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Original Article

Effects of Dietary Supplementation of arginine-silicate- Inositol and Phytase Complex on Egg Quality, Egg Shell Strength, and Blood Biochemical Characteristics of Laying Hens

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Abstract

This study aimed to determine the effects of Arginine silicate inositol complex (ASI; Arg=49.47 %, silicone=8.2 %, inositol=25%) supplementation on egg quality, shell strength, and blood biochemical traits of laying hens, as well as the effects of substituting inositol with varying concentrations of phytase on the traits as mentioned above. 90 Lohmann Brown laying hens, 26 weeks old, were randomly distributed in 6 treatments with 3 replicates (cage) and 5 birds per replicate. The isocaloric and isonitrogenic diets are used according to the age period requirements of the Lohmann Brown Classic management guideline. The treatments were as follows: 1ST treatment T1: received basal diet without additives, T2 received basal diet +1000 mg/kg arginine-silicate mixture (49.5±8.2 % respectively),T3 received basal diet +1000 mg/kg arginine-silicate- inositol (ASI) mixture (49.5, 8.2 , 25 % respectively), T4 received basal diet +1000 mg/kg arginine-silicate mixture (49.5±8.2% respectively) +500 FTU/kg, T5 received basal diet +1000 mg/kg arginine-silicate mixture (49.5±8.2% respectively) +1000 FTU/kg and T6 received basal diet +1000 mg/kg arginine-silicate mixture (49.5±8.2% respectively) +1000 FTU/kg +2000 FTU/kg. Results indicate a significant increase (P<0.05) in the relative yolk weight in T4, T5, and T6 (26.93, 26.83, 26.77%) compared to T1 (25.84%) and a significant increase ($P\leq$ 0.05) in T4, T5 compared to T3 (26.02%), while no differences observed between T2 (26.17%) compared to other experimental treatments. The relative albumin weight significantly decreased ($P\leq$0.05) in phytase supplementation treatments T4, T5, and T6 (63.21, 63.05, 63.22%) compared to T1, T2, T3 (64.99, 64.30, 64.08%), while a significant decrease ($P\leq$0.05) observed in T3 compared to T1. The relative shell weight significantly increased ($P\leq$0.05) in T3, T4, T5, and T6 (9.90, 9.86, 10.12, 10.02%), respectively, compared to T1, T2 (9.17, 9.53%) with a significant increase ($P\leq$0.05) in relative shell weight in T2 compared to T1. The eggshell thickness significantly increased ($P\leq$0.05) in T3, T4, T5, T6 treatments (0.409, 0.408, 0.411, 0.413 mm), respectively compared to T1, T2 (0.384, 0.391 mm). A significant increased ($P\leq$0.05) was observed in eggshell thickness in T2 compared to T1. A significant increase ($P\leq$0.05) was observed in the egg shell breaking strength in T3 and T5 treatments (59.40, 58.83) compared to T1 and T2 (46.20, 48.23). No significant differences were observed between T4 and T6 (53.90, 53.57) compared to other experimental treatments. Non HDL, calcium, and phosphorus levels in blood serum significantly increased ($P\leq$0.05) in T3, T4, T5, and T6 treatments compared to T1 and T2 treatments.

Keywords: Blood parameter, laying hens, poultry, Nutrition

1. Introduction

The eggshell quality is one of the most critical issues in the egg industry. A large proportion of the economic losses in egg production are caused by cracked and/or broken eggs during the dealing of collection, storage, and transportation. Approximately 8–10% of a

produced egg can be damaged during routine handling and were estimated to be around 247 million US dollars in the USA. Another importance of the eggshell is observed in the effective growth of a chicken embryo in hatcheries units since it is the primary source for skeleton forming and strength, As well as its importance in preventing the entry of external pathogens and the prevent losses of water from the egg contains (1).

The shell is made of calcium carbonate in the form of calcite, and hundreds of proteins interact with the mineral phase controlling its formation and structural organization and thus determining the mechanical properties (2). Each eggshell contains about 2.3 g of Ca. A modern laying hen produces approximately 426 eggs in a 90 wk production cycle (3), which means the deposit of 980 g of Ca. The hen deposits around 5.2 times its skeleton weight as Ca in the eggshell, perhaps due to the unique development of the medullary bone (4).

Calcium plays a vital role in the formation of eggshell. Increasing calcium absorption might lead to increased eggshell strength. The transcellular (active) and paracellular (passive) pathways are involved in intestinal calcium absorption (5).

Adequate dietary Ca, available phosphorus (avP), and vitamin D3 levels are essential to achieve Ca homeostasis and maximize long-term egg production, eggshell quality, and bone health in laying hens (6). supplementing laying hens' diets with ASI complex (arginine-silicate-inositol; 49.5, 8.2, 25 %), respectively, significantly improved eggshell quality through improving calcium utilization as reflected by up-regulation of genes related to calcium metabolism) during the peak of the laying period in laying hens and significantly improved eggshell quality in quail (5). Several roles for silicon have been defined and clinically suggested for metabolic disturbance in calcium absorption, growth, ossification defects, osteomalacia, rachitism, and decalcification (7). Silicon, with calcium, can regulate calcium turnover, controlling bone calcification and decalcification (8). The silicon and inositol in the complex increase the bioavailability of arginine (9).

Arginine is involved in synthesizing collagen and producing nitric oxide, which increases blood flow and reproductive activity. Some research demonstrated that silicon is associated with calcium metabolism and extracellular bone matrix formation (10). Approximately 60% of Phosphorous in plant feed ingredients is bound to myo-inositol, forming myo-inositol phosphates (phytate); phytate molecules can combine with up to five calcium atoms and form complexes, making phytate a limiting factor not only for P but also Ca (11). Phytase enzyme is commonly supplemented in laying hen diets to increase the bioavailability of P and Ca (12) and increases blood myo-inositol concentration (13). The present study aims to investigate the ability to add graded levels of phytase to liberate the required amounts of inositol and forming (arginine-silicate-inositol complex) rather than the additional effects on minerals, amino acids, and starch digestion due to removing the negative impact of phytate.

2. Materials and Methods

90 Lohmann Brown pullets reared in laying hen cages (54×57 cm and 47 cm height) and distributed as 5 birds per cage in the Poultry Research Station- department of animal production - office of Agricultural Research/ Abu- Ghraib under controlled climate conditions with ambient temperature approximately 22°C. The light period was increased from 14L:10D to 16L:8D. At the peak of production (26 weeks old), hens were randomly distributed to one of the six dietary experimental treatments. Each treatment was replicated 3 times, and 5 hens were used per cage (replicate) in a completely randomized design (CRD).

2.1. Experimental Diets

Diet was formulated to satisfy age stage requirements according to Lohmann Brown management guide 2016. Corn- wheat--soybean meal-based Diets were equal in energy (2753 kcal), crud protein (16. 97 %), Calcium (3.62 %) and Phosphorus (0.4%) and dietary

treatments was as follow T1: basal diet without additives , T2: basal diet +1000 mg/kg arginine-silicate mixture (49.5±8.2 % respectively) ,T3: basal diet +1000 mg/kg arginine-silicate- inositol (ASI) mixture (49.5, 8.2 , 25 % respectively) , T4:T2 +500 FTU/kg ,T5:T 2 +1000 FTU/kg and T6:T2+2000 FTU/kg, mixture was completed with corn starch as a carrier to the 100%. The experiment was conducted from 26 to 41 weeks of age. Feed was provided as restrictive mash (115g/bird/day), and water was *ad libitum*. Dietary energy, crude protein, Av. phosphorous, calcium, lysine, and methionine were determined according to National Research Council (14).

2.2. Feed Additives

Phytase enzyme was derived from Buttiauxella sp name Axtra® PHY 10000 TPT (6-phytase) from Danisco Animal Nutrition, DuPont Industrial Biosciences, a heat thermos table phytase (95 ^{o}C) with Optimal pH (2.5 - 5.5). Pure L-Arginine, pure Inositol 500 mg provided from Bulk supplements.com, 7511 Estage road, Henderson Nv 89011 with Lot number 1903212, 1901012 respectively. Silicone was fine powder with (99.6) purity from (Loudwolf) industrial & Scientific WWW.loudwolf.com.

2.3. Egg Quality Measurements

At the end of the period, two eggs from each replicate were collected for 2-day and subjected to egg quality determination within 24 h after oviposition. The egg yolk was separated using an egg yolk separator and then weighed, and albumin weight was also recorded using a digital electronic scale (Sartorius BL210S). Eggshell weight was recorded after 2-day of washing and drying, shell thickness was determined using a digital micrometer (0.01 mm), and the Haugh unit (an index of albumen quality) was determined with previous traits as described by Mountney and Parkhurst (15). Two eggs from each replicate were used to estimate eggshell strength upon the vertical axis using an instrument from the Tech lab system- Spain TLS-CDM.01/O5.

2.4. Blood Sample Collection and Laboratory Analyses

Six hens from each treatment (2 hen per replicate) were randomly selected. At the end of the experiment, blood samples were collected from the wing vent and centrifuged for 15 min (1500 rpm) to separate serum and determine serum levels of HDL, Non-HDL, calcium, and Phosphorus.

HDL was measured using a commercial kit (Syrbio-serya) as an enzymatic method and read using a spectrophotometer at 600 nm as described by Toro and Ackermann (16). Then Non-HDL is estimated according to Grundy, Cleeman (17). The phosphorus concentration in blood serum was measured as described by Gupta, Gangoliya (18) using a spectrophotometer at 660 nm. Moreover, the calcium concentration in blood serum was measured as described by Verma and Alim (19) using a spectrophotometer at 450 nm.

2.5. Statistical Analysis

The experimental design was a complete randomized (CRD) to analyze the variations of the six treatments using the Statistical Standard SAS (20), General Linear Model. The differences were tested using the Duncan Multilevel Test Duncan (21) the mean values of the different traits at the mean of 0.05 to compare using the mathematical model:

$$Y_{ij} = \mu + T_i + E_{ij}$$

3. Results and Discussion

3.1. Egg Quality and Eggshell Strength

Results in table 1 indicate a significant increase (P<0.05) in the relative yolk weight of T4, T5, and T6 (26.93, 26.83, 26.77%) compared to T1 (25.84%) and a significant increase (P≤0.05) observed in T4, T5 compared to T3 (26.02%), while no differences between T2 (26.17%) compared to other experimental treatments. The relative albumin weight significantly decrease (P≤0.05) T4,T5,T6 (63.21, 63.05, 63.22%) compared with T1,T2,T3 (64.99, 64.30, 64.08%), a

significant decrease. ($P\leq0.05$) was observed in T3 compared with T1.

A reflex in table 2 shows s a significant increase ($P\leq0.05$) in relative shell weight of T3, T4, T5, and T6 treatments (9.90, 9.86, 10.12, 10.02%) respectively compared with T1, T2 (9.17, 9.53%) with a significant increase ($P\leq0.05$) in relative shell weight in T2 compared to T1. Similar results showed in eggshell thickness, a significant increase ($P\leq0.05$) in T3, T4, T5, and T6 treatments (0.409, 0.408, 0.411, 0.413 mm), respectively, compared with T1, T2 (0.384, 0.391 mm), a significant increased ($P\leq0.05$) observed in eggshell thickness of T2 compared to T1. At the end of the experiment, a significant increase ($P\leq0.05$) was observed in the egg shell breaking strength of T3 and T5 (59.40, 58.83) compared with T1 and T2 (46.20, 48.23), no significant differences were observed in the egg breaking strength between T4 and, T6 (53.90, 53.57) and compared to other experimental treatments. No significant differences were observed in the Haugh unit between the experimental treatments.

Table 1. Effect of dietary supplementation with Arginine-Silicate and Inositol or Phytase Enzyme on egg quality, the strength of laying Hens from 26 -41 w

Treat	Relative yolk weight /g	Relative albumin weight /g	Relative shell weight /g	Shell thickness Mm	Broken Strength	HU unit
T1	25.84±023^{c}	64.99±0.27^{a}	9.17±0.12^{c}	0.384±0.002^{c}	46.20±2.55^{b}	81.64±1.27^{a}
T2	26.17±0.22^{abc}	64.30±0.22^{ab}	9.53±0.12^{b}	0.391±0.002^{b}	48.23±2.72^{b}	84.49±0.79^{a}
T3	26.02±0.29^{bc}	64.08±0.30^{b}	9.90±0.08^{a}	0.409±0.002^{a}	59.40±1.04^{a}	82.90±0.93^{a}
T4	a26.93±0.24^{a}	63.21±0.27^{c}	9.86±0.10^{a}	0.408±0.002^{a}	53.90±3.03^{ab}	83.53±0.84^{a}
T5	26.83±0.19^{a}	63.05±0.19^{c}	10.12±0.07^{a}	0.411±0.002^{a}	58.83±4.82^{a}	83.60±0.77^{a}
T6	26.77±0.32^{ab}	63.22±0.37^{c}	10.02±0.07^{a}	0.413±0.002^{a}	53.57±2.54^{ab}	83.71±0.63^{a}
sig	*	*	*	*	*	ns

Means within a column with different superscripts differ significantly ($P<0.05$)

Table 2. Effect of dietary supplementation with Arginine-Silicate and Inositol or Phytase Enzyme on some blood biochemical traits of Laying Hens from 26-41 w

Treatment	HDL	Non HDL	Ca	Phos
T1	35.17±1.54^{a}	235.67±2.62^{b}	25.65±0.55^{b}	6.65±0.35^{b}
T2	33.83±1.42^{a}	232.67±4.16^{b}	27.51±1.05^{b}	6.91±0.27^{b}
T3	35.67±1.20^{a}	249.00±3.65^{a}	31.13±0.94^{a}	7.90±0.26^{a}
T4	37.67±1.41^{a}	248.67±2.17^{a}	31.33±0.98^{a}	8.15±0.37^{a}
T5	37.50±1.18^{a}	254.33±3.78^{a}	33.07±0.66^{a}	8.31±0.32^{a}
T6	37.17±1.47^{a}	248.17±6.77^{a}	31.8±1.23^{a}	8.36±0.30^{a}

Means within a column with different superscripts differ significantly ($P<0.05$)

These results are consistent with Xia, Fouad (22) in a study conducted with supplementation of arginine to ducks' diets that resulted in a significant increase in egg yolk percentage ($P\leq0.05$) and also agree with the results of Al-Bayar, Al-Daraji (23), where the results showed a significant increase in relative yolk weight ($P\leq0.01$) with arginine supplementation in turkey diets with a significant decrease ($P\leq0.01$) in relative albumin weight. While the recorded data in the current study disagree with Kalvandi, Sadeghi (24) when adding arginine, Dersjant-Li, Millán (25), and Englmaierová, Skřivan (26) with the addition of phytase enzyme, where no significant differences were observed in relative yolk and albumin weight in the treated animals.

Regarding eggshell weight, shell thickness and shell breaking strength results are consistent with Sahin, Orhan (5), as their results showed a significant increase in eggshell weight ($P\leq0.001$) and shell thickness

($P\leq0.001$) in ASI supplementation treatments. It also agrees with the results of Skřivan, Englmaierová (27), Englmaierová, Skřivan (26) when phytase enzyme supplemented with diets results in a significant increase ($P\leq0.05$) in shell thickness, relative shell percentage, and shell breaking strength. Also, Lim, Park (28) found that silica supplementation in diets results in a significant increase in eggshell thickness and breaking strength compared with the control treatment. Xia, Fouad (22) results came to express a significant increase ($P\leq0.01$) in relative shell percentage and the thickness in arginine supplemented diets compared with control treatment. While results disagree with the results of Kalvandi, Sadeghi (24) with arginine supplementation where the shell thickness was not affected, Pongmanee (29) and Ren, Sun (30) with phytase enzyme supplementation as no significant differences were observed in eggshell thickness and shell breaking strength. Also, Bello and Korver (6) and Taylor, Bedford (31) did not notice any significant effect of dietary phytase enzyme supplementation on eggshell thickness, weight, and eggshell breaking strength. Sommerfeld, Künzel (13) indicated that dietary supplementation of pure myo-inositol 0.1% did not affect on. Eggshell weight, thickness, and eggshell breaking strength.

The yolk precursors are very low-density lipoproteins (VLDL) and vitellogenin (VTG) that are synthesized by the liver and secreted into the bloodstream, transported to the ovary, and absorbed via VLDL receptors (VLDLR-b) in the follicles. Xia, Fouad (22) observed that the abundance of VLDLR expressing in the second large follicle membrane increased in a quadratic with increasing dietary arginine levels. Blood biochemical traits in table 2 showed a significant increase ($P\leq 0.05$) in serum Non-HDL levels, which includes LDL and VLDL, this may explain the high relative yolk weight, Al-Bayar, Al-Daraji (23) indicated that arginine supplementation increased hormonal secretion LH and FSH, these hormones play a significant role in increasing estrogen hormone level in the blood, which effect on the synthesis of the precursor of yolk protein Vitellogenin and stimulate yolk Vitellogenesis synthesis by acting directly on the liver and increasing the synthesis of yolk proteins. Also, it is possible that the improvement in the yolk percentage can be attributed to the effectiveness and action of the additives as antioxidants that inhibit the activity of free radicals and protect the hepatocytes membranes from oxidative damage, thus maintaining the vital cellular metabolic functions of the liver. Antioxidants work to protect lipoproteins and other fatty compounds from oxidation, which are involved in yolk formation, which leads to an increase in the abundance of these substances and the maturation of ovarian follicles in a shorter time. Arginine acts as an antioxidant, and phytase can act as an antioxidant throw Phytate degradation intermediate compounds (13), like inositol triphosphate IP1,2,3 and inositol tetraphosphate IP1,2,3,6, that well documented as antioxidants by Phillippy and Graf (32).

Also, Jiang, Liu (33) indicated that MYO inositol could inhibit the generation of free radicals and prevent oxidative damage. It is expected that the increase in the levels of available phosphorous due to the addition of phytase enzyme will lead to an increase in the percentage of yolk since most of the phosphorous results from the decomposition of phytate incorporated into the yolk as phosphorylated lipids or proteins. It is noticed in table 2 that there is a significant increase ($P\leq0.05$) in the concentration of calcium and phosphorous in the blood serum in ASI and phytase enzyme supplementation treatments. It was previously reported by Sahin, Orhan (5) that dietary supplementation of ASI in laying hens' diets during the peak production period improved eggshell quality by improving calcium utilization by regulating genes related to calcium metabolism, whereby ASI supplementation increased significantly ($P<0.0001$).) gene expressions of calcium transporters (calbindin-D28k, sodium-calcium N exchanger, plasma membrane calcium ATPase, vitamin D receptor (VDR), and tight

junction proteins (zonula occludens-1 and occludin) in the duodenum which can help in increasing Calcium absorption to achieve calcium homeostasis. These mechanisms can positively affect eggshell traits. The Eggshell consists of calcium carbonate crystals, approximately two-thirds of the shell; these crystals are arranged in the organic matrix consisting of protein and polysaccharides.

Sahara, Despedia (34) demonstrated that increasing eggshell weight as a result of the increasing phytase level is a reflection of the facilitation of the body metabolism, including the availability of organic matter and calcium carbonate as main components of the eggshell, the increase in eggshell percentage in the current experiment may be due to the Significant increase ($P\leq0.05$) in the level of calcium and phosphorous in the blood serum, which led to an increase in the calcium content of eggshell as a result of calcium balance. Ribeiro, Barreto (35) indicates that the main factor for good eggshell quality is maintaining Ca balance during the egg production period. Because of the significant increase in relative yolk weight during the study with a significant increase in the relative shell weight, it is normal to be a significant decrease in the relative albumin weight.

3.2. Physiological Characteristics

Results in Table 2 indicate a significant increase ($P<0.05$) in the level of non- HDL in serum of T3, T4, T5, and T6 treatments (249.00, 248.67, 254.33, 248.17 mg/dl) compared with T1 and T2 treatment (235.67, 232.67 mg/dl. No significant differences were observed in the level of HDL in serum between the experimental treatments at the end of the experiment. Regarding the calcium level in blood serum, a significant increase ($P\leq0.05$) was noted in T3, T4, T5, and T6 treatments (31.13, 31.33, 33.07, 31.8 mg/dl), respectively, compared with T1 and T2 treatment (25.65, 27.51 mg/dl) with no significant differences between them. Phosphorous levels in serum were similar to calcium, a significant increase ($P\leq0.05$) was recorded in serum phosphorous levels for T3, T4, T5, and T6 treatments (7.90, 8.15, 8.31, 8.36 mg/dl), respectively compared with T1 and T2 treatment (6.65, 6.91 mg/dl), with no significant differences between them. No significant differences were observed in serum HDL levels between the experimental treatments.

Arginine and phytate degradation intermediates, (inositol 1,2,3-triphosphate, inositol 1,2,3,6-tetraphosphate) acts as antioxidants (36). Jiang, Liu (33) indicated that MYO could inhibit the generation of free radicals and prevent oxidative damage. Therefore these supplements may prevent the negative impact of free radicals and protect the hepatocyte membranes from oxidative damage, which results in maintaining the release of VLDL, lipoproteins, and Vitellogenin from the liver and transported to the ovary by the bloodstream. It is also noted from the results of blood biochemical traits (Table 2) that there are significant increases in calcium and phosphorous levels in the blood serum for T3, T4, T5, and T6 treatments, compared with T1and T2, which results in a significant increase in eggshell thickness and the relative shell weight (Table 1), as these results coincided with the high concentration of calcium in the blood. These results can be attributed to dietary ASI mixture supplementation increasing calcium and phosphorous availability and enhancing the anabolic processes in calcium utilization through regulating calcium transport proteins CaBP-D28k, OCLN, NCX1, ZO-1, and vitamin D receptor (VDR) (5).

Increasing eggshell weight can be attributed to the increase of phytase enzyme level, which is a reflection of the body's metabolism, including the availability of organic matter, phosphorus, and calcium as major components of the egg shell (34). The increase in the percentage of eggshell in the current experiment may be due to the significant increase ($P\leq0.05$) in the level of calcium and phosphorous in the blood serum as a result of the increase in its release from the phytate complex, which led to the calcium balance in the body.

In conclusion, supplementing laying hens' diets with ASI or arginine-silicate-phytase mixture enhances egg quality and eggshell strength in laying hens' peak production.

Authors' Contribution

Study concept and design: B. I. H. and H. H. N.
Acquisition of data: B. I. H. and H. H. N.
Analysis and interpretation of data: F. M. H. and B. I. H.
Drafting of the manuscript: B. I. H. and H. H. N.
Critical revision of the manuscript for important intellectual content: H. H. N.
Statistical analysis: F. M. H.
Administrative, technical, and material support: B. I. H.

Ethics

We hereby declare all ethical standards have been respected according to the ethics committee of the Office of Agricultural Research, Ministry of Agriculture, Baghdad, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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