

The Synergistic Role of Multiple Enzymes and Curcumin in Enhancing the Physiological Efficiency and Productive Performance of Laying Hens

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ABSTRACT. *This study aimed to evaluate the effect of adding multiple enzymes and curcumin as an antioxidant and their interaction on some biochemical indicators and productive performance. Using an integrated mixture of enzymes and antioxidants in the diet of laying hens is an effective strategy to improve physiological performance, enhance productive efficiency and increase egg quality. However, studies that evaluate this integrated effect on performance under multiple production conditions are still limited and few, which necessitates further research in this area. The experiment involved using 132-day-old (four months and 12 days) Meso laying hens. A total of 144 birds (124 hens + 20 roosters) were housed in the coop. They were weighed and then randomly divided into four experimental groups, with three replicates of 12 birds per replicate (10 hens + 2 roosters). Treatment T1 (control): standard diet without any additives. Treatment T2: standard diet containing added enzymes at a rate of 2 g/kg of feed. Treatment T3: standard diet containing added curcumin at a rate of 2 g/kg of feed. Treatment T4: standard diet containing added enzymes and curcumin (2 g enzymes/kg of feed) and (2 g curcumin/kg of feed). Regular drinking water was freely available in all treatments. The experiment involved using 132-day-old (four months and 12 days) Meso laying hens. A total of 144 birds (124 hens + 20 roosters) were housed in the coop. They were weighed and then randomly divided into four experimental groups, with three replicates of 12 birds per replicate (10 hens + 2 roosters). Treatment T1 (control): standard diet without any additives. Treatment T2: standard diet containing added enzymes at a rate of 2 gm/kg of feed. Treatment T3: standard diet containing added curcumin at a rate of 2 gm/kg of feed. Treatment T4: standard diet containing added enzymes and curcumin (2 gm enzymes/kg of feed) and (2 gm curcumin/kg of feed). Regular drinking water was freely available in all treatments. An increase in red blood cell (RBC) count was observed in the second treatment group compared to the control group. Significant increases in hemoglobin (Hb) concentration and packed cell volume (PCV) were also observed in both the second and third treatments compared to the control group. Furthermore, glucose levels decreased in the third and fourth treatments*

compared to the second treatment group and the control group. All treatments showed significant increases in total protein, globulin, and globulin/albumin ratio compared to the control group. The enzymes and curcumin showed a positive effect on serum lipid parameters, with a significant decrease in triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), and atherosclerosis index, along with decreased liver enzyme levels (AST and ALT) in all treatments compared to the control at 31 weeks of age. Conversely, at 29 weeks of age, a significant increase in both high-density lipoprotein (HDL) and low-density lipoprotein (LDL) was observed in all treatments compared to the control. The statistical analysis revealed a significant improvement at the probability level ($P \leq 0.05$) in most of the studied traits as a result of the nutritional additives, The chemical composition of the eggs also improved with an increase in protein and amino acid content and a decrease in cholesterol levels.

Keywords: Multiple-enzymes, Curcumin phyto-genic additive, Nutrient digestibility, physiological performance, amino acids.

1. INTRODUCTION

Laying hens are an important source of livestock production, characterized by high productivity and the ability to lay eggs throughout their lives. This makes them a primary source of high-quality animal protein, necessitating the adoption of appropriate methods to maintain and develop the poultry sector [1]. Eggs are distinguished by their complete composition of essential amino acids, fatty acids, vitamins, and minerals necessary for human health, which enhances their nutritional and economic importance [2], Therefore, improving the productivity of laying hens and maintaining the sustainability of their production performance requires the adoption of advanced and safe feeding strategies that simultaneously support the enhancement of physiological processes and the maintenance of flock health and production sustainability [3] In this context, recent years have witnessed a growing interest in using natural feed additives as safe, effective, and performance-enhancing alternatives due to their role in promoting digestive and immune health and increasing nutrient utilization efficiency, which has positively impacted the flock's productive and physiological performance [4].

One of the most important additives used is curcumin, a potent phenolic compound extracted from turmeric. It is a key natural antioxidant with anti-inflammatory and antimicrobial properties, as well as playing a role in improving immunity and reducing oxidative stress[5] It also contributes to improving egg quality, particularly by enhancing yolk pigmentation and giving it the desired orange color, in addition to its positive effect on reducing fat accumulation and regulating fat metabolism [6,7]. Recent studies have also shown that adding curcumin to laying hen diets improves production performance, increases feed conversion efficiency, and reduces blood lipid levels, in addition to enhancing antioxidant status [8],Furthermore, curcumin contributes to improved gastrointestinal health and function by enhancing intestinal tissue structure, increasing absorption efficiency, improving gut microbial balance, and boosting the immune response [9]. Studies have also shown that curcumin improves intestinal health, increases intestinal villus height,

and reduces damage caused by heat stress, which positively impacts digestive efficiency and overall performance [10,11]

On the other hand, feed enzymes, particularly polysaccharides, are among the modern nutritional tools that aim to improve the digestion of complex food components, such as non-starchy carbohydrates, fats, and proteins, leading to increased nutrient availability and absorption in the digestive tract [12]. These enzymes break down complex food compounds, resulting in improved digestibility and feed conversion efficiency, as well as reduced feed loss and an improved gut microbiome. They also contribute to stimulating the secretion of endogenous enzymes and enhancing the efficiency of physiological functions, which is reflected in improved production performance, reduced mortality rates, increased antioxidant activity, and enhanced overall health [13,14]. Furthermore, scientific evidence suggests that combining natural antioxidants with feed enzymes may produce a significant synergistic effect, improving nutrient utilization efficiency, enhancing digestive and immune functions, and boosting productive and physiological performance [15,16,17]. This synergy is also reflected in improved egg quality, increased egg weight, and regulation of blood biochemical parameters, as well as a positive impact on hormonal balance related to production [18].

Based on the above, this study aims to evaluate the effect of enhancing laying hen diets with a mixture of multiple enzymes and curcumin on improving physiological and productive performance, as well as studying the impact of this on egg characteristics and some vital indicators, in order to contribute to the development of modern and sustainable nutritional strategies that support the poultry industry and improve its economic and productive efficiency.

2. MATERIALS AND METHODS

The study was conducted in the animal production fields, College of Agriculture and Forestry/University of Mosul. 144 birds (124 hens + 20 roosters) aged 132 days were raised and randomly distributed into 4 groups, with each group having 3 replicates of 10 females and 2 males (12 birds per replicate). The treatments were as follows: Group 1 (control): The birds were fed a standard ration and normal drinking water. Group 2: The birds were given a standard ration with 2g of multi-enzymes/kg of feed added. Group 3: The birds were given a standard ration with 2g of curcumin/kg of feed added. Group 4: The birds were given a standard ration with 2g of multi-enzymes and 2g of curcumin/kg of feed added. The birds were raised in a semi-open hall and divided into 12 cages along the length of the hall, each measuring (3 x 1.5) m, separated by a 2 m wide service corridor. The feed for all treatments was formulated according to the recommendations of [19], and the proportions of the feed components were as shown in Table (1).

The birds and eggs were weighed, and monthly weight gain and feed intake were calculated. Blood samples were collected at the end of the study, and red blood cell count (RBC), hemoglobin concentration (Hb), and packed cell volume (PCV) were calculated according to [20]. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were also calculated according to [21].

In addition, glucose, total protein, globulin, lipid profile, AST and ALT enzyme levels, as well as FSH and LH hormones, were measured using a pre-prepared kit. Eggs were collected daily

to study their quality characteristics. Chemical analyses of egg content were performed, including protein [22], fat content as a percentage [23], cholesterol concentration carbohydrate content [24]. Amino acid content was measured in two stages: first, amino acid extraction from the samples using the method described [25], derivatization (injection and analysis) using Clarity chromatography software (2015). Vitamin concentration was determined using high-performance liquid chromatography (HPLC). The feed conversion ratio was calculated based on egg production.

The difference between groups was determined using Duncan's test and a completely randomized design (CRD) with one-way randomization. Data was analyzed using the [26].

3. RESULTS AND DISCUSSION

3.1. THE EFFECT OF ADDING CERTAIN ENZYMES AND CURCUMIN ON BLOOD COUNT

The results of the statistical analysis in Table (1) showed the effect of adding multiple enzymes, curcumin, and their mixture to the diet of laying hens on the blood picture. A significant increase in the number of red blood cells (RBC) was observed for the second treatment compared to the control. A significant increase in the concentration of hemoglobin (Hb) and the volume of packed red blood cells (PCV) was also observed in the second and third treatments compared to the control. However, no significant differences were observed between the second, third, and fourth treatments. It was also found that the mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) decreased in treatments 2 and 4 compared to the control group (treatment 3), while the mean corpuscular hemoglobin (MCH) increased in treatment 2 compared to the other treatments. Our results were consistent with those of [27], who observed a significant increase in red blood cell count and hemoglobin levels when polyenzymes were added at 80 and 100 g/ton of feed to laying hens, respectively, compared to the control group. However, our results did not agree with those of researcher [28], Adding multi-enzymes at a rate of 2 g/kg, antibiotic at 2 g/kg, and probiotic at 0.5 g/kg to the diet of laying hens led to a significant decrease in the number of red blood cells in the multi-enzyme supplementation treatment compared to the control group. This effect may be attributed to the active role that enzymes play in enhancing the efficiency

Table 1. Effect of adding multiple enzymes, curcumin and their mixture to the diet on the blood profile of laying hens (means ± standard error)

Treatment	T ₁ Control	T ₂ (2 gm Enzyme/Kg diet)	T ₃ (2 gm Curcumin/Kg diet)	T ₄ (2gm Enzyme+ 2gm Curcumin/Kg diet)
parameters				

Red blood cell (RBCs) (10⁶/mm)	2.66±0.16b	3.09±0.06a	2.86±0.06ab	2.96±0.07ab
Hemoglobin concentration (g/dl)	16.46±0.63b	18.33±0.17a	18.40±0.40a	17.20±0.15ab
Backed Blood Volume (PCV) %	33.43±0.52 b	35.66±0.26a	36.53±0.56a	35.00±0.79ab
Mean corpuscular volume (MCV) μ³	126.11±0.79a	115.25±0.86c	127.92±0.90a	118.26±0.96
Mean corpuscular hemoglobin (MHC) (Pg)	62.37±0.88a	59.22±0.60b	64.34±0.47a	55.89±0.73b
Mean corpuscular hemoglobin ratio (MCHC) (g/dl)	49.30±0.48a	51.40±0.53a	50.41±0.68ab	49.12±0.38b

* Different letters horizontally indicate significant differences at a probability level ($P \leq 0.05$).

of metabolic processes and improving nutrient absorption in the intestines of laying hens, by reducing the viscosity of the contents of the digestive tract and breaking down anti-nutritional compounds. Enzymes also contribute to increasing the bioavailability of essential nutrients, especially proteins and minerals, which enhance and supports hemoglobin formation processes and stimulates the production of red blood cells [29,30].

Our results are consistent with those of [31], who observed that adding curcumin to the diet of laying hens at levels of 50, 75, and 100 mg/kg significantly increased red blood cell count at 100 mg/kg and packed cell volume at 75 mg/kg compared to the control group. However, our results differed from theirs, as they found no significant differences in hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, or mean corpuscular hemoglobin ratio between the curcumin-enriched and control treatments. Perhaps the reason is that the effect of adding curcumin raises the levels of antibodies, including antioxidant enzymes (GPX, SOD), and prevents oxidative stress, without affecting some properties of hemoglobin, maintaining that it maintains iron levels, and does not cause negative effects on blood health and oxygen transport function [32].

3.2. ADDING CERTAIN ENZYMES AND CURCUMIN TO BLOOD SERUM GLUCOSE AND PROTEIN LEVELS

Table (2) shows a significant decrease in serum glucose levels in treatments three and four compared to treatment two and the control group, and a significant increase in total protein and globulin levels in all treatments compared to the control group. Albumin concentration was significantly decreased in treatments two and three compared to treatment four and the control group. Conversely, the results in the same table showed a significant improvement in the globulin/albumin ratio in all treatments compared to the control group. Our results are consistent with those of [33] regarding the effect of adding enzymes and oregano essential oil at a rate of 200 mg/kg of feed each to laying hen diets; these additions positively increased total serum protein levels in the enzyme and oil treatment group compared to the control group. The improvement in

nutrient utilization efficiency in laying hens may be attributed to the effect of multi-enzyme feed additives, which contribute to increasing nutrient availability by breaking down complex-structured substances and reducing the effect of anti-nutritional factors. In addition, the reason may be due to the role that enzymes contribute to positive morphological changes in the intestines, such as increasing the length and width of intestinal villi, which leads to an enhanced surface area for absorption and improved nutrient utilization efficiency [29,30] Our study was similar to that of [34] showing that supplementing laying hen diets with curcumin at levels of 50, 100, and 150 mg/kg significantly increased total protein levels and reduced glucose levels. This can be attributed to curcumin's effective role in promoting avian health due to its antioxidant and anti-inflammatory properties, which contribute to reducing oxidative stress and increasing the functional efficiency of physiological systems [35].

Table 2: Effect of adding multiple enzymes, curcumin, and their mixture to the diet on the glucose and protein levels in the blood serum of laying hens at 31 weeks of age.

Treatment	T ₁ Control	T ₂ (2 gm Enzyme/Kg diet)	T ₃ (2 gm Curcumin/Kg diet)	T ₄ (2gm Enzyme+ 2gm Curcumin/Kg diet)
Parameters				
Concentration of Glucose	227.00±1.15a	228.66±0.88a	213.00±1.15c	217.00±1.15b
Total Protein (g/dl)	3.00±0.05c	3.53±0.08b	3.33±0.08b	4.26±0.08a
Albumin (g/dl)	2.03±0.03a	1.70±0.05b	1.43±0.06c	1.93±0.08a
Globulin (g/dl)	0.96±0.08c	1.83±0.13b	1.90±0.15b	2.33±0.13a
Globulin/ Albumin	0.47±0.05c	1.08±0.11a	1.34±0.17a	1.21±0.11a

* Different letters horizontally indicate significant differences at a probability level ($P \leq 0.05$).

3.3. ADDING SOME ENZYMES AND CURCUMIN TO THE FAT IMAGE

The results shown in Table (3) show a significant decrease in the concentrations of triglycerides TG, total cholesterol TC, very low-density lipoproteins (VLDL), and the atherosclerosis index in all experimental treatments compared to the control treatment. High-density lipoproteins (HDL) and low-density lipoproteins (LDL) recorded a significant increase in all treatments of enzyme and curcumin addition compared to the control group. Our findings are consistent with those of [16], who observed an improvement in lipoprotein balance in the serum of laying hens fed multi-enzyme supplements. They noted that supplementing the diet with enzymes at different levels (150, 300, and 600 g/ton of feed) yielded positive results. Our findings also align with those of [36] whose study demonstrated a decrease in cholesterol and triglyceride concentrations due to improved lipid metabolism when curcumin was added to laying hen diets at a rate of 25 mg/kg. This reduction in lipid profile may be attributed to curcumin's antioxidant properties, its role in regulating lipid metabolism, and its contribution to improved liver health [37].

Results can be attributed to the active role of enzyme supplementation in regulating liver fat metabolism. This resulted in increased lipid metabolism efficiency and reduced lipid accumulation, leading to improved liver physiology [38], This reduction in lipid profile can also be attributed to the bioactive properties of curcumin, particularly its antioxidant properties, which contribute to regulating lipid metabolism and enhancing liver function, thus reducing fat deposition in tissues [37].

Table 3 Effect of adding multiple enzymes, curcumin and their mixture to the diet in the form of fat at 31 weeks of age (end of the experiment)

Treatment	T ₁ Control	T ₂ (2 gm Enzyme/Kg diet)	T ₃ (2 gm Curcumin/Kg diet)	T ₄ (2gm Enzyme+ 2gm Curcumin/Kg diet)
parameters				
Concentration of Triglycerides (mg/dl)	572.33±1.20a	456.00±1.00b	428.00±1.00d	433.00±1.52c
Concentration of Cholesterol (mg/dl)	235.33±0.88a	266.83±0.60b	222.16±0.72c	223.66±0.88c
HDL (mg/dl)	48.33±0.34c	50.86±0.31a	52.76±0.69a	53.43±0.80a
LDL (mg/dl)	72.53±0.95b	84.76±0.89a	83.80±0.98a	83.63±1.72a
VLDL (mg/dl)	114.46±0.24a	91.20±0.20b	85.60±0.20d	86.60±0.30c
Stress guide	4.86±0.02a	4.45±0.03b	4.21±0.05c	4.18±0.07c

* Different letters horizontally indicate significant differences at a probability level (P ≤ 0.05).

3.4. ADDING CERTAIN ENZYMES AND CURCUMIN TO LIVER ENZYMES

As shown in Table (4), the liver enzyme levels of AST and ALT decreased in all treatments compared to the control group at 29 weeks of age. AST levels were lowest in the third treatment, which was supplemented with curcumin, while ALT levels were lowest in the fourth treatment, which was supplemented with a mixture of enzymes and curcumin, compared to the control group. These results are consistent with those reported by [18], who found that AST levels decreased as a result of adding multi-enzymes to the laying hen diet. However, these results contradict those of [39], who found that adding 500 mg/kg of multi-enzymes to the laying hen diet did not produce significant differences in AST and ALT levels. These results may be attributed to the vital role of curcumin in reducing the effects of stress on the endoplasmic reticulum, in addition to its role in regulating lipid metabolism disorders. The decrease is also attributed to the reduced fat production in the liver of laying hens, which is reflected in the reduction of fat accumulation in the abdominal area [40].

Table 4: Effect of adding multiple enzymes, curcumin, and their mixture to the diet on AST and ALT enzymes at 31 weeks of age (end of the experiment).

Treatment	T ₁ Control	T ₂ (2 gm Enzyme/Kg diet)	T ₃ (2 gm Curcumin/Kg diet)	T ₄ (2gm Enzyme+ 2gm Curcumin/Kg diet)

parameters				
AST (U/L)	172.13±0.59a	160.29±1.36b	157.53±1.12b	159.16±1.08b
AST (U/L)	39.91±0.22a	36.06±0.37b	35.13±0.69b	32.66±0.88c

* Different letters horizontally indicate significant differences at a probability level ($P \leq 0.05$).

3.5. ADDING CERTAIN ENZYMES AND CURCUMIN TO THE FAT, CHOLESTEROL, CARBOHYDRATE, AND PROTEIN CONTENT OF EGGS

Table (5) which deals with the effect of additives that enhance the diet of laying hens with enzymes and curcumin, shows a significant effect of these additives on the chemical composition of the egg. It shows a significant increase in the percentage of fat in the egg content in favor of the third and fourth treatments compared to the control, without differing significantly from the second treatment. Meanwhile, the data obtained showed that the amount of cholesterol decreased in all treatments compared to the control group. This may be explained by the regulatory role of curcumin in fat metabolism, which contributes to modifying the gene expression of enzymes responsible for the synthesis of fatty acids, which may lead to an increase in the efficiency of fat deposition in the yolk [41].

Furthermore, the improved digestion and absorption of nutrients by the added enzymes may increase the availability of energy for fat synthesis within the liver and its transfer to the egg. Regarding carbohydrate content, the results indicated a significant increase in the third and fourth treatments compared to the second treatment and the control. This may be attributed to the improved digestion and absorption of complex sugars by the added enzymes, in addition to the role of curcumin in improving gut health and the gut microbiome, which positively impacted carbohydrate metabolism and storage in egg components [42]. On the other hand, it was found that the percentage of proteins in the fourth treatment was significantly higher compared to the control, while it did not differ in the second and third treatments from the fourth treatment, and that this improvement may be due to the synergistic effect between the enzymes and curcumin in improving the physiological condition and reducing oxidative stress, which increases the efficiency of protein use in egg formation. The current results are consistent with those reported by [43], who showed that adding enzymes at a level of 250 mg/kg of feed reduced cholesterol levels while not affecting lipid levels. However, our results differ from those of [28], who reported that adding enzymes at a rate of 2 g/kg to the diet of laying hens led to an increase in yolk cholesterol levels, reaching 10.65 mg/g, compared to the control group. This may be due to the role of enzymes in enhancing the digestion and absorption of nutrients, including fats, in the diets provided to laying hens, and the

resulting increase in absorption, which provides the liver with ample fat for cholesterol synthesis [28].

Table 5: Effect of enzyme and curcumin additives and their mixture on the content of fat, cholesterol, carbohydrates and proteins of the egg

Treatment	T ₁ Control	T ₂ (2 gm Enzyme/Kg diet)	T ₃ (2 gm Curcumin/Kg diet)	T ₄ (2gm Enzyme+ 2gm Curcumin/Kg diet)
parameters				
Fat %	11.42± 0.04b	12.26±0.02 ab	13.00± 0.58a	13.28± 0.56a
Cholesterol (mg/100 g)	225.03± 1.12a	211.76± 0.92 b	212.90± 0.17 b	201.90± 0.28 c
Carbohydrates %	1.30± 0.11 c	1.17± 0.01 c	1.56± 0.01b	1.77± 0.01a
Proteins %	13.73± 0.77b	14.99± 0.35 ab	15.03± 0.59 ab	15.77± 0.19a

* Different letters horizontally indicate significant differences at a probability level ($P \leq 0.05$).

3.6. ADDING CERTAIN ENZYMES AND CURCUMIN TO THE VITAMIN CONTENT IN EGG COMPOSITION

The results of the statistical analysis shown in Table (6) indicate that the addition of enzymes and curcumin, either alone or in combination, led to an increase in the egg's content of certain vitamins. The concentration of both vitamin A and vitamin B6 was observed to be higher in the third treatment compared to the other treatments. The egg's content of these vitamins was also significantly higher in the second and third treatments compared to the control in terms of the content of these two vitamins. This effect is attributed to curcumin's antioxidant properties, as it helps protect fat-soluble vitamins, especially vitamin A, from oxidation, leading to increased vitamin A deposition in the egg [44,5]. It may also be due to improved enzyme digestion, which leads to increased availability and absorption of micronutrients, positively impacting vitamin content [45]. In addition to the improved vitamin B2 (riboflavin) content of the egg, which was highest in the third treatment compared to the fourth and control treatments, the vitamin content in the egg did not differ significantly from that of the second treatment. This improvement in the concentration of certain vitamins in the egg may be attributed to curcumin's role in enhancing liver function and promoting vitamin metabolism, as well as its positive impact on the balance of the gut microbiome, which contributes to the production of certain vitamins [46]. This is explained by the contribution of combining enzymes and curcumin to achieving a synergistic effect, as the enzymes increase the release of associated food compounds, while curcumin increases the efficiency of liver metabolism, leading to improved synthesis and deposition of these bioactive compounds in the egg [47,48].

In general, our results confirm that curcumin played an important role in enhancing vitamin stability through oxidative stress, while enzymes improved nutrient bioavailability. These

improvements reflect the synergistic role of food additives in enhancing physiological status, absorption efficiency, and metabolism, which positively impacts the nutritional value of the egg [49,42].

Table 6: Effect of enzyme and curcumin additives and their mixture on the vitamin content of the egg

Treatment	T ₁ Control	T ₂ (2 gm Enzyme/Kg diet)	T ₃ (2 gm Curcumin/Kg diet)	T ₄ (2gm Enzyme+ 2gm Curcumin/Kg diet)
parameters				
Vitamin A	430.73± 0.18 d	449.83±0.08 c	470.70± 0.78a	458.93± 0.37 b
Vitamin B2	10.46± 0.81 c	15.26± 0.65 ab	17.26± 1.27a	13.30± 0.43b
Choline	257.93±0.20 c	265.90±0.80 b	253.10± 2.91 c	278.90± 1.15 a
Vitamin B6	4.23± 0.01 d	5.65± 0.01 c	7.36± 0.02 a	6.12± 0.03 b
Vitamin B12	0.51± 0.01 d	0.86± 0.01b	0.69± 0.01 c	0.98± 0.01 a

* Different letters horizontally indicate significant differences at a probability level ($P \leq 0.05$).

3.7. ADDING CERTAIN ENZYMES AND CURCUMIN TO AMINO ACIDS

Table (7) illustrates the effect of adding multiple enzymes, curcumin, and their mixture to the egg-laying hen diet on the contents of some amino acids. The table shows a significant improvement in the content of amino acids in the egg, as the highest concentration of methionine, glycine, cysteine, and leucine was in the fourth treatment compared to the second and third treatments and the control group. The amount of these amino acids also increased in the second and third treatments compared to the control group. It was also found that the amino acid phenylalanine increased in the third and fourth treatments compared to the second treatment and the control group. The increased amino acid content of the egg may be due to the combined effect of enzymes and curcumin, as the added enzymes, including proteases, improve protein digestion and increase the availability of amino acids in the intestine, thus increasing their absorption and transport to egg-forming sites [50].

On the other hand, curcumin plays a role in improving the physiological state of birds and reducing oxidative stress, thus increasing the utilization of amino acids in protein synthesis, particularly in the liver and ovaries, which are the primary sites for egg component synthesis[51], Sulfur-containing amino acids such as methionine and cysteine are also key elements in egg production and quality, playing a vital role in the synthesis of structural and enzymatic proteins. Furthermore, they contribute to enhancing antioxidant status through glutathione formation, which positively impacts egg quality and nutritional value [52], Additionally, increased leucine, a branched-chain amino acid, has been shown to improve protein synthesis efficiency and regulate its pathways by activating the mTOR pathway.

Table 7. Effect of enzyme and curcumin additives and their mixture on egg content of certain amino acids

Treatment	T ₁ Control	T ₂ (2 gm Enzyme/Kg diet)	T ₃ (2 gm Curcumin/Kg diet)	T ₄ (2gm Enzyme+ 2gm Curcumin/Kg diet)
parameters				
Methionine	66.96±0.29 c	70.76± 0.98 b	71.90± 0.60 b	79.30±0.83a
Claycine	187.20±0.62 d	192.33±0.88 c	197.86± 0.40 b	203.90±0.17a
Cysteine	118.40±0.98 d	120.73±0.52 c	124.63± 0.24 b	130.93±0.24a
Leucine	420.20±0.80 d	424.83±0.92 c	430.86± 0.75 b	441.76±0.75a
Phenylalanine	149.36±0.29 c	151.66±1.05 c	154.76±1.32b	162.66±0.46a

* Different letters horizontally indicate significant differences at a probability level ($P \leq 0.05$).

4. CONCLUSION

The results of this study demonstrated that dietary supplementation with multiple enzymes and curcumin, either individually or in combination, positively improved the physiological and productive performance of Meso laying hens. Significant improvements were observed in hematological and biochemical parameters, including enhanced protein profile, reduced glucose and lipid levels, and lower liver enzyme activity. In addition, egg quality improved through increased protein and amino acid content and reduced cholesterol levels. Therefore, the combined use of enzymes and curcumin can be considered an effective nutritional strategy for enhancing health status, productive efficiency, and egg quality in laying hens.

CONFLICT OF INTEREST

None of the authors will claim a conflict of interest.

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