Antioxidant enzymes activity in meat of poultry fed selenium supplemented diet.

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Abstract— **The aim of this work was to determine the effect of selenium supplementation of poultry diets, enriched with omega-3 fatty acids and containing vitamin E as fat-soluble antioxidant, on the antioxidant enzyme system in thigh and breast muscles. For that purpose, two isoenergetic and isoproteic diets were designed, based on corn and soy bean with the addition of linseed oil (4%), and 200 mg/kg of all rac-tocopheryl acetate. Selenium diet contained additional 200 mg/kg of sodium selenite. The overall antioxidant activity was enhanced (P<0.05) in thigh compared to breast muscle. Alpha tocopherol content was 8.38±0.19 vs. 5.21±0.13 micrograms/g fresh tissue respectively. Also higher levels of CAT, SOD and GPX activity (2.73 vs. 0.7; 2.68 vs. 1.68; 16.75 vs. 6.03 Units/g respectively), and lower TBARS values (0.09 vs. 0.28 mg MDA/kg) were found for both types of muscles. Dietary selenium enhanced the activity of the three enzymes, as expected for a coupled antioxidant system, with a positive interaction between diet and muscle for GPX and CAT. The antioxidant effect could be attributed to selenium, and not to differences in glutathione levels (the substrate for GPX), as they were similar in both muscles and its content was not affected by dietary selenium.**

Conclusions: dietary selenium, together with the incorporation of vitamin E, modulated the activity of antioxidant enzymes in breast and thigh muscles and enhanced the resistance to the oxidation in fresh poultry meat.

Keywords— **Antioxidant enzymes, Poultry meat, Selenium.**

I. INTRODUCTION

There is a global interest in increasing the consumption of omega-3 fatty acids from different food sources in order to prevent chronic diseases such as cardiovascular syndromes and some cancer types. Poultry meats are widely consumed and their fatty acid profile and tocopherol content can be easily modified through different dietary strategies thus making them excellent models to improve their nutritional value and oxidative stability [1]. However, poly-unsaturated fatty acids are prone to oxidation which affects colour, flavour, texture, nutritional value and subsequently, quality during storage [2]. These undesirable effects can be ameliorated by the presence of antioxidants incorporated within the tissues. Antioxidant systems are both enzymatic and non-enzymatic. Concentrations of endogenous antioxidants depend on animal species, muscle type and diet [3]. At this point, it is important to consider if their levels are sufficient in order to minimise the susceptibility to oxidation.

It was reported that 200 mg/kg of all rac-tocopheryl acetate lowered the oxidation in thigh and breast raw meat from chickens fed the supplement [4], [5]. Antioxidant enzymes are also implicated in reactions mediated by free radicals within tissues. Their activity is also important in meat quality since they impact the shelf-life of foods such as meat products [6].The main species are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). SOD and CAT are coupled enzymes. SOD scavenges superoxide anion by forming hydrogen peroxide and CAT decomposes hydrogen peroxide into water and oxygen. GPX can decompose both hydrogen and lipid peroxides formed during oxidation reactions. This enzyme requires reduced glutathione as substrate. Also, selenium is a co-factor of one isoform of this enzyme. For this reason, the addition of selenium in animal feed is used as a strategy to improve antioxidant activity in tissues. Therefore, the inclusion of vitamin E and selenium within poultry diets may improve both enzymatic and non enzymatic antioxidant systems.

II. MATERIALS AND METHODS

A. Animals and diets

Twelve Cobb`s chicken (21 days) were distributed among two dietary treatments. The treatments were part of an assay destinated to determine the importance of omega-3 fatty acids in poultry feed. Diets were isoenergetic and isoproteic, based on corn and soy bean with the addition of linseed oil (4%), and 200 mg/kg vit.E (VITE; n=6). Selenium diet contained additional 200 mg/kg of sodium selenite (VITESE; n=6). After 6 weeks, all animals were slaughtered under commercial conditions. Breast and thigh meat was removed from each carcass upon slaughter. All samples were immediately transported on ice and were kept at -80ºC until analysis. All procedures used complied with national regulations concerning experimentation on farm animals.

B. Oxidation determination

Lipid oxidation was determined by Thiobarbituric Reactive Susbtances (TBARS) [7] and expressed in malonaldehyde equivalents per kg muscle (mg MDA/kg).

C. Tocopherol levels in muscle

Vitamin E was extracted from muscle homogenates with n-hexane after a saponification step and the alpha isomer was determined by HPLC-UV/vis and fluorescence detection as described previously [7]

D. Antioxidant enzyme assays

Ten grams of minced meat were mixed with 20 ml of apotassium phosphate buffer (0.05 M, pH 7.7) and homogenised (Ultraturrax, IKA, Germany at 3000 rpm for 2 min) with addition of 2% Triton X-100. Two millilitres of this homogenate were extracted with cold ethanol: chloroform (1:4 vol/vol) for SOD determination. The rest of the homogenate (25 ml) were centrifuged at 4 ºC at 9500g and the supernatant kept at -80 ºC until processing. Enzyme activity was measured as stated previously [7]. Total SOD activity was measured according to the procedure of Misra and Fridovich [8]. CAT activity was measured by the rate of disappearance of 10 mM H2O2 at 240 nm [9]. GPX activity was assayed with a GSH reduction coupled to a NADPH oxidation by glutathione reductase [10]. Enzyme activities were referred to protein content within the homogenate [11].

All standards and chemicals were purchased from Sigma-Aldrich Argentina and solvents were analytical grade from Sintorgan, Argentina. Glutathione content was determined as stated in [7]. Supernatants were tested for total and oxidized glutathione (GSSG) content [12], measuring the continuous formation of TNB from DTNB (5,5'-dithio-bis (2-nitrobenzoic acid), dependant on the original concentration of GSH (glutathione reduced form), in the presence of NADPH and glutathione reductase. In order to calculate GSH and GSSG molar concentration, tissue volume was estimated after subtraction of intramuscular fat (1 % for breast samples and 4% for thigh samples) and protein content (23% for all treatments) according to the procedure stated previously [7].

E. Statistical analysis.

All data are expressed as mean and standard deviation for six animals per group. Data were analyzed using two-way ANOVA for a fixed effect model with two basal diets and two muscle types. Principal component analysis (PCA) was performed to describe the relationship between variables and their influence over muscle type and the addition of selenium with the SPSS® Advanced Statistics 12 software (SPSS Inc., Chicago, IL).

III. RESULTS AND DISCUSSION

Alpha tocopherol levels were significantly higher in thigh than in breast samples, regardless the incorporation of selenium in the diet (8.38±0.19 vs. 5.21±0.13 micrograms/g fresh tissue respectively). Also, glutathione content was not affected by dietary selenium. All samples showed similar values for both reduced (GSH) and oxidized (GSSG) species. Therefore, the redox potential in the muscles was not affected by the addition of selenium to the diet (table 1).

Table 1: Determination of total, reduced and oxydized glutathione content and redox portential in breast and thigh muscles.

Treatment/ muscle [a]	GSHt (M^{-4})	GSSG (M^{-4})	GSH (M^{-4})	Redox potential [b]
VITESE-T	2.33±0.94	1.38±0.36	0.96±0.53	-237.17± 1.24
VITESE-Br	2.82±0.68	1.40±0.42	1.43±0.68	-237.66±1.24
VITE-T	2.45±0.78	1.25±0.43	1.21±0.41	-237.06±1.76
VITE-Br	2.17±0.21	1.17±0.25	1.00±0.44	-237.44±0.42

[a] VITE: Diet without Selenium; VITESE: Selenium added (200mg/kg); Br: Breast; T: Thigh.
[b] Calculated using the The Nearnst equation for the reduction potential of the redox couple GSSG/2GSH [7].

One form of the glutathione peroxidase (GPX) is a seleno-protein enzyme. In this assay, GPX activity was enhanced by the content of selenium in the diet (table 2) with an interaction between diet and muscle type. Also, the activity was higher in thigh than in breast samples. This feature could not be attributed to a difference on GSH as a co-factor of the enzyme, as its concentration resulted similar among treatments and muscle types. Therefore, it can be observed that the addition of selenium could enhance the activity of GPX. As observed for GPX, CAT also presented an interaction between diet and muscle type. Again, a higher activity was observed for thigh muscle in comparison to breast samples. SOD activity was also higher in thigh muscle than in breast samples. However, this enzyme showed no interaction between muscle and diet.

Lipid oxidation was lower in thigh muscles than in breast samples. Therefore, the activity of the three enzymes, together with the presence of vitamin E, contributed to lower the prooxidant effect of the linolenic acid, as a dietary source of omega-3 PUFA, within the muscles.

It is important to remark that an insufficient vitamin E content (50 mg/kg) combined with an omega-3 rich source could trigger lipid oxidation in both types of muscle [13].

Principal component analysis (PCA) was applied to the set of antioxidant enzymes together with total glutathione content.

Table 2: Antioxidant enzyme activity and lipid oxidation in breast and thigh muscles.

Treatment/ muscle	GPX	SOD	CAT	TBARS (mgMDA/kg)
VITESE-T	20.70±2.83	2.48±0.23	3.29±0.29	0.06±0.01
VITESE-Br	7.78±2.89	1.95±0.28	0.74±0.21	0.25±0.02
VITE-T	13.07±5.93	2.04±0.19	2.41±0.25	0.12±0.01
VITE-Br	5.75±1.04	1.49±0.08	0.66±0.09	0.31±0.04
Significance level of ANOVA (P>F)				
Diet	0.000087	0.000039	0.000042	0.000010
Muscle	0.000000	0.000004	0.000000	0.000000
Diet x muscle	0.010119	0.944778	0.000350	0.743271

The first two principal components accounted for 90.92% of the total variation. As shown in figure 1, PCA scores for thigh samples were described by higher contents of antioxidant enzyme activity, separated along the principal component 1 (65.1% of the total variation). Also the addition of selenium in the diet was associated to higher scores of antioxidant enzyme activity, independently of the glutathione content within the muscles.

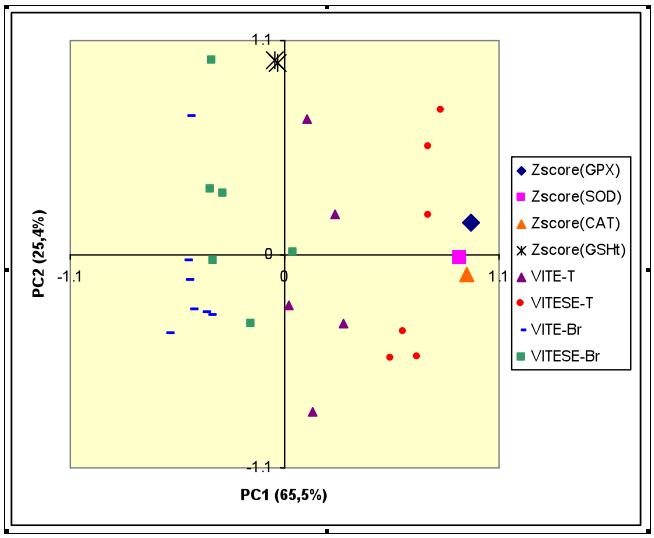

Figure 1 Principal Components assay for GPX, SOD, CAT and GHSt parameters.

IV. CONCLUSIONS

These results indicate that the oxidative stability and the enzymatic antioxidant system in poultry meat

depended on the muscle type. The addition of selenium to omega-3 and vitamin E enriched diets enhanced the activity of GPX, CAT and SOD enzymes, regardless the glutathione content within the muscles.

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