Using nutritional supplements as a management technique to improve hatching indicators and egg quality in Iraqi Mizo hens

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Abstract:

The study was carried out at the University of Mosul's College of Agriculture and Forestry's Animal Production Department's laying hen field. During the production phase, from the start of week 44 to the conclusion of week 54, 216 local laying hens (Mizo) were employed for research. They were grown on the ground and given a typical meal that included protein (17%) and energy (2800 kcal/kg), with betaine and glycine added to the treatment diets. Treatments were as follows: T1: Control group, T2: Use of betaine 2.4 g/kg in the diet, T3: Use of glycine 2.4 g/kg in the diet, T4: Use of betaine 1.2 g + glycine 1.2 g / kg in the diet. The experimental period lasted eleven week, during which hatchability indicators and egg quality measurements were collected, and internal egg contents were examined. The results of the statistical analysis ($P \leq 0.05$) are as follows: a significant increase in the weight of chicks at hatching, chick weight to egg weight %, fertility and hatching percentage, and a decrease in embryonic mortality %, a significant increase in the yolk color index and Hue unit, an increase in the protein % and carbohydrates % with a decrease in cholesterol of the egg content, and a significant increase in methionine, glycine, cysteine, leucine, and phenylalanine and the percentage of vitamin A and choline in treatments T2, T3, T4 compared to the control, and T4 was superior compared to control in vitamins B2 and B6.

Keyword: Betaine, Egg ingredients, Glycine, Laying Hens ,

Introduction

Since the beginning of the industrial and economic revolution, which began to take rapid steps at the start of the twentieth century, the poultry industry was and still is one of the basic and important pillars of the economy of many countries in the world, as poultry production projects are characterized by a rapid capital cycle and also their contribution to meeting consumers' demands for meat and eggs. In the last years of this century, poultry farming has witnessed rapid development compared to other animal production projects [1], followed at the same time by an increase in demand for it as a result of the increase in the world's population and the rise in health and cultural awareness and their awareness of the high nutritional value of poultry products [2]. Due to rapid economic growth, egg consumption is increasing worldwide. Eggs are consumed

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globally by people of all age groups, and eggs play an important role in a healthy global diet [3,4] . Eggs provide valuable nutrients as part of a balanced diet. They are an excellent source of high-quality animal protein at an affordable price, as they contain biologically beneficial components for the body. Eggs are characterized by a high content of iron, calcium, and phosphorus [5]. They are also rich in vitamins and are an important source of vitamin D. They are also rich in carotenes and contain most minerals [6.[

Egg production and quality depend on the close relationship between poultry nutrition and health [7] . Laying hens often experience various physiological changes in the late stages of production, such as excessive accumulation of reactive oxygen species (ROS) and redox imbalance [8] . These impair the reproductive function of chickens, accompanied by reduced egg quality and decreased egg density [9]. Furthermore, the immune system efficiency declines, and the intestinal microbiota of laying hens is disturbed during the late stage of production [10]. Therefore, countries around the world have turned their specialized scientists to research modern, practical ways to improve poultry performance by developing poultry feed and all management methods related to the production process. The use of nutritional supplements such as betaine and glycine is a modern nutritional technique that has been applied in recent years to maintain the productive performance of laying hens, improve egg quality, and prevent what is known as ovarian senescence. It also acts as an antioxidant [11,12, 13, 14]. Betaine is a trimethyl glycine naturally occurring in some grains and plant products. It has antioxidant properties, a methyl group donor, and an osmotic regulator [15,16]. It also improves ovarian aging by affecting the gene factor (EGR1) responsible for ATP6 [17] and responsible for cell renewal [18,19.[

Materials And Methods

This study was carried out in the laying hens field of the Animal Production Department - College of Agriculture and Forestry - University of Mosul, and 216 local Mezo laying hens, These hens were used for the study in the production stage from 44 weeks to 54 week, and the treatments included supplementation the ration with the amino acids betaine and glycine, were used for the study in the production stage from the beginning of week 44 until the end of week 54, and the treatments included preparing the feed with the amino acids betaine and glycine as follows:

T1: Control
T2 :Use of betaine 2.4 g/kg ration
T3 :Use of glycine 2.4 g/kg ration
T4: Use of betaine 1.2 g + glycine 1.2 gm/kg ration

The study used a laying hens production field with a floor covered with plastic mesh. It was divided into 12 pen , each representing a replicate at a density of 8 hens/m², and a rate of 3 replicates/treatment .

The hall was equipped with the necessary supplies for laying hens, and all appropriate environmental conditions were provided. The temperature ranged between 18-22°C, taking into account humidity, ventilation, and lighting. Veterinary supervision was available

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throughout the experiment in the fields of the Animal Production Department .

The feed ingredients for the ration were purchased from the local market, crushed, thoroughly mixed, and formulated according to the recommendations of [20]. Betaine and glycine were added to the ration according to the experimental parameters.

The study period lasted 11 weeks, during which data were collected on hatching parameters, such as chick weight at hatching and the ratio of chick weight to egg weight. Fertility and hatchability were also calculated.

Measurements of egg quality were taken, and chemical analyses of the egg content were conducted to estimate the percentage of protein, fat, and carbohydrates, as well as the content of some amino acids and vitamins, in the central laboratory of the Scientific Research Authority - Environment and Water Research Center/Ministry of Higher Education/Baghdad. At the end of the experimental period, three eggs were taken from each replicate and broken separately, removing the shell and shell membranes. The yolks were then mixed with the whites in a glass dish to prepare samples for each replicate, and samples were taken for testing .

Statistical analysis: A complete randomize-ed design (CRD) was used. data were analyzed using [21], Duncan multiplies Rang was applied to determine differences between treatments [22] significant range ($P \leq 0.05$. (

Table (1) shows the percentages of ration components

Feed Component	Its Percentage In The ration
Yellow Corn	47.5
Soybean meal	24
Wheat	15.5
Sunflower oil	1
Premix	2.5
Mono calcium phosphate	1
Limestone	8.250
Salt	0.250
Total	100
Chemical Composition (**)(*)	
M.E (kcal/kg feed)	2865.65
Crude Protein (%)	89. 16
Crude Fiber (%)	3.08
Calcium (%)	0.72
Methionine	0.41
Methionine + Cysteine (%)	0.7
Lysine (%)	0.9

*Calculated according to [20 [

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Results and Discussion

-1Use of betaine and glycine in ration and their effect on some hatching indicators of eggs produced by local Mizo hens

Table (2) shows that the use of betaine and glycine in the ration significantly increased the chick's weight hatching in the supplement treatments (1.2g betaine + 1.2g glycine)/kg feed) compared to the control (free of the supplement). The values were (45.43 and 40.93) gm, respectively. And from the table, it was shown that the percentage of chicks weight / egg weight increased significantly in the treatments in which betaine and glycine were used (T2, T3, T4) as they reached (72.72, 75.89, 74.22%), respectively, compared to the control, in which of chick weight% reached (67.23)%, as this percentage increased by (5.49, 8.66, 6.99)% more than the control group in the percentage of chicks' weight at hatching, and the increase in this percentage may be the result of the abundance of amino acids in the diet of mothers that were subjected to betaine and glycine supplements, which increased the deposition of protein and nutrients (Table 6 for amino acids), as betaine and glycine can increase the availability methionine by providing it with a methyl group, thus increasing the rate of utilization of methionine, purine, and glutathione, as they are considered structural units in the formation of elastin, collagen, and keratin they mentioned that [20, 21, 23], which enter into connective tissues and endothelial cells of blood vessels, and they also contribute to cell renewal and protection from damage, as well as in some biological molecules and compounds, and they participate in the metabolic processes of fat and amino acids metabolism and renewal [24 .[

This increased the activity and growth of embryonic tissues and increased the weight of chicks as an indicator of hatching, it is also consistent with Table (7) regarding vitamins, especially vitamin A, in the internal components of the egg, as the amount of vitamin A present in the egg content increased as a result of adding betaine and glycine to the feed, which increased the metabolic processes and consequently increased the embryonic mass, which in turn was reflected in the average weight of the chicks and their percentage of the egg weight [25 .[

Table (2) it was noted that the fertility rate of total eggs increased in the addition treatments compared to the control, which was 62%, as this rate increased significantly to 78%, 78%, and 82% for the second, third, and fourth treatments, respectively. This may be due to the increase in the health and physiological level of the flock, as these two amino acids have important antioxidant properties that work to remove and repair damaged cells and regulate the immune system [26]. They also have a role in the formation of steroids, such as cortisone-like hormones, androgenic and estrogenic hormones [27], and this was reflected in the fertility rate.

Table (2), found that the hatching rate of fertilized eggs increased significantly in the treatments of added betaine and glycine, as it was (79.49, 89.75, and 95.12) % for the second, third, and fourth treatments, respectively, compared to the control 67.74% . The hatching rate increased as a result of the increase in the egg content of vitamin B2

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(riboflavin) and vitamin B6 (pyridoxine) in the treatments to which betaine and glycine were added, compared to the control , as it enhanced their concentration in the egg, and this was confirmed (Table 7). They also play an important role in the growth and development of the fetus and its ability to survive, In addition, riboflavin has antioxidant properties that protect the fetus from oxidative respectively, compared to control, in which the percentage of total embryonic mortality 32.26%. The percentage of mortality decreased as a result of the increased concentration of vitamins B2 and B6, which led to an increase in the hatching% for the same treatments compared to the control. It is worth mentioning that the three periods of embryonic mortality were different from each other if the average period of embryonic .

stress and death [28, 29, 30, 31]. In the embryonic mortality trait, a significant decrease was found in the percentage of total embryonic mortality from the total fertilized eggs, reaching (20.51, 10.26, 12.20)% in the treatments that contained the two amino acids (2.4 g betaine, 2.4 g glycine, 2.4 g betaine + glycine),

deaths of the treatments was compared with each other, and in a noticeable way, as this period is greatly affected by the feeding of the laying hens during egg laying and reached (12.5, 0, and 0) % compared to the control, which reached (60)% of the total embryonic deaths rate, and this is attributed to the presence of betaine and glycine in the feed provided

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Table (2) Use of betaine and glycine in ration and their effect on some hatching indicators of eggs produced by local Mizo hens

Treatments Hatching Indicators	T1 (Control)	T2 2.4 gm Betaine	T3 2.4 gm Glycine	T4 1.2 gm Betaine +1.2 gm Glycine)
chick weight (gm)	40.93±1.12b	44.60 ±0.77ab	42.86 ±1.01 ab	45.43 ±1.34 a
chick weight to egg weight %	67.23± 1.4 b	72.72 ± 1.2 ab	68.89 ± 1.07 ab	74.22 ± 2.34 a
Fertility % of total eggs	62 b	78 a	78 a	82 a
Hatching% of total eggs	67.74	79.49	89.75	95.12
Total mortality of fertile eggs %	32.26	20.51	10.26	12.20
Early mortality to total mortality %	20	50	75	60
Middle mortality to total mortality%	60	12.5	Zero	Zero
Late mortality to total mortality %	20	37.5	25	40

Different letters in a row indicate significant differences between means (P≤0.05) (mean ± S.E.(

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use of betaine and glycine in ration and their effect on some quality indicators of eggs produced by hens:

- In the yolk specifications: Table (3) shows no significant effect of using betaine and glycine on relative yolk weight, yolk height, and yolk index of the supplementation treatments compared to the control. However, significant differences were observed regarding yolk color between the betaine and glycine supplementation treatments and the control treatment. In the week (54), the treatment fourth was the best, followed by the third and second treatment. The yolk color degree reached (10.66, 10.33, 9.66, and 8.33) degrees, respect-ively. The improvement in the color index is due to an improvement in the yolk formation and the deposition of beta-carotene

pigments, this was evident in the table 7 (the vitamin content), where the vitamin A deposited in the yolk was significantly higher in the supplementations compared to the control .

These two amino acids (betaine and glycine) activate metabolic processes [24, 32, 33], also they are essential requirements for supporting yolk formation, as the nutritional content and deposition efficiency affect the color of the yolk according to the number of carotenes it contains.

These results are consistent with [34], who found no effect of added betaine (0.7, 1.5) g/kg on yolk weight and index, while the yolk color of hen's eggs at 20 weeks of age improved compared to control, and also [35] who added betaine 0.5% to the ration of laying hens at (38) week of age.[21] found no significant effect of added (1000) mg betaine/kg of ration on yolk weight, percentage, and index,[36, 18] found no significant effect of added (3 and 6) gm betaine/kg feed on yolk color. Also, [37] indicated that added betaine didn't affected yolk characteristics at (45 and 57) weeks of age. Researcher [38] indicated that added betaine to drinking water at a conc-entration of 0.55 gm/L significantly affected yolk weight in ISA Brown at 25 weeks of age and Lohmann at 26 weeks of age. The results of this study on the use of glycine were consistent with [39] regarding the use of glycine at concentrations of 0.05 and 0.10%, with no significant differences in yolk weight, egg weight, or yolk color. As well as, [40] found no significant differences in yolk weight, height, and color when using glycine at different rates for 71-78 weeks, [41] found no significant effect of added glycine at 0.341%, and 0.683% to the feed of Lohman compared to the control on yolk color over a 12-week rearing period. These results differed from those of [41], who observed an increase in the yolk % in the eggs of local Mandarah hens when glycine was added to the feed .

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Table (3) Use of Betaine and Glycine in laying hen's diets and their effect on some yolk traits .

Age of hens (week)	Treatments T1	T2	T3	T4
Average Relative Weight of Yolk %				
Week 49	28.85 ± 1.70 a	27.22 ± 0.84 a	27.50 ± 1.34 a	26.01 ± 0.74 a
Week 54	26.05 ± 1.34 a	26.20 ± 1.73 a	25.16 ± 0.47 a	25.68 ± 1.94 a
Yolk Index				
Week 49	36.58 ± 0.81 a	39.28 ± 1.89 a	35.42 ± 1.45 a	38.39 ± 0.03 a
Week 54	48.74 ± 1.35 a	45.42 ± 0.91 a	46.98 ± 1.64 a	44.27 ± 2.36 a
Yolk Color Degree				
Week 49	6.66 ± 0.88 a	6.33 ± 0.33 a	6.66 ± 0.33 a	7.65 ± 0.33 a
Week 54	8.33 ± 0.33 c	9.66 ± 0.33 b	10.33 ± 0.33 ab	10.66 ± 0.88 a

Different letters in a row indicate significant differences between means (P≤0.05) (mean ± S.E.(

-In albumin properties: Table (4) shows no significant difference between the use of betaine and glycine in weight of albumin %. However, the albumin index and Hue unit improved in the addition treatments (T2,T3 and T4) compared to the control. The albumen index for the addition and control treatments reached (9.37, 10.88, 9.93 and 8.73), respectively. The Hue unit for the addition treatments (92.5, 94.05, and 93.40), respectively, compared to the control (84.80 .(

This is due to the dependence of these two indicators on the height of the egg white, which is linked to its quality, as it is believed that the height of the egg white is directly proportional to the amount of collagen in the egg white deposited in the egg white, which is the reason for the tension in the egg white. This is due to the high content of amino acids in the egg compound, especially in the egg white. This is consistent with Table (12), which is related to the egg's content of amino acids, These results are consistent with [42], who found a significant increase in the egg albumin index using betaine at (8) gm. As [38, 43] also reported that betaine supplementation in feed increased the Hue unit, which led to improved internal egg quality in chickens. However, they differed from [39, 34, 21, 36, 43, 41, 40, 44,38] , they reported no significant difference in egg white percentage when betaine or glycine was added to the ration .

-In egg shell specifications, Table (4) shown no significant differences between treatments in relative weight, shell thickness, or shape index when betaine and glycine were added. These results are consistent with those of [34,37], they who indicated that adding betaine or glycine. The results of this study differed from those of [21,18, 44.[

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Table (4) Use of Betaine and Glycine in laying hens diets and their effect on some albumin traits.

Age of hens (week)	Treatments T1	T2	T3	T4
Average Relative Weight of Albumin				
Week 49	60.62 ± 1.75 a	62.12 ± 0.57 a	62.03 ± 1.81 a	64.23 ± 0.84 a
Week 54	64.09 ± 2.00 a	63.31 ± 1.29 a	64.50 ± 0.48 a	63.91 ± 1.34 a
Albumin Shape Index				
Week 49	8.73 ± 0.80 b	9.37 ± 0.48 a	10.88 ± 0.63 a	9.93 ± 0.56 a
Week 54	8.99 ± 0.87 b	10.96 ± 1.40 a	9.32 ± 1.32 a	11.83 ± 0.99 a
Haugh Unit				
Week 49	84.78 ± 1.63 a	90.10 ± 2.19 a	91.22 ± 1.32 a	89.14 ± 2.04 a
Week 54	84.80 ± 1.29 b	90.50 ± 3.54 a	94.05 ± 1.94 a	93.40 ± 4.10 a

Different letters in a row indicate significant differences between means (P≤0.05) (mean ± S.E.(

Table (5) Use of Betaine and Glycine in laying hens diets and their effect on some shell egg traits.

Age of hens week	Treatments T1	T2	T3	T4
Week 49	0.46±0.02 **a**	0.47±0.03 **a**	0.45±0.01 **a**	0.47±0.02 **a**
Week 50	0.45±0.04 **a**	0.46±0.02 **a**	0.44±0.02 **a**	0.44±0.04 **a**
Week 51	0.43±0.01 **a**	0.44±0.02 **a**	0.43±0.03 **a**	0.43±0.03 **a**
Week 52	0.40±0.01 **ab**	0.38±0.02 **b**	0.42±0.01 **a**	0.41±0.03 **ab**
Week 53	0.40±0.02 **a**	0.43±0.01 **a**	0.41±0.04 **a**	0.40±0.01 **a**
Week 54	0.36±0.03 **a**	0.38±0.01 **a**	0.38±0.04 **a**	0.39±0.02 **a**

Different letters in a row indicate significant differences between means (P≤0.05) (mean ± S.E.(

-2use of betaine and glycine in ration and their effect on egg content

Table (6) shows that the highest protein content in the egg was in the fourth treatment, reaching (0.14)%, with a protein percentage increase of (3.45)% compared to the control treatment, which had a lower protein value of (11.15%). while, the difference in protein percentage for the second treatment, which reached (13.87%), and the third treatment (13.05%) compared to the control treatment, was (2.72 and 1.90% .(

We find that the fourth treatment had a significant increase in the protein percentage , and it is believed that this was due to the synergistic effect of the two amino acids glycine and betaine together, as they worked to provide the methyl group instead of the amino acid methionine, which increased its abundance in nutrients and increased the rate of its absorption and utilization, and this was reflected in production [45,21,22] they are considered as primary molecules of structural units in the formation of elastin, collagen and keratin [20]. This mixture of betaine and glycine improves the mucous membrane in the small intestine, improving the absorption rate and the rate of utilization of nutrients. This is consistent with (Table 13) regarding the

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content of amino acids in the egg, due to the fact that betaine has a role in influencing the genetic gene (OCLN, CLDN-1) responsible for cell renewal [44,17,19].The rate of utilization of feed increased, which was reflected in production in production [45,21,22 .[

There were no significant differences in the percentage of fat in the egg content between the first treatments and the T2,T3,T4 treatments of adding betaine and glycine to the feed. The values reached (10.58, 11.10, 11.45, 10.85) %. Consecutive, While the carbohydrate content was higher in the fourth treatment compared to the control treatment, the second and third treatments did not differ significantly from the aforementioned treatments, reaching values of 1.99, 1.12, 1.56, and 1.75%, respectively.

As for the amount of cholesterol, we observed an improvement in this trait. Its quantity decreased significantly in the fourth treatment, followed by the third treatment, and then the second treatment, compared to the control. The values reached (155.9, 177.63, 196.83, and 230.6 mg/100g, respectively. It is believed that the improvement in the amount of cholesterol in the egg content and its decrease were due to the activation of metabolic processes and lipid metabolism by betaine and glycine [24], as the addition of betaine increases the synthesis of low-density lipoprotein, which reduces fat absorption from the liver [37.[

Table (6) Use of Betaine and Glycine in laying hens diets and their effect on the egg content.

Contents	Treatments			
	T1	T2	T3	T4
Proteins %	11.15 ± 1.06 b	3.87± 0.56 ab	13.05 ±1.00 ab	14.60 ± 0.77 a
Fats %	10.58 ± 0.84 a	11.10 ± 0.60 a	11.45 ± 0.35 a	10.85 ± 0.73 a
Cholesterol %	230.600 ±1.13 a	196.833 ±2.17 b	177.630 ±1.80 c	155.900 ±1.61 d
Carbohydrates%	1.12 ± 0.24 b	1.56 ± 0.18 ab	1.75 ± 0.18 ab	1.99 ± 0.20 a

Different letters in a row indicate significant differences between means (P≤0.05) (mean ± S.E.(

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Use of betaine and glycine in laying hens' diets and their effect on the egg content of some amino acid .

Table (7) shows that the amount of methionine in egg content increased significantly in the fourth treatment, followed by the third and second treatments, with values reaching (76.07, 70.9, 64.50, and 59.8 ppm), respectively. It was also found that the amount of the amino acid glycine in egg content increased significantly in the fourth treatment, followed by the third and second treatments, compared to the control (196.00, 187.90, 181.40, and 170.59) ppm, respectively. The same applies to cysteine, leucine, and phenylalanine, where the values were (0.142.6, 1.33.50, 0.125.6, 0.112.5) ppm and (451.87, 432.60, 418.90, 399.80) and (168.70, 160.20, 149.96, 137.54) ppm for the above-mentioned treatments and fatty acids, respectively.

This confirms the fact that betaine and glycine work, as betaine can increase the availability of methionine by donating a methyl group, thus increasing the utilization rate of methionine [45,21,22].Glycine also

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contributes to the formation of some amino acids as a precursor for the synthesis of betaine, purine, and glutathione. It is also considered a structural unit in the formation of elastin, collagen, and keratin [20]. Researcher [46] indicated that glycine improves the metabolic efficiency of essential amino acids in addition to increasing the utilization of feed protein. It also contributes to some biologically important molecules and compounds [26] which is why the concentration of these amino acids in the egg has increased .

Table (6) Use of betaine and glycine in laying hens' diets and their effect on the egg content of some amino acids.

Amino acids	Treatments T1	T2	T3	T4
Methionine ppm	59.80 ± 2.09 d	64.50 ± 1.62 c	70.90 ± 1.67 b	76.00 ± 0.75 a
Glycine ppm	170.59 ± 0.98 d	181.40 ± 1.05 c	187.90 ± 0.60 b	196.00 ± 1.04 a
Cystine ppm	112.500 ± 0.85 d	125.600 ± 1.13 c	133.500 ± 1.17 b	142.600 ± 0.55 a
Leucine ppm	399.800 ± 1.35 d	418.900 ± 1.50 c	432.600 ± 1.10 b	451.867 ± 0.93 a
Phenylalanine ppm	137.540 ± 1.18 d	149.796 ± 1.70 c	160.200 ± 1.38 b	168.700 ± 1.58 a

Different letters in a row indicate significant differences between means (P≤0.05) (mean ± S.E.(

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Use of betaine and glycine in laying hens' diets and their effect on the egg content of some vitamins.

The effect of using betaine and glycine on the egg content of some vitamins. Table (8) shows that the use of betaine and glycine significantly increased the content of vitamin A and choline in the second, third, and fourth treatments compared to the control treatment, noting that the highest values for these two vitamins were in the fourth treatment, where the values reached (471.00, 462.22, 447.90, 432.48) international units. The values of choline concentration reached (288.0, 278.10, 269.70, 0251.6) international units for the fourth, third, second, and control treatments, respectively. This is due to the presence of betaine and glycine in the feed, which play a role in increasing the amount of vitamin A deposited in the yolk. This is consistent with Table (2) regarding chick hatching weight. The reason for the increased hatching weight of chicks in the betaine and glycine treatments was the role of vitamin A in increasing the activity of the thyroid-stimulating hormone (thyrotropin) in the body, which in turn increases the secretion of thyroxine by the thyroid gland, which works to increase metabolic rates in body tissues and skeletal muscles [25]. From Table (7), it was found that the concentration of riboflavin B2 increased significantly in the fourth and third treatments compared to the control, reaching (21.50, 19.80, and 12.70) international units for those treatments, respectively. While there were no significant differences between the fourth and third treatments, on the one hand, and between the second and control treatments, on the other hand, the values of the

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latter two treatments reached (14.90 and 12.70) international units, respectively. Similarly, regarding the concentration of B6 (pyridoxine), the fourth and third treatments outperformed the control treatment, while the second treatment did not differ from the aforementioned treatments, with values reaching (8.30, 7.39, 4.96, and 5.32) international units, respectively. These two vitamins (B2 and B6) play an important role in regulating embryo growth and vitality and in the hatching process, as their deficiency in the mothers' diet can lead to increased embryo mortality. [47] reported that a deficiency in riboflavin during the incubation period leads to poor embryo growth and, consequently, a reduced hatchability rate. It also plays an important role in the growth and development of the embryo and its ability to survive. Additionally, B6 possesses antioxidant properties, protecting the embryo from oxidative stress and mortality [29,31,44,47] indicated that fertilized eggs containing sufficient amounts of vitamin B6 resulted in a lower rate of embryonic mortality, with a higher hatchability and hatched chick weight (Table 2). This is due to pyridoxine's important functions in the metabolism of protein, amino acids, lipids, and phospholipids, and its key role in the energy-producing citric acid cycle. These results were consistent with the results in Table (2), which relate to reducing mortality and increasing hatchability. Vitamins B2 and B6 play important roles in basic metabolic processes, such as energy metabolism, the citric acid cycle, protein metabolism, amino acid metabolism, and phospholipid metabolism, as well as protecting the embryo from oxidative stress. These biological activities are essential for embryo growth and development [47,31] . Meanwhile, the concentration of B12 in the egg was not affected by the addition of betaine and glycine to the feed, as the concentration values of this vitamin in the experimental treatments were comparable to the initial treatment.

Conclusion

This study indicated that using betaine and glycine as nutritional supplements significantly increased the average weight of hatched chicks, which is considered an important pillar for building a laying flock, as offspring production depends on it. Their use also significantly increased the hatchability and fertilization rate of eggs, with a significant decrease in embryonic mortality, especially during the middle period, which relied on maternal nutrition. Cholesterol levels in the egg decreased a quality indicator consumers, especially those with diabetes, desire. This study indicated that these eggs can be considered programmed organic eggs, containing a desirable characteristic among consumers, addressing a nutritional health problem such as vitamin and amino acid deficiencies. We observed that the percentage of proteins and important amino acids increased in the eggs treated with betaine and glycine. Therefore, this method can be considered a pharmaceutical technique.

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