Effect of Supplementing a Mixture of Digestive Enzymes and a Probiotic to a Low-Energy and Low-Protein Diet on Egg Quality Traits and the Chemical and Biochemical Characteristics of Egg Yolk in Laying Hens

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***Abstract*—** The present study aimed to investigate the effect of supplementing a mixture of multi-enzymes and a probiotic to a low-energy and low-protein diet of laying hens (Lohmann Brown) on selected productive performance parameters and egg quality traits. The experiment was conducted over three consecutive periods covering the age range from 46 to 54 weeks. A total of 80 laying hens were randomly allocated to four experimental treatments T1, control diet with standard energy and protein levels. T2, low-energy and low-protein diet without additives. T3, low-energy and low-protein diet supplemented with enzymes and T4, low-energy and low-protein diet supplemented with a probiotic. results indicated that T4 showed a significant improvement ($P \leq 0.05$) in internal egg quality traits, particularly albumen index, Haugh unit , and yolk index, compared with T2 and the control treatment. Furthermore, the findings demonstrated that dietary supplementation played a significant role in maintaining productive performance stability and improving feed conversion efficiency despite the reduced nutrient density of the diet. In addition, a noticeable improvement was observed in oxidative indicators along with a reduction in yolk cholesterol levels. These results confirm the efficacy of this biological mixture as a strategic nutritional alternative for reducing feed costs without adversely affecting the qualitative and physiological standards of the final product.

***Keywords* —** laying hens, Enzymes, Probiotic.

INTRODUCTION

The poultry industry represents one of the fundamental pillars of global food security, with eggs constituting a high-quality and affordable protein source compared with other animal-derived products (1). In light of the current shift toward economic sustainability, producers face substantial challenges associated with escalating feed costs. This has prompted researchers to explore nutritional strategies capable of maintaining productive performance and final product quality through the use of low-protein and energy diets (2). Achieving optimal egg quality, particularly during peak production stages, requires a precise balance of dietary components to ensure the integrity of physiological processes and the biosynthesis of egg constituents (3). In this context, digestive enzymes have emerged as effective feed additives that compensate for the inherent limitations of the gastrointestinal tract in secreting certain endogenous enzymes, especially those responsible for degrading complex fiber fractions such as xylanase and β-glucanase. These enzymes facilitate the breakdown of plant cell wall structures and enhance nutrient release and bioavailability (4, 5). Supplementation with multi-enzyme complexes improves the digestibility of major nutrients, particularly starch and protein, thereby enhancing feed utilization efficiency and positively influencing egg quality (6). These effects are further potentiated by the inclusion of probiotics, which contribute to the modulation of intestinal microflora and stimulation of the intestinal mucosa (7, 8). Such improvements are directly associated with enhanced eggshell thickness (9) and reduced yolk cholesterol levels (10, 11), thereby increasing the nutritional and functional value of the

final product. Accordingly, the combined use of multi-enzymes and probiotics represents a promising nutritional strategy to mitigate the adverse effects associated with low-energy and low-protein diets, while maintaining optimal qualitative and physiological characteristics in laying hens (12).

Therefore, the present study aimed to evaluate the efficacy of a combined enzyme–probiotic mixture in compensating for reduced dietary protein and energy levels in laying hen diets, with the dual objective of lowering feed costs and reducing environmental ammonia emissions, while assessing its impact on preserving egg quality traits and improving the chemical and biochemical indicators of egg yolk.

MATERIALS AND MTHODS

A commercial multi-enzyme preparation (Mpzyme) produced by Promois International was used in this study. The product is supplied in powder form and contains seven major enzymes with defined activity levels. In addition, a commercial probiotic manufactured by golden peak joint stock company was utilized, this product is also available in powder form and consists of a blended composition of probiotic strains, vitamins, and other nutritional components.

Table 1. Active ingredients of the enzyme preparation and probiotic supplement

enzymes		Probiotics	
Amylase	2700 – 3400 u/g	**Lactobacillus acidophilus (min)**	3×10^8 CFU
Protease	<1200 HUT/g	**Bacillus subtilis (min)**	3×10^8 CFU
Cellulase	525 – 700 u/g	**Vitamin B1**	200 mg
Beta – Glucanase	375 – 500 u/g	**Vitamin B6**	200 mg
Xylanase	750 – 1050 u/g	**L-Lysine HCl**	2 g
Pectinase	200 – 300 u/g	**Beta glucan**	30 g
Phytase	75 – 150 u/g	**Excipients**	1 kg

Experimental design

The study was conducted in the laying hen house operated under a cage system at the Animal Field Station, Department of Animal Production , College of Agriculture - University of Anbar, from 2 June 2025 to 25 August 2025. The experimental period consisted of three consecutive phases of 28 days each, preceded by a 21 day adaptation period . Total of 80 laying hens of the (Lohmann Brown) strain, aged 46 weeks, were randomly assigned to four dietary treatments, with five replicates per treatment and four hens per replicate (20 hens per treatment). Birds were housed in 40 cages measuring 44 × 49 × 43 cm. Experimental diets were formulated as presented in Table (1) and offered daily at 08:00 am. at a fixed allowance of 100 g feed per hen per day. The treatments T1 (control), basal diet without additives ; T2 , low-energy and low-protein diet without additives ; T3 , low-energy and low-protein diet supplemented with a multi-enzyme mixture at 0.5 g kg feed ; and T4, low-energy and low-protein diet supplemented with a probiotic at 1 g kg feed. A lighting schedule of 16 h light and 8 h darkness per day was maintained throughout the experiment. Natural ventilation was supported by electric exhaust fans, a desert cooling system was applied to regulate ambient temperature, and fresh drinking water was provided ad libitum via a nipple drinking system.

Table 2. Composition of the Experimental Diets.

Ingredients	Diet (1)	Diet (2)	*Chemical composition	Diet (1)	Diet (2)
Yellow corn	43.6	37.9	**Metabolizable Energy (kcal/kg^{-1})**	2720	2650
Wheat bran	14	15	**Crude Protein %**	16.2	14.9
Soybean meal	19.4	14.82	**Digestible Protein**	15.9	14.25
Flour	10	20	**Calcium %**	3.68	3.7
Vitamin and mineral premix	2.5	2.5	**Total Phosphorus %**	0.671	0.775
Limestone	9.7	9.7	**Available Phosphorus %**	0.65	0.75
Dicalcium phosphate	0.07	0.07	**Lysine %**	0.87	0.73
Anti-toxin	0.01	0.01	**Methionine %**	0.28	0.32
Sunflower oil	1.3	0	**Methionine + Cystine %**	0.53	0.57
Total	100				

*According to the values of the chemical composition of the feed materials included in the omposition of the diet, according to what was stated in (NRC). (13)

Egg Quality and Biochemical Analyses

Egg quality traits were measured every 21 days throughout the three experimental periods. One egg per replicate was collected and stored at 4°C for 24 hours to allow the albumen to attain a gelatinous consistency, facilitating accurate measurement of albumen height. Each egg was individually weighed, and relative weight was calculated. Eggs were then broken onto a flat glass surface (mirror) to evaluate internal quality parameters, including Haugh unit (HU), yolk index, albumen index, as well as the percentage of albumen, yolk, and shell. Egg surface area and the shell weight per unit surface area (SWUSA) were calculated according to the equations described by (14). Eggshell thickness was measured using a micrometer. Egg yolk analyses included determination of cholesterol concentration following (15) and triglyceride levels according to (16), after lipid extraction from yolk as described by (17). Malondialdehyde (MDA) levels were assessed according to (18). Free fatty acids (FFA) percentage and peroxide value (PV) were also measured. All analyses were performed at the Central Laboratory, College of Agriculture, University of Anbar.

STATISTICAL ANALYSIS

Data were statistically analyzed using a one-way ANOVA to evaluate the effects of the four treatments, following the General Linear Model procedure in SAS software, version 9.1 (19). Multiple comparisons between means were conducted using Duncan's multiple range test at a significance level of 0.05 (20), and results are presented as means.

RESULT AND DISCUSSION

Statistical analysis across the four experimental periods (46–57 weeks) revealed variations in the response of egg quality

traits to supplementation. The supplemented treatments (T3 and T4) showed significant improvements ($P \leq 0.05$) in internal egg quality. Specifically, T3 exhibited superior performance in haugh unit and yolk index during the first period, while T4 showed higher yolk index values in the second period. In the third period, T4 outperformed in egg surface area and albumen index, and in the fourth period, T3 had a higher albumen percentage, resulting in a cumulative enhancement of albumen and yolk quality in favor of the supplemented treatments. In contrast, the control group (T1) showed a significant advantage in yolk percentage during the third period and in shell traits (relative shell weight, SWUSA, and shell thickness) during the fourth period only. However, cumulative overall analysis revealed no significant differences among treatments in shell quality, indicating that the enzyme–probiotic supplementation effectively maintained egg quality stability despite the reduced dietary energy and protein levels.

Table 3. Effect of Supplementing a Multi-Enzyme and Probiotic Mixture to a Low-Energy, Low-Protein Laying Hen Diet on the Cumulative Means of Selected Egg Quality Traits (46–57 Weeks of Age).

Studied Traits	Treatments				Mean	Std Error Mean	Sig. Level
	T1	T2	T3	T4			
Haugh Unit	53.24 ^{b}	53.57 ^{b}	55.74 ^{a}	56.19 ^{a}	54.69	1.176	P≤0.05
Yolk Index	38.68 ^{b}	39.12 ^{ab}	39.80 ^{a}	39.68 ^{a}	39.32	0.559	P≤0.05
Relative Shell Weight (%)	9.93	9.38	9.48	9.53	9.58	0.214	N.S.
Surface Area (cm^2)	70.20 ^{b}	71.91 ^{ab}	72.41 ^{ab}	73.60 ^{a}	72.03	1.033	P≤0.05
Shell Thickness (mm)	0.341	0.328	0.340	0.297	0.326	0.009	N.S.
Albumen Percentage (%)	64.19 ^{b}	65.99 ^{a}	66.45 ^{a}	66.75 ^{a}	65.84	0.550	P≤0.05
Yolk Percentage (%)	25.88 ^{a}	24.64 ^{b}	24.07 ^{b}	23.72 ^{b}	24.58	0.476	P≤0.05
Albumen Index	21.92 ^{b}	23.86 ^{ab}	25.43 ^{a}	26.98 ^{a}	24.55	1.166	P≤0.05
SWUSA	0.0825	0.0791	0.0797	0.0810	0.0806	0.0017	N.S.

NS: Indicates no significant differences among treatments at a significance level of ($P \leq 0.05$).
a, b, c: Different letters within the same row indicate significant differences among treatment means at a significance level of ($P \leq 0.05$).Experimental treatments:T1: Diet according to company recommendations without any additives.T2: Low-energy and low-protein diet without any additives. T3: Low-energy and low-protein diet supplemented with an enzyme mixture at 0.5 g/kg of feed. T4: Low-energy and low-protein diet supplemented with a probiotic at 1 g/kg of feed.

Chemical and Biochemical Traits of Laying Hen Egg Yolk

Table (4) results show, significantly affected yolk cholesterol percentage ($P \leq 0.05$), with T2 and T3 exhibiting higher values 3.8567 - 3.8667%, respectively compared to T4 3.5033% . No significant differences were observed between T2 and T3 and the control group (T1). Regarding other measured yolk parameters, including triglycerides, malondialdehyde (MDA), free fatty acids (FFA), and peroxide value (PV), no significant differences were detected among all experimental treatments and the control.

Table 4. Effect of Supplementing a Multi-Enzyme and Probiotic Mixture to a Low-Energy, Low-Protein Laying Hen Diet on Chemical and Biochemical Traits of Egg Yolk

Parameter	Treatments				Mean	Std. Error	Sig Level
	T1	T2	T3	T4			
Cholesterol (%)	3.5533^{ab}	3.8567^{a}	3.8667^{a}	3.5033^{b}	3.695	0.0657	P ≤ 0.05
Triglycerides (%)	12.773	13.433	11.557	13.850	12.903	0.702	NS
MDA1 (mg/kg)	0.1491	0.2167	0.1335	0.5581	0.264	0.0895	NS
FFA1 (%)	0.3567	0.3167	0.2900	0.4233	0.347	0.0342	NS
PV1 (meq/kg)	0.7767	0.9467	0.7233	1.2100	0.914	0.115	NS

Notes: - NS = Not significant ($P > 0.05$). - Different superscripts (a, b) within a row indicate significant differences at $P \leq 0.05$. - MDA = Malondialdehyde; FFA = Free Fatty Acids; PV = Peroxide Value.

The results presented in the tables indicate that dietary supplementation with bio-additives, whether multi-enzymes (T3) or probiotics (T4), effectively compensated for nutrient deficiencies caused by the reduced dietary energy and protein content. The significant improvement in albumen and haugh unit index in T3 can be attributed to the pivotal role of exogenous enzymes, such as xylanase and protease, in breaking down (NSPs). This enzymatic activity reduces digesta viscosity in the intestine, releasing trapped nutrients and increasing the availability of essential amino acids required for albumen protein synthesis in the oviduct. These findings are consistent with those reported (28 , 18), who emphasized the role of enzymes in compensating for limited endogenous secretions and enhancing internal egg components in energy-restricted diets.

T4 , supplemented with probiotics , demonstrated superior yolk index values and improved biochemical indicators, particularly during the later production. This effect is explained by the ability of probiotics to enhance intestinal villi integrity and promote a balanced gut microbiota , thereby improving calcium and carotenoid absorption necessary for yolk membrane strength and eggshell formation. These results align with the conclusions of (16) and (19) , who reported that probiotics act as physiological modulators maintaining egg quality under nutritional or age-related stress .

The reduction in yolk cholesterol in T4 reflects the capacity of probiotic strains , such as Bacillus subtilis, to inhibit hepatic enzymes responsible for lipid biosynthesis or to utilize cholesterol via beneficial intestinal bacteria , thereby enhancing the health value of the produced eggs (32) .

CONCLUSION

Multi-enzymes T3 primarily enhance digestibility and albumen quality, while probiotics T4 support physiological functions and reduce cholesterol . These findings indicate that both additives represent economically viable strategies for maintaining final product quality while reducing feed costs .

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N/A

Conflict of Interest

The authors declare no conflict of interest.

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