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Effects of antioxidant supplementation on meat quality and carcass yield in broilers fed commercial-type diets.

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.

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RESUMEN

Se realizó un experimento para evaluar los efectos de la suplementación de antioxidantes en la calidad de la carne de pollos de engorde. Se utilizaron un total de 200 aves Cobb 500, machos, de un día de edad en un diseño experimental completamente al azar. Se asignaron aleatoriamente a las aves en 4 tratamientos con 5 réplicas por tratamiento (10 aves por jaula) durante un período de alimentación de 49 días. Los tratamientos experimentales consistieron en 1) control sin suplementación de antioxidantes (Ctrl); 2) Suplementación con Vitamina E (Vit E; Acetato de tocoferol, 30 UI en la dieta); 3) Suplementación de extracto de Espirulina (Esp; 2% en la dieta; resulta en 40 mg/kg de Ficocianina); y 4) Suplementación de Vitamina E + Espirulina (Vit E + Esp 30 UI/ kg en la dieta de Vit E + 2% de la dieta de Espirulina). Las aves fueron alimentadas usando dietas comerciales ad libitum en forma de migajas en 3 fases: 1-10 días (iniciador), 11-21 días (crecimiento), y 22-49 días (finalizador). En el d 49, se evaluó el pH, parámetros de color (0.25 h, 0.75 h, 5 h, 24 h, 78 h, 192 h), capacidad de retención de agua, fuerza Warner-Bratzler (1, 3, 8 d), características y rendimiento de la carcasa. La suplementación de antioxidantes disminuyó la capacidad de retención de agua después de 192 h (P= 0.009). A los 15 minutos, a* disminuyó y h* incrementó con la suplementación de antioxidantes y a los 45 minutos, h* disminuyó ($P \leq 0.045$). Al tercer día, la fuerza Warner-Bratzler incrementó con la suplementación de Espirulina y Vitamina E comparado con la suplementación de Vitamina E (P=0.029). Adicionalmente, existió un mayor porcentaje de grasa en la carcasa total con suplementación de Vitamina E comparado con la suplementación de Espirulina, y hubo un menor porcentaje de grasa con la suplementación de Espirulina que con ambos antioxidantes ($P \leq 0.023$). En la pechuga, el porcentaje de hueso disminuyó con la suplementación de antioxidantes ($P = 0.053$). En el ala, existió un mayor porcentaje de grasa con la suplementación de Vitamina E comparado con la suplementación de Espirulina y Vitamina E (P=0.034). Los datos de este experimento sugieren un beneficio limitado de la suplementación de antioxidantes en la calidad de la carne. Sin embargo, el impacto de la Espirulina en el porcentaje de grasa requiere futura investigación.

ABSTRACT

An experiment was conducted to evaluate the effects of antioxidant supplementation on meat quality and carcass yield of broilers. A total of 200 day-old Cobb 500 male broilers were used in a completely randomized design. Broilers were assigned into 4 treatments, 5 replications each for 49 d. Experimental treatments were 1) Control, without antioxidant supplementation; 2) Vitamin E supplementation (Tocopheryl acetate, 30 UI/kg of diet; 3) Spirulina supplementation (2% of the diet; 40 mg/kg of phycocyanin); 4) Vitamin E + Spirulina supplementation (30 UI/kg of diet of Vitamin E + 2% of diet of Spirulina. Commercial diets were restrictedly fed in a crumble form in 3 phases. At 49 d, we evaluated pH, color parameters (0.25 h, 0.75 h, 5 h, 24 h, 78 h, 192 h), water holding capacity (WHC; 24 h, 78 h, 192 h), Warner-Bratzler shear force (1, 3, 8 d), carcass characteristics and carcass yield. Data were analyzed by ANOVA using the GLM procedure of SAS. Responses across time were evaluated as repeated measures. Antioxidant supplementation decreased WHC after 192 h (P= 0.009). At 15 minutes, $a^{\square}$ decreased and $h^{\square}$ increased with antioxidant supplementation, and at 45 minutes, $h^{\square}$ decreased ($P \leq 0.045$). Warner-Bratzler shear force increased with Spirulina and Vitamin E supplementation compared to Vitamin E supplementation at day 3 (P=0.029). There was a greater fat percentage in the total carcass with Vitamin E compared to Spirulina supplementation, and there was less fat percentage with Spirulina than with both antioxidants ($P \leq 0.023$). Breast bone percentage decreased with antioxidants (P = 0.053). In the 3-joint wing, Vitamin E supplementation increased fat percentage compared to Vitamin E and Spirulina (P=0.034). Data from this experiment suggest limited benefit of feeding antioxidants on meat quality. However, impact of Spirulina on fat percentage requires further exploration.

Key words: antioxidant, broilers, carcass yield, meat quality

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INTRODUCTION

Animal health is significant to achieve sustainable, responsible and efficient production system. Application of natural alternatives to encourage animal health and performance are needed (OIE, 2018). In addition, there is more interest in natural feed supplements to positively influence various aspects regarding productivity like palatability, performance, optimal immunity and meat quality (Dalle, et al., 2003). Madeira et al. (2017) published a review on microalgae as feed ingredients for livestock production and meat quality. In the last decade, there have been limited nutritional studies testing the benefits of microalgae like Spirulina on poultry (Peiretti and Meineri, 2008, Madeira et al, 2017), and scarce information relative to broiler meat quality. Spirulina algae consists of 20 g/kg of phycocyanin (Andes Spirulina, 2016), which has been associated with antioxidant properties (Romay, et al., 2003).

Spirulina has immune-enhancer activities, anticarcinogenic properties and can improve health. The algae have been reported to reduce hyperlipidemia, glucose levels in serum and hypertension control (Belay, et al., 1993). According to Dalle, et al. (2003), the use of Spirulina in rabbit and poultry nutrition improves bone development and strength, positively influencing carcass quality overall. In addition, Spirulina has high levels of carotenoid pigments such as zeaxanthin, xanthophylls and β-carotene. These accumulate in egg yolks and muscle tissue of chickens, increasing meat yellowness and redness (Takashi, 2003). According to Holman and Malau-Aduli (2012), there has been muscle tissue pigmentation that meets consumers preferences when 1% Spirulina was supplemented one week before slaughter. In the poultry industry, Spirulina supplementation is related with an improved feed efficiency. Particularly, when Spirulina is supplemented at high levels (up to 17%), it can eventually replace fish meal, vitamin and mineral premixes commonly used. Additionally, birds supplemented with the algae have shown a better health status in general

than unsupplemented birds (Venkataraman et al., 1994). In the same way, Vitamin E has an important antioxidant function. Like Spirulina, Vitamin E has been shown to be an immunity enhancer (Karadas, et al., 2016).

To our knowledge, there is not information available contrasting Vitamin E and Spirulina supplementation directly on broiler carcass and meat quality. Therefore, the objective of this experiment was to evaluate the effects of supplementation of Spirulina extract, Vitamin E and their combination on meat quality and carcass yield in broilers fed commercial diets.

MATERIALS AND METHODS

All procedures and methods were approved by the Institutional Animal Care and Use Committee by the University, following FASS (2010) guidelines.

Birds and Plane of Nutrition

A total of 200 one-day-old Cobb 500 broiler birds were obtained from commercially hatched eggs and were used to examine their meat quality, carcass characteristics, and carcass yield. The birds were reared and grown to market age under standard commercial conditions. Birds were provided free access to feed and water and were kept at an altitude of 2600 m above the sea level.

Broilers were randomly assigned into 4 treatments, 5 replications per treatment (10 birds/pen) for 49 d. Experimental treatments were 1) Control, without antioxidant supplementation (Ctrl); 2) Vitamin E supplementation (VitE; Tocopheryl acetate, 30 UI/kg of diet; 3) Extract of Spirulina supplementation (Spi; 2% of the diet; resulting on 40 mg/kg of phycocyanin); and 4) Vitamin E + Spirulina supplementation (VitE+Spi, 30 UI/kg of diet of Vit. E + 2% of spirulina on the diet. Commercial diets were fed ad libitum in a crumble form in 3 phases: 1–10 d (starter; 21% CP, and 3100 Mcal/kg ME), 11–21 d (grower; 20% CP and 3200 Mcal/kg ME), and 22–49 d (finisher; 18% CP and 3300 Mcal/kg ME). Carcass characteristics, carcass yield, meat quality parameters (pH, color, water holding capacity) and Warner-Bratzler shear values were measured.

Experiment and Procedures

All birds were slaughtered and processed at 49 d of age by cervical dislocation. Birds were immediately exsanguinated by manually severing both the carotid arteries and at least 1 jugular vein with a knife and were allowed to bleed for 2 minutes. After bleeding, birds were

scalded at 65°C in a rotary scalder followed by carcass defeathering. After slaughter and plucking operations, the head and legs were removed, and broiler chickens were eviscerated. The whole carcasses were weighted approximately 15 min after slaughter to obtain the hot carcass weight (HCW). Carcasses were divided following the U.S. Trade description for Poultry (USDA, 2000). An 8-piece tradition cut-up chicken was produced by cutting a whole bird into 2 split breasts with and rib portions, 2 drumsticks, 2 thighs with back portions, and 2 wings. The cuts from the left half-carcass were individually weight, vacuum packaged and frozen at -30ºC for subsequent dissection. The proportion of each cut in carcass was calculated as the weight of the sum of same cuts divided by HCW (x 100).

The right breast from each carcass was divided into 3 cuts and stored at 4ºC for technological meat quality evaluation during 8 days. All samples were transported to carcass and meat quality laboratory at Universidad Nacional de Chimborazo, where carcass component distribution and meat quality measurements were performed.

Meat Quality Measurements

Breast and leg colors were evaluated using a Chromameter (Minolta CR 400, Minolta GmbH, Langenhagen, Germany) and expressed using the CIE-LAB dimensions of redness (a^*), yellowness (b^*), lightness (L^*), chroma (C^*), and hue angle (h^*); for each bird, three readings were performed, and the averages were calculated and recorded (Karadas, 2016). Immediately after cutting the carcasses, the internal muscle pH was measured directly at 15 min, 45 min, 5 h, 24 h, 78, h, 192 h postchilling through an incision made by a knife in the right pectoralis major muscle by means of a portable pH meter (Boeco BT-600, Germany) (Abdullah and Matarneh, 2010). We measured at those time periods to simulate different points of chicken meat production industry from slaughter to consumer purchase. At Ecuadorian conditions takes approximately one or two days for the meat to get from the slaughterhouse to commercial distribution points, depending on their distance. This way, the

products will be on the racks 3 to 4 days post slaughter. Finally, the time the product will remain on display, depends on the quality and the appropriate refrigeration. Whole chicken properly processed and stored below 4°C has up to 9 days of shelf life (G. Romo, personal communication).

One chicken from each pen was collected to measure meat quality by using one of its breasts to measure pH and color whereas the other breast was used to measure cooking loss and Warner-Bratzler shear values. While the frozen muscles were still on their plates, they were thawed overnight in a refrigerator at 4°C. The muscles were then removed from the plates and manually deboned (left and right pectoralis major muscles without skin). Breast thawing loss was recorded by the weight of the breast before and after thawing. Both muscles were removed by severing the humeral-scapular joint and pulling it downward to strip the meat from the breast. The deboning process was performed by the same person, and care was taken to ensure that all fillets were removed in the same manner so that the meat quality variables would not be affected by the deboning procedure. For meat quality analysis, 1 pectoralis muscle was chosen randomly and used for measurements of cooking loss and shear force, whereas the other was used for color, pH, and water-holding capacity measurements. After breasts were deboned, 1 of the pectoralis muscles was weighed (initial weight) and then placed in a labeled polythene bag. The bags were placed in a thermostatically controlled water bath and cooked for 25 min at 85°C to achieve a maximum internal temperature of 80°C. After cooking, the bags were cooled at room temperature (25°C) before opening to drain the liquid, and the cooked samples were dried with a paper towel to remove excess surface moisture and reweighed. Cooking loss was reported as the weight lost during cooking divided by the fresh sample weight and was expressed as a percentage.

Within 3 h of cooking, the dried samples from each pectoralis major muscle were cut to obtain 6 cores (20 × 13 × 13 mm) of similar sizes, parallel to a line beginning at the

humoral insertion and ending at the point adjacent to the keel, including the complete depth of each cooked muscle sample. Each core was sheared perpendicularly to the longitudinal orientation of the muscle fiber, using a Warner-Bratzler shear blade with the triangular slot-cutting edge mounted on a shear force device (model 235, Salter Brecknell, USA) to determine the peak force (in kilogram-force) when shearing the samples. Shear force was determined as the average of the maximum force of the 6 replicates from each pectoralis major muscle sample (Karadas, 2016).

Carcass yield measurements

Carcass and carcass parts yield data were collected from 1 bird/pen (n=5 broilers/treatment) and included carcass yield (postchilled), thighs, breasts, wings, legs, abdominal fat (including fat around gizzard), kidneys and whole liver. Combined total mass was recorded for all parts considered as pairs (e.g., legs, thighs, both sides of the breast). Kidney and liver weights were expressed as percentages of whole live bird weight. Carcass yield was expressed as the percentage of whole live bird weight, and parts yields were expressed as the porcentage of postchilled dressed carcass weight. Bird carcasses and remaining diets were disposed of by composting, conforming to local regulations.

Statistical Analysis

Data were analyzed by ANOVA using the GLM procedure of SAS (SAS Institute, 1996), with the pen being defined as the experimental unit. Means were separated using pre-planned contrast (Ctrl vs average of all antioxidants; Vit E vs Spi; Vit E vs VitE+Spi). Responses across time were evaluated as repeated measures (i.e meat quality parameters pH, and color). Results were expressed as the least squares means and SEM. Probability values less than 0.05 were considered significant.

RESULTS AND DISCUSSIONS

Carcass characteristics

The effects of antioxidative supplementation on eviscerated weight, carcass weight and carcass parts percentages are shown on Table 1. There were no significative differences in carcass characteristics between treatments ($P \geq 0.083$). Results from this experiment agrees to previous data. Cheong, et al. (2015), fed Spirulina at 4% of the diet in quails during the whole feeding period and presented similar results to described above.

Carcass yield

The effects of antioxidant supplementation on carcass yield, muscle, bone, fat and skin percentages of each carcass part are shown on Table 2. Antioxidant supplementation did not alter breast, thigh, drumstick or total carcass yields ($P \geq 0.053$). However, there was a greater total fat percentage in the carcass with VitE supplementation compared to Spi supplementation. There was less total fat percentage with Spi supplementation than with Spi+VitE ($P \leq 0.023$). Birds supplemented with VitE increased fat percentage compared to VitE + Spi supplementation in the 3-joint wing (P=0.034). According to Cheong, et al. (2015), there was a significant decrease of fat percentage on quails fed up to 4% Spirulina, associated with lipid reduction properties. In addition, Peiretti and Meineri (2011) reported a lower lipid content in rabbit meat with Spirulina supplementation at different concentrations (50-150 g/kg). Spirulina has shown an ability to modulate lipid concentration in the body because its water soluble portion reduces the low-density lipoproteins (LDP) and high-density lipoproteins (HDP) ratio in blood (Ravi et al., 2010). This could be associated with the decreased fat deposition in the total carcass and the 3-joint wing in birds supplemented with Spirulina observed in our experiment.

Breast pH

The effects of antioxidant supplementation on pH are shown on Table 3. There were no significant differences in pH between treatments ($P \geq 0.057$). These results are in accordance with a study conducted by Raach-Moujahed et al. (2011) who found no significant changes in post mortem broilers pH at 0.25, 1, 3 and 24 h. This occurred when birds were fed diets with up to 5% Spirulina supplementation (Raach-Moujahed et al., 2011).

Breast Water Holding Capacity

The effects of antioxidant supplementation on water holding capacity (WHC) are shown on Table 3. There were no significant differences on WHC at 24 and 78 h ($P \geq 0.072$), however, antioxidant supplementation decreased WHC after 192 h ($P = 0.009$). In a study conducted by Dal Bosco et al. (2014), WHC was evaluated up to 9 d. They observed that antioxidant supplementation (Thyme and Spirulina) had a significant impact on WHC that contrast results explained above. On days 3 and 6, thyme supplementation increased approximately 5% the WHC of rabbit meat. Finally, there were no significant differences in WHC regarding Spirulina supplementation. At 9 d, WHC was not significantly altered by antioxidant supplementation. The results obtained by Dal Bosco et al. (2014) completely differ to our data. WHC is a meat quality parameter that decreases when refrigeration periods increase because of membrane catabolism. We observed a decreased WHC probably because of a lower water content in meat as previously noted on the fat content (Dal Bosco et al., 2014). When evaluating the effects of Spirulina supplementation on carcass yield parameters, we observed that the total carcass fat percentage decreased significantly. This decrease in fat percentage could be related to the observed decrease in WHC.

Breast Color Parameters

The effects of antioxidant supplementation on color parameters are shown on Table 4. Antioxidant supplementation did not alter lightness (L^*), yellowness (b^*) and chroma (c^*) during 0.25, 0.75, 5, 24, 78 and 192 h after slaughter ($P \geq 0.151$). At 0.25 h, redness (a^*) decreased and hue angle (h^*) increased with antioxidant supplementation, and at 0.75 h, h^* decreased ($P \leq 0.045$). There were no significant differences in redness (a^*) and hue angle (h^*) across treatments during 5, 24, 78 and 192 h ($P \geq 0.095$). In a study conducted by Cheong et al. (2015), 4% inclusion of Spirulina in a quail diet increased redness (a^*) and yellowness (b^*) significantly immediately after slaughter, and there were no significant changes in lightness (L^*). In a study conducted by Raach-Moujahed et al. (2011), redness was not affected by Spirulina inclusion in broiler diet at different concentrations. However, in contrast to our experiment, yellowness increased with 2.5 and 5% Spirulina supplementation. Spirulina can affect meat color due to the accumulation of pigments such as zeaxanthin and β-carotenes on tissues (Toyomizu, et al., 2001). However, in our experiment, Spirulina did not alter meat color parameters overall. This difference between our results and other previous investigations, could be explained by a difference of Spirulina inclusion and diet composition (Raach-Moujahed et al., 2011). In a study conducted by Karadas, et al. (2016), antioxidant supplementation (100 mg/kg lutein, 100 mg/kg lycopene, and Vitamin E 200 mg/kg) affected meat color parameters significantly. Yellowness (b^*) was higher with lutein supplementation, and lycopene supplementation did not significantly alter color parameters. In addition, 200 mg/kg of Vitamin E in the diet decreased yellowness (b^*) significantly. Redness (a^*) and lightness (L^*) values were not significantly affected by antioxidant supplementation.

Breast Warner-Bratzler shear force

The effects of antioxidant supplementation on Warner-Bratzler shear force are shown in Table 5. Antioxidant supplementation did not alter Warner-Bratzler shear force during d 1, and d 8 ($P \geq 0.175$). At day 3, Warner-Bratzler shear force increased with VitE + Spi supplementation compared to VitE supplementation (P=0.029). In a study conducted by Raach-Moujahed et al. (2011), diet incorporation of Spirulina at 1, 2.5 and 5% did not alter meat tenderness significantly. In contrast to our results, Cheong, et al., (2015), reported that Spirulina in quail diets decreased shear force, associated with improved meat tenderness. In both of these previous studies, tenderness measurement times are not clearly specified. Thus, differences between literature and our experiment could be explained since measurements were performed in various time periods.

Conclusion

Data from this experiment suggest limited benefit of feeding antioxidants on meat quality parameters. However, impact of Spirulina on fat percentage requires further exploration. Additionally, this experiment demonstrates how the addition of a natural antioxidant such as Spirulina interacts with a synthetic antioxidant used in the industry. With a similar antioxidant activity, an additive effect would be expected, thus, Spirulina can fulfill a role as antioxidant while Vitamin E can fulfill its other physiological activities. Vitamin E plays a non-antioxidant role in various molecular processes such as gene expression and enzymatic activity. In addition, by enhancing Vitamin E availability, broiler lots can avoid consequences of Vitamin E deficiencies such as muscular degeneration and anemia due to erythrocyte destruction (Schubert, et al., 2018). All this translating in an optimized broiler production. With the need of the industry to find new natural alternatives to enhance production, the use of natural antioxidants such as Spirulina is encouraged.

Table 1. Effects of antioxidant supplementation on carcass characteristics

Item	Treatments[a]				SE[c]	Contrasts[b]		
	Control	Spi	VitE	Spi+VitE		1	2	3
Live weight, g	3077.5	3225.24	3310.20	3204.7	154.312	0.356	0.702	0.636
Carcass weight, g	2140.4	2190.6	2052.2	2191.8	88.218	0.966	0.284	0.280
Carcass (%)	69.97	68.09	62.95	68.42	3.37	0.384	0.297	0.268
Breast (%)	36.73	36.50	35.54	36.40	1.186	0.678	0.577	0.616
Thigh (%)	20.76	20.36	20.60	22.59	0.960	0.710	0.860	0.163
Drumstick (%)	14.85	15.66	14.02	15.33	0.625	0.833	0.083	0.158
3-joint wing (%)	10.02	9.95	9.72	9.92	0.302	0.663	0.594	0.642

[a]Experimental treatments were 1) Control, without antioxidant supplementation (**Ctrl**); 2) Vitamin E supplementation (**VitE**; Tocopheryl acetate, 30 UI/kg of diet; 3) Extract of Spirulina supplementation (**Spi**; 2% of the diet; resulting on 40 mg/kg of phycocyanin); and 4) Vitamin E + Spirulina supplementation (**VitE+Spi**, 30 UI/kg of diet of Vit. E + 2% of the diet of Spirulina.
[b] Contrasts included: 1) Control vs average antioxidants; 2) Spi vs VitE; 3) VitE vs Spi+VitE
[c] SE of the treatment means (n = 5).

Table 2. Effects of antioxidant supplementation on carcass yield

	Treatments[a]					Contrasts[b]		
Item	Control	Spi	VitE	Spi+VitE	SE[c]	1	2	3
Total carcass								
Muscle (%)	57.27	57.64	55.84	56.52	1.469	0.727	0.398	0.746
Bone (%)	16.28	15.13	16.12	14.93	0.626	0.237	0.281	0.196
Fat (%)	9.92	8.47	12.15	8.25	1.032	0.809	0.023	0.017
Skin (%)	8.21	9.09	8.90	10.59	0.713	0.130	0.846	0.112
Breast (%)								
Muscle (%)	78.48	77.06	77.35	76.61	1.828	0.496	0.910	0.778
Bone (%)	8.60	6.97	8.07	6.39	0.605	0.053	0.215	0.066
Fat (%)	4.21	4.55	5.08	4.08	0.955	0.750	0.700	0.467
Skin (%)	4.98	5.53	5.86	7.65	0.958	0.235	0.812	0.205
Thigh (%)								
Muscle (%)	55.74	55.37	55.02	55.63	1.399	0.808	0.864	0.762
Bone (%)	19.66	18.92	18.81	18.96	1.066	0.541	0.943	0.921
Fat (%)	9.23	8.06	11.81	8.47	1.613	0.910	0.120	0.163
Skin (%)	6.39	6.96	6.62	7.13	0.764	0.572	0.753	0.643
Drumstick (%)								
Muscle (%)	55.62	57.52	56.60	56.4	1.162	0.376	0.586	0.903
Bone (%)	19.26	19.42	18.97	19.15	0.985	0.948	0.749	0.901
Fat (%)	3.60	.039	4.25	4.08	0.762	0.726	0.435	0.876
Skin (%)	7.76	7.70	8.23	8.35	0.536	0.592	0.497	0.870
3-joint wing (%)								
Muscle (%)	41.12	39.91	38.75	40.23	1.245	0.314	0.522	0.415
Bone (%)	27.15	26.24	28.18	27.83	1.080	0.836	0.220	0.818
Fat (%)	4.42	4.36	7.68	2.77	1.471	0.763	0.129	0.031
Skin (%)	17.52	19.33	17.27	19.45	1.664	0.553	0.395	0.368

[a]Experimental treatments were 1) Control, without antioxidant supplementation (**Ctrl**); 2) Vitamin E supplementation (**VitE**; Tocopheryl acetate, 30 UI/kg of diet; 3) Extract of Spirulina supplementation (**Spi**; 2% of the diet; resulting on 40 mg/kg of phycocyanin); and 4) Vitamin E + Spirulina supplementation (**VitE+Spi**, 30 UI/kg of diet of VitE + 2% of the diet of Spirulina.
[b] Contrasts included: 1) Control vs average antioxidants; 2) Spi vs VitE; 3) VitE vs Spi+VitE
[c] SE of the treatment means (n = 5).

Table 3. Effects of antioxidant supplementation on breast pH and Water Holding Capacity

	Treatments[a]					Contrasts[b]		
Item	Control	Spi	VitE	Spi+VitE	SE[c]	1	2	3
0.25 h								
pH	6.52	6.59	6.57	6.57	0.082	0.657	0.838	0.999
0.75 h								
pH	6.34	6.32	6.23	6.22	0.083	0.288	0.437	0.906
5 h								
pH	5.97	5.90	5.87	5.88	0.077	0.381	0.841	0.927
24 h								
pH	5.80	5.69	5.69	5.68	0.043	0.057	0.922	0.794
WHC I[d]	20.38	20.72	20.16	20.62	1.192	0.847	0.743	0.789
WHC II[e]	21.46	20.52	21.17	20.99	1.935	0.737	0.815	0.948
78 h								
pH	5.81	5.76	5.71	5.76	0.055	0.211	0.573	0.539
WHC I[d]	22..42	25.06	21.02	21.83	2.125	0.393	0.198	0.790
WHC II[e]	26.96	23.11	30.03	26.65	4.109	0.517	0.251	0.570
192 h								
pH	5.78	5.75	5.73	5.68	0.032	0.219	0.628	0.334
WHC I[d]	27.92	23.80	24.14	20.96	2.55	0.072	0.927	0.392
WHC II[e]	32.46	19.58	27.51	22.63	3.068	0.009	0.086	0.277

[a]Experimental treatments were 1) Control, without antioxidant supplementation (**Ctrl**); 2) Vitamin E supplementation (**VitE**; Tocopheryl acetate, 30 UI/kg of diet; 3) Extract of Spirulina supplementation (**Spi**; 2% of the diet; resulting on 40 mg/kg of phycocyanin); and 4) Vitamin E + Spirulina supplementation (**VitE+Spi**, 30 UI/kg of diet of VitE + 2% of the diet of Spirulina.

[b] Contrasts included: 1) Control vs average antioxidants; 2) Spi vs VitE; 3) VitE vs Spi+VitE

[c] SE of the treatment means (n = 5).

[d] WHC I = cooking

[e]WHCII= pressure

Table 4. Effects of antioxidant supplementation on breast color parameters

Item	Treatments[a]				SE[c]	Contrasts[b]		
	Control	Spi	VitE	Spi+VitE		1	2	3
15 min[d]								
L□	47.64	51.44	49.97	49.41	1.748	0.130	0.555	0.822
a□	4.53	2.59	2.88	3.12	0.538	0.045	0.710	0.748
b□	11.94	11.12	13.72	13.23	1.284	0.330	0.157	0.788
c□	12.82	11.45	14.13	13.76	1.299	0.461	0.150	0.840
h□	69.15	77.75	77.88	76.42	1.875	0.005	0.962	0.588
45 min[d]								
L□	46.16	48.75	46.38	49.08	1.748	0.243	0.342	0.279
a□	5.26	3.40	4.19	3.89	0.544	0.095	0.319	0.704
b□	14.84	14.31	16.36	15.40	1.284	0.407	0.264	0.788
c□	15.79	14.77	16.89	15.43	1.299	0.551	0.252	0.429
h□	70.64	77.23	75.58	76.00	1.594	0.010	0.477	0.855
5 hrs[d]								
L□	48.12	50.49	48.52	50.15	1.748	0.342	0.428	0.511
a□	7.95	6.51	6.45	7.37	0.722	0.165	0.951	0.382
b□	18.62	17.09	17.60	17.71	1.284	0.405	0.780	0.955
c□	20.30	18.36	18.80	19.14	1.299	0.293	0.808	0.855
h□	67.66	69.18	70.58	67.78	1.812	0.271	0.593	0.291
78 hrs[d]								
L□	48.16	48.90	48.25	49.64	1.748	0.554	0.792	0.577
a□	6.69	7.08	6.84	8.05	0.868	0.285	0.845	0.340
b□	14.02	15.77	15.29	14.97	1.284	0.340	0.791	0.861
c□	15.93	17.39	16.94	17.11	1.299	0.431	0.809	0.930
h□	63.15	66.23	65.06	60.98	3.339	0.524	0.808	0.401
192 hrs[d]								
L□	48.46	49.78	46.66	48.84	1.748	0.597	0.212	0.380
a□	8.93	7.21	7.89	8.81	0.810	0.151	0.561	0.433
b□	12.55	12.25	14.77	14.79	1.284	0.223	0.170	0.994
c□	15.52	14.28	16.83	17.31	1.299	0.334	0.170	0.792
h□	55.30	59.75	61.83	57.80	3.073	0.153	0.639	0.368

[a]Experimental treatments were 1) Control, without antioxidant supplementation (**Ctrl**); 2) Vitamin E supplementation (**VitE**; Tocopheryl acetate, 30 UI/kg of diet; 3) Extract of Spirulina supplementation (**Spi**; 2% of the diet; resulting on 40 mg/kg of phycocyanin); and 4) Vitamin E + Spirulina supplementation (**VitE+Spi**, 30 UI/kg of diet of VitE + 2% of the diet of Spirulina.

[b] Contrasts included: 1) Control vs average antioxidants; 2) Spi vs VitE; 3) VitE vs Spi+VitE

[c] SE of the treatment means (n = 5).

[d] a□=redness, b□=yellowness, L□=lightness, C□=chroma, h□= hue angle

Table 5. Effects of antioxidant supplementation on breast Warner-Bratzler shear values (kilogram-force)

	Treatments[a]					Contrasts[b]		
Item	Control	Spi	VitE	Spi+VitE	SE[c]	1	2	3
Shear force								
Day 1	1.10	1.11	1.15	1.12	0.134	0.797	0.857	0.895
Day 3	0.82	0.73	0.95	1.38	0.134	0.509	0.269	0.029
Day 8	1.09	0.95	1.03	0.83	0.134	0.175	0.701	0.545

[a]Experimental treatments were 1) Control, without antioxidant supplementation (**Ctrl**); 2) Vitamin E supplementation (**VitE**; Tocopheryl acetate, 30 UI/kg of diet; 3) Extract of Spirulina supplementation (**Spi**; 2% of the diet; resulting on 40 mg/kg of phycocyanin); and 4) Vitamin E + Spirulina supplementation (**VitE+Spi**, 30 UI/kg of diet of Vit. E + 2% of the diet of Spirulina.

[b] Contrasts included: 1) Control vs average antioxidants; 2) Spi vs VitE; 3) VitE vs Spi+VitE

[c] SE of the treatment means (n = 5).

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