PEDRO RIGHETTI ARNAUT

EFFECTS OF SELENIUM SOURCE ON PERFORMANCE, SELENIUM RETENTION AND PHYSIOLOGICAL VARIABLES IN BROILERS

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Zootecnia, para obtenção do título de *Magister Scientiae.*

Orientadora: Melissa Izabel Hannas

Coorientadores: Luiz Fernando Teixeira Albino
Horácio Santiago Rostagno

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“A vida não dá e nem empresta, não se comove e nem se apieda. Tudo quanto ela faz é retribuir e transferir aquilo que nós lhe oferecemos”. (Albert Einstein)

ABSTRACT

ARNAUT, Pedro Righetti, M.Sc., Universidade Federal de Viçosa, July, 2020. **Effects of selenium source on performance, selenium retention and physiological variables in broilers.** Adviser: Melissa Izabel Hannas. Co-advisers: Luiz Fernando Teixeira Albino and Horacio Santiago Rostagno.

Given its participation in physiological processes such as antioxidant defense systems and thyroid hormone secretion, selenium (Se) has received attention in poultry nutrition and supplementation of organic trace mineral in practical broiler feeds has been defended due to its bioavailability, which is beneficial on environmental grounds, as well because the results on response variables of interest in animal production. A dose-response assay was conducted to evaluate the growth performance, tissue mineralization, retention, and physiological responses of growing broilers fed diets containing different levels and sources of selenium. A total of 500 10-d-old male Cobb 500 broiler chicks were randomly assigned to 10 treatments with 10 replicates and five birds per replicate. Treatments were obtained from a 2×5 factorial arrangement where 2 sources of Se (sodium selenite [SS] and selenium yeast [SY]) were supplemented in semi-purified diets at 0, 0.08, 0.16, 0.24 and 0.32 mg Se/kg feed. Chicks fed SY diets had body weight (BW), and average daily gain (ADG) optimized at 0.133 and 0.130 mg Se/kg, respectively. Both Se sources linearly increased ($P<0.05$) the glutathione peroxidase (GSH-Px) activity in chick blood but higher values were observed in SS fed chicks ($P<0.05$). Both Se sources influenced the thyroid hormones serum concentration ($P<0.05$). Chicks fed SY exhibited greater retention of Se in the feathers ($P<0.05$). Organic Se as SY showed to be more bioavailable than SS. SY supplementation resulted in lower liver Se concentration as Se supplementation increased ($P<0.05$). Based on performance traits, the supplemental level of organic Se as SY to support the proper growth of broiler chicks is 0.133 mg Se/kg.

Keywords: Glutathione peroxidase. Organic trace minerals. Selenium balance. Selenium yeast. Tissue mineralization.

RESUMO

ARNAUT, Pedro Righetti, M.Sc., Universidade Federal de Viçosa, julho de 2020. **Efeito de fontes de selênio no desempenho, retenção de selênio e variáveis fisiológicas em frangos de corte**. Orientadora: Melissa Izabel Hannas. Coorientadores: Luiz Fernando Teixeira Albino e Horacio Santiago Rostagno.

Diante da sua participação em processos fisiológicos como defesa antioxidante e hormônios tireoidianos, o selênio (Se) tem recebido atenção na nutrição de frangos de corte e sua suplementação na forma micromineral orgânico em dietas práticas de frangos tem sido defendida devido a sua biodisponibilidade, a qual é benéfica para o meio-ambiente, bem como em razão dos resultados sobre variáveis resposta de interesse na produção animal. Um ensaio dose-resposta foi conduzido para avaliar o desempenho, mineralização de tecidos, retenção e respostas fisiológicas de frangos de corte em crescimento alimentados com dietas contendo diferentes fontes e níveis de selênio. Um total de 500 frangos de corte macho da linhagem Cobb 500 com 10 dias de idade foram aleatoriamente designados a 10 tratamentos com 10 repetições e 5 aves por repetição. Os tratamentos foram obtidos de um arranjo fatorial 2×5 onde 2 fontes de Se (selenito de sódio [SS] e selênio-levedura [SL]) foram suplementados em dietas semi-purificadas em 0, 0,08, 0,16, 0,24 e 0,32 mg Se/kg de ração. Aves alimentadas com SL tiveram seu peso corporal (PC) e ganho de peso diário (GPD) otimizados em 0,133 e 0,130 mg Se/kg, respectivamente. As duas fontes de Se aumentaram linearmente ($P<0,05$) a atividade da glutationa peroxidase (GSH-Px) no sangue de aves, porém valores maiores foram observados em aves supplementadas com SS ($P<0,05$). As duas fontes de Se infuenciaram a concentração dos hormônios tireoideanos no soro ($P<0,05$). Aves alimentadas com SL exibiram maior retenção de Se nas penas ($P<0,05$). Se orgânico suplementado na forma de SL resultou em maior biodisponibilidade do que SS. A suplementação de SL resultou em menor concentração de Se no fígado conforme o aumento dos níveis de Se na dieta ($P<0,05$). Baseado nas características de desempenho, a suplementação de Se orgânico como SL que garante o crescimento adequado de frango de corte é 0,133 mg Se/kg.

Palavras-chave: Glutationa peroxidase. Microminerais orgânicos. Balanço de selênio. Selênio-levedura. Mineralização de tecidos.

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1 INTRODUCTION

Selenium (**Se**) is an essential trace mineral that plays an important role as a cofactor of the antioxidant enzyme glutathione peroxidase (**GSH-Px**) and thioredoxin reductase which are responsible for the protection of the body against the reactive oxygen species (**ROS**) that are produced in the cellular energetic metabolism (Choct et al., 2004; Rao et al., 2013; Surai and Fisinin, 2014; Wang et al., 2018). Selenium also acts as a cofactor of the 5'-deiodinase enzyme required for the conversion of the thyroxin (**T4**) into triiodothyronine (**T3**), hormone involved in the protein, carbohydrate and lipid metabolism (Jianhua et al., 2000; Smith et al, 2002; Upton et al., 2008; Madkour et al., 2015; Wang et al., 2015). Although its importance in the body protection and growth, several studies did not report influence of Se supplementation on the broiler growth performance (Wang et al., 2010; Cai et al., 2012; Rao et al., 2013; Couloigner et al., 2015). In this scenario, nutritionist worldwide utilize the levels estimated by the NRC (1994) of 0.15 mg Se/kg, which may not correspond to the requirements of the modern poultry strain, once the fast growth rate lead to increased ROS production. Moreover, even when no differences in the growth performance are noticed, Se supplementation may increases the quality of meat products (Peric, et al., 2009; Cai et al., 2012).

Traditionally, Se is supplemented in diets either as inorganic salts such as sodium selenite (**SS**) or as the organic form of selenium enriched-yeast (**SY**), which is majorly composed of selenomethionine (**Se-Met**) (Rayman, 2004), a Se source where the Se molecule is a structural component of the sulfur amino acid methionine. Due to its biding with an amino acid, the Se present in the SY is considered less toxic, more digestible, retained and bioavailable than the SS due to its incorporation in different proteins in the body (Choct et al., 2004; Wang et al., 2008; Briens et al., 2012; Surai and Fisinin, 2014; Couloigner et al., 2015) and, thus, it dietary provision would enhance the Se benefits. When supplied in practical diets, SS is typically used in supplements with inorganic sources of other microminerals and selenium yeast is typically used with organic sources of other microminerals.

Moreover, studies that investigated the effect of the Se supplementation on the chickens' performance were usually done with maize and soybean-meal based diets (Cai et al., 2012; Rao et al., 2013; Cemin et al., 2018), which may not allow the reduction of the Se content in the diet to a concentration low enough to evidence Se deficiency symptoms. Thus, the objective of this study was to determine the effect of the selenium levels and source as inorganic or organic

forms on the growth performance, selenium retention, antioxidant enzymes and thyroid hormones concentration in broilers.

2 MATERIAL AND METHODS

All the animal care procedures described along this section were previously approved by the Institutional Animal Care and Use Committee of the Federal University of Viçosa, Viçosa, Minas Gerais, Brazil, prior to the beginning of the assay, (Register number 111/2014) .

2.1 Birds and husbandry

A total of five hundred 1-d-old male Cobb 500 chickens were obtained from a local commercial hatchery (Rivelli, Mateus Leme, Brazil) and used in the current assay. From 1 to 7 d of age, birds were fed a pre-starter diet formulated to meet or exceed Rostagno et al. (2011) nutritional recommendations, except for Se, whose dietary supplementation was provide according to the NRC (1994). Throughout the entire pre-experimental period, all chicks had free access to water and feed (mash). At 8 d of age, chicks were housed in an environmentally controlled room and allotted into 49cm × 27cm × 33cm (length x height x width) plastic cages with raised wire floors until the end of the feeding assay. Feed and demineralized water were provided *ad libitum* throughout the 10-day experimental period. Photoperiod was set at 12 h natural light/12 h artificial light. Prior to experimental period, all chicks were weighed and assigned to treatment groups so initial body weight (180.3 ± 1.18 g) was similar among experimental treatments.

2.2 Experimental diets and treatments

A 2×5 factorial arrangement was used to investigate the effect of 2 sources of microminerals and Se (as organic and inorganic sources) and 5 selenium supplementation levels (0, 0.08, 0.16, 0.24, and 0.32 mg Se/kg). Ten replicate cages of 5 chicks were randomly assigned to each of 10 treatment groups. Each cage was considered an experimental unit. Selenium sources included supplemental Se from SS (45.85% Se - Metalloys & Chemicals commercial Ltda - Cotia, SP) and selenium enriched yeast (0.2405% Se – Sel-Plex® - Alltech, Maringá, Brazil). The micromineral sources were chosen according to the Se source, consider that practical diets are usually supplemented with one micromineral source. Thus, diets supplemented with SS or SY received an inorganic and organic micromineral premix, respectively. A semi-purified basal diet, based on casein, albumin, corn, and dextrose (Table 1), was formulated to meet or exceed nutritional requirements of Rostagno et al. (2011) for

starter broilers (8-21 days of age), except for microminerals. From the basal diet, 4 different diets were produced. The 4 diets differed from each other with regard to amount and source of Se supplemented (SS or SY) and to the trace minerals supplemented (organic microminerals or inorganic microminerals) as follows: 1) basal diet supplemented with organic trace mineral (OM) without Se supplementation; 2) OM + 0.32 mg Se/kg feed supplemented as SY; 3) basal diet supplemented with inorganic trace microminerals (IM) without Se supplementation; and 4) IM + 0.32 mg Se/kg of feed supplemented as SS. Diets supplemented with organic microminerals containing supplemental Se at 0 and 0.32 mg/kg as SY were mixed to produce five dilution series, which, in turn, resulted in five different organic Se concentrations: 0, 0.08, 0.16, 0.24, and 0.32 mg Se/kg of feed. The same procedure was adopted with diets supplemented with inorganic trace minerals, resulting in the same levels of inorganic Se and a total of 10 diets. Except for Se, all microminerals were supplied to meet NRC (1994) required estimates. Sodium phytate was added to the semi-purified diet to simulate practical diets based on corn and soybean meal. All test diets were supplemented with a commercial phytase enzyme.

2.3 Performance and Sample Collection

At 17 d of age, all chicks and feed leftovers from each experimental unit were weighed to determine body weight (**BW**) and average daily feed intake (**ADFI**). Average daily gain (**ADG**) and feed conversion ratio (**FCR**) were calculated from such data. Mortality rate was also monitored, to adjust FCR. At the end of the assay, one bird per cage (10 birds/treatment) was randomly selected and slaughtered by cervical dislocation. A longitudinal cut was made in the abdominal cavity to collect liver and left breast muscle. Collected tissue samples were lyophilized (Liobras– São Carlos, SP), ground in a ball mill (Tecnal Equipamentos para Laboratório, TE-350, São Paulo, Brazil) and stored for further analysis.

2.4 Concentration of Selenium

The Se concentration of the feed ingredients, experimental diets and tissues were analyzed at the Mineral Laboratory of the Animal Science Department of the Universidade de São Paulo (São Paulo, SP) following the methodology proposed by Olson et al (1975). The Se levels of all the ingredients were determined before diet provision to safely estimate the minimum mineral values.

2.5 Selenium Retention

In order to determine the Se retention in the whole body, ten chicks were randomly collected and slaughtered at 8-d of age after a 12 h fasting (water was provided *ad libitum*). Similarly, one chick of each experimental unit was randomly collect and slaughtered at the end of the experimental period (17-d of age). In both procedures, the birds were weighed before and

after plucking to determine the weight of feathers. The carcasses and feathers were lyophilized and stored for further analysis. Afterwards, the carcasses were frozen in liquid nitrogen and ground in an industrial mixer (Spolu – Benesse do Brasil - Itajobi, SP) and stored for further analysis. The whole-body Se retention (**SeR**) and balance (**SeBal**) were calculated as follows:

$$SeR = SeTf - SeTi \quad (1)$$

$$SeBal(\%) = \frac{SeR}{SeI} \times 100 \quad (2)$$

where SeTf and SeTi are the Se amount (mg) in the carcass (**SeC**) and feathers (**SeF**) at the end and beginning of the experimental period, respectively, and **SeI** is the selenium intake (mg) during the same period.

The Se source bioavailability was calculates as the slope ratio between the linear regression equations when linear behavior was statistically observed for the two Se sources for the same variable.

2.6 Antioxidant Enzymes and Hormones

At the end of the experimental period one animal per cage was randomly selected to collect blood samples through heart puncture. The samples were collect in three tubes, heparinized vacutainer tubes containing Na heparin to analyze the whole blood activity of GSH-Px, vacuntainer tubes containing EDTA to analyze the activity of superoxide dismutase (**SOD**), and vacutainer serum tubes for the thyroid hormones (T3 and T4) analysis, respectively. The enzymatic activity were determined through the kits of Randox Laboratories Ltda. (County Antrim, UK) Ransel® and Ransod®, respectively, following the manufacturer guidelines. Total thyroxine and free and total triiodothyronine in the serum were analyzed at Diagnóstico do Brasil (São José do Rio Preto, SP) laboratory through the immunoassay kits of Beckman Coulter Diagnostics, Access Total T4®, Access Free T3® and Access Total T3®, respectively.

2.7 Statistical Analysis

Data were analyzed as a completely randomized design under a two-way pseudo (source x levels) factorial ANOVA. However, we take into account some particularities related to inorganic and organic trace mineral sources and Se microminerals levels. In this context, given the implied issues of level zero from both sources, the traditional two-way factorial analysis was generalized to a fractional factorial design, which consist in a carefully chosen subset (fraction) under an experimental treatments framework (Box et al., 2005). This approach is easily accomplished by using common statements from PROC MIXED of SAS ® (SAS Institute Inc., Cary, NC) software. According to the previously mentioned analysis, the significance ($P<0.05$) of source effect (only two levels) was evaluated through F-test; whereas

orthogonal contrasts were applied to perform the analysis between linear and quadratic responses of dependent variables in function of increasing Se levels. Also, the effects of Se levels were compared using Tukey´s multiple comparison test. The cages average served as the experimental unit for growth performance, while the single chicks (one per cage) served as the experimental unit for tissue mineral contents, retention, thyroid hormone concentration and enzyme activities. Relative bioavailability values of Se as selenium yeast were estimated by slope ratio comparison based on independent linear regressions using SS as the standard source. The regressions were calculated using the Se intake (adjusted by feed intake during the whole experiment) as the independent variable rather than added Se level. A tendency was considered for P-value between 0.05 and 0.1.

Table 1. Proximate and nutritional composition of semi purified diets used in experimental period (8-17 days), as-fed basis.

Ingredients, g/kg	
Corn	300
Albumin[1]	120
Starch	127.4
Dextrose	134
Casein[1]	40
Soy protein isolate	40
Broken rice	80
Soybean oil	20
Cellulose[1]	40
Calcium carbonate[1]	17.6
Potassium phosphate[1]	15.80
Magnesium chloride[1]	6.50
Potassium chloride[1]	4.68
Choline chloride, 60%	3.75
Mixture of amino acids[2]	35.55
Micronutrients[3]	12.65
Microminerals[4]	2.00
Phytase	0.10
Calculated nutrients	
AMEn, kcal/kg	3122
Crude protein[6], %	22.58
SID amino acids, %	
Lysine, %	1.254
Methionine, %	0.552
Methionine + Cystine, %	0.913
Threonine, %	0.831
Calcium[6], %	0.878
Total P[6], %	0.581
Available P, %	0.420
Se[6], mg/kg	0.138

[1] P.A purism reagent exceeds standard ACS specification in trace metals analysis.

[2] 0.03% L-lysine (79%); 0.27% L-arginine (98.5%); 0.40% L-glycine (98.5%); 0.85% L-alanine (99%); and 2.0% L-glutamic acid (99%). The amino acids alanine, glycine, and glutamic acid were added to maintain the ratio of essential nitrogen to total nitrogen at 0.50.

[3] 0.055% coccidiostatic; 0.010% avilamycin; 0.030% BHT; 1.02% sodium phytate and 0.150% vitamin blend supplemented per kg of feed: vitamin A, 7500 IU; vitamin D_3, 1900 IU; vitamin E, 28 IU; vitamin B_1,2 mg; vitamin B_2, 5 mg; vitamin B_6, 1.2 mg; vitamin B_{12}, 12 mcg; vitamin K, 1.5 mg; nicotinic acid, 0.03 mg; pantothenic acid, 0.01 mg; folic acid, 0.7 mg; and biotin, 0.07mg.

[4] Mineral blend supplemented per kg of feed: 80 mg Fe; 1 mg I; 60 mg Mn; 40 mg Zn; and 10 mg Cu, except for Se added according each experimental treatment. Inorganic micromineral premix sources: $FeSO_4$, $MnSO_4$, $ZnSO_4$, CuSO4 and Na_2SeO_3; organic micromineral premix sources: Bioplex® Mn, Zn, Cu and Fe and Selplex® (Alltech, Maringá, Brazil).

[5] Microbial phytase – 600 FTU/kg.

[6] Analyzed value in ingredients.

3 RESULTS

The analyzed concentration of Se in SS supplemented diets were 0.123, 0.192. 0.245, 0.356 and 0.428 mg Se/kg, whereas the concentration in the diets supplemented with SY were 0.131, 0.213, 0.271, 0.350, 0.418 mg Se/kg. These values for Se agree with the calculated values for both sources of 0.138, 0.218, 0.298, 0.378 and 0.458 mg of Se/kg.

No interactions were observed between the microminerals source and Se level (P>0.05) in any of the variables measured in this assay. Differences were observed (P<0.05) in the main effects of ADG among the supplemented levels where the diets with no supplementation resulted in greatest numerical ADG (Table 2). Despite the difference (P<0.05) observed in the BW main effects, no differences were observed with the Tukey test at 5%. A positive linear response was observed in the FC (P<0.05) following the increasing supplementation levels. A tendency in the quadratic response was noticed in the BW (P=0.088) and ADG (P=0.092) of birds fed SY and the optimal supplementation levels were estimated at 0.133 and 0.130 mg Se/kg, respectively, through the derivative of the fitted polynomial quadratic models $BW_{(SY)} = -501.8x^2 + 133.2x + 500.5$, $r^2 = 0.08$, and $ADG_{(SY)} = -48.4899x^2 + 12.6437x + 32.0393$, $r^2 = 0.08$. Greater values of BWG, ADG and ADFI were observed (P<0.05) at the supplementation level of 0.16 mg Se/kg when the diets were supplemented with SY and organic microminerals.

Sodium selenite supplementation resulted in greater values of GSH-Px activity (P<0.01) in the blood and a linear increase (P<0.01) was observed in the activity regardless the source (Table 3). The linear regression equations of the GSH-Px activity in the blood is shown in Figure 1. The slope ratio between sources shows that the bioavailability of the SS with inorganic microminerals was 19.5% higher than the SY and organic microminerals.

Se levels affected free T4 (**fT4**) and tended to affect the total T3 concentration (P=0.056) and free T3:free T4 (P=0.078). Increased SS levels promoted a quadratically (P<0.05) reduction response in total T3 concentration. At 0.08 mg Se/kg, birds fed SS and inorganic trace minerals had the lowest T3 concentration (P<0.05). Differences between mineral sources in the T3 concentration were observed only when no Se was added to the diets (P<0.05). A trend (P=0.089) in the interactive effects of Se levels and sources was noticed in the free T3 concentration in the serum. The difference between Se sources in the free T3 (**fT3**) concentration was observed at 0.16 mg Se/kg. An fT3 concentration in broiler chicks fed SY and organic trace microminerals showed a trend to quadratic increasing (P=0.091) according to the supplemented Se levels. Birds fed SY had the lowest free T3 concentration at 0.08 mg Se/kg

(P<0.05). A quadratic (P<0.05) or trend to quadratic (P=0.057) increase in free T4, were observed in the blood of birds fed SS and SY, respectively. A linear increase (P=0.037) in the free T3 to free T4 ratio was noticed with SY supplementation.

A linear response (*P*<0.01) was observed in the main effects of SeI, SeC, SeT and SeR for both Se sources (Table 4) where the concentration increased following the increased supplemented levels. A tendency (*P*=0.075) in a decreasing linear response was observed in the SeBal with the increasing Se levels. A quadratic response was observed in the SeF (*P*<0.05) in the birds fed SY and organic microminerals and, according the fitted polynomial quadratic model $SeF_{(SY)} = -0.2641x^2 + 0.1824x + 0.0245$, $r^2 = 0.26$, the optimal Se retention in the feathers was estimated at the dietary Se concentration of 0.30 mg Se/kg.

The increased supplementation levels tended (*P*=0.073) to decrease the Se liver concentration (Table 5) for both main effects and for the birds fed diets with SY and organic microminerals (*P*<0.05). Meanwhile, neither the Se sources nor levels influenced the Se concentration in the broilers breast muscle.

Multiple linear regression equations were not utilized to evaluate the relative bioavailability in this experiment because the basal diets were different, with different mineral sources, so it would be invalid to force a common intercept. Significant (P<0.05) linear regression equations in the same variable for the two Se sources were observed for the GSH-Px activity, SeC, SeF, SeT as well as SeR (Table 6). When the response to SS was set to 100%, the estimated relative bioavailability of SY was 126%, 117%, 126% and 126% based on the retention variables SeC, SeF, SeT and SeR , respectively, indicating greater SY availability to improve these corresponding parameters. However, the estimated relative bioavailability of SY for the GSH-Px activity was 81%.

Table 2. Growth performance of broiler chicks fed different dietary supplementation levels of selenium provided by organic and inorganic sources

Selenium source[1]	Selenium levels, mg/kg					Means	SEM[2]	P-value				
	0.0	0.08	0.16	0.24	0.32			Source	Level	S*L[3]	L[4]	Q[5]
Body weight, g/bird												
Means	510	490	508	502	494		6.84	0.432	0.019	0.144	0.180	0.977
SS	514	487	497 b	500	498	499					0.378	0.104
SY	506 AB	492 AB	521 Aa	502 AB	490 B	502					0.308	0.088
Average daily gain, g/bird/day												
Means	32.9 A	31.0 B	32.9 AB	32.1 AB	31.4 AB		0.672	0.429	0.017	0.132	0.165	0.977
SS	33.3	30.7	31.6 b	31.9	31.7	31.9					0.366	0.097[e]
SY	32.6 AB	31.3 B	34.1 Aa	32.2 AB	30.9 B	32.2					0.283	0.093[f]
Average daily feed intake, g/bird/day												
Means	47.3	45.5	47.2	46.4	45.9		0.790	0.661	0.122	0.107	0.847	0.160
SS	48.5 a	45.6	46.1 b	46.4	46.0	46.6					0.090	0.122
SY	46.0 b	45.3	48.2 a	46.4	45.8	46.3					0.847	0.160
Feed conversion rate, g/g												
Means	1.43	1.45	1.45	1.45	1.47		0.015	0.238	0.185	0.335	0.018	0.809
SS	1.44	1.46	1.46	1.46	1.45	1.46					0.661	0.449
SY	1.42 B	1.44 AB	1.44 AB	1.44 AB	1.48 A	1.44					0.004	0.616

[1] *SS* sodium selenite, *SY* selenium enriched-yeast.
[2] Standard error of means.
[3] Interactive effects between selenium and microminerals sources and selenium levels.
[4] Linear effect of supplementation selenium levels.
[5] Quadratic effect of supplementation selenium levels.
[A-B] Different uppercase letters in the same line and lowercase letters in the same column are different by Tukey test at 5%.
[ef] Different superscripts letter indicates difference in the orthogonal contrasts between equations.

Table 3. Antioxidant enzyme activity in the blood and hormonal concentration in the serum of broiler chicks fed different dietary supplementation levels of selenium provided by organic and inorganic sources

Selenium source[1]	Selenium levels, mg/kg					Means	SEM[2]	P-value				
	0.0	0.08	0.16	0.24	0.32			Source	Level	S*L[3]	L[4]	Q[5]
Glutathione peroxidase, U/L												
Means	79.1 D	153 C	216 BC	266 AB	319 A		24.3	<0.01	<0.01	0.121	<0.01	0.406
SS	93.8 C	165 BC	240 AB	335 Aa	334 A	233					<0.01[e]	0.174
SY	62.8 C	126 BC	194 B	198 Bb	304 A	183					<0.01[e]	0.904
Superoxide dismutase, U/L												
Means	98.7	88.9	92.1	81.9	92.9		7.17	0.524	0.647	0.394	0.647	0.243
SS	98.5	86.4	91.8	88.0	93.4	91.6					0.887	0.187
SY	98.9	91.5	92.5	75.8	92.3	90.2					0.420	0.549
Total T3, ng/ml												
Means	3.32	2.30	3.01	2.51	2.73		0.125	0.419	0.056	0.134	0.278	0.241
SS	3.99 Aa	2.41 B	3.39 AB	2.59 AB	2.84 AB	2.87					0.136	0.018
SY	2.76 b	2.16	2.75	2.45	2.59	2.68					0.816	0.682
Free T3, ng/ml												
Means	0.0056	0.0045	0.0055	0.0057	0.0054		0.0002	0.336	0.270	0.089	0.344	0.786
SS	0.0059	0.0048	0.0050 b	0.0060	0.0055	0.0053					0.648	0.163
SY	0.0052 AB	0.0042 B	0.0068 Aa	0.0059 AB	0.0053 AB	0.0056					0.343	0.091
Free T4, ng/ml												
Means	31.6	36.4	38.8	32.9	35.9		0.942	0.416	0.050	0.403	0.627	0.140
SS	34.0	37.5	42.7	34.5	37.5	35.2					0.604	0.048[e]
SY	29.3	36.0	37.2	31.0	34.3	36.6					0.805	0.057[e]
Free T3:Free T4[6]												
Means	1.6	1.3	1.4	1.8	1.7		0.1	0.230	0.078	0.735	0.070	0.125
SS	1.6	1.4	1.6	2.0	2.0	1.5					0.575	0.531
SY	1.6	1.3	1.3	1.9	1.5	1.7					0.037	0.451

[1] *SS* sodium selenite, *SY* selenium enriched-yeast.
[2] Standard error of means.
[3] Interactive effects between selenium and microminerals sources and selenium levels.
[4] Linear effect of supplementation selenium levels.
[5] Quadratic effect of supplementation selenium levels.
[6] Free T3:Free T4 x 10000
[A-B] Different uppercase letters in the same line and lowercase letters in the same column are different by Tukey test at 5%.
[ef] Different superscripts letter indicates difference in the orthogonal contrasts between equations.

Table 4. Selenium balance of broiler chicks fed different dietary supplementation levels of selenium provided by organic and inorganic sources

Selenium source[1]	Selenium levels, mg/kg					Means	SEM[2]	P-value				
	0.0	0.08	0.16	0.24	0.32			Source	Level	S*L[3]	L[4]	Q[5]
Intake, mg/bird												
Means	0.065 E	0.099 D	0.139 C	0.176 B	0.210 A		0.003	0.794	<0.01	0.794	<0.01	0.882
SS	0.067 E	0.098 D	0.138 C	0.175 B	0.211 A	0.138					<0.01[e]	0.435
SY	0.064 E	0.099 D	0.141 C	0.176 B	0.209 A	0.138					<0.01[e]	0.321
Carcass, mg/bird												
Means	0.050 C	0.062 BC	0.073 AB	0.075 AB	0.082 A		0.006	0.118	<0.01	0.717	<0.01	0.221
SS	0.046 B	0.062 AB	0.073 A	0.067 AB	0.078 A	0.065					<0.01[e]	0.261
SY	0.054 B	0.062 AB	0.074 AB	0.083 A	0.085 A	0.072					<0.01[e]	0.539
Feathers, mg/bird												
Means	0.0015 C	0.0020 BC	0.0023 AB	0.0025 A	0.0025 A		0.002	0.049	<0.01	0.725	<0.01	<0.01
SS	0.0015 C	0.0018 BC	0.0022 AB	0.0024 AB	0.0025 A	0.0021					<0.01[e]	0.331
SY	0.0015 B	0.0021 AB	0.0025 A	0.0027 A	0.0025 A	0.0023					<0.01[e]	<0.01
Total, mg/bird[6]												
Means	0.051 C	0.064 BC	0.077 AB	0.077 AB	0.084 A		0.006	0.136	<0.01	0.698	<0.01	0.168
SS	0.047 B	0.064 AB	0.077 A	0.069 AB	0.081 A	0.068					<0.01[e]	0.208
SY	0.055 B	0.064 B	0.077 A	0.085 A	0.088 A	0.074					<0.01[e]	0.488
Retention, mg/bird[7]												
Means	0.024 C	0.037 BC	0.050 AB	0.050 AB	0.057 A		0.006	0.136	<0.01	0.698	<0.01	0.168
SS	0.020 B	0.037 AB	0.050 A	0.042 AB	0.054 A	0.041					<0.01[e]	0.208
SY	0.028 B	0.037 AB	0.050 AB	0.058 A	0.061 A	0.047					<0.01[e]	0.488
Balance, %												
Means	34.1	34.0	35.3	25.6	27.4		4.55	0.393	0.393	0.487	0.075	0.421
SS	30.3	37.0	35.8	24.0	25.8	30.6					0.133	0.252
SY	37.9	31.1	34.7	33.2	29.0	33.2					0.301	0.997

[1] *SS* sodium selenite, *SY* selenium enriched-yeast.
[2] Standard error of means.
[3] Interactive effects between selenium and microminerals sources and selenium levels.
[4] Linear effect of supplementation selenium levels.
[5] Quadratic effect of supplementation selenium levels.
[6] Sum of the Se content in the feathers and carcass.
[7] Whole body Se in the birds in the beginning of the experiment: 0.0027 mg Se/kg.
[A-B] Different uppercase letters in the same line are different by Tukey test at 5%.

[ef] Different superscripts letter indicates difference in the orthogonal contrasts between equations.

Table 5. Selenium concentration (dry matter) on tissues of broiler chicks fed different dietary supplementation levels of selenium provided by organic and inorganic sources

Selenium source[1]	Selenium levels, mg/kg					Means	SEM[2]	P-value				
	0.0	0.08	0.16	0.24	0.32			Source	Level	S*L[3]	L[4]	Q[5]
Liver, mg/kg												
Means	1.55	1.47	1.41	1.37	1.33		0.06	0.288	0.504	0.185	0.074	0.764
SS	1.41	1.50	1.50	1.54	1.44	1.47					0.790	0.600
SY	1.69 A	1.45 AB	1.36 AB	1.18 B	1.24 AB	1.38					0.006	0.343
Breast muscle, mg/kg												
Means	0.296	0.279	0.278	0.292	0.267		0.08	0.311	0.516	0.501	0.293	0.999
SS	0.307	0.258	0.272	0.292	0.253	0.277					0.214	0.700
SY	0.285	0.299	0.284	0.293	0.281	0.288					0.811	0.700

[1] *SS* sodium selenite, *SY* selenium enriched-yeast.
[2] Standard error of means.
[3] Interactive effects between selenium and microminerals sources and selenium levels.
[4] Linear effect of supplementation selenium levels.
[5] Quadratic effect of supplementation selenium levels.
[A-B] Different uppercase letters in the same line are different by Tukey test at 5%.

Table 6. Relative bioavailability (RBV) of different selenium sources based on the slope ratio between linear regressions of GSH-Px, SeC, SeF, SeT and SeR on analyzed Se intake[1]

Dependent variables[2]	Selenium source[3]	Regression equations[4]	RBV	R^2	P-value
GSH-Px	SS	Y = 1756.8x – 8.6073	100	0.54	<0.01
	SY	Y = 1415.2x – 17.477	81	0.58	<0.01
SeC	SS	Y = 0.1879x + 0.041	100	0.20	<0.01
	SY	Y = 0.2368x + 0.039	126	0.27	<0.01
SeF	SS	Y = 0.006x + 0.0013	100	0.30	<0.01
	SY	Y = 0.007x + 0.0013	116	0.29	<0.01
SeT	SS	Y = 0.1939x + 0.0419	100	0.21	<0.01
	SY	Y = 0.2439x + 0.0404	125	0.28	<0.01
SeR	SS	Y = 0.1939x + 0.0148	100	0.21	<0.01
	SY	Y = 0.2439x + 0.0133	125	0.28	<0.01

[1] Dietary analyzed Se intake = feed intake during the whole experimental period times analyzed Se content (basal + supplemental) for each respective Se source.

[2] *GSH-Px* glutathione peroxidase, *SeC* selenium content in the carcass, *SeF* selenium content in the feathers, *SeT* selenium content in whole bird (carcass + feathers), *SeR* selenium retention.

[3] *SS* sodium selenite, *SY* selenium enriched-yeast.

[4] x is the analyzed Se intake (mg).

4 DISCUSSION

The objective of this experiment was to compare the performance and Se balance of broilers fed different Se levels and sources of selenium and microminerals. Increased supplementation levels resulted in an increase in the FC, which differs from the results found by Wang et al. (2015). Although no differences in the main effects of the others growth performance variables, data summarized in Table 3 show that among birds fed diets supplemented with SY and organic minerals, BW and ADG were greatest at the level of 0.16 mg Se/kg. Also, at the same dietary level, greater BW, ADG and ADFI were observed in birds fed SY compared to those fed SS. The optimal supplementation level estimated for BW and ADG of birds fed SY, 0.133 and 0.130 mg Se/kg, respectively are lower than the NRC (1994) estimates of 0.15 mg/kg, but similar to the values recommended by Rostagno et al. (2017) of 0.138 mg Se/kg for organic Se and microminerals. The values found in this experiment are also lower than those estimated by Cemin et al. (2018) of 0.75 mg Zn-L-SeMet/kg, respectively. Differently, Wang et al. (2010), Cai et al. (2012), Rao et al. (2013) and Couloigner et al. (2015) did not observe influence of neither level nor source on the growth performance. Interestingly, the main effects response shows that birds fed diets without Se supplementation had higher values of ADG when compared to other levels. Wang and Xu (2008) suggested that the lack of response in the growth performance may be explained by the fact that the Se level in the basal diet (0.138 mg Se/kg) was close to the NRC (1994) requirements of 0.15 mg Se/kg, which would be enough to maintain proper growth. Moreover, the semi-purified diets in this experiment were composed of 30% of corn, in which the Se content is majorly composed of Se-Met and, thus, the birds with no Se supplementation would be fed a certain amount of Se from Se source with high bioavailability (Rayman, 2004). In addition, Wang and Xu (2008) also suggested that a short experimental period may not be enough to evidence the Se deficiency symptoms in the birds.

As a cofactor for 5'-deiodinase (5'-ID), Se participates in the conversion of T4 to the biologically active T3, affecting the metabolism of protein, lipids and carbohydrates (Bckett et al., 1992; Smith et al., 2002; Madkour et al., 2015; Wang et al., 2015). The influence of Se on thyroid functioning was well illustrated by Jianhua et al. (2000) who noticed a decrease in the synthesis of T3 in chicks fed diets supplemented with iopanoic acid, a monodeiodinase inhibitor, which depresses the hepatic 5'-ID activity. Yet, the authors noticed a negative correlation of T3 plasmatic concentration with chick growth performance. In the current study,

a positive relationship was noticed between the thyroid hormones and growth performance. As detailed in Table 3, serum concentration of free T3 of chicks fed SY and organic trace mineral premix exhibited a similar pattern to those noticed for ADG (Table 2). The linear increase in the free T3: free T4 ratio in chicks fed SY supplemented diets indicates an increase in the conversion of free T3 from the inactive free T4. Such findings support those reported by Madkour et al. (2015) and Wang et al. (2015) that noticed higher T3 and low T4 serum concentrations in response to Se supplemental levels and organic Se sources (selenium yeast and dl-selenomethionine). The influence of selenium yeast supplementation in the thyroid hormone concentration agrees with the findings of Upton et al. (2008) who reported a possibly more efficient conversion of T4 into T3 after selenium yeast supplementation.

The GSH-Px enzyme is a component of the antioxidant system in the chickens and play an important role neutralizing reactive oxygen species responsible for causing damages to the DNA structure and cellular membrane which results in cellular death (Choct et al., 2004; Wang et al., 2018). Although some authors considered the SY more digestible and more bioavailable than SS (Briens et al., 2012; Wang et al., 2018), the SY supplementation resulted in lower values of GSH-Px activity in the blood in this experiment, which are in agreement with previous reports that observed higher values of GSH-Px after SS supplementation in the plasma, kidneys, pancreas and breast when compared with SY supplementation (Choct et al., 2004; Yoon et al., 2007; Dlouha et al., 2008; Wang et al., 2008; Wang et al., 2011; Liao et al., 2012; Rao et al., 2012). The possible metabolic pathways followed by different dietary Se sources differs. The Se metabolism was well described by Combs (2001), Rayman (2004), Rayman et al. (2008) and Dalto and Matte (2017). After absorption, selenite is converted in the liver into selenodiglutathione (GSSeSG) and further to hydrogen selenide (H_2Se), which has a central role in the Se metabolism. The H_2Se formed is either methylated and excreted or converted to selenophosphate ($HSePO_3^{2-}$) and its Se incorporated into tRNA Se-cysteinyl with further translation to selenoproteins, such as GSH-Px. On the other hand, Se-Met follow a transsulfuration into selenocysteine (Se-Cys) through a B_6-dependent reaction prior to its conversion into H_2Se (Dalto and Matte, 2017). Thus, the conversion of Se-Met into H_2Se requires more steps than the conversion of selenite, which may reduce its conversion into selenoproteins. In addition, Forstrom et al., (1978) and Sunde and Hoekstra (1980) hypothesized that the greater conversion of SS into GSH-Px might be related to the body attempt to store Se as selenoproteins. Meanwhile, Dalto and Matte (2017) stated that SS must be immediately metabolized to be either excreted or incorporated in an organic moiety in order

to reduce its toxicity. Moreover, Se-Met, which is the major form of the Se present in the SY (Rayman, 2004), can be non-specifically incorporated as a building block into different proteins throughout the body and, thus, less Se would be available to be incorporated into selenoproteins (Schrauzer, 2008). Data collected in this experiment (Table 4) evidence that chicks fed SY had more Se retained in the feathers than those fed SS. Thus, instead of being converted into selenoproteins such as GSH-Px, we hypothesized that Se-Met might have been redirected to other tissues to act as an amino acid, decreasing its incorporation into GSH-Px when compared with birds fed SS.

Nevertheless, it is worth mentioning that although recognized for its antioxidant properties, Se can also act as a pro-oxidant agent by increasing the hydrogen peroxide (H_2O_2) levels and leading to DNA damage (Ramoutar and Brumaghim, 2007; Hao et al., 2014). Moreover, the diets supplemented with SS contained inorganic microminerals such as Fe and Cu, which are also known for its pro-oxidant effects (Park and Imlay, 2003; Surai et al., 2003; Surai, 2005). Thus, it is still unclear whether the increase in the GSH-Px activity after Se supplementation represents either a greater antioxidant capacity or a physiological response to the increased ROS production induced by the supplemented inorganic microminerals.

Although a linear increase in the Se content in the carcass, feathers and in the Se retention of broiler fed graded Se levels (Table 5), influence of the Se source was only observed in the Se content of the feathers, in which broiler fed SY had an increase of 9.5% in the feathers Se content. These results are in agreement with the reports of Mahan and Parret (1996), Edens et al. (2001), Choct et al. (2004), Yoon et al. (2007) and Couloigner et al. (2015). Similarly, Edens et al. (2001) and Choct et al. (2004), reported not only increased Se retention, but also improvement in the feathering score of birds fed SY, which was addressed to the utilization of Se-Met in the synthesis of the keratin in feathers.

The linear regression equations for the Se content in the carcass, feathers and retention for both SS and SY are described in the Figures 2 to 5 and, according to the slope ratio between sources, the SY bioavailability was, respectively, 26.02, 16.66, 25.79 and 25.79% higher when compared to SS. Ammerman et al. (1995) define the term “bioavailability” as the degree to which an ingested nutrient is absorbed and utilized in the metabolism by the animal. Furthermore, these authors also stated that an increased bioavailability influence not only the dietary level but also the body tolerance of a certain nutrient. In this scenario, SS is considered the Se source with less benefits when compared to SY once, by not being stored, it would not be available to aid the body upon environmental challenge (Surai and Fisinin, 2014). Moreover,

due to its pro-oxidant properties, the excess of SS in diets lead to hepatic (Ashouri et al., 2015) and intestinal damages (Attia et al., 2010). On the other hand, the supplementation of Se-Met in the form of SY would build-up a Se storage in the muscles that not only would reduce the Se pro-oxidant activity but also aid the broiler in stressful conditions (Surai and Fisinin, 2014; Surai et al., 2017). Nevertheless, the greater Se retention in the feathers and greater SY bioavailability for the retention variables, indicates that the Se-Met was rather utilized as methionine than selenium itself. Upon increasing the methionine dietary concentration, Fatufe and Rodehutscord (2005) noticed a reduction in the methionine efficiency of utilization on broilers which may indicates greater nitrogen excretion of broiler fed diets with SY. This issue, however, remains unclear and need further investigation.

The Se concentration in the tissues is level and source dependent (Liao et al., 2012; Couloigner et al., 2015; Cemin et al., 2018). In the present study, neither the source nor the supplemented levels influenced the Se deposition in the breast muscle, whereas the main effects and the SY and organic microminerals supplementation showed linear reduction in the Se concentration in the liver of broiler chickens. As mentioned previously, the liver is a central organ in the Se metabolism and its mineral concentration is a sensitive criterion to assess the mineral status in the body (Liao et al., 2012). The Se-Met that is not converted into selenoproteins may be stored in organs with high protein synthesis such as the skeletal muscle, liver and kidney (Schrauzer, 2000) whereas SS cannot be stored in the body (Surai and Fisinin, 2014). Our data (Table 5) indicates that not only the Se retained in the chicks body increased with the increased Se levels, but also the Se retained in the feathers were greater when birds were fed SY, which suggest that the reduction in the Se concentration in the liver was due to its deposition as methionine in other tissues. Moreover, the reduced concentration in the liver might lead to other benefits. The study of Hao et al. (2014) noticed that the increased liver Se storage also increases the production of H_2O_2 after the supplementation of 0.62 mg nano-Se/kg, which, in turn, resulted in greater values of the serum concentration of aspartate transaminase (AST) and alanine transaminase (ALT), enzymes that are biomarkers of hepatic lesions (Biasi et al., 1991; Ashouri et al., 2015). In addition, Peric et al. (2009) observed that after 21 days feeding birds with diets containing different SS and Sel-Plex® proportions but same Se levels, the birds fed diets containing higher proportions of SS had increased serum concentrations of AST and ALT. These results combined demonstrate that, although no effect of the Se source in the Se liver concentration in this experiment, the organic Se supplementation can mitigate hepatic damage.

On the other hand, the increased Se concentration in the muscle may enhance the meat quality by increasing the GSH-Px concentration, which preserves the cellular integrity and consequently reduces the drip loss percentage (Choct et al., 2004; Cai et al., 2012). Different of the results found in this experiment, Sevcikova et al (2006), Wang and Xu (2007) and Hendl et al. (2010) who observed an increase in the Se concentration in the liver and muscle after the Se supplementation. According to Sevcikova et al. (2006) and Briens et al. (2012), the differences in the tissue Se concentration found by different authors can be explained by the experimental period length, calculation method and analytical method to determine the Se concentration.

5 CONCLUSIONS

To the best of our knowledge, this is the first research conducted to investigate supplemental Se levels and trace mineral sources on growing chick responses, where the other trace minerals in supplement had the same nature of the Se source assessed, i.e. organic or inorganic. Irrespective of the source, based on performance traits, the supplemental Se level for growing chicks under the conditions of this study is lower than the requirement estimate of NRC (1994). The ideal level of selenium yeast, provided as SY and organic trace minerals, for growth was 0.133 mg Se/kg diet, whereas for SS, the benefits of its supplementation on growth were not so clear. Selenium, regardless of the source, proved to be essential in antioxidant responses and thyroid hormone activation. Selenium utilization by chicks differed between the sources assessed, and outcomes suggested that selenium yeast is less efficiently used in antioxidant pathways compared with SS, but more efficiently retained into bodily proteins. Selenium yeast, provided as SY, showed a higher relative bioavailability compared to SS.

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UNIVERSIDADE FEDERAL DE VIÇOSA
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Viçosa, 08/01/15

CERTIFICADO

A comissão de ética no uso de animais de produção da universidade federal de viçosa certifica que o **processo nº 111/2014**, intitulado **"Exigência e biodisponibilidade de diferentes fontes de microminerais para frangos de corte"**, coordenado pelo **prof(a). Melissa Izabel Hannas,** está de acordo com os princípios éticos da experimentação animal, estabelecido pelo Conselho Nacional de Controle de Experimentação Animal - CONCEA e com a legislação vigente, tendo sido aprovado por esta Comissão em **17/Dez/2014**.

CERTIFICATE

The ethic commission in use of production animals of universidade federal de viçosa certifies that the **process number 111/2014**, named **"Requirement and bioavailability of different sources of trace minerals for broilers"**, coordinated by **prof(a). Melissa Izabel Hannas,** is in agreement with the Ethical Principles for Animal Research established by the National Council of Animal Experimentation Control (CONCEA) and with actual Brazilian legislation, and was approved by this commission on **Dec, 17th, 2014.**