



RESEARCH ARTICLE

Assessment of Dietary Selenium Sources in Commercial Male Broiler Breeders: Effects on Semen Quality, Antioxidant Status and Immune Responses

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ARTICLE HISTORY (19-130)

Received: April 01, 2019
Revised: May 18, 2019
Accepted: May 23, 2019
Published online: June 12, 2019

Key words:

Antioxidant status
Immune response
Male broiler breeders
Selenium sources
Semen quality

ABSTRACT

Objective of this experiment was to evaluate the effects of different dietary sources of selenium on semen quality, antioxidants status and immune response in commercial male broiler breeders (Ross-308). A total of 180 50-wk-old Ross-308 male broiler breeder birds were randomly distributed to 4 treatments, each of which had 5 replicates with 9 male birds each, with a 2-week pretreatment and 14-week trial period. In one treatment birds were fed a basal corn-soybean diet supplemented with inorganic i.e. Sodium Selenite (SS) and other three treatments birds were fed diet supplemented with one of the three organic selenium sources i.e. Selenium enriched yeast (SY), L-Seleno-methionine (L-Se-Meth) or Seleno-hydroxy-methionine (OH-Se-Meth), 0.3 mg/kg of diet. Performance of male broiler breeder was evaluated measuring their body weights, semen volume, sperm concentration and motility at end of 7th and 14th week of experiment (59th and 66th weeks of birds' age, respectively). Glutathione peroxidase activity, total antioxidant capacity and antibodies titer against Newcastle disease virus was also measured. Semen ejaculation volume (0.32, 0.34, 0.33 vs. 0.23 and 0.34, 0.33, 0.35 vs. 0.19 ml/ejaculate), spermatozoa count (2968, 3010, 3054 vs. 2366 and 2854, 3174, 2816 vs. 1700 10⁶/ml), percentages of live (93.3, 92.8, 92.9 vs. 88.6 and 93.0, 92.3, 92.4 vs. 87.9%) and dead spermatozoa (6.72, 7.24, 7.12 vs. 11.4 vs. and 7.0, 7.7, 7.56 vs. 12.1%), glutathione peroxidase status (14.1, 17.7, 16.5 vs. 11.8 and 15.3, 17.8, 16.9 vs. 10.5%) and total antioxidant capacity (7.14, 7.07, 6.98 vs. 5.75 and 6.64, 7.12, 6.84 vs. 4.86%) were improved in male broiler breeders fed diets supplemented with organic selenium sources (SY, L-Se-Meth, OH-Se-Meth) than those fed inorganic selenium (SS) at end of 7th and 14th week of experiment, respectively. Dietary selenium sources did not affect body weight, flock uniformity and antibodies titers against Newcastle disease virus at any phase of experiment. It was concluded that organic selenium supplementation improved semen quality characteristics and antioxidant status, however, did not affect body weight, flock uniformity and immune responses in commercial male Ross-308 broiler breeders.

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To Cite This Article: Ashraf S, Bhatti SA, Nawaz H and Khan MS, 2019. Assessment of dietary selenium sources in commercial male broiler breeders: effects on semen quality, antioxidant status and immune responses. Pak Vet J. <http://dx.doi.org/10.29261/pakvetj/2019.081>

INTRODUCTION

Selenium has been considered as a vital dietary nutrient that plays a significant role in productivity and health of poultry birds (Choct *et al.*, 2004); it provides a shield against oxidative stress, improves immune function, and reproductive performance (Kaur and Bansal, 2005). Severe deficiency of selenium leads to decreased production and reproduction performance of poultry.

Commercial poultry birds are under various types of stresses, and selenium, being an integral part of several seleno-proteins, can help in maintaining the antioxidant defenses preventing the tissue damage (Surai and Fisinin, 2014).

Selenium is an indispensable constituent of glutathione peroxidase. This enzyme family is important in integrated antioxidant system; it neutralizes potential threats to the integrity of cellular macromolecules by

eliminating hydrogen peroxide and detoxifying lipid hydroperoxides (Brigelius-Flohé, 1999). Selenium also plays an important role in immune function and production of immunoglobulin (Spallholz *et al.*, 1973). Selenium influences immune responses through its incorporation into selenoproteins such as selenocysteine (Hoffmann *et al.*, 2007). Supplementation of selenium in chicken diet increases antibody titers against Newcastle disease (Hegazy and Adachi, 2000).

Selenium supplementation in male broiler breeders is important for maintaining semen fertility (Surai, 2006). Selenium has been considered to be a constituent of spermatozoa and an essential element for spermatogenesis (Ebeid, 2012). The influence of dietary selenium on enhancing semen quality has been demonstrated in avian species (Maysa *et al.*, 2009). Seminal plasma can protect spermatozoa against lipid peroxidation in avian species (Cecil and Bakst, 1993). Dietary inclusion of selenium in male chicken's diet improved selenium-dependent glutathione peroxidase activity in the testes, seminal plasma, and spermatozoa, which resulted in decreased susceptibility of sperms to lipid peroxidation (Surai *et al.*, 1998). Selenoproteins including glutathione peroxidases protect against oxidative damage to spermatozoa throughout the process of sperm maturation, whereas mitochondrial glutathione peroxidase 4 and sperm nucleus glutathione peroxidase 4 serve as structural components of mature spermatozoa. Thus, selenium and selenoproteins ensure viability of spermatozoa by providing protection against reactive oxygen species (Ahsan *et al.*, 2014). Selenium deficiency may slow down the development of the testis and epididymis, decreasing sperm density in semen, and increasing the production of abnormal sperm cells (Graupner *et al.*, 2015).

Selenium is added in poultry ration because it is usually below animal requirement levels in feed ingredients and has variable bioavailability (Whanger, 2002). Traditionally, selenium has been supplemented in poultry rations as sodium selenite, which is an inorganic source. In comparison to inorganic sources of selenium, organic forms of selenium, such as seleno-cysteine and seleno-methionine, are absorbed by the same active-transport mechanism used for protein absorption, and therefore are more available to the body than inorganic selenium sources (Shanmugam *et al.*, 2015), moreover, organic sources of selenium are less excreted in the environment than inorganic sources (Wang *et al.*, 2009). Inclusion of organic selenium in cockerels' diet increased both count and motility of sperms, and also decreased dead sperm percentage in comparison to control birds fed inorganic selenium (Ebeid, 2012).

Traditional way of producing organic selenium is to grow yeast colonies of *Saccharomyces cerevisiae* on media enriched with sodium selenite. (Schrauzer, 2003). Most yeast products enriched with selenium have seleno-methionine as predominant form of selenium; nevertheless, its proportion may vary markedly (Whanger, 2002). Additionally, molecular differences can exist between commercial presentations which can potentially affect the availability of selenium to birds.

Many researchers attributed higher biological influence of selenium-enriched yeast to seleno-methionine, a synthetic analog of selenium with

methionine, which is a major form of selenium in Selenium-enriched yeast. Seleno-methionine is also a predominant form of selenium in some forage crops and cereals. Besides, seleno-methionine is the only seleno-amino acid that is non-specifically incorporated into tissue proteins in place of methionine, allowing the build-up of selenium reserves in organism. Moreover, selenomethionine has frequently been suggested as a supplementary source, for broiler breeders (Schrauzer, 2003). Despite extensive research that has been conducted comparing the effects of inorganic selenium with organic selenium, a little work is reported on comparing different forms of organic selenium in male broiler breeders and their effects on semen quality.

Therefore, objective of this experiment was to evaluate the effects of dietary sources of selenium on semen quality, antioxidant capacity and immune responses in commercial male broiler breeders (Ross-308).

MATERIALS AND METHODS

The study was conducted at Research and Development Farm of Faisal Chicks Pvt. Ltd., Multan, Pakistan. All procedures followed in the conduct of this experiment were in compliance with ethical standards of Animal Care of Institutional Biosafety Committee, University of Agriculture Faisalabad, Pakistan.

Birds, housing and environmental conditions: A total of 180 50-wk-old Ross-308 male broiler breeder birds were randomly distributed to 4 treatments, each of which had 5 replicates with 9 male birds each, with a 2-week pretreatment and 14-week trial period. Birds were reared in double sided flat deck type cage units measuring 8" x 16" x 18" and having 1 male bird in each. Fresh and clean water was offered round the clock. Birds were vaccinated against ND, IB, IBD, HPS, coryza, fowl pox etc. for multiple times during their whole life span of 0-66 weeks, according to recommendations of Breeder Company. guide lines; temperature, humidity were also provided according to the Breeders' recommendations for the same.

Experimental diets: In treatment one, the birds were fed a basal corn-soybean diet supplemented with inorganic i.e. Sodium Selenite (SS) and in other three treatments birds were fed diet supplemented with one of the organic selenium sources i.e. Selenium enriched yeast (SY), L-Seleno-methionine (L-Se-Meth) or Seleno-hydroxymethionine (OH-Se-Meth), 0.3 mg/kg of diet (Table 1). Experimental diets were fed following the Breeders' recommendations.

Data recording

Body weight and uniformity: Birds were individually weighed at the start of experiment (50th weeks of birds' age) and then, at end of 7th and 14th week of experiment (59th and 66th weeks of birds' age, respectively). Flock uniformity was determined as the percentage of pullets that had a body weight within $\pm 10\%$ of the flock average at a given age.

Semen quality: Semen samples were collected on weekly basis using abdominal massage technique by squeezing

the copulatory organs (Kharayat *et al.*, 2016). Ejaculation volume was measured by graduated tubes. Sperm concentration, viability, motility and deformity were determined during 7th and 14th week of experiment (59th and 66th weeks of birds' age, respectively). Spermatozoa concentration was estimated using a haemocytometer (Kharayat *et al.*, 2016). Sperm motility was subjectively assessed by visual estimation with contrast phase microscopy (Kharayat *et al.*, 2016). Sperm viability was determined as the percentages of live and dead spermatozoa that were impermeable and permeable to eosin stain, respectively (Kharayat *et al.*, 2016). Sperm deformity was measured using the in vivo crystal violet staining techniques (Santiago-Moreno *et al.*, 2009). After staining, air-dried slides were examined at 400× magnification to identify sperms displaying morphological abnormalities (including abnormalities in the head, connecting piece, tail and end piece).

Table 1: Ingredients and nutrients composition of male broiler breeders' diets

Ingredient Name	Male
Maize	52
Rice polish	25.95
Canola meal	8
Soybean meal	3
Sunflower meal	3.78
Guar meal	2
Calcium carbonate	1.5
Di-Calcium phosphate	1.3
Salt	0.2
Molasses	2
DL-Methionine	-
Sunflower Oil	-
Vitamin premix*	0.05
Mineral premix**	0.1
Sodium bicarbonate	0.12
Total	100
Nutrients composition, % (calculated)	
Crude protein	14
Metabolisable energy, Kcal/Kg	2800
Ether extract	5.3
Crude fiber	5.3
Ash	7.2
Calcium	1.2
Av. Phosphorus	0.4
Sodium	0.23
Chlorine	0.23
Dig. Lysine	0.49
Dig. Methionine	0.2
Dig. Methionine + Cysteine %	0.41
Threonine	0.4

*Mineral premix provides 10 mg Copper, 2 mg Iodine, 50 mg Iron, 120 mg Manganese and 100 mg Zinc per kg of diet. ** Vitamins premix provides 11000 IU Vitamin A, 3500 IU Vitamin D3, 100 IU Vitamin E, 5 mg Vitamin K, 3 mg thiamin, 12 mg Riboflavin, 55 mg nicotinic acid, 15 mg Ca Pantothenate, 4 mg Vitamin B6, 0.25 mg d-biotin, 2 mg Folic acid, 0.03 mg Vitamin B12 and 250 mg Choline chloride, per kg of diet.

Antioxidant status and immune responses: Blood samples were collected from wing vein of the birds from each replicate, at end of 7th and 14th week of experiment (59th and 66th weeks of birds' age, respectively). Each blood sample was divided into two equal parts. One part was transferred to EDTA containing vacutainer and was used for plasma collection by centrifuging the sample at 6,000 rpm for ten minutes. The supernatant plasma was collected, placed in plastic eppendorf tubes, and stored at -20°C until further analysis. Plasma antioxidant concentrations was measured by glutathione peroxidase

activity (Gajčević *et al.*, 2009) and total antioxidant capacity (Rizk *et al.*, 2017). Second part of blood samples were transferred to gel-activated tubes and serum samples were collected. The supernatant serum was collected, placed in plastic eppendorf tubes, and stored at -20°C until further analysis. For immune responses, antibodies titer against Newcastle disease virus was measured by hemagglutination-inhibition test by the method of OIE (2012).

Statistical analysis: Data collected were analyzed using GLM Procedures of Minitab Statistical Software 18. Means were compared using Tukey's Test.

RESULTS

Body weight and uniformity: Live body weight and flock uniformity was not influenced ($P>0.05$) in birds fed diets supplemented with inorganic or three forms of organic Selenium (Table 2) at the end of 7th and 14th week of experiment (59th and 66th weeks of birds' age, respectively).

Semen quality: Inclusion of organic sources of selenium (SY, L-Se-Meth, OH-Se-Meth) increased ($P<0.05$) the ejaculation volume, sperm concentration and percentage of live spermatozoa, and reduced ($P<0.05$) dead spermatozoa percentage in male birds as compared to inorganic selenium (SS) supplemented birds, at end of 7th and 14th week of experiment (59th and 66th weeks of birds' age, respectively; Table 3). However, sperm mobility score was not influenced ($P>0.05$) by dietary sources of selenium at any phase of experiment (Table 3). The percentages of normal and abnormal sperms were also not influenced ($P>0.05$) by dietary sources of selenium at end of 7th week of experiment (59th weeks of birds' age), however, percentage of normal sperms increased, and of abnormal sperms decreased by inclusion of organic sources of selenium (SY, L-Se-Meth, OH-Se-Meth) at end of 14th week of experiment (66th weeks of birds' age).

Antioxidant status: Inclusion of organic sources of selenium (SY, L-Se-Meth, OH-Se-Meth) improved ($P<0.05$) glutathione peroxidase activity (Fig. 1) and total antioxidant capacity (Fig. 2) in male breeder birds as compared to those fed inorganic selenium supplemented diet.

Immune responses: Antibodies titers against NDV was not different ($P>0.05$) in male broiler breeders fed different dietary sources of selenium at any phase of experiment (Fig. 3).

DISCUSSION

Dietary selenium sources did not affect ($P>0.05$) body weight and flock uniformity in commercial male Ross-308 broiler breeders at any phase of experiment. Results of this study are in agreement with those of Mohanty *et al.* (2018), who reported that body weights of coloured broiler breeders were not different ($P>0.05$) among different treatments fed diets with inorganic or organic sources of selenium, from 40th to 52nd week of age. In other studies (Jiang *et al.*, 2009; Niu *et al.*, 2009),

Table 2: Effects of various dietary selenium sources on body weight and uniformity in Ross-308 male broiler breeders

Parameters	Experimental groups*				SEM	P-Value
	SS	SY	OH-Se Met	L-Se Met		
	50 Weeks of age					
Body weight, Kg	5.16	5.14	5.15	5.15	0.01	0.688
Uniformity ($\pm 10\%$ of average), %	100	100	100	100	-	-
	7 th week of experiment (59 th weeks of birds' age)					
Body weight, Kg	5.47	5.39	5.42	5.29	0.12	0.168
Uniformity ($\pm 10\%$ of average), %	89.1	91.3	90.4	92.2	0.43	0.089
	14 th week of experiment (66 th weeks of birds' age)					
Body weight, Kg	5.61	5.52	5.73	5.59	0.26	0.098
Uniformity ($\pm 10\%$ of average), %	90.1	90.3	93.4	89.7	0.56	0.092

*SS = Sodium Selenite @ 0.3mg/kg of feed; SY= Selenium enriched yeast @ 0.3mg/kg of feed; OH-Se Met = Hydroxy-Selenomethionine @ 0.3mg/kg of feed; L-Se Met = L-Selenomethionine @ 0.3mg/kg of feed.

Table 3: Effects of various dietary selenium sources on semen quality parameters in Ross-308 broiler breeders

	7 th week of experiment (59 th weeks of birds' age)						14 th week of experiment (66 th weeks of birds' age)					
	SS	SY	OH-Se Met	L-Se Met	SEM	P-Value	SS	SY	OH-Se Met	L-Se Met	SEM	P-Value
Volume, ml/ejaculate	0.23 ^b	0.32 ^a	0.34 ^a	0.33 ^a	0.01	0.0001	0.19 ^b	0.34 ^a	0.33 ^a	0.35 ^a	0.01	0.0001
Concentration, 10 ⁶ /ml	2366 ^b	2968 ^a	3010 ^a	3054 ^a	70	0.0001	1700 ^b	2854 ^a	3174 ^a	2816 ^a	157	0.0001
Live Sperm %	88.6 ^b	93.3 ^a	92.8 ^a	92.9 ^b	0.66	0.0001	87.86 ^b	93.0 ^a	92.26 ^a	92.44 ^a	0.61	0.0001
Dead Sperm %	11.4 ^a	6.72 ^b	7.24 ^b	7.12 ^b	0.66	0.0001	12.14 ^a	7.04 ^b	7.74 ^b	7.56 ^b	0.61	0.0001
Motility, score	4.01	4.07	4.04	4.17	0.27	0.528	4.1	4.04	4.44	4.0	0.29	0.429
Normal Sperms %	91.1	92.04	92.02	92.14	6.16	0.09	89.64 ^b	91.8 ^a	91.92 ^a	92.01 ^a	0.15	0.001
Abnormal Sperm %	8.90	7.96	7.98	7.86	6.16	0.09	10.36 ^a	8.20 ^b	8.08 ^b	7.99 ^b	0.15	0.001

SS = Sodium Selenite @ 0.3mg/kg of feed; SY= Selenium enriched yeast @ 0.3mg/kg of feed; OH-Se Met = Hydroxy-Selenomethionine @ 0.3mg/kg of feed; L-Se Met = L-Selenomethionine @ 0.3mg/kg of feed; ^{a-c} Values not followed by a common superscript differ significantly (P<0.05).

it has also been reported that dietary sources (inorganic or organic) of selenium did not influence ($P>0.05$) body weights of male poultry birds. No influence of selenium on body weights of birds may be due to the reason that selenium has no direct role on body weight and growth responses, when birds are reared under non stress environment (Zadeh *et al.*, 2018). In contrast, Rajashree *et al.* (2014) found improvement in the body weights of commercial broiler breeder birds fed diets supplemented with organic selenium than those fed inorganic selenium or control, at 29th and 34th week of age. In the present experiment, no change in the body weight was observed, as these birds had passed over 50 weeks of their age. The variation in the results could also be due to different strains of birds, composition of diets, seasons, and weather conditions during the experimental periods (Zadeh *et al.*, 2018). Flock uniformity was also not influenced by different selenium sources during the experimental period; it was because birds with 100% uniformity were selected at the start of experiment and body weights of birds were not influenced by dietary treatments. Quant *et al.* (2010) also reported no effect of dietary organic selenium on pen uniformity over 19 week period, in Cobb-500 broiler breeders.

Semen ejaculation volume, sperm concentration, percentages of live and normal spermatozoa, were improved by dietary inclusion of organic selenium in male broiler breeders. These results are in accordance with Maysa *et al.* (2009) who concluded that semen amount, concentration, and live percentage of sperms were improved ($P<0.05$) in males breeders fed organic selenium than those fed inorganic selenium. Ebeid (2012) and Rizk *et al.* (2017) also reported that organic selenium supplementation in diet of male breeder birds improved ($P<0.05$) semen quality traits, by increasing the semen volume, and count of spermatozoa, and reduced dead and abnormal spermatozoa percentages. Surai and Fisinin (2014) reported that dietary supplementation of selenium is very much crucial for maintaining the high semen quality in avian species. In contrast, Slowinska *et al.*

(2011) reported that semen sperm concentration was not different in male birds fed diet containing inorganic or organic sources of selenium.

In the present study, sperm motility score was not influenced ($P>0.05$) by dietary sources of selenium at any phase of the experiment. Similar results have been reported by Slowinska *et al.* (2011) who reported that percentage of motile spermatozoa was not different ($P>0.05$) in male birds fed inorganic or organic sources of selenium. However, Ebeid (2012) and Rizk *et al.* (2017) found that organic selenium supplementation improved ($P<0.05$) motility of spermatozoa in male breeder birds. The variations in semen quality traits may be due to differences in birds' strains and age, composition of diets, and weather conditions during the experimental periods.

Glutathione peroxidase activity and total antioxidant capacity was improved by inclusion of organic sources of selenium in male broiler breeders. Maysa *et al.* (2009) observed an increase in glutathione peroxidase status in male breeder birds fed organic selenium compared with those on inorganic selenium. Rizk *et al.* (2017) reported that supplementation of organic selenium in diet of 22-week male Sinai breeder birds improved total antioxidant capacity, during early production period (22-34 weeks). Selenium is an essential trace element that up-regulates a major component of the antioxidant defense mechanism by controlling the glutathione pool of the body and its major selenium-containing antioxidant enzymes (Jiang *et al.*, 2009). Possible reason for improvement in antioxidant status is could be that organic selenium has better bioavailability than inorganic selenium, and thus improved antioxidants status and reduced production of lipid peroxidation products (Zadeh *et al.*, 2018). However, Li *et al.* (2018) reported that supplementation of inorganic or organic sources of selenium in Lingnan Yellow broiler breeders, had no influence ($P>0.05$) on glutathione peroxidase and total antioxidant capability in serum. These differences might be due to differences in birds' strains and age, analytical procedure and weather conditions during the experimental periods.

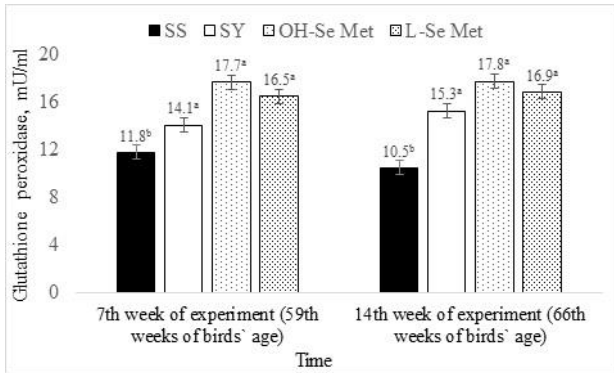


Fig. 1: Effects of various dietary selenium sources on plasma glutathione peroxidase of Ross-308 broiler breeders. SS = Sodium Selenite@ 0.3mg/kg of feed; SY= Selenium enriched yeast @ 0.3mg/kg of feed; OH-Se Met = Hydroxy-Selenomethionine @ 0.3mg/kg of feed; L-Se Met = L-Selenomethionine @ 0.3mg/kg of feed. ^{a-c} Values not followed by a common superscript differ significantly (P<0.05).

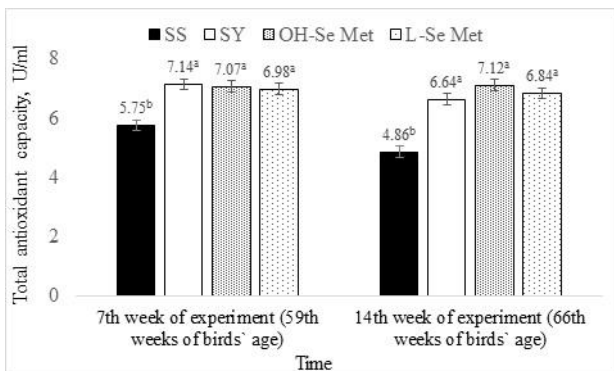


Fig. 2: Effects of various dietary selenium sources on plasma total antioxidant capacity of Ross-308 broiler breeders. SS = Sodium Selenite@ 0.3mg/kg of feed; SY= Selenium enriched yeast @ 0.3mg/kg of feed; OH-Se Met = Hydroxy-Selenomethionine @ 0.3mg/kg of feed; L-Se Met = L-Selenomethionine @ 0.3mg/kg of feed. ^{a-c} Values not followed by a common superscript differ significantly (P<0.05).

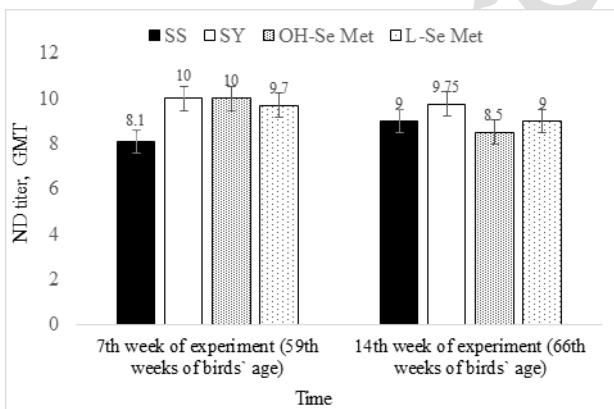


Fig. 3: Effects of various dietary selenium sources on immune responses of Ross-308 broiler breeders. SS = Sodium Selenite@ 0.3mg/kg of feed; SY= Selenium enriched yeast @ 0.3mg/kg of feed; OH-Se Met = Hydroxy-Selenomethionine @ 0.3mg/kg of feed; L-Se Met = L-Selenomethionine @ 0.3mg/kg of feed.

Antibodies titer against NDV was not influenced by different dietary sources of selenium in male broiler breeders. Similar results have been reported by Selim *et al.* (2015) who concluded that antibodies titer against new castle disease virus was not influenced (P>0.05) by supplemental selenium source in male birds. However, Niu *et al.* (2009) reported that titer of total antibody

significantly increased (P<0.05) with increasing dietary selenium level in male broilers. Bakhshalinejad *et al.* (2018) reported that total anti-sheep red blood cell titers were enhanced (P<0.05) by using organic sources of selenium than inorganic selenium in Ross-308 male broilers. These variations might be due to the differences in birds' strains and age, vaccination program, stress conditions, and farm facilities in which the birds were reared in present study.

Conclusions: It is concluded that dietary organic selenium supplementation improved semen quality characteristics and antioxidant status; however, it did not affect body weight, flock uniformity and immune responses in commercial male Ross-308 broiler breeders.

Acknowledgments: The authors acknowledge the material support provided by Dr. Faisal Shahid, Faisal Chicks Pvt. Ltd., Multan, Pakistan, for conducting the research. The authors also acknowledge the fellowship provided by the Higher Education Commission of Pakistan to Shahzad Ashraf under the framework of HEC Indigenous PhD Fellowship Program.

Authors contribution: SA, Experimental work and Manuscript writing; SAB, Experimental designing; HN, Manuscript preparation; MSK, Data analysis

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