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# Oxidative, antioxidant, selenium status, and consumers acceptability of poultry meat enriched with selenium by dietary supplementation

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INFORMATION

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# INTRODUCTION

Chicken meat is a valuable source of high biological value protein 20-22% (Marangoni et al., 2015), vitamins, minerals, and other interesting peptides as anserine (Fukada et al., 2016), and low contribution of total lipids (del Puerto et al., 2017). Depending on the birds diet, lipids could be enriched in polyunsaturated fatty acids (25–30%, PUFA) and in monoun-

### SUMMARY

The effect of selenium supplementation with an organic form (Se/Net) and an inorganic form (Se/Na) in the poultry finishing diet, on the oxidative and antioxidative status of Pectoralis and Gastrocnemius muscles, refrigerated at 2 °C during 0, 3 and 7 days, was studied. Also, selenium enrichment and sensory evaluation of each muscle was studied in fresh (O days) meat. At 35 days old, chicks were distributed into three groups (30 birds each), and were fed with a corn-soybean basal diet without Se supplementation (Control) or with 0.30 ppm of selenium as selenomethionine (SeMet) or as sodium selenite (SeNa) until day 56 d-old. In Pectoralis muscle, significantly lower TBARS values were found at day 0 in meat from birds supplemented with both sources of Se (P<0.05). A higher GPx activity and selenium content was observed in meat from birds supplemented with SeMet compared with the Control (P<0.05). In SeMet raw meat from both muscles, selenium is increased and conserved after cooking in Gastrocnemius. The organic source enhanced the antioxidant enzyme activity in fresh Gastrocnemius muscle, and the inorganic source enhanced it in the fresh and in the refrigerated stored Pectoralis muscle (P<0.05). Inorganic selenium in poultry diet increase textural attributes as cohesiveness and chewed need to form a bolus. Perception of an aromatic aftertaste in Gastrocnemius (P<0.05) and Pectoralis (P<0.06) muscle felt as a metallic taste was perceived with SeMet diet. Swallowing ability was increased by SeNa and SeMet sources only in Gastrocnemius. In conclusion, selenium in the poultry diet improved antioxidant status, nutritional value by Seenriching and textural and taste sensory perception in meat but differences are found depending on the muscle type.

# Estatus oxidativo, antioxidante, del selenio, y aceptabilidad del consumidor de la carne de ave enriquecida con selenio dietario

# RESUMEN

Se estudió el efecto de la suplementación con selenio con una forma orgánica (SeMet) y una forma inorgánica (SeNa) en la dieta de acabado de aves de corral, sobre el estado oxidativo y antioxidante de los músculos Pectoralis y Gastrocnemius, refrigerados a 2 ° C durante 0, 3 y 7 días. Además, se estudió el enriquecimiento de selenio y la evaluación sensorial de cada músculo en carne fresca (O días). A los 35 días de edad, los polluelos se distribuyeron en tres grupos (30 aves cada uno), y fueron alimentados con una dieta basal de maíz y soja sin suplementos de Se (Control) o con 0,30 ppm de selenio como selenometionina (SeMet) o como selenito de sodio (SeNa) hasta el día 56 dedad. En el músculo pectoral, se encontraron valores significativamente más bajos de TBARS en el día O en la carne de aves suplementadas con ambas fuentes de Se (P<0,05). Se observó una mayor actividad de GPx y contenido de selenio en la carne de aves suplementadas con SeMet en comparación con el Control (P<0,05). En la carne cruda SeMet de ambos músculos, el selenio se aumenta y se conserva después de la cocción en Gastrocnemius. La fuente orgánica mejoró la actividad de la enzima antioxidante en el músculo Gastrocnemius fresco, y la fuente inorgánica la mejoró en el músculo Pectoralis fresco y refrigerado almacenado (P<0.05). El selenio inorgánico en la dieta de las aves de corral aumenta los atributos texturales como la cohesión y la necesidad masticada de formar un bolo. La percepción de un regusto aromático en el músculo Gastrocnemius (P<0,05) y Pectoralis (P<0,06) se percibió como un sabor metálico con la dieta SeMet. La capacidad de deglución fue aumentada por las fuentes de SeNa y SeMet solo en Gastrocnemius. En conclusión, el selenio en la dieta avícola mejoró el estado antioxidante, el valor nutricional por el enriquecimiento de Se y la percepción sensorial textural y gustal en la carne, pero se encuentran diferencias según el tipo de músculo.

saturated fatty acids (40%, MUFA) (del Puerto et al., 2017). This makes chicken meat beneficial for human health, as PUFA, particularly n-3, have protective role against CVD (cardiovascular diseases), while MUFA gives more stability to oxidation processes (del Puerto et al., 2017). However, PUFA present in poultry meat makes it more sensible to lipid and protein oxidative processes, which can alter its nutritional value and quality attributes (Dominguez et al., 2019). Among the quality attributes of poultry meat, color, flavor, and water retention capacity are the ones in which consumers' acceptance and industry demands pay the most attention (Cao et al., 2018). These attributes are largely determined by the cell membrane integrity, affecting the loss of water and the consequent loss of nutrients. The stability of the membrane is mainly affected by the oxidative damage caused by free radicals on the lipid and protein components of the meat (Dominguez et al., 2019). The oxidative process induces rancidity and affects the color of meat due to its effect on myoglobin, causing discoloration of the breasts, (Livingston and Brown, 1981; Xiong et al., 2020) both frequently not accepted by the consumer (Ryu et al., 2005). Also, stored meat with optimal quality properties is being limited by the oxidation process (Xiong et al., 2020). Poultry meat has endogenous antioxidants that limit the oxidative processes and preserves meat quality. The antioxidant defense enzymes glutathione peroxidase (GPx), catalase and superoxide dismutase play an important role intercepting reactive oxygen substances and thus reducing levels of oxidative damage on both lipids and proteins of meat (Muhlisin et al., 2016; Zou et al., 2019). Besides, the antioxidants incorporated through the diet contribute to the protection of oxidative damage.

In this context, antioxidant supplementation in the poultry diet is an interesting and affordable strategy to protect the meat from oxidation. Among the antioxidant supplements used, seleni-um (Se) is a micronutrient required mostly by animals and humans, that has attracted attention in recent years because of its dual role as a cofactor of the antioxidant enzyme GPx (Pascual and Aranda, 2013) and its important activity of strengthening the immune system as a component of selenoproteins and enzymes, its influence on the thyroid gland and anti-carcinogenic capacity (Hariharan and Dharmaraj, 2020). In previous works, it was shown that the expression of certain GPx enzymes could be strongly linked to selenium availability (Blas et al., 2013) in the diet, so Se supplementation could promote its activity in tissues, as previously reported in several species (Khalili et al., 2020; Wang and Xu, 2008). Besides, it was demonstrated that skeletal muscle is one of the major sites for selenium storage (Pascual and Aranda, 2013). Particularly, in avian species, Zhou and Wang (2011) reported an increase of GPx activity in chickens supplemented up to 0.30 ppm of selenium, however, this increase in activity is not exceeded when increasing the supplementation doses from 0.30 to 0.50 ppm, which would indicate a maximum limit of increase. Besides, toxicity related to an elevated dose of selenium is clearly established in broilers (Kim and Kil, 2020). Maximal levels for selenium supplementation in the animal diets are also regulated (Zanetti et al., 2016).

By its particularity, dietary selenium supplementation not only can increase GPx activity but also can increase the levels of this nutrient in eggs and meat (Fisinin et al., 2009; Payne and Southern, 2005) and it is a good way to improve the selenium status in the population (Fisinin et al., 2009). Selenium has many chemical derivatives such as selenomethionine, selenocysteine, selenite, and selenate, being the first one mentioned, the most consumed through foods. Whereas when selenium is supplemented, selenomethionine, selenium-rich yeast, and sodium selenite are the organic and inorganic forms used, and they vary in the absorption, efficiency, and metabolism mechanisms (Hariharan and Dharmaraj, 2020). Several studies in different species have indicated that selenomethionine could be more effective than inorganic sources, selenite or selenate (Ali et al., 2020; Juniper et al., 2008; Vignola et al., 2009; Yanyan et al., 2011).

Although selenium daily recommendations in humans are low, it has been proved that dietary deficiencies negatively affect health status (Hariharan and Dharmaraj, 2020). Poultry meat, an affordable food, enriched with selenium is a good way to improve the contribution of animal proteins to selenium status in human people.

Considering all these aspects, it was decided to study the effect of supplementing a finishing broiler diet with 0.3 ppm of organic or inorganic selenium, on lipid and protein oxidative status and GPx activity of meat, during the refrigerated storage in vacuum bags at 2 °C for 7 days, as well as the effect on muscle enrichment in this mineral and the variations induced by cooking and consumers acceptability.

#### MATERIALS AND METHODS

#### Animals and diets

Two hundred Ross male chickens of 1 day old were grown-up until 35 days on the floor with a litter of wood husk in a heated room with a photoperiod of 23 hours light, one dark. During this period, they received a starter corn-soybean diet (Crude Protein, CP, 21.20%; Metabolizable Energy, ME 3191 kcal/kg) and water *ad libitum*. Temperature and photoperiod of 23 hours of light were controlled until slaughter. At 35 days of age, ninety birds were selected by weight uniformity and health conditions, housed in experimental cages of 90 cm long and 90 cm wide, on the floor of wood husk and randomized allotted into 3 treatments (30 birds in each treatment, located in 10 cages with 3 birds by cage by treatment) according to the experimental diets received until slaughter which are detailed below.

a) A basal diet, based on ground corn-soybean (21.9% CP and ME 2931 kcal/kg diet), considered as Control.

b) Basal diet supplemented with 0.30 ppm of selenium from an organic source, selenomethionine (SeMet).

c) Basal diet supplemented with 0.30 ppm of selenium from an inorganic source, sodium selenite (SeNa). In all cases, isoproteic and isocaloric diets were formulated. Experimental diet composition is shown in **Table I**. On day 56, age of commercial sacrifice, all the animals were sacrificed in a commercial slaughterhouse, in accordance with good animal welfare practices approved by the Honorary Committee on Experimental Animals (CHEA, ID 702) of the Universidad de la República (Udelar), Montevideo, Uruguay.

#### MEAT SAMPLES, STORAGE AND COOKING

At 24 hours *post mortem*, the *Pectoralis* and *Gastrocnemius* muscles were extracted, and each one was divided into three pieces and placed in vacuum bags (105 µm, Lacor, LR69454, Spain), using domestic vacuum equipment. One sample of each muscle was frozen rapidly at -30 °C (day 0 samples), and the other two were kept in refrigerated storage at 2 °C, for 3 and 7 days, respectively, and then frozen at -30 °C until analysis (3 and 7 days samples). For cooking, meat samples from *Pectoralis* and *Gastrocnemius* were placed in polyethylene bags. The bags were then submerged

 Table I. Composition of experimental diets (Composición de dietas expermentales).

	Experimental diets			
Items (%)	Control	SeMet (0.30 ppm Se)	SeNa (0.30 ppm Se)	
Ground corn	60.0	60.0	60.0	
Soybean meal	32.5 32.5		32.5	
Meat and bone meal	4.2	4.2	4.2	
Calcium carbonate	1.0	1.0	1.0	
Dicalcium phosphate	0.80	0.80	0.80	
Vegetal oil	0.50	0.50	0.50	
Salt	0.30	0.30	0.30	
Lysine	0.25	0.25	0.25	
DL-Methionine	0.10	0.10	0.10	
Premix (*)	0.25	0.25	0.25	
Chemical composition				
CP (%) <sup>(**)</sup>	21.9	21.9	21.9	
ME (kcal/kg)	2931	2931	2931	
CF (%) <sup>(**)</sup>	3.7	3.7	3.7	
Ca (%) <sup>(**)</sup>	1.2	1.2	1.2	
P (%) <sup>(**)</sup>	0.42	0.42	0.42	
Se (ppm) (**)	0.010	0.297	0.301	

<sup>(1)</sup>Broiler mineral and vitamin premix. Provided (per 1.5 kg of diet): 12.000.000 U.I. of vitamin A, 2.000.000 UI of vitamin D3, 25.000 U.I of vitamin E, 7.6 g of vitamin K, 5 g of vitamin B2, 10 g of calcium D pantothenate, 30 g of niacin, 0.5 g of folic acid, 13 mg of vitamin B12, 500 g of choline chloride, 0.5 g of vitamin B1, 1 g of vitamin B6, 90 g of Mn, 35 g of Zn, 25 g of Fe, 2 g of Cu, 2 g of I, 0.1 g of Co and 0.1 g of Se; <sup>(\*\*)</sup> Analyzed values; CP: crude protein; ME: metabolizable energy; CF: crude fiber.

in an 80 °C pre-set water bath until the internal temperature reached 78 °C (Digital thermometer, Lacor, Spain). After cooling, meat samples were stored at -30 °C until analysis.

#### DETERMINATION OF LIPID OXIDATION

Lipid oxidation was measured in meat stored during 0, 3, and 7 days from both muscles (n=10) according to Lynch and Frei (1993) with modifications as described in Terevinto et al. (2019). For this, 5 g frozen meat were homogenized in a Waring-Blender (Fisher Inc. USA) with 100 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 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 (Fisher Inc. USA) for 30 min. After cooling in ice for 5 min and kept at room temperature for 45 min, the pink chromogen was extracted with 3 ml of n-butanol, and phase separation was done by centrifugation at 3000 g for 10 min (Sorvall ST16-R, USA). The absorbance of the supernatant was measured at 535 nm in a Genesys-6 spectrophotometer (Thermo Scientific Inc. USA). The molar extinction coefficient of the malondialdehyde (MDA, 156,000 M<sup>-1</sup> cm<sup>-1</sup>) was used to calculate the concentration of MDA.

#### DETERMINATION OF PROTEIN OXIDATION

Protein oxidation was determined in meat from Pectoralis and Gastrocnemius muscles (n=10) stored refrigerated during 0, 3 and 7 days, by the carbonyl protein assay according to Mercier et al. (2004) with modifications as described in Terevinto et al. (2019). The same homogenate for lipid oxidation, kept frozen since the day before, was thawed and then centrifuged at 2000 g for 10 min (Sorvall ST16-R, USA). Two ml from the supernatant was removed and incubated with 2 ml of 2 M HCl (blank). Another aliquot of 2 ml sample was incubated with 2 ml of 0.02 M dinitrophenylhydrazine (DNPH) in 2 M HCl. Incubation was done for one hour at room temperature with regular stirring. Then, 2 ml of 20 % TCA was added and left at room temperature for 15 min with regular stirring. Centrifugation was done at 2000 g for 10 min (Sorvall ST16-R, USA) and pellets were washed three times with 4 ml of ethanol: ethyl acetate (1:1). Pellets were dissolved in 6 ml of 6 M guanidine HCl with 0.02 M KH<sub>2</sub>PO<sub>4</sub> (pH 6.5) and then tubes were left at room temperature for 15 min with regular stirring. Afterward, they were centrifuged at 2400 g for 10 min (Sorvall ST16-R, USA). Absorbance was measured at 370 nm in a Genesys-6 spectrophotometer (Thermo Scientific Inc. USA) and DNPH concentration 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 homogenate using bovine serum albumin (BSA) from Sigma chemicals Co (St Louis, USA) as a protein standard, as described by Stoscheck (1990).

#### DETERMINATION OF GPX ACTIVITY

To determine the GPx activity, Paglia and Valentine (1967) method adapted and described in Terevinto et al. (2015) was followed, in muscles at day 0 and 7. A sample of meat of 2.5 g were homogenized in an Ultra Turrax T18 (IKA Co, Germany) with 12.5 ml of 50 mM KH<sub>2</sub>PO<sub>4</sub> buffer and 0.5 mM EDTA (pH 7.0) for 1 min at 18,000 rpm, centrifuged at 2000 g for 2 min at 4 °C (Sorvall ST16-R, USA) and filtered. The assay mixture contained 50 mM KH<sub>2</sub>PO<sub>4</sub> 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<sub>3</sub> (Sigma S-2002). The incubation mixture contained 1980  $\mu$ l of the assay mixture and 20  $\mu$ l 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). An extinction coefficient of 6300 M<sup>-1</sup> cm<sup>-1</sup> was used to calculate NADPH concentration. The GPx activity was expressed as nmoles of oxidized NADPH/min/ mg protein. Protein content was determined at 280 nm in the homogenate using bovine serum albumin (BSA) from Sigma chemicals Co (St Louis, USA) as protein standard, as described by Stoscheck (1990).

#### Selenium content in raw and cooked samples

Selenium content was determined in raw (fresh, 0 days) and cooked meat (fresh, 0 days). Samples of 5 g were taken from each muscle, Pectoralis and Gastrocnemius, raw or cooked, and were dried at 105 °C in an oven, with circulating air, to obtain a constant weight. Subsequently, the samples were ashed in a covered crucible at 550° C in a muffle furnace, with a temperature ramp (Thermolyne, USA) for 16 h, to obtain white residual ash. The ash was subjected to an acid digestion process in an Erlenmeyer flask, covered with a micro glass-ball, with 6 M HCl and 1 M HNO<sub>3</sub> on a hot plate, filtered with ashless Whatman filter paper, and diluted with deionized water reaching 20 ml. A blank (without samples) was also digested as the meat sample. Calibration solutions of Se (35, 70, 140, and 280 µg Se/l) were prepared immediately before use by dilution (with 0.2% distilled HNO<sub>3</sub> 65% in distilled and deionized water) of a 1000 µg Se/l, HNO<sub>3</sub>2% standard solution for AA (certified, N93000149, Perkin Elmer, USA) and stored in PTFE bottles at 4 °C.

Se measurements in acidic aqueous dilution (blanks, samples, and calibration curve) were performed as total Se content (Cabrera et al., 2010; Almani et al., 2020), with an atomic absorption spectrometer (Perkin Elmer, Analyst 300) (Norwalk, CT, USA), equipped with deuterium background correction, and graphite furnace HGA-800 (Perkin Elmer), and a pyro coated graphite tube with an integrated platform and an autosampler AS-800 (Perkin Elmer). The determinations were conducted using matrix modifiers based on magnesium nitrate and palladium nitrate. Argon (99% purity) was used as a carrier gas, and a selenium HCL lamp was used as a light source. All determinations were performed in triplicate. The detection limit was  $11 \mu g/l$ (IUPAC, 1998; calculated as 3 × standard deviations of blanks + average of 10 blanks), and precision was 5.1% (calculated as RSD, %, of 10 measures of  $35 \mu g/l$ ).

Sub-boiling distilled HNO<sub>3</sub> 1 M, prepared with HNO<sub>3</sub> 65%, puriss. p.a. (84378, Merck, Germany); HCl 6 M, prepared with HCl 37%, EMSURE, puriss. p.a. (30721, Merck, Germany); Mg (NO<sub>3</sub>)<sub>2</sub>, in 17% HNO<sub>3</sub>, magnesium matrix modifier 1% (63043, puriss. p.a. for graphite furnace-AAS, Fluka, Chemika, Switzerland); and Pd (NO<sub>3</sub>)<sub>2</sub>, in 15% HNO<sub>3</sub> palladium nitrate modifier 1% (B0190635, puriss. p. a. for graphite furnace-AAS, Perkin Elmer, Germany) were used for sample preparation and analysis. Millipore-MilliQ distilled deionized water (18 M $\Omega$  cm<sup>-1</sup> resistivity) was used throughout. Glassware was soaked in dilute (50 ml/l) distilled nitric acid and then, rinsed thoroughly in distilled deionized water. The result was expressed as µg Se / 100 g of raw meat or cooked meat.

#### Sensory evaluation

Descriptive sensory tests were performed in cooked pieces of 4 x 4 cm skinless from fresh chicken Pectoralis and Gastrocnemius, without spicing oil or spices, on a stainless plate in a commercial oven at 180 °C for 17 minutes, to an endpoint temperature of 78-80 °C. Endpoint temperatures were verified in the thickest part of one piece used as a reference, with a digital thermometer fitted with a stainless probe (Lacor, LR 62498, RPC). Cooked samples were allowed to rest 3 min before serving for sensory testing. Eight texture and six flavor attributes were evaluated by 24 trained descriptive panelists using a 0-6 universal intensity scale (Yeu et al., 2008). The numerical intensity scale for each attribute ranged from 0=none, to 6=very much (Meilgaard et al. 1991; Yeu et al., 2008) related to each attribute. The sensory test used in this study included three descriptive textural attributes for tenderness characteristics in the first few bites stage, as cohesiveness, hardness, and juiciness inspired since Zhuang et al. (2009) and Escobedo del Bosque et al. (2020). In the textural second phase, chewed needed to form bolus and bolus moisture were evaluated, in the third phase, bursting speed bolus and swallow ability and in the fourth phase residual swallowed particles.

For the flavor phase one, as after feel-aftertaste, the aromatics taste as cooked chicken taste and metallic taste were used, and for the flavor phase two, the basis tastes as sweet taste, salty taste, bitter taste, and acid taste were used. The attributes, definition and the numerical attribute intensity was defined following Escobedo del Bosque et al. (2020) and Zhuang et al. (2009).

Each panelist was offered a piece of *Pectoralis* and *Gastrocnemius* simultaneously (Stokes et al., 2018) coming from each one of the diets, in a tray containing small dishes with the samples marked with a letter, number or symbol. Panelists were asked to evaluate the intensity of the attributes of texture, juiciness, tenderness, and flavor within the numerical scale. Sensory evaluation was carried out through a panel of 24 people selected and trained to evaluate according to international standards (ISO 8586-2012. Sensory analysis — General guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors). The evaluators were instructed to clean the palate with a sip of mineral water between the evaluations of each meat sample.

#### DATA ANALYSIS

Results of TBARS, carbonyl content, and GPx activity, are reported as means ± standard error of the mean (SEM) for fresh and aged meat. To evaluate the effect of diet, muscle type, and storage time, a repeated-measures ANOVA was used. To evaluate the effect of diet within each muscle, one-way ANOVA was used .. Selenium content results were analyzed by ANOVA GLM, with diet, muscle type, and cooking as main effects. Data obtained in the sensory panel were analyzed for each muscle separately, using a one-way ANOVA to evaluate the differences due to the diet for each sensory attribute. A level of significance of P<0.05 was established. A post hoc multiple comparison Tukey and Kramer test (p<0.05) was applied when significances in the ANOVAs analysis were obtained. The data were analyzed with the NCSS program (NCSS software release 2006, 329 North 1000 East, Kaysville, UT84037, USA2011). Also, data about sensory evaluation was represented by the spider diagram for each attribute of the meat and for each diet received by poultry.

#### RESULTS

#### LIPID AND PROTEIN OXIDATION

Lipid and protein oxidation results in refrigerated storage muscles at 0, 3, and 7 days, are shown in **Table II** and, respectively. No effect of selenium supplementation was found on the oxidation of lipids or proteins as TBARS and carbonyls (P>0.05). However, in fresh (day 0) *Pectoralis* muscle, levels of TBARS were lower when Se was supplemented compared to the Control group (P<0.05). A muscle effect was observed on lipid oxidation results, where the *Gastrocnemius* muscle presented a higher level of TBARS compared to *Pectoralis* (P<0.001). No storage effect was observed for lipid and protein oxidation in meat (P>0.05).

#### **GP**X ACTIVITY

Results of GPx activity in *Pectoralis* and *Gastrocnemius* muscles refrigerated during 0 and 7 days of chicken supplemented with Se are shown in **Table IV**. These data show a clear positive effect of selenium supplementation with SeMet on the antioxidant enzyme activity (P<0.05). In Pectoralis, GPx activity was higher (at 0 and at 7 days) in meat from chicken supplemented with SeNa, and in *Gastrocnemius* (at day 0), it was higher with SeMet. Also, *Pectoralis* muscle presented a higher GPx activity compared to *Gastrocnemius* (P<0.001) and the antioxidant activity of this enzyme significantly decreased with storage time (7 days<0 days) (P<0.001).

#### Selenium content in raw and cooked meat

Selenium content was determined in raw and cooked muscles without storage (fresh muscles) and the results are presented in **Table V**. When the main effect of diet was evaluated, chickens from the Se-Met group presented a higher Se content in meat than the Control group (P>0.05), but it was not different from SeNa group (P<0.01) (**Table V**). *Gastrocnemius* deposited more selenium than *Pectoralis* (P<0.05) and cooked meat presented a significantly lesser content of selenium than raw meat (P<0.0001). When fresh meat

Table II. Lipid oxidation (TBARS, mg MDA/kg meat) in *Pectoralis* (PM) and *Gastrocnemius* (GM) muscles refrigerated during 0, 3 and 7 days, of chicken supplemented with selenomethionine (SeMet) and sodium selenite (SeNa) in a finishing diet (Oxidación lipídica (TBARS, mg MDA/kg de carne) en músculos Pectoralis (PM) y Gastrocnemius (GM) refrigerados durante 0, 3 y 7 días, de pollo suplementado con selenometionina (SeMet) y selenito de sodio (SeNa) en una dieta final).

Muscles	Storage (days)	Experimental diets			
		Control	SeMet	SeNa	
	0	0.30 ± 0.02ª	0.25 ± 0.02 <sup>b</sup>	0.26 ± 0.02 <sup>b</sup>	
PM	3	0.31 ± 0.03	0.26 ± 0.04	0.23 ± 0.05	
	7	0.33 ± 0.07	0.25 ± 0.03	0.23 ± 0.02	
	0	0.25 ± 0.02	0.34 ± 0.06	0.36 ± 0.07	
GM	3	0.38 ± 0.05	$0.40 \pm 0.04$	0.35 ± 0.04	
	7	0.39 ± 0.03	0.39 ± 0.04	0.35 ± 0.10	

Main effects:

Diet: NS

Muscle: P<0.001 GM>PM

Storage: NS
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Data represent means  $\pm$  SEM of n=10. Main effects were analyzed by ANOVA GLM followed by a Tukey-Kramer Test (P<0.05). <sup>a,b</sup> indicate significant differences between diets by ANOVA one way and Tukey-Kramer test (P<0.05).

from chickens fed different diets was compared in each muscle studied (*Pectoralis* and *Gastrocnemius*), Se content was higher in the SeMet group compared with Control and SeNa group. Also, in cooked *Gastrocnemius*, selenium content was higher with the SeMet diet (P<0.05). Thus, the organic source significantly favored the deposit of this mineral in both muscles. Besides, *Gastrocnemius* presented a greater Se content compared to *Pectoralis* (P<0.05), even when cooked, maintaining near 50 % of this nutrient, whereas *Pectoralis* maintains near 40% of it.-

#### SENSORY EVALUATION

Sensory evaluation was performed only in muscles without storage (day 0, fresh samples). Results obtained with trained panelists are presented in **Figure 1**. In cooked *Pectoralis*, no effect of Se supplementation was observed (P>0.05) for textural attributes, except for SeMet diet on metallic taste (P<0.06). Contrarily, in cooked *Gastrocnemius*, some significant differences between diets were observed for the attributes related to the texture and flavor. Meat from SeNa group presented higher cohesiveness (P<0.05) and chewed needed to form a bolus (P<0.05) score compared to the Control group.

Table III. Protein oxidation (carbonyl content, nmoles DNPH/mg protein) in *Pectoralis* (PM) and *Gastrocnemius* (GM) muscles refrigerated during 0, 3 and 7 days, of chicken supplemented with selenomethionine (SeMet) and sodium selenite (SeNa) in a finishing diet (Oxidación de proteínas (contenido de carbonilo, nmoles DNPH/ mg de proteína) en músculos Pectoralis (PM) y Gastrocnemius (GM) refrigerados durante 0, 3 y 7 días, de pollo suplementado con selenometionina (SeMet) y selenito de sodio (SeNa) en una dieta fina).

Muscles	Storage(days) ——	Experimental diets			
		Control	SeMet	SeNa	
РМ	0	0.16 ± 0.03	0.20 ± 0.04	0.20 ± 0.03	
	3	0.18 ± 0.02	$0.20 \pm 0.02$	$0.22 \pm 0.04$	
	7	0.17 ± 0.04	$0.20 \pm 0.03$	0.18 ± 0.02	
GM	0	0.21± 0.04	0.18 ± 0.01	$0.19 \pm 0.04$	
	3	0.21± 0.05	$0.20 \pm 0.05$	0.22 ± 0.03	
	7	$0.22 \pm 0.02$	$0.19 \pm 0.05$	$0.19 \pm 0.02$	
Main effects:					
Diet: NS					
Muscle: NS					
Storage: NS					

Data represent means ± SEM of n =10. Main effects were analyzed by ANOVA GLM followed by a Tukey-Kramer Test (P <0.05).

Meat from the SeMet and SeNa groups presented greater values of the score in swallow ability compared to the Control group (P<0.05) and a significant score for metallic taste for SeMet diet in comparison with Control and SeNa groups (P<0.05).

#### DISCUSSION

Effect on lipid and protein oxidation and GPx activity.

In this work, selenium supplementation improved the protein oxidative stability in poultry meat, only in *Pectoralis* muscle at day 0 of the display, decreasing significantly the MDA values. However, the difference among the muscles was more significant than the response to the supplementation. The clearest effect was the increase in the GPx activity. The GPx is a natural antioxidant enzyme that protects cells and the organism as a whole against oxidative damage caused by free radicals (Mates et al., 1999). In the present work, no significant difference in meat GPx activity was found between SeMet and SeNa supplementation, but meat from the organic source (SeMet) showed greater activity than meat from the Control diet. In this way, Van Ryssen (1989) in lambs, Zhan et al. (2007) in pigs, and Payne & Southern (2005) in chickens, reported equal activity in animals supplemented with organic and inorganic forms. In a work published by Leeson et al. (2008), muscle's GPx activity was not affected by dietary selenium, but in the liver was higher in animals supplemented with sources of selenite and enriched yeast, whereas in plasma it was higher in birds fed

Table IV. Glutathione peroxidase (GPx) activity (nmoles NADPH/min/mg proteín) in *Pectoralis* (PM) and *Gastrocnemius* (GM) muscles refrigerated during 0 and 7 days, of chicken supplemented with selenomethionine (SeMet) and sodium selenite (SeNa) in a finishing diet (Actividad de la glutatión peroxidasa (GPx) (nmoles NADPH/min/mg proteín) en músculos Pectoralis (PM) y Gastrocnemius (GM) refrigerados durante 0 y 7 días, de pollo suplementado con selenometionina (SeMet) y selenito de sodio (SeNa) en una dieta final).

Muscles	Storogo (dovo)	Ex	Experimental diets		
	Storage (days)	Control	SeMet	SeNa	
PM	0	11.05 ± 0.60 <sup>b</sup>	11.07 ± 0.57 <sup>b</sup>	14.02 ± 1.04 ª	
	7	11.83 ± 0.13 <sup>b</sup>	11.90 ± 0.33 <sup>b</sup>	13.09 ± 0.60 ª	
GM	0	10.89 ± 0.29 <sup>b</sup>	$16.75 \pm 0.96$ <sup>a,A</sup>	$8.98 \pm 0.88$ <sup>b</sup>	
	7	9.57 ± 1.14	8.68 ± 0.73 <sup>в</sup>	9.00 ± 0.90	

Main effects

Diet: P<0.05 SeMet > Control; SeNa=SeMet, Control

Muscle: P<0.001 PM > GM

Storage: P<0.001 Day 0 > Day 7

Data represent means ± SEM of n=10. Main effects were analyzed by ANOVA GLM followed by a Tukey-Kramer Test (P <0.05). <sup>a,b</sup> indicate significant differences between diets, for each muscle and day of storage, by ANOVA one way and Tukey-Kramer Test (P<0.05). <sup>A,B</sup> indicate significant differences between days for each diet, by ANOVA one way and Tukey-Kramer Test (P<0.05).

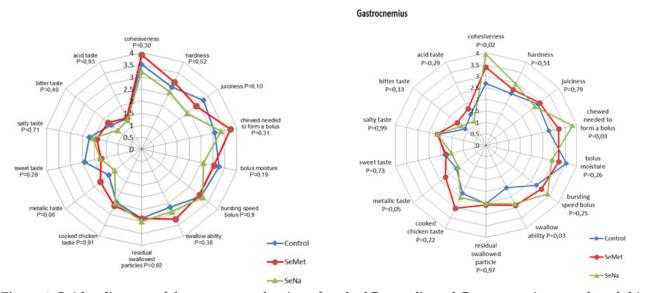


Figure 1. Spider diagram of the sensory evaluation of cooked Pectoralis and Gastrocnemius muscles of chickens supplemented with selenomethionine (SeMet) or sodium selenite (SeNa) in a finishing diet. Data represent means of n=24 panelists. Data were analyzed by a one-way ANOVA and post hoc Tukey-Kramer Test (P<0.05). For Gastrocnemius, SeNa>Control for cohesiveness, SeNa>Control for chewed needed to form a bolus, SeNa,SeMet>Control for swallow ability (P<0.05). For Pectoralis and Gastrocnemius, SeMet>SeNa,Control, for metallic taste, (P < 0.06 and 0.05) respectively (Diagrama de araña de la evaluación sensorial de los músculos Pectoralis y Gastrocnemius cocidos de pollos suplementados con selenometionina (SeMet) o selenito de sodio (SeNa) en una dieta final. Los datos representan medias de n=24 panelistas. Los datos se analizaron mediante un ANOVA unidireccional y una prueba post hoc de Tukey-Kramer (P<0.05). Para Gastrocnemio, SeNa>Control para la cohesión, SeNa>Control para masticado necesario para formar un bolo, SeNa,SeMet>Control para la capacidad de deglución (P<0.05). Para Pectoralis y Gastrocnemius, SeMet>SeNa, Control, para el sabor metálico, (P < 0.06 y 0.05) respectivamente).

selenite compared with B-Traxim and yeast (Zhan et al., 2007).

The chickens supplemented with inorganic forms only showed an increase in the activity of the GPx in blood while the combination of the organic and inorganic forms with vitamin E increased the activity of GPx, free thyroxine, and triodothyroxine (Arthur et al., 1992). Wang and Xu (2008) reported that selenium supplementation in chicken diets increased the activity of plasma GPx and that this increase is less pronounced when supplementation is made with an inorganic source. In contrast, studies in chicken blood (Zhan et al., 2007) and chicken meat (Ghazi Harsini et al., 2012) report that GPx activity was not affected by the source of supplementation and increased faster when the source of supplementation was selenite compared to the organic form. Recent reviews explained that selenium, independently of the form it is administered, must be transformed into selenocysteine before being incorporated into GPx (Hariharan and Dharmaraj, 2020), and inorganic forms are metabolized less efficiently than the organic ones. Besides, there are recent findings of other effects that limiting the use of inorganic sources in the field of the therapeutic in humans (Hariharan and Dharmaraj, 2020). It has been established in previous investigations that selenium is a mineral associated to the GPx enzyme (Hariharan and Dharmaraj, 2020).

In this work, GPx activity was higher in fresh and stored *Pectoralis* muscle from chickens supplemented with SeNa compared with the other two treatments, while in *Gastrocnemius* it is SeMet that increased the

GPx activity, only in fresh samples. This may explain the lower level of lipid oxidation found in the Pectoralis muscle at day 0 (fresh samples) with Se supplementation compared with the Control group. Similar results are found by Dhumbal et al. (2013) who reported a decrease in the oxidative deterioration of lipids in the *Pectoralis* when supplementing with 0.30 ppm Se. Also, Dlouha et al. (2008) and Rajashree et al. (2014) reported decreased values of TBARs between 69-41% in meat supplemented with selenium. Li, et al. (2011) established a correlation of 0.82 between the value of TBARS and the activity of the GPx, which supports the theory that the improvement in the oxidative stability of the meat is due to the protective effect of the Se via an improvement in the activity of the GPx. Rhee et al. (2005) showed that, when supplementing with 8 ppm of Se, oxidative stability was improved and this was much better with the combination of 100 IU of alphatocopherol retarding the oxidation associated with the formation of metmyoglobin. Interestingly, in the present study, in *Gastrocnemius* muscle, GPx activity was higher in chickens supplemented with the organic source, compared with the other two treatments. When different muscles are incorporated in the research frequently different responses can be obtained, likely related to the muscle characteristics (Terevinto et al., 2015). According to Brigelius-Flohé (1999), the presence of selenium in the diet increases the activity of GPx in the meat of several species including rats, chickens, lamb, cattle, horses, pigs and fish. On the other hand, De Vore and Greene (1987), Gatellier et al. (2004), and Zho and Wang (2011) established that a linear correlation between the activity of GPx and dietary selenium (in blood or tissues), while Gruzauskas et al. (2014) found that GPx activity increased in supplemented diets of broilers with 0.5 ppm Se. In a recent work, Shourrap et al. (2018) found a higher catalase and glutathione peroxidase activity in birds supplemented with 0.48 ppm Se-enriched yeast in fresh meat. According to Tavarez et al. (2014), the GPx activity values in the supplemented groups were 7 and 12% higher than the control, and an increase in the concentration of selenium was also observed, which suggests an effect of selenium on the enzyme and this consequently had less oxidative damage. The storage effect was different according to the type of muscle in the present study, when a separate analysis was performed for each muscle. Indeed, in the Gastrocnemius muscle, a more marked decrease in GPx activity with storage was observed in the SeMet group than in the SeNa or Control group. In Pectoralis, GPx activity is maintained after 7 days of refrigerated storage. This result indicates that GPx is more sensible to oxidation processes that occur in a more oxidative muscle as Gastrocnemius. Similar findings were reported by Fellemberg et al. (2019), Insani et al. (2008), and Renerre et al. (1996) in refrigerated broiler meat and in beef meat during display, probably related to increased protein oxidation (Terevinto et al., 2015; Terevinto et al., 2015). However, in the present work, no protein oxidation was observed during refrigerated storage, in neither of the two muscles studied, Pectoralis or Gastrocnemius. These results show that there is not a single and unique effect of selenium on the enzymatic activity of GPx. It depends on the type of muscle, the source of Se and doses used in the supplementation, and the interaction between both, probably due to the different conformation and function of each muscle, more glycolytic or more oxidative, that may affect this response.

### EFFECT ON SELENIUM CONTENT IN RAW AND COOKED MEAT

Previous works of Behme and Wolters (1983) reported that Se is found mainly in the kidney, testes, liver, adrenal plasma erythrocytes, spleen, pancreas, lungs, heart, thymus, gastrointestinal tract, skeleton, brain and muscle. Although selenium is present in all tissues and cells of the body, the concentration and distribution of this mineral will depend on the chemical form and the amount in which it is supplied in the diet (Hariharan and Dharmarj, 2020). Squeletic muscle store 28-46% of the total pool and the role of selenium in health and nutrition is integral (Pascual and Aranda, 2013). Selenium has several promising roles in the human and animal body such as antioxidant, anti-inflammatory, anti-mutagenic, anti-carcinogenic, antiviral, antibacterial, and antifungal effects (Maiyo and Singh 2017). Increasing the level of selenium in affordable foods, contribute to food nutrition and security, and wellbeing, and health status (Hariharan and Dharmaraj, 2020). In our study, chicken meat from the Gastrocnemius and Pectoralis muscles of the SeMet group presented a higher Se content compared with Control and SeNa groups, and no differences between these last two groups were observed. Shorrap et al. (2018), recently, also found that organic sources presented better retention in breast and thigh and this deposition would depend on the selenium levels in

the diet up to 0.67 ppm, varying from the inorganic to organic sources (Payne and Southern, 2005) in blood and muscle. However, upper levels of selenium must be limited due to toxic effects when supplemented for a long term (Hararihan and Dharmarj, 2020). A narrow margin exists between an ideal and toxic intake of selenium for poultry and for its derived animal protein for human nutrition (Suchy et al., 2014). Differences between sources are related also to the potential toxicity and the bioaccumulation in tissues of birds (Kim and Kil, 2020). According to Sunde (1997) and a previous work of Beilstein and Whanger (1986) in sheep, this is because the organic source (selenomethionine) can be incorporated into proteins in a quantity similar to methionine because Se and S (sulfur) have similar atomic properties. The ability of Se to occupy the site of S and that of selenomethionine, to replace methionine, can be incorporated into the protein when it is metabolized. The selenium fraction of the organic source that does not occur as selenomethionine is metabolized as selenite and incorporated into plasma and other selenoproteins. In a concept of sustainable poultry production, safe and environmentally, a moderate level of selenium must be used.

In the present study, a muscle effect can be seen, where Pectoralis presented a higher GPx activity compared with the Gastrocnemius muscle. This result may explain in part, the lower level of lipid oxidation found in the former muscle. Despite this, Se content found in this muscle was lower than in Gastrocnemius. Li et al. (2011) did not observe differences in Se concentration or antioxidant activity in the different muscles in the baseline, but the concentrations of Se in the plasma, muscle, and liver increased as a function of the dietary contribution of Se. Besides, the activity of GPX1 in the liver and muscle and of GPX 3 in plasma in the group Se deficient was lower compared to the one that obtained the adequate diet, while the group that received the overdose had a greater activity of GPX1 in the liver. These authors established that diets deficient in Se induce a lower activity of GPX1 and greater oxidative stress in the tissue, and increase the content of TBARS in the liver and muscle. In chicks deficient in selenium, the antioxidant activity in poultry muscles decreased significantly and that in blood serum and immune tissues (thymus, pouch, and spleen) a higher level of TBARS and lower activity of GPx and SOD compared to those supplemented with selenium was observed (Zhang et al., 2012). In cooked muscles, a hard decrease was observed in the selenium content, but in Gastrocnemius the decrease is lesser than in Pectoralis, and particularly, in SeMet meat selenium content is conserved and significantly higher than in Control and SeNa groups. Selenium supplementation in the poultry diet may contribute to obtaining meat with an enhanced nutritional value even when cooked, and this is important for human nutrition. A portion of 100 g of enriched poultry meat with selenium contributes, as cooked, with a higher percentage of daily requirements of selenium for adults and children. Considering the RDA recommendations for selenium for adults (55 ug daily), 100 g of Gastrocnemius enriched with SeMet, contribute with the 50% of the RDA and 100 g of Pectoralis enriched with SeMet, and contribute with 30%

of the RDA in a cooked presentation. In conclusion, selenium content increased in poultry meat with moderate doses of 0.3 ppm SeMet, and it is conserved after cooking at 50%, resulting in a better option to supplement the poultry diet.

#### EFFECT ON CONSUMERS ACCEPTABILITY

In *Gastrocnemius* muscle, the inorganic source (SeNa) determined more cohesiveness of meat and more chewing need to form a bolus, than the Control group. However, meat from animals receiving selenium, either organic or inorganic sources, showed to be swallowed more easily than Control, characteristics well evaluated by the panelists. Recently, Permatilleke et al. (2020) observed that even if cohesiveness of cooked beef correlated positively with particle size of bolus resulting in larger bolus particle size, cohesiveness did not show significant correlation with any of the oral processing parameters. However, saliva incorporation is key factor for the agglomeration of particles as demonstrated many years ago by Lucas and Luke (1983b) and it depends on the individual factors (Permatilleke et al., 2020). If meat enriched with selenium was more easily swallowed it was likely possible that selenium modified structure and that it was favorable to ready swallow by a better bolus structure (Guo, 2021). Although inter individual variability related to panelist play in this response and this need further research (Permatilleke et al., 2020). Cohesiveness is a very important attribute associate to a safe swallowing, attribute necessary for elderly and for persons suffering dysphagia (Cichero, 2016). Considering the important contribution of poultry meat in protein for elderly, it is necessary to carry up more investigation to associate Se-enriched meat to structure and textural attributes for oral processing. Also, at the level of the aromatic aftertaste, a sensation of metallic taste was felt in the Gastrocnemius and in the Pectoralis muscle only with the organic source (SeMet), but it did not affect the overall acceptability. It is in concordance with a higher deposition of selenium with SeMet source found in this study in cooked meat. Other research, as reported by Miezeliene et al. (2011) did not found significant differences of the selenium supplementation in the odor characteristics neither in the coloration, nor in the fibrousness, juiciness, or tenderness, but an aftertaste was detected with 0.5 ppm of (organic + inorganic) selenium only in fresh cooked breast. Also, Haug et al. (2007) reported that an unusual flavor was perceived in poultry meat supplemented with selenium. Recent research did not found differences in taste or odor for selenium-enriched poultry meat (Khan et al., 2018) with similar doses of organic and inorganic selenium. Even though this aftertaste did not negatively affect the overall acceptability of the panelists, more research is necessary to improve the doses or the cooking method used.

Juiciness is a factor of high contribution to the sensory quality of meat, and the relationship between juiciness and physical and chemical properties have been previously studied (Miezeliene et al., 2011) accepting that supplementation with selenium at 0.1 to 0.5 mg/kg does not have an effect on this parameter. However, Khan et al. (2018) reported an increased positive effect of selenium on poultry diet on the juiciness.

Miezeliene et al. (2011), reported that meat from Gastrocnemius showed a greater color intensity and hardness when chewing, as selenium supplementation increases but did not report negative effects on the acceptability of the meat, so it was concluded that the supplementation with selenium in the diets had no negative effect on the acceptability of chicken meat. Selenium in the poultry diet modifies many attributes of meat, like color, pH, glycogen and lactate content, and drip loss (Miezeliene et al., 2011; del Puerto et al., 2016; Khan et al., 2018), which could influence the organoleptic and sensory characteristics of poultry meat (Alnahhas et al., 2015). Oral processing and resulting textural and taste perception of Se-enriched meat by panelist requires more investigation about impact on food structure of selenium supplemented in poultry finishing diet.

#### CONCLUSIONS

Supplementation with 0.30 ppm of selenium in the finishing diet of chickens was able to enhance the GPx activity in meat, where selenium source affected differently, in each muscle studied. The organic source (SeMet) favored the deposit of selenium in both raw and cooked Gastrocnemius, adding value to the avian protein as food. There was not a clear effect of selenium supplementation, in this work, on the lipid and protein oxidation during 7 days of refrigerated storage. Poultry meat, particularly Gastrocnemius muscle, from chicks receiving organic and inorganic selenium in diet, showed that textural attributes as cohesiveness and chewing were more pronounced with SeNa as a source, and were detected by the panelists. Se-Met source gave an aromatic aftertaste that was felt as a metallic taste in both muscles, but overall acceptability was not impacted. Selenium supplementation improved swallowed ability. In conclusion, selenium supplemented in a moderate dose as 0.30 ppm in diet chicks, produced a Se-enriching poultry meat, with improved technological properties and textural attributes depending on the type of muscle considered.

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