standard (standard no. U-42-M, Nu-Chek Prep, Inc., Elysian, MN). The 2-step methylation procedure outlined by Cruz-Henandez et al. (2004) was conducted, first using 0.3 mL of sodium methoxide (0.5 M in methanol) incubated in a water bath at 50°C for 15 min followed by 1 mL of 5% methanolic hydrochloric acid (HCl) incubated at 80°C for 30 min. After cooling the samples to room temperature (~21°C), 1 mL of deionized water and 3 mL of hexane were added, mixed thoroughly, and centrifuged at $1{,}000 \times g$ for 5 min to clearly separate phases. The upper organic phase containing fatty acid methyl esters (FAMEs) was transferred to a vial containing sodium sulphate to remove any water. The analysis of FAMEs was performed using gas chromatography (6890 N, Agilent Technologies, Santa Clara, CA) coupled to a flame ionization detector (Palo Alto, CA) with a highly polar Rt-2560 capillary column (100 m, 0.25 mm ID, 0.20 μmol film thickness; Restek, USA). The carrier gas was hydrogen with a flow rate of 1 mL/min; the injector and detector temperature was 250°C. One microliter of the sample was injected into the machine in 5:1 split ratio. The oven temperature was set at 45°C for 4 min and increased at a rate of 13°C/min for 1 min to 175°C for 27 min and increased to 215°C at a rate of 4°C/min for 35 min, with a total run time of 86 min. FAMEs were identified by comparison of retention times with reference standards (GLC 463 and UC-59 M, Nu-Chek Prep, Inc.). The FAME results were expressed as mg/100 g meat.

Shelf-display stability

Color was measured using a BYK Gardner Color-Guide 45/0 (6807) portable colorimeter (BYK-Chemie GmbH, Wesel, Germany), set at D65 illuminance, 10° viewing angle and aperture of 8 mm, was calibrated prior to color measurements starting with the green, black, and white standards for calibration, in that order. The 3 LL portions (with SCF and bone removed) allotted for shelf stability test were randomly assigned to black Styrofoam trays to evaluate the effect of different shelf storage display times (0, 3, and 7 d) on drip loss, color, and protein and fat oxidation. Muscle cuts were removed ~72 h postmortem, and day 0 implied the first measurement after processing the cuts. Trays were covered with a 10 μm polyvinyl chloride film (Versafilm, Crown National, Montague Gardens, Cape Town, South Africa) and displayed under a continuous, cool and white, fluorescent illumination (4,600 lm; Philips TL-D 58 W/33–640 1SL/25, Bielsko-Biala, Poland) at 4°C ± 2°C, simulating retail display conditions. The samples were removed from the packaging and the surface was patted dry with a paper towel before recording end-weights. Drip loss was measured at 3-d and 7-d shelf display by expressing the weight loss of the respective cuts between day 0 and days 3 and 7, respectively, as a percentage of the initial weight (day 0).

Color was measured at 4 positions on the meat surface on the day of cutting (day 0) and subsequently after 3 and 7 d to determine color shelf stability.

Measurements were taken on the first cut for 0 d measurement after processing the 4 chops and allowing the muscle to bloom for 1 h at 4°C ± 2°C and directly after removing of the cling film for subsequent measurements on days 3 and 7. The CIE color convention (Commission International de 1'Eclairage, 1976) was followed where the 3 fundamental outputs are lightness (L^*) on a scale of 0 (all light absorbed) to 100 (all light reflected); redness (a^*) spans from +60 (red) to −60 (green); and yellowness (b^*) values span from +60 (yellow) to −60 (blue). Saturation index, also known as chroma (C), was calculated as $C = (a^{*2} + b^{*2})^{0.5}$ and hue angle $(h°) = \tan^{-1}(b^*/a^*)$. For statistical analysis, the mean values of 4 measurements from different locations on the samples were used.

After color and drip loss assessments, the samples were homogenized at 4°C ± 2°C for ~15 s using a water-cooled knife mill (Knifetec 1095) and divided into 2 equal parts for the analysis of lipid and protein oxidation. These samples were vacuum-sealed and kept at −80°C until analyzed. Thiobarbituric acid reactive substances (TBARS) assay was used to estimate the secondary products of lipid peroxidation (Gatellier et al., 2005). In short, 20 mL of 150 mM potassium chloride buffer containing 0.1 mM BHT was mixed with 1 g of lamb meat sample and homogenized at 3,000 rpm for 20 s. A 500 μL aliquot of the homogenate was mixed with 0.25 mL of a 1% (w/v) thiobarbituric acid solution containing 0.05 M sodium hydroxide and 0.25 mL of a 2.8% (w/v) trichloroacetic acid (TCA). After vortexing, the mixture was incubated at 100°C in a water bath for 1 h. After cooling to room temperature, 2 mL of 1-butanol was added and samples centrifuged at 2,575 × g for 30 min. Absorbance readings were measured in triplicate at 532 nm with a spectrophotometer (SPECTROstar Nano, BMG, Ortenberg, Germany). The concentration of TBARS was calculated using 1,1,3,3-tetramethoxypropane (TMP) standard curve (0–20 μM; $R^2 > 0.99$) and presented as mg malondialdehyde (MDA)/kg meat.

Protein oxidation was evaluated in triplicate by calorimetrically measuring total carbonyl content and protein concentration as described by Uushona et al. (2023). Briefly, the homogenate was divided into 2 equal aliquots of 0.1 mL and precipitated with 10% TCA (w/v) in 1 mL after homogenizing with 10 mL of 0.15 M potassium chloride. One pellet was suspended in 1 mL of 2 N HCl, while the other was suspended in 0.5 mL of 2 N HCl and 0.5 mL of 0.2% solution of 2,4-dinitrophenylhydrazine (DNPH; w/v) in 37% HCl. Both pellets were then incubated at room temperature (~21°C) for 1 h, after which the proteins precipitated again in 0.5 mL of TCA. Carbonyl content was estimated from the difference in absorbance between the DNPH-treated pellet and the HCl-treated pellet measured at 375 nm (SPECTROstar Nano, BMG, Ortenberg, Germany) based on the adsorption coefficient of was expressed in nmol of carbonyl/mg of protein using a molar extinction coefficient of 22 $mM^{-1} \cdot cm^{-1}$ (for protein hydrazones). Protein concentration was quantified calorimetrically using a QuantiPro BCA (bicinchoninic acid) Assay Kit (Sigma-Aldrich, St. Louis, MO).

Statistical analyses

Data analyses were performed in Statistica Software 14 (TIBCO, Palo Alto, CA). Data were analyzed using a linear mixed-effects model approach with diet as a fixed effect (days on display added for shelf stability analyses) and carcass nested within diet as random effects. A one-way analysis of variance (ANOVA) was performed, except where shelf display was involved, when a two-way ANOVA was used, and the fixed effects of diet and days on display as well as their interactions were tested. Individual lambs were the experimental units. Statistical significance was determined at a level of $P < 0.05$.

Results

Summary of growth and slaughter results

Diet had no effect on growth performance and carcass characteristics ($P > 0.050$). Mean values (and standard error of mean) for daily feed intake, average daily gain, slaughter weight, cold carcass weight, and back fat thickness (9th and 10th rib 2.5 cm from midline) were 1.68 kg/day (0.041), 0.32 kg/day (0.019), 48.8 kg (1.295), 23.05 kg (0.629), and 7.4 mm (0.843), respectively.

Physicochemical properties: proximate analyses and pH measurements

Results for muscle physicochemical properties are presented in Table 3. No differences ($P > 0.050$) were recorded for proximate analyses or muscle pH and temperature across the treatment groups.

Shelf-display stability

Diet had no effect on any of the color parameters ($P > 0.050$), while the effect of shelf display days was significant for all parameters ($P < 0.05$; Table 4). Lightness reached its highest value on day

Table 3. Effect of plant oil supplementation and type of antioxidant on physicochemical properties of lamb *longissimus* muscle

Item	Grain ($n = 10$)	Oil ($n = 10$)	OilCaps ($n = 10$)	OilNat ($n = 10$)	SEM	*P* value
Proximate composition (%)						
Ash	1.14	1.16	1.13	1.14	0.028	0.815
Crude protein	19.44	20.46	20.07	19.44	0.478	0.362
Crude fat	5.09	5.45	4.98	5.29	0.475	0.897
Moisture	73.64	73.12	73.80	73.75	0.230	0.160
pH and temperature						
pH 1 h	6.20	6.22	6.18	6.16	0.038	0.711
Temperature 1 h (°C)	36.0	33.9	35.8	35.4	0.626	0.079
pH 24 h	5.66	5.62	5.62	5.62	0.018	0.204
Temperature 24 h (°C)	4.7	4.6	4.6	4.6	0.025	0.214

SEM = standard error of mean.

[a,b]Means within a row with different superscripts differ significantly at $P < 0.050$.

Grain: Control, base mix feed supplemented with yellow maize, did not contain soybean-sunflower unrefined oil blend.

Oil: Treatment, base mix feed supplemented with soybean-sunflower unrefined oil blend only.

OilCaps: Treatment, supplemented with soybean-sunflower unrefined oil blend and the synthetic antioxidant, Capsoquin.

OilNat: Treatment, supplemented with soybean-sunflower unrefined oil blend and the natural antioxidant, Biored.

3 and then declined, showing meat becoming darker by day 7 ($P = 0.003$). The exception was the Oil treatment, where lightness continued to increase between 3 and 7 d, although no differences ($P > 0.050$) were recorded between diet groups at day 7. Redness declined consistently over the 7 d ($P < 0.001$) on display and the Oil diet reached the lowest value, significantly lower than OilCaps on day 7 (Interaction: $P = 0.013$). The values for yellowness remained stable between day 0 and 3 and then declined significantly to day 7 for all treatments ($P < 0.001$). For chroma, day 3 values were lower than day 0 values, except for the Grain diet, but then declined by ∼6 units between days 3 and 7 ($P < 0.001$). Hue angle showed almost a mirror image of chroma showing an initial slower color drift between days 0 and 3, which accelerated between days 3 and 7 ($P < 0.001$) for all treatments, but in particular for the Oil diet, which recorded numerically higher

Table 4. Effects of plant oil supplementation and type of antioxidant on color stability of lamb *longissimus* muscle

	Day 0				Day 3				Day 7				
Item	Grain	Oil	OilCaps	OilNat	Grain	Oil	OilCaps	OilNat	Grain	Oil	OilCaps	OilNat	SEM
Lightness	40.1	39.9	40.4	40.2	40.8	41.2	42.1	41.4	39.2	41.8	39.7	38.9	0.798
Redness	15.5^{abc}	16.5^{a}	15.8^{ab}	16.9^{a}	14.2^{d}	14.9^{bcd}	14.1^{cd}	13.8^{d}	9.1^{ef}	8.3^{f}	9.8^{e}	9.5^{ef}	0.518
Yellowness	13.9	14.1	13.6	14.4	13.2	14.0	13.2	13.1	10.0	11.3	10.9	10.7	0.441
Chroma	20.8^{abc}	21.7^{a}	20.8^{abc}	22.2^{a}	19.4^{cde}	20.4^{bcde}	19.4^{de}	19.1^{e}	13.7^{f}	14.2^{f}	14.7^{f}	14.4^{f}	0.525
Hue angle	41.6	40.7	40.8	40.4	43.0	43.3	43.3	43.6	48.1	53.9	48.1	48.4	1.674

***P* values**	Diet (D)	Days (d)	D × d
Lightness	0.656	0.003	0.119
Redness	0.889	<0.001	0.013
Yellowness	0.338	<0.001	0.563
Chroma	0.604	<0.001	0.032
Hue angle	0.492	<0.001	0.366

SEM = standard error of mean.

[a–f]Means within a row with different superscripts differ significantly at $P < 0.050$.

Days: 0, 3, 7.

Grain: Control, base mix feed supplemented with yellow maize, did not contain soybean-sunflower unrefined oil blend.

Oil: Treatment, base mix feed supplemented with soybean-sunflower unrefined oil blend only.

OilCaps: Treatment, supplemented with soybean-sunflower unrefined oil blend and the synthetic antioxidant, Capsoquin.

OilNat: Treatment, supplemented with soybean-sunflower unrefined oil blend and the natural antioxidant, Biored.

($P > 0.050$) values for hue angle on day 7 compared to the other 3 treatment groups.

Oxidation as measured by TBARS and free carbonyls increased between days 0 and 7 on retail display ($P < 0.010$; Table 5). Neither fat oxidation nor protein oxidation were affected by diet ($P > 0.050$).

Meat fatty acids

No differences were recorded across treatments for total fatty acid content (mg/100 meat; $P = 0.536$), total saturated fatty acids (SFA; $P = 0.420$), monounsaturated fatty acids (MUFA; $P = 0.733$), or PUFA ($P = 0.890$). However, some minor fatty acids showed differences within the MUFA group. Petroselinic acid (*c*6–18:1) was higher ($P = 0.037$) in the OilCaps treatment groups compared to all the other groups. Elaidic acid (*t*9-18:1) was lowest in the Oil treatment group compared to all other treatments ($P = 0.038$). Total CLA was higher ($P = 0.028$) for oil treatments compared to the Grain treatment. The *t*10,*c*12–18:2 CLA isomer was highest in OilNat, intermediate in Oil and OilCaps, and lowest in Grain ($P = 0.001$), while the *c*11,*t*13–18:2 CLA isomer was higher ($P = 0.019$) in all Oil treatments compared to the Grain treatment.

Discussion

Physicochemical properties

The pH and temperature values recorded at 1 h postmortem suggest a normal glycolysis pathway for electrically stimulated lambs, and the final pH of ~5.6 was within the accepted pH range of 5.4 to 5.8 for lamb meat (Warner, 2016; Holman et al., 2021). Although higher levels of dietary fat were expected to increase IMF and total fatty acids, no differences were found between the Grain treatment and Oil treatments, which agrees with other studies wherein oil inclusions were much higher (Manso et al., 2009, Francisco et al. 2015; Miltko et al., 2019). However, Quiñones et al. (2019) recorded higher IMF and total fatty acids when adding canola oil to the diet, at levels that were difficult to calculate (50 mL of canola oil 3 times per week). Bessa et al. (2005) recorded increased SCF and IMF levels only when lucerne was included as dietary component but no effect with pure concentrate diet. Moreover, fatty acids composition was affected by type of diet (lucerne vs. no lucerne; Bessa et al., 2005). The high SCF and IMF was attributed to higher fat content of the lucerne-oil diet and higher feed intake compared to the concentrate-oil diet and is therefore a function of higher intake of oil (Bessa et al., 2005). In the current study, oil supplementation did not affect feed intake.

Shelf-display stability

Color parameters showed little variation across treatments over the 7-d shelf display period and followed a normal pattern of deterioration toward 7 d as oxidation of oxymyoglobin to metmyoglobin progressed (King et al., 2023). The numerically higher hue angle value ($P > 0.05$) for the Oil treatment at 7 d

Table 5. Effect of plant oil supplementation and type of antioxidant on oxidative stability of lamb *longissimus* muscle

	Day 0				Day 7				
Item	Grain	Oil	OilCaps	OilNat	Grain	Oil	OilCaps	OilNat	SEM
TBARS	0.43	0.49	0.51	0.54	0.98	1.02	0.92	0.88	0.085
FC	5.59	6.05	5.17	5.04	8.97	8.22	8.10	7.15	0.611
***P* values**	Diet (D)	Days (d)	D × d						
TBARS	0.96	<0.01	0.48						
FC	0.43	<0.01	0.27						

SEM = standard error of mean.

[a,b]Means within a row with different superscripts differ significantly at $P < 0.05$.

Fat oxidation: TBARS (thiobarbituric acid reactive substances) expressed as mg malondialdehyde/kg meat.

Protein oxidation: FC = free carbonyls expressed as nmol carbonyl/mg protein.

Grain: Control, base mix feed supplemented with yellow maize, did not contain soybean-sunflower unrefined oil blend.

Oil: Treatment, base mix feed supplemented with soybean-sunflower unrefined oil blend only.

OilCaps: Treatment, supplemented with soybean-sunflower unrefined oil blend and the synthetic antioxidant, Capsoquin.

OilNat: Treatment, supplemented with soybean-sunflower unrefined oil blend and the natural antioxidant, Biored.

may suggest higher levels of metmyoglobin (MMb) formation, which could be linked to higher lipid oxidation in this treatment group, causing aldehyde products to initiate conformational changes in myoglobin (Alderton et al., 2003). However, despite significant differences in TBAR values between day 0 and 7 of display (Table 5), no differences were recorded across treatments to support the suggestion of higher lipid oxidation in the oil treatment samples. Variation in hue angle values within treatment groups were also much higher at day 7 (standard deviation 5.75–10.23) than at days 0 and 3 (s.d. 0.95–2.69), probably explaining the lack of significance at day 7. Nevertheless, the fact that both antioxidants maintained mean hue angle values similar to that of the Grain treatment compared to the Oil treatment without antioxidants may suggest the involvement of oxidation in the color deterioration even though the effect on hue angle was not significant between the oil diet and the other 3 diet groups at day 7. Inai et al. (2014) suggested that the reduction of the flavanol structure and rapid conversion of the quinone form of polyphenols (in natural antioxidants) to the phenol form plays a role in the oxymyoglobin reduction and higher stability.

Results on the effect of natural antioxidants on oxidative and color stability in the literature are inconsistent. Cho et al. (2010) reported a more desirable color for goat meat when quercetin (flavonoid) was used as antioxidant in goat meat production. Andrés et al. (2014) reported lower values for yellowness and hue angle accompanied by lower TBARS in an extended shelf stability study with lamb supplemented with quercetin, whereas Muela et al. (2014) found no effect on color despite lower levels of MDA in meat of lambs supplemented with a mixed flavonoid product. Bodas et al. (2012) found no effect of naringin (flavonoid) on either color or TBARS over 9 d of shelf stability. Booyens et al. (2012) compared the effect of synthetic and natural antioxidants similar to the present study in feeds supplemented with either soybean oil or tallow (3% DM) and found no effect of type of antioxidant on color or fat oxidative stability, although the antioxidants were not tested against a control. According to the review by Estévez (2021), several studies have indicated that phenolic-rich extracts are efficient in counteracting the negative impact of protein oxidation on product quality. A plausible explanation for the lack of antioxidant effect in our study could be the low inclusion level of plant oils, which suggests that both the base diet (Grain) and supplemented diet (Oil) were relatively shelf-stable under the conditions tested in this study.

Meat fatty acids

The effect of oil supplementation on fatty acid profile of meat depends on the composition of the basal diet, fatty acid profile of the oil, level of inclusion, and duration of feeding (Shingfield et al., 2013; Gómez-Cortés et al., 2014). Although the fatty acid profile of the oil blend in the current study was not determined, crude soybean and sunflower oils are generally high in linoleic and oleic acids. In contrast to the study of Quiñones et al. (2019), oleic acid was not affected but the minor MUFA elaidic acid (*t*9–18:1) was lower in muscle of the Oil treatment compared to all other treatments. Petroselinic acid (*c*6–18:1), which has anti-inflammatory properties (Alaluf et al., 2002), was higher in all oil treatments (for OilCaps) compared to the Grain group. Francisco et al. (2015) recorded higher levels (g/100 g meat) of total *trans*-MUFA and total n-3 PUFA when feeding an oil blend (4% and 8% DM) but found no effect on SFA, total *cis*-MUFA, long chain n-3 PUFA, total n-6 PUFA, or CLA. The concentration of the affected fatty acids corresponded to the level of oil inclusion (Francisco et al., 2015). On the other hand, Miltko et al. (2019) recorded lower SFA and MUFA and higher n-3 PUFA with 5% inclusion of linseed in lamb feedlot diets but no effect with rapeseed oil. The different effects were owed to the differences in fatty acid makeup of the 2 oils, with linseed having less oleic acid (MUFA) and linoleic acid (n-6 PUFA) and more alpha linolenic (n-3 PUFA) than rapeseed oil. Bessa et al. (2008) reported similar distinctions when feeding sunflower or linseed with the former increasing rumenic acid (RA; *c*9, *t*11–18:2) and its precursor vaccenic acid (VA; *t*11–18:1) more than the latter. Linoleic acid (LA) is less likely to be exposed to biohydrogenation than alpha-linolenic acid (ALA; 90%; Hur et al., 2017). Neither LA, RA, the most common natural form of CLA, or VA were affected by oil supplement in our study, which agrees with the studies of Manso et al. (2009) and Miltko et al. (2019). In contrast, higher values for the *t*10, *c*12–18:2 isomer were recorded and could be explained by a "*trans*10 shift" in FA from RA and VA toward *t*10, *c*12–18:2 and *t*10–18:1 (not in our study) when high cereal grain diets are supplemented with LA-rich feed components. Apparently, high fermentable starch diets cause the lowering of rumen pH resulting in a change in rumen microbe composition towards sugar-fermenting species and a "*trans*10 shift" when an LA supplement is added (Mapiye et al., 2015; Bravo-Lamas et al., 2016). Bessa et al. (2005) and Manso et al. (2009) also recorded higher *t*10, *c*12–18:2 levels when feeding

soybean and sunflower oil, respectively. According to Vahmani et al. (2020) the effects of t10,c12–18:2 on human health are varied across literature. t10,c12–18:2 is known to have health benefits including anti-obesity (body fat reduction), anti-atherosclerotic, and anti-diabetic properties, while other studies associated it

Table 6. Effect of plant oil supplementation and type of antioxidant on fatty acid content of lamb *longissimus* muscle (mg fatty acid/100 g of meat)

Fatty acid		Grain	Oil	OilCaps	OilNat	SEM	*P* value
Total PUFA		314.7	327.3	311.5	338.1	26.74	0.890
Selected PUFA							
LA	18:2n–6	257.8	273.8	259.5	277.1	23.3	0.910
ALA	18:3n–3	17.0	17.7	17.2	20.6	1.69	0.405
GLA-AA	18:3n–6/ 20:0	4.8	5.3	5.0	5.8	0.51	0.484
ESA	20:2n–6	2.4	2.5	2.4	3.1	0.23	0.157
D-GLA	20:3n–6	2.0	2.3	1.8	1.9	0.61	0.941
DPA	22:5n–3	15.3	12.2	13.3	14.6	1.14	0.237
DHA	22:6n–3	7.9	5.5	5.8	6.8	0.73	0.105
Total CLA		55.9[a]	78.7[b]	75.5[b]	87.8[b]	7.29	0.028
Rumenic acid	c-9, t-11–18:2	23.5	29.3	29.7	32.3	3.07	0.246
Linolelaidic acid	t-9, t-12–18:2	4.8	5.2	5.1	6.2	1.16	0.845
	t-10, c-12–18:2	2.9[a]	4.4[bc]	4.3[b]	5.5[c]	0.42	0.001
	c-11, t-13–18:2	24.7[a]	40.6[b]	37.4[b]	43.8[b]	4.33	0.019
Total MUFA		1,253	1,386	1,368	1,454	128	0.733
Selected MUFA							
Myristoleic acid	c-9–14:1	3.9	3.8	4.0	3.9	0.44	0.995
Pentadecenoic acid	c-10–15:1	5.4	3.3	4.6	5.5	1.26	0.573
Palmitoleic acid	c-9–16:1	54.0	61.1	63.9	60.4	5.76	0.668
Palmitelaidic acid	t-9–16:1	9.75	8.44	9.69	12.11	2.63	0.798
Petroselinic acid	c-6–18:1	24.9[a]	45.1[ab]	51.9[b]	49.5[ab]	8.80	0.037
Oleic acid	c-9–18:1	1054	1156	1155	1212	108	0.774
Elaidic acid	t-9–18:1	18.7[a]	10.8[b]	11.6[ab]	13.2[ab]	2.61	0.038
Cis-Vaccenic acid	c11–18:1	55.3	60.1	58.4	63.4	4.65	0.667
	t10/t11–18:1	19.7	28.7	25.1	29.4	4.77	0.464
Eicosenoic acid	c-5–20:1	0.56	0.46	0.54	0.73	0.12	0.386
	c-8/c-11–20:1	2.6	3.00	2.9	3.8	0.34	0.125
Total SFA		1,216	1,468	1,395	1,531	139	0.420
Lauric acid	12:0	4.2	4.5	4.2	4.3	0.46	0.966
Tridecylic acid	13:0	7.7	6.8	6.6	7.2	0.64	0.614
Myristic acid	14:0	71.4	90.7	85.5	90.3	8.62	0.367
Pentadecylic acid	15:0	18.5	20.9	19.5	21.7	1.81	0.589
Palmitic acid	16:0	735.9	883.9	842.8	907.3	83.0	0.484
Margaric acid	17:0	63.11	72.91	71.43	76.01	6.93	0.599
Stearic acid	18:0	313.1	386.7	372.7	421.8	42.7	0.352
Behenic acid	22:0	2.5	1.7	2.1	2.5	0.41	0.513
Total fatty acids		2,840	3,262	3,164	3,412	281	0.536

ALA = alpha-linolenic acid; c = cis; CLA = conjugated linoleic acid; D-GLA= dihomo-gamma-linolenic acid; DHA = docosahexaenoic acid; DPA = docosapentaenoic acid; ESA = eicosapentaenoic acid; GLA-AA = gamma-linolenic acid co-eluted with arachidonic acid; LA = linoleic acid; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SEM = standard error of mean; SFA = saturated fatty acids; t = trans; t10/t11–18:1 = trans vaccenic acid co-eluded.

[a,b]Means within a row with different superscripts differ significantly at $P < 0.050$.

Grain: Control, base mix feed supplemented with yellow maize, did not contain soybean-sunflower unrefined oil blend.

Oil: Treatment, base mix feed supplemented with soybean-sunflower unrefined oil blend only.

OilCaps: Treatment, supplemented with soybean-sunflower unrefined oil blend and the synthetic antioxidant, Capsoquin.

OilNat: Treatment, supplemented with soybean-sunflower unrefined oil blend and the natural antioxidant, Biored.

with increased insulin resistance, hepatic steatosis, pro-carcinogenic properties, adverse effects on blood lipids, and inflammation.

CLA isomer *c*11, *t*13–18:2 was as abundant in our study as the more common RA (*c*9, *t*11–18:2) and was higher in all oil treatments; this is the first report of this isomer in meat from lambs fed plant oils. However, Collomb et al. (2004) reported that levels of *c*11, *t*13–18:2 in milk corresponded with high intake of ALA, which does not correspond with the lower levels of ALA in sunflower and soyabean oil. Although there is limited knowledge on bioactivity and health effects of *c*11, *t*13–18:2, it has been found to inhibit the growth of several human cancer cell lines.

Antioxidants did not have any effects on fatty acid levels in meat from lambs fed oil diets, except for minor *t*10, *c*12–18:2 that was higher in OilNat compared to OilCap treatments, but the relevance of such a small difference is questionable. These findings are comparable to a report by Booyens et al. (2012) that found no effects of antioxidants on fatty acid profile in oil and tallow supplemented diets.

Conclusion

The inclusion of low levels of plant oil (soybean-sunflower blend) in a high-energy lamb feedlot diet to mitigate the risk of metabolic disorders has limited effects on meat quality. Although color stability was not significantly affected by oil supplement numerically higher values for hue angle indicating color deterioration beyond 3 d for this treatment (Oil) may be worthwhile exploring further. Although fatty acid composition did not show major changes in response to low supplementation levels of oil, CLA levels were increased and could contribute to the nutritional value of supplemented lamb.

Acknowledgments

The authors are grateful to Chalmar Beef and Lamb for sponsoring the research and providing the growth and slaughter facilities.

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