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Effect of extruded flaxseed supplementation during the indoor fattening of yearling bulls on beef carcass, meat composition, and fatty acid profile

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Abstract: This study investigates the impact of supplementing extruded flaxseed to the diet of yearling bulls during the indoor fattening on beef carcass quality, meat composition, and fatty acid profile. Twenty male crossbred calves (Holstein × Simmental) were divided into two groups: control group and flaxseed-supplemented group, each with 10 calves. The control group received a conventional diet, while the flaxseed group was fed the same diet enriched with 5% extruded flaxseed on a dry-matter basis. The study revealed no significant differences in growth performance, carcass weight, or yield between the two groups. However, the flaxseed-supplemented group exhibited a higher intramuscular fat content, which was likely due to the increased energy intake from the flaxseed lipid content. Additionally, flaxseed supplementation improved the fatty acid profile of beef by increasing the percentage of monounsaturated fatty acids (MUFA) and reducing the saturated fatty acid (SFA) percentage and the n-6/n-3 polyunsaturated fatty acid ratio. Despite these improvements, the study did not reveal any significant increase in the proportion of n-3 fatty acids in the meat. These findings suggest that while flaxseed supplementation enhances the nutritional profile of beef, further research is needed to optimise the balance of energy intake to maximise the increase in n-3 fatty acids.

Keywords: extruded flaxseed; Holstein-Simmental crossbreds; indoor fattening; intramuscular fat; monounsaturated fatty acids; n-6/n-3 ratio

Meat, as a rich source of proteins, minerals, and vitamins, is an essential part of the human diet (Long et al. 2020). Meanwhile, increasing health consciousness has led meat consumers worldwide to become concerned about healthy eating habits. In this context, numerous studies have demonstrated that an adequate intake of n-3 fatty acids (FAs) is associated with a reduced risk of coronary heart disease, autoimmune and inflammatory disorders, osteoporosis, rheumatoid arthritis, and certain types of cancer (Simopoulos 2002). The most abundant n-3 FA in food is linolenic acid

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(C18:3 n-3), which is a precursor to docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), two long-chain n-3 FAs involved in many metabolic and physiological processes (Skrivan et al. 2020; Van Dael 2021). Accordingly, an adequate intake of C18:3 n-3 has been established as 1.6 g/d for men and 1.1 g/d for women aged 19–50 years, with the possibility of achieving up to 10% of this intake through longer n-3 FAs (Gebauer et al. 2006). In addition, research has also indicated that the recommended ratio of n-6/n-3 polyunsaturated fatty acids (PUFA) should be 4–5 or less (Skrivan et al. 2020).

Among consumed meats, ruminant meat can be a relevant source of n-3 FAs, which is especially high when the animal is grass-fed (Davis et al. 2022). When cattle are produced indoors, i.e. not grass-fed, the beef sector faces an increased pressure to offer beef with a healthier FA profile (Leiva et al. 2021). Increased levels of the n-3 FAs in indoor-reared beef can be achieved with varying degrees of success by adding rich sources of C18:3 n-3 to cattle diets (Mir et al. 2018; Leiva et al. 2021).

Flaxseeds (*Linum usitatissimum*) and their oil are natural sources of polyunsaturated FA (PUFA) characterised by their high content of C18:3 n-3. There are 35–45% of lipids in flaxseeds, 45–52% of which are C18:3 n-3. Consequently, both flaxseeds and flaxseed oil can be added to ruminant diets to increase the C18:3 n-3 proportion in beef and to decrease the PUFA n-6 to PUFA n-3 ratio, and such additions have resulted in decreased saturated fatty acids (SFA) and increased PUFA percentages in beef lipids (Barton et al. 2007; Gomez et al. 2016; Leiva et al. 2021). Moreover, flaxseed can also be used as an alternative source of protein to soy in ruminant diets. However, the flaxseed high oil content limits its use, as research has shown that the fat content within cattle diet should be limited (Petricevic et al. 2020). In addition, high levels of flaxseed in the diet may reduce feed intake and pose a risk to animal safety due to anti-nutritional factors, which can be reduced with previous heating or fermentation (Xu et al. 2022). Due to these factors, the maximum amount of flaxseed in the diet of cattle should not exceed 5–14% of dry matter.

The effect of feeding flaxseed or flaxseed oil on the lipid profile of beef varies depending on feeding factors such as the ratio of concentrate or forage in the diet (Vahmani et al. 2015). It appears that the efficacy of flaxseed in increasing C18:3 n-3 is higher in concentrate-rich diets. This finding seems to be mediated by the effect of the concentrate/forage ratio on the FA biohydrogenation reactions that take place in the rumen. Although numerous studies have examined the effects of flaxseed supplementation on beef quality, the existing research has mostly focused on specialised beef breeds, under outdoor or mixed feeding conditions, and commonly whole or rolled flaxseed rather than extruded forms was used. Thus, little information is available regarding the use of extruded flaxseed in indoor fattening systems based on maize silage, which represents the predominant production model in Bosnia and Herzegovina and much of Central and Southeastern Europe. Furthermore, data on Holstein × Simmental crossbreds are scarce, despite their major contribution to regional beef production and their distinct physiological characteristics affecting lipid deposition and fatty acid metabolism.

This study aims to assess the effects of including a limited amount of extruded flaxseed in the fattening diet of crossbred yearlings fed concentrate and silage, specifically examining its influence on beef quality and fatty acid profile. The present study therefore provides an additional insight by evaluating this supplementation strategy under a production context of particular relevance for the Western Balkan region, where improving the beef nutritional quality has growing economic and public-health importance.

MATERIAL AND METHODS

Animals and feeding conditions. Twenty 6-month-old male calves of the crossbred Holstein × Simmental (50%) cows were selected on a local commercial dairy cow farm (Draga, Podzvizd, municipality of Velika Kladuša, Bosnia and Herzegovina; latitude 45°11'02.3''N, longitude 15°52'10.6''E). All calves were purchased from an external dairy farm and the number of sires represented in each group could not be determined. The animals used in the trial were not castrated. The fattening period lasted exactly 275 days, which corresponds to the minimum duration required for farmers to meet eligibility criteria for national livestock subsidies. All animals were slaughtered on the same day, under identical pre-slaughter handling and fasting conditions, in order to eliminate variability associated with slaughter timing. The calves were divided into

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two homogeneous groups of 10 animals each and allocated on the basis of body weight to two feeding treatments carried out in separate 50 m^2 barns on the farm: control (CON) and flaxseed-supplemented group (FXS), with the respective initial body weights of 189 ± 19.2 kg and 186 ± 19.2 kg. After a 10-day adaptation period to concentrate, the animals were fattened for the nine-month trial period on maize silage offered *ad libitum* and 4 kg per day of the respective commercial concentrate.

The CON concentrate was a complete pelleted feed normally used on the farm and it consisted of maize, maize gluten feed, wheat feed, sunflower meal, barley, sugar beet molasses, calcium carbonate, sodium bicarbonate, sodium chloride, and a mineral–vitamin premix (see Table 1 for the nutrient composition). The FXS concentrate contained the same ingredients but it was formulated with the inclusion of 5% extruded flaxseed, replacing an equivalent proportion of the CON concentrate on a dry-matter basis. Both the CON and FXS concentrates were produced commercially and specifically for this experiment by the Emona Krmila Company (Ljubljana, Republic of Slovenia). Flaxseed was subjected to an extrusion process to deactivate cyanogenic glycoside-based antinutritional factors (Xu et al. 2022).

The silage was sampled twice during the experiment and analysed for dry matter (ISO 1999), crude protein (ISO 2009), crude fat (ISO 2015), crude fibre (ISO 2000), and ash (ISO 2022) in the laboratory of the Biotechnical Faculty of the University of Bihać. Analyses of the commercial concentrates for dry matter (ISO 1999), crude protein (ISO 2009), crude fat (ISO 2015), crude fibre (ISO 2000), crude ash (ISO 2022), phosphorus (ISO 1998), calcium, sodium, and magnesium (ISO 2000) were performed at the Emona Development Centre for Nutrition (Ljubljana, Republic of Slovenia). The chemical composition of the maize silage and concentrates is also presented in Table 1. Moreover, the fatty acid composition of the concentrates is shown in Table 2.

At the end of the trial, the animals were transported from the farm to a regional slaughterhouse, MS-ALEM, Bosanska Krupa (Bosnia and Herzegovina), where they were offered feed and water. The animals were fasted for 24 h before being weighed and then they were slaughtered. The animals were slaughtered in an approved slaughterhouse using the classical method of slaughter according to halal ritual slaughter, which consists of cutting the trachea, oesophagus, and exsanguination by severing the main artery and vein of the neck (*v. jugularis externa* and *a. carotis communis*). All handling practices involving the animals prior to slaughter followed the Law on the Protection and Welfare of Animals (2009) of Bosnia and Herzegovina (Official Gazette of BiH,

Table 1. Chemical composition (%) of the feeds used in this study

Compound	Corn silage	Commercial concentrate[&]	Commercial concentrate[&] + flaxseed[$]
Moisture (%)	64.6	9.6	9.4
Crude protein (%)	2.50	15.1	15.7
Crude fat (%)	1.10	3.90	5.00
Crude fibre (%)	14.1	7.3	8.5
Crude ash (%)	1.20	6.20	6.00
Phosphorus (%)	nd	0.60	–
Calcium (%)	nd	0.90	–
Sodium (%)	nd	0.30	–
Magnesium (%)	nd	0.30	–
ME (MJ/kg DM)	6.5*	12.8	13.2

*Estimated from typical values for maize silage at similar dry matter and fibre contents; [&]The commercial concentrate also contained the following nutritional supplements (per 1 kg): 117.5 mg copper (II) sulphate pentahydrate, 110 mg manganese (II) oxide, 115 mg zinc sulphate monohydrate, 6 mg coated granular cobalt (I) carbonate, 6 mg potassium iodide, 2 mg sodium selenite, and a vitamin premix containing 1 500 IU Vitamin A, 1 250 IU Vitamin D_3, and 50 mg Vitamin E; [$]Estimated from the composition of the commercial concentrate (95%) and the composition of extruded flaxseed (5%), the latter extracted from Giacomino et al. (2013)

DM = dry matter; nd = not determined

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Table 2. Fatty acid profile of the concentrates offered to the animals, expressed as percentage of total fatty acids

Fatty acid	CON	FXS
C 12:1	0.23	0.15
C 14:0	0.13	0.11
C 16:0	14.65	11.31
C 16:1	0.17	0.15
C 17:0	0.11	0.09
C 18:0	2.28	2.69
C 18:1	22.70	21.53
C 18:2 n-6	54.99	41.18
C 18:3 n-3	3.16	21.90
C 20:0	0.30	0.23
C 20:1	0.47	0.33
C 22:0	0.19	0.15
C 24:0	0.31	0.17

CON = control, a commercial concentrate (see Table 1); FXS = flaxseed, the same commercial concentrate enriched with 5% flaxseed

No. 25/09) and the recommendations of Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes (European Union 2010). After slaughtering, processing, and labelling, the hot beef half-carcasses were weighed, then cooled for 24 h at a temperature of 4 °C, and the weight of the cold beef half-carcasses was measured again.

Meat sampling and quality analysis. Twenty-four hours after slaughter, the left-side carcass was jointed and the 6th rib steak was obtained, weighed, and dissected into muscle, bones, fat (subcutaneous and intermuscular fat), and remainder tissues, as described in Robelin and Geay (1975) and Serra et al. (2004).

The proportion of each main tissue was expressed as the percentage of the sum of muscle, bones, and fat. The *longissimus thoracis* (LT) muscle was separated from the ribeye steak that was divided into two similar portions. One of the portions was covered with plastic cling film, cooled, and stored in a chamber at 4 °C, with the pH being measured on day 0, 3, 6, and 9 of storage. The other portion was immediately collected, labelled, ground with a meat grinder for homogenisation, packaged in a vacuum, and immediately transported in a mobile refrigerator at 4 °C to the laboratory of the Faculty of Biotechnology and the laboratory of the Emona Development Centre for Nutrition in Ljubljana (Republic of Slovenia), where meat samples were stored at a temperature of –20 °C until further analysis for their proximate composition and fatty acid profile.

The pH value of the LT muscle was determined using a pH/ORP Meter LX210PPO (LabDex, London, UK) equipped with a puncture electrode. The pH meter was calibrated using standard buffer solutions at pH 4.0 and pH 7.0. A duplicate measurement of moisture, fat, protein and ash contents in the LT muscle samples was conducted according to AOAC International guidelines: Official Methods 950.46, 991.36, 981.10 and 920.153, respectively (AOAC 1999).

Moisture was determined by weight difference after drying 5 g of homogenised sample at 100 °C for 4 h to a constant weight. Fat content was determined using a Soxhlet extraction apparatus, and protein by the Kjeldahl method. Ash content was determined by heating the sample in a muffle furnace (high-temperature oven) at 550 °C until a constant weight was achieved.

Analysis of the FA composition of the beef LT muscle, as well as of the commercial concentrate offered to the CON and FXS animals, was performed at the Emona Development Center for Nutrition (RCP), Ljubljana (Republic of Slovenia). Fatty acids were analysed using the *in situ* transesterification technique according to Park and Goins (1994). The resulting FA methyl esters (FAME) were quantified following the method of Joseph and Ackman (1992). Gas chromatographic analysis was performed using an Agilent 6890N Network GC System (Agilent Technologies, USA) equipped with a flame ionisation detector (FID) and a Supelco OMEGAWAX 320 capillary column (30 m × 0.32 mm × 0.25 µm). The injector was maintained at 250 °C and the detector at 270 °C. The column temperature program started at 170 °C (5 min), followed by a 1 °C/min increase to 215 °C and a final hold at 215 °C for 15 minutes. Helium served as the carrier gas at 1.8 ml/min, while hydrogen (30 ml/min) and synthetic air (400 ml/min) supplied nitrogen as the make-up gas (25 ml/min) to the detector. The total run time was approximately 65 minutes. Identification of FAME was achieved by comparing retention times with those of authenticated standards (Nu-Check Prep Inc., USA).

All analyses were carried out in duplicate. Due to the limited ability of gas chromatography with a flame ionisation detector (GC-FID) to fully separate positional and geometric isomers of PUFA,

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individual chromatographic peaks could not always be resolved into their distinct isomeric forms. Therefore, each PUFA peak was assigned to its most abundant natural isomer (e.g. C18:2 n-6, C18:3 n-3) based on retention-time matching with certified FAME standards, following the standard analytical approach described by Vahmani et al. (2015). Consequently, the PUFA values reported represent the major isomers associated with each chromatographic peak. The sums of SFA, MUFA, PUFA, n-6 and n-3 FAs were calculated from all peaks detected in the chromatogram, including trace FAs (<0.1%) and unresolved components were not individually listed.

Statistical analysis. The data was subjected to a general linear model analysis of variance (ANOVA) using the software package SPSS v24.0 (IBM, Somers, NY, USA). The analysis was conducted to assess the effect of the treatment on the quality traits studied. Treatment was the fixed factor. The ANOVA result was considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Growth performance, carcass, and meat quality traits. Feeding effects on growth performance, carcass weight and yield, and ribeye steak tissue composition are shown in Table 3. No significant differences were detected between the two feeding groups for any of the traits studied, though a trend towards significance was observed for slaughter weight ($P = 0.075$) and weight of the ribeye steak ($P = 0.07$) in favour of the CON group. These numerical differences may reflect random variability associated with the relatively small sample size, or they may indicate a tendency towards statistically significant differences. In the latter case, the slightly lower slaughter weight in the FXS group might be attributable to differences in actual energy intake, given that the diets were not isoenergetic. However, this could not be confirmed with certainty because silage intake was not individually recorded.

Conversely, the LT muscle chemical composition (Table 4) showed significant differences between the two feeding treatments for intramuscular fat content ($P \leq 0.05$), which was higher in the FXS group ($P = 0.050$). A higher intramuscular fat content was to be expected in the FXS beef, as the FXS diet contained more fat (suggesting higher energy intake).

This result reflects that found by Kim et al. (2004), who reported that the marbling score of Hanwoo bulls tended to increase as the dietary level of flaxseed increased from 0% to 15%.

The initial values of pH (day 0) of the beef from both groups were within the average values for beef at around 5.5 (Monson et al. 2004; Ripoll et al. 2014). During aerobic chilled storage, the pH value in-

Table 3. Growth performance, carcass traits, and tissue composition of the 6th rib steak obtained from a half carcass

Characteristic	CON (n = 10)	FXS (n = 10)	P-level
Growth performance			
Initial weight (kg)	189 ± 19.2	186 ± 19.2	0.816
Weight at slaughter (kg)	577 ± 40.5	544 ± 41.7	0.075
Average daily gain (kg/day)	1.44 ± 0.19	1.33 ± 0.18	0.166
Carcass traits			
Hot carcass weight (kg)	332 ± 22.5	312 ± 34.7	0.108
Cold carcass weight (kg)	329 ± 22.6	308 ± 35.1	0.114
Dressing percentage (%)	57.1 ± 3.34	56.5 ± 3.73	0.696
Rib steak tissue composition			
Weight of the rib steak (kg)	1.56 ± 0.21	1.26 ± 0.49	0.074
Muscle (%)	65.9 ± 4.10	66.6 ± 4.00	0.293
Bone (%)	24.2 ± 4.10	22.5 ± 3.60	0.307
Subcutaneous fat (%)	5.60 ± 3.00	6.40 ± 2.70	0.703
Intermuscular fat (%)	4.30 ± 2.00	4.70 ± 2.60	0.806

CON = control beef; FXS = flaxseed beef

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Table 4. Chemical composition (%) and pH value of the *longissimus thoracis* muscle and pH changes during storage

Characteristic	CON (n = 10)	FXS (n = 10)	P-level (treatment)
Moisture (%)	71.3 ± 0.81	70.7 ± 1.40	0.256
Protein (%)	22.2 ± 0.80	21.6 ± 1.00	0.129
Fat (%)	4.80 ± 0.85	5.70 ± 0.89	0.050
Ash (%)	0.91 ± 0.06	0.85 ± 0.15	0.255
pH			
day 0	5.50 ± 0.11^{2}	5.51 ± 0.07^{3}	0.884
day 3	5.65 ± 0.10^{1}	5.55 ± 0.07^{23}	0.009
day 6	5.66 ± 0.11^{1}	5.63 ± 0.07^{2}	0.457
day 9	5.70 ± 0.10^{1}	5.74 ± 0.08^{1}	0.364

All chemical composition values are expressed as % of fresh tissue; $^{1-3}$Different superscripts within the same column indicate significant differences between storage days ($P < 0.05$); P-level (treatment): effect of feeding treatment; P-level (day): effect of storage time within each treatment
CON = control beef; FXS = flaxseed beef

creased by up to two-tenths in both groups. This increase was attributed to the production of basic substances by enzymatic processes related to microbial growth and it is associated with quality deterioration, being an indicator of the loss of meat freshness (Lee and Shin 2019). Notably, however, on day 3 of storage, the pH of the FXS beef was lower and more similar to that of day 0 than the pH of the CON beef. This result suggests that FXS meat might be more resistant to a pH increase during the first stages of storage. Since microbial metabolism is the main factor responsible for the rise in meat pH once residual glucose is exhausted during storage (Casaburi et al. 2015), these findings suggest that there may have been either lower microbial growth or higher residual glucose content in the FXS meat.

Fatty acid profile. The FA composition of the concentrates offered to the CON and FXS yearlings (Table 2) shows that the main changes resulting from the inclusion of 5% flaxseed in the concentrate were an almost sevenfold increase in the C18:3 n-3 percentage and a reduction in the percentage of linoleic acid (C18:2 n-6) by 13 percentage points.

The use of dietary flaxseed in beef cattle has been mainly aimed at modifying and improving the fat composition of beef since flaxseed is a rich source of PUFA, and more specifically of C18:3 n-3 FA (Vahmani et al. 2015). The intramuscular fatty acid composition of beef from the two feeding treatments is shown in Table 5. The used chromatographic method did not separate the isomers from the different PUFA isomers; therefore, the different PUFA shown in the table are the result of assigning the PUFA chromatographic peaks to their most abundant isomer.

As shown in Table 5, feeding resulted in notable changes in the intramuscular fatty-acid profile of beef. Regarding the three most abundant fatty acids in beef (palmitic, stearic and oleic acid, i.e. C16:0, C18:0 and C18:1, respectively), which together typically account for the majority of intramuscular fat in cattle (Aldai et al. 2009), flaxseed supplementation did not affect C16:0 (P = 0.512) but significantly decreased C18:0 (P = 0.030) and increased C18:1 (P = 0.025). Flaxseed supplementation also significantly increased the overall MUFA percentage (P = 0.012), with a corresponding tendency towards a lower SFA proportion (P = 0.058) and PUFA proportion (P = 0.052). The increase in MUFA was largely attributable to the higher proportion of C18:1, which is considered nutritionally beneficial for consumers (EFSA 2004).

Results regarding the effect of flaxseed supplementation on MUFA content in beef have been inconsistent across the literature. Some studies reported increases in MUFA and intramuscular fat (Kim et al. 2004; Alberti et al. 2013), while others found no effect (Barton et al. 2007) or even a reduction in *cis*-MUFA (Nassu et al. 2011). These discrepancies likely stem from differences in forage type, concentrate proportion, and the physiological capacity of different breeds for lipid deposition.

In the present study, the higher proportion of C18:1 in the FXS group can be explained by two mechanisms. First, intramuscular fat content was significantly higher in flaxseed-fed animals.

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Table 5. Fatty acid profile of the *longissimus thoracis* muscle expressed as percentage of total fatty acids

Fatty acid	CON (n = 10)	FXS (n = 10)	P-level
SFA			
C10:0	0.07 ± 0.03	0.05 ± 0.03	0.186
C12:0	0.07 ± 0.04	0.08 ± 0.04	0.569
C13:0	0.08 ± 0.03	0.07 ± 0.03	0.673
C14:0	2.64 ± 0.39	2.83 ± 0.56	0.376
C15:0	0.40 ± 0.06	0.40 ± 0.07	0.879
C16:0	22.65 ± 1.39	23.17 ± 2.00	0.512
C17:0	0.99 ± 0.08	0.88 ± 0.10	0.015
C18:0	20.76 ± 3.13	17.21 ± 3.57	0.030
C19:0	0.13 ± 0.03	0.11 ± 0.02	0.156
C20:0	0.17 ± 0.04	0.15 ± 0.03	0.256
MUFA			
C14:1	0.32 ± 0.13	0.51 ± 0.25	0.042
C16:1	2.27 ± 0.40	3.08 ± 0.66	0.004
C18:1	40.12 ± 3.30	44.69 ± 3.12	0.025
C19:1	0.12 ± 0.11	0.13 ± 0.02	0.060
C20:1	0.38 ± 0.04	0.44 ± 0.11	0.111
PUFA n-6[&]			
C18:2	6.03 ± 1.13	4.82 ± 1.15	0.029
C20:2	0.08 ± 0.03	0.06 ± 0.04	0.277
C20:3	0.17 ± 0.08	0.08 ± 0.05	0.014
C20:4	1.63 ± 0.33	1.31 ± 0.25	0.043
C22:4	0.07 ± 0.04	0.04 ± 0.04	0.156
PUFA n-3[$]			
C18:3	0.48 ± 0.05	0.56 ± 0.15	0.137
C20:5	0.08 ± 0.04	–	–
C22:5	0.31 ± 0.11	0.29 ± 0.09	0.653
Sums and ratios[&]			
SFA	47.96 ± 2.96	44.97 ± 3.61	0.058
MUFA	45.46 ± 3.73	50.06 ± 3.64	0.012
PUFA	6.58 ± 1.74	4.97 ± 1.72	0.052
n-6[&]	5.95 ± 1.57	4.31 ± 1.72	0.029
n-3[$]	0.63 ± 0.19	0.65 ± 0.22	0.811
PUFA/SFA	0.137 ± 0.051	0.111 ± 0.037	0.023
n-6/n-3	9.44 ± 1.65	6.63 ± 0.79	<0.001

[&]PUFA n-6 values correspond to the dominant naturally occurring n-6 isomer for each chromatographic peak; [$]PUFA n-3 values correspond to the dominant naturally occurring n-3 isomer for each chromatographic peak

CON = control beef; FXS = flaxseed beef; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; n-6: ω-6 fatty acids; n-3: ω-3 fatty acids; SFA = saturated fatty acids

It is well established that increases in intramuscular fat are accompanied by proportional increases in oleic acid, the predominant FA in bovine triacylglycerols (Wood et al. 2008). As neutral lipids accumulate, they dilute phospholipid-rich PUFAs, resulting in a relative increase in MUFAs, especially C18:1. Second, the combination of reduced C18:0 and increased C18:1 observed here suggests

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enhanced Δ-9 desaturase activity, which converts C18:0 to C18:1. Dietary flaxseed, through its higher lipid and energy content, may have stimulated lipogenic pathways in muscle tissue, leading to a higher desaturation index.

In the present study, moreover, flaxseed supplementation significantly reduced the percentages of most n-6 PUFA, the sum of n-6 PUFA ($P = 0.023$), and the PUFA/SFA ratio ($P = 0.023$) in beef. This can be explained by the higher intramuscular fat content in the FXS beef ($P = 0.05$). The higher the amount of intramuscular fat in the muscle, the higher the percentage of triacylglycerols in the fat (which are rich in SFA), and the lower the percentage of phospholipids (which are rich in PUFA) (Wood et al. 2008). Additionally, the higher dietary intake of n-3 PUFA, especially of C18:3, would also result in lower n-6 PUFA proportions (Pouzo et al. 2015). Furthermore, the lower PUFA content could also be explained by higher ruminal biohydrogenation in the FXS group (Nassu et al. 2011).

Although the flaxseed-supplemented diet contained approximately seven times more C18:3 than the control diet, neither its percentage nor the total n-3 PUFAs in intramuscular fat were significantly increased. This result contrasts with previous findings showing an increase in the percentage of n-3 PUFAs (mainly C18:3) in intramuscular fat when flaxseed was added to cattle diets (Kronberg et al. 2006; Nassu et al. 2011; Alberti et al. 2013). Conversely, and in agreement with previous studies, a lower n-6/n-3 ratio was found in the FXS beef. This is likely related to the higher C18:3 content of the flaxseed concentrate (resulting in higher intake of this FA) and, consequently, to the higher relative proportion of this FA (and total n-3 PUFAs) compared to C18:2 n-6 (and total n-6 PUFAs). The decrease in the n-6/n-3 PUFA ratio in beef intramuscular fat is a well-recognised observation when flaxseed is used in cattle diets.

The discrepancy regarding the absence of the C18:3 increase following flaxseed feeding could be explained by the confounding effect of higher intramuscular fat in FXS beef, which dilutes phospholipid-rich PUFA within triacylglycerol-rich neutral lipids, as previous studies reported no significant change in intramuscular fat content, a result which may be attributed to the use of isoenergetic diets for the control and flaxseed-supplemented cattle. Another explanation for that discrepancy could be the higher ruminal biohydrogenation of dietary C18:3 n-3 in the flaxseed group. Ruminal biohydrogenation represents a major metabolic bottleneck for the transfer of dietary PUFA, particularly of C18:3 n-3, into ruminant tissues. Unsaturated fatty acids entering the rumen are rapidly hydrogenated by the rumen microbiota into stearic acid (C18:0), with several transient intermediates such as *trans*-11 C18:1 being formed in the process. The efficiency of this saturation process is influenced by the forage-to-concentrate ratio, rumen pH, rumen retention time, and the physical form of the fat source (Nassu et al. 2011). Diets with higher proportions of energy, like those used in this study, may reduce rumen pH and modify microbial activity, which can increase the extent of biohydrogenation, thereby decreasing the amount of C18:3 n-3 reaching the duodenum for absorption.

CONCLUSION

The use of a concentrate supplemented with 5% extruded flaxseed in stall-fattened yearlings on a forage-and-concentrate diet showed no significant effect on growth performance or carcass quality, but it resulted in increased intramuscular fat, most likely due to the higher energy intake associated with the flaxseed lipid content. Flaxseed supplementation improved the nutritional quality of beef lipids by decreasing the proportion of SFA, increasing MUFA, and lowering the n-6/n-3 ratio. However, unlike many previous studies, neither the proportion nor the sum of n-3 fatty acids in intramuscular fat increased in flaxseed-fed cattle.

An important limitation of this study is that the experimental diets were not isoenergetic or isonitrogenous, meaning that the higher intramuscular fat content observed in the flaxseed group was likely influenced by the increased dietary energy intake rather than by the specific effect of flaxseed itself.

Future research should therefore focus on evaluating isoenergetic diets, different inclusion levels of extruded flaxseed, and strategies to reduce ruminal biohydrogenation, for example through protected lipid sources or alternative feeding systems, to more effectively enhance the n-3 PUFA content of beef.

Conflict of interest

The authors declare no conflict of interest.

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