Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X 2021 / v.23 / n.3 / 001-008

http://dx.doi.org/10.1590/1806-9061-2020-1277

Original Article

Impact of Selenium-Supplemented Diets on Egg Morphometry and Quality in Four Varieties of Indigenous Aseel Chicken

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■Keywords

Aseel, egg morphometry, egg quality, selenium supplementation.

Submitted: 10/October/2020
Approved: 14/March/2021

ABSTRACT

The present study aimed at evaluating the effects of dietary selenium (Se) supplementation on egg morphometric and quality traits in Aseel chicken. In total, 84 adult molted Aseel hens, comprising 4 varieties (Lakha, Mushki, Peshawari, and Mianwali), were divided into 3 dietary treatments (organic at 0.30 ppm, inorganic at 0.30 ppm, and without selenium) with 7 replicates (1 bird in each) under randomized complete block design. Data were analyzed using factorial ANOVA and means were compared using Duncan's Multiple Range Test. The results indicated greater egg length in Mushki and Mianwali, lower egg breadth in Lakha, the highest ($p \leq 0.05$) egg volume and surface area in Mianwali, and higher shape index in Lakha and Peshawari. Lakha had the highest Haugh unit and lower eggshell thickness with and without membrane than the other three varieties. Interaction of Se sources and Aseel varieties exhibited the highest ($p \leq 0.05$) egg volume and surface area in Mianwali variety when fed on organic Se supplemented diet. Similarly, the interaction showed the highest eggshell thickness with membrane in Peshawari and without membrane in Mianwali when fed on inorganic Se supplemented diet. From the findings, it can be concluded that egg physical characteristics of Mianwali variety hens can be improved by the supplementation of Se-enriched yeast, as an organic Se source, in the diet.

INTRODUCTION

Egg morphometric parameters of the fertile eggs have gained a tremendous importance as they influence the hatching traits, including chick weight (Narushin *et al.,* 2002; Narushin, 2005), egg hatchability (Narushin & Romanov, 2002a; Narushin, 2005), eggshell quality (Narushin 2001b; Narushin, 2005), and egg interior quality (Narushin & Romanov, 2002b; Narushin, 2005). The morphometric characteristics of the egg play an essential role in embryo development and successful hatching (Narushin & Romanov, 2002). Various species of birds lay eggs with different shapes and sizes (Sekeroglu & Altuntas, 2009), as different genetic groups (Nwachukwu *et al.,* 2006; Yakubu *et al.,* 2008) or Aseel varieties (Ahmad, 2013) stated to be responsible for changes in egg length and breadth. Furthermore, it is believed that shape index responds differently for different breeds (Ali *et al.,* 2012), varieties (Ahmad, 2013), and strains (Anderson, 1996). Egg volume is another essential egg morphometric parameter described with discrepancies in values due to different Aseel varieties (Ahmad, 2013) and layer strains (Rayan *et al.,* 2010). Egg surface area, likewise, also varies with the variations in genetic makeup (Anderson, 1996; Anderson *et al.,* 2004; Ahmad, 2013). The internal quality of eggs is also vital for both table and hatching eggs (Sekeroglu & Altuntas, 2009). Different breeds (Ali *et al.,* 2012), varieties (Shafiq *et al.,* 2013), and strains (Monira *et al.,* 2003) of hens are among the significant factors that influence egg quality. The Aseel variety has heavy bodyweight (Ahmad *et al.,* 2014) and strong muscular thighs (Dohner, 2001), can have the ability to withstand and survive in harsh tropical climatic conditions. Despite these excellent features, its potential regarding fertility and hatchability is not fully exploited because of poor egg physical characteristics and internal quality. Therefore, there is a dire need to improve the egg morphometric and quality traits of Aseel hens to achieve a reasonable progeny size. A balanced diet with dietary supplementation of selenium may improve egg morphometric and quality traits of Aseel eggs.

The appearance of compounds as nano-sized elements can result in unique properties, showing interactions with other ingredients, and change coordination with other compounds in biological systems, consistent with demonstration of compounds as larger elements or in solution (Gangadoo *et al.,* 2020). Selenium is amongst the essential minerals compulsory for optimum development and production in birds. It maintains numerous functions linked to poultry rearing, production, and prophylaxis. Selenium is a fundamental portion of the enzyme glutathione peroxidase that works as an antioxidant enzyme and helps to govern levels of hydrogen peroxide and lipid peroxides (Ahmadi *et al.,* 2018). Nowadays, selenium is known to modulate a wide spectrum of key biological processes and play a vital role in immune system functioning, protecting from oxidative injury, and carcinogenesis reticence as well. On the other hand, it is generally known that greater selenium quantities are dangerous causing several grave fitness complications (Ahmadi *et al.,* 2019). The nano-selenium supplementation may improve feed intake, feed conversion, and weight gain in poultry. Moreover, nano-selenium stimulates immune system of broiler chickens in comparison with the other sources of selenium, as at nano-scales selenium shows novel and new potent properties. Consequently, supplementing poultry feed with nano-selenium is recommended to propagate poultry with enhanced performance (Shabani *et al.,* 2019).

Dietary selenium (Se) is an essential trace mineral for poultry (NRC, 1994), identified to increase and maintain shell quality in older hens. Selenium, either inorganic as sodium selenite or organic such as selenocysteine, selenomethionine, and / or Se-enriched yeast (Foster & Sumar, 1997) is reported to have a positive effect on various egg characteristics. Organic Se being more bio-available (Cruz & Fernandez, 2011) has been preferred to improve shell quality and maintenance of egg freshness during storage (Papazyan *et al.*, 2006). However, others still differ and state no difference in the performance of both organic and inorganic Se (Paton *et al.,* 2000b). Very little work, so far, has been published on the evaluation of different egg physical and morphometric characteristics in Aseel chicken. Therefore, a study was designed to investigate the main and interactive effects of Se supplementation on egg morphometric and quality traits in different varieties of Aseel chicken. There is a hypothesis that dietary Se supplementation improves egg morphometric (egg length, breadth, volume, surface area, and shape index), and quality (egg weight, shell thickness, Haugh unit, and yolk index) characteristics in Aseel hens.

MATERIALS AND METHODS

Experimental birds, housing, and ration

The present study was conducted at the Indigenous Chicken Genetic Resource Centre (ICGRC), Department of Poultry Production, University of Veterinary and Animal Sciences (UVAS), Lahore, for eight weeks duration. The ethical committee of the University of Veterinary and Animal Sciences, Lahore, approved the protocol of the trial. In total, 84 adult females from four varieties of indigenous Aseel were used. From each array, 21 hens were further divided into three groups (7 each) according to the type of Se-supplemented diets in a Randomized Complete Block Design. Each of the seven hens was kept in an individual cage to get a separate record of egg production. The experiment was conducted in a well-ventilated open housing system. A corn-soybean meal basal diet was formulated for females keeping in view their nutritional requirement (NRC, 1994). The basal diet was iso-nitrogenous and iso-caloric containing 15.1% CP and 2,682 ME Kcal/kg, respectively. Before the initiation of the trial, the basal diet was analyzed for Se content. The quantity of Se in the basal diet was 0.04 mg/kg. Se from sodium selenite and Se-enriched yeast (Se-yeast Sel-plex® Alltech, Nicholasville, KY) was supplemented at 0.30 ppm into the basal diet. Control diet was without selenium supplementation (Table 1).

Measurements

In total, 84 eggs (21 from each variety), according to dietary Se treatments, were picked to study egg morphometric (egg length, egg breadth, shape index, egg surface area, and egg volume) and quality (egg weight, shell thickness with and without membrane,

Table 1 – Ingredient and nutrient composition of basal diet.

Ingredients (%)	Quantity
Corn	42.6
SBM[1]	15.6
Corn Gluten (60%)	1.0
Rice Tips	19.0
Wheat bran	13.0
DCP[2]	1.2
$CaCO_3$	7.4
DL-Methionine	0.2
Nutrient composition (%)	
CP	15.1
ME (kcal/kg)	2682
Calcium	2.8
Phosphorus	0.4
Lysine	0.9
Methionine	0.5
Selenium (mg/kg)	0.04
Vitamin premix[3]	0.20
Mineral premix[3]	0.30

[1]Soybean meal.

2Dicalcium Phosphate.

3Vitamin-mineral premix provided per kg of diet: Vitamin A (retinyl acetate), 12,000 IU; vitamin D3 (cholecalciferol), 5,000 IU; vitamin E (DL-α-tocopheryl acetate), 50 mg; vitamin K3, 3 mg; thiamin, 2 mg; riboflavin, 7 mg; vitamin B6, 5 mg; vitamin B12, 15 µg; pantothenic acid, 50 mg; folic acid, 1 mg; biotin, 200 µg; Fe, 80 mg; Cu, 10 mg; Zn, 80 mg; I, 1 mg.

Haugh unit, and yolk index) parameters. These eggs were weighed using electronic balance capable of measuring up to 0.1 g. In contrast, egg length and breadth were measured with the help of a digital vernier caliper capable of measuring up to 0.1 cm. The shape index was taken as the ratio between egg width and egg length (Anderson *et al.,* 2004), whereas egg volume and egg surface area were determined using two separate formulae for each parameter and to take the average of the results (Etches, 1996). Eggs were then broken one by one, and the egg contents were carefully poured into a petri dish. Eggshell thickness was measured with and without vitelline membranes with the help of a dial pipe gauge and was taken as the average of three separate measurements from three different places without cracking the shell. Yolk index was chosen as the ratio between yolk height and yolk width whereas the Haugh unit score was measured using the egg weight and albumen height values in the formula $H_{unit} = 100 \times \log (H + 7.57 - 1.7 \times W^{0.37})$, where H = albumin height (mm), and W = egg weight (g).

Data were analyzed using factorial ANOVA with the help of GLM procedure of Statistical Analysis System (SAS Institute Inc., 2002-04) and means were separated using Duncan's Multiple Range Test at 5% probability level, considering each bird as an experimental unit.

RESULTS AND DISCUSSION

Egg morphometric traits

Mushki and Mianwali hens indicated significantly ($p \leq 0.05$) greater egg length than those of Lakha and Peshawari (Table 2). This greater egg length may be due to variations in genotype and bodyweight as different strains, or varieties lay eggs of varying weight and size because of different bodyweights (Bell & Weaver, 2005). Similarly, it was also observed that strains with varying bodyweight lay eggs of varying weight and size, which have their unique egg length (Arafa *et al.,* 1982). Mianwali variety in interaction with inorganic Se supplemented diet revealed the highest egg length (56.04±0.7) indicating synergistic effect.

Similarly, significant differences ($p \leq 0.05$) were observed in egg breadth among varieties and their interaction with Se sources (Table 2). Birds of Lakha variety exhibited significantly lower ($p \leq 0.05$) egg breadth than the rest of the three types, which may be due to genetic variations like different strains or varieties which lay eggs of varying egg weight and size (Bell & Weaver, 2005). Similar results were reported in a study on strains of varying bodyweights, which lay eggs of varying egg weight and size, having their unique egg breadth (Arafa *et al.,* 1982). A study conducted on Aseel, likewise, indicated maximum egg breadth in Mushki Aseel (Ahmad, 2013). Egg breadth was the highest when Mushki was fed diet supplemented with inorganic Se showing a positive relationship between Mushki and inorganic Se. However, the Se sources alone could not produce any effect on egg breadth.

Mianwali variety demonstrated the highest egg volume followed by Mushki, Peshawari, and Lakha (Table 2) that may be the result of more top egg length and breadth in several ranges. Another study on Aseel, however, indicated higher egg volume in Lakha and Mushki than than in the other two types (Ahmad, 2013). Brown breeder hens laid eggs of significantly higher egg volume as compared to those of white egg layers (Rayan *et al.,* 2010). Interaction of organic Se and Aseel varieties resulted in the highest egg volume in Mianwali variety, which may be due to its trend and synergistic effect with organic Se. However, Se sources could not produce any effect on egg volume. Results indicated the highest egg surface area in Mianwali variety followed by Mushki, Peshawari, and Lakha (Table 2). This may be due to strain variation (Anderson, 1996; Anderson *et al.,* 2004) and comparatively higher egg length and breadth in that variety. Significant differences in egg surface area, likewise, have already

Table 2 – Effects of dietary selenium supplementation on egg morphometric traits in four varieties of Aseel chicken.

Parameters Variables		Egg Length (mm)	Egg Breadth (mm)	Egg Volume (mm^3)	Surface Area (mm^2)	Shape Index
Se Sources						
Organic Se		52.54±0.9	40.25±0.4	44.36±1.6	61.38±1.5	76.81±1.2
Inorganic Se		51.75±1.4	40.47±0.7	45.27±1.9	62.17±1.8	78.52±1.2
Control		51.79±0.8	40.25±0.4	43.22±1.16	60.36±1.1	77.83±0.8
Varieties						
Lakha		49.08±1.6^{b}	38.99±0.8^{b}	38.75±1.6^{d}	56.07±1.5^{d}	79.73±1.1^{a}
Mushki		53.84±0.6^{a}	40.66±0.5^{a}	45.75±1.3^{b}	62.72±1.2^{b}	75.55±0.7^{b}
Peshawari		50.71±0.9^{b}	40.27±0.5^{a}	42.81±1.1^{c}	59.99±1.0^{c}	79.57±1.4^{a}
Mianwali		54.49±0.7^{a}	41.37±0.4^{a}	49.81±0.9^{a}	66.42±0.8^{a}	76.03±1.2^{b}
Se Sources × Varieties						
Organic Se	Lakha	52.82±2.6^{abc}	40.95±1.0^{ab}	42.61±3.2^{dc}	59.76±3.1^{dc}	77.73±2.0^{ab}
	Mushki	53.85±0.9^{abc}	39.68±0.3^{b}	43.52±0.8^{dc}	60.69±0.7^{dc}	73.73±1.4^{b}
	Peshawari	49.53±2.2^{c}	39.23±0.2^{b}	39.26±1.4^{de}	56.63±1.3^{d}	79.54±3.7^{ab}
	Mianwali	53.97±0.2^{abc}	41.15±1.1^{ab}	52.04±1.0^{a}	68.42±0.9^{a}	76.23±1.9^{ab}
Inorganic Se	Lakha	44.35±1.3^{d}	36.65±0.9^{c}	34.69±1.1^{e}	52.14±1.1^{e}	82.74±0.8^{a}
	Mushki	54.93±0.5^{ab}	42.20±0.2^{a}	50.21±0.0^{ab}	66.81±0.0^{ab}	76.83±0.8^{ab}
	Peshawari	51.71±1.1^{abc}	41.52±0.4^{ab}	45.95±0.3^{bc}	62.95±0.3^{bc}	80.39±2.5^{ab}
	Mianwali	56.04±0.7^{a}	41.51±0.7^{ab}	50.21±0.9^{ab}	66.80±0.8^{ab}	74.10±1.8^{b}
Control	Lakha	50.10±2.0^{bc}	39.36±0.8^{b}	38.95±2.1^{de}	56.32±2.1^{de}	78.70±1.8^{ab}
	Mushki	52.73±1.4^{abc}	40.11±0.8^{ab}	43.52±1.9^{dc}	60.67±1.8^{dc}	76.09±0.7^{ab}
	Peshawari	50.88±1.5^{abc}	40.06±1.1^{ab}	43.21±1.1^{dc}	60.40±1.0^{dc}	78.78±1.5^{ab}
	Mianwali	53.45±1.9^{abc}	41.46±0.4^{ab}	47.17±1.7^{abc}	64.05±1.5^{abc}	77.75±2.5^{ab}

Superscripts on different means within rows differ significantly ($p \leq 0.05$).

been reported in Aseel varieties (Ahmad, 2013). The highest egg surface area was observed again in Mianwali variety when coupled with organic Se whereas Se sources alone could not show any effect on the egg surface area.

Among varieties, shape index was significantly higher ($p \leq 0.05$) in Lakha and Peshawari type compared with Mushki and Mianwali (Table 2), which may be due to differences in genotype. In contrast, another study on indigenous Aseel reported a higher shape index in Mianwali and Peshawari than the rest of the two varieties (Ahmad, 2013). Different strains also have clear indications of having a significant effect on egg shape index (Anderson, 1996; Ali *et al.,* 2012), resulting in higher egg shape index in brown layers than white egg layers (Brand *et al.,* 2004; Rayan *et al.,* 2010). The interaction of Se sources and varieties showed the highest shape index in Lakha when fed diet containing inorganic Se, which might be due to positive relationship between Lakha variety and inorganic Se. Different Se sources alone could not show any effect on shape index. Shape index, likewise, remained unchanged despite supplementation of Se in diet (Pavlovic *et al.,* 2010), showing no response of sodium selenite or selenium-enriched yeast on shape index (Invernizzi *et al.,* 2013). Organic Se in the form of Sel-Plex™ also did not influence shape index (Renema, 2004; Hanafy *et al.,* 2009).

Egg quality traits

Enhanced egg weight was observed in Mianwali variety followed by Mushki, Peshawari, and Lakha (Table 3), which might be due to higher bodyweight of the respective birds as speculated that bodyweight and egg weight are positively associated. Interaction of different treatments revealed that Mianwali variety produced eggs with an improved weight when diet was supplemented with organic Se, which might be attributed to Mianwali variety based on its higher bodyweight, further enhanced by supplementation of organic Se showing synergistic impact with Mianwali variety. However, Se sources alone could not exert any influence on egg weight. In a similar study on quail, no significant effect of different Se sources was reported on egg weight (Cruz & Fernandez, 2011), which clearly showed that egg weight remained unchanged despite dietary supplementation of Se in the diet (Attia *et al.,* 2010; Arpasova *et al.,* 2012).

Significantly lower eggshell thickness ($p \leq 0.05$; with and without membrane) was observed in birds of Lakha variety in comparison to the other three types (Table 3), which might be due to genetic variations.

Table 3 – Effects of dietary selenium supplementation on egg quality traits in four varieties of Aseel chicken.

Parameters Variables		Egg wt. (g)	Shell Thickness with membrane (mm)	Shell Thickness without membrane (mm)	Haugh Unit	Yolk index
Se Sources						
Organic Se		48.58±1.8	0.37±0.0	0.35±0.0	70.47±3.2	0.49±0.0
Inorganic Se		49.58±2.1	0.37±0.0	0.35±0.0	73.16±2.1	0.45±0.0
Control		47.33±1.3	0.37±0.0	0.34±0.0	72.96±1.9	0.53±0.0
Varieties						
Lakha		42.44±1.8^{d}	0.35±0.0^{b}	0.31±0.0^{b}	79.99±0.8^{a}	0.52±0.1
Mushki		50.11±1.4^{b}	0.36±0.0^{ab}	0.35±0.0^{a}	70.04±2.1^{bc}	0.45±0.0
Peshawari		46.89±1.2^{c}	0.39±0.0^{a}	0.35±0.0^{a}	74.52±2.1^{ab}	0.52±0.0
Mianwali		54.55±1.0^{a}	0.38±0.0^{ab}	0.36±0.0^{a}	64.23±2.8^{c}	0.47±0.0
Se Sources × Varieties						
Organic Se	Lakha	46.67±3.5^{dc}	0.36±0.0^{abc}	0.32±0.0^{bc}	81.04±1.1^{a}	0.49±0.0^{ab}
	Mushki	47.67±0.9^{dc}	0.37±0.0^{abc}	0.36±0.0^{ab}	72.42±4.8^{abc}	0.46±0.0^{ab}
	Peshawari	43.00±1.5^{de}	0.39±0.0^{abc}	0.36±0.0^{ab}	71.98±5.6^{abc}	0.55±0.1^{ab}
	Mianwali	57.00±1.2^{a}	0.36±0.0^{abc}	0.35±0.0^{ab}	56.43±2.5^{d}	0.45±0.0^{b}
Inorganic Se	Lakha	38.00±1.2^{e}	0.33±0.0^{c}	0.28±0.0^{c}	80.36±0.7^{a}	0.43±0.0^{b}
	Mushki	55.00±0.0^{ab}	0.34±0.0^{bc}	0.33±0.0^{abc}	69.83±3.3^{abc}	0.40±0.0^{b}
	Peshawari	50.33±0.3^{bc}	0.41±0.0^{a}	0.37±0.0^{ab}	76.64±2.0^{abc}	0.54±0.0^{ab}
	Mianwali	55.00±1.0^{ab}	0.40±0.0^{ab}	0.39±0.0^{a}	65.79±4.4^{dc}	0.45±0.0^{b}
Control	Lakha	42.67±2.3^{de}	0.37±0.0^{abc}	0.33±0.0^{abc}	78.57±2.2^{ab}	0.63±0.1^{a}
	Mushki	47.67±2.2^{dc}	0.37±0.0^{abc}	0.35±0.0^{ab}	67.87±3.7^{bc}	0.49±0.0^{ab}
	Peshawari	47.33±1.2^{dc}	0.36±0.0^{abc}	0.33±0.0^{bc}	74.92±3.2^{abc}	0.47±0.0^{ab}
	Mianwali	51.67±1.8^{abc}	0.38±0.0^{abc}	0.35±0.0^{ab}	70.47±4.4^{abc}	0.52±0.0^{ab}

Superscripts on different means within rows differ significantly ($p \leq 0.05$).

Interaction of Se sources and types resulted in the lowest eggshell thickness with and without membrane in Lakha variety when fed with inorganic selenium supplemented diet. The Se sources, however, could not influence the eggshell thickness. Pavlovic *et al.* (2010) in laying hens and Cruz & Fernandez (2011) in quail breeders as well, reported a similar response of Se on eggshell thickness, concluding that there is no difference between SS and SY groups for eggshell thickness (Invernizzi *et al.,* 2013). The shell thickness, likewise, remained unaffected in layers when fed with organic selenium supplemented diet (Correia *et al.,* 2000; Paton *et al.,* 2000a).

Among varieties, the highest Haugh unit score was seen in Lakha, followed by Mushki, and then Mianwali (Table 3). It might be due to comparatively higher albumen height in that variety. A significant difference in Haugh unit score, likewise, was also found in a study on different breeds of poultry (Monira *et al.,* 2003; Ali *et al.,* 2012). Interaction of Se sources and varieties revealed maximum (81.04±1.1) Haugh unit score in Lakha hens using the diet supplemented with organic Se, which might be attributed to the trend of Lakha variety for higher Haugh unit score. The Se sources alone, however, could not affect Haugh unit score. Likewise, Haugh unit score in layers was not changed even if the diet was supplemented with Se from an organic source (Correia *et al.,* 2000) or regardless of the source (Patton, 2000; Cruz & Fernandez, 2011). There is another claim that the administration of selenium (Mohiti-Asli *et al.,* 2008), or organic trace minerals individually or in combination (Scatolini, 2007) did not affect Haugh unit score.

The highest yolk index was observed in Lakha when fed with a control diet. However, Se sources alone could not show any difference in yolk index (Table 3). Yolk index remained unaffected despite supplementation of Se in the diet (Mohiti-Asli *et al.,* 2008; Attia *et al.,* 2010; Cruz & Fernandez, 2011) either in the form of organic or inorganic (Arpasova *et al.,* 2012; Invernizzi *et al.,* 2013). Varieties alone also could not show any difference in the yolk index. Non-significant differences ($p > 0.05$) were reported in the yolk index among different types of Aseel (Shafiq *et al.,* 2013; Ahmad, 2013), and between Vanaraja and White Leghorn breeds of chicken (Haunshi *et al.,* 2011).

CONCLUSIONS

From the findings, it can expediently be concluded that Mianwali variety, for its good egg physical characteristics and positive interaction with the organic Se, can be selected for further improvement if uninterruptedly fed on organic Se supplemented diet. This novel approach might help the farmers in producing quality eggs and chickens, fetching premium returns from the elite class, reviving a prestigious agro-based rural poultry culture, and having livelihoods for their households.

DISCLOSURE OF POTENTIAL CONFLICT OF INTEREST

No competing conflict of interest was reported by the authors.

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ERRATAS

In the article Impact of Selenium-Supplemented Diets on Egg Morphometry and Quality in Four Varieties of Indigenous Aseel Chicken, Doi: 10.1590/1806-9061-2020-1277, published in the Revista Brasileira de Ciência Avícolas/Brazilian Journal of Poultry Science, v23 (2):001-008,

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