Research Article

Performance and Physiological Response of Chickens Supplemented with Dietary L-Glutamic Acid During the Late Phase of Egg Production

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Abstract | Dietary influence of L-Glutamic acid (Glu) on productive performance, egg quality, hatchability, intestinal morphology, tibia quality and blood parameters for Inshas chickens during the late laying period was performed. A total of 330 of Inshas chickens aged 50 weeks were randomly divided into five treatment groups. The birds of control group (Glu 1) were not supplied with Glu acid. Rest birds groups were supplemented with Glu as additional doses (0.4, 0.6, 0.8 and1%) for Glu2, Glu 3, Glu 4 and Glu 5, respectively. Egg production percentage and egg mass for Glu 3 and Glu 4 groups were significantly improved compared to those for the rest groups. Also, supplementing the diets with all concentrations of Glu acid significantly improved feed conversion ratio besides, eggshell calcium and phosphorus compared to those for control. Moreover, both Glu 3 and Glu 4 groups represented best significant improvement of hatchability and hatched chick weight. Highest significant increase of villus height and villus area was observed for chickens of Glu3 group (0.6%) compared with the others groups. While chickens of Glu3 and Glu4 (0.6 and 0.8%) represented the significant increases of villus width compared with those for the rest groups. Also, supplemented concentrations of 0.6, 0.8 and 1 % Glu significantly improved Seedor index for tibia. This study implies that dietary supplementation of 0.6 or 0.8 % Glu during late laying phase for chickens has a functional role on the intestine and subsequent improvement of egg production, egg quality, hatchability, tibia bone quality and thyroid hormones.

Keywords | L-Glutamic acid, Layers, Egg production, Intestine, Bone quality

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INDRODUCTION

Glutamate or L-glutamic acid (Glu) plays a vital role in intestinal health through glutamine synthetase enzyme (D' Mello, 2003). Glu has a productive role as a main source of energy and maintaining of the intestinal barrier (Xiao *et al*., 2016). Glu mechanism has impact on endocrine system through transformation into arginine and ornithine thus stimulates secretion of insulin-like growth hormone and growth hormone (Jazideh *et al*., 2014). Also, dietary Glu enhanced ovarian angiogenesis by elevating plasma arginine and nitric oxide concentrations and ultimately improved follicular development in the chickens (Ma *et al*., 2020).

Zavarize *et al.* (2011) showed that 1% Glu ameliorated feed consumption and conversion rate beside eggshell formation depends on the modularly bone quality and affects eggshell quality. Also, Pereira *et al.* (2019) pointed that Glu supplementation increased Ca amount in shell suggesting Ca excretion increase and mineral fixation in eggshell without skeletal injury. Moreover, Gholipour *et al.* (2019) stated that 1% Glu has positive effect on production traits and fertility in Guinea fowls.

It is well known that hen's age has negative influence on egg production including laying rate, egg weight, egg mass and eggshell quality (Zita *et al.*, 2009). Loss of structural bone during the late laying period was documented by (Chen and Kim, 2020) leading to skeletal problem such as osteoporosis (Bain *et al.*, 2016). Bone strength reduction with considerable cortical bone loss for elder chickens was previously documented by (Whitehead and Fleming, 2000). A long-term supplementation of Glu in the form of ketoglutaric acid was especially effective in improving the microstructure of trabeculae of medullary bone (Tomaszewska *et al.*, 2020).

For overcome the deterioration in the performance of chickens during the late stage of egg production, we used different concentrations of glutamic acid for trials of improving intestinal health and some physiological factors and in turn improvement in eggshell quality, egg production, fertility and hatchability.

MATERIALS AND METHODS

Experimental design

Three hundred and thirty Inshas chickens (a local Egyptian chicken strain) aged 50 weeks were used in this experiment and commenced three months in winter season at Kafr El-Sheikh Animal Production Research Station, Animal Production Research Institute, Agricultural Research Center, Egypt. The Institutional Animal Ethics Committee of Animal Production Research Institute approved the field experiment.

Birds were fed diet containing 2730 kcal/Kg ME and 16.1% crude protein. The ingredient profile and nutrient composition of the basal diet are shown in Table 1, the diet was formulated by ingredients at Kafr El-Sheikh Animal Production Research Station, Animal Production Research Institute, Agricultural Research Center, Egypt. Feed and water were provided ad-libitium throughout the experimental period. Birds were randomly assigned to 5 treatments with 6 replicates (10hens +1 male per replicate) in floor pens under a 16-h light: 8h dark lighting schedule.

The birds of the first group were served as control (Glu1) without any additional supplementation of L-glutamic acid (1.7% Glu). The four rest chicken groups were supplemented with (Glu) as additional doses (0.4, 0.6, 0.8 and1%) to the basal diet to be 2.1, 2.3, 2.5 and 2.7% for Glu2, Glu 3, Glu 4 and Glu 5, respectively.

Table 1: Ingredients and chemical analysis of the layer diet.

Ingredients	**%**
Yellow corn	64.00
Soy bean meal (44 %)	22.50
Corn gluten (60%)	1.58
Wheat bran	1.68
Di-calcium phosphate	1.40
Limestone	8.14
Vit+Min Premix [1]	0.30
Sodium chloride	0.30
DL- Methionine (99%)	0.10
Total	100
Chemical analysis	
Crude protein %	16.10
ME (Kcal/ kg)	2730
Crude fiber %	3.30
Ether extract %	2.87
Calcium (%)	3.43
Av. Phosphorus (%)	0.39
Methionine %	0.40
Lysine	0.84
Methionine + Cystine %	0.68

1-Vit+Min mixture provides per Kilogram of diet: Vit. A, 1200 IU; Vit. E, 10 IU; menadione, 3 mg; Vit. D3, 2200 ICU; riboflavin, 10mg; Ca pantothenate, 10mg; nicotinic acid, 20 mg; Choline chloride, 500mg, Vit. B12, 0.01mg; Vit.B6, 1.5mg; Vit.B1, 2.2mg; Folic acid, 1mg; Biotin, 0.05mg. Trace mineral (milligrams per kilogram of diet) Mn.55; Zn. 50; Fe. 30; Cu. 10; Se. 0.10; Anti-oxidant. 3mg.

Data collected

Productive and egg quality parameters

Daily egg production percentage, egg weight (g) and egg mass (g egg/hen/day were recorded in each treatment group. As well, daily feed consumption (g/hen/day) with feed conversion ratio (g feed/g egg) were detected. At the end of experimental five eggs representing each replicate were randomly taken to estimate eggshell weight (g), eggshell as a percentage for egg weight. Eggshell thickness without membranes (mm) was measured by a micrometer. Eggshell strength (Newton, N) was done by (Digital Force Gauge-FGC-50) at Faculty of Veterinary Medicine, Alexandria University according to (Bennett *et al.*, 1988). Crude ash for eggshells was determined by burning for one night in ash oven and analyzed for calcium (Ca) % and phosphorus (P)% using atomic absorption.

Hatching parameters

One thousand hatching Inshas eggs representing the replicates and groups were settled in the incubator. Eggs were weighed before setting in incubator at 37.5 °C and 55% Relative humidity (RH). Eggs were individually weighed again on 9th and 18th days of incubation to obtain weight loss percentages. At 18th day of incubation, the eggs were candled and those live embryos were transferred to the hatcher and at 37°C and 70% RH. Fertility and hatchability of fertile eggs percentages were determined. All percentages data were subjected to arcine square root percentages transformation prior to analyses. Eggs that failed to hatch at the end of incubation and having full opportunity for hatch were broken out and then examined macroscopically to estimate embryonic development and assigned according to their times of death by days as possible. Embryonic mortality percentages expressed as a percentage of fertile eggs set were recorded every day and classified in three periods (1-6, 7-15 and 16-21 days). Chick weight at pull out is the weight (g) of the chicks at the time of removal from the hatcher.

Intestinal villi morphology

At the experimental end, 3 hens from each replicate were randomly chosen and sacrificed. The chicken intestines were collected and isolated from the same previous laying hens. Middle portion part of jejunum was opened longitudinally. Ten sections representing each hen were measured for detection of villus height, width and crypt depth (μm). Villus height was measured from the top to the junction of villus–crypt, while width was measured in the middle part. Apparent villus surface area (mm^2) was calculated using the formula [(2π) × (villus width/2) × (villus height)] (Sakamoto *et al*., 2000).

Bone analysis

Tibia bones were taken from the same previous slaughtered hens at the end of experiment. Tibia weight was measured and divided by its length for detecting Seedor index (SI) as described by (Seedor *et al*., 1991). Breaking strength was evaluated for right tibia used (Newton, N) in Faculty of Veterinary Medicine, Alexandria University according to (Park *et al*., 2003). The left tibia was dried in oven at 105°C and crude ashes quantities were detected according to the method of (Yi *et al*., 1996) then dissolved, filtered and calcium and phosphorus percentages were assayed by atomic absorption.

Blood parameters

Blood samples were collected from the same previous slaughtered hens at the end of experiment. Blood samples were centrifugation at 3000 rpm for 20 minutes for obtained Serum and subsequently stored at -20 °C for biochemical analyses and hormonal levels. Serum Triiodothyronine (T_3) and Thyroxine (T_4) (ng/ml) were detected by radioimmunoassay according to the method of (Darras *et al*., 1992). In addition, total calcium (Ca, mg/dl) and ionized calcium (ICa, mg/dl) , phosphorus (P, mg/dl) and alkaline phosphatase (ALP, LU/L) alkaline phosphatase (ALP) by available commercial bio-diagnostic kits, Egypt. Total protein concentrations (g/dI) were measured using Biuret method as described by (Armstrong and Carr, 1964).

Statistical analysis

Data were statistically analyzed according to one-way ANOVA implemented in SAS program (SAS, 2016) using GIM procedure and mean differences were tested by Tukey's test and used for comparisons at P≤0.5 significant level.

The following model was used:

$$Y_{ij} = \mu + H_i + e_{ij}$$

Where; Y_{ij} = observed traits, μ = the overall mean, H_i = the effect of dietary Glu and e_{ij} = random error.

RUSULTS AND DISCUSSION

Productive performance

Table 2 represents the dietary effect of Glu acid supplementation on productive performance of Inshas chickens during late phase of egg production. Egg production % and egg mass had significantly increased for both concentrations (Glu3 and Glu4) compared with those for Glu1, Glu2 and Glu5 groups. All Glu acid concentrations significantly increased egg weight compared with control. Feed consumption was not affected by Glu administration through experiment period. Moreover, all concentrations of Glu acid supplementations significantly improved feed conversion ratio compared to control.

Egg quality

Effects of dietary Glu acid concentration on some eggshell quality traits and egg shell minerals of Inshas chickens during late phase of production are shown in Table 3. Groups of the highest concentrations of Glu acid (0.6, 0.8 and 1 %) represented significant increase in eggshell weight compared with those for Glu1 and Glu 2 groups (0 and 0.4 %). The same trend of the significant increase was observed in eggshell percentage with non-statistical increase in Glu 2. Eggshell thickness without membranes and eggshell strength had been significantly increased for groups of Glu3 and Glu4 compared to Glu1 and Glu2. All concentrations of Glu supplementation significantly improved Ca concentration in egg shell compared with control. The same trend of increase for Ca concentration was observed for P with highest significant record in Glu3 group (0.6%) compared with all other experimental groups.

Table 2: Effect of dietary Glutamic acid supplementation on productive performance of Inshas chickens during late phase of production.

Traits	Glutamic acid concentration (Glu)					SEM	P value
	Glu 1 (control)	Glu 2 (0.4 %)	Glu 3 (0.6 %)	Glu 4 (0.8 %)	Glu 5 (1 %)		
Egg production (%)	60.75^{b}	62.26^{b}	74.96^{a}	70.71^{a}	65.08^{b}	1.692	<0.000
Egg weight (g)	51.55^{b}	53.58^{a}	54.53^{a}	54.00^{a}	53.85^{a}	0.522	0.017
Egg mass (g/hen/day)	34.80^{c}	37.07^{bc}	45.40^{a}	42.50^{a}	38.88^{b}	1.088	<0.000
Feed consumption (g/hen/day)	119.15	119.27	118.49	119.29	118.78	0.168	0.180
Feed conversion ratio (g egg mass/g feed)	2.31^{a}	2.23^{b}	2.18^{b}	2.21^{b}	2.21^{b}	0.022	<0.01

$^{a,b \text{ and } c}$ means having different letters in the same row are significantly different (P≤0.05).

Table 3: Effects of dietary Glutamic acid supplementation on some eggshell quality traits and shell minerals of Inshas chickens during late phase of production.

Traits	Glutamic acid concentration (Glu)					SEM	P value
	Glu 1 (control)	Glu 2 (0.4 %)	Glu 3 (0.6 %)	Glu 4 (0.8 %)	Glu 5 (1 %)		
Eggshell weight (g)	5.42^{b}	5.74^{b}	6.26^{a}	6.14^{a}	6.13^{a}	0.125	0.0257
Eggshell percentage	10.63^{b}	10.81^{ab}	11.42^{a}	11.39^{a}	11.38^{a}	0.176	<0.000
Eggshell thickness without membranes (mm)	0.33^{c}	0.33^{c}	0.37^{a}	0.36^{a}	0.35^{b}	0.004	<0.000
Eggshell strength (N)	4204.33^{c}	4288.33^{c}	4647.00^{a}	4585.00^{ab}	4553.00^{b}	24.99	<0.000
Eggshell calcium (%)	30.00^{c}	38.10^{b}	39.20^{a}	39.00^{a}	38.11^{b}	0.28	<0.001
Eggshell phosphorus (%)	0.105^{c}	0.130^{b}	0.148^{a}	0.139^{b}	0.139^{b}	0.036	<0.001

$^{a,b \text{ and } c}$ means having different letters in the same row are significantly different (P≤0.05).

Table 4: Effect of dietary Glutamic acid supplementation on hatching traits of Inshas chickens during late phase of production.

Traits	Glutamic acid concentration (Glu)					SEM	P value
	Glu 1 (control)	Glu 2 (0.4 %)	Glu 3 (0.6 %)	Glu 4 (0.8 %)	Glu 5 (1 %)		
Fertility (%)	84.02^{c}	90.11^{b}	90.00^{b}	94.67^{a}	90.66^{b}	0.604	<0.000
Hatchability of fertile egg (%)	88.05^{b}	88.16^{b}	96.30^{a}	95.80^{a}	88.97^{b}	1.002	<0.000
Egg weight loss (%)							
0-9 day	7.96^{a}	7.49^{ab}	6.49^{b}	7.04^{ab}	7.19^{ab}	0.454	0.06
10-18 day	6.37^{a}	5.85^{a}	3.84^{b}	4.81^{ab}	5.08^{ab}	0.484	<0.000
0-18 day	13.38^{a}	13.36^{a}	10.12^{c}	11.55^{bc}	12.24^{ab}	0.532	<0.000
Embryonic mortality (%)							
Early (1-6 day)	3.99 ^{a}	3.69^{ab}	1.46^{b}	2.11^{ab}	2.93^{ab}	0.602	<0. 000
Mid (7 -15 day)	1.61	1.48	0.76	0.69	1.48	0.746	0.874
Late (16 – 21day)	6.35^{a}	4.46^{a}	0.72^{b}	1.39^{b}	3.67^{a}	0.845	0.05
Total embryonic mortality (%)	11.94^{a}	9.63^{ab}	2.95^{c}	4.20^{c}	8.09^{b}	0.974	0.006
Hatched chick weight (g)	32.67^{d}	35.66^{cd}	41.33^{a}	39.76^{ab}	36.66^{bc}	0.498	0.05

$^{a,b,c \text{ and } d}$ means having different letters in the same row are significantly different (P≤0.05).

Hatching performance

Effects of dietary Glu acid concentration on some hatching traits of Inshas chickens during late phase of production are shown in Table 4. The best significant percentage of fertility was detected for eggs of Glu4 compared with the other rest groups. Moreover, both concentrations for Glu3 and Glu4 groups represented best significant improvement of hatchability of fertile eggs. Highest numerical percentage of egg weight loss were observed for Glu1 and Glu2 groups among the experimental incubation intervals compared with the others, while the least percentage of loss were detected for egg of Glu3 and Glu4 groups. Moreover, Glu3 and Glu4 groups represented significant reduction of embryonic mortality percentages among the late phase of incubation (16-21days) and total embryonic mortality (0-21days) compared to rest groups. Whereas, Glu1 group

showed highest records of embryonic death among the same studied incubation intervals. Furthermore, the best significant hatched chick weight was observed for Glu 3 group supplemented with 0.6 % followed by Glu 4 and Glu 5 compared with those of Glu1 and Glu2.

Intestinal villi morphology

The histological changes of intestinal jejunum for hens of the experimental groups supplemented with different concentrations of Glu acid during the late phase of egg production are shown in Figure 1a, b, c, d, e. Figure 1a illustrates the histology of intestinal jejunum for hens of Glu 1 group which showed thin villi lined with pseudo stratified epithelium with goblet cells. Also, Figure 1b demonstrated that intestinal jejunum histology of hens for group (0.4% Glu acid) which demonstrates the normal intestinal villi with mild increase of their length. Moreover, Figure 1c illustrates that intestinal jejunum of hens for group (0.6 % Glu acid) possessed marked increase of intestinal villi length with decrease of the inter-villus spaces. Figure 1d showed that intestinal jejunum of hens for group (0.8 % Glu acid) had an increase of villi length. Besides, Figure 1e Intestinal jejunum of hens for group (0.1% Glu 5) had an increase of intestinal villi length.

Table 5 shows the effect of dietary Glu acid concentration on intestinal morphology of Inshas chickens during late laying phase. Chickens supplemented with 0.6 % Glu acid for Glu3 group represented highest significant records of villus height, crypt depth and villus area compared with other groups. Moreover, chickens of Glu3 and Glu4 represented the highest significant increase of villus width compared with the rest groups.

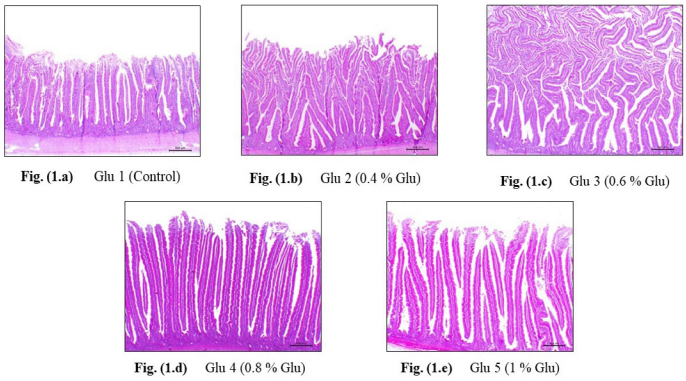

Figure 1: Histology of intestinal jejunum for hens of the groups supplemented with different concentration of Glutamic acid during the phase of egg production.

Tibia bone quality

Data of Table 6 show the effect of dietary Glu acid on tibia bone quality parameters for Inshas chickens during the late phase of egg production. Tibia of Glu3 hens represented significant increase of breaking strength (N) followed by that of Glu 4 comparing with the other experimental groups. Also, it obvious from this table that supplementing the diet with concentration of 0.6, 0.8 and 1 % Glu acid significantly improved SI and increased concentration of tibia ash % and P % in the tibia ash compared with those supplemented with 0.4 % Glu acid and control. Moreover, all Glu acid concentrations significantly increased Ca % in the tibia ash compared with control.

Table 5: Effect of dietary Glutamic acid supplementation on intestinal morphology of Inshas chickens during late phase of production.

Traits	Glutamic acid concentration (Glu)					SEM	P value
	Glu 1 (control)	Glu 2 (0.4 %)	Glu 3 (0.6 %)	Glu 4 (0.8 %)	Glu 5 (1 %)		
Villus height (µm)	1081.08^{d}	1393.29^{c}	2386.49^{a}	1788.95^{b}	1550.59^{c}	39.77	0.0795
Villus width (µm)	96.12^{c}	104.59^{bc}	140.31^{a}	134.90^{a}	109.84^{b}	2.374	0.0693
Crypt depth (µm)	119.40^{b}	111.02^{bc}	103.60^{c}	105.19^{c}	153.14^{a}	1.67	0.0958
Villus area (mm^2)	0.32^{e}	0.46^{d}	1.05^{a}	0.76^{b}	0.53^{c}	0.011	0.0145

$^{a,b,c,d \text{ and } e}$ means having different letters in the same row are significantly different (P≤0.05).

Table 6: Effect of dietary Glutamic acid supplementation on Tibia bone quality for parameters Inshas chickens during late phase of egg production.

Traits	Glutamic acid concentration (Glu)					SEM	P value
	Glu 1 (control)	Glu 2 (0.4 %)	Glu 3 (0.6 %)	Glu 4 (0.8 %)	Glu 5 (1 %)		
Tibia Breaking strength (N)	250.11^{d}	262.70^{c}	319.6^{a}	298.80^{b}	279.7^{c}	0.02	<0.001
Seedor index (mg/mm)	58.13^{b}	59.76^{b}	62.60^{a}	62.20^{a}	62.30^{a}	0.02	<0.001
Tibia ash (%)	44.80^{c}	54.70^{b}	56.21^{a}	56.10^{a}	55.00^{ab}	0.02	<0.001
Calcium in the tibia ash (%)	19.50^{b}	24.20^{a}	25.00^{a}	25.80^{a}	24.00^{a}	0.124	0.05
Phosphorus in the tibia ash (%)	8.16^{e}	10.05^{d}	10.64^{a}	10.30^{b}	10.12^{c}	0.02	0.05

$^{a,b,c,d \text{ and } e}$ means having different letters in the same row are significantly different (P≤0.05).

Table 7: Effect of dietary Glutamic acid supplementation on some blood parameters of Inshas chickens during late phase of production.

Traits	Glutamic acid concentration (Glu)					SEM	P value
	Glu 1 (control)	Glu 2 (0.4 %)	Glu 3 (0.6 %)	Glu 4 (0.8 %)	Glu 5 (1 %)		
Total protein (g/dl)	6.14^{d}	7.32^{c}	8.33^{a}	7.99^{ab}	7.53^{bc}	0.168	0.082
Calcium (mg/dl)	11.45^{d}	14.10^{c}	17.55^{a}	17.35^{a}	15.45^{b}	0.216	<0.000
Ionized calcium (mg/dl)	10.65^{c}	11.35^{b}	14.35^{a}	11.90^{b}	11.90^{b}	0.190	0.015
Phosphorus (mg/dl)	5.35^{b}	6.45^{a}	7.10^{a}	6.85^{a}	6.75^{a}	0.254	0.008
Alkalin phosphatase (I.U./L)	28.05^{b}	34.30^{a}	38.20^{a}	38.10^{a}	36.55^{a}	1.116	<0.001
T_3 (ng/ml)	2.00^{d}	2.72^{c}	3.90^{a}	3.77^{a}	3.56^{b}	0.041	<0.000
T_4 (ng/ml)	4.66^{d}	6.35^{c}	8.00^{a}	8.04^{a}	6.99^{b}	0.137	<0.000

$^{a,b,c \text{ and } d}$ means having different letters in the same row are significantly different (P≤0.05).

Blood parameters

The findings of Table 7 showed that Glu acid supplementation affected serum biochemical traits and thyroid hormones. It apparently from this table that supplementing the diet with both concentrations of Glu3 and Glu4 represented the highest significant values of serum total protein compared with those for Glu1 and Glu2 groups. The same mentioned groups (Glu3 and Glu4) demonstrated the same significant increase of Ca compared to all experimental groups. Moreover, serum of birds for group Glu3 represented the same significant increase of ICa comparable to those for the all-rest groups. All treated groups with Glu acid represented significant increase of P and ALP compared with control. Referring to the data of thyroid hormones, both Glu3 and Glu4 showed significant increase of T_3 and T_4 hormones comparing with those for the other groups.

The apparent amendment of egg production percentage, egg weight and egg mass due to Glu acid supplementation with doses of 0.6 and 0.8 % is in accordance with results reported by (Dong *et al*., 2010) who referred that the diet with 0.8 % glutamine was able to increase the productive performance of laying hens through stimulating hormone secretion and better development of both the duodenum and oviduct structure in laying hens. Also, Bezerra *et al.* (2015) supported the same results and mentioned that Glu acid is the reason of the developing of the digestive system and consequently egg production. Zavarize (2011) drawn the same conclusion of feed conversion improvement but with 1% Glu only.

Referring to the results of egg quality traits, the improvement of eggshell weight, shell thickness and shell strength in this study could be related to the increase of Ca and P as shown in Table 3. Contrary to our results, (Dong *et al*., 2010) found that dietary glutamine supplementation had no significant influence on egg shell weight and egg shell thickness.

It is concluded from the current results that the increase of Ca and P due to supplementing the diet with Glu had a cardinal role in improvement of eggshell weight, shell thickness and strength especially for the late phase of egg production. These results and conclusions are in good agreement with the finding of (Pereira *et al*., 2019) who showed that Glu acid provided Ca increase in eggshell. Also, the same authors mentioned that the circulating Ca increase during shell deposition is related with Ca intestinal absorption and medullar bone deposition and consequently preserved cortical bone.

The improvement of fertility in this study due to using Glu acid had been previously documented by (Gholiipour *et al*., 2019) who found that 1% Glu has the most positive effect on fertility parameters in Guinea fowls. This improvement could interpret from a physiological point of view by (Dong *et al*., 2010) who mentioned that dietary inclusion of Glu increased FSH and LH in laying hens and showed that glutamine improves gonadal hormone levels and consequently the productive and reproductive performance.

Hatchability increases for all bird groups supplemented with Glu acid was mainly due to diminishing the number of embryonic deaths in these treatments. Also, the combined results of increasing hatchability % and decreasing embryonic mortality due to Glu acid supplementation may be related to improvement of eggshell measurements as detected in Table 3. This explanation is in harmony with previous notion cited by (Rizk *et al*., 2008) who stated that hatchability % could be affected by eggshell thickness. Furthermore, (Wesam *et al*., 2017) showed that eggs with less egg shell thickness had higher pore number and egg weight loss and consequently eggshell conductance increase leading to hatchability diminish.

Burton and Tullett (1985) suggested that fresh eggs and weight loss of incubated eggs determine the hatched

chick weight. The notion of increase chick body weight with the increase of egg weight had been documented by (Rizk *et al*., 2006) who mentioned that there is a positive linear correlation between egg weight and hatched chick weight. Moreover, Oliaei *et al*. (2014) indicated that in ovo administration of Gln increased chick weight at hatch.

It is concluded from the current results that using 0.6 and 0.8 % Glu acid could be a good tool for optimizing the egg quality traits and in turn the egg weight loss and embryonic mortality and finally hatchability improvement.

Improvement of intestinal villus morphology due to Glu supplementations with respect to all studied parameters such as villus height and width, crypt depth and villus area extended the earlier findings of (Maiorka *et al*., 2000). Fischer da silva *et al*. (2007) have asserted that an increase in number of intestinal villi due to 1% Glu supplementation in broilers. Also, Wang *et al*. (2008) reported that intestinal proliferation had been increased and morphological improved due to glutamine supplementation. Moreover, Burrin and Stoll (2009) stated that Glu acts as an oxidative fuel and plays cardinal role for ameliorated the function intestine through the turnover of the intestinal mucosa.

Generally, the improvement of breaking strength, SI, Ca and P in the tibia ash resulted from using of 0.6, 0.8 and 1 % Glu acid and these results are parallel with the results of (Pereira *et al*., 2019) who reported that 0.8% Glu acid resulted in improvement of bone parameters for elder laying chickens. Hunter and Goldber (1994) found that Glu acid can work as endogenous factors to control bone cell activity. Hinoi *et al*. (2004) reported that Glu acid enters the cells via inotropic and metabotropic receptors and transporters in the osteoblasts and osteoclasts. Brakspear and Mason (2012) mentioned that Glu plays a role in bone formation and mass maintenance. Also, Blais *et al*. (2019) found that increase in Gln plasma concentration stimulates osteoblast activity.

There is a paucity of information concerning the direct effect of Glu acid on serum total protein, Ca, ICa, P and ALP, besides T3 and T4 hormones. The noticed increase of total protein, Ca, Ica, P and ALP in the current results could be the reason of improvement for egg weight and egg production % as illustrated in Table 2, as well as shell quality in Table 3 and bone quality in Table 6.

The significant increase of serum total protein due to supplementing all experimental Glu acid concentrations could be related to the function of Glu and Gln which be substrates of protein synthesis as documented by (Newsholme *et al*., 2003). In addition, all mentioned increase of Ca, ICa, P and ALP enzyme due to Glu acid supplementations did not agree with reported by (Pereira *et al*., 2019) who mentioned that Glu supplementation did not affect the level of Ca, P and ALP in hens' serum. The difference between the mentioned results and our results may be attributable to strain, hens age and physiological status of birds. The significant increase of serum Ca and ICa by addition of Glu acid in our study could be explained on light of those mentioned by different research workers as (Newsholme *et al*., 2003) stated that Glu acid caused permeability increase of intestine. Also, He *et al*. (2021) mentioned that Gln is a major energy source for the intestine. Moreover, in the study of (Yang *et al*., 2008) found that addition of ploy $^{\gamma}$ glutamic to rats diet increased amount of soluble Ca in small intestine and finally bone Ca increase. Moreover, the same pervious authors added that chelation of Ca by $^{\gamma}$ poly-Glu acid prevents the precipitation of Ca salts, thereby increasing Ca solubility in the small intestine and increasing the passive transport of Ca to the bone. Furthermore, (Ma *et al*., 2020) showed that the improvement of eggshell quality for laying chickens supplemented with N-carbamyl glutamate affects gene expression of Ca metabolism and secretion of related hormones to promote eggshell formation.

In conclusion, the detected increase of Ca and ICa in eggshell and the chicken bone in our results and earlier results by authors could be due to the absorption increase of Ca in the intestine due to dietary Glu supplementation.

The significant elevation of ALP enzyme due to Glu addition as shown in the current results could be a good indicator of good bone formation and this conclusion is approach with documented by (Li *et al*., 2014) who stated that ALP is a sign of mature osteoblasts. Also, better quality and breaking strength of eggshell and bone could be due to the higher blood Ca, ICa and P as stated by (Jiang *et al*., 2010).

Oure results referring the increasing level of T_3 and T_4 hormones with all levels of Glu acid are in accordance with the results of Alfonso *et al*. (2000) who found that L- Glu increased serum T_3, T_4 and TSH concentrations in rats. Furthermore, Aizawa *et al*. (2012) found that Glu and Gln acid enhanced thyroid stimulating hormone for rats. The foregoing results of increasing thyroid hormone by Glu supplementation could be the reason of increasing egg production. These results may shed some lights as reported by (Harlap *et al*., 2021) who stated that thyroid hormones affect the productive potential of laying chickens.

In the future researches are needed to assess the direct role of glutamic acid on the digestive enzymes and reproductive system for chickens during late phase of egg production.

CONCLUSION

This study implies that dietary supplementation with concentration of 0.6 or 0.8 % Glu for late laying chickens has an improvement functional role in the intestine and subsequent improvement of egg production, egg quality, hatchability, tibia bone quality and T_3 and T_4 hormones.

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NOVELTY STATEMENT

The authors have developed the composition of laying chickens diets during the late phase of egg production by Glutamic acid. The results of the dietary supplementation with Glutamic acid for late phase of egg production and its effect on production and physiological traits are published for the first time.

AUTHOR'S CONTRIBUTION

REK, FAT and WAF: Experiment idea and design. MRE, MRA and HAS: Performed the farm experiment. AMK, HAS, MRE and MRA: Performed the laboratory analysis. FAT: Statistical analysis. REK, WAF and AMK: Wrote and revised the manuscript with approval of all authors.

Ethical approval

All procedures and husbandry guidelines were performed according to the Experimental Animal Care Committee Ethics of Animal Production Research Institute, Agriculture Research Center, Giza, Egypt.

Conflict of interest

The authors have declared no conflict of interest.

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