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ANIMAL PRODUCTION

Intramuscular fatty acid composition of steers finished on oat pastures mixture with vetch or cornmeal supplementation

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ABSTRACT: *The effects of energetic supplementation and mixture vetch (Vica sativa L.) were studied to evaluate the intramuscular fatty acid profile of steers finished on oat (Avena sativa L.) pastures. Eighteen 21-month-old crossbred steers were evaluated. The experiment was carried out in three treatments and six repetitions: oat pasture (OA), oat pasture + vetch (OA + VET), and oat pasture + supplementation (OA + SUP). Supplementation comprised the addition of corn meal at a daily dose of 1% of the animals' body weight. Steers receiving supplementation had higher lipid content in Longissimus lumborum than did those fed with OA + VET (1.25 vs 1.02%), whereas those fed with OA (1.15%) showed intermediate values. Conjugated linoleic acid levels were higher in steers fed OA (0.57%) and OA + SUP (0.59%), whereas the highest amount of omega-3 fatty acids was observed in animals fed OA + VET (3.32%). Pasture-finishing without supplementation resulted in a lower ratio of n-6:n-3 (3.14). Steers finished in oat mixture with vetch showed healthier intramuscular fat than did those finished with cornmeal supplementation; importantly, the higher the amount of PUFAs, the better n-6:n-3 ratio.*
Key words: *conjugated linoleic acid, energetic supplementation, grass, legume.*

Composição de ácidos graxos da gordura intramuscular de novilhos terminados em pastagem de aveia consorciada com ervilhaca ou suplementados com grão de milho moído

RESUMO: *Os efeitos da suplementação energética e da ervilhaca (Vica sativa L.) foram estudados para avaliar o perfil de ácidos graxos intramusculares de novilhos terminados em pastagens de aveia (Avena sativa L.). Foram avaliados 18 novilhos mestiços com 21 meses de idade. O experimento foi realizado em três tratamentos e seis repetições: pastagem de aveia (OA), pastagem de aveia + ervilhaca (OA + VET) e pastagem de aveia + suplementação (OA + SUP). A suplementação compreendeu a adição de fubá na dose diária de 1% do peso corporal dos animais. Os novilhos que receberam suplementação apresentaram maior teor lipídico no Longissimus lombar do que aqueles alimentados com OA + VET (1,25 contra 1,02%), enquanto aqueles alimentados com AO (1,15%) apresentaram valores intermediários. Os níveis de ácido linoléico conjugado foram maiores em novilhos alimentados com AO (0,57%) e OA + SUP (0,59%), enquanto a maior quantidade de ácidos graxos ômega-3 foi observada em animais alimentados com OA + VET (3,32%). Animais terminados em pastagens sem suplementação apresentaram menor proporção de n-6: n-3 (3,14). Novilhos terminados em pastagem de aveia com ervilhaca apresentaram gordura intramuscular mais saudável do que aqueles terminados com suplementação de milho moído, uma vez que apresentaram maior quantidade de PUFAs e melhor a relação n-6: n-3.*
Palavras-chave: *ácido linoleico conjugado, gramíneas, leguminosas, suplementação energética.*

INTRODUCTION

Mixture between grasses and temperate legumes has proved to be important for feeding beef cattle because these species possess excellent forage quality and provide high yields per area, allowing for long grazing periods, and improving the performance of animals during the critical production period (LISBINSKI et al, 2019). This increase in production is because of the long and later cycle of legume as compared to oat (WAGHORN & CLARK, 2004). Oats have low dry matter content, which may cause physical limitation of the rumen (VAN SOEST, 1994). Furthermore, energy supplementation is an alternative often used for finishing cattle in temperate pasture because these supplement assist in nutrient balance and increase the total dry matter intake (MOORE et al., 1999).

The beef cattle production aiming for high meat quality depends on the nutritional value of the diet offered to the animals. Factors that influenced the quality of animal products can be controlled at various stages of their production through manipulation of parameters such as nutrition that directly affect the growth rate of animals (WOOD et al, 2003).

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Studies have shown that increased inclusion of forage in the diet is advantageous to the animals' fat composition, and cattle finished on pastures have lower omega-6/omega-3 ratio and higher amounts of conjugated linoleic acid (CLA), both of which are beneficial to human health (VARELA et al, 2004.). According to VARELA et al. (2004) the polyunsaturated omega-6/omega-3 ratio is an index of the role of fatty acids in human arteriosclerosis. Fatty acids of the omega-3 series tend to decrease the levels of arachidonic acid in the tissues, inhibiting cyclogenase and lipogenase activities, with effect antithrombotic, reducing lipid levels in the plasma and increasing prostaglandin synthesis. According to MULVIHILL (2001), in animal studies, CLA reduced the risk of breast tumors and inhibited the induction of skin tumors and, because it has immuno-mediating antiteratogenic properties, increases growth efficiency, reduced body fat and prevents diabetes. Grain diets decrease ruminal biohydrogenation rate by the growth of microorganisms (GARCIA et al, 2008).

Recent research has focused on beef cattle fed with a temperate pasture mixture of legumes. However, there is a scarcity of literature discussing the effects of mixture pastures in temperature climates with legumes on the composition and profile of fatty acids in the meat of cattle in the finishing stage. Against this background, the present study investigated the effects of energetic supplementation and mixture vetch (*Vicia sativa* L.) to evaluate the intramuscular fatty acid profiles of steers finished on oat (*Avena sativa* L.) pastures.

MATERIALS AND METHODS

Eighteen castrated steers (crossbred ¼ Marchigiana, ¼ Aberdeen Angus, 1/2 Nellore) at 19 ±1.2 months of age and a mean initial body weight of 360 ± 34.3 kg, were evaluated. Seven hectares, subdivided into nine paddocks of 0.78 ha each, were used for the study, (3 paddocks each treatment and 2 steers each paddock). Animals (6 per treatment) were assigned to the following treatments: oat pasture (OA), oat pasture + vetch (OA + VET), and oat pasture + supplementation with corn meal at 1% of the body weight per day, based on dry matter (OA + SUP).

A continuous grazing system with variable stocking rate was adopted by following the methodology described by MOTT & LUCAS (1952). The forage mass was evaluated based on the visual estimation technique described by CAMPBELL & ARNOLD (1973). Average forage mass (893 kg DM/ha), stocking rate (1360.5 kg live weight (LW)/ha) and herbage allowance (7.1 kg DM/100 kg LW) showed no difference between treatments (HIRAI et al., 2015). More information on pasture implementation and pasture management were as described by HIRAI et al. (2015).

All the animals were slaughtered at the end of the grazing cycle (75 grazing days). The animals were transported by truck on a paved road about 30 km away to commercial l abattoir, with state inspection, where they were slaughtered following the conventional practices of the Brazilian beef industry. They were weighed after 12 h of fasting. On average, the final body weight of steers was 413 ± 5.2 kg with 2.4 ± 0.3 mm fat thickness values. Carcass and meat characteristic of the animals are presented by Hirai et al. (2014).

The carcasses were identified and weighed before cooling at 4 °C for 24 h. After cooling, the right half of the carcass was used to determine the quantitative traits. Twenty-four hours later, *longissimus lumborum* samples were obtained by a complete cross-section between the 11th and 13th ribs. The fat layer was discarded and the muscle portion was vacuum packaged, identified, frozen in the freezing tunnel (-30 °C to -35 °C), and maintained in a freezer at -20 °C until further analysis (storage = 90 days).

At the laboratory, the samples of foods (Table 1) and meat were subjected to extraction and methylation analyses, quantitative determination of the lipid fraction, and qualitative determination of fatty acids. The beef was ground in a food processor with dry ice to prevent heating and possible chemical changes. The fraction of total lipids was extracted from the samples according to the methodology of BLIGH & DYER (1959). Fatty acids were esterified according to the technique described by HARTMAN & LAKE (1973) and analyzed using a gas chromatograph, Agilent brand (model HP6890), equipped with a flame ionization detector (FID) and Supelco SP2560 capillary column (100m x 0.25mm x 0.2μm). Injector and detector temperatures were maintained at 250 °C and 280 °C, respectively. Temperature gradient used to separate the fatty acid esters was 140 °C for 5 min., increasing 1.6 °C/min to 210 °C, maintained for 10 min.; increasing 10 °C/min to 240 °C, maintained for a further 15 minutes, totaling a 76-minute run. The carrier gas flow rate (N2) was 30 mL/min. The injection volume was 1μL with a split ratio of 1:50. The fatty acids were identified by comparing the retention time of the fatty acids from the samples with known standards.

Table 1 - Fatty acids profile (percentage of fatty acids) of the experimental diets of oats and vetch pastures and corn meal.

Fatty acid	--------------------------------------Food--------------------------------------		
	Oat	Vetch	Cornmeal
C12:0	1.79	0.47	0.06
C14:0	1.19	0.86	0.16
C15:0	0.61	0.27	0.04
C16:0	19.74	16.81	13.47
C17:0	0.75	0.51	0.10
C18:0	2.68	3.76	2.54
C20:0	1.22	1.53	0.62
C22:0	1.09	0.83	0.20
C24:0	0.85	0.94	0.23
Total Saturated (AGS)	29.91	25.98	17.40
C15:1 cis (n-5)	0.42	0.20	0.00
C16:1 *cis* (*n*-7)	2.38	2.84	0.14
C17:1 cis (*n*-7)	0.34	0.00	0.05
C18:1 *trans* (*n*-7)	1.09	0.49	0.62
C18:1 cis (n-9)	3.57	6.01	32.40
C20:1 cis (*n*-11)	0.00	0.31	0.29
Total Monounsaturated	7.80	9.85	33.50
C18:2 *cis* (*n*-6)	15.97	24.67	47.56
C18:3 *cis* (*n*-3)	46.32	37.71	1.04
C20:2 cis (n-6)	0.00	0.18	0.03
20:3 cis (n-3)	0.00	0.28	0.00
C22:4 cis (n-6)	0.00	0.00	0.31
C22:5 cis (n-3)	0.00	1.32	0.17
Total Polyunsaturated (PUFA)	62.29	64.16	49.11
Total Unsaturated	70.08	74.02	82.60
n-6, %	15.97	24.85	47.90
n-3, %	46.32	39.31	1.21
n-6/ n-3	0.34	0.63	39.71
PUFA/AGS	2.08	2.47	2.82

Fatty acids were quantified by incorporating internal standard, methyl tricosanoic (C23:0) acid, into each sample during methylation.

Diet was considered the main effect with three levels including OA, OA + VET and OA + SUP. Analysis was conducted using GLM procedure of SAS (Version 9.3, Cary, NC). Residual normality and homogeneity were assessed by Shapiro-Wilk and Levene's tests (P>0.05). When significance (P<0.05) was indicated by ANOVA, means separations were performed using the LSMEANS function and individual differences were determined by the Tukey's test (P<0.05).

RESULTS

Animals finished with OA + SUP, showed significantly higher (P<0.05) total lipid content in *longissimus lumborum* than those finished with OA

+ VET, whereas those finished with OA did not show significant differences (Table 2).

The Lignoceric acid (24:0) was the only saturated fatty acid showed variation (Table 2). Animal that received vetch in the diet presented higher content of 24:0 than those who received supplementation. This variation was not sufficient to affect the total amount of saturated fatty acids (SFA) present in *longissimus lumborum*.

The sum of monounsaturated fatty acids (MUFAs) was not affected by treatments (Table 3). Myristoleic acid content (14:1 *cis*) was higher (P<0.05) in the meat of animals received supplementation than in that received only oat pasture. The oleic acid (18:1 *cis*) levels was higher (P<0.05) in oat then Oat and Oat + Vetch treatment. The Nervonic acid was higher in Oat + Vetch treatments.

CLA content was lower (P<0.05) in *longissimus lumborum* of animals fed with legumes (Table 2). But the deposition of total intramuscular poly-unsaturated fatty acids (PUFA), linoleic (18:2 *cis*), alpha linolenic (18:3 *cis* n-3), eicosatetraenoic (20:3 *cis*), arachidonic (20:4 *cis*) and eicosapentaenoic (20:5 *cis*) acids was found to be higher (P<0.05) in oat + vetch fed cattle than in those fed with oat + supplementation.

The animals oat + supplementation fed showed lower PUFA/SFA ratio, n-6, n-3 sum and higher n-6:n-3 ratio (Table 3).

DISCUSSION

It is possible that cornmeal supplementation with high starch content fosters the production of

Table 2 - Fatty acid profile (g/100 g FAME) in *longissimus lumborum* of steers finished on oat pastures with vetch or cornmeal supplementation.

Fatty acids	---------------------------------Diets*-------------------------------			Standard error	P - value
	OA	OA+ VET	OA + SUP		
Lipids, %	1.15 ab	1.02 b	1.25 a	0.06	0.0425
6:0	X'0.05	0.05	0.04	0.01	0.6965
8:0	0.04	0.04	0.08	0.03	0.6503
10:0	0.25	0.29	0.28	0.03	0,6260
12:0	0.24	0.19	0.21	0.04	0.6411
14:0	2.94	2.26	2.73	0.26	0.1849
15:0	0.50	0.44	0.56	0.05	0.2673
16:0	25.2	22.9	24.6	1.30	0.4450
17:0	1.05	0.93	1.03	0.09	0.5931
18:0	15.80	18.75	19.74	2.10	0.3962
20:0	0.14	0.14	0.15	0.01	0.8604
24:0	0.07 ab	0.09 a	0.05 b	0.01	0.0506
14:1 *cis* (n-5)	0.38 b	0.38 b	0.42 a	0.05	0.0298
16:1 *cis* (*n*-7)	2.76	2.40	2.81	0.29	0.5656
18:1 *trans* (n-9)	0.32	0.32	0.38	0.03	0.2535
18:1 *cis* (*n*-9)	36.1	34.8	36.0	0.90	0.5448
18:1 *trans* (*n*-7)	2.95 a	1.84 b	2.78 ab	0.27	0.0223
20:1 *cis* (*n*-11)	0.13	0.12	0.13	0.01	0.7515
24:1 *cis* (*n*-9)	0.04 b	0.06 a	0.03 b	0.01	
C18:2 *cis*9, *trans*11 (CLA)	0.57 a	0.40 b	0.59 a	0.04	0.0096
C18:2 *cis* (*n*-6)	5.43 ab	6.55 a	4.11 b	0.47	0.0102
C18:3 cis (n-6)	0.03	0.04	0.03	0.01	0.3139
C18:3 *cis* (*n*-3)	1.31 ab	1.71 a	0.77 b	0.15	0.0026
20:3 cis (n-6)	0.51 ab	0.74 a	0.36 b	0.07	0.0099
C20:4 *cis* (*n*-6)	2.23 ab	3.03 a	1.47 b	0.22	0.0009
20:5 *cis* (*n*-3)	0.76 b	1.35 a	0.47 b	0.11	0.0002
C22:6 cis (n-3)	0.16	0.26	0.13	0.04	0.0738

OA = oat pasture; OA + VET = oat pasture + vetch and OA + SUP = oat pasture + supplementation *Means followed by different letters in the same line differ significantly; Tukey test (P<0.05).

Table 3 - Total fatty acids profile (g/100 g FAME) in *longissimus lumborum* of steers finished on oat pastures with vetch or cornmeal supplementation.

	---------------------------Diets-------------------------				
	OA	OA+ VET	OA + SUP	Standard error	P-value
Total saturated fatty	46.3	46.1	49.5	1.20	0.1171
Total monounsaturated fatty acids	39.7	37.9	39.7	1.16	0.4426
Total polyunsaturated fatty acids	13.98 [a]	15.98 [a]	10.74 [b]	0.84	0.0024
PUFA/SFA ratio[**]	0.30 [a]	0.35 [a]	0.22 [b]	0.02	0.0035
n-6	8.20 [ab]	10.36 [a]	5.98 [b]	0.73	0.0036
n-3	2.23 [b]	3.32 [a]	1.37 [b]	0.26	0.0004
n-6:n-3 ratio	3.74 [ab]	3.14 [b]	4.54 [a]	0.22	0.0022

OA = oat pasture; OA + VET = oat pasture + vetch and OA + SUP = oat pasture + supplementation. [*]Means followed by different letters in the same line differ significantly; Tukey test (P<0.05). [**]PUFA: Polyunsaturated fatty acid; SFA: saturated fatty acid.

propionic acid in the rumen and enabled higher amounts of available glucose to the animal, thereby increasing the meat ether extract content (SCOLLAN et al., 2006). SCOLLAN et al. (2001), reported that SFAs such as myristic (14:0), palmitic (16:0), and stearic (18:0) acids are predominant in beef, wherein 18:0 represents approximately 30% of total SFAs. As well as our results FRUET et al. (2018) reportex low effect of pasture supplementation compared to legume-associated pastures, probably due to the short time of supplementation.

Changes in intramuscular fat occur mainly by the increase of triglycerides during fattening (WOOD et al., 2008). Thus, there is a propensity for a greater accumulation of SFA and MUFA according to the increase of intramuscular fat and proportional decrease of PUFA (CHAIL et al., 2016). The animals of present study didn’t show difference for marbling (HIRAI et al., 2014), justifying, in part, the lack of variation in SFA and MUFA.

Animals fed exclusively on pasture have higher PUFA than those fed the concentrate because of the large presence of fatty acids, especially n-3, in pastures compared to that in the concentrate. In addition, long-chain PUFAs in the muscle are associated with phospholipids of cell membranes, which are minimally influenced by nutrition.

The OAT + VET showed higher linoleic, alpha linolenic (ALA), eicosatetraenic, arachidonic and eicosapentaenoic contents in comparation to OAT + SUP. Higher transfer efficiency of PUFA to milk fat from legume-fed cattle compared to grass-fed are reported by (KALAČ & SAMKOVA, 2010). Dewhurst et al. (2003) observed 240% increase in the proportion of ALA passing through the rumen of animals fed red clover silage, even with a higher biohydrogenation in those fed red clover silage comporated to grass silage (86.1 versus 94.3).

Polyphenols are constantly identified as responsible for limited lipolysis and biohydrogenation. CABIDDU et al. (2010) observed that vetch has higher level of tannic polyphenols than crimson clover. These authors state that tannic phenols had a negative effect on lipolysis and biohydrogenation.

CLA consisted of geometric isomers of linoleic acid and has distinct bioactive characteristics (anticarcinogenic, hypocholesterolemic, antiobesity, and immune-modulatory effects) (KIM et al. 2016). Lower concentrations of CLA were observed in the meat of animals fed OA + VET. MAIA et al. (2007) reported that in relation to MUFAs, PUFAs are more toxic to bacteria involved in the bio-hydrogenation. In ruminants that ingested large quantities of PUFAs, such as high *n-3* (PUFA) contained in legumes, bio-hydrogenation facilitates the survival of *Butyrivibrio fibrisolvens* bacteria. High PUFA intake could exceed the bio-hydrogenation capacity of ruminal microorganisms, resulting in higher intestinal absorption of PUFAs, which might explain the lower CLA content of the meat in animals fed with OA + VET.

It was expected that it would have a lower CLA participation in OAT + SUP, but the lower content was in OAT + VET. For NIELSEN et al. (2006) only the presence of grain does not significantly alter the PUFA biohydrogenation in the rumen, CLA and trans-C18: 1 isomers. For this to occur there was a need for the presence of a forage naturally rich in starch and linoleic acid, such as corn silage., which wasnot demonstrated in the forages of the present study. VAN DORLAND et al. (2008) observed reduced proportions of CLA, including rumenic acid in milk fat of dairy cows fed with clovers.

The content of vaccenic acid was 2-2.5 times higher than of rumenic acid – CLA, with a strong relationship between them (ELGERSMA et al., 2006). Vaccenic acid is an isomer produced in significant amounts by bio-isomerization and bio-hydrogenation of linolenic (18:3 n-3) and linoleic (18:2 n-6) acids by the ruminal anaerobic bacteria B. fibrisolvens (GRIINARI & BAUMAN 1999). The vaccenic acid can be transformed into CLA by the action of Δ9-desaturase enzyme in the tissues (HAYASHI et. al. 2007) or yield stearic acid by bio-hydrogenation. As well as CLA there was low participation of vaccenic acid in OAT+VET.

Eicosapentaenoic acid (C20:5 n-3, EPA), considered one of the most important 20-carbon fatty acids for human consumption, was formed by the desaturation and elongation of α-linolenic acid (SMITH, 2007), which was present in higher levels in leguminous diets, confirmed in the present study.

Total n-3 PUFA was higher in OAT + VET. Grass and legume contain high proportions of C18:3n3, which is the main n-3 series of essential fatty acids (DEWHURST et al. 2006). The literature reported similar responses (SCHMIDT et al., 2013; WRIGHT et al., 2015). The lower linoleic acid content of legumes compared to grasses may explain changes in rumen biohydrogenation in grazing steers (WRIGHT et al., 2015). This reduction in linolenic acid biohydrogenation increased the flow of linolenic acid and decreases the flow of stearic and vaccine acids to the duodenum (LEE et al., 2006).

The n-3 fatty acids might help to prevent or treat a variety of diseases, including heart disease, cancer, arthritis, depression, and Alzheimer's disease (KIM et al., 2016). The n-6 have important physiological functions as cell membrane constituents, in regulation of blood viscosity, permeability of blood vessels, maintaining blood pressure, inflammation, and platelet function (KIM et al. 2016). Per the nutritional recommendation by the Department of Health (1994), bovine meat is considered a healthy food for humans if the n-6:n-3 ratio does not exceed the limit of 4. In the present study, this ratio was greater than 4 in animals fed with OA + SUP, which was significantly higher ($P<0.05$) than those finished with OA + VET, which averaged approximately at 3.

CONCLUSION

Steers finished in oat mixture with vetch showed healthier intramuscular fat than did those finished with corn meal supplementation; importantly, the higher the amount of PUFAs, the better n-6:n-3 ratio. Animals receiving vetch showed relatively low amounts of CLA.

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BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The animal rearing and management activities were approved by the animal research ethics committee – CEUA, of the Universidade Tecnológica Federal do Paraná (UTFPR), filed under number 2014-009.

DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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