EFECT OF DIET AND STORAGE ON OXIDATIVE STABILITY OF BEEF

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Abstract – The effect of corn grain (0.7% of live weight) and flaxseed (0.125 and 0.25%) supplementation of grazing steers on antioxidant status and colour stability in beef was investigated. To simulate retail conditions, M. *Longissimus thoracis* samples were randomly distributed between treatments of refrigerated ageing times (3, 14 and 56 days under vacuum) with or without five days of aerobic exposure. Diet, ageing and exposure affected oxidative stability (TBARS augmented between 3 to 4-fold, P< 0.05), antioxidant status (decrease of FRAP and sum of antioxidant vitamins up to 20 and 80%, respectively) and redness (loss of a* value, P< 0.05) during retail display conditions. The use of flaxseed to improve the fatty acids composition is possible, but to counteract the effect of oxidative damage in beef, it is necessary to modulate the antioxidant status through dietary delivery.

Key Words – Argentine beef, ageing, retail display, oxidation, natural antioxidants.

I. INTRODUCTION

The link between nutrition and health is a hot topic. Current dietary recommendations suggest the need to increase the proportion of polyunsaturated fatty acids (PUFAs), especially the n-3 series at the expense of PUFA of the n-6. Meat from animals finished on pastures has a healthier fatty acid profile than those finished on concentrates [1]. Argentine beef has been traditionally produced on pasture. However, in order to increase energy intake, the supplementation of pasture-based diets with sources based on the use of 0,5 to 1,0% (BW, body weight) of cereal grains is becoming more common among producers. This practice could have a negative impact on fatty acid profile, especially on n6:n3 PUFA, hence reducing the nutraceutical properties of beef from grazing [2]. New strategies of supplementation, as use of flaxseed, has been proposed for increase the concentration of beneficial fatty acids, especially highly unsaturated n-3 fatty acids in muscle from ruminant species [3]. In a previous study we observed that the beef n6:n3 ratio could be improved, through the addition of increasing levels of flaxseed to corn grain supplement [4]. However, while increasing the content of beneficial fatty acids in meat is commendable from a human health perspective, such changes in fatty acid profile may have deleterious effects on the appearance and shelf-life of meat. The diet of animals can significantly affect the inherent susceptibility of meat lipids to oxidative deterioration, by modifying both the antioxidant and the pro-oxidant components of muscle. Pasture consumed by cattle is known to supply vitamin E requirements in addition to other natural antioxidants [1], while Flaxseed is one of the richest sources of α-linolenic acid (45-52%), but also it is high in anti-oxidants nutrients such as lignans, fenolic compounds and tocopherols [5].

We hypothesized that the incorporation of natural antioxidants from pasture or from pasture plus dietary supplements could generate in muscle an adequate antioxidant capacity to shield the oxidation given by the highest dietary levels of PUFA in beef.

II. MATERIALS AND METHODS

Animals and diets

Twenty four Angus steers from the same herd and back grounded on a rotational grazing system without supplementation were randomly assigned to four dietary treatments: (no-supplement, **CNTRL**; supplemented: 0.7% live weight (LW) of cracked corn grain, **FLAX-0**; FLAX-0 plus 0.125% LW of whole flaxseed,

FLAX-1; FLAX-0 plus 0.250% LW of whole-flaxseed, **FLAX-2**). Throughout the study, steers from the four dietary treatments grazed as one group, but received 0.5 kg (as-fed) of wheat bran and their dietary treatment (supplement) individually, therefore animal was used as experimental unit.

Sample collection and storage treatments

Animals were harvested in a commercial slaughter house after 70 d on trial with an average of 508 kg BW.

The *longissimus thoracic* muscle was removed cut into six 2.5 cm thick steaks that were vacuum packaged and randomly distributed among six treatment combinations, generated by three postmortem aging periods at 2 ºC (**PM, 3; 14 and 56 days**) on vacuum and two aerobic exposition periods (**AE, 0 and 5 days**). For aerobic exposition (retail display) steaks were placed on Polyfoam trays, overwrapped with an oxygen-permeable polyvinylchloride film and stored under simulated retail display conditions: illumination (2000 Lux) and 2ºC. After completing their assigned aging and aerobic exposition periods, steaks vacuum packed were stored at -25 ºC for later analysis.

Antioxidant vitamins content

Antioxidant vitamins (α and γ tocopherols, β-carotene, Retinol and Lutein) of meat samples aged (PM-3, 14 and 56) and retail displayed (EA-0, EA-5) were extracted following the extraction procedure described by Descalzo, et al., [6]. All samples and standards (external standards for each vitamin) were analyzed by reverse phase high performance liquid chromatography (HPLC).

FRAP assay

Antioxidant compounds such as alpha-tocopherol, trolox, vitamin C, uric acid, bilirubin, among others, are able to reduce ferric to ferrous-TPTZ which develops a blue color [7].

For meat samples, this assay was modified to measure endogenous ions that could react with TPTZ developing blue color (i.e. endogenous Fe^{II}), following the procedure described by Descalzo et al., [6].

Determination of the color stability and lipid oxidation

Meat color was measured daily during the five days of retail display (at day 0, 1, 2, 3, 4 and 5 of AE) using a Minolta CR 310 Chroma meter (Minolta Corp., Ramsey, NJ). The instrumental conditions were: large area aperture (5 cm diameter), D65-artificial and 10º standard angle observed. To assess the amount of lipid peroxidation TBARS assays were performed according to Jo and Anh [8] at the being AE-0 and the end EA-5 of each retail display. Results were expressed as mg of malonaldehyde (MDA) /kg meat.

Statistical analysis

The ANOVA was generated using the Proc Mixed procedure (SAS Inst. Inc., Cary, NC) with the main effects of DIETS, PM and AE, as well as their interaction, included in the statistical model. Least squares means was computed for main and interactive effects and was separated statistically using F-protected ($P < 0.05$) t-tests (PDIFF option). Meat redness, as denoted by the a* value, was subjected to the Mixed Procedure of SAS (SAS, 1998) as design with repeated measures.

III. RESULTS AND DISCUSSION

Tables 1 and 2 have shown the effect of diet and postmortem ageing on antioxidant and oxidative parameters of beef retail displayed, respectively.

The interactive effect of diet x AE was significant ($p< 0.05$) only for TBARS and Total concentration of antioxidant. In addition, as is observed in the bottom side of table 1 the addition of increasing levels of flax seed to diet of steers increased peroxidation index in beef, due to an increase of PUFAs with two or more double bond. The interactive effect AE x PM (Table 2) was highly significant for all parameters studied. The triple interaction diet x PM x AE and double interaction diet x PM resulted no significant ($p > 0.05$).

The incorporation of flaxseed or corn grain to the pasture diet, did not affected substantially the content of total antioxidants in muscle (Table 1). In absence of oxygen (AE=0), CNTRL and FLAX-1 treatments showed slightly higher concentration of antioxidant vitamins in beef, probably due to a better incorporation or less consumption of the vitamin in the tissue, or the presence of other factors in the supplement that

interact with its incorporation; however at 5 days of aerobic exposition the level was drastically reduced and showed no differences between treatments due to consumption of vitamins that interact to counteract lipid oxidation.

FRAP value determinates the total antioxidant capacity of samples. Mahecha et al [9] showed that hydrophilic extracts of Longissimus muscle had higher antioxidant values than lipophilic extracts, either using FRAP or TEAC methods. In this work, FRAP was measured in aqueous fractions of muscle extracts. A positive correlation between FRAP and the level of α-tocopherol in muscle have been reported previously [6]. Therefore, it was measured as an indicator of total antioxidant capacity of meat homogenates. In concordance with antioxidant levels, FRAP value was highest in CNTRL and FLAX-1 diets, while as expected after 5d of AE, TBARS level was highest in FLAX-2 and lowest in FLAX-1. This data suggests that could be an additional contribution of natural antioxidants by the inclusion of flax seed in FLAX-1 diet, which may have offset the increased level of peroxidation that could be generated by elevated levels of n3-PUFA in muscle. While in FLAX-2 diet, this would not have been enough.

Table 1. Effect of diet and Aerobic exposition (days) on oxidative stability parameters

Item	AE (d)	Diet					Significance		
		CNRL	FLAX-0	FLAX-1	FLAX-2	SE	Diet	AE	Diet*AE
TBARS (mg MDA/kg)	0	0.1 aB	0.1 aB	0.1 aB	0.1 aB	0.02	***	***	***
	5	0.3 bA	0.4 bA	0.3 cA	0.5 aA				
FRAP (μM)	-	509 a	471 b	515 a	478 b	10.3	**	***	ns
Sum of antioxidant (μg/g)[1]	0	2.2 aA	1.6 bA	2.2 aA	1.8 bA	0.10	**	**	**
	5	0.5 aB	0.5 aB	0.5 aB	0.5 aB				
Peroxidation index[2]	nd	10	9.8	11	14	1.23	*L	nd	nd

[1]α, γ-Tocopherol + β-Carotene + Retinol + Lutein.[2]Calculated as Hu, et al, [10] from fatty acid profile of fresh beef [4]. Means with different capital letter within the same column (AE effect) and different non capital letters within the same row (diet or *PM* effect) indicate significant differences (P <0.05). nd: not determinate. [L] linear flaxseed level effect.

Ageing on vacuum reduced vitamins levels in beef, this phenomena occurred in parallel with a gradual reduction of FRAP across postmortem ageing on vacuum (Table 2). Besides, increasing ageing on vacuum reduced lipid stability and FRAP activity in beef after 5 d of retail display (AE-5).

Table 2. Effect of Post-mortem ageing on vacuum (days) and aerobic exposition (days) on oxidative stability parameters

item	AE (d)	PM (d)				Significance	
		3	14	56	SE	PM	PM*AE
TBARS (mg MDA/kg)	0	0.1 aB	0.1 aB	0.1 aB	0.03	***	***
	5	0.2 cA	0.3 bA	0.6 aA			
FRAP (μM)	0	577 aA	540 bA	467 cA	12.7	***	*
	5	525 aB	427 bB	425 bB			
Sum of antioxidant (μg/g)	0	2.2 aA	1.9 bA	1.6 bA	0.09	**	**
	5	0.5 aB	0.5 aB	0.5 aB			

The oxidation of myoglobin to (brown) metmyoglobin is associated with deterioration of red color or reduction of the parameter a* (redness) in meat. As can be seen in figure 1 (A), redness decreased ($P < 0.05$) as retail display continued from 2 to 5 days for all dietary treatments. The more aged the meat, the more rapid decrease of a* value, with the lowest value recorded (12.52) at day 56 ($p<0.05$)(Figure 1 B). This could be associated to increased TBARS and reduced FRAP values of beef. As expected, we observed no significant differences in redness between dietary treatments within each day of display. This means that it is possible to alter the fatty acid composition of the meat without affecting significantly the observable red color at a given display time.

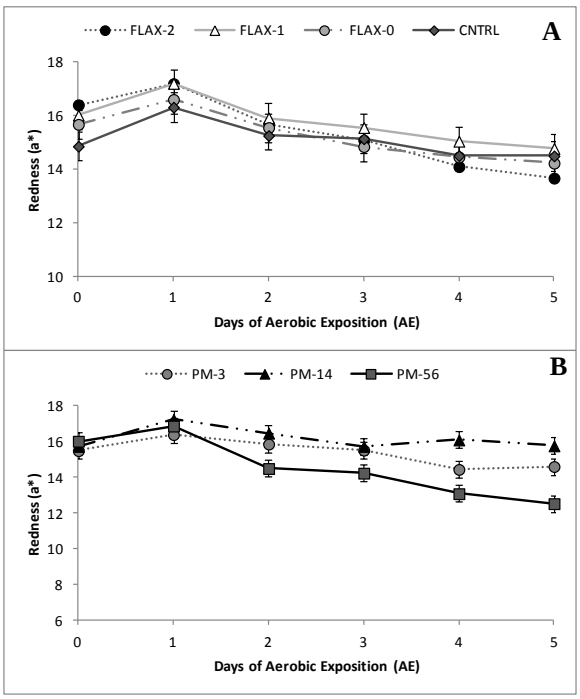

Figure 1. A) Interactive effect of diet and aerobic exposition (AE) on beef redness value. **B)** Interactive effect of postmortem ageing in vacuum (PM) and aerobic exposition on beef redness value.

IV. CONCLUSION

Extended aging in vacuum reduced the oxidative stability of lipids in beef subsequently displayed under aerobic conditions. This occurred in parallel with a decrease of redness, which was more noticeable after 5 days of air exposure. Concomitantly, a significant reduction in antioxidant vitamins and the reducing power (FRAP) and an increase in lipid oxidation (TBARS) occurred, no significant differences were appreciable in meat quality between samples from grazing animals fed high-PUFA diets. This means that it is possible to use flaxseed to improve the fatty acid composition in meat, taking into account the additional necessity to modulate the antioxidant status through dietary delivery.

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REFERENCES

1. Daley, C.A., Abbott, A., Doyle, P.S., Nader, G.A., & Larson, S. (2010). A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. Nutrition Journal 9, 10.
2. Garcia, P.T., Pensel, N.A., Sancho, A.M., Latimori, N.J., Kloster, A.M., Amigone, M.A., & Casal, J.J., (2008). Beef lipids in relation to animal breed and nutrition in Argentina. Meat Science, 79, 500-508.
3. Noci, F., French, P., Monahan, F. J., & Moloney, A. P. (2007). The fatty acid composition of muscle fat and subcutaneous adipose tissue of grazing heifers supplemented with plant oil-enriched concentrates. Journal of Animal Science, 85, 1062-1073.
4. Pouzo, L., Fanego, N., Santini, F & Pavan, E. (2013). Estrategias para mejorar el perfil de ácidos grasos en carne. In Proceedings XIV Congreso Nacional de Ciencia y Tecnología de alimentos. (pp.1-6), 23-26 October 2013, Rosario, Argentina.
5. Touré, A., & Xueming, X. (2010). Flaxseed Lignans: Source, Biosynthesis, Metabolism, Antioxidant Activity, Bio-Active Components, and Health Benefits. Comprehensive Reviews in Food Science and Food Safety, 9, 261-269.
6. Descalzo, A. M., Rossetti, L., Grigioni, G., Irurueta, M., Sancho, A. M., Carrete, J., & Pensel, N.A. (2007). Antioxidant status and odour profile in fresh beef from pasture or grain-fed cattle. Meat Science, 75, 309-317.
7. Benzie, I.F.F., & Strain, J. J. (1999). Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. New York Academic Press.
8. Jo, C. y Ahn, D. U. (1998). Fluorometric analysis of 2-Thiobarbituric acid reactive substances in Turkey. Pout Sci. 77:475-480
9. Mahecha, L., Nuernberg, K., Nuernberg, G., Martin, J., Hubbermann, E.M., Knoeller, S., Claeys, E., De Smet, S., & Dannenberger, D.(2011). Antioxidant enzyme activities and antioxidant capacity in longissimus muscle from bulls fed diets rich in polyunsaturated fatty acids. Food Chemistry, 127, 379-386.
10. Hu, M. L., Frankel, E. N., Leibovitz, B. E., & Tappel, A. L. (1989). Effect of dietary lipids and vitamin E on in vitro lipid peroxidation in rat liver and kidney homogenates. Journal of Nutrition, 119, 1574–1582.