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***Original Article***

# Effects of Dietary Supplementation of Arginine-Silicate-Inositol and Phytase Complex on Performance and Blood Biochemical Traits of Laying Hens

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**Abstract**

Arginine silicate inositol complex (ASI; Arg = 49.47%, silicone = 8.2%, inositol = 25%) is a novel, bioavailable source of Si and Arg and may offer potential benefits for laying hens' performance. The aim of this study was to evaluate the effect of Arginine-Silicate and inositol/phytase on the performance of laying hens. A total of 90 laying hens, 25 weeks old, were randomly assigned to 6 treatments with 3 replicates (5 birds per replicate). The treatments were as follows: 1ST treatment PC: positive Control group (basal diet without additives) , 2nd treatment: basal diet +1000 mg/kg arginine-silicate complex (49.5±8.2 % respectively), 3d treatment: basal diet +1000 mg/kg arginine-silicate- inositol (ASI) complex (49.5, 8.2 , 25 % respectively) , 4th treatment: T 2 +500 FTU/kg , 5th treatment: T2 +1000 FTU/kg and 6th treatment :T2+2000 FTU/kg . Results indicate a significant increase ($P$<0.05) in hen house production (H.H. pro.%) of T5 (95.06 %)compared with T1(91.67%) and no significant differences between T2, T3, T4, T6 (91.84, 93.21, 93.46, 92.98%) and compared with T1 and T5. were no significant difference observed in average egg weight and egg mass between the experimental treatments all over the period. Daily feed intake (DFI) significantly decreased ($P$<0.05) with supplementing diets with deferent levels of phytase with arginine–silicate mixture T4, T5, andT6 (113.56،113.06 ،112.10 g) compared with T1 (114.34 g ) which has no significant differences compared with T2 and T3 (113.96, 113.92 g). Phytase supplementation significantly ($P$<0.05) improved FCR g feed/egg in T5 (119.02) compared with T1 and T2 (124.89, 124.32), while no significant differences between T3.T4.T6 treatments (122.39, 121.80, 120.69) respectively and compared with other treatments. The experimental treatments observed no significant difference in g feed/ g egg.

**Keywords:** Arginine-Silicate, Inositol, Phytase Enzyme, Blood biochemical traits, Laying Hens

## 1. Introduction

Arginine silicate inositol complex (ASI; Arg= 49.47%, silicone= 8.2%, inositol= 25%) is a novel, bioavailable source of Si and Arg and may offer potential benefits for laying hens' performance. Previous studies reported the positive effects of the ASI complex on egg production in laying hens and quill (1). The silicon and inositol in the complex increase arginine bioavailability. L-arginine (L-Arg) is classified as an essential amino acid for birds because of the disability to synthesize in the body due to an incomplete urea cycle because of the lack of carbamoyl phosphate synthase enzyme (2). L-Arg is involved in the biosynthesis of various molecules, including protein, nitric oxide, ornithine, creatine, glutamate, proline, polyamine, glutamine, agmatine, and dimethylarginine, and therefore plays crucial biological and physiological roles (3). Serum nitric oxide (NO) is linked to high egg production. Arginine also induces

ovulation through enhancing luteinizing hormone (L.H.) secretion (4). Silicon is an important mineral associated with calcium metabolism and the formation of the extracellular bone matrix (5). Inositol is a cyclical isomer of glucose, which is involved in many physiological processes and is suggested to be involved in energy and lipid metabolism, reproduction, general metabolic performance, and bone and muscle formation (6). Tow third of phosphorous in the plant-based feed ingredients sources is bound to myo-inositol, forming myo-inositol phosphate and known as the phytic acid, which can bind with other minerals and nutrients, making complex compounds, severely reduce their availability, reducing the efficiency of digestion in the digestive tract and negatively affecting the absorption processes (7). Phytase enzymes hydrolyzed phytate in graded steps to release phosphorous, inositol, calcium, other bonded minerals, and nutrients such as amino acids, fats, and starch in laying hens' diets (8, 9). The aim of this study was to evaluate the effect of Arginine-Silicate and inositol/phytase on the performance of laying hens.

## 2. Materials and Methods

### 2.1. Animals and Housing

A total of 90 Lohmann Brown pullets 12 weeks of age were reared in laying hen cages under controlled climate conditions at the Poultry Research Station- department of animal production - office of Agricultural Research. Five birds were placed in each cage (cage dimensions 54×57 cm with a height of 47 cm). The light period was increased from 14L:10D to 16L:8D .The ambient temperature was maintained at approximately 22 ℃ throughout the trial. At 26 weeks old (peak of production), the hens were randomly assigned to one of the six dietary treatments (Table 1). Each dietary treatment was replicated 3times with 5 hens per cage (replicate) in a completely randomized design (CRD).

### 2.2. Experimental Diets

Diet was formulated according to the Lohmann Brown management guide 2016 age stage. The ingredient and nutrient composition of the experimental diets is shown in table 1. Corn-wheat-soybean meal based diets were formulated as follow: 1ST treatment PC: positive Control (basal diet without additives) T2: basal diet +1000 mg/kg arginine-silicate complex (49.5±8.2 % respectively), T3:basal diet+1000 mg/kg arginine-silicate- inositol (ASI) complex (49.5, 8.2 , 25 % respectively),T4: T2+500 FTU/kg, T5:T2+1000 FTU/kg and T6:T2+2000 FTU/kg, the percentage of the mixture was completed with corn starch as a carrier. All Diets were equal in protein and metabolic energy. The feeding trial was conducted for 16 wk. from 26 to 41 weeks of age. Feed was restrictive (115 g/bird/day), as mash and water were provided *ad libitum*. Experimental diets of crude protein, energy, calcium, Av. phosphorous, methionine, and lysine were calculated according to National Research Council (10).

### 2.3. Feed Additives

Phytase enzyme was a 6-phytase derived from Buttiauxella sp name Axtra® PHY 10000 TPT from Danisco Animal Nutrition, DuPont Industrial Biosciences, Marlborough, U.K. is a heat stable at a high-temperature degree 95 °C, Optimal pH (2.5 - 5.5). Pure L-Arginine (35.27oz) and pure Inositol 500 mg (17.64 oz) were obtained from Bulk supplements.com, 7511 Estate Road, Henderson Nv 89011 with Lot number 1903212, 1901012, respectively. Silicone fine powder (99.6) purity obtained from (Loudwolf) industrial& Scientific WWW.loudwolf.com.

### 2.4. Laying Performance

Eggs were collected daily at 1 pm throughout the experiment's duration (16 weeks). The egg production percentage was calculated according to the number of chickens housed at the beginning of the experiment (Hen House production-HH%). Daily egg weight measurements were taken with a digital electronic scale (Sartorius BL210S), and each replicate's average egg weight and mass were calculated accordingly. Feed was restricted (115g/bird/day), and rested feed was weighed at the end of each week to determine average daily feed intake and Feed conversion ratio (gm feed per egg; gm

feed per g egg). H.H. egg production percentage, average egg weight, egg mass, feed intake (F.I.), and feed conversion ratio (FCR) were calculated according to the method of Mountney and Parkhurst (11).

**2.5. Blood Sampling and Laboratory Analyses**

At the end of the experiment (41 wk), 6 hens from each treatment (2hen per replicate) were randomly selected, and blood samples were collected from the wing vent and centrifuged for 15 min (1500 rpm) to separate serum. to analyse serum levels of glucose, total protein (T.P.), total cholesterol (CHO) and triglyceride (T.G.).

Total protein and triglycerides were estimated using a commercial kit (Biolabo-France) as an enzymatic method and read using a spectrophotometer at 546 nm as described by Moss, Baron (12); Toro and Ackermann (13), respectively.

The cholesterol level is estimated according to the kit instructions and Richmond (14). The method of enzymatic glucose analysis was followed by Al-Jubori (15) using the Biosystems-Spain kit.

**2.6. Statistical Analysis**

A complete randomized design (CRD) was performed to analyze the effect of six treatments, using the Statistical Standard SAS (16) and following General Linear Model. Differences between treatments tested using the Duncan Multilevel Test Duncan (17) at the mean of 0.05 to compare the mean values of the different traits according to the mathematical model:

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

**Table 1.** Feed components and calculated chemical composition of maize strains used in the experiment

| Content | Stage of age | | |
|---|---|---|---|
| | **Pre-production 9-16 w** | **Pre-production 16 – 5% pro** | **Production** |
| Yellow corn | 35.25 | 32.3 | 32.1 |
| Wheat | 34 | 32 | 32 |
| Soybean meal | 12 | 23.5 | 24 |
| Premix | 1 | 1 | 1 |
| Wheat bran | 15 | 5 | - |
| Limestone | 0.7 | 3.4 | 7.65 |
| Calcium diphosphate | 1.45 | 1.8 | 1.55 |
| Plant oil | 0.5 | 0.9 | 1.6 |
| Salt | 0.1 | 0.1 | 0.1 |
| Total | 100 % | 100 % | 100 % |
| M.E. kcal/kg | 2749 | 2751 | 2753 |
| Crude protein % | 14.54 | 17.55 | 16. 97 |
| Methionine and cysteine% | 0.64 | 0.91 | 0.89 |
| Lysine % | 0.73 | 0.84 | 0.84 |
| Calcium % | 0.94 | 2.06 | 3.62 |
| Phosphorus% | 0.39 | 0.45 | 0.4 |

* layer Premix 1% produced by the company Intraco LTD Belgian-free of calcium and phosphorus, ** Soya bean used from the source of Argentine crude protein ratio of 48%, and the metabolic energy 2440 kcal/kg, *** Chemical composition of other feed ingredients calculated based on (NRC, 1994).

## 3. Results and Discussion

### 3.1. Egg Production

Results in table 2 indicate a significant increase ($P$<0.05) in the H.H. pro.% of T5 (95.06%) compared with (T1) control treatment (91.67.%), there were no significant differences between T2, T3, T4, T6 (91.84, 93.21, 93.46, 92.98.%) and compared with T1 and T5. These results concur with Sahin, Orhan (1), who reported that adding ASI at 500 or 1000 mg/kg results in a significant ($P$<0.001) increase in egg production; another study conducted by Kalvandi, Sadeghi (18) on Quail breeders, Ahmed and Salad (19) on laying hens demonstrated that supplementation diets with L-arginine significantly ($P$<0.05) increase egg productionFernández, Chárraga (20); and finally, these results are consistent with the findings of Sahin (1), who reported With phytase supplementation, Fernández and Chárraga (20) and Lim, Park (21) with silicon supplementation, egg production increased significantly ($P$<0.0005; $P$<0.05, respectively).

This result disagrees with Onderci, Sahin (22) with (500 or 1000 mg/kg) of ASI, Ren, Sun (23) when adding phytase enzyme (2000 FTU / kg feed), Herwig, Walk (24) with (0.16%) inositol, Fascina, Pasquali (25) with arginine supplementation as results indicates no significant effects on egg production.

These significant improvements in egg production may be due to the role of arginine in stimulating gonadotropin-stimulating hormone secretion (GnRH) and thus increasing L.H. and FSH hormones secretion responsible for follicle growth and ovulation. These hormones also increase the secretion of estrogen hormone by the theca cells of small follicles (26). NO can be synthesized from L-Arg by nitric oxide synthase (NOS) enzyme, and the NO concentration in chicken serum increased significantly when diets were supplemented with arginine (27). (NO) plays a significant role in the physiology of the reproductive system by controlling the activity of the reproductive organs and increasing blood flow to the ovary, thus providing nutrients.

As indicated Kim, Pitargue (28), high doses of phytase have a positive impact on egg production may be due to the improvement in the availability and utilization of nutrients such as carbohydrates, fats, and proteins (7) which agree with our biochemical traits results reported in table 3 and phosphorous with other minerals such as calcium, zinc, and copper. Matuszewski, Łukasiewicz (29) has indicated that zinc deficiency can interfere with the growth of sexual organs and the reproductive process in females.

**Table 2.** Effect of supplementation with Arginine-Silicate and Inositol or Phytase Enzyme on Performance of laying Hens from 26-41w

| Treatment | H.H. pro.% | Average egg Weight /g | Egg mass g/hen/day | Av. Daily F.I. g/day | FCR g feed/egg | FCR g feed/egg |
|---|---|---|---|---|---|---|
| T1 | 91.67±0.93$^{b}$ | 58.44±0.66$^{a}$ | 53.60±1.08$^{a}$ | 114.34±0.27$^{a}$ | 124.89±0.97$^{a}$ | 2.14±0.04$^{a}$ |
| T2 | 91.84±1.32$^{ab}$ | 58.52±1.04$^{a}$ | 53.83±2.03$^{a}$ | 113.96±0.23$^{ab}$ | 124.32±2.56$^{a}$ | 2.13±0.08$^{a}$ |
| T3 | 93.21±1.08$^{ab}$ | 58.39±1.25$^{a}$ | 54.49±1.57$^{a}$ | 113.92±0.22$^{ab}$ | 122.39±1.38$^{ab}$ | 2.10±0.06$^{a}$ |
| T4 | 93.46±1.10$^{ab}$ | 58.41±0.81$^{a}$ | 54.64±1.18$^{a}$ | 113.56±0.31$^{bc}$ | 121.80±1.20$^{ab}$ | 2.09±0.04$^{a}$ |
| T5 | 95.06±0.73$^{a}$ | 58.62±0.41$^{a}$ | 55.75±0.69$^{a}$ | 113.06±0.08$^{c}$ | 119.02±0.92$^{b}$ | 2.04±0.02$^{a}$ |
| T6 | 92.98±0.48$^{ab}$ | 59.36±0.78$^{a}$ | 55.25±0.62$^{a}$ | 112.10±0.17$^{d}$ | 120.69±0.44$^{ab}$ | 2.04±0.03$^{a}$ |
| Sig | * | ns | ns | * | * | ns |

* a,b,c,d - Values within a row with different letters differ significantly at $P$<0.05. N.S. - not significant.
Experimental treatments: T1control group (basal diet), T2: basal diet +1000 mg/kg arginine-silicate complex (49.5±8.2 % respectively, T3:basal diet +1000 mg/kg arginine-silicate- inositol (ASI) complex (49.5, 8.2 , 25 % respectively) ,T 4 : T2 + 500 FTU/kg , T5 : T 2 +1000 FTU/kg and T6: T2+2000 FTU/kg.

**Table 3.** Effect of supplementation with Arginine-Silicate and Inositol or Phytase Enzyme on blood biochemical traits of laying Hens from 26 -41 w

| Treatment | CHOL mg /dl | Tri mg /dl | Glucose mg /dl | T protein g /dl |
|---|---|---|---|---|
| T1 | 270.8±1.87$^{b}$ | 915.0±16.07$^{a}$ | 197.5±2.81$^{c}$ | 5.17±0.12$^{d}$ |
| T2 | 266.5±3.22$^{b}$ | 911.7±14.0$^{a}$ | 205.83±3.75$^{bc}$ | 5.33±0.14$^{cd}$ |
| T3 | 284.7±4.48$^{a}$ | 956.7±13.33$^{a}$ | 211.67±6.41$^{bc}$ | 5.67±0.09$^{bc}$ |
| T4 | 286.3±2.70$^{a}$ | 920.0±9.66$^{a}$ | 2183.3±5.27$^{ab}$ | 6.02±0.19$^{ab}$ |
| T5 | 291.8±3.72$^{a}$ | 941.7±10.14$^{a}$ | 229.17±6.25$^{a}$ | 6.35±0.13$^{a}$ |
| T6 | 285.3±7.53$^{a}$ | 920.0±19.66$^{a}$ | 217.5±4.43$^{ab}$ | 6.22±0.11$^{a}$ |
| Sig | * | ns | * | * |

* a,b,c,d - Values within arrows with different letters differ significantly at $P<0.05$. N.S. - not significant.
Experimental treatments: T1control group(basal diet), T2: basal diet +1000 mg/kg arginine-silicate complex (49.5±8.2 % respectively, T3:basal diet +1000 mg/kg arginine-silicate- inositol (ASI) complex (49.5, 8.2 , 25 % respectively) ,T 4 : T2 + 500 FTU/kg , T5 : T 2 +1000 FTU/kg and T6: T2+2000 FTU/kg

### 3.2. Average Egg Weight and Egg Mass

There was no significant difference in average egg weight and egg mass between experimental treatments; these results are consistent with those of Ren, Sun (23) when supplemented with diets with phytase, Herwig, Walk (24) with inositol (0.16%) supplementation and Yuan, Bu (30) with arginine supplementation when results indicate no significant differences in the average egg weight and egg mass. While disagreeing with Sahin, Orhan (1), when supplementation diets with 500 or 1000 mg/kg of ASI result in a significant increase in the average egg weight of laying hens, and it also disagrees with the results of Ahmed and Salad (19) with the addition of L-Arg, Baghban-Kanani, Hosseintabar-Ghasemabad (31) with the addition of phytase, where a significant increase was observed in egg weight and egg mass respectively.

The absence of significant differences between treatments may be due to the fact that similar egg weights reflexed the positive correlation between dietary sulfur amino acids and egg weight; in this study, the levels of methionine + cysteine in all treatments were equaled and in accordance with particular requirements of hens .there were no significant differences in the egg mass between the different treatments and this may reflect the absence of significant difference in the average weight.

### 3.3. Average Daily Feed Intake (F.I.)

Daily (DFI) significantly decreased ($P<0.05$) with adding deferent levels of phytase with arginine–silicate mixture T4, T5, and T6 (113.56 ، 113.06 ، 112.10 g) compared with T1 (114.34 g) which has no significant differences compared with T2 and T3 (113.96 ، 113.92 g), the lowest significant decrease ($P<0.05$) was in T6 compared with other treatments followed with T5 which was comparable to T4 and significantly decreased compared with T1, T2, and T3.

These results agree with Herwig, Walk (24) supplementing (0.16%) inositol, Żyła, Mika (32) supplementing 0.1% myo-inositol, and Pereira, Junqueira (33) supplementing 500 FTU/ kg feed, where a significant decrease ($P<0.05$) observed in (DFI). These results disagree with Ren, Sun (23), and phytase supplementation, as there was no significant effect on (DFI). In contrast to our results, Lim, Park (21) with the addition of silicate, Sahin, Orhan (1) with the addition of ASI observed a significant increase in (DFI).

Birds eat to satisfy their energy needs and reach satiety; one of the most important factors influencing the regulation of (F.I.) is the energy content of their diet; a decrease in energy content leads to an increase in (F.I.) and *vice versa*. The glucose level in the blood is a crucial factor in (F.I.) regulating, as a high level of glucose leads to a decrease in (F.I.), and birds respond

to changes in glucose level in the long term. Improvements in protein and energy digestion due to the addition of phytase enzyme, as reported in table 3 expected to improve energy utilization in laying hens. Whereas Dersjant-Li, Evans (34) indicated that phytase supplementation at doses of 300 FTU/kg could replace 55 kcal/kg of AMEn, 0.325% C.P. and a dose of 600 FTU /kg can replace 60 kcal/kg of AMEn, 0.607% C.P. The decrease in feed consumption can also be attributed to the significant increase in the level of glucose in the plasma shown in table 3 for phytase supplementation treatments. In addition, MI also may promote increased energy utilization and reduced F.I. (35).

**3.4. Feed Conversion Ratio (FCR)**

Phytase supplementation in T5 (1000FTU/ kg) to arginine–silicate mixture significantly ($P$<0.05) improved FCR g feed/egg (119.02) compared with T1 and T2 (124.89, 124.32 g feed/egg), while no significant differences observed between T3, T4, T6 treatments (122.39, 121.80, 120.69 g feed/egg) respectively and compared with other treatments. The experimental treatments observed no significant difference in g feed/ g egg.

(North and Bell 1990) FCR is closely related to feeding consumption, egg weight, and production. In this study, the improvement in the FCR may be attributed to the presence of a significant decrease in F.I. for phytase treatments, especially the T5 and T٦ treatments, with the presence of a significant increase in egg production for the T5 compared to the control treatment, which results in a significant decrease in FCR gm feed/egg in this treatment, while eggs weight were almost identical and no significant differences observed between the treatments result in the absence of Significant differences in FCR g feed/ g egg. This improvement in FCR can be attributed to the releasing of chelated phytate complex nutrients and minerals (P, Ca, Mg, Mn, Fe, K, Na, and S), which have important physiological and metabolic roles like manganese (mn) that contribute in the many activating enzymes involved in protein, carbohydrate, lipid, and energy metabolism (36). These results are consistent with Herwig, Walk (24) and inositol supplementing (0.16%), Pongmanee, Kühn (37) with phytase supplementing, Lim, Park (21) with silicate supplementing, as there were no significant differences observed in FCR, gm. feed / gm. egg. While it disagrees with the results of Kalvandi, Sadeghi (18) with arginine supplementation, Baghban-Kanani, Hosseintabar-Ghasemabad (31) with phytase supplementation, there was a significant ($P$<0.05) increase in the FCR of gm feed / g egg.

**3.5. Physiological Characteristics**

The results in table 3 showed a significant increase ($P$<0.05) in serum cholesterol in T3, T4, T5, and T6 treatments (284.7, 286.3, 291.8, 285.3 mg /dl), respectively, compared with T1 and T2 (270.8, 266.5 mg /dl ). Blood serum glucose significantly increased ($P$<0.05) in T5 (229.17 mg /dl ) compared with T1, T2 and T3 (197.5, 205.83 , 211.67 mg /dl) respectively with no differences compared withT4 and T6 (2183.3, 217.5 mg /dl) respectively. Total protein significantly increased ($P$<0.05) in phytase supplemented treatments T5 and T6 (6.35, 6.22 g /dl) compared with T1, T2, and T3 (5.17, 5.33, 5.67 g /dl), respectively and did not differ to T4 (6.02 mg /dl ).

No significant differences were observed in tri-G between the experimental treatments. T.P. is the critical index that reflects the construction of liver proteins. L-Arg supplementation increased the rate of hepatic protein synthesis in laying hens (30). Results of this study table 3 showed that dietary LArg supplementation tends to increase the levels of T.P. in the blood,

Cowieson, Ptak (38) reported that adding 500 FTU/kg to broiler diets results in increased blood glucose concentration in the broiler, and this effect may be related to sodium-dependent glucose transport mechanisms. as well as liberated phytate-binding carbohydrates, lipids, and proteins may be explained the increasing of Glucose, (T.P.), (CHO) and t (T.G.) In

the serum. Arginine acts as an antioxidant, and the phytate degradation intermediates, like inositol 1,2,3-triphosphate and inositol 1,2,3,6-tetraphosphate, are well documented as antioxidants by Phillippy and Graf (39). Antioxidants protect liver tissue from the effects of free radicals and protect the hepatocyte membranes from oxidative damage, thus maintaining the vital cellular metabolic functions of the liver, which leads to the promotion of the release of Vitellogenin, VLDL, and other components of the egg yolk from the liver to the ovary by the bloodstream and maintaining the lipoproteins and stimulate liver cells to manufacture proteins.

In conclusion, providing ASI or arginine-silicate - phytase mixture to laying hens' diets enhance egg production, feed utilization, and FCR g feed/egg in the peak period of laying hens.

## Authors' Contribution

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

## Ethics

The animal study was approved by the ethics committee of the University of Anbar, Baghdad, Iraq.

## Conflict of Interest

The authors declare that they have no conflict of interest.

## References

1. Sahin K, Orhan C, Tuzcu M, Hayirli A, Komorowski JR, Sahin N. Effects of dietary supplementation of arginine-silicate-inositol complex on absorption and metabolism of calcium of laying hens. PloS One. 2018;13(1):0189329.
2. Yuan C, Ding Y, He Q, Azzam M, Lu J, Zou X. L-arginine upregulates the gene expression of target of rapamycin signaling pathway and stimulates protein synthesis in chicken intestinal epithelial cells. Poult Sci. 2015;94(5):1043-51.
3. Khajali F, Wideman R. Nutritional approaches to ameliorate pulmonary hypertension in broiler chickens. J Anim Physiol Anim Nutr. 2016;100(1):3-14.
4. Duan X, Li F, Mou S, Feng J, Liu P, Xu L. Effects of dietary L-arginine on laying performance and anti-oxidant capacity of broiler breeder hens, eggs, and offspring during the late laying period. Poult Sci. 2015;94(12):2938-43.
5. Croissant JG, Fatieiev Y, Almalik A, Khashab NM. Mesoporous silica and organosilica nanoparticles: physical chemistry, biosafety, delivery strategies, and biomedical applications. Adv Healthc Mater. 2018;7(4):1700831.
6. Gonzalez-Uarquin F, Rodehutscord M, Huber K. Myo-inositol: its metabolism and potential implications for poultry nutrition—a review. Poult Sci. 2020;99(2):893-905.
7. Gautier A, Walk C, Dilger R. Effects of a high level of phytase on broiler performance, bone ash, phosphorus utilization, and phytate dephosphorylation to inositol. Poult Sci. 2018;97(1):211-8.
8. Higgins NF, Crittenden PD. Phytase activity in lichens. New Phytologist. 2015;208(2):544-54.
9. Englmaierova M, Skrivan M, Skrivanova E, Bubancova I, Cermak L, Vlckova J. Effects of a low-phosphorus diet and exogenous phytase on performance, egg quality, and bacterial colonisation and digestibility of minerals in the digestive tract of laying hens. Czech J Anim Sci. 2015;60(12):542-9.
10. Council N-NR. Nutrient requirements of poultry. National Academy Press Washington; 1994.
11. Mountney GJ, Parkhurst CR. Poultry products technology: Routledge; 2017.
12. Moss D, Baron D, Walker P, Wilkinson J. Practical clinical biochemistry. William Heinemann Medical, London; 1980.
13. Toro G, Ackermann PG. Practical clinical chemistry: Little Brown & Company; 1975.
14. Richmond W. Preparation and properties of a cholesterol oxidase from Nocardia sp. and its application to the enzymatic assay of total cholesterol in serum. Clin Chem. 1973;19(12):1350-6.
15. Al-Jubori O. Synergistic effect of ginger, propolis added to concentrated diet on some production and

physiological characteristics of the Awassi ewes. Iraqi J Agric Sci. 2017;48(2).
16. SAS J. Statistical Analysis System, v. 10.0. 2. Cary, North Carolina USA. 2012.
17. Duncan DB. Multiple range and multiple F tests. Biometrics. 1955;11(1):1-42.
18. Kalvandi O, Sadeghi A, Karimi A. Arginine supplementation improves reproductive performance, antioxidant status, immunity and maternal antibody transmission in breeder Japanese quail under heat stress conditions. Ital J Anim Sci. 2022;21(1):8-17.
19. Ahmed M, Salad E. Effect of Dietary Supplementation with L-Arginine and Vitamin C on Reproduction Performance and Economical Efficiency for Matrouh Strain Hens under Egyptian Summer Conditions. Ann Agric Sci Moshtohor. 2021;59(3):417-24.
20. Fernández S, Chárraga S, Avila-Gonzalez E. Evaluation of a new generation phytase on phytate phosphorus release for egg production and tibia strength in hens fed a corn-soybean meal diet. Poult Sci. 2019;98(5):2087-93.
21. Lim CI, Park JE, Kim SE, Choe HS, Ryu KS. Effects of dietary silicate based complex mineral on performance, egg quality and immunological competence in laying hens. Korean J Poult Sci. 2017;44(4):267-74.
22. Onderci M, Sahin N, Sahin K, Balci T, Gursu M, Juturu V, et al. Dietary arginine silicate inositol complex during the late laying period of quail at different environmental temperatures. Br Poult Sci. 2006;47(2):209-15.
23. Ren Z, Sun W, Cheng X, Liu Y, Han D, Yan J, et al. The adaptability of Hy-Line Brown laying hens to low-phosphorus diets supplemented with phytase. Poult Sci. 2020;99(7):3525-31.
24. Herwig E, Walk C, Bedford M, Schwean-Lardner K, Classen H. Contrasting the effects of phytase and pure myo-inositol on the performance, digestibility, blood and egg yolk inositol levels and digestion physiology of laying hens. Br Poult Sci. 2021;62(4):517-27.
25. Fascina VB, Pasquali GAM, Berto DA, Silva AdL, Garcia EA, Pezzato AC, et al. Effects of arginine and phytogenic additive supplementation on performance and health of brown-egg layers. Revista Brasileira de Zootecnia. 2017;46:502-14.
26. Johnson A. Ovarian follicle selection and granulosa cell differentiation. Poultry science. 2015;94(4):781-5.
27. Zhang X, Zhu M, Yuan C, Zou X. Proteomic analysis of hypothalamus and liver proteins affected by dietary l- arginine supplementation in laying hens. J Anim Physiol Anim Nutr. 2018;102(6):1553-63.
28. Kim JH, Pitargue FM, Jung H, Han GP, Choi HS, Kil DY. Effect of superdosing phytase on productive performance and egg quality in laying hens. Asian-Australas J Anim Sci. 2017;30(7):994.
29. Matuszewski A, Łukasiewicz M, Niemiec J. Calcium and phosphorus and their nanoparticle forms in poultry nutrition. Worlds Poult Sci J. 2020;76(2):328-45.
30. Yuan C, Bu X, Yan H, Lu J, Zou X. Dietary L-arginine levels affect the liver protein turnover and alter the expression of genes related to protein synthesis and proteolysis of laying hens. Poult Sci. 2016;95(2):261-7.
31. Baghban-Kanani P, Hosseintabar-Ghasemabad B, Azimi-Youvalari S, Seidavi A, Laudadio V, Mazzei D, et al. Effect of dietary sesame (Sesame indicum L) seed meal level supplemented with lysine and phytase on performance traits and antioxidant status of late-phase laying hens. Asian-Australas J Anim Sci. 2020;33(2):277.
32. Żyła K, Mika M, Duliński R, Świątkiewicz S, Koreleski J, Pustkowiak H, et al. Effects of inositol, inositol-generating phytase B applied alone, and in combination with 6-phytase A to phosphorus-deficient diets on laying performance, eggshell quality, yolk cholesterol, and fatty acid deposition in laying hens. Poult Sci. 2012;91(8):1915-27.
33. Pereira AA, Junqueira OM, Griep DN, Lima DMd, Sgavioli S, Moraes SdS. Phytase in diets with crude protein levels for commercial layers. Acta Sci - Anim Sci. 2017;39:163-8.
34. Dersjant-Li Y, Evans C, Kumar A. Effect of phytase dose and reduction in dietary calcium on performance, nutrient digestibility, bone ash and mineralization in broilers fed corn-soybean meal-based diets with reduced nutrient density. Anim Feed Sci Technol. 2018;242:95-110.
35. Chatree S, Thongmaen N, Tantivejkul K, Sitticharoon C, Vucenik I. Role of inositols and inositol phosphates in energy metabolism. Molecules. 2020;25(21):5079.
36. Xiao J, Wu S, Zhang H, Yue H, Wang J, Ji F, et al. Bioefficacy comparison of organic manganese with inorganic manganese for eggshell quality in Hy-Line Brown laying hens. Poult Sci. 2015;94(8):1871-8.
37. Pongmanee K, Kühn I, Korver DR. Effects of phytase supplementation on eggshell and bone quality, and phosphorus and calcium digestibility in laying hens from 25 to 37 wk of age. Poult Sci. 2020;99(5):2595-607.

38. Cowieson A, Ptak A, Maćkowiak P, Sassek M, Pruszyńska-Oszmałek E, Żyła K, et al. The effect of microbial phytase and myo-inositol on performance and blood biochemistry of broiler chickens fed wheat/corn-based diets. Poult Sci. 2013;92(8):2124-34.
39. Phillippy BQ, Graf E. Antioxidant functions of inositol 1, 2, 3-trisphosphate and inositol 1, 2, 3, 6-tetrakisphosphate. Free Radic Biol Med. 1997;22(6):939-46.