

# Effect of Dried Extract of Boldo (*Peumus Boldus* Mol.) on Growth and Oxidative Tissue Status of Broiler Chickens

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## ■ Keywords

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## ABSTRACT

The addition of antioxidants to broiler diets has been shown to enhance their antioxidant status. Since boldo (*Peumus boldus* Mol.) leaves contain highly antioxidant molecules, a dried extract of boldo (DEB) was added to broiler diets to improve "in vivo" antioxidant tissue status and to favor animal growth. A DEB standardized for antioxidant content was prepared and added to poultry diets at three different levels (low-DELB, medium-DEMB, and high-DEHB) for a period of 6 weeks. A single negative control (no added antioxidant) and one positive control (supplementation with 200 mg/kg vitamin E) were used. Plasma antioxidant capacity (PAC), thiol content (GHS), and basal and induced lipoperoxidation of liver, leg and breast tissues were determined in birds at 2, 4, and 6 weeks of age. PAC increased with chicken age until week 6, but was unaffected by DEB addition at any level. However, DEB increased hepatic GSH content. No data indicated that DEB improved the resistance against induced lipoperoxidation in the assayed tissues. DEB contains compounds exhibiting high antioxidant activity "in vivo", as evidenced by the increase in liver thiol content.

Regarding broiler performance, no differences in poultry body weight and feed consumption were detected during the assay.

## INTRODUCTION

Living organisms generate various reactive oxygen species (ROS), including oxygen singlets, peroxides, and free radicals (i.e. superoxide, hydroxyl, and peroxy radicals). The oxidative damage caused by these substances can be prevented and/or counteracted "in vivo" by a wide range of antioxidant defense mechanisms, including vitamins A, C, and E, glutathione (GSH), and various enzymatic mechanisms. The "in vivo" antioxidant status corresponds to a balance between pro-oxidative substances and the corresponding antioxidant defense mechanisms. When this balance is not achieved and the generation of pro-oxidants surpasses the antioxidant mechanisms, the body enters into oxidative stress (Yu, 1994; Smythies, 1998), which impairs growth, development, and productive parameters. Dietary supplementation with antioxidants (AOXs) improves the antioxidant status of poultry (Sheehy *et al.*, 1994; Fellenberg and Speisky, 2006), lowering lipoperoxidation in the tissues (Woodall *et al.*, 1996; Applegate and Sell, 1996; Maraschiello *et al.*, 1999; Öztürk-Ürek *et al.*, 2001). Polyphenols (a class of compounds present in plants) are phytoquinones that can act as AOXs (Benzie and Szeto, 1999). In fact, the addition of tea polyphenols to a broiler diet protects liver and muscle tissues from the oxidative stress induced by corticosterone (Eid *et al.*, 2003). Green tea polyphenols greatly reduce the lipoperoxidation of broiler breast (Biswas and Wakita, 2001), and grape pomace polyphenols have been shown to decrease

lipoperoxidation in poultry breast and thigh (Goñi *et al.*, 2007). Studies addressing the potential of natural AOXs, other than Vit E, to improve oxidative status in poultry are scarce (Fellenberg and Speisky, 2006).

Boldo (*Peumus boldus* Mol.) is a native Chilean species, which infusion has been ingested for centuries by the Chilean population (Speisky and Cassels, 1994). The addition of boldo to substrates containing riboflavin and tryptophan protects people from light exposure due to the singlet oxygen sequestration by AOXs present in this plant (Silva *et al.*, 2002). Many molecules contribute to boldo's antioxidant activity. The most important include alkaloids (boldine) (Speisky *et al.*, 1991) and polyphenols (flavonoids) (Speisky and Cassels, 1994). The polyphenol content in an aqueous extract of boldo leaves represents about 12 to 36% of the total solids (Schmeda-Hirschmann *et al.*, 2003).

In view of the high amount of antioxidants in boldo leaves, the effect of supplementing broiler feed with dried aqueous extract of boldo (with standardized antioxidant activity) on growth and antioxidant tissue status of broilers was evaluated.

## MATERIALS AND METHODS

### Dried extract of boldo (DEB)

Boldo leaves were collected in August, in the central area of Chile. A sample was kept at the Herbario of Escuela de Química y Farmacia (SQF: N° 22.239), at Universidad de Chile.

The vegetal material was dehydrated in the dark, up to 4% humidity, before being pulverized to a powder in a knife mill. An 8% (P/V) boldo infusion was prepared from the powdered material using boiling distilled water. The infusion was filtered and dehydrated by spray-drying, and a fine, light-reddish-brown powder (hereafter termed "DEB") was obtained with a 6% yield in weight.

Thin-Layer Chromatography was used to detect the flavonoids and alkaloids (boldine) in the DEB (Wagner and Bladt, 1996). Total polyphenols and flavonoid contents were quantified by the Glories method (Mazza *et al.*, 1999), using as standards galic acid and quercetine, respectively. The antioxidant ability was measured by the ferric reducing ability of plasma (FRAP) (Langley-Evans, 2000). Free radical trapping ability was determined using the DPPH decolorizing method, with quercetine as a standard (Feresin *et al.*, 2002; Amié *et al.*, 2003). The concentrations used were 3.3, 16.7, 33.3, and 66.7 g/mL. An equivalent containing a water-soluble analog of vitamin E

(Trolox®), was made with the antioxidant ability obtained through FRAP.

### Poultry and treatment

Two hundred twenty-five, one-day old male chickens (line ROSS 208), were randomly assigned to 25 poultry battery cages, with nine birds each. They were fed five different diets, each of which was replicated five times. The birds were grown to day 21 at controlled temperature (initially 30 °C, and gradually decreasing to 24 °C). From day 22 to 42, the chickens were kept in cages at room temperature (24 °C) and fed ad libitum with a commercial feed that was modified according to the treatments. Daily feed intake was measured, and average animal weight was determined every three days. The experimental design was a factorial 5\*3, i.e., five diets (treatments) to three growing ages (2, 4 and 6 weeks). At each age, three chickens from each cage were sacrificed by cervical dislocation. Samples of intracardiac blood were taken (using heparinized syringes), together with representative samples of liver and muscle (breast and leg) tissues. Blood was immediately centrifuged at 2000 x g, and plasma was stored at -70 °C for later analysis (earlier than 45 days). Liver, breast and leg samples were washed in a solution of 0.9% NaCl at 4 °C, packed in plastic bags, and then immediately frozen in dry ice prior to storage at -70 °C for later analysis.

### Diets

The experimental diets were commercial ones. The initial diet contained 20% crude protein and 2.85 Mcal/kg AME. The final diet contained 18% crude protein and 2.90 Mcal/kg AME. The following five treatments were applied: 1) Control Treatment (CON), basal diet; Basal diet supplemented with 2) Dried Extract of Low Boldo (DELB), DEB equivalent to 100 mg/kg Trolox®; 3) Dried Extract of Medium Boldo (DEMB), DEB equivalent to 200 mg/kg of Trolox®; 4) Dried Extract of High Boldo (DEHB), DEB equivalent to 400 mg/kg of Trolox®; 5) vitamin E (Vit E), 200 mg/kg -tocopherol.

### Chemical Analyses

#### Plasma Antioxidant Capacity (PAC)

PAC was determined by the FRAP method (Langley-Evans, 2000). The PAC was determined against a FeSO<sub>4</sub> (1-30 µM) standard curve.

#### Content of Soluble Thiols

Soluble thiol content was determined using Ellman's technique (Ellman, 1959). Thiol content was measured

against a GHS standard curve (1-60  $\mu$ M) and expressed in  $\mu$ mol/g of tissue.

#### Basal lipoperoxidation and susceptibility to lipoperoxidation

The analysis of thiobarbituric acid-reactive substances (TBARS) is widely used to measure the extent of oxidation in biological samples. Samples of 500 mg liver tissue were homogenized in 4 mL phosphate buffer, pH 7.4. In a flask, 3.5 mL of the homogenized sample was combined with 1.5 mL phosphate buffer; while in another flask, 300  $\mu$ L of homogenized sample was mixed with 9.65 mL phosphate buffer and 50  $\mu$ L of 5.05 mM  $\text{FeCl}_3$ . Both flasks were incubated for 0 min (basal lipoperoxidation) and 30 min (susceptibility to lipoperoxidation, induced by temperature or  $\text{Fe}^{3+}$  plus temperature) in a 37 °C water bath with gentle stirring. 600  $\mu$ L total homogenate was added to a tube containing 100  $\mu$ L of a solution of 60% TCA and 2mM EDTA. Agitation and centrifugation were performed at 12000xg for 5 min at 24 °C. In a test tube, 500  $\mu$ L of supernatant was mixed with 1000  $\mu$ L of a solution of 0.67% 2-thiobarbituric acid (TBA), in 0.3 M HCl. The mixture was incubated at 100 °C, for 10 min. The absorbance was read at 535 nm. The results were expressed as absorbance at 535 nm/g of tissue.

For leg and breast muscle, 1000 mg of tissue was homogenized in 10 mL of phosphate buffer, pH 7.4. The total homogenate was separated into two flasks of 5 mL each. Next, 50  $\mu$ L of 5.05 mM  $\text{FeCl}_3$  were added to one of the flasks and 50  $\mu$ L of phosphate buffer were added to the other. The flasks were then incubated for 0 min (basal lipoperoxidation) and 20 min (susceptibility to lipoperoxidation induced by temperature or Fe plus temperature) in a 37 °C water bath with gentle stirring. The remainder of the protocol is identical to that performed on the liver samples.

#### Statistical Analysis

The model used,  $y = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \epsilon_{k(ij)}$ , corresponded to a factorial design of two factors ( $\alpha$  y  $\beta$ ) with i and j levels for each factor. The effect of each factor and their interaction ( $\alpha\beta$ ) were analyzed separately. The analysis of variance, ANOVA, was performed using the Statistical Analysis System (SAS Institute Inc., 1999) software package. The Student t-test was used to determine differences between LSMean values with a significance level of  $p < 0.05$ .

## RESULTS

### Dried Extract of Boldo

The DEB obtained contained 10% total polyphenols and 2% flavonoids. The highest radical trapping ability of DEB was 79% at 66.7 g/mL.

### Weight gain and food intake

No differences in bird body weight ( $p > 0.85$ ) were detected during the entire experimental period (Figure 1). In addition, there was no effect of the treatments on feed intake, which was 220 g/d ( $p > 0.75$ ) at the end of the trial (Figure 2).

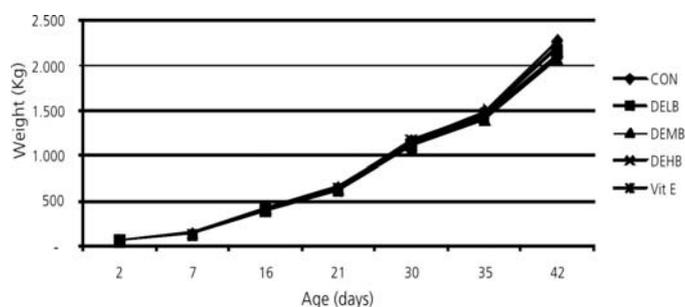


Figure 1 – Bird live weight between 2 and 42 days of age.

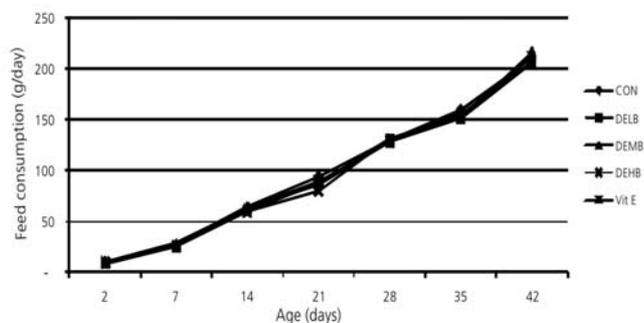


Figure 2 – Bird feed intake between 2 and 42 days of age

### Plasma antioxidant capacity

As shown in Table 1, the PAC of 6-week old chicks was the highest as compared with the PAC of chickens with other ages. According to the treatments, the highest PAC was observed in chickens supplemented with Vit E. However, the value was not significantly different from CON chickens. The PAC of chickens supplemented with DEB was the lowest, but not statistically different from CON chickens. The PAC of chickens supplemented with Vit E, was statistically different of those supplemented with different doses of DEB.

**Table 1** - Effect of different dietary concentrations of DEB (Dried Extract of Boldo) on Plasma Antioxidant Capacity values in 2-, 4-, and 6-week-old broiler chickens (mM FeSO<sub>4</sub>).

Age/Treatment	Control	DELB (low)	DEMB (medium)	DEHB (high)	Vit E	LSMeanAge
2 weeks	8.29±0.75 <sup>ABb</sup>	7.81±1.22 <sup>Bb</sup>	7.92±1.31 <sup>Bb</sup>	7.40±0.83 <sup>Bb</sup>	8.77±0.35 <sup>Ab</sup>	7.83
4 weeks	8.67±2.62 <sup>ABb</sup>	7.57±1.28 <sup>Bb</sup>	7.32±0.60 <sup>Bb</sup>	8.22±2.01 <sup>Bb</sup>	9.31±0.91 <sup>Ab</sup>	8.20
6 weeks	10.23±1.39 <sup>ABa</sup>	9.08±0.75 <sup>Ba</sup>	9.33±1.36 <sup>Ba</sup>	9.11±1.14 <sup>Ba</sup>	10.38±0.96 <sup>Aa</sup>	9.62
LSMean Treatments	9.06	8.16	7.95	7.95	9.49	

A,B,C: Significant differences in PAC values between treatments (rows); a,b,c: significant differences in PAC values between periods (columns) (p<0.05). Each result is expressed as mean + SD and corresponds to the analysis performed in 15 chickens in duplicate.

### Tissue thiol content (GSH)

#### Liver

GSH content decreased significantly between 4 and 6 weeks of age (p=0.008) in birds fed the CON diet (Table 2). Liver GSH content in chickens fed DELB and Vit E did not vary with age and, in the DEMB treatment, it increased in weeks 2 and 6.

In 2-week-old birds, the addition of DEHB resulted in the highest content of liver GSH (21%) (p=0.0038) relative to the CON. Even though treatments with DEMB, DELB, and Vit E increased GSH content up to 12%, these values were not significantly different from CON. In 6-week-old chickens, treatments with DELB and DEMB significantly increased (19% and 32%, respectively) GSH content as compared to the CON (p=0.0134 and p=0.0001, respectively).

#### Breast muscle

GSH content diminished with respect to age for all treatments.

#### Leg muscle

GSH content decreased until birds were 4-weeks-old. Then, it increased between 4 and 6 weeks for all treatments. Vit E supplementation decreased GSH content in leg muscle.

**Table 2** - Effect of different dietary concentrations of DEB (Dried Extract of Boldo) on thiol values (GSH) in the liver, leg, and breast of 2-, 4-, and 6-week-old broiler chicken (mM GSH).

Liver						
Age/Treatment	Control	DELB (low)	DEMB (medium)	DEHB (high)	Vit E	LSMeanAge
2 weeks	5.29±0.44 <sup>abB</sup>	5.97±0.37 <sup>aAB</sup>	5.68±0.75 <sup>bAB</sup>	6.41±0.96 <sup>aA</sup>	5.82±0.60 <sup>aAB</sup>	5.83
4 weeks	5.90±0.55 <sup>aA</sup>	5.49±0.68 <sup>aA</sup>	5.91±0.39 <sup>abA</sup>	5.60±0.60 <sup>bA</sup>	5.47±0.24 <sup>aA</sup>	5.68
6 weeks	4.88±0.47 <sup>bC</sup>	5.83±0.70 <sup>aAB</sup>	6.47±0.44 <sup>aA</sup>	5.01±0.59 <sup>bC</sup>	5.43±0.64 <sup>abC</sup>	5.52
LSMean treatments	5.36	5.77	6.02	5.68	5.57	
Breast						
2 weeks	1.41±0.07 <sup>a</sup>	1.27±0.26 <sup>a</sup>	1.33±0.10 <sup>a</sup>	1.34±0.14 <sup>a</sup>	1.27±0.11 <sup>a</sup>	1.32
4 weeks	1.17±0.14 <sup>b</sup>	1.17±0.14 <sup>b</sup>	1.12±0.10 <sup>b</sup>	1.23±0.10 <sup>b</sup>	1.15±0.16 <sup>b</sup>	1.17
6 weeks	0.99±0.12 <sup>c</sup>	0.97±0.08 <sup>c</sup>	0.92±0.18 <sup>c</sup>	0.97±0.10 <sup>c</sup>	1.07±0.08 <sup>c</sup>	0.96
LSMean treatments	1.20	1.13	1.12	1.18	1.16	
Leg						
2 weeks	1.48±0.18 <sup>Aa</sup>	1.37±0.14 <sup>Ba</sup>	1.39±0.14 <sup>ABa</sup>	1.40±0.18 <sup>Aa</sup>	1.26±0.11 <sup>Ba</sup>	1.38
4 weeks	1.13±0.17 <sup>Ab</sup>	1.06±0.17 <sup>Bb</sup>	1.08±0.11 <sup>ABb</sup>	1.25±0.20 <sup>Ab</sup>	1.07±0.15 <sup>Bb</sup>	1.12
6 weeks	1.53±0.11 <sup>Aa</sup>	1.29±0.22 <sup>Ba</sup>	1.42±0.12 <sup>ABa</sup>	1.50±0.29 <sup>Aa</sup>	1.35±0.17 <sup>Ba</sup>	1.41
LSMean treatments	1.38	1.24	1.29	1.38	1.22	

A,B,C: Significant Differences in thiol values between treatments (columns); a,b,c: significant differences in thiol content between ages (rows) (p<0.05). Each result is expressed as mean + SD and corresponds to the analysis performed in 15 chickens in duplicate.

### Effect on basal and induced lipoperoxidation

#### Liver

The basal lipoperoxidation of the liver tissue of birds fed the CON, DEB and Vit E diets increased until 4 weeks of age, and then decreased at 6 weeks. As shown in Table 3, DEB supplementation did not protect the liver tissue from basal or induced (temperature) lipoperoxidation at any of the evaluated ages. Only Vit E supplementation significantly protected against both lipoperoxidations (p=0.0025) as compared to CON.

A similar effect was observed in induced (Fe plus temperature) lipoperoxidation. There was no antioxidant protection with DEB supplementation, but there was with Vit E supplementation (p<0.0001).

The basal lipoperoxidation of both breast and leg tissues was lower than that of the liver (data not shown). DEB supplementation did not protect these tissues from oxidation (as compared to the CON) in any of the lipoperoxidations (basal or induced). Only Vit E supplementation decreased TBARS content of these tissues.

### DISCUSSION

The obtained DEB exhibited a high antioxidant activity. One g of DEB was equivalent to 0.2 g of Trolox<sup>®</sup>

**Table 3** - Effect of different dietary concentrations of DEB (Dried Extract of Boldo) on TBARS values of the liver tissue of 2-, 4-, and 6-week-old broiler chickens (DO<sub>535</sub> nm/g of tissue).

Basal TBARS (0 min incubation)						
Age/Treatment	Control	DELB (low)	DEMB (medium)	DEHB (high)	Vit E	LSMeanAge
2 weeks	0.37±0.03 <sup>Ab</sup>	0.43±0.10 <sup>Ab</sup>	0.40±0.11 <sup>Ab</sup>	0.37±0.22 <sup>Ab</sup>	0.27±0.05 <sup>Bb</sup>	0.37
4 weeks	0.83±0.39 <sup>Aa</sup>	0.55±0.10 <sup>Aa</sup>	0.66±0.07 <sup>Aa</sup>	0.76±0.34 <sup>Aa</sup>	0.36±0.04 <sup>Ba</sup>	0.63
6 weeks	0.49±0.14 <sup>Ab</sup>	0.53±0.08 <sup>Ab</sup>	0.48±0.13 <sup>Ab</sup>	0.48±0.13 <sup>Ab</sup>	0.30±0.11 <sup>Bb</sup>	0.46
LSMean treatments	0.56	0.51	0.51	0.54	0.31	
Induced TBARS (30 min incubation at 37°C)						
2 weeks	0.24±0.07 <sup>Ac</sup>	0.36±0.15 <sup>Ac</sup>	0.28±0.18 <sup>Ac</sup>	0.37±0.19 <sup>Ac</sup>	0.15±0.11 <sup>Bc</sup>	0.28
4 weeks	0.71±0.34 <sup>Aa</sup>	0.55±0.05 <sup>Aa</sup>	0.72±0.23 <sup>Aa</sup>	0.73±0.25 <sup>Aa</sup>	0.30±0.02 <sup>Ba</sup>	0.60
6 weeks	0.48±0.07 <sup>Ab</sup>	0.68±0.28 <sup>Ab</sup>	0.44±0.27 <sup>Ab</sup>	0.46±0.21 <sup>Ab</sup>	0.20±0.13 <sup>Bb</sup>	0.45
LSMean treatments	0.48	0.53	0.48	0.52	0.22	
Induced TBARS (30 min incubation at 37°C and Fe 25 mM)						
2 weeks	51.70±9.98 <sup>bA</sup>	41.23±14.72 <sup>bA</sup>	39.99±11.37 <sup>bA</sup>	45.10±14.82 <sup>bA</sup>	18.67±4.26 <sup>aB</sup>	39.33
4 weeks	72.45±7.37 <sup>aA</sup>	67.05±3.00 <sup>aA</sup>	72.41±8.53 <sup>aA</sup>	68.41±17.80 <sup>aA</sup>	3.6±1.34 <sup>bB</sup>	56.78
6 weeks	61.88±4.50 <sup>abA</sup>	59.65±8.83 <sup>aA</sup>	62.95±8.14 <sup>aA</sup>	59.73±4.87 <sup>aA</sup>	12.10±7.28 <sup>abB</sup>	51.26
LSMean treatments	62.00	55.97	58.45	57.74	11.45	

A,B,C: Significant Differences in TBARS values between treatments (columns); a,b,c: significant differences in TBARS values between ages (rows) (p<0.05). Each result is expressed as mean + SD and corresponds to the analysis performed in 15 chickens in duplicate.

(Fellenberg *et al.*, 2003). The highest radical-trapping capacity of DEB was 79% at 66.7 g/mL, which corresponds to the activity of 1.8 g/mL quercetine. This capacity was similar to that reported by Schmeda-Hirschmann *et al.* (2003).

No previous reports had addressed how DEB supplementation affects antioxidant status of poultry. In the present study, there were no significant differences in weight gain using any of the applied treatments (e.g. boldo, Vit E). This observation agrees with several scientific reports. For example, Woodall *et al.* (1996) found no significant differences in weight gain of broiler fed Vit E or several supplementary carotenoids. Jakobsen *et al.* (1994) found no differences in weight gain of poultry supplemented with Vit E. Bailey *et al.* (1996) found no differences in weight gain in poultry whose food had been supplemented with ethoxyquin. In addition, Biswass and Wakita (2001) found that supplementation with green tea polyphenols maintained or lowered weight gain in broilers, and Goñi *et al.* (2007) reported that broilers supplemented with grape pomace exhibited no differences in growth performance. The above studies suggest that neither DEB nor other AOXs supplemented in the feed would affect weight gain in poultry. Furthermore, no significant differences were found in feed intake of the diets with DEB or Vit E. These results agree with studies of Jakobsen *et al.* (1994) for Vit E, and Biswass and Wakita (2001), for polyphenols. Our results suggest that DEB addition, even at the highest dose, does not alter the palatability or final feed intake.

In order to evaluate the *in vivo* antioxidant status in poultry, PAC was measured using the FRAP method

(Langley-Evans, 2000), since this technique has been shown to correlate well with ORAC in human plasma (Cao and Prior, 1998).

No previous reports have tested how PAC varies with chicken age. In this trial, it was shown that PAC increased with age. It is possible that the protein content in muscles and lipid content in all tissues increase as birds grow (Grey *et al.*, 1983). The higher PAC in chicken tissues could indicate that the body is prepared to protect this higher amount of potentially oxidizable tissue substrate.

Vitamin E or DEB supplementation did not cause a significant increase in PAC relative to the CON treatment, a result that is similar to that published by Goñi *et al.* (2007). The fact that Vit E supplementation did not significantly increase PAC may be due to the presence of Vit E in lipoproteins, not contributing to PAC. In contrast, the lack of effect of DEB on PAC could be due to interaction between DEB components and proteins in the diet. In this respect, Serafini *et al.* (1996) found, in tests performed in humans, that tea polyphenols had a low antioxidant effect in plasma when they were ingested with milk. The authors attributed this effect to the formation of complexes between tea polyphenols and milk proteins. In addition, Langley-Evans (2000) found that not only cow milk, but also soy milk, could diminish the antioxidant ability of tea polyphenols. In broiler feeds, soybean meal (protein > 40%) is the main protein source, and it could form complexes with DEB polyphenols; thus, the lack of effect may be due to the lack of bioavailability of the supplemented polyphenols.

GSH is an important endogenous antioxidant that protects the cell against the attack of free radicals and

oxygen-reactive species. However, there is little information addressing GSH content in broiler tissues. In the present study, liver GSH content markedly decreased with growth. This result differs from those reported by Enkvetchakul and Bottje (1995), who found that liver GSH content increased with age (3.1 and 4.5  $\mu\text{mol/g}$  at 5 and 7 weeks, respectively), and also from those of Enkvetchakul *et al.* (1995), who reported no significant differences in liver GSH of 5 and 7-week-old chickens. Although these results are contradictory, our study contributes with data on liver GSH content, which was measured by spectrophotometry (Ellman, 1959), a technique different from that (HPLC) used in previous studies.

An intriguing finding in this study is that DEB treatments increased liver GSH content in 2- and 6-week-old birds as compared to the CON treatment. However, the treatment with Vit E resulted in the same GSH content as CON. Further investigation will be required to determine how DEB increases liver GSH content. It is possible that the mechanisms that account for the intestinal absorption and liver retention of DEB AOX components promote "lower GSH intake". If this is the case, in order to explain the lack of GSH increase in other tissues and also in PAC, we propose that, although the AOX components of DEB are absorbed, their systemic bioavailability would be extremely low due to biotransformation and/or utilization at the hepatic level. This interpretation would depend on the fact that the boldo AOX metabolites are subjected to modifications that could lead to a loss of their antioxidant activity (Cheynier, 2005).

Regarding muscle tissues (leg and breast), it should be noted that no previous reports have characterized GSH content in these tissues in relation to age. In the present study, muscle GSH content was 3.5 times lower than that in liver tissue. In contrast, GSH content was higher in the leg muscle than in the breast muscle. This is likely to be the result of the oxidative nature of the leg tissue, which would produce higher levels of free radicals because of its higher mitochondria content (Von Lengerken *et al.*, 2002). As a consequence, the higher GSH content in leg could represent a physiological adaptation of that tissue to better protect itself against oxidation.

In this study, no defined pattern was detected in the variation of GSH with age. In the liver, GSH levels tended either to be stable or to decrease. Whereas in the breast tissue, GSH content decreased with age, and, in the leg, GSH decreased during the first 4 weeks, prior to increasing at week 6. In broilers,

previous reports found that serum GSH increased until 3 weeks of age (Wang *et al.*, 1998), or decreased between weeks 3 and 6 (Enkvetchakul *et al.*, 1995). Although GSH content variation with age would apparently be dependent on the nature of the tissue/fluid, existing information remains both scarce and controversial. Thus, additional studies will be required to determine GSH variation as a function of age.

Vitamin E addition to feeds either decreased (leg) or maintained (breast and liver) GSH content as compared to the CON treatment, whereas DEB supplementation increased (liver) or maintained (breast and leg) the GSH content. The former result agrees with the report of Husveth *et al.* (2000), who found that Vit E supplemented in broiler feeds decreased serum GSH. A possible explanation for this phenomenon is that the higher Vit E intake requires a lower presence of GSH to recycle this antioxidant vitamin in tissues. The increase in liver GSH content by DEB supplementation cannot be clearly explained, and further studies are required to clarify this issue.

Although there was some protective effect of DEB on liver tissues at the various ages of slaughtering, it was not significant. The fact that the different levels of DEB did not significantly protect the liver tissue suggests that this AOX did not reach liver concentrations sufficient for detection, or, alternatively, biotransformation could have produced less powerful metabolites (Cheynier, 2005).

In contrast, Vit E treatment effectively and significantly protected hepatic tissue from both basal and induced (temperature + presence/absence of Fe) lipoperoxidation at all slaughtering ages. This effect could be due to the higher contribution of Vit E to the diet; such AOXs become concentrated in the liver (Mezes, 1994; Applegate and Sell, 1996; Surai *et al.*, 1998; Husveth *et al.*, 2000), thereby protecting the membranes of this organ from lipoperoxidation. The oxidative protection of the liver tissue by Vit E detected in this study confirms several previous reports ((Applegate and Sell (1996); Madabushi *et al.* (1996); Woodall *et al.* (1996); Husveth *et al.* (2000); Surai and Sparks (2000)).

The muscle tissue exhibited a lower TBARS content than the liver tissue, which is probably due to the lower lipid content in leg and breast as compared with liver.

In this study, the basal oxidative tone of chicken breast increased (depending on the type of lipoperoxidation) between 2.5 and 5.4 times with age. This agrees with the fact that, as chickens grow, there is greater fat deposition in tissues (Grey, 1983). The

extent of lipoperoxidation increase depends on a balance between stressors and oxidative protectors present in the tissue. Thus, the higher lipoperoxidation became more significant at a younger age when more oxidative stressors (Fe and temperature) were used.

As with the liver tissue, DEB provided no protection against lipoperoxidation in chicken breast, at any dose. This result contrasts the greater protection in chicken breast afforded by feed supplementation with green tea polyphenols (Biswas and Wakita, 2001).

In conclusion, the main effect of the DEB was reflected in the liver GSH content, which increased relative to the control. Our results also indicated that DEB used at any concentration protected the tissues from lipoperoxidation.

## REFERENCES

- Amié D, Davidović-Amié D, Beslo D, Trinajstić N. Structure-radical scavenging activity relationships of flavonoids. *Croatica Chemica Acta* 2003; 76:55-61.
- Applegate TJ, Sell JL. Effect of dietary linoleic to linolenic acid ratio and vitamin E supplementation on vitamin E status of poult. *Poultry Science* 1996; 75:881-890.
- Bailey CA, Srinivasan LJ, McGeachin RB. The effect of ethoxyquin on tissue peroxidation and immune status of single comb white leghorn cockerels. *Poultry Science* 1996; 75:1109-1112.
- Benzie I, Szeto Y. Total antioxidant capacity of teas by the ferric reducing antioxidant power: assay. *Journal of Agricultural and Food Chemistry* 1999; 47:633-636.
- Biswas AH, Wakita M. Effect of dietary Japanese green tea powder supplementation on feed utilization and carcass profiles in broilers. *Journal of Poultry Science* 2001; 38:50-57.
- Cao G, Prior R. Comparison of different analytical methods for assessing total antioxidant capacity of human serum. *Clinical Chemistry* 1998; 44:1309-1315.
- Cheynier V. Polyphenols in foods are more complex than often thought. *The American Journal of Clinical Nutrition* 2005; 81(Suppl): 223S-229S.
- Eid YZ, Ohtsuka A, Hayashi K. Tea polyphenols reduce glucocorticoid-induced growth inhibition and oxidative stress in broiler chickens. *British Poultry Science* 2003; 44:127-132.
- Ellman GL. Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics* 1959; 82: 70-77.
- Enkvetchakul B, Bottje WG. Influence of diethyl maleate and cysteine on tissue glutathione and growth in broiler chickens. *Poultry Science* 1995; 74:864-873.
- Enkvetchakul B, Anthony NB, Bottje WG. Liver and blood glutathione in male broiler chickens, turkeys and quail. *Poultry Science* 1995; 74:885-889.
- Fellenberg MA, Delporte C, Backhouse N, Gómez M, Speisky H. Preparación de un extracto estandarizado de boldo *Peumus boldus* Mol. para su utilización como antioxidante natural. *Proceedings del Congreso Internacional Farmacéutico*; 2003; Santiago, Chile. p.42.
- Fellenberg MA, Speisky H. Antioxidants: their effects on broiler oxidative stress and its meat oxidative stability. *Worlds Poultry Science Journal* 2006; 62:53-70.
- Feresin G, Tapia A, Gutiérrez A, Delporte C, Backhouse N, Schmeda-Hirschmann G. Free radical scavengers, antiinflammatory and analgesic activity of *Acaena magellanica*. *Journal of Pharmacy and Pharmacology* 2002; 54:835-844.
- Goñi I, Brenes A, Centeno C, Viveros A, Saura-Calixto F, Rebolé A, Arija I, Estevez R. Effect of dietary grape pomace and vitamin E on growth performance, nutrient digestibility, and susceptibility to meat lipid oxidation in chickens. *Poultry Science* 2007; 86:508-516.
- Grey TC, Robinson D, Jones JM, Stock SW, Thomas NL. Effect of age and sex on the composition of muscle and skin from a commercial broiler strain. *British Poultry Science* 1983; 24:219-231.
- Husveth F, Manilla HA, Gaal T, Vajdovich P, Balogh N, Wagner L, Loth I, Nemeth K. Effects of saturated and unsaturated fats with vitamin E supplementation on the antioxidant status of broiler chicken tissues. *Acta Veterinaria Hungarica* 2000; 48:69-79.
- Jakobsen K, Engberg R, Andersen J, Jensen SK, Lauridsen C, Sorensen P, Henckel P, Bertelsen G, Skibsted L, Jensen C. Supplementation of broiler diets with all-rac-  $\alpha$ - or a mixture of natural source RRR-  $\alpha$ - $\gamma$ - $\beta$ - tocopheryl acetate. 1. Effect on vitamin E status of broilers in vivo and at slaughter. *Poultry Science* 1994; 74:1984-1994.
- Langley-Evans S. Consumption of black tea elicits an increase in plasma antioxidant potential in humans. *International Journal of Food Sciences and Nutrition* 2000; 51:309-315.
- Madabushi HT, De Mulder CL, Tappel AL. Vitamin E protects chick tissues against ex vivo oxidation of heme protein. *Lipids* 1996; 31: 43-46.
- Maraschiello C, Sarraga C, Garcia Regueiro JA. Glutathione peroxidase activity, TBARS, and alpha-tocopherol in meat from chickens fed different diets. *Journal of Agricultural and Food Chemistry* 1999; 47:867-872.
- Mazza G, Fukumoto L, Dalaguis P, Girard B, Ewerst B. Anthocyanins, phenolics and color of cabernet franc, merlot and pinot noir wines from British Columbia. *Journal of Agricultural and Food Chemistry* 1999; 47:4009-4017.
- Mezes M. Effect of vitamin E treatment on early postnatal changes of vitamin E status of chicken. *Acta Veterinaria Hungarica* 1994; 42:477-480.
- Öztürk-Ürek R, Bozkaya LA, Tarhan L. The effects of some

antioxidants vitamin -and trace elements- supplemented diets on activities of SOD, CAT, GSH-Px and LPO levels in chicken tissues. *Cell Biochemistry and Function* 2001; 19:125-132.

Schmeda-Hirschmann G, Rodriguez JA, Theoduloz C, Astudillo SL, Feresin GE, Tapia A. Free-radical scavengers and antioxidants from *Peumus boldus* Mol. "Boldo". *Free Radical Research* 2003; 37: 447-452.

Serafini M, Ghiselli A, Ferro-Luzzi A. In vivo antioxidant effect of green and black tea in man. *European Journal of Clinical Nutrition* 1996; 50:28-32.

Sheehy PJ, Morrissey PA, Flynn A. Consumption of thermally-oxidized sunflower oil by chicks reduces alpha-tocopherol status and increases susceptibility of tissues to lipid oxidation. *British Journal of Nutrition* 1994; 71:53-65.

Silva E, Jopia M, Edwards AM, Lemp E, De la Fuente JR, Lissi E. Protective effect of boldo and tea infusions on the visible light-mediated pro-oxidant effects of vitamin B2, riboflavin. *Photochemistry and Photobiology* 2002; 75:585-590.

Smythies J. Recent advances in oxidative stress and antioxidants in medicine. *Journal of Orthomolecular Psychiatry* 1998; 13:11-18.

Speisky H, Cassels B. Boldo and boldine: an emerging case of natural drug development. *Pharmacological Research* 1994; 29:1-12.

Speisky H, Cassels B, Lissi E, Videla L. Antioxidant properties of the alkaloid boldine in systems undergoing lipid peroxidation and enzyme inactivation. *Biochemical Pharmacology* 1991; 41:1575-1581.

Surai P, Kostjuk I, Wishart G, Macpherson A, Speake B, Noble R, Ionov I, Kutz E. Effect of vitamin E and selenium supplementation of cockerel diets on glutathione peroxidase activity and lipid peroxidation susceptibility in sperm, testes, and liver. *Biological Trace Element Research* 1998; 64:119-132.

Surai PF, Sparks NH. Tissue-specific fatty acid and alpha-tocopherol profiles in male chickens depending on dietary tuna oil and vitamin E provision. *Poultry Science* 2000; 79:1132-1142.

Von Lengerken G, Maak S, Wicke M. Muscle metabolism and meat quality of pigs and poultry. *Veterinarija ir Zootechnika* 2002; 20: 82-86.

Wagner H, Bladt S. *Plant drug analysis*. 2nd ed. Heidelberg.: Springer-Verlag; 1996.

Wang S, Cawthon D, Bottje WG. Age-related changes of plasma glutathione and cysteine in broilers: effect of dithiothreitol reduction *vitro* on free and bound pools. *Poultry Science* 1998; 77:1234-1240.

Woodall AA, Britton G, Jackson MJ. Dietary supplementation with carotenoids: effects on -tocopherol levels and susceptibility of tissues to oxidative stress. *British Journal of Nutrition* 1996; 76: 307-317.

Yu BP. Cellular defenses against damage from reactive oxygen

species. *Physiology Review* 1994; 74:139-162.