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"Effects of Different Levels of Soybean Bioactive Peptides and Vitamin E on Performance, Egg Quality, and Serum Metabolites of Laying Hens"

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Abstract

An experiment was carried out to find out how laying hen performance, egg quality, and serum metabolites were affected by adding soybean bioactive peptide (SBP) and vitamin E to their diet. Ninety Lohmann Extra hens that were 45 weeks old were divided into five groups at random. Each group consisted of three replicates, each with six birds. The dietary treatments included 250 mg/kg of vitamin E added to the baseline diet, 2.5, 5, and 7.5 g/kg of diet for the SBP groups, and the basal diet as the control.

The findings demonstrated that egg production rate and egg weight significantly increased with SBP supplementation ($P \leq 0.05$). While there were no appreciable changes between the vitamin E-treated groups and the control group, there was a noticeable difference in feed efficiency between the SBP groups and the control group. Peptides-supplemented groups showed a definite advantage in terms of egg quality attributes, as evidenced by the weights of egg components, yolk index, and albumin index. The SBP supplement also resulted in an increase in glutathione peroxides (GSH-PX) and superoxide dismutase (SOD). Additionally, compared to the positive control treatment (adding vitamin E), there was a substantial ($P \geq 0.05$)

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drop in the cholesterol levels in the yolk among the groups supplemented with peptides. The group that got an excessive amount of soybean peptide (5 g/kg) had the highest serum total antioxidant activity. The findings concluded that adding SBP to the diets can enhance egg quality and production while also influencing the hens' serum's total antioxidant activity during the laying phase.

Keywords: Soybean Bioactive Peptide, Egg Quality, Laying Hen, Serum Metabolites.

Introduction

Due to its well-balanced necessary amino acid characteristics, soybean meal is the ideal source of protein in chicken feed composition (Ravindran 2013). Due of its abundance and affordability, it is the most significant source economically. As stated by Singh et al. (2014), soy protein has a high biological value and is nutritionally equivalent to animal proteins. In addition to being high in fiber and minerals, soybeans are a superior source of protein. Using a range of technological and biological techniques, soybeans can be processed into a wide range of food products (Lule et al. 2015). According to Hou et al. (2017), bioactive peptides are organic compounds composed of amino acids bound together by amide or peptide bonds, which have a low molecular weight. Recently, there has been a lot of interest in learning more about the bioactivities and functional characteristics of peptides, particularly those produced from soybean proteins.

Bioactive peptides are protein morsels that are typically 2–20 amino acids long. As a result, they can be highly diverse, but their lowest common denominator is a behavior that influences and improves health (Bravo et el., 2023). According to Susy et al. (2018), these sequences are often inactive when encrypted in the parent protein, and the bioactivity is only visible during hydrolysis. And bioactive peptides are defined as peptides that are separated from various protein sources and that, when they have certain physiologically beneficial effects in vitro and in vivo, (Bhandari et el. 2020).

These peptides can be made in a variety of ways, including microbial fermentation (Xu et al. 2019), in vitro enzymatic hydrolysis of alkali or acid (Rizzello et al. 2016, Samperi et al. 2015), or gastrointestinal digestion via proteolytic enzymes (Zenezini et al. 2016). Protein quality is determined by the kind of amino acids present, their absorptive capacity, and the absence of anti-nutrients that may limit protein digestion. Functional foods that enhance health are becoming more and more popular these days. In particular, the continuous use of synthetic antibiotics as growth promoters can lead to bacterial resistance. Due to their ability to fight germs and their lack of harmful residues that endanger human health or the environment, antimicrobial peptides have gained importance in recent years (Roque-Borda et al. 2021). Rich sources of bioactive peptides can be found in dietary proteins. These peptides are hidden in a latent state inside native proteins and must be released through enzymatic proteolysis (Saavedra, et al. 2013).

Bioactive peptides have been shown in numerous studies to have a high antimicrobial capacity (Roque-Borda et al. 2021; Osman et al. 2016; Wald et al. 2016), therapeutic antioxidant (Power et al. 2013; Hisham et al. 2018), immunomodulatory (Kotzamanis et al. 2007; Osho et al. 2019), and enhanced digestive enzyme activity (Jin et al. 2008). They also appear to promote the digestive and absorptive abilities in poultry (Abdollahi et al., 2017; Xiao et al., 2017). The biological activities dependent on the structural features of peptides, including amino acid composition, sequence, molecular weight, hydrophobicity on the surface and within molecules, net charge, and spatial conformation (Udenigwe & Fogliano, 2017).

According to Abdollahi et al. (2017), broiler chickens' productive performance and feed conversion ratio were altered by the addition of bioactive peptides made from

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soybean meal that was synthesized by enzymatic hydrolysis (SBP). In a similar vein, broiler diets supplemented with SBP may benefit performance standards (Abdollahi et al. 2018). It was reported that adding 5 g of SBP/kg to the diet of laying hens could improve their productivity and egg quality by enhancing the morphology of their intestines and the digestibility of fat and protein, The percentage of eggs produced also increased very quickly, but feed consumption did not increase proportionately (Pan et al. 2019).

According to Olukosi et al. (2018), adding SBP to laying hens' diets during their peak producing phase did not improve their productivity, but it did positively support the maintenance of the hens' body weight (BW) and egg quality during storage. On the other hand, Osho et al. (2019) reported that graded SBP concentrations had linear effects ($P < 0.01$) on dietary enhanced broiler chicken growth performance, nutrient digestibility, and jejunal morphology. Additionally, Landy and Faghani (2020) suggested that adding bioactive peptides from cottonseed to broiler diets could have a positive impact on immune responses, growth performance, and serum total antioxidant activity. It could also be used as an antibiotic substitute in broiler diets.

Research on bioactive peptides has advanced quickly, however there is still a lack of understanding about several laying hen performance indicators and metrics (such as serum biochemical indices, egg quality features, and performance requirements). As a result, the purpose of this study was to ascertain how varying doses of vitamin E and soybean bioactive peptides affected the laying hens' performance, egg quality, and serum metabolites.

Materials and Methods

Birds and Experimental Diets

The 12-week experiment ran from March 25, 2022, to June 25, 2022, at the Poultry Farm, College of Agriculture, University of Basrah. Ninety Lohmann Extra at 45-

weeks-old hens were divided into five treatment groups at random. Each treatment group consisted of three replicates, each with six hens. The dietary treatments included adding 250 mg/kg of vitamin E to the basal diet, as well as giving the 2.5, 5, and 7.5 g/kg diet to the SBP groups. The 12-week experiment (45 to 56 weeks of age) included a 16-hour lighting program and free-flowing feed and water for the chickens. Table (1) provides a detailed description of the feeding regimen used.

Table (1): Base Diet Components and Composition (%)

Ingredients	**Ration (%)**
Yellow corn	52
Soybean meal (48% CP)	24
Wheat	10
limestone	9
Dicalcium phosphate	0.7
Premix (6% CP)[a]	2.5
sunflower oil	1.5
Salt	0.3
Total	100
Calculated analysis [b]	
Metabolisable energy, kcal/kg	2815
Crude protein	17.62
Crude fibre	3.12
Calcium	4.20
Available phosphorus	0.50
Lysine	0.85
Methionine	0.42
Methionine + Cystine	0.73
Energy: protein	159.76

[a] The premix produced by Belgian company Intraco, contains 5% crude protein, 1250 kcal / kg metabolizable energy, 1.36% lysine, 6.65% methionine, 7.50% methionine + cysteine, 22% calcium and 10.10% available phosphorus.

[b] The computation was done using feed ingredient data from NRC (1994).

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The birds were weighed using an electronic scale PNORAMA (ACS-A30k/2g) for each replicate separately at the beginning of the experiment, then, at the conclusion of the experiment, it was weighed for each copy, and the body weight was ascertained according to the equation mentioned by Al-Fayyad et al. (2011).

The daily feed was provided freely at eight o'clock in the morning every day, and the feed was taken from a pre-weighted quantity. At the end of the week, the rest of the feed is collected and weighed. Thus, we have the amount of feed consumed during the week, and the amount of feed consumed (gm feed/bird/week) was determined. For each duplicate according to the equation mentioned by Al-Yassin and Abdel-Abbas, 2010)). Also, The Feed efficiency was determined on the basis of the amount of feed (gm) needed to produce one gram of eggs, according to the method mentioned by Ibrahim and Saleh (2013). The Hen Day (H.D.) approach, as described by Al-Zubaidi (1986), was used to calculate the percentage of egg production.

Evaluation of Egg Quality

A sensitive Lamis-type balance was used to determine the weight of the yolk and the shell.However, the following formula was used to determine the albumen's weight:

$$\text{Albumen weight (gm)} = \text{egg weight} - (\text{yolk weight} + \text{shell weight with the membranes})$$

Yolk diameter and Albumen diameter were measured using an electronic Vernier calipe, while the Albumen and yolk height were measured using a metal ruler. As for the yolk index, its value was determined according to the equation indicated by Rose (1997):

$$\text{yolk index} = \frac{\text{yolk weight}}{\text{yolk diameter}}$$

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The ratio of the weight of the yolk to the Albumen was determined according to the equation mentioned by Al-Fayyad and Naji (1989):

$$\text{Yolk: albumin ratio} = \frac{\text{yolk weight}}{\text{Albumen weight}}$$

Haugh unit was determined based on the method referred to by Selle et al, (2000). As for egg yolk cholesterol concentration: The method mentioned by Elias and Francy, (1968) was followed. As for Blood and meat spot, they were observed immediately after the egg was broken and their appearance was calculated as a percentage of the number of eggs tested.

Determination of Serum Biochemical Parameters

Blood samples were collected from the Caudal tibial vein in standard tubes and then placed in a centrifuge at 3000 rpm to separate the blood components from the serum. The concentration of total protein, albumin, globulin, cholesterol, triglycerides, and HDL in serum was assayed using ready-made estimation kit (Kit) supplied by BIOLABO-SAS Company and the assay was done by following the steps indicated by the company in each manual attached to the kit and using a device spectrophotometer. The activity of SOD and GSH-P enzymes in serum was assayed using the standard ready-made kit produced by IBM International GMBH, Germany, using the Enzyme Linked Immune Sorbent Assay. So, using a commercial kit from BioAssay Systems, total antioxidant activity was measured in the serum samples (Re et al. 1999).

LDL is derived mathematically by means of a special equation based on the values of cholesterol, triglycerides and HDL. The LDL concentration was assayed according to the equation indicated by Wu (2006):

$$\text{LDL} = \text{holesterol} - \text{HDL} - \text{VLDL}$$

very low density lipoproteins were assayed using the following equation:

$$\text{VLDL} = \frac{\text{triglycerides}}{5}$$

Statistical Analysis

The information was subjected to statistical analysis utilizing a completely randomized design (C.R.D). using the ready-made statistical program (SPSS 2022) and the results were compared using Duncan's test for one-factor and two-factor traits, and a T-test to compare some traits, statistically significant differences were taken at the 0.05 level.

Results and Discussion

The laying performance of hens is displayed in Table (2). When compared to the Control and Treatment groups, it was evident that the SBP-supplemented groups did not have a substantial increase in body weight gain.

Table (2): Impact of Vitamin E and Dietary Soybean Bioactive Peptide on Laying Hen Performance at 45–56 wk. of Age

Laying hens performance	Control	Soybean bioactive peptide g/kg diets			Vit. E 250 (mg/k)	SEM	*P*<
		2.5	5.0	7.5			
Initial live weight (g)	1520	1519	1543	1581	1570	12.22	0.407
Final body weight (g)	1711	1722	1738	1781	1762	11.96	0.352
Body weight change (g)	191	202	195	200	192	1.81	0.199
Hen day egg production (%)	75.19^{b}	81.28^{a}	82.14^{a}	78.90^{ab}	78.96^{ab}	0.85	0.058
Egg weight (g)	65.48^{c}	67.80^{b}	68.53^{ab}	69.32^{a}	67.19^{b}	0.38	0.001
Egg mass (g/bird/d)	49.25^{b}	55.14^{a}	56.31^{a}	54.74^{a}	53.06^{a}	0.75	0.005
Feed intake (g/bird/d)	115.33	118.66	115.33	114.00	115.00	1.28	0.872
Feed efficiency (g feed/g egg)	2.34^{b}	2.16^{ab}	2.06^{a}	2.08^{a}	2.17^{ab}	0.03	0.072
Mortality (%)	0	0	0	0	0	0	

SEM: standard error of mean, $^{a, b}$: Values that do not share a common superscript in the same row are different (P < 0.05).

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While Landy and Kheiri (2021) mentioned that there is a significant weight gain when supplementing diets with cottonseed bioactive peptides.

The results of this study showed that the peptide treatments significantly ($P < 0.05$) increased the performance indicators (hen day egg production, feed efficiency, egg weight, and egg mass) when compared to the vitamin E supplementation therapy. Our findings are consistent with those of (Abdollahi et al., 2018), who found that bioactive soybean seed peptides might increase feed conversion efficiency and be used in chicken feed as a new functional protein. Similarly, Abdollahi et al. (2017) found that improving the diet of broilers by adding 6 g SBP/kg of feed led to an improvement in FCR through changes in intestinal histology and optimal nutrient digestion. However, there were no appreciable variations in mortality or feed consumption between the treatments.

Due to the biological functions, the wide range of activity and quickness of action and Absorbability, bioactive peptides have received much attention as a growth promoter and an active ingredient in poultry diets. Pan et al. (2019) suggest that intestinal shape, as well as the apparent availability of crude protein and ether extract in the diet, may be the cause of this improved production performance.

The number of eggs produced for each replicate daily was calculated by collecting eggs twice at eight in the morning and one in the afternoon. The mass of eggs produced by the bird was calculated weekly for the replications of the transactions according to the equation mentioned by Al-Fayyad and Naji (1989). As for Mortality, no Mortality was recorded in every repeater and treatment from the beginning of the experiment to its end. The increase in the rate of egg production and weight may be due to the readiness of the protein and the high coefficient of digestion and ability to absorb it in the digestive system .

It is noticed from Table (3) a clear superiority of the egg quality traits represented by the yolk index, the Albumen index, Albumen weight, yolk weight, yolk height, yolk diameter, albumen height, and albumen diameter for the peptide addition treatments compared to the control treatment and the vitamin E addition treatment.

Table (3): Impact of Vitamin E and Dietary Soybean Bioactive Peptide on the Traits of Egg quality in laying chickens at 56 weeks of age

Egg quality criteria	Control	Soybean bioactive peptide g/kg diets			Vit. E 250 (mg/kg)	SEM	*P*<
		2.5	5.0	7.5			
Shell weight (g)	7.28	7.27	7.32	7.30	7.31	0.010	0.441
Yolk weight (g)	16.52 ^{c}	18.32 ^{ab}	18.07 ^{ab}	18.83 ^{a}	17.14 ^{bc}	0.212	0.002
Yolk Height (mm)	16.66 ^{c}	20.23 ^{a}	18.67 ^{b}	20.41 ^{a}	17.29 ^{c}	0.245	0.00
Yolk diameter (mm)	38.94 ^{a}	37.25 ^{b}	34.28 ^{c}	35.52 ^{c}	38.43 ^{ab}	0.310	0.00
Yolk index (%)	0.42 ^{b}	0.54 ^{a}	0.54 ^{a}	0.57 ^{a}	0.45 ^{b}	.0008	.000
Albumen weight (g)	42.28 ^{c}	43.31 ^{ab}	42.64 ^{bc}	43.65 ^{a}	42.57 ^{bc}	0.157	0.028
Albumen Height (mm)	4.15^{b}	5.95 ^{a}	5.80 ^{a}	6.19 ^{a}	4.43^{b}	0.154	0.00
Albumen diameter	60.68 ^{c}	65.95 ^{a}	63.04^{b}	66.82 ^{a}	61.63 ^{c}	0.336	0.00
Albumen index (%)	0.06 ^{b}	0.08 ^{a}	0.09 ^{a}	0.09 ^{a}	0.07 ^{b}	0.002	0.00
Yolk: albumin ratio	0.39	0.42	0.42	0.43	0.40	0.005	0.17
Haugh unit score	77.25 ^{b}	88.17 ^{a}	87.50 ^{a}	89.26 ^{a}	79.28 ^{b}	0.913	0.00
Blood spots (%)	0.00	0.44	0.00	0.00	1.77	0.292	0.23
Meat spots (%)	0.88	0.00	0.00	0.88	1.33	0.258	0.37

SEM: standard error of mean, $^{a, b}$: Values that do not share a common superscript in the same row are different ($P < 0.05$).

This increase in production performance can be explained providing the protein and fat materials necessary for the formation of egg parts (Pan et al. 2019), and it is evident by the superiority of peptide treatments with a significant increase of high-density lipoproteins in the bloodstream compared to the control treatment (Table 4). This could also be because the biological activity of the soybean peptide addition has

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a major impact on the health and function of the intestines and speeds up the body's metabolic processes. Our results differed from Landy and Kheiri (2021), who did not obtain a significant effect on the height of the white and the height of the yolk when feeding laying hens (Bovans White) a diet containing biologically active cottonseed peptides at a level of (5 g/kg) compared to the control treatment. The yolk:albumin ratio and shell weight did not significantly differ among the five groups in our investigation. Additionally, for the two traits—blood spots and meat spots—there were no statistically significant differences between any of the treatments.

Table (4): Some Serum Biochemical Indices and Cholesterol Egg Yolk at 56 wk. of Age of Hens fed Soybean Bioactive Peptide and Vitamin E

Parameters	**Control**	**Soybean bioactive peptide g/kg diets**			**Vit. E 250 (mg/kg)**	**SEM**	***P<***
		2.5	**5.0**	**7.5**			
Total Protein (g/dl)	4.67	5.38	5.14	5.47	5.69	0.154	0.29
Albumen (g/dl)	2.49	2.59	2.74	2.61	2.80	0.045	0.21
Globulin (g/dl)	2.17	2.73	2.37	2.80	2.86	0.136	0.47
Glucose (mg/dl)	257.83	250.20	250.33	226.50	248.16	5.086	0.40
Total cholesterol (mg/dl)	295.66	279.00	279.46	277.16	284.40	3.856	0.54
Triglycerides (mg/dl)	146.40	123.03	124.60	122.23	146.30	5.131	0.32
HDL-c (mg/dl)	132.73 ^{ab}	143.88 ^{ab}	142.03 ^{ab}	147.33 ^{a}	129.76 ^{b}	2.481	0.08
LDL-c (mg/dl)	133.65 ^{a}	110.51 ^{ab}	109.51 ^{ab}	105.39 ^{b}	125.37 ^{ab}	4.134	0.12
VLDL-c (mg/dl)	29.28	24.60	24.92	24.44	29.26	1.026	0.32
Total antioxidant activity	227.66 ^{b}	254.00 ^{ab}	269.00 ^{a}	252.33 ^{ab}	251.33 ^{ab}	4.708	0.05
SOD (IU/l)	8.14 ^{b}	9.75 ^{ab}	9.69 ^{ab}	10.40 ^{a}	9.04 ^{ab}	0.280	0.08
GSH-PX (IU/l)	9.28 ^{b}	11.39 ^{a}	9.79 ^{b}	9.90 ^{b}	10.74 ^{a}	0.216	0.00
Cholesterol egg yolk (mg/g)	7.73 ^{ab}	7.38 ^{ab}	6.17 ^{b}	6.23 ^{b}	8.54 ^{a}	0.317	0.05

SOD: Superoxide dismutase, GSH-PX: Glutathion peroxides, SEM: standard error of mean, $^{a,\,b}$: Values that do not share a common superscript in the same row are different (P < 0.05).

The biochemical blood parameters in this experiment, which are represented by total protein, albumin, globulin, glucose, cholesterol, and triglycerides, were not affected by the treatments ($P > 0.05$). The group supplemented with 7.5 g of soybean peptides / kg feed had the greatest level ($P > 0.05$) of high-density lipoproteins (HDL). In contrast to the other treatments, the group receiving 7.5 gm of soybean peptides had a lower level ($P > 0.05$) of low-density lipoproteins. The group that had the highest total antioxidant activity of serum was the one that received an excessive amount of soybean peptide (5 g/kg). Numerous antioxidant enzymes and the linked biomolecules that work intricately to scavenge free radicals make up total antioxidant activity (Ren et al. 2012).

Given their amino acid composition, the physiologically active peptides might be able to confer hydrogen, As indicated by Girgih et al. (2015), antioxidant peptides possessing a high concentration of important amino acids, including Tyr, Trp, and Phe, show the ability to transfer electrons.

Our findings showed that a soybean bioactive peptide supplement boosted the amount of SOD activity in plasma. Additionally, superoxide is the primary free radical produced in biological systems during normal mitochondrial respiration. According to Wu et al. (2018), SOD convert superoxide to hydrogen peroxide, which is then further detoxified by GSH-Px or Catalase using the H2O configuration. This procedure will prevent the harmful effects of superoxide (Surai, 2016).

GSH-Px activities in serum were elevated in the current study when laying hens' diets supplemented with soybean bioactive peptides, suggesting that dietary soybean bioactive peptide supplementation boosted the activity of antioxidant enzymes. Additionally, a substantial ($P<0.05$) reduction in yolk cholesterol concentration was seen between the peptide-supplemented groups and the positive control treatment (adding vitamin E). In this regard, Landy and Kheiri (2021) confirmed that a

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significant increase was achieved in the effectiveness of total antioxidants in the blood serum of laying hens fed diets containing biologically active cottonseed peptides compared to the control diet. Zhao et al., (2022) reported an improvement in antioxidant indicators (SOD, GSH-Px) for laying hens when small peptides were added at different levels (0, 1.5, 3.0, 4.5, 6.0) g/kg of laying hens' diet during the incubation and growth periods.

Conclusion

The findings concluded that adding 7.5 g of soybean bioactive peptide/kg to the diets of laying hens may have a positive impact on the growth performance, characteristics of egg quality, and serum total antioxidant activity.

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