American Journal of Food and Nutrition, 2015, Vol. 3, No. 5, 118-124
Available online at http://pubs.sciepub.com/ajfn/3/5/2

DOI:10.12691/ajfn-3-5-2

Catalase, SOD and GPx Activities in *Triceps brachii* Muscle from Aberdeen Angus Steers Finished on Pasture, Pasture and Concentrate, or Concentrate

A. Terevinto[1], M.C. Cabrera[1,2], A. Saadoun[2,*]

[1]Dpto Producción Animal & Pasturas, Laboratorio Calidad de Alimentos, Facultad de Agronomía, Universidad de la República. Garzón 809. Montevideo. Uruguay
[2]Fisiología & Nutrición, Facultad de Ciencias, Universidad de la República. Calle Igua 4225. Montevideo. Uruguay
*Corresponding author: asaadoun@fcien.edu.uy

Abstract Three feeding system were investigated to determine if any of them is more suitable to ensure a better antioxidant protection to meat from *Triceps brachii* muscle of Aberdeen Angus steers. They were finished on pasture, pasture + concentrate with corn grain, or concentrate alone. TBARS, protein carbonyls and antioxidant enzymes activities have been determined in fresh and aged meat (14 days in vacuum, 1-2°C). The level of TBARS ranged between 0.59-0.64 mg MDA/kg meat, whereas levels of protein carbonyl ranged between 0.18-0.22 mmoles DNPH/mg protein. The activities of catalase and superoxide dismutase are higher in fresh meat of animals fed pasture, compared to those finished on pasture and concentrate, or on concentrate alone. Glutathione peroxidase (GPx) showed higher activities in meat of animals finished on concentrate in comparison those finished on pasture and concentrate, or pasture. However, the results of the investigation do not allow the advising, for now, for the most suitable feeding system for finishing steers that help producers to preserve meat from lipids and protein oxidation. More investigations are necessary to highlight the feeding conditions, which could influence the activities of antioxidant enzyme catalase, SOD and GPx in meat.

Keywords: *pasture, concentrate, tbars, carbonyls, catalase, SOD, GPx, aberdeen angus meat*

Cite This Article: A. Terevinto, M.C. Cabrera, and A. Saadoun, "Catalase, SOD and GPx Activities in *Triceps brachii* Muscle from Aberdeen Angus Steers Finished on Pasture, Pasture and Concentrate, or Concentrate." *American Journal of Food and Nutrition*, vol. 3, no. 5 (2015): 118-124. doi: 10.12691/ajfn-3-5-2.

1. Introduction

Meat is a valuable food that contains important nutrients that are necessary to maintain health and well-being in human. Meat contains high quality proteins, fatty acids, vitamins and minerals, like heme iron, zinc, selenium and copper. However, meat is perishable product that can be altered by internal and external factors like storage temperature, oxygen, and light. Oxidation induces modifications of muscle lipids and proteins and, therefore, affects the organoleptic and nutritional properties of meat and meat products [1]. Oxidation cause lower industrial and nutritional values of meat and economical lost for producers. In addition, consumers generally reject oxidized meat and meat products [1,2]. Oxidant stressors arise from both internal and external sources and initiate lipid oxidation in muscle foods. The most important stressors are the reactive oxygen species (ROS) including free radicals and peroxides [1,2]. However, there are different ways to preserve meat from oxidation. One way is the consumption by the animals of antioxidant components present naturally in food like vitamins E and C, phenolic substances, antioxidant pigments, etc. All these natural antioxidants act as chain breaking in radical production and are considered as a line of protection against radical attack [2]. Another way is that, physiologically, the organism builds different adaptive strategies to protect its own tissues from the radical attack and oxidation. This last way are represented by an interlinked, complex and very efficient enzymatic system composed by the catalase, superoxide dismutase (SOD) and glutathione peroxidase (GPx) [2]. That antioxidant enzymatic group constitutes the primary mechanism for protecting cells from oxidative damage in vivo [2]. 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 [2].

Furthermore, current meat production operation implies often the ageing process as a way to improve meat quality. The ageing process consisted in the storage of meat vacuum-packaged at temperature between 1-2°C in the dark, for 14-60 days. The ageing duration depends of the market conditions, local or international. The ageing is conducted as a way to improve the tenderness of meat. In addition [3], most of the sensorial parameters also improved by ageing, help to ensure the acceptability of meat by consumers. However, ageing is another oxidant factor for meat.

The oxidative stability of meat depends upon balance between oxidant (ROS) and antioxidant from food (vitamins) and endogenous sources (enzymes). Food offered to the animals could be a good way to improve the antioxidant defense of the animal tissues that will be transferred to meat after slaughtering [4]. Then, the proposed investigation consisted in comparing the lipids and proteins oxidation and the activities of catalase, SOD and GPx in meat from animals fed one of the three feeding systems worldwide used to produce beef meat: pasture alone, pasture and supplementation with concentrate and concentrate alone.

2. Materials and Methods

2.1. Animal Diets, Samples and Chemicals

Aberdeen Angus steers with a live weight of 479 ± 28 kg (n=30) were fed on pasture. Ten of them were randomly assigned to remain on this diet (Pasture), other ten were assigned to remain on pasture and received *ad libitum* supplementation with concentrate (Pasture + Concentrate). The last group of ten were finished on feedlot system, without access to pasture, and fed concentrate alone. The animals received the corresponding diet (Pasture or Pasture + Concentrate or Concentrate) the last 110 days until slaughter. Pasture consisted in tall fescue (*Festuca arundinacea*), white clover (*Trifolium repens*) and birdsfoot trefoil (*Lotus subbiflorus*). Concentrate was composed by roughage of whole plant sorghum silage and wet grain sorghum, soybean hulls and wheat bran, minerals and urea. The animals were slaughtered with 24-30 months of age in an authorized abattoir following rules by Ministry of Agriculture of Uruguay (MGAP). After 36 hours of carcasses chilling, *Triceps brachii* muscle (TB) was removed from each carcass. Each muscle (approximately 1000 g) was divided in two pieces, one was vacuum packaged, aged during 14 days at 1-2°C and then frozen at -20°C, and the other 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 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 homogenate was frozen overnight at -20 ºC to be used for carbonyl and protein content assays, and part was used the same day for the TBARS (thiobarbituric acid reactive species) test. The TBARS procedure for the determination of lipid oxidation was followed according to [5 and 2]. Briefly, the homogenate was centrifuged at 2000 g at 4°C for 10 minutes (Sorvall ST16-R, 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 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 by centrifugation at 3000 g during 10 min. The absorbance of the supernatant was measured at 535 nm in a Genesys-6 spectrophotometer (Thermo inc.). 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 according to [6]. The homogenate samples, frozen the 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 (Sorvall ST16-R, USA). The absorbance of the supernatant was measured at 370 nm in a Genesys-6 spectrophotometer (Thermo inc.) 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 [7].

2.4. Determination of Antioxidant Enzyme Activities

A 18 g frozen sample 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 9000g at 4°C for 10 min (Sorvall ST16-R, USA). The supernatant was used for the determination of catalase and SOD activities. The expression of the enzymes activities were presented by g of fresh meat and by mg of protein contained in the extraction media. This exclude any biased observation due to the extraction methods.

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 Inc.), following the method described by [8]. 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.

Total SOD activity was determined as proposed by [9], with the modifications of [2], by measuring the inhibition of pyrogallol autoxidation. 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 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 were homogenized with an Ultra Turrax T18 (IKA Co, Germany) with 25 ml of 50 mM KH_2PO_4 buffer and 0.5mM EDTA (pH 7.0) for 1 min at 18,000 rpm. The homogenate was centrifuged at 2000 g for 2 min at 4°C (Sorvall ST16-R, USA). 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 NaN_3 (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. USA) [10,11]. An extinction coefficient of 6300 M^{-1} cm^{-1} was used to calculate NADPH concentration. The GPx activity was expressed as µmoles of oxidized NADPH/min/g of fresh meat and as nmoles of oxidized NADPH/min/mg protein. As for Catalase and SOD, the expression of the GPx activity was presented by g of fresh meat and by mg of protein contained in the extraction media. This exclude any biased observation due to the extraction methods.

2.5. Statistical Analysis

The effect of feeding system and ageing on TBARS and carbonyl content on catalase, SOD and GPx activities were analyzed by ANOVA using the General Linear Model (GLM) procedure (NCSS software release 2006, 329 North 1000 East, Kaysville, UT 84037) for a fixed effect model with three feeding systems and two processes. Only when significant feeding system x process interaction was noted for each parameter studied, ANOVA one- way or T-test were used to determine differences between feeding systems within the same processing and between processing (fresh and aged meat) for each feeding system. When treatment effects were significant ($P < 0.05$) means were compared with Tukey-Kramer test (NCSS software release 2006, 329 North 1000 East, Kaysville, UT 84037).

3. Results and Discussion

3.1. Lipid Oxidation

There is no a main effect of feeding system ($P>0.05$) on lipids oxidation (TBARS) of fresh meat from TB (Figure 1). However, when only fresh meat is considered, feeding pasture and concentrate seems to cause more lipids oxidation ($P<0.05$) than feeding concentrate (Figure 1). This observation is in accord with a previous observation from our laboratory, using the *Biceps femoris* muscle from steers of the same breed (unpublished data). Both results are, however, not in accord to other two investigations. In a first study, using *Longissimus dorsi* muscle from Charolais breed steers, the trial showed that feeding with concentrate caused more lipids oxidation in fresh meat [12]. The same conclusion was reached in the second investigation, using *Psoas major* muscle, from crossbreed animals fed exclusively pasture or concentrate [13]. However, this same last group, reported recently [14], using *Longissimus dorsi* muscle, that they do not detected different lipids oxidation in meat from Aberdeen Angus steers, the same breed used in the present investigation.

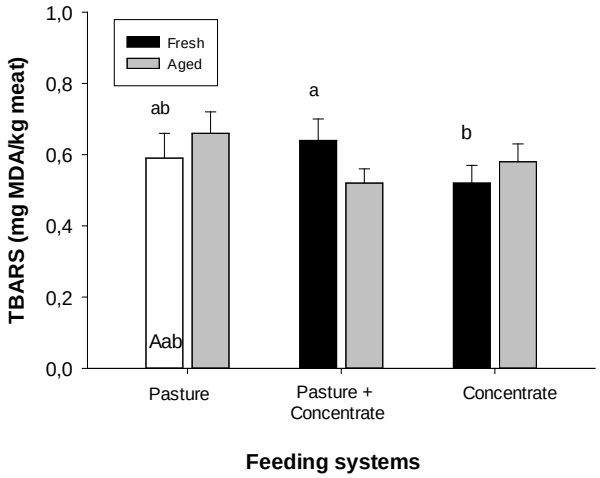

Figure 1. Lipid oxidation (TBARS, mg MDA/kg meat) in fresh and aged *Tricep brachii* muscle of steers from pasture, pasture + concentrate and concentrate based feeding systems. Data are mean ±SEM (n=10). Different lowercase letters means significant differences among feeding systems in fresh meat ($P<0.05$). Main effects: Feeding systems No significant. Ageing No significant

The breed of animals and the type of muscles used in each investigation could explain the different results reported between the described investigations and our own work. For example, muscles are not identical, and show individual characteristics in their biochemical parameters, like their different oxidative and glycolytic metabolism [15]. As well as in their nutritional composition [16,17]. More comparative studies should be conducted to highlight the importance of the feeding system on the oxidative stability of meat and meat products.

When ageing process is considered, there is no a main effect of the feeding system on the lipids oxidation when meat is submitted to 14 days of ageing that include vacuum packaging and chilling at 1-2°C (Figure 1). However, in a previous work from our laboratory using *Biceps femoris* muscle (unpublished data), the ageing caused more lipids oxidation, in spite of the vacuum packaging and the chilling. This difference between our experiments with different muscles reinforces our hypothesis that in oxidation studies, the type of muscle used in the investigation could determine the kind of responses.

Interesting data attracted our attention in the present experiment. In Table 1 the total lipids content of TB was presented, and the steers fed concentrate showed a level twice compared to animals fed pasture and almost twice compared to the animals fed pasture and concentrate. This observation is in accord with other report not only for beef meat [13], but also for lamb [18]. Thus, it seems that a higher level of lipids in TB muscle is not necessarily associated with a higher lipids oxidation status. This point should be considered in future investigation.

Table 1. Lipids content (%) in *Triceps brachii* muscle of Aberdeen Angus steers from pasture, pasture and concentrate or concentrate based feeding systems

Feeding system	Lipids(%)
Pasture	3.25 ± 0.29 b
Pasture + Concentrate	4.13 ± 0.58 b
Concentrate	7.29 ± 1.28 a
Signification	P<0.01

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

3.2. Protein Oxidation

To the contrary to the results for lipids oxidation, protein oxidation showed a significant main effect (P<0.03) of the feeding system. The meat from animals fed pasture presented a lower protein oxidation compared to both, animals fed pasture and concentrate, and animals fed concentrate (Figure 2). After ageing, there is a significant main effect (P<0.01) showing that the aged meat have a higher protein oxidation when compared to the unaged meat (Figure 2). Like for lipids, protein oxidation is associated with the radical attack of meat in presence of oxygen through a chain reaction [19]. That reaction could occurs with reduced forms of transition metals like iron and copper present in meat [20]. The oxidation of protein of muscle result of a denaturation and proteolysis-induces changes in meat quality, which include colour, aroma, flavour, water-holding capacity. Protein oxidation also induces multiple physico-chemical changes and nutritional value in meat proteins including a decrease in the bioavailability of amino acid and impaired digestibility of protein [19]. Our results presented differences with the investigations of Mercier [6], when fresh meat is considered. However, in that experiment, the muscles, the breed and the sex were different, *Longissimus dorsi* versus *Triceps brachii*, Charolais cows versus Aberdeen Angus steers, respectively Limited information on that point is available in the scientific literature. The relation between protein oxidation and meat quality received little attention from meat scientist, thus more investigation are needed.

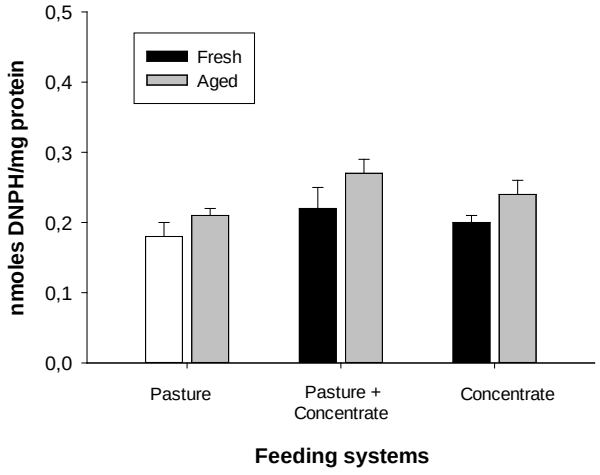

Figure 2. Carbonyl proteins (nmoles DNPH/mg protein) in fresh and aged *Triceps brachii* muscles of steers from pasture, pasture + concentrate, and concentrate. Data are mean ±SEM (n=10). Main effects: Feeding systems P<0.03 Pasture < Pasture + concentrate, concentrate; Ageing P<0.01 Aged > Fresh

3.3. Antioxidant Enzyme Activities

3.3.1. Catalase

There is a significant main effect (P<0.05) of catalase, when activity is expressed by g of meat. Indeed, meat from animals fed pasture presented more activity than animals fed pasture and concentrate or concentrate alone (Figure 3). However, this significant effect turn not significant when the activity of the enzyme was expressed by mg of protein. This opposed results make difficult to conclude about the effect of feeding system on the activity of catalase. In previous investigation showed no differences between animals fed pasture and other one fed concentrate using *Longissimus dorsi* [2], and *Psoas major* [21].

In both experiments, the expression of catalase activity was by mg of protein. Furthermore, in a previous experiment for our laboratory, the catalase activity of *Biceps femori* muscle from Aberdeen Angus steers (unpublished data) showed no main effect of the feeding system similar to the present experiment. In that experiment, the activity of catalase was also expressed by mg of protein. Together, the four experiments seems to shows that catalase probably did not respond differently to feeding systems when pasture and concentrate were compared.

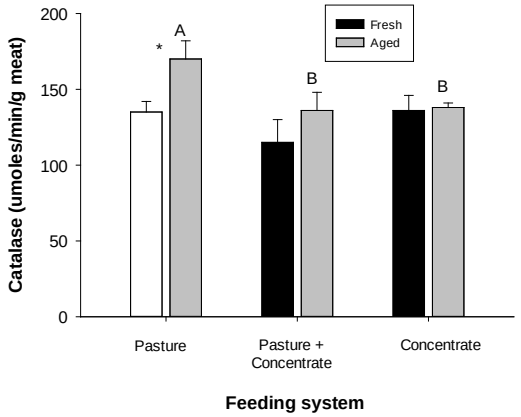

Figure 3. Catalase activity (µmoles/min/g carne) in fresh and aged *Triceps brachii* muscle of steers from pasture, pasture + concentrate and concentrate. Data are mean ±SEM (n=10). Different uppercase letters means significant differences among feeding systems in aged meat (P<0.05). *shows difference between fresh and aged meat (P<0.05). Main effects: Feeding system P<0.05 Pasture > Pasture + concentrate and Concentrate. Ageing P<0.05 Aged > Fresh

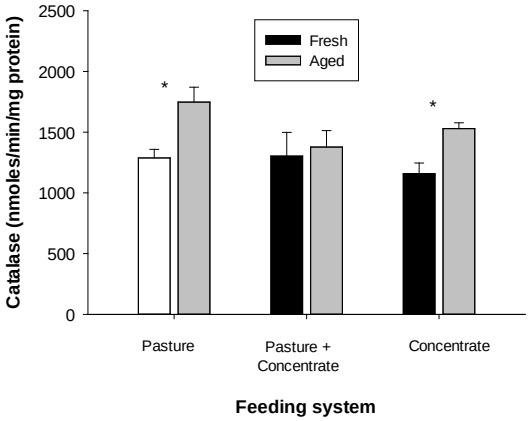

Figure 4. Catalase activity (nmoles/min/mg protein) in fresh and aged *Triceps brachii* muscle of steers from pasture, pasture + concentrate and concentrate. Data are mean ± SEM (n=10). *shows difference between fresh and aged meat (P<0.05). Main effects: Feeding system No significant; Ageing P<0.01 Aged >Fresh

Independently of the unit of expression of the activity of the enzyme, ageing caused a higher activity of catalase (Figure 3 and Figure 4).

Activities of catalase, as antioxidant enzyme in vacuum packaging meat is poorly documented and this make difficult to conclude about the results obtained here. There is not an explanation about the higher level of catalase after ageing. More investigations are required to understand this point.

3.3.2. Superoxide Dismutase

A significant main feeding system effect was found in SOD activity (P<0.05), meat from pasture fed animals showed a higher SOD activity independently of the unit of expression of the activity (Figure 5 and Figure 6). These activities are in accord to the results reported in early studies in fresh meat from *Longissimus dorsi* and *Psoas major* muscles [2,6,21]. SOD and catalase are coupled as antioxidant enzymes. Then, it would be expected that the two enzymes cooperate to fight against oxidant factor and could be influenced similarly by the feeding system.

However, in the present experiment and previously published reports, the two enzymes seem to have different responses to feeding system, [2,21]. The activity of SOD can be affected by the content of copper and zinc in meat [2]. In a previous investigation from our laboratory, it has been found that meat from Aberdeen Angus steers fed pasture contain more Zinc than ones fed concentrate. This observation could explain the higher activity of SOD in animals fed pasture in comparison to other fed concentrate [22]. Other naturally occurring antioxidant, like vitamins present in pasture, can complete the enzymatic action [2].

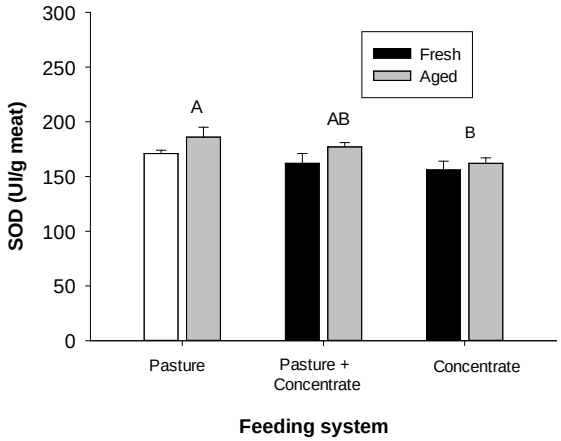

Figure 5. SOD activity (UI/g meat) in fresh and aged *Triceps brachii* muscle of steers from pasture, pasture + concentrate and concentrate. Data are mean ± SEM (n=10). Different uppercase letters means significant differences among systems in aged meat (p<0.05). NS: no significant. Main effects: Feeding system: P<0.05 Pasture > Concentrate, Pasture + concentrate = Pasture, Concentrate; Ageing P < 0.05 Aged > Fresh

After ageing, SOD showed a main effect of ageing (P<0.05) independently to the expression of enzymatic activity (Figure 5 and Figure 6). These responses could be explained by the responses to the ROS attack occurring in meat during refrigerated ageing in vacuum. There are very few reports, which studied the effect of ageing on the activity of antioxidant enzymes in meat. Antioxidant enzymes continue to be active in meat, even after various days post mortem. Enzymes and antioxidant nutrients (mainly vitamins and peptides) continue to fight against ROS various days after dead of animals [2]. This effect, more pronounced in meat produced on pasture, help to extend the shelf life of the meat during commercial display. This point need to be considered in future investigation.

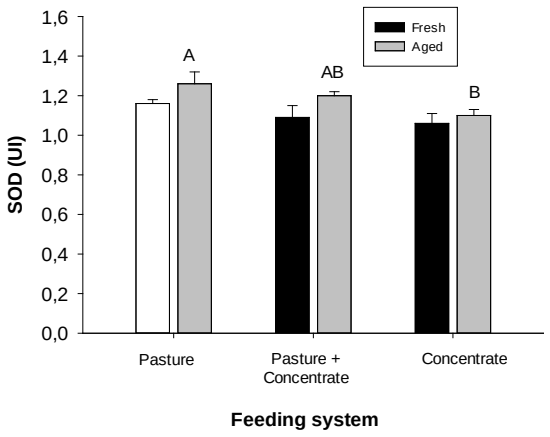

Figure 6. SOD activity (UI) in fresh and aged *Triceps brachii* muscle of steers from pasture, pasture + concentrate and concentrate. Data are mean ± SEM (n=10). Different uppercase letters means significant differences among systems in aged meat (P<0.05). Main effects: Feeding system P<0.05 Pasture > Concentrate, Pasture + concentrate = Pasture, Concentrate; Ageing P<0.05 Aged > Fresh

3.3.3. Glutathion Peroxidase

For GPx activities, a significant main feeding effect (P<0.0001) was found independently of the unit of expression of the activity, showing that animals receiving pasture and concentrate or concentrate alone have a higher activities of the enzyme (Figure 7 and Figure 8). The animals fed pasture seems have a lower activity of GPx. This is in accord with results reported previously in animals produced in Europe and fed pasture or grain [2,6]. It has been hypothesized that the difference between animals fed pasture or concentrate could be associated with the fact that concentrate are richer in selenium in comparison with pasture [2].

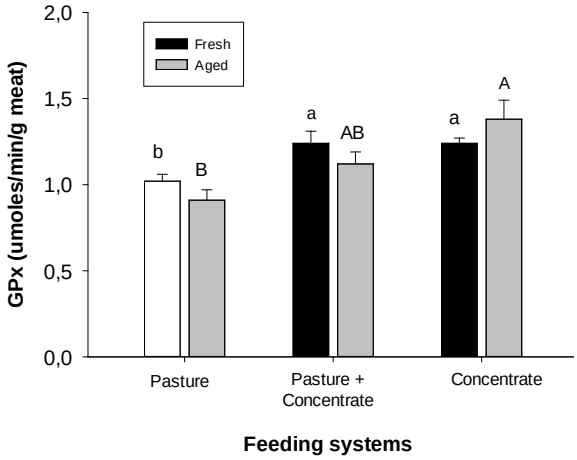

Figure 7. GPx activity (µmoles/min/g meat) in fresh and aged *Triceps brachii* muscle of steers from pasture, pasture + concentrate and concentrate. Data are mean ± SEM (n=10). Different lowercase and uppercase letters means significant differences among feeding systems in fresh and aged meat, respectively (P<0.05). Main effects: Feeding system P<0.0001, Pasture + concentrate, concentrate > Pasture; Ageing: No significant

This is linked to the geographical difference of the selenium content in plant [22,23]. This explanation could

be acceptable for animals produced in Europe, but no for those produced in South America. Indeed, pasture produced in South America is rich in selenium when compared those pasture produced in Europe [22,23]. However, two investigations realized in South America (Argentina) showed opposed results using crossbreed steers. British X indicus in one report and crossbreed without more details in the other one [1,21].

The breed effect in the responses to selenium in GPx activity cannot be ruled-out. No effect of ageing has been observed in the GPx activities, independently of the unit of expression of the activity (Figure 7 and Figure 8).

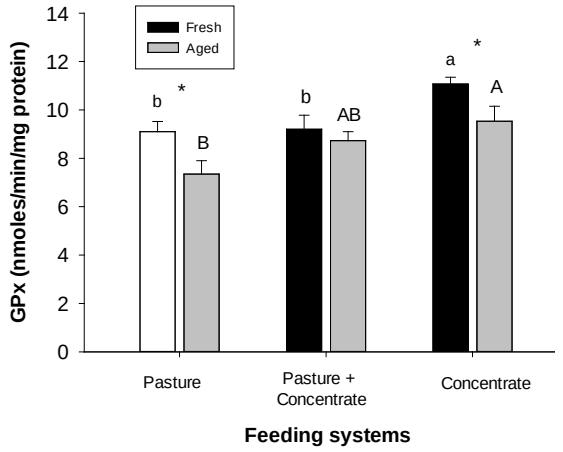

Figure 8. GPx activity (nmoles/min/mg protein) in fresh and aged *Triceps brachii* muscle of steers from pasture, pasture and concentrate and concentrate. Data are mean ±SEM (n=10). Different lowercase and uppercase letters means significant differences among feeding systems in fresh and aged meat, respectively (P<0.05). *shows difference between fresh and aged meat (p<0.05). Main effects: Feeding systems P<0.001 Concentrate > Pasture, Pasture + concentrate; Ageing P<0.01 Fresh > Aged

4. Conclusion

The investigation results point towards that the feeding system seems not to have direct effect on the resistance to lipids oxidation, of meat from *Triceps brachii* muscle of Aberdeen Angus steers. However, in the case of protein oxidation, pasture alone protect more efficiently meat compared to pasture and concentrate, and concentrate alone. These results could be probably associated, globally, to the action of catalase and SOD, which present higher activities in animals fed pasture. It is well known that these two antioxidant enzymes work associatively to fight against ROS. As expected and previously described in other report, GPx present more activity in animals fed concentrate in comparison to ones fed pasture. This behavior of the GPx remain unclear and cannot be explained only by the fact that the concentrate is richest in selenium than pasture. This is true for European pasture, but not for South American one, like in the present investigation. This point has to be deeply studied in future investigation. Of course, no-enzymatic antioxidant present in food, like vitamins, probably completed the antioxidant action of enzymes in reducing the oxidation. However, the enzymatic action of catalase, SOD and Gpx against ROS, remain the first line of defense. Future studies have to highlight precisely the antioxidant action of those enzymes in the protection of meat from lipids and protein oxidation. For now, and in regard to the results observed here, it's not possible to advice what kind of feeding system is the most suitable to preserve meat from lipids and protein oxidation.

Acknowledgement

To Alejandro Sans (Frigorifico Solis) and Ricardo Palma (Uruguayan Society of Aberdeen Angus Breeders) for their help.

Conflict of Interest

The Authors declare that there is no conflict of interest.

References

[1] Insani, E. M, Eyherabide, A, Grigioni, G, Sancho, A. M, Pensel, N. A, and Descalzo, A. M, Oxidative stability and its relationship with natural antioxidants during refrigerated display of beef produced in Argentina, Meat Science, 2008, 79, 444-452.

[2] Gatellier, P, Mercier, Y, and Renerre, M, Effect of diet finishing mode (pasture or mixed diet) on antioxidant status of Charolais bovine meat, Meat Science, 2004, 67: 385-394.

[3] Ouali, A, Herrera-Mendez, C. H, Coulis, G, Becila, S, Boudjellal, A, Aubry, L, and Sentandreu, M. A, Revisiting the conversion of muscle into meat and the underlying mechanisms, Meat Science, 2006, 74, 44-58.

[4] Daley, D.A, Abbott, A, Doyle, P.S, Nader, G.A, and Larson, S, A review of fatty acid profiles and antioxidant content in grass-fed and grainfed beef. Nutrition Journal, 2010, 2- 12.

[5] Lynch, S. M, and Frei, B, Mechanisms of copper- and iron-dependent oxidative modification of human low-density lipoprotein, Journal of Lipid Research, 1993, 34, 1745-1751.

[6] Mercier, Y, Gatellier, P, and Renerre, M, Lipid and protein oxidation in vitro, and antioxidant potential in meat from Charolais cows finished on pasture or mixed diet, Meat Science, 2004, 66, 467-473.

[7] Stoscheck, C. M, Quantitation of Protein, Method of Enzymology, 1990, 182, 50-68.

[8] Aebi, H, Catalase in vitro. In L. Packer (Ed), Oxygen radicals in biological systems, Method of Enzymology, 1984, 105, 121-126.

[9] Marklund, S, and Marklund, G, Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase, European Journal of Biochemistry, 1974, 47, 469-474.

[10] DeVore, V. R. and Greene, B. E, Glutathione peroxidase in post-rigor bovine semitendinosus muscle, Journal of Food Science, 1982, 47, 1406-1409.

[11] Günzler, A, and Flohé, L, Glutathione peroxidase. In R. A. Greenwald (Ed), CRC Handbook of methods for oxygen radical research, 1985, 1, 285-290.

[12] Gatellier, P, Mercier, Y, Juin, H, and Renerre, M, Effect of finishing mode (pasture- or mixed diet) on lipid composition, colour stability and lipid oxidation in meat from Charolais cattle, Meat Science, 2005, 69, 175-186.

[13] Descalzo, A. M, Insani, E. M, Biolatto, A, Sancho, A. M, García, P. T, Pensel, N. A, and Josifovich, J. A, Influence of pasture or grain-based diets supplemented with vitamin E on antioxidant/oxidative balance of Argentine beef, Meat Science, 2005, 70: 35-44.

[14] Pouzo, L.B, Descalzo, A.M, Zaritzkya, N.E, Rossetti, N, Pavan, E, Antioxidant status, lipid and color stability of aged beef from grazing steers supplemented with corn grain and increasing levels of flaxseed, Meat Science, 2016,111, 1-8.

[15] Talmant, A, Monin, G, Briand, M, Dadet, M, and Briand, Y, Activities of metabolic and contractile enzymes in 18 bovine muscles, Meat Science, 1986,18, 23-40.

[16] Cabrera, M. C, Ramos, A, Saadoun, A, and Brito, G, Selenium, copper, zinc, iron and manganese content of seven meat cuts from

Hereford and Braford steers fed pasture in Uruguay. Meat Science, 2010, 84, 518-528.

[17] Ramos, A, Cabrera, M.C, and Saadoun, A, Bioaccessibility of Se, Cu, Zn, Mn and Fe, and haem iron content in unaged and aged meat of Hereford and Braford steers fed, Meat Science, 2012, 91, 116-124.

[18] Popova, T, Effect of rearing system on the fatty acid composition and oxidative stability of the *M. Longissimus lumborum* and *M. semimembranosus* in lambs, Small Ruminants Research, 2007, 150-157.

[19] Lund, M. N., Heinonen, M., Baron, C. P., & Estevez, M. (2011). Protein oxidation in muscle foods: A review. Molecular, Nutrition and Food Research, 55, 83–95.

[20] Falowo, A. B, Fayemi, P.O, and Muchenje, V, Natural antioxidants against lipid–protein oxidative deterioration in meat and meat : A review, Food Research International, 2014, 64, 171-181.

[21] Descalzo, A.M, Rossetti, L, Grigioni, G, Irurueta, M, Sancho, A.M, Carrete, J, and Pensel, N.A, Antioxidant status and odour profile in fresh beef from pasture or grain-fed cattle, Meat Science, 2007, 75, 309-317.

[22] Cabrera MC, Ramos A and Saadoun A, Selenium, zinc, copper and manganese in fresh Angus steers meat from pastures to concentrated-based feeding systems in Uruguay. IUNS 20th. International Congress of Nutrition. Granada. España. Annals of Nutrition & Metabolism, 64:1531.

[23] Cabrera, M.C, and Saadoun, A, An overview of the nutritional value of beef and lamb meat from South America, Meat Science, 2014, 98,3, 435-444.