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**Abstract** Grass-fed and grain-fed production systems are common production systems applied to beef production, each production system encloses benefits and weakness. In order to counterbalance benefits from both production systems, animals should be raised on pasture to acquire the nutritional benefits associated with grass ingestion and finished on grain to improve sensorial attributes. Grass natural abundance in antioxidants (tocopherols and carotenes) confers beef antioxidant protection, delaying lipid oxidation and colour oxidation. However, grain diets are scarce in these natural antioxidants, offering little antioxidant protection. It was this paper objective to assess the effect of different finishing options on liposoluble antioxidant vitamins ( $\alpha$ -tocopherol,  $\gamma$ -tocopherol and  $\beta$ -carotene) contents in *Longissimus lumborum* muscle.

Thirty two pure bred Alentejano young bulls were randomly allocated, accordingly to age, into four groups: 1) group CCC fed on concentrate; 2) group PPP raised on pasture; 3) group PPC was raised on pasture but received 2 months of finishing diet (concentrate) and 4) group PCC, raised on pasture but with a finishing period of 4 months.

The meat vitamin E homologues and  $\beta$ -carotene were simultaneously quantified by normal-phase HPLC, using fluorescence (tocopherols) and UV-visible photodiode array ( $\beta$ -carotene) detections in tandem.

Considering the antioxidant properties of both tocopherols and  $\beta$ -carotene, the results of this study show that beef finished on pasture (PPP group) contains higher contents of  $\alpha$ -tocopherol and  $\beta$ -carotene than other groups finished on concentrate diet independently of the finishing period. Therefore, beef from the PPP groups encloses a superior antioxidant potential than any other group. Taking into account previously studies, the  $\alpha$ -tocopherol contents in beef from PPC group seems to be enough to retard beef oxidation, while  $\alpha$ -tocopherol contents in both PCC and CCC groups are below the contents considered necessary to delay meat oxidation in food chain. It is therefore advisable that longer finishing periods on concentrate should receive vitamin E supplementation.

**Index Terms** – Alentejana bulls,  $\alpha$ -tocopherol,  $\gamma$ -tocopherol,  $\beta$ -carotene

## I. INTRODUCTION

Grass-fed and grain-fed production systems are common production systems applied to beef production worldwide. Both production systems enclose advantages and disadvantages to consumer. Grass-fed production systems offer improved nutritional quality due to increased contents of n-3 fatty acids [1], i.e., eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA), which have been shown to exert various beneficial health effects [2]. On the other hand, grain-fed beef is considered superior concerning sensorial characteristics, due to higher amounts of intramuscular lipid contents, which confer it higher tenderness and flavour [3-6]. Therefore, to counterbalance benefits from both production systems, animals should be raised in pasture to acquire the benefits associated with grass ingestion and finished on grain to improve sensorial attributes. Achievement of beef best characteristics requires studies to assess the effect of different finishing periods on several beef characteristics. It was the present work objective to study the effect of different finishing periods on the contents of beef major lipid-soluble vitamins ( $\alpha$ -tocopherol,  $\gamma$ -tocopherol and  $\beta$ -carotene).

## II. MATERIAL AND METHODS

Thirty two young bulls from Alentejano breed were used in this study. After weaning (7-11 months old, with live body weights ranging between 200 and 300 kg), the young bulls were randomly allocated, accordingly to age, into four groups: 1) group CCC fed on a diet consisting of 70% concentrate feed and 30% molasses-fibrous cubes, during 14-15 months and slaughtered with approximately 600 kg of live body weight; 2) group PPP was maintained for 15 months on pasture, grazing on spontaneous pastures, summer tritcalis and maize stubbles, receiving no finishing diet; 3) group PPC was maintained in pasture in similar conditions as PPP but received 2 months of finishing diet (with equal composition of group CCC); 4) group PCC, raised in similar conditions as group PPC but with a finishing period of 4 months (one of the animals in this group died during the study period and was not included in the results).

All meat samples, obtained by a transversal cut from the *longissimus lumborum* muscle were trimmed of connective, adipose tissue before blending in a food

processor, afterwards, meat samples were vacuum-packed and stored under freezing (-20° C.) until analysis, which was performed within a month.

The simultaneous analysis of tocopherols and  $\beta$ -carotene in meat was performed using a normal-phase silica column, with fluorescence detection for tocopherols (excitation wavelength of 295 nm and emission wavelength of 325 nm) and UV-Vis photodiode array detection for  $\beta$ -carotene (450 nm) in series. The injection volumes used varied between 10

and 100  $\mu$ l in order to get values inside the linearity range of the standard curves, as was previously described by Mestre Prates *et al.* [7].

Statistical treatment of data was conducted by ANOVA at a significance level of 5% ( $H_0$ :  $p < 0.05$ ), using the one-way ANOVA procedure of Statistix for Windows. When the F-test was significant, the comparison of means was assessed by the LSD method also at a significance level of 5%.

### III. RESULTS AND DISCUSSION

Table 1 – Vitamin E homologues ( $\alpha$ -tocopherol,  $\gamma$ -tocopherol) and  $\beta$ -carotene contents ( $\mu$ g /g of meat) present in *longissimus lumborum* muscle from young bulls fed different finishing diets

	PPP	PPC	PCC	CCC	P
$\alpha$ -tocopherol	6.56 <sup>a</sup>	4.78 <sup>b</sup>	2.83 <sup>c</sup>	1.77 <sup>d</sup>	<0.0001
$\gamma$ -tocopherol	0.07	0.08	0.08	0.09	n.s.
$\beta$ -carotene	0.07 <sup>a</sup>	0.03 <sup>b</sup>	0.01 <sup>c</sup>	0.01 <sup>c</sup>	<0.0001

Different superscripts are significantly different in between ( $P < 0.05$ )

Despite the existence of eight vitamin E natural isomers, only two homologues,  $\alpha$ - and  $\gamma$ -tocopherol were detected in *longissimus lumborum* beef, which are the only vitamin E homologues with significant accumulation in bovine muscle tissues, as was previously observed in Barrosã veal, by Prates *et al.* [7]. The muscle superior contents of  $\alpha$ -tocopherol relatively to  $\gamma$ -tocopherol is dependent of: 1) higher contents of  $\alpha$ -tocopherol in green forages, as pasture and grass silage, as well as, in grain (wheat and oat) [8] and 2) superior affinity of  $\alpha$ -tocopherol transfer protein ( $\alpha$ -TTP) towards  $\alpha$ -tocopherol comparatively to other tocopherols and tocotrienol isomers [9].

The results of this study show that both  $\alpha$ -tocopherol and  $\beta$ -carotene were negatively influenced by the extent of concentrate feeding. The  $\alpha$ -tocopherol and  $\beta$ -carotene contents, decreased in the proportion of concentrate feeding period ( $P < 0.05$ ), while  $\gamma$ -tocopherol showed no significant differences between the finishing groups ( $P > 0.05$ ). Total contents of  $\alpha$ -tocopherol in *longissimus lumborum* beef from the group PPP (6.55  $\mu$ g/g) was the highest of all groups in this analysis. It was also observable a continuous and significant decrease in beef  $\alpha$ -tocopherol contents with the increase in the length of concentrate in the finishing period (PPP > PPC > PCC > CCC). The inverse association observed between the  $\alpha$ -tocopherol levels and the increase of concentrate feeding in the finishing period is correlated with the higher contents of  $\alpha$ -tocopherol in grass [8, 10], that are 5-10 times higher in green roughages than in cereals [11].  $\alpha$ -Tocopherol concentration in muscle depends mainly on muscle characteristics, level of vitamin E in the diet and period of ingestion [12, 13].  $\alpha$ -Tocopherol in meat is indispensable for the preservation of meat overall

quality, which is achieved through its antioxidant properties, inhibiting fatty acid oxidation and loss of desirable colour during refrigeration and frozen storage [14]. It was reported that  $\alpha$ -tocopherol concentration to retard metmyoglobin formation in beef was 3.0  $\mu$ g/g in minced muscle [15] or 3.8  $\mu$ g/g in whole muscle [16]. The PPP group in our study achieved values of total  $\alpha$ -tocopherol that are on the upper limit of those previously registered in similar studies (1.03-7.00  $\mu$ g/g) [16, 17]. Our values of  $\alpha$ -tocopherol are near the limit for the accumulation of  $\alpha$ -tocopherol in muscle foods [16, 17] and in *longissimus lumborum* muscle in particular, which should be around 7  $\mu$ g/g [18]. Beef from both PPP and PPC groups contain an  $\alpha$ -tocopherol concentration (6.56 and 4.78  $\mu$ g/g, respectively) that has been considered enough to retard meat oxidation in both minced and whole beef [16], while the  $\alpha$ -tocopherol concentration in beef from PCC and CCC groups is below the desirable value.

In cattle,  $\beta$ -carotene is essentially the only carotenoid absorbed at the intestinal level and is, therefore, the predominant carotenoid form found in beef [19, 20]. Of all four different groups in study, the PPP group displayed the highest  $\beta$ -carotene (0.073  $\mu$ g/g), while the lowest  $\beta$ -carotene values were observed in the PCC group (0.01  $\mu$ g/g) and CCC group (0.01  $\mu$ g/g), which shown no significant differences in between. The PPC group displayed an intermediary value of total and specific  $\beta$ -carotene contents, below the PPP group but above the PCC and the CCC groups (0.03  $\mu$ g/g). This results are to some extent in agreement with Descalzo *et al.* [21], who have previously studied  $\beta$ -carotene deposition in muscle tissues and observed that pasture-fed cattle incorporate significantly higher amounts of

$\beta$ -carotene into muscle tissues than grain-fed cattle [21].

Considering the antioxidant properties of both tocopherols and  $\beta$ -carotene, the results of this study show that beef finished in pasture (PPP group) contains higher contents of  $\alpha$ -tocopherol and  $\beta$ -carotene than other groups finished on concentrate diet independently of the finishing period. Therefore, beef from the PPP groups encloses a superior antioxidant potential than any other group. Taking into account previously studies, the  $\alpha$ -tocopherol contents in beef from PPC group seems to be enough to retard beef oxidation, while  $\alpha$ -tocopherol contents in both PCC and CC groups are below the contents considered necessary to delay meat oxidation in food chain. It is therefore advisable that longer finishing periods on concentrate should receive vitamin E supplementation.

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