VETERINARSKI ARHIV 93 (6), 627-640, 2023 DOI: 10.24099/vet.arhiv.1889

Increasing long-chain n-3 fatty acids in beef from forage-finished cattle through supplementation with the calcium salts of fish oil

Osman Kucuk[1*], Bret William Hess[2], Steve Paisley[3], Keith Underwood[4] and Daniel Clark Rule[3]

[1]Department of Animal Nutrition and Nutritional Diseases, School of Veterinary Medicine, Erciyes University, Kayseri, Turkey
[2]Western Association of Agricultural Experiment Station Directors, Reno, NV, USA
[3]University of Wyoming, Department of Animal Science, Laramie, WY, USA
[4]South Dakota State University, Department of Animal Science, Brookings, SD, USA

KUCUK, O., B. W. HESS, S. PAISLEY, K. UNDERWOOD, D. C. RULE: Increasing long-chain n-3 fatty acids in beef from forage-finished cattle through supplementation with the calcium salts of fish oil. Vet. arhiv 93, 627-640, 2023.

ABSTRACT

Two experiments were conducted to determine the effects of dietary fish oil Ca salts supplementation on the muscle deposition of fatty acids, with the major focus on eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) in beef cattle. For Experiment 1, thirty-eight grazing LowLine Angus steers were divided into three groups and supplemented either control, fish oil (FO)- or palm oil (PO)-based fatty acid Ca salts, supplemented individually in dry form. In Experiment 2, 14 Angus heifers and 14 Angus steers were fed free-choice harvested forage and supplemented either PO or FO Ca salts, delivered as a suspension within dried molasses lick tubs. Growth performances, sensory characteristics and the concentrations of EPA and DHA of the *M. Longissimus thoracis* (LT) were evaluated. *M. Longissimus thoracis* fatty acid concentrations of EPA and DHA were greater ($P<0.001$) for cattle fed FO; whereas C18:2 *n-6* and the ratio of *n*-6 to *n*-3 were greater ($P<0.001$) for cattle fed PO in both experiments. Sensory evaluation of LT steaks obtained from the carcasses of the LowLine steers of Experiment 1 did not reveal any adverse effects of the FO supplementation ($P\geq0.2695$). We conclude that supplementing FO Ca-salts to forage-fed beef cattle increases muscle deposition of EPA and DHA, with no adverse effects on flavor. Supplementing fish oil to beef cattle is a way to meet the omega fatty acid requirements of humans.

Key words: omega-3 fatty acids; fish oil; muscle; Low-Line Angus; grazing

Introduction

Omega-3 (*n-3*) polyunsaturated fatty acids (PUFA), namely eicosapentaenoic acid (EPA; 20:5 *n-3*), docosahexaenoic acid (DHA; 22:6 *n-3*) and alpha-linolenic acid (ALA; 18:3 *n-3*) are beneficial in the prevention of chronic diseases, including cardiovascular problems, cancer, Alzheimer's, arthritis, and glucose intolerance (WALL et al., 2010; SHAHIDI and AMBIGAIPALAN, 2018; INNES and CALDER, 2020; ELAGIZI et al., 2021; SHIBABAW, 2021). Seafood is an important

*Corresponding author:
Osman Kucuk, DVM, MSc, PhD, Professor of Nutrition, Department of Animal Nutrition and Nutritional Diseases, School of Veterinary Medicine, Erciyes University, Kayseri, 38039, Turkey, phone: +90 505 6287251; fax: +90 352 337 2740; e-mail: osmankucukwy@yahoo.com

ISSN 0372-5480
Printed in Croatia

dietary source of EPA and DHA, while plant oils, such as flaxseed, contain ALA. Marine fish species contain 200-440 mg EPA+DHA /100 g raw fillet (RINCON-CERVERA et al., 2019). Conversion of ALA to EPA and EPA to DHA occurs, but the process is not efficient (4-8%) in humans (BURDGE et al., 2002; BAKER et al., 2016). The recommended daily intake of EPA and DHA is 100-250 mg for children up to 10 years, 250 mg for healthy adults, and 300 mg for pregnant women, consumed in fish and/or supplements of fish oils (FAO, 2010).

The concentration of *n-3* PUFA in beef is very low, although beef from grass/forage-fed cattle has greater concentrations of *n-3* PUFA compared with beef of grain-finished cattle (5-33 mg vs 2-19 mg per 100 g fresh beef) (RULE et al., 2002; LEHESKA et al., 2008; DUCKETT et al., 2013; VAN ELSWYK and MCNEILL, 2014). Attempts to increase ruminant tissue concentrations of EPA and DHA by FO supplementation have shown mixed results (MANDELL et al., 1997; VATANSEVER et al., 2000; HENNESSY et al., 2021), largely because of the ruminal biohydrogenation of PUFA (SCOLLAN et al., 2001). The calcium salts of fatty acids provide variable protection from biohydrogenation, but the greater benefit is the provision of inert fatty acids, as described by PALMQUIST (2009). Biohydrogenation can be reduced to 33 to 50% with calcium salts, and the negative effects of lipid supplementation are minimized (PALMQUIST, 2009). Additionally, the salts are in powder form, which enables easier mixing. We hypothesized that supplementation of beef cattle grown on forage diets with the calcium salts of FO will increase EPA and DHA in the beef. The objectives of this study were to determine EPA and DHA deposition and sensory traits in the *Longissimus thoracis* (LT; 12th rib) from forage-finished beef cattle supplemented with the calcium salts of FO. Additionally, the efficacy of dried molasses lick tubs as a delivery system for supplementation of FO calcium salts was investigated.

Materials and methods

The two experiments were conducted at the James C. Hageman Sustainable Agriculture Research and Extension Center, Lingle, Wyoming. The University of Wyoming Institutional Animal Care and Use Committee approved all procedures.

Experiment 1, experimental design. A total of 40 half-blood LowLine Angus steers with initial body weights (BW) of 290.5±6.62 kg were divided into either control (CON; no supplemental fat), the calcium salts of palm oil (PO), or the calcium salts of fish (FO) treatments. Calcium salts of the fatty acids were provided by Virtus Nutrition (Corcoran, CA) as their EnerGII (palm oil) or StrataG (fish oil) products. Calcium salts were mixed with dried beet pulp to contain 7.6% molasses, 18.2% fatty acid calcium salts, 4.4% vitamin and mineral mix, and 1.8% Poloxalene (a bloat preventer). The CON supplement contained 4.0% $CaCO_3$ to balance for Ca in the calcium salt supplements.

Supplements were fed at 0.25% of BW as a dry mix and formulated to limit supplemental fat to 2.0% for PO and FO treatments, to avoid any potential negative associative effects of supplemental fat in cattle consuming high-forage diets (BROKAW et al., 2001; PAVAN and DUCKETT, 2008). The steers were fed supplements every other day. They were allowed ad libitum access to irrigated pasture composed of 25% bromegrass, 25% wheatgrass, and 50% alfalfa (crude protein (CP) = 20.9%; available forage was 36.7 kg dry matter (DM)/head/d) pasture forage. They were weighed monthly. Individual intake of grazed forage was not determined, however, pasture forage was available for consumption at 12.7% of starting BW. Four animals died in early October, and each group had 12 steers per treatment. The pasture rotation was weekly over a period beginning in early June and ending mid-October. The steers were fed forage harvested from the same pastures until early December when steers were shipped 137 km for harvest at a private slaughterhouse. The duration of the experiment was 187 d.

Experiment 1, tissue sampling and fatty acid analysis. Fabrication and vacuum packaging of primals occurred 48 h postmortem. Final fabrication occurred 12 d postmortem when a 2.54-cm thick cross section of *Longissimus thoracis* (LT; 12th rib) was obtained from each carcass, vacuum-packaged, and stored at -20° C. While still frozen, the LTs were diced into small cubes and placed into

plastic cups, weighed, and then lyophilized for 5 d using a Virtis Genesis 25 ES freeze dryer (The Virtis Co, Gardiner, NY). The cups were removed, immediately weighed, then the dried LT cubes were ground and homogenized using a home-style coffee grinder. Fatty acid methyl esters (FAME) were prepared by direct transesterification with 0.2 *N* KOH in methanol (MURRIETA et al., 2003), while forage and supplement FAMEs were prepared using methanolic-HCl (WESTON et al., 2008). For the LT samples, 1.0 mg of C13:0 served as the internal standard, whereas, for feed and supplement samples 1.0 mg of C21:0 served as the internal standard. Fatty acid methyl esters were separated using Agilent Technologies 6890 Gas–Liquid Chromatography (GLC) (Avondale, PA) equipped with an Agilent 7683 automatic sample injector, flame ionization detector, and Agilent ChemStation software for integration of peaks. A 100-m × 0.25-mm (i.d.) fused silica capillary column (SP-2560, 0.2-m film thickness, Supelco, Bellefonte, PA) was used, with an oven temperature of 140°C to 240°C at 4°C/min. Injector and detector temperatures were 250°C. The carrier gas was helium, with a split ratio of 50:1 and a column flow of 2 mL/min. Fatty acids were identified by comparing retention times (Nu-Chek Prep, Inc., Elysian, MN).

Experiment 1, sensory evaluation. Thirty-six LT cross sections were analyzed in duplicate for moisture, fat, crude protein and ash (AOAC, 2007). The LT beef samples (100 g) were cooked at 80°C to a core internal temperature of 71°C for cooking loss. Cooking loss was calculated as the percentage of weight loss upon cooking (HONIKEL, 1998). The Warner–Bratzler shear force (WBSF) analysis was also applied to the beef samples cooked in the same way as in the cooking loss method. After cooking, 8 cores (1.27 cm diameters) were used for meat tenderness using an Instron Universal Testing Machine (G146 Warner-Bratzler Meat Shear Fixture, USA) equipped with a Warner–Bratzler shearing device.

Nine trained panelists were selected for the sensory quality evaluation of sliced LT cube (1.3 cm) samples served with no spices. The panelists were served water during the evaluation to refresh their mouth between the samples. A total of 25 sessions with 6 samples for each were held for a total 72 individual meat samples (36 animals × 2 replicates). Cooked beef samples were evaluated for four attributes: initial tenderness, juiciness, flavor intensity, and off flavor intensity (MEILGAARD et al., 1991; AMSA, 1995).

Experiment 1, statistical analysis. Data were analyzed as a completely randomized design. Live weight changes, LT FAMEs, and sensory characteristics were analyzed using PROC MIXED of SAS (SAS Inst. Inc., Cary, NC, USA), with diet as the fixed effect and initial body weight as a covariate. When the ANOVA was considered significant ($P<0.05$), the means were separated by pairwise comparison using the Duncan's multiple comparison test.

Experiment 2, experimental design. Experiment 2 was conducted to address the variation in supplement intake that occurred during Experiment 1. The dry mix supplement had a fish aroma, which might have affected palatability and desirability for some of the cattle. To address this issue we tested the use of dried molasses lick tubs to determine if the variation in tissue concentration of FO *n-3* fatty acids lessened. Fourteen Angus heifers (initial BW of 235.3±0.2 kg) and 14 Angus steers (initial BW of 267.3±3.3 kg) were randomly divided by BW into either a FO- or a PO-based fatty acid Ca salt treatment, so that seven steers and heifers were represented in each of the two dietary supplement treatments. Fatty acids Ca salts of PO and FO were the same as in Experiment 1, however, they were mixed and suspended in dried molasses lick tubs (Ridley Block, Whitewood, SD) which contained 30% by weight of fatty acid calcium salts. Our original design was for pasture grazing, but the dry conditions did not support the adequate growth of forage. The 14 cattle on each lick tub treatment were raised in a single dry lot pen for both supplements. Two lick tubs (114-kg) in each pen were offered to the cattle for 220 d, and the lick tubs were replaced when 95% of the supplement was consumed. Cattle were provided *ad libitum* access to harvested forage, composed of 65% hay and 35% haylage (50% alfalfa, 25% brome-grass, and 25% wheat grass mixed forage), as well as with water and the trace mineral salt blocks.

Only two treatments were conducted, FO and PO. The primary purpose of Experiment 2 was to evaluate the variations in the tissue concentrations of fatty acids, mainly EPA and DHA, in response to delivery of the fatty acid calcium salts via lick tubs. In production, the tubs are placed in lots or pasture for group feeding. The PO treatment provided a fat-containing control for appropriate evaluation of growth performance, as well as a fatty acid contrast to FO.

Experiment 2, sampling and analyses. The cattle were weighed every 45 days, and then harvested at 220 days at the University of Wyoming Meats Laboratory. At each weighing, forage samples were obtained and composited for acid detergent fiber (ADF) and neutral detergent fiber (NDF) (GOERING and VAN SOEST, 1970), crude protein (Method 990.03; AOAC, 2007), and FA analysis (WESTON et al., 2008). The lick tubs were sampled by drilling cores and collecting the resulting ground molasses and calcium salts for fatty acid analysis.

Carcass fabrication followed the same schedule as for Experiment 1. Muscle samples were prepared and analyzed as described in Experiment 1. Concentrations of fatty acids in LT (mg/100 g of fresh LT) were reported.

Experiment 2, statistical analysis. Live weight changes, average daily gain, and fatty acid concentrations were analyzed as a 2 x 2 factorial design experiment, using a generalized linear model of SAS to determine the effects of supplement (FO or PO) and sex (heifer or steer) on LT fatty acids and to calculate least squared means (LSD). The LSDs were compared using the Tukey critical difference. The level of probability was regarded as significant when $P \leq 0.05$.

Results

Experiment 1, growth performance, and fatty acid concentrations of feeds and LT. The initial and final body weights, as well as the average daily gain (ADG) of steers were similar for all treatments ($P \geq 0.1713$; Table 1). Intake of the CON supplement was greatest, whereas FO was consumed the least ($P < 0.0001$; Table 1).

Table 1. Growth performance of grazing steers supplemented either palm oil-based or fish oil-based fatty acid Ca salts

	Treatment			Statistical parameter	
Parameter	CON	PO	FO	SEM	P value
Initial BW (kg)	292.75	283.56	290.98	6.810	0.6036
Final BW (kg)	425.12	406.58	412.03	8.928	0.3324
Weight gain (kg)	132.37	123.02	121.05	4.429	0.1713
ADG (kg/day) (187 d)	0.708	0.658	0.647	0.024	0.1713
Supplement intake (g/d)	615.33[a]	415.17[b]	294.37[c]	17.813	<.0001

Column means with different superscript letters differ significantly ($P < 0.05$) as assessed by Tukey's Multiple Range Test
BW - body weight; ADG - average daily gain; SEM - standard error of mean; CON - control; PO - palm oil-based fatty acid Ca salts; FO - fish oil-based fatty acid Ca salts

The PO calcium salts contained palmitate (C16:0; 48.88%), palmitoleate (C16:1 *n*-7; 0.32%), stearate (C18:0; 3.71%), oleate (C18:1 *n*-9; 33.95%), linoleate (C18:2 *n-6*; 10.14%), and alpha-linolenate (ALA, C18:3 *n-3*; 0.53%). The FO calcium salt contained myristate (C14:0; 8.24%), pentadecanoate (C15:0; 0.57%), C16:0 (28.90%), C16:1 *n*-7 (8.76%), C18:0 (3.78%), C18:1 *n*-9 (14.65%), C18:2 *n-6* (4.62%), ALA (1.09%), stearidonate (C18:4 *n-3*; 1.17%), arachidonate (C20:4 *n-6*; 0.61%), eicosapentaenoate (EPA, C20:5 *n-3*; 10.07%), docosapentaenoate (C22:5 *n-3*; 1.10%), and docosahexaenoate (DHA, C22:6 *n-3*; 2.90%).

Forage contained (mg/g DM): C16:0, 3.07 (12.31% of total fatty acids); C16:1 *n*-7, 0.25 (0.98%); C18:0, 0.35 (1.42%); C18:1 *n-9*, 0.29 (1.14%); C18:2 *n-6*, 3.60 (14.47%); and ALA, 14.34 (57.58%).

Steers fed FO consumed modest amounts of C16:0 and C18:0 as well as total FA (P<0.0001; Table 2), and were the only treatment offered EPA or DHA. Steers fed PO consumed substantial amounts of C16:0, C18:0, total FA, and modest amounts of C18:3 *n-3* (P<0.0001).

The fatty acid concentrations (mg/100 g) in the LT of the steers are shown in Table 3. Concentrations of C18:1 *trans*-11 tended to be greater in FO-fed steers compared with PO- or CON-fed steers (P=0.0624), whereas, concentrations of C18:2 *n-6* were greatest in PO-fed steers (P<0.0001).

Dietary treatment did not influence concentrations of ALA or C18:2 *cis*-9, *trans*-11 (P≥0.3483). However, steers supplemented with PO had greater (P<0.0001) C20:3 *n-6* and C20:4 *n-6* concentrations in their LT compared with both CON and FO. Concentrations of EPA and DHA (P<0.0001), as well as C22:5 *n-3* (P=0.0040) were greater for FO compared with those of CON and PO. The sum of EPA+DHA concentrations in the LT were greater in FO-fed steers compared with those of CON- and PO-fed steers (P≥0.0003).

Of the combined fatty acid concentrations, only total polyunsaturated FA (PUFA) concentrations were greater in the LT of PO- and FO-fed steers (P=0.0013) compared with CON-fed steers. Total *n-3* PUFA concentrations were greatest with FO (P<0.0001), whereas, total *n-6* PUFAs were greatest with PO (P<0.0001), resulting in a greater ratio of *n-6* to *n-3* in PO-fed steers (P<0.0001).

Experiment 1, sensory evaluation. The chemical composition and sensory characteristics of LTs from grazing steers are shown in Table 4. Water content was lower in FO-fed steers compared with those of CON- and PO-fed steers (P=0.0329). Dietary treatment had no influence on fat or protein percentages (P≥0.1251). Ash percentage was greatest in FO-fed steers (P=0.0482). Juiciness, tenderness, flavor, and off flavor scores were similar for all dietary treatments (P=0.2695). Peak internal temperature, as well as WBSF were similar across dietary treatments (P≥0.2645). The cooking yield of LTs from FO-fed steers was less (P=0.0426) with greater cooking loss (P=0.0426) compared with LTs from CON- and PO-fed steers.

Table 2. Intake of fatty acids (g/d) from supplements by grazing steers offered either control or Ca-salts of fish oil or Ca-salts of palm oil supplements

Fatty acid	Treatment			Statistical parameter	
	CON	PO	FO	SEM	P value
C14:0	0	0	3.48	0.150	<.0001
C15:0	0	0	0.24	0.002	<.0001
C16:0	2.46^{c}	40.26^{a}	12.21^{b}	0.194	<.0001
C16:1 *n-7*	0.20^{b}	0.26^{b}	3.70^{a}	0.084	<.0001
C18:0	0.21^{c}	3.06^{a}	1.60^{b}	0.016	<.0001
C18:1 *cis*-9	1.40^{c}	27.97^{a}	6.19^{b}	0.120	<.0001

Table 2. Intake of fatty acids (g/d) from supplements by grazing steers offered either control or Ca-salts of fish oil or Ca-salts of palm oil supplements (continued)

Fatty acid	Treatment			Statistical parameter	
	CON	PO	FO	SEM	P value
C18:2 *n-6*	2.01[b]	8.35[a]	1.95[b]	0.051	<.0001
C18:3 *n-3* (ALA)	0.21[c]	0.43[b]	0.46[a]	0.006	<.0001
C18:4 *n-3*	0	0	0.49	0.005	<.0001
C20:4 *n-6*	0	0	0.26	0.000	<.0001
C20:5 *n-3* (EPA)	0	0	4.25	0.003	<.0001
C22:5 *n-3*	0	0	0.46	0.015	<.0001
C22:6 *n-3* (DHA)	0	0	1.23	0.030	<.0001
Total	7.86[c]	82.37[a]	42.26[b]	0.486	<.0001

Column means with different superscript letters differ significantly (P<0.05) as assessed by Tukey's Multiple Range Test
SEM - standard error of mean; CON - control; PO - palm oil-based fatty acid Ca salts; FO - fish oil-based fatty acid Ca salts; ALA - alpha-linolenic acid (18:3 *n-3*); EPA - eicosapentaenoic acid (20:5 *n-3*); DHA - eicosapentaenoic acid (20:5 *n-3*)

Table 3. Effects of supplementing grazing steers with palm oil-based or fish oil-based fatty acid Ca salts on fatty acid concentrations (mg/100 g) of *Longissimus thoracis*

Fatty acid	Treatment			Statistical parameter	
	CON	PO	FO	SEM	P value
C14:0	51.32	59.24	56.57	6.221	0.6608
C14:1	5.48	6.89	6.72	0.835	0.4371
C15:0	20.73	24.85	23.91	2.629	0.5179
C16:0	567.73	711.38	649.62	61.157	0.2636
C16:1	48.27	55.47	52.31	5.882	0.6895
C17:0	30.23	37.19	38.22	3.942	0.3091
C18:0	355.93	481.32	440.84	44.751	0.1455
C18:1 *trans*-11	38.34	53.59	69.23	8.884	0.0624
C18:1 *cis*-9	651.35	814.84	708.25	68.041	0.2406
C18:1 *cis*-11	24.73	28.91	29.51	2.073	0.2216
C18:2 *n-6*	93.67[c]	122.75[a]	105.37[b]	4.058	<.0001
C18:3 *n-3* (ALA)	35.54	37.03	39.11	2.357	0.5645
C18:2 *cis*-9 *trans*-11 (CLA)	8.56	10.27	11.51	1.417	0.3483
C20:3 *n-6*	10.06[b]	14.59[a]	10.10[b]	0.393	<.0001
C20:4 *n-6*	33.05[b]	41.48[a]	33.48[b]	1.069	<.0001
C20:5 *n-3* (EPA)	12.20[b]	12.44[b]	21.01[a]	0.849	<.0001
C22:5 *n-3*	19.54[b]	20.61[b]	22.13[a]	0.507	0.0040
C22:6 *n-3* (DHA)	2.01[b]	2.17[b]	6.50[a]	0.310	<.0001

Table 3. Effects of supplementing grazing steers with palm oil-based or fish oil-based fatty acid Ca salts on fatty acid concentrations (mg/100 g) of *Longissimus thoracis* (continued)

Fatty acid	Treatment			Statistical parameter	
	CON	PO	FO	SEM	P value
EPA+DHA	14.21[b]	14.61[b]	27.51[a]	1.131	<.0001
Total SFA	1025.94	1313.99	1209.18	116.620	0.2246
Total USFA	982.82	1221.06	1115.26	91.815	0.2001
Total MUFA	768.18	959.71	866.04	84.182	0.2877
Total PUFA	214.64[b]	261.35[a]	249.22[a]	8.462	0.0013
Total *n-3* PUFA	69.30[b]	72.25[b]	88.76[a]	2.985	<.0001
Total *n-6* PUFA	136.78[b]	178.82[a]	148.96[b]	4.823	<.0001
n-6/*n-3*	1.98[b]	2.48[a]	1.69[c]	0.051	<.0001
PUFA/SFA	0.22	0.203	0.24	0.018	0.4074

Column means with different superscript letters differ significantly (P<0.05) as assessed by Tukey's Multiple Range Test
SEM - standard error of mean; CON - control; PO - palm oil-based fatty acid Ca salts; FO - fish oil-based fatty acid Ca salts; ALA - alpha-linolenic acid (18:3 *n-3*); EPA - eicosapentaenoic acid (20:5 *n-3*); DHA - eicosapentaenoic acid (20:5 *n-3*); CLA - conjugated linoleic acid (18:2, *cis*-9 *trans*-11); Total SFA - a total of C14:0, C15:0, C16:0, C17:0 and C18:0; Total USFA - a total of 14:1, 16:1, C18:1 *trans*-11, C18:1 *cis*-9, C18:1 *cis*-11, C18:2 *n-6*, C18:3 *n-3* (ALA), CLA *cis*-9 *trans*-11, C20:3 *n-6*, C20:4 *n-6*, 20:5 *n-3* (EPA), C22:5 *n-3* and C22:6 *n-3* (DHA); Total MUFA - a total of C14:1, C16:1, C18:1 *trans*-11, C18:1 *cis*-9, and C18:1 *cis*-11; Total PUFA - a total of C18:2 *n-6*, C18:3 *n-3* (ALA), CLA *cis*-9 *trans*-11, C20:3 *n-6*, C20:4 *n-6*, 20:5 *n-3* (EPA), C22:5 *n-3* and C22:6 *n-3* (DHA); Total *n-3* PUFA - a total of C18:3 *n-3* (ALA), 20:5 *n-3* (EPA), C22:5 *n-3* and C22:6 *n-3* (DHA); Total *n-6* PUFA - a total of C18:2 *n-6*, C20:3 *n-6* and C20:4 *n-6*

Experiment 2, diet analysis and cattle growth. The harvested forage contained 15.2% CP, 49.1% ADF, and 53.9% NDF. The fatty acid concentrations (mg/g DM) of the harvested forage were: C16:0, 2.6 (21.1% of total fatty acids); C18:0, 0.4 (3.3%); C18:1 *n-9*, 0.4 (3.3%); C18:2 *n-6*, 1.8 (14.6%); ALA, 2.2 (17.9%); total fatty acids, 12.3. The pen average intake of FO and PO lick tubs was 220 g/d and 230 g/d, respectively. The lick tub concentration of ALA was 10.2 mg/g of the lick tub for PO, and 14.0 mg/g for the FO tubs. The concentrations of EPA and DHA were 182.4 mg/g and 52.4 mg/g, respectively, in the FO lick tubs.

The growth performance of cattle supplemented with the calcium salts of PO and FO delivered in dried molasses lick tubs is shown in Table 5. Weight gain and ADG were greater (P=0.0033) for cattle supplemented with PO. Initial and final BW were greater for steers than for heifers (P≥0.0073). However, the total weight gains and ADG of steers and heifers were similar (P=0.1224).

Experiment 2, LT fatty acids. The effects of lick tub supplement and sex on concentrations (mg/100 g of fresh muscle) of fatty acids in LTs are shown in Table 6. As observed in Experiment 1, no saturated fatty acids were affected by dietary fat source (P>0.025). Concentrations of C18:1 *trans*-11 were greater (P=0.0016) in FO-supplemented cattle, whereas concentrations of C18:2 *n-6* were greater (P=0.0010) in PO-supplemented cattle. Although the effect of fat source only showed a trend towards greater C18:1 *trans*-11 in Experiment 1, the effect was consistent for this fatty acid and C18:2 *n-6* in both experiments. Concentrations of all *n-3* fatty acid reported were greater (P<0.002) for FO-supplemented cattle, whereas, all *n-6* fatty acids were greater (P<0.0001) in the LTs of PO-supplemented cattle. These differences resulted in greater (P<0.0001) ratios of *n-6* to *n-3* fatty acids. The only sex effect observed was for C20:3 *n-6,* where concentrations were greatest (P=0.0308) for steers. This difference was consistent with the

magnitude of difference between heifers and steers for C18:2 *n*-6; however, no sex effect for C18:2 *n*-6 occurred (P=0.135).

Neither dietary treatment (P≥0.2619) nor sex (P≥0.0933) influenced LT concentrations of total SFA, total USFA, total MUFA or total MUFA. Cattle supplemented with FO had greater total *n-3* PUFA concentrations (P<0.0001), but lower concentrations of total *n-6* PUFA (P<0.0001) compared with PO-supplemented cattle, resulting in a lower ratio of *n-6* to *n-3* (P<0.0001). There were no interactions between treatment and sex (P≥0.1650) for LT fatty acid concentrations.

Table 4. Effects of supplementing grazing steers palm oil-based (PO) or fish oil-based (FO) fatty acid Ca salts on the chemical composition and sensory characteristics of *Longissimus thoracis*

	Treatment			Statistical parameter	
	CON	PO	FO	SEM	P value
H_2O (%)	74.81[a]	74.85[a]	74.02[b]	0.241	0.0329
Fat (%)	2.04	2.16	2.39	0.203	0.4766
Protein (%)	22.63	22.55	22.96	0.144	0.1251
Ash (%)	1.12[ab]	1.11[b]	1.15[a]	0.010	0.0482
Juiciness*	5.49	5.53	5.34	0.209	0.8019
Tenderness*	5.28	5.93	5.61	0.351	0.4379
Flavor intensity*	5.17	4.87	5.01	0.125	0.2695
Off flavor intensity*	1.33	1.24	1.28	0.053	0.4674
Peak Internal Temperature (°C)	71.74	72.32	74.27	1.123	0.2645
Cooking Yield (%)	76.34[a]	76.71[a]	73.24[b]	1.021	0.0426
Cooking loss (%)	23.66[b]	23.29[b]	26.76[a]	1.021	0.0426
WBSF	5.27	5.47	5.70	0.484	0.8219

Column means with different superscript letters differ significantly (P<0.05) as assessed by Tukey's Multiple Range Test
SEM - standard error of mean; CON - control; PO, palm oil-based fatty acid Ca salts; FO - fish oil-based fatty acid Ca salts; WBSF - The Warner–Bratzler shear force
*1 = extremely tough, dry, dislike, off flavor; 9 = extremely tender, juicy, like, good flavor.

Table 5. Growth performance of cattle supplemented with either palm oil-based (PO) or fish oil-based (FO) fatty acid Ca salts offered in dried molasses lick tubs

	Treatment		Sex		SEM	P value		
	PO	FO	Heifer	Steer		Trt	Sex	Trt x Sex
Initial BW (kg)	253.07	252.65	238.19[b]	267.54[a]	7.248	0.9669	0.0077	0.5438
Final BW (kg)	410.49	389.25	380.11[b]	419.63[a]	9.687	0.1277	0.0073	0.9721
Weight gain (kg)	157.41[a]	136.60[b]	141.92	152.09	4.573	0.0033	0.1224	0.3039
ADG (kg/day) (220 d)	0.715[a]	0.621[b]	0.645	0.691	0.020	0.0033	0.1224	0.3039

Column means with different superscript letters differ significantly (P<0.05) as assessed by Tukey's Multiple Range Test
BW - body weight; ADG - average daily gain; SEM - standard error of mean; CON - control; PO, palm oil-based fatty acid Ca salts; FO - fish oil-based fatty acid Ca salts

Table 6. Effects of supplementing forage finished beef cattle palm oil (PO)-based or fish oil (FO)-based fatty acid Ca salts in dried molasses lick tubs on fatty acid concentrations (mg/100 gr) of *Longissimus thoracis*

Fatty acid	Treatment		Sex		SEM	P value		
	PO	FO	Heifer	Steer		Trt	Sex	Trt x Sex
C14:0	73.39	72.98	78.39	67.98	6.914	0.9673	0.2973	0.5531
C14:1	10.69	9.23	10.73	9.19	1.110	0.3623	0.3343	0.6723
C15:0	15.32	17.22	16.44	16.10	1.559	0.3979	0.8801	0.5797
C16:0	796.90	744.66	826.05	715.51	65.321	0.5769	0.2431	0.5316
C16:1	56.76	62.24	66.16	52.84	5.101	0.4549	0.0771	0.7990
C17:0	31.60	36.89	35.33	33.16	2.871	0.2050	0.5978	0.3747
C18:0	410.29	416.09	429.80	396.58	34.732	0.9071	0.5053	0.3170
C18:1 *trans*-11	8.51	13.96	11.24	11.23	1.088	0.0016	0.9941	0.7033
C18:1 *cis*-9	778.02	664.62	786.07	656.58	52.858	0.1423	0.0961	0.5420
C18:1 *cis*-11	28.77	34.52	33.42	29.87	2.264	0.0851	0.2786	0.5074
C18: 2 *n-6*	101.27[a]	77.03[b]	94.15	84.15	4.571	0.0010	0.1350	0.7765
C18:3 *n-3* (ALA)	24.48[b]	31.58[a]	29.31	26.76	1.458	0.0021	0.2280	0.3048
C18:2 *cis*-9 *trans*-11 (CLA)	6.61	6.35	6.72	6.24	0.609	0.7654	0.5860	0.8685
C20:3 *n-6*	11.48[a]	6.86[b]	9.78[a]	8.56[b]	0.374	<.0001	0.0308	0.3617
C20:4 *n-6*	32.87[a]	21.66[b]	27.46	27.08	0.775	<.0001	0.7306	0.6052
C20:5 *n-3* (EPA)	10.58[b]	29.17[a]	19.80	19.95	0.879	<.0001	0.9022	0.9073
C22:5 *n-3*	19.58[b]	30.98[a]	25.20	25.37	0.771	<.0001	0.8809	0.3402
C22:6 *n-3* (DHA)	5.49[b]	9.39[a]	7.86	7.02	0.544	<.0001	0.2875	0.0646
EPA+DHA	16.07[b]	38.56[a]	27.65	26.97	1.067	<.0001	0.6549	0.2888
Total SFA	1327.51	1287.84	1386.02	1229.33	109.907	0.8007	0.3235	0.4490
Total USFA	1095.13	997.62	1127.91	964.84	65.972	0.3064	0.0933	0.5710
Total MUFA	882.75	784.58	907.63	759.71	60.417	0.2619	0.0962	0.5522
Total PUFA	212.38	213.04	220.28	205.14	7.722	0.9526	0.1783	0.8503
Total *n-3* PUFA	60.14[b]	101.13[a]	82.17	79.10	2.841	<.0001	0.4524	0.2380
Total *n-6* PUFA	145.63[a]	105.55[b]	131.39	119.79	5.287	<.0001	0.1340	0.6993
n-6/*n-3*	2.44[a]	1.04[b]	1.82	1.67	0.076	<.0001	0.1715	0.1650
PUFA/SFA	0.17	0.18	0.17	0.18	0.014	0.6147	0.4348	0.4324

Column means with different superscript letters differ significantly (P<0.05) as assessed by Tukey's Multiple Range Test
SEM - standard error of mean; CON - control; PO - palm oil-based fatty acid Ca salts; FO - fish oil-based fatty acid Ca salts; ALA - alpha-linolenic acid (18:3 *n-3*); EPA - eicosapentaenoic acid (20:5 *n-3*); DHA - eicosapentaenoic acid (20:5 *n-3*); CLA - conjugated linoleic acid (18:2, *cis*-9 *trans*-11); Total SFA - a total of C14:0, C15:0, C16:0, C17:0 and C18:0; Total USFA - a total of 14:1, 16:1, C18:1 *trans*-11, C18:1 *cis*-9, C18:1 *cis*-11, C18:2 *n-6*, C18:3 *n-3* (ALA), CLA *cis*-9 *trans*-11, C20:3 *n-6*, C20:4 *n-6*, 20:5 *n-3* (EPA), C22:5 *n-3* and C22:6 *n-3* (DHA); Total MUFA - a total of C14:1, C16:1, C18:1 *trans*-11, C18:1 *cis*-9, and C18:1 *cis*-11; Total PUFA - a total of C18:2 *n-6*, C18:3 *n-3* (ALA), CLA *cis*-9 *trans*-11, C20:3 *n-6*, C20:4 *n-6*, 20:5 *n-3* (EPA), C22:5 *n-3* and C22:6 *n-3* (DHA); Total *n-3* PUFA - a total of C18:3 *n-3* (ALA), 20:5 *n-3* (EPA), C22:5 *n-3* and C22:6 *n-3* (DHA); Total *n-6* PUFA - a total of C18:2 *n-6*, C20:3 *n-6* and C20:4 *n-6*

Discussion

Growth performance. The body weight gains and ADG of steers in Experiment 1 were not influenced by supplementing PO or FO fatty acid Ca salts, although supplement intakes from FO were lower compared with those of CON and PO. Forage intake, which was not measured, would have provided the greater proportion of energy intake needed for growth. The supplemental oils in Experiment 1 were fed at 0.25% of BW to avoid potential negative associative effects of supplemental fat (BROKAW et al., 2001; PAVAN and DUCKETT, 2008). Fish oil supplementation of grazing cattle reduced growth rate, but the oil was not modified (WISTUBA et al., 2005). In contrast, no differences in ADG or dry matter intake was observed in heifers supplemented with fish oil at 140 g/heifer/day (CHILDS et al., 2008). The cattle from Experiment 2 in the present study supplemented with PO had greater ADG compared with those fed FO. These results were surprising since the PO and FO energy levels (271% total digestible nutrients (TDN) vs 266% TDN for PO and FO, respectively), as well as the pen average intake of FO and PO lick tubs (0.22 kg/d and 0.23 kg/d for FO and PO, respectively) were similar. The similar live weight gains and ADG between steers and heifers in Experiment 2 were consistent with the results of STEEN et al. (2003), who reported no differences in the live weight gains of steers and heifers grazed on perennial ryegrass pasture. The fatty acid concentrations of forages from both experiments were of similar magnitude to those previously reported (WESTON et al., 2008), with a concentration of total fatty acids of 23.7 mg/g DM, (2.4% of DM as fatty acids). Therefore, the impact of the forages fed for both experiments on LT fatty acid compositions was comparable.

LT fatty acid concentrations. We observed consistencies between the two experiments in LT fatty acid concentrations. Concentrations of C18:1 *trans*-11, EPA, C22:5 *n-3*, DHA, total *n-3* PUFA, and EPA+DHA increased, whereas, C18:2 *n-6*, C20:3 *n-6*, C20:4 *n-6*, total PUFA, total *n-6* PUFA, and the ratio of *n-6* to *n-3* were lower in FO-supplemented cattle in both experiments. Moreover, C14:0, C14:1, C15:0, C16:0, C16:1, C17:0, C18:0, C18:1 *n-9*, C18:1 *cis*-11, conjugated linoleic acid (CLA; *cis*-9 *trans*-11), total SFA, total USFA, and total MUFA remained similar between FO- and PO-supplemented cattle in both experiments. In Experiment 2, only C20:3 *n-6* was affected by sex, where it was greater in heifers than in steers.

Our results were contrary to the results from HENNESSY et al. (2021), who reported similar C16:0 but lower C18:0 concentrations in the muscle in heifers supplemented with 140 g/heifer/day of partially rumen protected fish oil compared with controls. Supplementing *n-3* PUFA to ruminants was reported to cause a competition between PUFA with SFA for uptake by the intestine (BALLOU et al., 2009). Additionally, inhibition of the elongation process and *de novo* fatty acid synthesis in tissues (SANTOS-SILVA et al., 2004) due to a greater proportion of exogenous fatty acids in the metabolic pool (YANG et al., 1978) resulted in a lower rate of SFA deposited in the tissues (WORTMAN et al., 2009). However, these effects would be attenuated if cattle were fed a rumen inert fatty acid, such as fatty acid calcium salts, because of the higher level of protection from rumen biohydrogenation and metabolism of the long-chain PUFA. The contrast in the supplement effect on C18:1 *trans*-11 and C18:2 *n-6* suggests that a greater rate of biohydrogenation could have occurred in FO supplemented cattle, such that greater C18:1 *trans*-11 was associated with lesser C18:2 *n-6* in the LT. However, this observation was not consistent with the effects on CLA in the LT. Fish oil calcium salt consumption did not affect ALA in Experiment 1, but this fatty acid was greater in the LTs of FO supplemented cattle in Experiment 2, suggesting that biohydrogenation of ALA was less or not affected by FO calcium salts. Nevertheless, ALA in the LT would reflect consumption of this fatty acid, which could have been affected by forage source and intake in Experiments 1 and 2.

Trans-vaccenic acid, a precursor of CLA synthesis in tissues (KADEGOWDA et al., 2013), was not associated with LT CLA concentrations in the present study. The conversion of *trans*-vaccenic acid to CLA in tissues is catalyzed by stearoyl-CoA desaturase, which is inhibited by EPA (LIN et al., 2004; GRUFFAT et al., 2005; RENAVILLE

et al., 2006). The greater concentrations of C18:1 *trans*-11 without greater CLA concentrations in the LT in FO-supplemented cattle might have been due to the EPA effects from FO.

Lower LT concentrations of C18:2 *n-6* were associated with lower intake from the supplements in FO-supplemented cattle compared with those of PO. Similarly, the greater content of ALA in FO supplements resulted in greater LT concentrations of ALA, but this was only observed in Experiment 2 in FO-supplemented cattle. As the main USFA of forages (GLASSER et al., 2013), ALA from the diet can be converted to EPA and DHA in the liver, although the process is not highly efficient (BRENNA, 2002). The observation of EPA and DHA in the LT of CON- and PO- supplemented cattle reflects the absorption and subsequent conversion of a portion of dietary ALA, where the increase in these fatty acids observed in the FO-supplemented cattle is a clear indication of provision from the FO-calcium salts. Feeding fish oil or fish meal to steers resulted in similar increases in these fatty acids in the meat (MANDELL et al., 1997; VATANSEVER et al., 2000; HENNESSY et al., 2021). In these studies, the magnitude of increase depended on the duration (50-168 d) and the amount of fishmeal fed (5 or 10%; MANDELL et al., 1997). Additionally, the particular lipid fraction evaluated (neutral lipid or phospholipid; VATANSEVER et al., 2000), as well as the supplement type (rumen protected fish oil; HENNESSY et al., 2021) impacts the outcome.

Consuming 100 g of LT from the FO-supplemented cattle of the present study would provide 88.76-101.13 mg *n-3* FA (ALA, EPA, and DHA) per day, compared with 60.14-72.25 mg *n-3* FA in PO-supplemented cattle. This would be a greater contribution from grass-finished beef, by contributing greater towards the recommended daily intake of 250 mg/d EPA and DHA. The benefits of consuming meat from the FO-supplemented cattle also would contribute to a lower ratio of *n-6* to *n-3* PUFA, which was 1.04-1.69 compared with those of PO-supplemented cattle at 2.44-2.48.

The results of Experiment 1 may have been confounded by variations in FO calcium salt intake. As the total long-chain PUFA (EPA + DHA) was plotted against the concentration sum of these fatty acids in LT (data not shown), the descriptive data accompanying the plot showed a linear relationship between intake of the fatty acids and their concentration in the LT. One of the main objectives of the present study was to compare variations in LT concentrations of EPA in Experiment 1 with variations in this fatty acid in LT found in Experiment 2. The changes in EPA concentrations in LT samples due to feeding FO compared with those of feeding PO in Experiment 1 and Experiment 2 were calculated by subtracting the amounts in the LTs from FO-fed cattle from the amounts in the LTs from PO-fed cattle. The mean, standard deviation, range, and coefficient of variations of LT EPA concentrations from Experiment 1 were 8.57, 4.53, 1.55 to 14.81, and 52.82%, respectively, whereas, the same values in the same order were 7.90, 3.01, 3.83 to 15.38, and 38.09%, respectively, for Experiment 2. Although mean concentrations of EPA in Experiment 1 and Experiment 2 were comparable, FO Ca salt supplementation through the lick tub yielded a lower standard deviation and coefficient of variation. Supplementing FO Ca salts via lick tubs decreased the variations, as evidenced by the comparable LT EPA concentrations yielded with upper ranges contrary to the fewer variations with lower ends in Experiment 1, probably because the cattle more willingly consumed the supplements than the dried molasses product. However, cattle were provided tubs while housed in pens as opposed to the larger area typical of pastures, which could also influence the outcome. Nevertheless, the lick tub delivery system reduced variations in LT concentrations of EPA.

Sensory characteristics of LT from cattle fed n-3 fatty acids. Experiment 1 included sensory evaluation of steaks to determine if any increase in long-chain *n-3* PUFA, or other attributes from a FO supplement would affect their flavor and other characteristics. The cooking loss and texture properties of the meat from the FO-supplemented cattle were similar to beef from PO- or CON-supplemented cattle. WISTUBA et al. (2006) and WOLF et al. (2019) reported no changes in cooking loss of the *biceps femoris* from beef cattle supplemented with fish oil. Similar values of Warner-

Bratzler shear force in LT from FO-supplemented cattle compared with those of PO-supplemented cattle indicated that the tenderness of steaks would not be impacted by this supplementation strategy. Most importantly, no fishy flavor was observed in steaks from the FO-supplemented cattle. Contrary to the results of the present study, WOLF et al. (2019) reported a stronger fishy flavor in beef and beef patties from heifers supplemented with 60 g/kg DM of a rumen protected fish oil for 8 weeks. The discrepancy between the present work and WOLF et al. (2019) could have been due to differences in intake and the duration of supplementation.

Conclusions

The present experiments showed that supplementing the calcium salts of fish oil to grazing or forage finished beef cattle did not impact growth performance and, importantly, the same supplementation resulted in increased muscle deposition of EPA and DHA, without negatively influencing the flavor and tenderness of the meat. Therefore, such a supplementation strategy could be used for grass-fed cattle to increase EPA and DHA in the meat, which are linked to prevention of several diseases in humans. The present experiments also showed that the calcium salts of fish oil delivered in dried molasses lick tubs is an effective means of supplementing long-chain *n-3* fatty acids to grazing cattle for grass-fed beef production.

References

AMSA (1995): American Meat Science Association. Research guidelines for cookery, sensory evaluation, and instrumental tenderness measurements of fresh meat of AMSA. Chicago, IL, USA, pp. 155-159.

AOAC (2007): Association of Official Analytical Chemists. Official Methods of Analysis of AOAC International. Association of Analytical Communities, Arlington, Virginia, USA, pp. 254-259.

BAKER, E. J., E. A. MILES, G. C. BURDGE, P. YAQOOB, P. C. CALDER (2016): Metabolism and functional effects of plant-derived omega-3 fatty acids in humans. Prog. Lipid Res. 64, 30-56.
DOI: 10.1016/j.plipres.2016.07.002

BALLOU, M. A., R. C. GOMES, S. O. JUCHEM, E. J. DEPETERS (2009): Effects of dietary supplemental fish oil during the peripartum period on blood metabolites and hepatic fatty acid compositions and total triacylglycerol concentrations of multiparous Holstein cows. J. Dairy Sci. 92, 657-669.
DOI: 10.3168/jds.2008-1196

BRENNA, J. T. (2002): Efficiency of conversion of α-linolenic acid to long chain n-3 fatty acids in man. Curr. Opin. Clin. Nutr. Metab. Care 5, 127-132.
DOI: 10.1097/00075197-200203000-00002

BROKAW, L., B. W. HESS, D. C. RULE (2001): Supplemental soybean oil or corn for beef heifers grazing summer pasture: effects on forage intake, ruminal fermentation, and site and extent of digestion. J. Anim. Sci. 79, 2704-2712.
DOI: 10.2527/2001.79102704x

BURDGE, G. C., A. E. JONES, S. A. WOOTTON (2002): Eicosapentaenoic and docosapentaenoic acids are the principal products of α-linolenic acid metabolism in young men. Brit. J. Nutr. 88, 355-364.
DOI: 10.1079/bjn2002662

CHILDS, S., C. O. LYNCH, A. A. HENNESSY, C. STANTON, D. C. WATHES, J. M. SREENAN, M. G. DISKIN, D. A. KENNY (2008): Effect of dietary enrichment with either n-3 or n-6 fatty acids on systemic metabolite and hormone concentration and ovarian function in non-lactating heifers. Animal 2, 883-893.
DOI: 10.1017/S1751731108002115

DUCKETT, S. K., J. P. S. NEEL, R. M. LEWIS, J. P. FONTENOT, W. M. CLAPHAM (2013): Effects of forage species or concentrate finishing on animal performance, carcass and meat quality. J. Anim. Sci. 91, 1454-1467.
DOI: 10.2527/jas.2012-5914

ELAGIZI, A., C. J. LAVIE, E. O'KEEFE, K. MARSHALL, J. H. O'KEEFE, R. V. MILANI (2021): An Update on Omega-3 Polyunsaturated Fatty Acids and Cardiovascular Health. Nutrients 13, 204.
DOI: 10.3390/nu13010204

FAO (2010): The Food and Agriculture Organization of the United Nations. Fat and Fatty Acids in Human Nutrition: Report of an Expert Consultation; Food and Agricultural Organization of the United Nations, Rome, Italy, pp. 143-153.

GLASSER, F., M. DOREAU, G. MAXIN, R. BAUMONT (2013): Fat and fatty acid content and composition of forages: A meta-analysis. Anim. Feed Sci. Technol. 185, 19-34.
DOI: 10.1016/j.anifeedsci.2013.06.010

GOERING, A. L, P. J. VAN SOEST (1970): Forage fiber analysis (apparatus, reagents, procedures, and some applications. In: Agricultural Handbook No. 379. ARS, USDA. (Goering, A. L, P. J. Van Soest, eds.), Washington, DC, USA, pp. 121-128.

GRUFFAT, D., A. DE LA TORRE, J. M. CHARDIGNY, D. DURAND, O. LOREAU, D. BAUCHART (2005): Vaccenic acid metabolism in the liver of rat and bovine. Lipids 40, 295-301.
DOI: 10.1007/s11745-005-1385-1

HENNESSY, A. A., D. A. KENNY, C. J. BYRNE, S. CHILDS, R. P. ROSS, R. DEVERY, C. STANTON (2021): Fatty acid concentration of plasma, muscle, adipose and liver from beef heifers fed an encapsulated n-3 polyunsaturated fatty acid supplement. Animal 15, 1751-7311.
DOI: 10.1016/j.animal.2020.100039

HONIKEL, K. O. (1998): Reference methods for the assessment of physical characteristics of meat. Meat Sci. 49, 447-457.
DOI: 10.1016/s0309-1740(98)00034-5

INNES, J. K., P. C. CALDER (2020): Marine Omega-3 (N-3) Fatty Acids for Cardiovascular Health: An Update for 2020. Int. J. Mol. Sci. 21, 1362.
DOI: 10.3390/ijms21041362

KADEGOWDA, A. K. G., T. A. BURNS, M. C. MILLER, S. K. DUCKETT (2013): Cis-9, trans-11 conjugated linoleic acid is endogenously synthesized from palmitelaidic (C16:1 trans-9) acid in bovine adipocytes. J. Anim. Sci. 91, 1614-1623.
DOI: 10.2527/jas.2012-5590

LEHESKA, J. M., L. D. THOMPSON, J. C. HOWE, E. HENTGES, J. BOYCE, J. C. BROOKS, B. SHRIVER, L. HOOVER, M. F. MILLER (2008): Effects of conventional and grass-feeding systems on the nutrient composition of beef. J. Anim. Sci. 8, 3575-3685.
DOI: 10.2527/jas.2007-0565

LIN, X., J. J. LOOR, J. H. HERBEIN (2004): Trans10,cis12-18:2 is a more potent inhibitor of de novo fatty acid synthesis and desaturation than cis9,trans11-18:2 in the mammary gland of lactating mice. J. Nutr. 134, 1362-1368.
DOI: 10.1093/jn/134.6.1362

MANDELL, I. B., J. G. BUCHANAN, B. J. HOLUB, C. P. CAMPBELL (1997): Effects of fish meal in beef diets on growth performance, carcass characteristics, and fatty acid composition of longissimus muscle. J. Anim. Sci. 75, 910-919.
DOI: 10.2527/1997.754910x

MEILGAARD, M., G. V. CIVILLE, B. T. CARR (1991): Sensory evaluation techniques. 3rd ed., CRC Press Inc, Boca Ranton, FL, USA, pp. 97-112.

MURRIETA, C. M., B. W. HESS, D. C. RULE (2003): Comparison of acidic and alkaline catalysts for preparation of fatty acid methyl esters from ovine muscle with emphasis on conjugated linoleic acid. Meat Sci. 65, 523-529.
DOI: 10.1016/s0309-1740(02)00244-9

PALMQUIST, D. L. (2009): Omega-3 fatty acids in metabolism, health, and nutrition and for modified animal product foods. Prof. Anim. Sci. 25, 207-249.
DOI: 10.15232/S1080-7446(15)30713-0

PAVAN, E., S. K. DUCKETT (2008): Corn oil or corn grain supplementation to steers grazing endophyte-free tall fescue. I. Effects on in vivo digestibility, performance, and carcass quality. J. Anim. Sci. 86, 3215-3223.
DOI: 10.2527/jas.2007-0703

RENAVILLE, B., A. MULLEN, F. MOLONEY, Y. LARONDELLE, Y. J. SCHNEIDER, H. M. ROCHE (2006): Eicosapentaenoic acid and 3,10 dithia stearic acid inhibit the desaturation of trans-vaccenic acid into cis-9, trans-11-conjugated linoleic acid through different pathways in Caco-2 and T84 cells. Br. J. Nutr. 95, 688-695.
DOI: 10.1079/bjn20061717

RINCON-CERVERA, M. A., V. GONZALEZ-BARRIGA, R. VALENZUELA, S. LOPEZ-ARANA, J. ROMERO, A. VALENZUELA (2019): Profile and distribution of fatty acids in edible parts of commonly consumed marine fishes in Chile. Food Chem. 274, 123-129.
DOI: 10.1016/j.foodchem.2018.08.113

RULE, D. C., K. S. BROUGHTON, S. M. SHELLITO, G. C. MAIORANO (2002): Comparison of muscle fatty acid profiles and cholesterol concentrations of bison, beef cattle, elk, and chicken. J. Anim. Sci. 80, 1202-1211.
DOI: 10.2527/2002.8051202x

SANTOS-SILVA, J., I, MENDES, P. PORTUGAL, R. BESSA (2004): Effect of particle size and soybean oil supplementation on growth performance, carcass and meat quality and fatty acid composition of intramuscular lipids of lambs. Livest. Prod. Sci. 90, 79-88.
DOI: 10.1016/j.livprodsci.2004.02.013

SCOLLAN, N. D., M, S. DHANOA, J. J. CHOI, W. J. MAENG, M. ENSER, J. D. WOOD (2001): Biohydrogenation and digestion of long chain fatty acids in steers fed on different sources of lipid. J. Agric. Sci. 136, 345-355.
DOI: 10.1017/S0021859601008796

SHAHIDI, F., P. AMBIGAIPALAN (2018): Omega-3 polyunsaturated fatty acids and their health benefits. Annu. Rev. Food Sci. Technol. 9, 345-381.
DOI: 10.1146/annurev-food-111317-095850

SHIBABAW, T. (2021): Omega-3 polyunsaturated fatty acids: anti-inflammatory and anti-hypertriglyceridemia mechanisms in cardiovascular disease. Mol. Cell. Biochem. 476, 993-1003.
DOI: 10.1007/s11010-020-03965-7

STEEN, R. W. J., N. P. LAVERY, D. J. KILPATRICK, M. G. PORTER (2003): Effects of pasture and high-concentrate diets on the performance of beef cattle, carcass composition at equal growth rates, and the fatty acid composition of beef. New Zealand J. Agric. Res. 46, 69-81.
DOI: 10.1080/00288233.2003.9513533

VAN ELSWYK, M. E., S. H. MCNEILL (2014): Impact of grass/forage feeding versus grain finishing on beef nutrients and sensory quality: The U.S. experience. Meat Sci. 96, 535-540.
DOI: 10.1016/j.meatsci.2013.08.010

VATANSEVER, L., E, KURT, M. ENSER, G. R. NUTE, N. D. SCOLLAN, J. D. WOOD, R. I. RICHARDSON (2000): Shelf life and eating quality of beef from cattle of different breeds given diets differing in n-3 polyunsaturated fatty acid composition. Anim. Sci. 71, 471-482.
DOI: 10.1017/S135772980005548X

WALL, R., R. P. ROSS, G. F. FITZGERALD, C. STANTON (2010): Fatty acids from fish: The anti-inflammatory potential of long-chain omega-3 fatty acids. Nutr. Rev. 68, 280-289.
DOI: 10.1111/j.1753-4887.2010.00287.x

WESTON, T. R., J. D. DERNER, C. M. MURRIETA, D. C. RULE, B. W. HESS (2008): Comparison of catalysts for direct transesterification of fatty acids in freeze-dried forage samples. Crop Sci. 48, 1636-1641.
DOI: 10.2135/cropsci2007.07.0376sc

WISTUBA, T. J., E. B. KEGLEY, J. K. APPLE, M. E. DAVIS (2005): Influence of fish oil supplementation on growth and immune system characteristics of cattle. J. Anim. Sci. 83, 1097-1101.
DOI: 10.2527/2005.8351097x

WISTUBA, T. J., E. B. KEGLEY, J. K. APPLE (2006): Influence of fish oil in finishing diets on growth performance, carcass characteristics, and sensory evaluation of cattle. J. Anim. Sci. 84, 902-909.
DOI: 10.2527/2006.844902x

WOLF, C., J. MESSADÈNE-CHELALI, S. E. ULBRICH, M. KREUZER, K. GILLER, J. BÉRARD (2019): Replacing sunflower oil by rumen-protected fish oil has only minor effects on the physico-chemical and sensory quality of Angus beef and beef patties. Meat Sci. 154, 109-118.
DOI: 10.1016/j.meatsci.2019.04.002

WORTMAN, P., Y. MIYAZAKI, N. S. KALUPAHANA, S. KIM, M. HANSEN-PETRIK, A. M. SAXTON, K. J. CLAYCOMBE, B. H. VOY, J. WHELAN, N. MOUSTAID-MOUSSA (2009): n3 and n6 polyunsaturated fatty acids differentially modulate prostaglandin E secretion but not markers of lipogenesis in adipocytes. Nutr. Metab. 6, 5.
DOI: 10.1186/1743-7075-6-5

YANG, Y. T., R. L. BALDWIN, W. N. GARRETT (1978): Effects of dietary lipid supplementation on adipose tissue metabolism in lambs and steers. J. Anim. Sci. 47, 686-690.
DOI: 10.2527/jas1978.473686x

Received: 15 February 2022
Accepted: 15 March 2022

KUCUK, O., B. W. HESS, S. PAISLEY, K. UNDERWOOD, D. C. RULE: Povećanje dugolančanih *n-3* masnih kiselina u mesu dobivenom od goveda tovljenih na paši uz dodatak kalcijevih soli iz ribljeg ulja. Vet. arhiv 93, 627-640, 2023.

SAŽETAK

Provedena su dva pokusa kako bi se procijenili učinci dodatka kalcijevih soli iz ribljeg ulja na nakupljanje masnih kiselina u mišićima, s posebnim naglaskom na eikozapentanoičnu (EPA) i dokozaheksanoičnu kiselinu (DHA) u govođem mesu. U prvom je pokusu ukupno 38 pašnih goveda, kastriranih junaca pasmine angus (linija malog uzrasta) podijeljeno u tri pokusne skupine, uključujući i kontrolnu skupinu. U pokusnim su skupinama u prehranu životinja kao suha tvar uvedene kalcijeve soli masnih kiselina iz ribljeg ulja (FO) i palmina ulja (PO). U drugom je pokusu 14 junica i 14 kastriranih junaca pasmine angus hranjeno po volji krmnim biljem kojemu su dodane kalcijeve soli palmina ili ribljeg ulja. Soli ulja su kao suspenzija u osušenoj melasi ponuđena u blokovima za lizanje. Procijenjeni su pokazatelji rasta, senzoričke značajke i koncentracije EPA-e i DHA-a mišića *m. longissimus thoracis* (LT). Koncentracije masnih kiselina EPA i DHA u *m. longissimus thoracis* bile su veće (P<0,001) u goveda hranjenih FO-om, dok su C18:2 *n-6* i omjer *n-6* prema *n-3* bili veći (P<0,001) u goveda hranjenih u oba pokusa. Senzorička procjena LT odrezaka dobivenih od trupova kastriranih junaca (linija malog uzrasta) iz prvog pokusa nije otkrila štetne učinke FO suplementacije (P≥0,2695). Na temelju rezultata ovih pokusa zaključujemo da dodatak kalcijevih soli ribljeg ulja u prehranu goveda hranjenih krmnim biljem povećava odlaganje EPA-e i DHA-a u mišićima bez štetnih učinaka na okus. Dodatak ribljeg ulja govođem mesu može biti način da se zadovolje potrebe ljudskog organizma za omega-3 masnim kiselinama.

Ključne riječi: omega-3 masne kiseline; riblje ulje; mišić; angus linija malog uzrasta; pašni uzgoj