



Short communication

Meat and fat quality traits of grazing steers supplemented with corn grain and increasing amounts of flaxseed[☆]M.M. Della Rosa^{a,b,1}, L.B. Pouzo^{a,b}, E. Pavan^{b,c,d,*}^a Consejo Nacional de Investigaciones Científicas y Técnicas CONICET, Av. Rivadavia 1917, C1033AAJ Ciudad Autónoma de Buenos Aires, Argentina^b Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata, Ruta Nac. 226 km. 73.5, 7620 Balcarce, Argentina^c Estación Experimental Agropecuaria Balcarce, Instituto Nacional de Tecnología Agropecuaria, Ruta Nac. 226 km 73.5, 7620 Balcarce, Argentina^d Department of Animal and Veterinary Science, University of Clemson, Clemson, SC 29634-0331, USA

ARTICLE INFO

Keywords:

Color
Shear-force
Glycogen
Troponin
Collagen
Sarcomere length

ABSTRACT

To evaluate the effect of corn and flaxseed supplementation at finishing on *longissimus thoracis* color and shear-force and on subcutaneous fat color, forty-eight angus steers were assigned to four dietary treatments (DIET): no-supplement, supplemented with 0.7% live weight (LW) of cracked corn grain plus 0.0%, 0.125% and 0.25% LW of whole flaxseed in two finishing trials (Trial-1, early spring and Trial-2, late spring). None of the evaluated variables were affected by DIET in either trial, nor in a combined statistical analysis of the pooled data. The lack of DIET effect observed on muscle color and shear-force, are in agreement with the similar *longissimus* muscle pH@45 min, temp@45 min, pH@24 h, and glycogen content at slaughter observed between DIET. Lack of shear force difference between supplementation treatments is in agreement with their similar muscle sarcomere length, total and soluble collagen content and proportion of intact troponin-T. Subcutaneous fat color was also similar between supplementation treatments. Corn or flaxseed supplementation of steers grazing a high-quality pasture did not improve meat or subcutaneous fat color and meat shear force.

1. Introduction

Beef color and tenderness are affected by the relative rate of muscle pH and temperature decline in carcass. A rapid temperature decline increases the incidence of darker beef colors (Page et al., 2001) and muscle cold shortening (Savell et al., 2005; Thompson et al., 2006). Due to its insulating effect on muscles, a subcutaneous fat thickness threshold of 0.76 cm would be required to reduce the incidence of dark-cutters and to guarantee meat tenderness (Tatum et al., 1982; Page et al., 2001; Savell et al., 2005). In addition, beef color and tenderness are also defined by its ultimate pH. Higher ultimate pH values result from lower muscle glycogen at slaughter and produce darker and tougher meats (Wulf et al., 2002).

Beef from pasture-finished cattle tend to be leaner (Neel et al., 2007; Pavan and Duckett, 2008) and to have lower glycogen content (Immonen et al., 2000) at slaughter than concentrate-finished ones. Nonetheless, corn oil or corn grain supplementation of pasture-fed cattle has shown to increase subcutaneous fat thickness (Pavan et al., 2007; Pavan and Duckett, 2008) and muscle glycogen (Knee et al.,

2007). Energy supplementation to grazing cattle could also improve fat color by reducing its yellowness. According to Dunne et al. (2009) this effect could be obtained through energy supplementation by lowering total carotenoid intake or by increasing fat accretion.

Increasing energy supplementation by adding flaxseed to the corn grain supplemented to grazing steers increased subcutaneous fat thickness while reducing the negative effect of corn supplementation on *longissimus* muscle polyunsaturated fatty acids n-6: n-3 ratio (Pouzo et al., 2015). The present study aims to evaluate if the effect observed by Pouzo et al. (2015) of increasing energy supplementation by adding flaxseed to the corn grain supplemented to grazing steers on subcutaneous fat thickness has a concomitant effect on beef color and shear-force, and on fat color.

2. Materials and methods

Two similar supplementation trials were conducted in accordance with Argentinean national recommendations for animal handling and those of the National Institute for Agricultural Technology (INTA) at

[☆] This study was funded by the Instituto Nacional de Tecnología Agropecuaria (INTA; PNPA-1126024) and the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT; PICT 2009-03).

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¹ Authors have no conflict of interest.

EEA-Balcarce, Province of Buenos Aires. Forty-eight Angus steers reared on a rotational grazing system without supplementation were randomly assigned to two trials (Trial-1 and Trial-2) and four dietary treatments (DIET: no-supplemented, CNTRL; supplemented with 0.7% LW of cracked corn grain plus no flaxseed, FLAX-0; plus 0.125% LW of whole flaxseed, FLAX-1; or plus 0.250% LW of whole-flaxseed, FLAX-2). In each trial, steers were supplemented during 70 d prior to harvest; those assigned to Trial-1 started the supplementation period on August, 3rd (366 ± 27 kg LW) and those assigned to Trial-2 started on October, 10th (458 ± 42 kg).

During the study the steers rotationally grazed on annual ryegrass (*Lolium multiflorum* cv. Billy Max and cv. Jack). Supplement (corn and flaxseed) was provided on individual feeders, and individual intake was measured (Pouzo et al., 2015). After the supplementation period, steers were harvested at a commercial slaughter house with an average of 474 and 508 kg LW for Trial-1 and Trail-2, respectively. Steers in Trial-1 were harvested the following morning and had free access to water and feed withdrawal; whereas, for reasons not associated with the study, steers in Trail-2 were kept an extra day in confinement with free access to fresh water and to grass hay.

At harvest pH and temperature were recorded and 45 min *post-mortem* (pH@45 m and Temp@45 m, respectively) were measured on *longissimus thoracis* muscle (LT) between the 12th and 13th ribs from the left side carcass (Portable pH-meter, Sper Scientific). At 24 h *post-mortem* muscle pH was recorded again (pH@24 h) and then loin samples from the left side of the carcasses were taken cutting between 8th and 9th, and 12th and 13th vertebrae.

The LT were obtained from the loins, cut into steaks and vacuum packaged. From caudal to cranial: two 0.5 cm and four 2.5 cm-thick steaks were obtained. One of the two 0.5 cm-thick steaks was immediately stored at -20 °C for sarcomere length determination, the other, was aged for 3 d at 4 °C and then stored at -20 °C for intact troponin-T (Tn-T) quantification. The first of the four 2.5 cm-thick steak was immediately stored at -20 °C for the estimation of total glycogen content at slaughter and for the determination of total and insoluble collagen content. The other three 2.5 cm-thick steaks were randomly assigned to one of three aging periods at 4 °C (AP; 3, 14 and 56 days), after completing the assigned aging period were stored at -20 °C for Warner–Bratzler shear force (WBSF) evaluation.

Instrumental color measurements were recorded for L* (lightness), a* (redness) and b* (yellowness) after ribbing on the exposed LT muscle between the 12th and 13th ribs and after 30 min of blooming. Readings were performed with a Minolta CR-310 (Minolta Corp, Ramsey, N.J.) with a 50 mm-diameter measuring area, a 10° standard observer and using a D65 illuminant. Values were recorded in three locations of the expose muscle area and of the subcutaneous fat at the 12th rib to obtain a representative reading.

Warner–Bratzler shear force analysis was conducted according to AMSA (1995)'s guidelines. Steaks were thawed at 4 °C for 12 h and cooked on preheated open heart electric grill (Farberware, Bronx, New York) to an internal temperature of 71 °C. Steaks were cooled at 4 °C for 1 h before six 1.27-cm-diameter cores were removed from each steak parallel to the muscle fiber orientation. Cores were sheared perpendicular long axis of the core using a Warner-Bratzler Shear Force machine (G-R Manufacturing, Manhattan, KS, US) with a digital dynamometer coupled.

Total collagen content in LT was estimated by the determination of hydroxyproline using the procedure described by Bergman and Loxley (1963). Insoluble collagen content was determined according to a procedure adapted from Hill (1966). Soluble content was estimated as the difference between total and insoluble collagen content.

Sarcomere length was measured according Cross et al. (1981) using a helium-neon laser (CVI Melles Gliot).

Muscle glycogen content was extracted by acid hydrolysis (Pighin et al., 2013) and glucose concentration in the extract was determined spectrophotometrically (505 nm; Spectrophotometer Thermo Fisher

ScientificUSA) using a commercial kit (Glicemia enzimática, GOD/POD; Wiener Lab., Rosario Argentina). The quantified glucose included free glucose and glucose product of glycogen hydrolysis (Pighin et al., 2013).

Lactate was determined spectrophotometrically (550 nm; spectrophotometer -Thermo Fisher Scientific, USA) following the procedure described by Neath et al. (2007) using a commercial kit (Randox kit LAC; Randox Laboratories Ltd, Crumlin, Co. Antrim, UK). Total glycogen content in LT at slaughter was then estimated by adding glycogen and glucose content and half of the lactate content.

Intact Tn-T was determined in muscle samples aged for 3 d using western blotting procedures according to Huff-Lonergan et al. (1995) with minor changes as described by Lucero-Borja et al. (2014).

2.1. Statistical analyses

Data from each trial were analyzed separately using R core team (2013) using a randomized complete design including DIET in the model as fixed effect and using animal as the experimental unit (n = 12). Shear-force data were analyzed using a split-plot design with the effect of DIET in the main plot and the effect of AP and the corresponding interaction in the sub-plot. Animal within DIET was used as the error term for the main plot effect. Least squares means were computed for main and interactive effects and separated statistically using F-protected ($P < 0.05$) *t*-tests. Orthogonal pre-planned contrasts were used to evaluate the linear and quadratic effects of flaxseed level added to the supplemented corn grain. As during Trial-1 a steer assigned to FLAX-2 died for reasons not associated with the study, data are presented as lsmeans ± standard error of the mean. Finally, data from both trials were pooled for a combined statistical analysis including TRIAL as fixed effect in the previous models.

3. Results

As none of the evaluated variables were affected by DIET ($P > 0.05$) in Trial-1 or in Trial-2, only results from the combined analysis of pooled data are presented (Table 1).

Animal performance, carcass characteristics as well as total and individual *longissimus* muscle fatty acid content data had been reported previously by Pouzo et al. (2015). In the current study no dietary treatment effects were observed ($P > 0.05$) for any of the LT characteristics evaluated. Subcutaneous fat color parameters were neither affected by dietary treatments ($P > 0.05$). No linear or quadratic effects for flaxseed levels were observed for any of the evaluated variables ($P > 0.05$; data not shown). Trend toward a linear increase of sarcomere length with increasing the level of flaxseed added to the supplemented corn. Sarcomere length tended to increase linearly ($P = 0.09$) with increasing level of flaxseed.

Shear force was not affected by dietary treatments ($P = 0.91$). Increasing aging period from 3 to 14 days reduced shear force by a 10%, from 38.4 to 34.8 N and extending the aging period to 56 days, reduced shear force to 29.6 N ($P < 0.01$).

4. Discussion

The main objective of the present study was to evaluate the effect of increasing level of energy supplementation through increasing the amount of unprocessed flaxseed added to the corn grain supplemented to grazing steers during the last 70 d of finishing on *longissimus thoracis* muscle color and shear force. The lack of DIET on color and shear force is not in agreement with the expected positive impact (higher L*, a* and b* values and lower WBSF values) associated to the increased subcutaneous fat thickness observed with supplementation; subcutaneous fat thickness increased from 5.17 mm in CNTRL to a maximum of 7.36 mm in FLAX-2 (Pouzo et al., 2015). According to Page et al. (2001) and Tatum et al. (1982) meat color and shear-force were negatively

Table 1
Longissimus muscle characteristics from grazing Angus steers supplemented with corn grain and increasing levels of flaxseed during the last 70 d of finishing (Ismeans \pm S.E.M.).

Items ^a	DIET ^b				P-value
	CNTRL (n = 12)	FLAX-0 (n = 12)	FLAX-1 (n = 12)	FLAX-2 (n = 11)	
Longissimus muscle					
pH@45 m	6.84 \pm 0.07	6.78 \pm 0.07	6.89 \pm 0.07	6.93 \pm 0.07	0.46
pH@24 h	5.39 \pm 0.06	5.47 \pm 0.06	5.46 \pm 0.06	5.44 \pm 0.06	0.83
Temp@45 m	35.8 \pm 0.3	36.1 \pm 0.3	35.8 \pm 0.3	36.2 \pm 0.3	0.74
Glycogen, μ mol glucose. g ⁻¹ fresh tissue	37.7 \pm 2.71	43.7 \pm 2.71	36.9 \pm 2.71	39.4 \pm 2.83	0.25
Color parameters					
L*	34.97 \pm 0.63	34.88 \pm 0.63	34.74 \pm 0.63	35.25 \pm 0.65	0.95
a*	18.02 \pm 0.54	18.86 \pm 0.54	18.16 \pm 0.54	18.95 \pm 0.57	0.53
b*	10.42 \pm 0.41	10.57 \pm 0.41	10.45 \pm 0.41	10.90 \pm 0.43	0.85
Collagen, mg g⁻¹ fresh tissue					
Total	4.35 \pm 0.32	4.18 \pm 0.32	4.12 \pm 0.32	3.98 \pm 0.34	0.89
Soluble	1.44 \pm 0.25	1.18 \pm 0.25	1.26 \pm 0.25	1.11 \pm 0.26	0.81
Insoluble	2.91 \pm 0.29	2.99 \pm 0.29	2.85 \pm 0.29	2.87 \pm 0.30	0.99
Sarcomere length, μ m	1.92 \pm 0.04	1.90 \pm 0.04	1.96 \pm 0.04	1.98 \pm 0.04	0.47
Intact troponin-T	2.52 \pm 0.80	2.20 \pm 0.80	2.35 \pm 0.80	2.78 \pm 0.84	0.96
WBSF, N ^c	33.2 \pm 1.9	34.7 \pm 1.9	35.1 \pm 1.9	34.1 \pm 2.0	0.91

^a pH@45 m, pH at 45 min *postmortem*; pH@24 h, pH at 24 h *postmortem*; Temp@45 m, Temperature at 45 min *postmortem*; L, lightness (0 = black, 100 = white); a*, redness (lower numbers = more green/less red, higher numbers = more red/less green); b*, yellowness (lower numbers = more blue/less yellow, higher numbers = more yellow/less blue).

^b CNTRL, no supplement, FLAX-0, supplemented 0.7% LW of corn plus no flaxseed, FLAX-1 y FLAX-2, supplemented 0.7% LW of corn plus 0.125% and 0.250% LW flaxseed respectively.

^c Ageing period effect (AP), $P < 0.01$; DIET \times AP effect, $P = 0.57$.

affected when carcass subcutaneous fat thickness was below a 7.6 mm threshold. The effect of subcutaneous fat thickness on color and on shear-force is associated with its insulation effect during carcass chilling (Aalhus et al., 2001). Fast muscle temperature declines, relative to muscle pH decline, produced darker meats and increased shear-force values. The latter would be the result of a greater sarcomere shortening (Thompson, 2002). Thus, the lack of DIET effect on shear-force is in line with the similar muscle temp@45 min, pH@45 min and sarcomere length observed in the present study between dietary treatments. The similar meat color and shear force observed between dietary treatments is further supported by their also similar pH@24 h (Purchas and Aungsupakorn, 1993; Wulf et al., 2002). Furthermore, the similar pH@24 h observed between dietary treatments is also in agreement with their lack of differences in post-mortem proteolysis, as suggested by the similar intact troponin-t index and aging response ($P = 0.57$).

The low, and similar between dietary treatments, pH@24 h (5.44 ± 0.03) observed suggest that muscle glycogen content at slaughter did not limit postmortem pH decline, not even in the carcasses from non-supplemented steers. Furthermore, muscle glycogen content observed at slaughter indicate that supplementation did not increase muscle buffer capacity against the apparent stressors factors in the post farm gate period leading to slaughter as suggested by Pethick et al. (1999). These lack of 70 d energy supplementation effect on muscle glycogen content at slaughter, contrast with the increased observed by Knee et al. (2007) in the semimembranosus and semitendinosus muscles of steers supplemented for a shorter period but with higher levels (~ 2% LW). The contrasting results between both studies can be explained by the higher supplementation level used by Knee et al. (2007) and by the negative energy balance in their control treatment as suggested by its negative live weight gain (LWG) that contrasted with the 1.1 kg/d obtained in the supplemented treatment. In the present study, despite an increase subcutaneous fat accretion with supplementation, no live weight gain (LWG) differences between dietary treatments were observed and the LWG obtained (1.0–1.1 kg LW/d) suggests that plane of nutrition was adequate in all treatments (Pouzo et al., 2015). Therefore, as suggested by Knee et al. (2007) energy supplementation has the potential to increase muscle glycogen when pasture quality is poor or when quantity is lacking, but the potential to increase muscle glycogen would diminish when pasture quality and quantity allows good gains even without energy

supplementation.

The observed increase subcutaneous fat thickness with increasing level of supplementation (Pouzo et al., 2015) was not enough to generate a change in subcutaneous fat color through a dilution of accumulated carotenoids as suggested by Dunne et al. (2009). According to Pouzo et al. (2016) carotenoids intake or muscle content were not affected by energy supplementation; probably as the result of the lack of energy supplementation effect on pasture intake (Pouzo et al., 2015). Wright et al. (2015) observed that corn supplementation to grazing steers had no effect on fat thickness but increased muscle carotenoids content and yellow color intensity of the subcutaneous fat. It seems that the effect of supplementation on fat yellowness would depend on its capacity to reduce carotenoids intake, mainly through a reduction of pasture intake. According to Bargo et al. (2003) the main effect of supplements on high-quality pasture intake is by a reduction on grazing time.

5. Conclusions

When pasture quality and quantity is not limiting if an improvement on color or shear-force is intended through supplementation greater levels of supplementation or longer period of supplementation than the ones used in the present study need to be evaluated. Similarly, if the objective of supplementation is to increase muscle glycogen of cattle finished on pastures, greater supplementation levels or longer periods should be evaluated. When trying to reduce fat yellowness of pasture-finished cattle through supplementation, the impact of supplementation on pasture intake need to be considered.

Acknowledgment

This study was funded by the Instituto Nacional de Tecnología Agropecuaria (INTA; PNPA-1126024) and the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT; PICT 2009-03). The support of Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) to MMDR and LBP is gratefully acknowledged. None of the sponsors had any role in the study design, in the collection, analysis, and interpretation of data, in the writing of the report, or in the decision to submit the paper for publication.

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