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Effects of Dietary Soybean Bioactive Peptides on Egg Quality Parameters in Laying Japanese Quail

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Abstract | This study was aimed to investigate the impact of soybean bioactive peptides (SBP) addition to female quail diets during the egg production stage on egg quality characteristics. For this purpose, ninety-six female quails were raised at eight weeks of age. The birds were distributed into four treatments including control group (without any addition). The additional therapies included three levels (1, 2, and 3 g/kg feed) for the second, third, and fourth treatments, respectively. The results of the experiment showed a significant increase in egg weight, albumin height, Haugh unit, yolk weight, yolk height, and yolk index. A substantial decrease in yolk diameter, triglycerides, and cholesterol were reported in the treatment groups. However, there were no significant differences in albumin weight, albumin pH, and yolk pH. Taken together, we conclude that the addition of SBP improved the quality of the egg and can be considered a valuable additive in the feed.

Keywords | Female quails, Soybean bioactive peptides, Egg quality, Haugh units, Yolk cholesterol, Albumin pH

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INTRODUCTION

Quails are characterized by their early growth, short generation times, and short incubation periods compared to other domesticated animal species. These features make them an essential source of animal protein, such as meat and eggs, in a short period (Lukanov and Pavlova, 2020; Al-Hindawi et al., 2021; Ismail et al., 2024). Domestic birds are considered an animal model and occupy an important place in experimental research (Morris et al., 2020).

SBP They are an excellent food source because they contain dietary fiber, high-quality protein, a high percentage of unsaturated fatty acids, and other substances that have various physiological effects (Omoni and Aluko, 2005; Kogut *et al.*, 2013; Udenigwe, 2014; Mohsen *et al.*, 2024).

Soybeans are a rich source of plant proteins and phytochemicals, such as isoflavones and phenolic compounds, due to their rich amino acid composition, high content, and low price, making them the second largest source of vegetable oils globally (Singh and Hati, 2014; Lule *et al.*, 2015). Based on the dry weight of their seeds, the composition of soybeans consists of protein (30–35%), fat (20%), dietary fiber (9%), and moisture (8.5%) (Li *et al.*, 2021).

They are considered functional foods, as they help prevent and treat various diseases while providing good nutrition. Experimental studies have demonstrated their role in enhancing blood circulation, regulating digestion, and promoting bowel movements. In addition, they contain phytochemicals known for their positive effects on health, such as fibre, isoflavones, phospholipids, saponins, phenolic acids, trypsin inhibitors, and phytic acid (Kogut *et al.*, 2013; Udenigwe, 2014; Wei *et al.*, 2024). Proteins are an important nutrient in chicken eggs. These proteins are characterized by a high rate of digestion and absorption, and they release complete proteins (Puglisi and Fernandez, 2022). Research studies have identified 371, 428, and more than 500 protein types in egg white, yolk, and cortical membrane proteins, respectively, through proteomic analyses (Kulshreshtha *et al.*, 2022; Sarantidi *et al.*, 2023). White proteins primarily include albumin, ovotransferrin, ovomucin, ovomucoid, and lysozyme (Kulshreshtha *et al.*, 2022). Yolk proteins primarily include lipofetellin, globular protein, phosvitin, and low-density lipoprotein (LDL) (Marcet *et al.*, 2022). Egg shell membrane proteins mainly contain collagen, keratin, agrin, and laminin (Makkar *et al.*, 2015). Egg proteins exhibit various functional activities, including antibacterial, immune-enhancing, and antioxidant properties (Liu *et al.*, 2015; Pimchan *et al.*, 2023).

The study aimed to incorporate plant-based bioactive soybean peptides, which provide essential amino acids, into the bird's diet, potentially influencing the chemical composition and quality of the egg fat and improving its overall quality.

MATERIALS AND METHODS

This study was conducted in the poultry farm of the College of Agriculture, Tikrit University. Ninety-six eight-week-old female brown quails were used in this study. They were randomly distributed into four treatments, with each treatment comprising 24 birds and eight replicates. SBP compounds were added as follows: The first treatment (T1) was the control treatment without any additives. The additional treatments included three levels (1, 2, and 3 g/kg) feed for the second, third, and fourth treatments, respectively.

Bird management

The production ration prepared by the Feed Laboratory of the Animal Field Department at Tikrit University was used to feed the birds. This ration contained 2,800 kcal/kg of feed and a protein content of 21%. Feed and water were freely available to the birds. SBP were hand-mixed for manual addition to the treatment rations.

Sample collection and characteristics

After the female Japanese quail reached 50% of her egg production, 100 eggs were taken within one week of each treatment. The eggs were cracked, and measurements were made to evaluate egg quality. Egg weight in grams and albumin and yolk quality were measured individually using a sensitive three-stage balance after separation. After cracking the eggs on a flat glass surface, the albumin height and diameter (in mm) were measured using an electronic Vernier scale, dye unit, and pH meter. Yolk quality was determined by measuring yolk weight, height (mm), diameter, and pH. Albumin and bile index were measured in dye units. Cholesterol concentration was measured according to the method of Elias and Francy (1968), and triglyceride concentration was measured according to the method of Kaya et al. (2001) using the following equations.

$$\textit{Albumin index} = \textit{Albumin height (mm)} / \textit{Albumin diameter (mm)}$$

$$\textit{Yolk index} = \textit{yolk height (mm)} / \textit{yolk diameter (mm)}$$

Haugh unit = 100 Log (H+7.57-1.7W0.37), H= height albumin mm, w= egg weight, 7.57= constant number

Egg yolk cholesterol (mg/g yolk) = sample reading/ standard cholesterol reading x 2

mm: means millimeter

Haugh unit = 100 Log (H+7.57-1.7W0.37), H= height albumin mm, w= egg weight, 7.57= constant number

Soybean peptides used in the study

SBP was obtained from Vanavaran Novin Gostar Biotechnology Co., Tehran, Iran (as yellow-brown powder). The molecular weight distribution of SBP was determined using a Superdex HR 10/300 GL peptide column connected to an HPLC system (Karimzadeh *et al.*, 2016).

Statistical analysis

Data were analyzed using a completely randomized design to determine the effect of the treatment ratios used on the studied traits. Significant differences between mean values were compared using Duncan's multiple range test (Duncan, 1955) at a significance level ($p \leq 0.05$), using SAS (2012) The following mathematical model worked:

$$Yij = \mu + Ti + Eij$$

Where; Yij = the observed value specified for treatment I; μ = the mean year of the studied recipe; Ti = the behavioral effect i; eij = the random experimental error, which is normally distributed with a mean equal to zero and a variance of σ^2.

Table 1: Effect of adding SBP to quail diets on egg weight, albumin content, Hugh unit, albumin weight, and pH of eggs (Mean ± Standard error).

Traits treatments	Egg weight (g)	Albumen height (mm)	Haugh units	Albumen weight (g)	Albumen pH
T1	10.14±0.005 c	3.96±0.005 b	87.65±0.02 b	6.03±0.008	9.31±0.58
T2	10.17±0.005 c	3.98±0.005 b	87.73±0.02 b	6.37±0.02	9.02±0.57
T3	11.59±0.33 b	4.37±0.32 b	88.77±1.92 b	6.45±0.33	9.05±0.58
T4	12.46±0.34 a	5.12±0.005 a	92.25±0.20 a	6.16±0.008	9.09±0.58
Significance	*	*	*	N.S	N.S

The letters a, b, c, and d within one column indicate statistically significant differences at the probability level (P ≤ 0.05). N.S.: means no significant differences at (P>0.05). * Indicates the presence of significant differences (P ≤0.05). T1 Control, T2 1 gm/kg of SBP, T3 2 gm/kg of (SBP), T4 3 gm/kg of SBP.

Table 2: Effect of adding SBP to quail diets on yolk weight, height, diameter, index, and pH of eggs (Mean ± Standard error).

Traits treatments	Yolk weight (g)	Yolk height (mm)	Yolk diameter (mm)	Yolk index (%)	Yolk pH
T1	2.83±0.02 b	9.72±0.005 d	24.62±0.005 a	0.39±0.003 b	6.40±0.58
T2	2.64±0.04 b	9.82±0.005 c	24.32±0.34 ab	0.40±0.006 ab	6.17±0.58
T3	2.94±0.15 b	9.85±0.005 b	24.33±0.33 ab	0.40±0.006 ab	6.13±0.58
T4	3.42±0.21 a	9.90±0.008 a	23.72±0.008 b	0.42±0.001 a	6.10±0.57
Significance	*	*	*	*	N.S

The letters a, b, c, and d within one column indicate statistically significant differences at the probability level (P ≤ 0.05). N.S.: means no significant differences at (P>0.05). * Indicates the presence of significant differences (P ≤0.05). T1 Control, T2 1 gm/kg of SBP, T3 2 gm/kg of (SBP), T4 3 gm/kg of SBP.

RESULTS

Effect of adding SBP to quail diets on egg weight, albumin content, Haugh unit, albumin weight, and albumin pH

The results of Table 1 showed a significant increase (P ≤ 0.05) in egg weight for the third and fourth treatments compared to the first and second treatments, while the results of the same Table 1 indicated a significant increase for the fourth treatment compared to the three experimental treatments in albumin height and Haugh unit, respectively. The results also showed no significant differences between the experimental treatments for both albumin weight and pH value.

Effect of adding SBP to quail diets on yolk weight, height, diameter, yolk index, and yolk pH

From the results of the statistical analysis in Table 2, we find a significant increase (P ≤ 0.05) in favor of the fourth addition treatment compared to the rest of the experimental treatments in yolk weight. At the same time, we note that there is a significant increase for the addition treatments compared to the control treatment in yolk height. The results also indicated a significant decrease in yolk diameter for the fourth treatment compared to the first treatment. The results of the Table 2 also showed a significant increase for the fourth treatment compared to the control treatment in yolk index, and we did not notice any significant differences between the experimental treatments in yolk pH.

The effect of adding SBP to quail diets on cholesterol and triglycerides in the yolk

The results of the statistical analysis, presented in Table 3, showed a significant decrease (P ≤ 0.05) in cholesterol levels between the third and fourth addition treatments compared to the first and second treatments. The results of the Table 3 also showed a significant decrease in triglycerides in the addition treatments compared to the control treatment.

Table 3: The effect of adding SBP to quail diets on cholesterol and triglycerides in the yolk (Mean±Standard error).

Trait treatments	Triglycerides (mg/100ml)	Cholesterol (mg/100ml)
T1	20.99±0.30 a	12.35±0.005 b
T2	20.36±0.18 b	12.45±0.005 a
T3	18.65±0.008 d	10.12±0.005 d
T4	19.34±0.008 c	10.22±0.005 c
Significance	*	*

The letters a, b, c, and d within one column indicate statistically significant differences at the probability level (P ≤ 0.05). N.S.: means no significant differences at (P > 0.05). * Indicates the presence of significant differences (P ≤ 0.05). T1 Control, T2 1 gm/kg of SBP, T3 2 gm/kg of (SBP), T4 3 gm/kg of SBP.

DISCUSSION

The reason for the increase in egg weight in the treatments where bioactive soybean peptides were added in the third and fourth treatments may be due to the role of peptides in the development of intestinal villi, increasing their ability to be absorbed in the intestine, increasing the stability of intestinal bacteria, and providing crude protein and fat in the feed, thus improving the digestibility of food, which contributes to improved egg production and weight gain. The reason for the increase in albumin and Haugh unit in the fourth addition treatment may be attributed to the fact that peptides have antioxidant properties that protect proteins and reduce the breakdown of albumin proteins due to oxidative stress, especially in the summer. Previous studies have indicated that the addition of SBP leads to an improvement in Haugh unit because the peptides work to maintain albumin weight by reducing moisture loss and retaining water within the egg albumin, thereby reducing water evaporation through the eggshell pores. In addition, they enhance the thickness of the albumin layers, as the peptides support the formation of albumin proteins such as ovalbumin and maintain the structural integrity, which increases albumin weight and reduces the decrease in its height during storage. On the other hand, peptides work to prevent the oxidative breakdown of proteins. These peptides contain compounds such as isoflavones and sulfur-containing amino acids, including methionine, which prevent the oxidation of albumin proteins, thereby preserving their structural integrity and weight. They also work to reduce protein degradation during long-term storage. Albumin proteins are susceptible to degradation by enzymes or heat, and peptides help inhibit these processes, thereby preserving the overall mass of albumin.

Preserving yolk quality may be attributed to the fact that peptides inhibit the oxidation of lipids in the yolk, thereby increasing the shelf life of eggs and reducing rancidity, especially in eggs stored for extended periods. On the other hand, peptides protect chicken liver cells (where yolk proteins such as vitellogenin are produced), which enhances the formation of egg yolk. These peptides offer health benefits for poultry, as they function as a prebiotic that supports the growth of beneficial bacteria in the intestine, thereby improving the overall absorption of nutrients. They also play a role in reducing oxidative stress in birds, which has a positive impact on egg quality and production. Among their physical properties is the ability to increase the consistency and moisture of the yolk, which reduces its tendency to dry out during storage. The decrease in the diameter of the yolk in the fourth treatment indicates the strength of the yolk membrane. The lower the quality of this membrane, the more susceptible the yolk becomes to damage. The strength of the yolk membrane is an important characteristic, especially in the food industry when separating albumin from the yolk, because it helps prevent the yolk part from mixing with the albumin part during separation. This is what Olukosi *et al.* (2018) found when adding biologically active soybean peptides to chicken feed: It improved the color and yolk index. However, the diameter of the yolk decreased significantly for the eggs collected after feeding the peptides for eight weeks.

According to Table 3, the decrease in triglyceride and cholesterol values may be due to the effect of peptides that lower LDL cholesterol and raise HDL cholesterol. These varying effects of peptides may be related to lipid metabolism and the presence of secondary metabolites, such as the concentration of essential amino acids, as well as the size and type of peptides produced (Tang *et al.*, 2012). The decrease in triglyceride and total cholesterol in egg yolk is due to soybean peptides lowering their levels in blood serum (Alahyaribeik et al., 2022).

CONCLUSIONS

We conclude from this study that soybean peptides improved egg quality traits: Egg weight, albumin content, Haugh unit, yolk weight, yolk height, yolk diameter, yolk index, and reduced yolk triglycerides and cholesterol. Therefore, peptides may play a role in producing healthier eggs for consumers.

RECOMMENDATIONS

We recommend adding 3g/kg of SBP to improve egg quality, reduce harmful cholesterol and triglyceride levels, and encourage consumers with atherosclerosis and hyperlipidemia to use levels higher than 3g/kg.

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New statements

By using SBP, intestinal health and digestive enzyme activity can be improved, thereby improving egg quality.

AUTHOR'S CONTRIBUTION

Ahmed A.D. AL-Doori: Initiated the idea for the submitted research project, contributed to sample collection and laboratory testing, and contributed to the preparation of the final version of the manuscript. Asaad D. S. AL-Doori: Supervised the formulation of the bird feed and the addition of (BSP), conducted statistical analysis of the data, and interpreted the results. Saif A. J. aldarraji: Verified the

data analysis and contributed to supervising and guiding the technicians in the field. Ahmed A. Allaw: Initiated the idea for the submitted research project and reviewed the final version of the manuscript. Haitham R. M. Al-Kaisi: Data Analysis

Generative AI or AI-assisted Technology Statement

The author(s) declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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