The balance between vitamin E and highly peroxidizable fatty acids in muscle and the oxidative stability of beef from cattle grown on forage- or concentrate-based rations

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Abstract— **The effect of different feeding systems on the levels of vitamin E and highly peroxidizable polyunsaturated fatty acids (HP-PUFA) in bovine muscle and on its associated oxidative stability was determined. One-hundred heifers were divided into 4 groups and fed: outdoors at pasture (P) or concentrates indoors (C) for 11 months, or were fed grass silage indoors for 5 months followed, for 6 months, by either grazed pasture (SiP) or grazed pasture plus 50% concentrates (SiPC). Vitamin E and fatty acids were measured in the *longissimus dorsi* muscle (LM) which was also used for lipid oxidation (TBARS), metmyoglobin (MMb%) and hue angle (*H**) values measurements during 11 days storage in a high oxygen atmosphere. Muscle vitamin E concentration decreased ($P < 0.0005$) in the order: P and SiP > SiPC > C, while HP-PUFA concentration in the C treatment was lower compared to P, SiP and SiPC ($P = 0.05$). The SiPC and C treatments reduced meat colour stability with higher MMb% ($P = 0.002$) and *H** values ($P < 0.0005$) in LD than P and SiP treatments. Compared to the other treatments, SiPC had lower LD lipid stability ($P < 0.0005$), with no differences observed between P, SiP and C. These results confirm a positive effect of grass-based diets on meat colour stability and underline that pasture-based diets supplemented with concentrates could significantly impair meat oxidative stability.**

Keywords— **Feeding system, Beef, Oxidative stability.**

I. INTRODUCTION

Oxidative stability of muscle depends on the balance between pro-oxidant and antioxidant components [1]. In meat, polyunsaturated fatty acids (PUFA) are susceptible to oxidative reactions and can be considered pro-oxidant components, while dietary antioxidants, such as vitamin E, can contribute to the antioxidant defences of muscle [2].

Compared to concentrate-based diets, pasture-based feeding systems generally produce higher levels of PUFA in muscle, but provide higher amounts of antioxidants [3]. However, cattle are rarely raised exclusively on pasture and often receive supplemental concentrates or receive grass silages during periods of housing. Such differences in the composition of the diet can alter the balance between pro- and antioxidants in muscle. Furthermore, little information on the effect of long term consumption of different diets on beef oxidative stability is available.

Here cattle were assigned for 11 months to different dietary treatments involving exclusive feeding of grazed grass or concentrates or a sequence of grass silage feeding followed by grazed grass with or without supplementation with concentrates. The objective was to study the effect of these different feeding systems on meat oxidative stability through modification of the balance between highly oxidizable PUFA and vitamin E in muscle.

II. MATERIALS AND METHODS

A. Animals and diets

One-hundred Charolais × Limousin heifers (average BW 275 kg ± SD 27.0 kg) were divided into four groups with 25 animals per treatment. Over an initial 5-month period, one group of heifers (P) grazed at pasture, while other two groups (SiP and SiPC) were kept indoors and offered grass silage *ad libitum*. During the remaining 6 months of experimental feeding, heifers in groups P and SiP grazed at pasture,

while heifers in the SiPC groups were offered a restricted allowance of pasture and, once daily, received concentrates for a 50% of the daily DM intake. Over the whole 11-month experimental period, another group of heifers (C) received a barley-based concentrate with supplemental straw in stall. The rations for the C and SiPC groups were adjusted to maintain similar rates of growth to those of the heifers at pasture (P).

B. Muscle sampling and analyses

Slaughtering and muscle sampling: After slaughtering, carcasses were kept at 4°C for 24 h, after which the *longissimus dorsi* muscle (LM) was excised, vacuum packaged and and stored at -20°C.

Muscle vitamin E concentration and fatty acid composition: Vitamin E was extracted from LM and measured by HPLC [4].

Extraction of fatty acids and gas-chromatography analysis of fatty acid methyl esters (FAME) were performed as described by Noci et al. [5]. The proportion and the absolute concentration of PUFA were calculated as the sum of all the identified polyunsaturated fatty acids expressed as percentage of the total FAME and as mg/g of muscle, respectively. The concentration of the highly peroxidizable PUFA (HP-PUFA) was calculated as the sum of PUFA with three or more double bonds expressed as absolute concentration (mg/g of muscle) [6].

Meat lipid and colour stability: A 100 g portion of LM was finely minced. Meat patties (thickness 2.5 cm) were placed into polyamide/polyethylene bags. The samples were packaged in modified atmosphere (MAP; 80% O_2 : 20% CO_2) and stored at 4°C. Meat colour, lipid oxidation and myoglobin oxidation were analysed after 2 h (day 0) and after 4, 7 and 11 days.

Meat lipid oxidation was measured as TBARS values [7].

Using a Minolta CM-2022 spectrophotometer, hue angle (H^*) values and the reflectance spectra from 400 nm to 700 nm were measured. Metmyoglobin (MMb%) at the meat surface was determined [8].

C. Statistical analysis

A repeated measures analysis was used to test the effect of the dietary treatment, time of storage and their interaction on the oxidative stability parameters (TBARS, H^* values and MMb%). A one-way ANOVA was performed to test the effect of the dietary treatment on the muscle fatty acid composition and vitamin E concentration. Mean values were compared by means of the Tukey's test.

III. RESULTS

D. Muscle vitamin E and fatty acid composition

Vitamin E concentration in muscle from heifers in the P and SiP groups was higher compared to SiPC and C groups ($P < 0.01$; Table 1). The SiPC group had higher vitamin E levels than the C group ($P < 0.01$).

The C treatment resulted in the lowest proportion of PUFA in the intramuscular fat compared to the P, SiP and SiPC treatments ($P < 0.001$, $P < 0.0005$ and $P = 0.01$, respectively). The ratio of the concentration of vitamin E to that of PUFA was higher in LM from heifers in both P and SiP groups compared to the SiPC and C group ($P < 0.001$; Table 1). The concentration of highly peroxidisable PUFA (HP-PUFA) was lower in the LM from animals in the C group compared to P, SiP and SiPC groups ($P < 0.05$), with no difference observed between the latter.

Table 1: Effect of the dietary treatment on muscle vitamin E concentration and fatty acid composition.
[1] Ratio between the concentration of vitamin E and that of PUFA (both expressed as mg/g of muscle)
[2] SEM: standard error of means
[a,b,c] within a raw, means without a common superscript differ

	Dietary treatment					
Item	P	SiP	SiPC	C	SEM [2]	*P* value
Vitamin E, µg/g	2.59 [a]	2.45 [a]	1.76 [b]	1.15 [c]	0.086	<0.0005
PUFA, % of tot FAME	9.62 [ab]	11.04 [a]	8.96 [b]	6.94 [c]	0.268	<0.0005
Vitamin E ÷ PUFA [1]	16.15×10^{-4} [a]	14.51×10^{-4} [a]	9.14×10^{-4} [b]	6.93×10^{-4} [b]	6.1×10^{-5}	<0.0005
HP-PUFA, mg/g	0.84 [a]	0.85 [a]	0.87 [a]	0.65 [b]	0.171	0.02

E. Meat oxidative stability

Lipid oxidation: Lipid oxidation increased over storage time ($P < 0.0005$; Fig. 1). However, the dietary treatment affected TBARS values ($P < 0.0005$). After 4 days, meat from P-fed animals had lower TBARS values compared to the SiPC group ($P = 0.002$). After 7 days, meat from heifers in the SiPC treatment reached higher TBARS values compared to the P and the SiP groups ($P = 0.001$ and P = 0.05, respectively). After 11 days, the SiPC treatment resulted in higher TBARS values compared to P and C (P < 0.05). Differences in TBARS values between the P, SiP and C groups were not detected .

Colour stability and myoglobin oxidation: Hue angle values (*H**; Fig. 2a) and metmyoglobin percentages (MMb%, Fig. 2b) increased over storage duration ($P < 0.0005$). However, the dietary treatment affected both parameters ($P < 0.0005$ and $P = 0.002$ for *H** and MMb%, respectively). After 7 days, beef from animals in the P group had lower *H** values than SiPC and C groups ($P < 0.05$), while the latter groups showed higher *H** values compared to P and SiP after 11 days ($P < 0.001$).

Lower MMb% were measured, after 11 days, in meat from heifers in the P and SiP groups compared to both SiPC and C groups ($P < 0.001$).

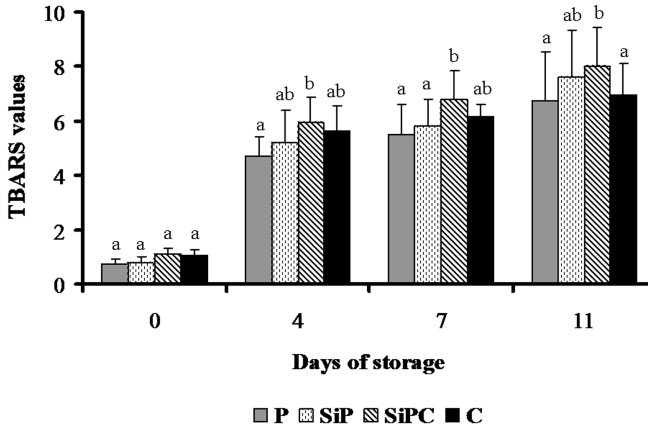

Fig. 1: Effect of dietary treatment and time of storage on lipid oxidation (TBARS values) of minced beef stored in a high oxygen modified atmosphere for 11 days at 4°C.

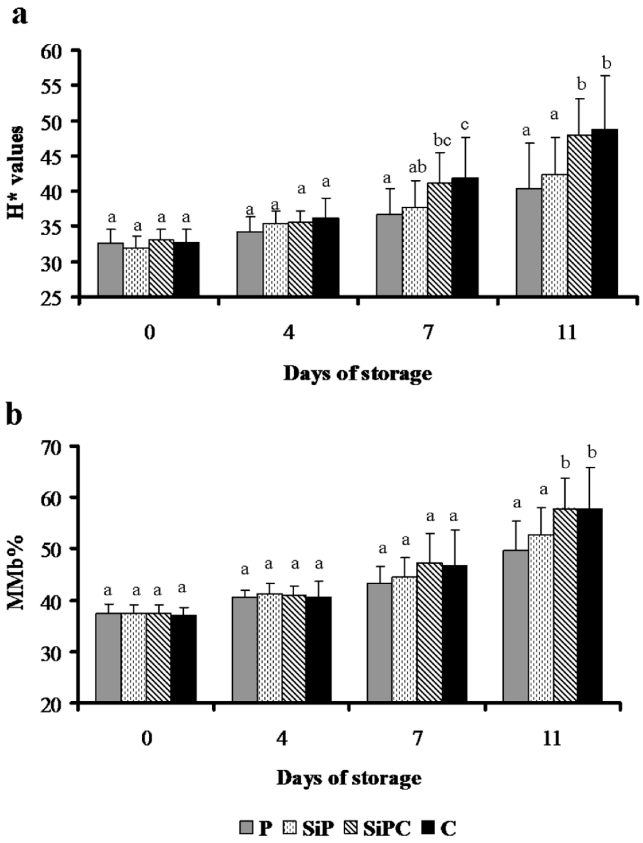

Fig. 2: Effect of dietary treatment and time of storage on hue angle (*H**) values (a) and on metmyoglobin (MMb) percentages (b) measured in minced beef stored in a high oxygen modified atmosphere for 11 days at 4°C.

IV. DISCUSSION

Pasture-based feeding systems have been frequently shown to improve meat oxidative stability compared to concentrate-based diets, due to the high concentration of antioxidants in green herbage [2]. Therefore, the decreasing concentration of vitamin E in muscle with increasing proportion of concentrates in the diet found here were not surprising.

The PUFA represent the substrates in which lipid oxidation is initiated and propagated and, generally, herbage-based diets increase the levels of PUFA in muscle compared to concentrate-based diets [3]. Therefore, the lower PUFA% found in LM from animals fed exclusively concentrates (C) compared to that from grass-fed animals (P, SiP and SiPC) were expected. Nevertheless, animals in the P and SiP

treatments had a higher ratio of the concentration of vitamin E to the concentration of PUFA compared to the SiPC and C treatments. This would suggest a higher oxidative stability of beef from animals in the P and SiP groups. However, no differences in TBARS values were found between meat from animals in the C, P and SiP groups, while the SiPC treatment impaired meat lipid stability.

Since the susceptibility of PUFA to oxidation increases with increasing degree of unsaturation, it may be of interest to focus on the concentration of highly unsaturated PUFA in muscle [6]. In the present study, a greater concentration of highly peroxidizable PUFA (HP-PUFA) was found in meat from animals fed grass (P, SiP and SiPC) compared to beef from heifers given exclusively concentrates (C). We hypothesize that a reduction in muscle antioxidants may have a detrimental effect on meat oxidative stability when HP-PUFA remain high (SiPC group). Conversely, the balance between pro- and antioxidants in muscle would be more favorable when the reduction in antioxidants is accompanied by a reduction in HP-PUFA (C group), or when the high concentration of HP-PUFA is counterbalanced by high levels of antioxidants (P and SiP groups).

Vitamin E is believed to exert an indirect positive effect on meat colour stability mediated by its direct activity against lipid oxidation [9]. However, the greater colour deterioration and myoglobin oxidation observed in beef from animals receiving concentrates (SiPC and C groups) compared to that from heifers in the P and SiP groups seems to be unrelated to muscle vitamin E, as no differences in TBARS were observed between P, SiP and C treatments. Some water-soluble antioxidants, such as polyphenols, may have contributed to enhance the colour stability of beef from grass-fed animals (P and SiP) without an involvement of vitamin E [10]. Moreover, modified atmosphere packaging may provide conditions in which the link between lipid and myoglobin oxidation is weakened [9]. In this study, the adoption of a high oxygen atmosphere could be another reason explaining the different trends observed for lipid oxidation and for colour stability parameters.

V. CONCLUSIONS

Our results confirmed the positive effect of grass-based feeding systems on meat colour stability compared to feeding strategies based exclusively on concentrates or including high supplementation of concentrates in grass-based diets. Moreover, the supplemetation with concentrates to herbage-based diets can impair meat shelf life if adequate amounts of antioxidants are not provided.

REFERENCES

1. Morrissey P A, Sheehy P J A, Galvin K, Kerry J P, Buckley D J (1998). Lipid stability in meat and meat products. Meat Sci 49:73-86.
2. Buckley, D J, Morrissey P A, Gray J I (1995). Influence of dietary vitamin E on the oxidative stability and quality of pig meat. J Anim Sci 73:3122–3130.
3. Wood J D, Enser M (1997). Factors influencing fatty acids in meat and the role of antioxidants in improving meat quality. Br J Nutr 78:49-60.
4. Röhrle F T, Moloney A P, Black A, Osorio M T, Sweeney T, Schmidt O, Monahan F J (2011). α-Tocopherol stereoisomers in beef as an indicator of vitamin E supplementation in cattle diets. Food Chem 124:935-940.
5. Noci, F, Monahan F J, French P, Moloney A P (2005). The fatty acid composition of muscle fat and subcutaneous adipose tissue of pasture-fed beef heifers: Influence of the duration of grazing. J Anim Sci 83:1167–1178.
6. Yang A, Lanari M C, Brewster M, Tume R K (2002). Lipid stability and meat colour of beef from pasture- and grain-fed cattle with or without vitamin E supplement. Meat Sci 60:41-50
7. Siu G M, Draper H H (1978). A survey of the malonaldehyde content of retail meats and fish. J Food Sci 43:1147–1149.
8. Krzywicki K (1979). Assessment of relative content of myoglobin, oxymyoglobin and metmyoglobin at the surface of beef. Meat Sci 3:1-10.
9. Faustman C, Sun Q, Mancini R,. Suman S P (2010). Myoglobin and lipid oxidation interactions: Mechanistic bases and control. Meat Sci. 86:86-94.
10. Luciano G, Vasta V, Monahan F J, López-Andrés P, Biondi L, Lanza M, Priolo A (2011). Antioxidant status, color stability and myoglobin resistance to oxidation of *longissimus dorsi* muscle from lambs fed a tannin-containing diet. Food Chem. 124:1036-1042.