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Influence of Feeding System on Lipids and Proteins Oxidation, and Antioxidant Enzymes Activities of Meat from Aberdeen Angus Steers

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Abstract Three feeding system were investigated to determine if any of them is more suitable to ensure a better antioxidant protection to meat. Animals were produced on pasture, pasture supplemented with corn grain, or feedlot. TBARS, protein carbonyls and antioxidant enzyme activities have been determined in fresh ad aged meat from *Biceps femoris* of Aberdeen Angus steers. No feeding system showed clear protection against lipids and protein oxidation in fresh meat. However, aged meat suffer always lipids and protein oxidation independently of the feeding system. There is not a clear pattern of the action of the catalase antioxidant enzyme (no significant main effect). However, pasture based feeding system present a lower SOD (0.96 UI) and GPx (9.22 nmoles/min/mg protein) activity, versus feedlot (1.12 UI and 11.48 nmoles/min/mg protein, respectively). Based on the results of the present investigation, it seems difficult to conclude about the best feeding system advised to minimize the lipids and protein oxidation of meat.

Keywords: *FAberdeen angus, meat, TBARS, protein carbonyls, catalase, SOD, GPx, pasture, feedlot*

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1. Introduction

Meat production implies a complex process, which consist mainly in the slaughtering of animal, the carcass cooling, cutting-up and the rapid chilling of the different meat cut for their preparation to be sold locally or exported. After that, depending of the cut and the destination market, the meat is aged during 14-60 days. The ageing process consisted in the maintaining of the different meat cuts in vacuum-packaged atmosphere and temperature between 1-2 ºC in the dark. The ageing is generally done as a way to improve the tenderness and most of the sensorial parameters to ensure the acceptability of meat by consumers [1]. During all these processes meat are sensitive to environmental factor like oxygen, light and all the handling conditions. This may result to oxidative damage of the meat that reduces the oxidative stability, quality and value of the product [2]. Particularly if it is destined for a supplementary industrial transformation process

The oxidation damage is the most important non-microbial factor that affects the conservation of meat and meat product. Oxidation induces modifications of muscle lipids and proteins and, therefore, affects the organoleptic and nutritional properties of meat and meat products [3]. Stressors arising from both internal and external sources initiate lipid oxidation in muscle foods. The most important stressors are the reactive oxygen species (ROS) including free radicals and peroxides. For example, during handling, processing and cold storage of fresh meat, released endogenous iron is partially responsible for the catalysis of lipid oxidation [4]. Protein oxidation is responsible for many biological modifications as protein fragmentation or aggregation and decrease in protein solubility affect the quality of meat and meat products that have to be forwarded to the restoration industry [5].

Fortunately, the physiology of animals presents endogenously an efficient biological system to protect tissue from the oxidative damage. The antioxidant enzymes including super oxide dismutase (SOD), catalase and glutathione peroxidase (GPX) constitute the primary mechanism for protecting cells from oxidative damage *in vivo* SOD scavenges superoxide anion by forming hydrogen peroxide and catalase safely decomposes hydrogen peroxide to water and O_2. GPx can decompose both hydrogen peroxide and lipoperoxides formed during lipid oxidation. These antioxidant enzymes are relatively stable in meat during refrigerated storage [6]. A second line of defence consisted in the presence of nutrients obtained from the food, the main of them are vitamins, particularly E, and antioxidant peptides. All of them cooperate with the antioxidant enzymes to minimize the damaging effect of oxidation.

In consequence, is of considerable importance to explore the parameters that modulate the action of the oxidation resulting in meat during its handling and conservation. One of the possible way is through the study of the effect of the different feeding system. According to this point, in the present work, the three feeding system used worldwide to produce meat, pasture, pasture and supplementation and feedlot, were compared about their effect on the lipids and protein oxidation of *Biceps femoris* muscle as biological model. Furthermore, the activities of the antioxidant enzymes including catalase, SOD and GPx, the first line of antioxidant defence, were determined.

2. Materials and Methods

2.1. Animal Diets, Samples and Chemicals

A group of 30 Aberdeen Angus steers (mean live weight 493 ± 28 kg) was divided up into three smaller groups. One for each feeding system: 1) *Pasture* group with animals produced on natural and improved pastures, 2) *Pasture + Supplement* group with animals fed natural and improved pasture and supplemented ad libitum with corn grain the last month before slaughtering. 3) *Feedlot* with animals fed a diet with roughage to concentrate ratio of 30:60 (on dry matter basis) for 90 days. Roughage consisted of whole plant sorghum silage and silo wet grain sorghum. The concentrate consisted by soybean hulls and wheat bran. Animals were slaughtered with 24-30 months of age and the *Biceps femoris* muscle was removed from each left half carcass. Each muscle (approximately 1000 g) was divided in two pieces, one was vacuum packaged, and aged for 14 days at 1-2 ºC and then frozen at -20 °C. The other piece was directly frozen at -20 °C, until further analysis. All chemicals used in the investigation are in analytical grade from Sigma chemicals Co (St Louis, USA). Ethyl acetate (Hplc grade), ethanol (Hplc grade), Butanol (Hplc grade) Trichloroacetic acid (analytical grade) and HCl (analytical grade) were from Merck Corporation (USA).

2.2. Determination of Lipid Oxidation

Samples (n=10) of 10 g frozen meat were homogenized in a Waring-Blender (Fisher Inc. USA) with 200 ml of an extraction buffer (0.15 M KCl, 0.02 M EDTA and 0.30 M BHT) at 12,000 rpm for 1 minute. Part of the homogenated was frozen (-20º C for carbonyl and protein content assays, and part was used for the TBARS (thiobarbituric acid reactive species) test. The TBARS procedure for the determination of lipid oxidation was done according to [6-7]. Briefly, the homogenated was centrifuged at 2000 g at 4 ºC for 10 minutes (Thermo Scientific Inc. USA), and 1 ml of the supernatant was incubated with 1 ml of a 2-thiobarbituric acid (TBA)-trichloroacetic acid (TCA) solution (35 mM TBA and 10% TCA in 125 mM HCl) in a boiling water bath (Fisher Inc. USA) for 30 min. After cooling in ice for 5 min and kept at room temperature for 45 min, the pink chromogen was extracted with 4 ml of n-butanol and phase separation done by centrifugation at 3000 g during 10 min (Sorvall ST16-R, USA). The absorbance of the supernatant was measured at 535 nm in a Genesys-6 spectrophotometer (Thermo Scientific Inc. USA). The concentration of malondialdehyde (MDA) was calculated using the molar extinction coefficient of the MDA (156,000 M-1 cm-1). Results were expressed as mg MDA/kg of fresh meat.

2.3. Determination of Protein Oxidation

The protein oxidation level was determined by the carbonyl protein assay [5]. The homogenate samples (n=10), frozen one day before, were thawed at room temperature. Two aliquots of 2 ml from each sample were put into two different tubes. These tubes were centrifuged at 2000 g for 10 min (Sorvall ST16-R, USA). One was incubated with 2 ml of 2 M HCl (blank) and the other one with 2 ml of 0.02 M dinitrophenylhydrazine (DNPH) in 2 M HCl, for one hour at room temperature with regular stirring. Then, 2 ml of 20 % TCA was added. After stirring, the mixture was left at room temperature for 15 min with regular stirring. The tubes were centrifuged at 2000 g for 10 min (Sorvall ST16-R, USA). The pellets were washed three times with 4 ml of ethanol:ethyl acetate (1:1), centrifuging each time, to eliminate traces of DNPH. The pellets were dissolved in 6 ml of 6 M guanidine HCl with 0.02 M KH_2PO_4 (pH 6.5). The tubes were incubated at room temperature for 15 min with regular stirring. Afterwards, they were centrifuged at 2400 g for 10 min. The absorbance of the supernatant was measured at 370 nm in a Genesys-6 spectrophotometer (Thermo Scientific Inc. USA) and the concentration of DNPH was calculated using the DNPH molar extinction coefficient (22,000 M-1 cm-1). Results were expressed as nmoles of DNPH/mg of protein. Protein content was determined at 280 nm in the extraction buffer using bovine serum albumin (BSA) as protein standard [8].

2.4. Determination of Antioxidant Enzyme Activities

A 18 g frozen sample (n=10) was homogenized in a Waring-Blender (Fisher Inc. USA) with 200 ml of an extraction buffer containing 0.15 M KCl and 0.79 M EDTA (pH 7.4) for 1 min at 12,000 rpm. The homogenate was centrifuged at 9000 g at 4 ºC for 10 min (Sorvall ST16-R, USA). The supernatant was used for the determination of catalase and SOD activities.

The activity of catalase was measured recording the H_2O_2 disappearance by the decrease in absorbance at 240 nm during 3.5 min using a Genesys-6 spectrophotometer (Thermo Scientific Inc. USA). The incubation mixture contained 2820 µl of the extraction buffer, 90 µl of the supernatant and 90 µl of H_2O_2 0.2 M. The activity was calculated using the molar extinction coefficient of H_2O_2 (39.4 M-1 cm-1) and results were expressed as µmoles of discomposed H_2O_2 min/g fresh meat and as nmoles of discomposed H_2O_2 min/mg protein [9].

Total SOD activity was determined by measuring the inhibition of pyrogallol autoxidation [6,7,8,9,10]. The incubation mixture contained 2850 µl of 50 mM phosphate buffer (pH 8.2), 75 µl of the supernatant and 75 µl of 10 mM pyrogallol. The increase in absorbance at 340 nm was recorded during 2 min in a Genesys-6 spectrophotometer (Thermo Scientific Inc. USA). One unit (U) was taken as the activity that inhibits the reaction by 50%, and results were expressed as U and U/g fresh meat.

For the determination of GPx activity, 5 g meat sample (n=10) were homogenized with an Ultra Turrax T18 (IKA-Werke, Germany) with 25 ml of 50 mM KH_2PO_4 buffer and 0.5 mM EDTA (pH 7.0) for 1 min at 18,000 rpm. The homogenate was centrifuged at 2000 g for 2 min at 4 °C and then the supernatant was filtered. The assay mixture contained 50 mM KH_2PO_4 buffer, 0.5 mM EDTA, 1 mM reduced glutathione (Sigma G4251), 0.15 mM NADPH (Sigma N1630), 1.5 U glutathione reductase (Sigma G3664), 0.15 mM H_2O_2 and 1 mM NaN3 (Sigma S-2002). The incubation mixture contained 1980 µl of the assay mixture and 20 µl of the filtered sample. The activity of GPx was measured at 22 °C recording the oxidation of NADPH by the decrease in absorbance of the incubation mixture at 340 nm during 3 min using a Genesys-6 spectrophotometer (Thermo Inc.). An extinction coefficient of 6300 M^{-1} cm^{-1} was used to calculate NADPH concentration [11-12]. The GPx activity was expressed as µmoles of oxidized NADPH/min/g of fresh meat and as nmoles of oxidized NADPH/min/mg protein.

2.5. Statistical Analysis

The data of TBARS, lipid content, carbonyl content, catalase, SOD and GPx activities, are reported as mean ± standard error of the media for fresh and aged meat in the three feeding systems. To evaluate feeding system and ageing effects, an analysis of variance using the GLM procedure was followed and *post hoc* Tukey-Kramer test. The three systems were also compared using a one-way ANOVA followed by the Tukey-Kramer multiple comparison test, for fresh or for aged meat. Fresh and aged meat results were compared using the *t*-Student test. The level of significance was established at $P < 0.05$, and the software used was the NCSS, 2007 (NCSS, 329 North 1000 East, Kaysville, UT 84037).

3. Results and Discussion

3.1. Lipid Oxidation

No feeding system main effect was found for lipids oxidation when fresh meat is considered. This is not in according with two reports one by using the *Longissimus dorsi* muscle from Charolais steers (less than 36 months) finished exclusively with grass or fed mixed food consisting in maize silage and hay [13]. The other used *Psoas major* muscle from crossbreed animals [4] fed exclusively pasture or on feedlot system (fed grain and hay). The two reports conclude that feeding animals fed pasture have better lipids stability when compared to other ones supplemented with grain. This discrepancy between the two report and the present investigation could be due to the difference in the breed, Aberdeen Angus here versus Charolais and crossbreed in the first report and the second one, respectively. Also the observed difference could be accounted for the muscle difference, *Longissimus muscle* and *Psoas major* versus *Biceps femoris* in the present investigation. However, there is not sufficient data in the literature to conclude about this point.

However, in a recent report using the *Longissimus thorasis* muscle of Aberdeen Angus steers, fed pasture and supplemented with flaxseed, the results showed that the level of TBARS is not different between control no supplemented and supplemented animals [14]. This last report is in accord with the observation of the present investigation. Furthermore, when the total intramuscular lipids content (the main target of the oxidation process) was considered, it is interesting to note that the Feedlot group have slightly but significantly more intramuscular lipids in comparison to the other two group (Table 1). In one report [4], the intramuscular lipids, of the feedlot group (grain and hays) showed a much more difference with the pasture group, 4.7 % and 2.7 % for feedlot and pasture, respectively. This difference in intramuscular lipids content between the two studies perhaps could explain the higher level in TBARS in the feedlot group presented in the investigation [4]. Unfortunately, no data about the intramuscular lipids are available to compare in the other two reports [13,14].

For ageing process, to the contrary, there is a significant main effect of ageing process ($P<0.01$) on the lipids oxidation. Indeed, aged meat suffered a significant lipids oxidation, in spite of the vacuum packaging and the chilling expected to minimize the oxidation. This is much more evident for the system feeding with pasture and supplement ($P<0.05$) in comparison to the other two systems (Figure 1). May be this higher lipid oxidation could be explained by the use of corn grain in the supplemented group. Corn grain is rich with polyunsaturated fatty acids, a target of oxidation. Nevertheless, the lipids content of meat from this feeding system showed a lower level (Table 1). A similar conclusion has been proposed in the investigation that which used corn grain (with and without flaxseed) to supplement the animals in their investigation [15].

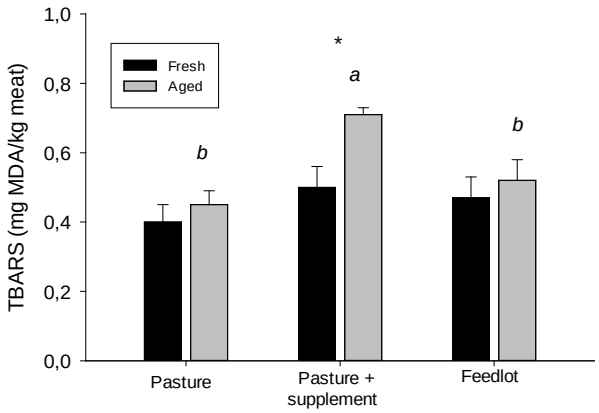

Figure 1. Lipid oxidation (TBARS, mg MDA/kg of meat) in fresh and aged *Biceps femoris* muscle in steers from pasture, pasture and supplement and feedlot based feeding systems. Data are mean ± SEM (n=10). Different lower letters means statistical significance among systems for aged meat ($P<0.05$). *shows statistical difference between fresh and aged meat ($P<0.05$). Main effects: Feeding system: No Significant Ageing: ($P<0.01$) Aged > Fresh

Table 1. Lipids content (%) in *Biceps femoris* muscle of Aberdeen Angus steers from pasture, pasture and supplement and feedlot based feeding systems

Feeding system	Lipids (%)
Pasture	1.51 ± 0.15 b
Pasture + supplement	1.13 ± 0.09 b
Feedlot	1.80 ± 0.18 a
Signification	$P<0.01$

Data are mean ±SEM. Different lower letters means significant differences among feeding systems by ANOVA and Tukey test ($P<0.05$).

There is limited information about the effect of ageing in meat quality in the scientific literature. Since the ageing process is a valuable process to improve meat quality, like tenderness, more investigation about this point is needed to resolve the particular effect of breed, types of muscles and the influence of the different diet.

3.2. Protein Oxidation

The effect of the different feeding system on the protein oxidation, as measured by carbonyl groups, was illustrated in Figure 2. There is a significant main effect of the feeding system. The fresh meat from pasture system showed a more protein oxidation effect when compared to the other two systems. This is particularly true when pasture system is compared to the feedlot system (Figure 2). Even though it is accepted that the protein oxidation is linked to lipid oxidation in beef meat, it does not have to be a similar pattern [5]. Is the case in the present experiment.

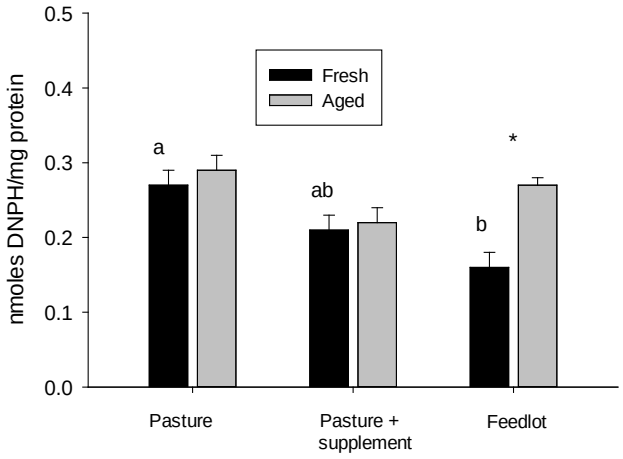

Figure 2. Carbonyls protein (nmoles DNPH/mg protein) in fresh and aged *Biceps femoris* muscle in steers from pasture, pasture and supplement, and feedlot feeding systems. Data are mean ± SEM (n=10). Different lower letters means statistical significance among feeding systems for fresh meat ($P<0.05$). *shows significant difference between fresh and aged meat ($P<0.05$). Main effects: Feeding systems ($P<0.01$). Pasture > Pasture + supplement, Feedlot. Ageing: ($P<0.01$) Aged > Fresh

Furthermore, the pasture system showed a higher effect on the protein oxidation (P<0.05) when compared to the feedlot system. This is an unexpected result, because generally it is observed that the animals fed pasture present no difference or a lower effect when compared with animals fed grain in a feedlot system [5]. We have to note again some differences between the investigations of Mercier [5] with the present study. The muscles are different, *Longissimus dorsi* versus *Biceps femoris*, and the breed and the sex too, Charolais cows versus Aberdeen Angus steers. The relation between protein oxidation and meat quality received little attention from meat scientist, thus more investigation is needed.

3.3. Antioxidant Enzyme Activities

3.3.1. Catalase

Catalase activity did not shows any effect of fed system nor ageing process as illustrated in Figure 3 and Figure 4. The same result is obtained when the enzyme activity is expressed by g of fresh meat and by mg of total protein. The expression of the enzymes activity by g of fresh meat and by mg of protein contained in the extraction media, exclude any biased observation due to the extraction methods. Interestingly, the no effect of feeding system observed here is in agreement with three different studies in steers [5,6], and [16].

Apparently, the activity of catalase as antioxidant enzymes seems to be associated with older animals fed n-3 rich sources [6]. Pasture is generally considered as rich n-3 sources, then there is no easy to conclude here about the no apparent effect of catalase when the three feeding system are compared.

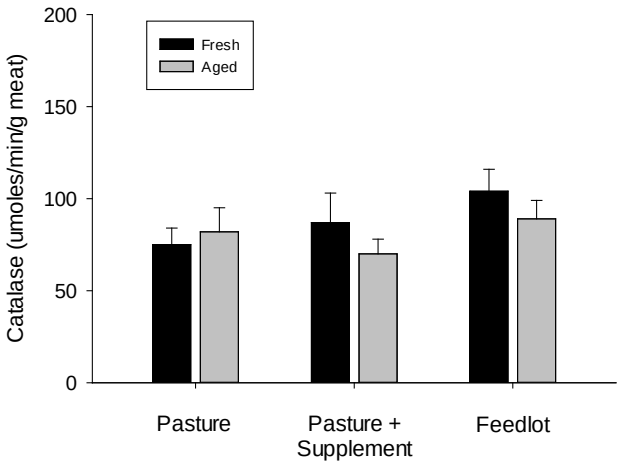

Figure 3. Catalase activity (µmoles/min/g meat) in fresh and aged *Biceps femoris* muscle of steers from pasture, pasture and supplement and feedlot based feeding systems. Data are mean ± SEM (n=10). Main effects: Feeding systems: No Significant Ageing: No Significant

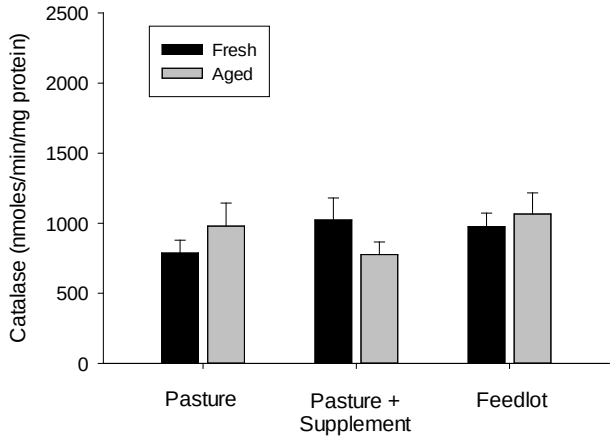

Figure 4. Catalase activity (nmoles/min/mg protein) in fresh and aged *Biceps femoris* muscle of steers from pasture, pasture and supplement, and feedlot based feeding systems. Main effects: Feeding system: No Significant. Ageing: No Significant

3.3.2. Superoxide Dismutase (SOD)

SOD is an enzyme which present an activity coupled with the catalase. At least to the biochemical and functional view point. However, that association is not observed in the present investigation, since the response of SOD and catalase are very different within the same experimental design. There are a significant main effect for the feeding system for SOD in fresh meat (Figure 5 and Figure 6). Indeed, pasture feeding system seems to have a lower activity in SOD when compared to the other

two feeding systems. However, two reports, [6] and [15], showed a higher level of SOD in animals fed pasture compared to animals in feedlot system. This difference could be explained by the fact that in one report analyzed the *Psoas major* muscle [6], a much more oxidative muscle than the *Biceps femoris* used in the present experiment.

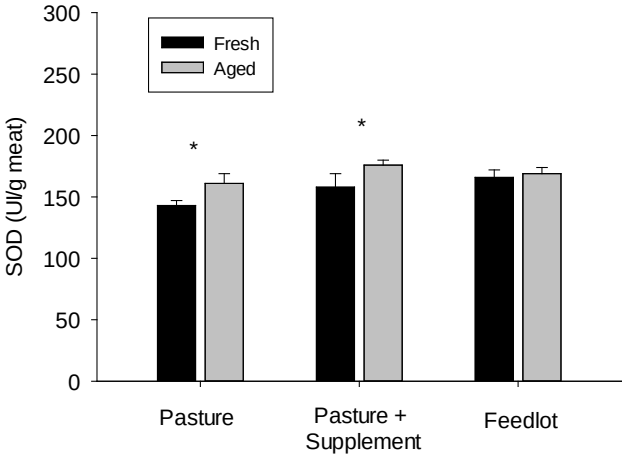

Figure 5. SOD activity (UI/g meat) in fresh and aged *Biceps femoris* muscle of steers from pasture, pasture and supplement and feedlot based feeding systems. Data are mean ± SEM (n=8). * shows significant differences between fresh and aged meat (P<0.05). Main effects: Feeding system: P<0.05 Pasture < Pasture + Supplement, feedlot. Ageing: P<0.01 Fresh < Aged

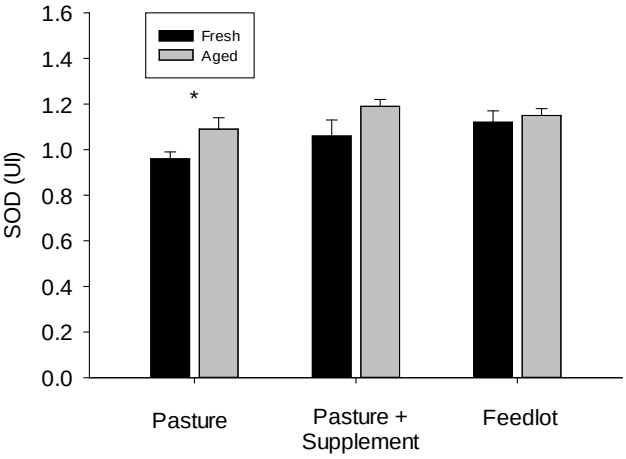

Figure 6. SOD activity (UI) in fresh and aged *Biceps femoris* muscle of steers from pasture, pasture and supplement and feedlot based feeding systems. Data are mean ± SEM (n=8). * shows significant differences between fresh and aged meat (P<0.05). Main effects: Feeding system: P<0.05 Pasture < Pasture + Supplement, Feedlot Ageing: P<0.01 Fresh<Aged

It has been observed that the SOD activity is higher in oxidative muscle than in glycolytic ones [6,16]. Unfortunately, this explaination have to be relativized, because the muscle used in another investigation [15], the muscle considered was *Longissimus dorsi*, similar to the *Biceps femoris* for its oxidability.

Also, it has been proposed that the minerals can modulate the SOD activity, since Cu and Zn are cofactor for SOD. More copper and zinc in food implies more SOD activity [6]. However, in a report from our investigation team using Hereford breed [17], the level of zinc is much more elevated in *Longissimus dorsi* than in *Psoas major* and results from the same investigation showed that SOD activity is similar [18]. Then, the association between SOD, oxidative muscle and copper and zinc, is not so evident to explain the activity of SOD in meat.

When the ageing process is considered for SOD activity, there is a significant main effect of ageing (Figure 5 and Figure 6) showing that aged meat present a higher activity (P<0.01) of SOD when compared to the fresh meat. For instance, there is no explanation for this observation.

3.3.3. Glutathion Peroxidase (GPx)

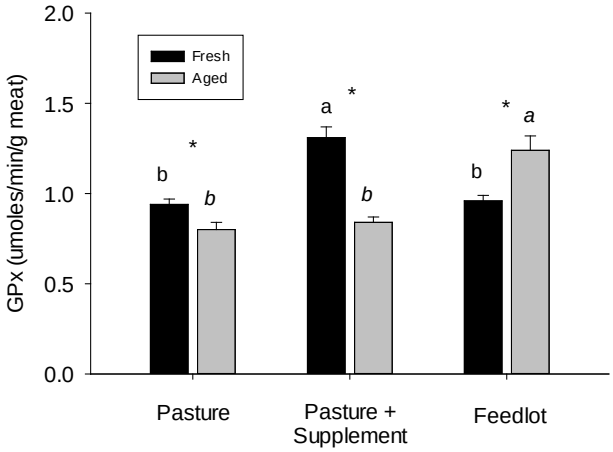

Figure 7. GPx activity (µmoles/min/g meat) in fresh and aged *Biceps femoris* muscle in steers from pasture, pasture and supplement and feedlot based feeding systems. Data are mean ± SEM (n=8). Different lower letters means significant differences among systems for fresh muscle and for aged muscles (italic) (P<0.05). *shows significant differences between fresh and aged (p>0.05). Main effects: Feeding systems: P<0.001 Pasture<pasture + supplement, feedlot Ageing: P<0.01 Fresh>Aged

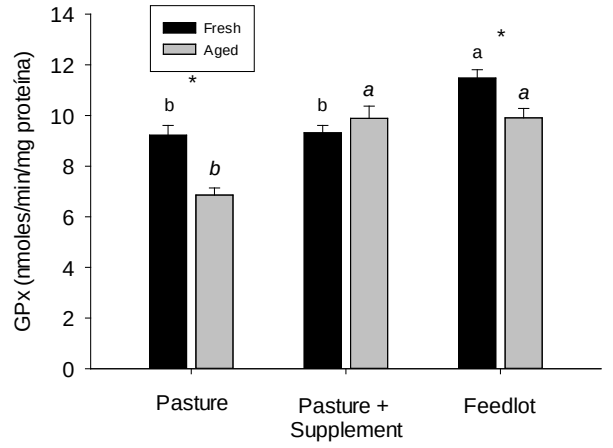

Figure 8. GPx activity (nmoles/min/mg protein) in fresh and aged *Biceps femoris* muscle of steers from pasture, pasture and supplement and feedlot based feeding systems. Data are mean ± SEM (n=8). Lower letters means significant differences among feeding systems for fresh and aged (italics), (P<0.05). * shows significatn differences between fresh and aged meat (P<0.05). Main effects: Feeding system: P<0.001 Pasture < Pasture and supplement < Feedlot. Ageing: P<0.001 Fresh > Aged

The activity of GPx showed a significant feeding system effect. The meats from animals feed pasture have showed a lower level of enzymes in comparison to the other feeding systems (Figure 7 and Figure 8). However, pasture and supplement, and feedlot, are similar when the activity is expressed by gram of fresh meat. When the activity of GPx is expressed by mg of protein, the feedlot system showed significantly more GPx than the other two feeding system. The decrease of level of GPx meat of

animals fed pasture compared to animals finished in feedlot system is in accord to the results reported in three investigations [5,6,15]. One of them concluded that the difference between pasture and feedlot system could be associated with the fact that grain and concentrate used in the feedlot system are richer in selenium in comparison with pasture [6]. This is linked to the geographical difference of the selenium content in plant. This explanation could be acceptable for animals produced in Europe, but no for those produced in South America because pasture is much more richer in selenium [19]. The relation between GPx activity and the meat oxidation and quality reported here and the scientific literature is very complex and need more future investigation.

4. Conclusion

There is no evidence which help to advice producer about a specific feeding system that protect efficiently lipids and protein from oxidation in fresh meat coming from *Biceps femoris* muscle of Aberdeen Angus steers. In addition, there is not a clear pattern of the action of the enzymes, catalase, SOD and GPx, that counteract the lipids and protein oxidation of meat studied here. It seems difficult to conclude about the best feeding system suitable to minimize lipids and protein oxidation of meat from *Biceps femoris.* More investigation is necessary using different muscles to compare and understand the biochemical relation behind the lipids and protein oxidation of meat, and the specific action of the antioxidant enzymes present in fresh and aged meat like catalase, SOD and GPx.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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