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Interactive effects of phytase and non-starch polysaccharide-degrading enzymes on productive performance, egg quality, serum biochemical indices and fecal viscosity in laying hens

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ABSTRACT

This study evaluated the effects of dietary phytase (PHY) and non-starch polysaccharide (NSP)-degrading enzyme supplementation, alone and in combination, on performance, egg quality, serum biochemical indices, and fecal viscosity in laying hens. Hens were assigned to dietary treatments comprising three PHY levels (0, 450, and 900 FTU/kg) with or without NSP-degrading enzyme supplementation. Phytase supplementation significantly improved eggshell characteristics, increasing eggshell thickness ($P = 0.029$) and eggshell breaking strength ($P = 0.001$), with the greatest responses generally observed at 900 FTU/kg. Eggshell breaking strength increased from 34.93 N in hens receiving no PHY to 39.80 and 42.47 N in hens receiving 450 and 900 FTU/kg PHY, respectively, representing increases of 13.9% and 21.6%. A significant PHY × NSP interaction was detected for eggshell ratio ($P = 0.010$) and eggshell breaking strength ($P = 0.041$), indicating that the responses of these traits depended on the combination of enzyme treatments. Neither PHY nor NSP supplementation significantly influenced Roche yolk color or instrumental yolk color parameters (L*, a*, and b*). NSP supplementation significantly reduced glucose ($P = 0.004$) and increased alkaline phosphatase activity ($P = 0.021$). A significant PHY × NSP interaction was observed for serum creatinine ($P = 0.002$). Serum phosphorus and hepatic enzymes were not significantly altered by either enzyme supplementation. Fecal viscosity remained unaffected by PHY, NSP, or their interaction at days 84 and 112. Overall, dietary phytase primarily improved eggshell structural characteristics, whereas NSP-degrading enzyme supplementation had limited effects on productive performance and egg quality but modified selected serum biochemical indices.

Introduction

Modern laying-hen production requires nutritionally precise diets that support sustained egg production, efficient nutrient utilization, and maintenance of bird health while minimizing feed costs and nutrient excretion (Sultan et al., 2025; Wahyuni et al., 2025; Sultana et al., 2025). The utilization of dietary phosphorus and other nutrients is particularly important because a considerable proportion of phosphorus in plant-derived feed ingredients is bound in the form of phytate, which has limited availability to poultry (Bello et al., 2022). Phytate can also interact with proteins and minerals and thereby reduce the availability of nutrients beyond phosphorus itself (Ciptaan et al., 2025). Consequently, dietary phytase supplementation has become an established nutritional strategy for improving phytate degradation and enhancing nutrient utilization in poultry (Jin et al., 2026).

Phytase supplementation may have benefits extending beyond phosphorus release (Selle et al., 2023). By hydrolyzing phytate and reducing its antinutritional effects, phytase can potentially improve the availability of calcium, amino acids, and other nutrients, with consequences for productive performance and egg characteristics (Nuamah et al., 2024). The response to phytase, however, can vary according to dietary composition, phytate concentration, calcium and phosphorus levels, enzyme dose, and the physiological stage of the bird (Adabi et al., 2025). Therefore, evaluation of different phytase inclusion levels in

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laying-hen diets remains relevant, particularly during the later stages of production when nutrient utilization and shell quality are important considerations.

Non-starch polysaccharides (NSPs) represent another important group of antinutritional components in cereal-based poultry diets (Nguyen et al., 2022). Soluble NSPs can increase intestinal digesta viscosity, interfere with nutrient diffusion and digestion, and alter the intestinal microbial environment (Hafeez et al., 2026). These effects may ultimately reduce nutrient utilization and compromise productive efficiency (Sztupecki et al., 2023). Supplementation with exogenous NSP-degrading enzymes, such as xylanase and β-glucanase-containing preparations, can hydrolyze complex NSP structures and reduce their adverse effects on the gastrointestinal tract (Papadopoulos and Lioliopoulou, 2023). Consequently, NSP-degrading enzyme supplementation has received considerable attention as a nutritional approach for improving feed utilization and intestinal function in poultry.

The potential interaction between phytase and NSP-degrading enzymes is of particular interest because these enzymes act through different but potentially complementary mechanisms (Bawish et al., 2024). Phytase primarily targets phytate, whereas NSP-degrading enzymes modify the physical and nutritional properties of plant cell-wall components. Improved degradation of phytate and NSPs could theoretically enhance nutrient availability and intestinal conditions simultaneously (Liu et al., 2023). Nevertheless, responses to enzyme combinations are not necessarily additive and may depend on diet composition, enzyme dose, bird age, and the physicochemical characteristics of the feed (Bedford and Apajalahti, 2022; Sureshkumar et al., 2023). Thus, factorial evaluation of phytase and NSP-degrading enzyme supplementation is necessary to distinguish their individual effects from possible synergistic or antagonistic interactions.

Despite the extensive use of individual feed enzymes in poultry nutrition, information regarding their combined application in laying hens, particularly under a factorial supplementation strategy involving different phytase concentrations and NSP-degrading enzymes, remains comparatively limited. This design enabled the assessment of the individual and interactive effects of these enzyme supplements on productive performance, egg quality, yolk color characteristics, serum biochemical indices, and fecal viscosity. Therefore, the present study aimed to evaluate the effects of dietary phytase and NSP-degrading enzyme supplementation, individually and in combination, on these responses in laying hens fed the experimental diets used in this study. We hypothesized that phytase and NSP-degrading enzyme supplementation would influence nutrient utilization and selected production and egg-quality traits, and that the responses to phytase might depend on the presence or absence of NSP-degrading enzymes.

Materials and methods

Animal care, experimental design, and nutrition

A total of 108 laying hens (Hy-line brown) having age 28 week were used in the experiment. The hens were housed in conventional wire laying cages measuring 42 × 40 × 46 cm, with two hens per cage. The cages were arranged in three tiers, comprising 54 cages in total. The experiment was conducted according to a 3 × 2 factorial arrangement of treatments, with three dietary phytase (PHY) supplementation levels (0, 450, and 900 FTU/kg diet) and two non-starch polysaccharide (NSP)-degrading enzyme supplementation levels (0 and 1.00 kg/t diet), resulting in six experimental treatments. Each treatment consisted of nine replicate cages, with two hens per replicate, giving 18 hens per treatment and 108 hens in total. During allocation, hens from each dietary treatment were distributed across different cage locations to minimize potential effects associated with cage position. Feeders were equipped with partitions to prevent hens from accessing feed allocated to adjacent cage sections. Fresh water was continuously available through nipple drinkers, and feed was provided ad libitum throughout the experimental period. A lighting program of 16 h light and 8 h darkness was maintained throughout the experiment, with the light period from 05:00 to 21:00 h and the dark period from 21:00 to 05:00 h. The experimental feeding period lasted 112 days (16 weeks) following a 10-day adaptation period, during which the hens were acclimated to the experimental diets and housing conditions. Feed intake and egg production were recorded at 14-day intervals throughout the experiment; however, the data were pooled across the entire 112-day experimental period to obtain an overall mean for each experimental unit before statistical analysis. Egg quality measurements were conducted periodically throughout the experiment.

Feed preparation and enzymes inclusion

The individual feed ingredients required for the experimental diets were purchased from a local commercial feed manufacturing company. A basal diet for laying hens was prepared according to the nutrient requirements of laying hens, and its ingredient composition and chemical characteristics are presented in Table 1. The diet was formulated to provide approximately 2700 Kcal ME/kg metabolizable energy, 17.0% crude protein, 3.80% calcium, and 0.28% available phosphorus (AP).

The basal diet was formulated based on the nutrient requirements of laying hens according to NRC (1994), with the nutrient specifications also considered in relation to the Hy-Line Brown recommendations. The dietary calcium concentration was maintained at 3.80%, while AP was set at 0.28% across all experimental treatments to provide a consistent basal dietary formulation for evaluating the effects of phytase and NSP-degrading enzyme supplementation. The interpretation of phytase responses depends on the dietary calcium and phosphorus concentrations, the amount of phytate-bound phosphorus, and the phytase activity supplied. Therefore, the phosphorus composition of the basal diet is important for interpreting the effects of phytase supplementation on eggshell quality and productive performance. A commercial phytase preparation (Phytase 450, with a declared activity of 4,500,000 FTU/kg of premix) was incorporated into the diets at 0, 0.100, or 0.200 kg/t, corresponding to calculated phytase activities of 0, 450, and 900 FTU/kg of complete feed, respectively. These values represent calculated activities based on the declared activity of the commercial preparation, not phytase activities measured in the finished diets. A commercial NSP-degrading enzyme mixture was incorporated at 1.00 kg/t diet in the NSP-supplemented treatments. The NSP enzyme preparation contained xylanase (145,000 U/kg premix), β-glucanase (30,000 U/kg premix), cellulase (64,000 U/kg premix), and hemicellulase (120,000 U/kg premix). The inclusion of an NSP-degrading enzyme complex was intended to target non-starch polysaccharides present in the cereal components of the basal diet, particularly wheat and barley. These ingredients can supply NSP substrates for enzymes such as xylanase and β-glucanase, while cellulase and hemicellulase target additional cell-wall polysaccharide components. The enzyme complex was supplemented at 1.00 kg/t of feed, providing the declared enzyme activities specified above. However, total and soluble NSP concentrations were not determined in the experimental diets; therefore, the actual NSP substrate concentrations and the extent to which they limited nutrient utilization could not be quantified. The selected supplementation level was intended to provide a combination of NSP-degrading activities appropriate for the cereal-based diet, although the magnitude of the biological response would depend on the composition and accessibility of the dietary NSP substrates.

Accordingly, the six experimental diets were: (1) 0 PHY + 0 NSP; (2) 0 PHY + NSP; (3) 450 PHY + 0 NSP; (4) 450 PHY + NSP; (5) 900 PHY + 0 NSP; and (6) 900 PHY + NSP. The enzyme preparations were thoroughly mixed into the basal diet according to their respective treatment groups to ensure homogeneous distribution.

The enzyme preparations were thoroughly mixed into the basal diet according to their respective treatment groups to ensure homogeneous distribution. The experimental diets were prepared and provided in

Table 1
Feed composition and chemical analysis.

Feed materials (g/kg)	0 Phytase	450 Phytase	900 Phytase
	Quantity	Quantity	Quantity
Corn	100	100	100
Barley	250	250	250
Wheat	251	251	251
Sunflower meal (32% CP)	97.99	97.99	97.99
Soybean meal (46% CP)	150.28	150.28	150.28
Limestone	97.06	97.06	97.06
Vegetable oil	40.20	40.20	40.20
Monocalcium phosphate	5.94	5.94	5.94
Salt	2.96	2.96	2.96
Vitamin and mineral premix	2.00	2.00	2.00
Phytase 450	0.0	0.100	0.200
D-L-Methionine	1.84	1.84	1.84
L-Lysine	0.73	0.73	0.73
Total, kg	1000	1000	1000
Analyzed chemical composition*			
Dry matter (DM, %)	93.48	93.48	93.48
Crude protein (CP, %)	17.00	17.00	17.00
Crude fat (CF, %)	5.74	5.74	5.74
Crude ash (%)	11.60	11.24	11.77
ADF (ADF, %)	0.66	0.66	0.66
NDF (NDF, %)	2.72	2.72	2.72
Methionine + cystine (%)	0.77	0.77	0.77
Methionine** (%)	0.47	0.47	0.47
Lysine** (%)	0.86	0.86	0.86
Calcium** (%)	3.80	3.80	3.80
Available phosphorus** (%)	0.28	0.28	0.28
Total Phosphorous (%)	0.55	0.55	0.55
Phytate phosphorus (%)	0.25	0.25	0.25
Non-phytate phosphorus (%)	0.30	0.30	0.30
Metabolic Energy Kcal/kg***	2700	2700	2700

AP: Available phosphorus, Per kg of vitamin-mineral premix ration: Vitamin A, 12000 I.U; Vitamin D3, 2000 I.U; Vitamin E, 20.0 mg; Vitamin K, 3.0 mg; Vitamin B1 (thiamin), 3.0 mg; Vitamin B2 (riboflavin) 3.0 mg; Vitamin B6, 5.0 mg; Vitamin B12, 0.15 mg; Vitamin C, 50 mg; niacin, 20.0 mg; biotin, 0.05 mg; calcium D-pantothenate, 6 mg; folic acid, 0.75 mg; choline chloride, 150 mg; manganese, 80.0 mg; iron, 60.0 mg; zinc, 60.0 mg; copper, 5.0 mg; iodine, 1.0 mg; cobalt, 0.2 mg; selenium provides 0.15 mg and canthaxanthin 20 mg. *Dry matter, crude protein, and crude ash were analyzed according to established procedures AOAC (2013), and other nutrient compositions were calculated based on NRC (1994). Phytase 450: contains 4500,000 FTU per kg premix. NSP enzymes mixture (xylanase 145000 U/kg, β-glucanase 30000 U/kg, cellulose 64000 U/kg, and hemicellulose 120000 U per kg premix).
Dry matter, crude protein, and crude ash were analyzed according to AOAC (2013) procedures. Other nutrient values were calculated from ingredient composition data using NRC (1994). The phytase preparation had a declared activity of 4,500,000 FTU/kg of premix and was included at 0.100 and 0.200 kg/t of feed, providing calculated activities of 450 and 900 FTU/kg of complete feed, respectively. The NSP enzyme mixture supplied xylanase (145,000 U/kg of premix), β-glucanase (30,000 U/kg), cellulase (64,000 U/kg), and hemicellulase (120,000 U/kg). AP, available phosphorus; FTU, phytase units.

mash form, without steam conditioning, pelleting, or other thermal processing. Fresh experimental diets were prepared every 28 days to minimize potential oxidation, microbial deterioration, and loss of enzyme activity during storage. The prepared feeds were placed in covered plastic containers and stored in a shaded, dry area until feeding. Because the diets were not subjected to steam conditioning or pelleting, they were not exposed to the elevated temperatures associated with these processing procedures. However, enzyme activity was not measured after mixing or during storage; therefore, the retention of enzyme activity in the finished diets could not be confirmed.

Determination of body weight, feed intake, and feed conversion ratio

Individual body weight (BW) was measured at the beginning and end of the experimental period using a precision balance (model EP114C, OhAUS Corporation, Pine Brook, NJ) with an accuracy of ±1 g. Hens were allocated to treatments to achieve comparable initial BW among groups. Body weight change was calculated as the difference between final and initial BW. Feed intake was determined for each experimental unit at the end of each 14-day period by subtracting the weight of residual feed from the amount of feed offered. Where mortality occurred, feed intake was adjusted according to the number of birds present during the corresponding period. Average daily feed intake was calculated by dividing feed consumed during each 14-day period by the number of feeding days and the average number of hens present during that period. Feed conversion ratio (FCR) was calculated for each consecutive 14-day period based on feed intake relative to egg mass. The FCR values from all eight 14-day periods were subsequently pooled to obtain an overall mean FCR for the entire 112-day experimental period, which was used for statistical analysis:

FCR = Feed intake (g/hen) / Egg mass (g/hen).

Egg mass was calculated from egg production and average egg weight.

Determination of egg production, egg weight, and internal egg quality

Egg production was recorded throughout the 112-day experimental period, and the data were summarized as an overall mean for the entire experimental period (Evci et al., 2025). Egg quality characteristics were evaluated during the experimental period, and the measurements obtained from the sampling dates were pooled to calculate an overall mean value for each experimental unit. Eggs were collected from the experimental units according to the predetermined sampling schedule. Egg weight and internal egg quality characteristics were determined using an Egg Analyzer® (ORKA Food Technology LLC, West Bountiful, Utah). The measured parameters included egg weight, albumen height, Haugh unit (HU), and Roche yolk color score. Yolk color was additionally assessed instrumentally using a Minolta CR-400 colorimeter (Konica Minolta, Inc., Osaka, Japan). Color coordinates were recorded as L* (lightness), a* (redness), and b* (yellowness).

Determination of eggshell characteristics and breaking strength

Following egg-quality measurements, eggshells were carefully separated, washed with tap water to remove adhering albumen, and dried overnight at 70°C. The dried eggshells were weighed using a precision balance with an accuracy of 0.1 g.

Eggshell ratio was calculated as:

Eggshell ratio (%) = [Eggshell weight (g) / Egg weight (g)] × 100.

Eggshell surface area (ESA) was estimated according to the equation proposed by Carter (1975):

ESA (cm^2) = 3.9782 × Egg weightˆ0.7056.

Eggshell weight per unit surface area was calculated as:

Eggshell weight per unit area (mg/cm^2) = Eggshell weight (mg) / Eggshell surface area (cm^2).

Eggshell thickness was measured at the sharp, blunt, and middle/equatorial regions of each eggshell using a digital micrometer with an accuracy of 0.01 mm. The mean of the three measurements was used as the eggshell thickness for each egg.

Eggshell-breaking strength was determined on days 28, 56, 84, 112 using an Instron Universal Testing Machine (Instron Universal Testing Machine, Model 4301 Instron Ltd., High Wycombe, UK), and the measurements were pooled to obtain an overall mean value for each experimental unit. Uncracked eggs were positioned vertically between the testing surfaces, and measurements were performed at room temperature using a constant crosshead speed of 10 mm/min and a 100-N load cell. Compression and penetration tests were conducted using a 35-mm-diameter compression plate and an 8-mm-diameter probe, respectively. Following the breaking-strength assessment, eggshells were cleaned, and eggshell thickness, including the cuticle and membrane, was measured at the equatorial, sharp, and blunt regions using a 550-501 digital micrometer (NSK, Tokyo, China). The mean of these three measurements was used as the final eggshell thickness value.

Determination of serum biochemical parameters

At the end of the 112-day experimental period, approximately 10 mL of blood was collected from a randomly selected laying hen from each experimental subgroup via the wing vein. Blood samples were transferred into appropriate tubes and centrifuged (Centrifuge 5810 (Eppendorf SE, Hamburg, Germany) to separate the serum. The separated serum was transferred into sterile Eppendorf tubes and stored at −20°C until biochemical analysis. On the day of analysis, serum samples were removed from the freezer and allowed to thaw before analysis. Serum concentrations of glucose, creatinine, triglycerides, total cholesterol, phosphorus, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were determined using an automated biochemical analyzer ((Siemens Healthcare GmbH, Erlangen, Bavaria, Germany) with commercially available diagnostic kits (Istanbol, Türkiye) specific for each parameter. All analyzes were performed according to the manufacturers' instructions.

Determination of fecal viscosity

Fecal viscosity on day 84 and 112 of the experiment was determined using a rotational Rheometer/Viscometer (DV3T Rheometer, Brookfield, Middleboro, MA). Fresh fecal samples were collected immediately after defecation using nylon sheets positioned beneath the cages. Samples were stored at 4°C until analysis. For analysis, 5 g of fecal material was transferred into a 10-mL glass tube and mixed with 5 mL of distilled water. The suspension was homogenized using a vibrator for 4 min and subsequently centrifuged at 5000 rpm for 4 min to separate the liquid and solid fractions. During viscosity measurements, a laboratory water bath (Memmert GmbH + Co. KG, Schwabach, Bavaria, Germany) was maintained at 41°C to ensure a constant sample temperature. Approximately 1 mL of the supernatant was transferred to the viscometer chamber, and viscosity was measured at 10 rpm using the appropriate spindle. The viscosity values were recorded according to the instrument output.

Statistical analysis

All data were analyzed according to a 3 × 2 factorial arrangement of treatments, with dietary phytase (PHY) supplementation at three levels (0, 450, and 900 FTU/kg diet) and NSP-degrading enzyme supplementation at two levels (0 and 1.00 kg/t diet). The experimental unit was the replicate cage, with two hens per cage. For variables measured repeatedly during the experimental period, including feed intake, FCR, egg production, egg weight, and egg mass, data from each experimental unit at each 14-day period were analyzed using a linear mixed model with repeated measures. The fixed effects included PHY level, NSP supplementation, time, and their two-way and three-way interactions. Experimental unit was included as the subject for repeated measurements to account for correlations among observations obtained from the same cage over time. An appropriate within-subject covariance structure was selected based on the Akaike information criterion (AIC). The factorial treatment effects and their interactions were evaluated while accounting for the repeated-measures structure.

Body weight measured only at the beginning and end of the experiment was analyzed according to the factorial design, with the cage as the experimental unit. Egg-quality and eggshell traits were analyzed according to their actual sampling structure; repeated-measures models were used only where observations across sampling periods could be linked to the same experimental unit. Fecal viscosity measured at the designated sampling times was analyzed using a repeated-measures model if samples from the same experimental units were collected at both time points; otherwise, the analysis reflected the actual sampling design.

Model assumptions were evaluated using residual diagnostics. Results are presented as means ± standard error (SE). Statistical significance was declared at \(P < 0.05\), and \(0.05\leq P < 0.10\) was considered a tendency. Where appropriate, multiple comparisons were adjusted for the comparisons being made.

Results

Table 2 presents the effects of dietary PHY and NSP-degrading enzyme supplementation on body weight parameters of laying hens. Neither PHY supplementation nor NSP-enzyme supplementation significantly affected initial BW, final BW, BW change, or BW change percentage. For PHY, the corresponding P-values were 0.238, 0.438, 0.697, and 0.730, respectively, while for NSP supplementation, the P-values were 0.542, 0.414, 0.253, and 0.199, respectively. No significant PHY × NSP interaction was observed for any body weight parameter, with P-values of 0.345, 0.760, 0.754, and 0.777, respectively.

Table 3 shows that dietary PHY supplementation did not significantly affect feed intake, FCR, egg production, egg weight, or egg mass, with P-values of 0.205, 0.143, 0.697, 0.151, and 0.270, respectively. Similarly, NSP supplementation did not significantly affect these parameters (P = 0.575, 0.059, 0.200, 0.453, and 0.242, respectively). Although FCR tended to differ between NSP treatments, the difference was not statistically significant (P = 0.059). No significant PHY × NSP interaction was detected for feed intake, FCR, egg production, egg weight, or egg mass (P = 0.761, 0.079, 0.934, 0.367, and 0.451, respectively).

Table 4 presents the effects of dietary PHY and NSP supplementation on egg quality and eggshell characteristics. PHY supplementation significantly affected eggshell thickness and eggshell breaking strength (P = 0.029) and (P = 0.001), respectively). Eggshell thickness was greater in hens receiving 900 FTU/kg PHY (0.38 mm) than in those

Table 2
Effects of phytase (PHY) enzyme supplementation and non-starch polysaccharide (NSP)-degrading enzyme supplementation, alone and in combination, on initial body weight, final body weight, and body weight change in laying hens.

Treatments	Initial BW (g)	Final BW (g)	BW Change (g)	BW Change, (%)
PHY Enzyme				
0	2004.93 ±28.39	1927.21 ±30.85	−77.71 ±37.10	−3.54±1.83
450	2027.79 ±28.39	1907.07 ±30.85	−120.71 ±37.10	−5.53±1.83
900	1960.29 ±28.39	1871.57 ±30.85	−88.71 ±37.10	−4.11±1.83
P	0.238	0.438	0.697	0.730
NSP				
-	2007.71 ±23.17	1887.33 ±25.19	−120.38 ±30.30	−5.76±1.49
+	1987.62 ±23.17	1916.57 ±25.19	−71.05 ±30.30	−3.03±1.49
P	0.542	0.414	0.253	0.199
Interactions				
0PHY×-NSP	1981.14 ±40.14	1896.86 ±43.63	−84.29 ±52.47	−4.12±2.58
0PHY×+NSP	2028.71 ±40.14	1957.57 ±43.63	−71.14 ±52.47	−2.96±2.58
450PHY×-NSP	2058.00 ±40.14	1891.57 ±43.63	−166.43 ±52.47	−7.91±2.58
450PHY×+NSP	1997.57 ±40.14	1922.57 ±43.63	−75.00 ±52.47	−3.15±2.58
900PHY×-NSP	1984.00 ±40.14	1873.57 ±43.63	−110.43 ±52.47	−5.25±2.58
900PHY×+NSP	1936.57 ±40.14	1869.57 ±43.63	−67.00 ±52.47	−2.96±2.58
P	0.345	0.760	0.754	0.777

BW: body weight, P0: no addition phytase enzyme, P450: 450 unit phytase enzyme addition, P900: 900 unit phytase enzyme addition, NSP: non-starch polysaccharide, [a,b,c:] Values with different superscript in a column differ significantly at $P < 0.05$.

Table 3
Effects of dietary phytase (PHY) and non-starch polysaccharide (NSP)-degrading enzyme supplementation, alone and in combination, on feed intake, feed conversion ratio, egg production, egg weight, and egg mass in laying hens.

Treatments	Feed intake (g)	FCR	Egg production (%)	Egg weight (g)	Egg mass (g/hen/day)
PHY Enzyme					
0	117.44 ±0.82	1.79 ±0.02	27.21±0.16	68.20 ±0.62	18.56 ±0.82
450	115.35 ±0.82	1.81 ±0.02	27.29±0.16	66.86 ±0.62	18.24 ±0.82
900	116.08 ±0.82	1.85 ±0.02	27.00±0.16	66.58 ±0.62	17.98 ±0.82
P	0.205	0.143	0.697	0.151	0.270
NSP					
-	116.56 ±0.67	1.80 ±0.02	27.29±0.13	66.95 ±0.51	18.27 ±0.67
+	116.02 ±0.67	1.84 ±0.02	27.05±0.13	67.49 ±0.51	18.25 ±0.67
P	0.575	0.059	0.200	0.453	0.242
Interactions					
0PHY×-NSP	116.67 ±1.16	1.78 ±0.03	27.14±0.22	68.41 ±0.88	18.57 ±1.16
0PHY×+NSP	118.20 ±1.16	1.81 ±0.03	27.29±0.22	67.99 ±0.88	18.55 ±1.16
450PHY×-NSP	115.32 ±1.16	1.86 ±0.03	27.14±0.22	65.89 ±0.88	17.89 ±1.16
450PHY×+NSP	115.37 ±1.16	1.77 ±0.03	27.43±0.22	67.84 ±0.88	18.61 ±1.16
900PHY×-NSP	116.07 ±1.16	1.89 ±0.03	26.86±0.22	66.54 ±0.88	17.87 ±1.16
900PHY×+NSP	116.10 ±1.16	1.81 ±0.03	27.14±0.22	66.62 ±0.88	18.08 ±1.16
P	0.761	0.079	0.934	0.367	0.451

[a,b,c] Values with different superscript in a column differ significantly at $P < 0.05$.

receiving 0 or 450 FTU/kg (both 0.37 mm, based on the rounded means). Eggshell breaking strength increased from 34.93 N in the unsupplemented group to 39.80 N and 42.47 N in the 450- and 900-FTU/kg PHY groups, respectively, representing increases of approximately 13.9% and 21.6%, respectively, relative to the unsupplemented group. These changes suggest a potentially meaningful improvement in shell mechanical strength, although biological relevance should be interpreted in the context of practical egg-handling and breakage outcomes. Egg weight, Haugh unit, eggshell ratio, eggshell surface area, eggshell weight per unit area, and yolk height were not significantly affected by PHY supplementation (P = 0.151), 0.101, 0.110, 0.137, 0.429, and 0.179, respectively).

NSP supplementation did not significantly affect any measured egg quality or eggshell characteristic (P = 0.453), 0.121, 0.688, 0.464, 0.840, 0.867, 0.235, and 0.542, respectively). A significant PHY × NSP interaction was observed for eggshell ratio (P = 0.010). The treatment means showed that eggshell ratio increased numerically with increasing PHY in the absence of NSP supplementation, from 9.50% at 0 FTU/kg to 9.62% at 450 FTU/kg and 9.96% at 900 FTU/kg. In contrast, with NSP supplementation, the corresponding values were 9.66%, 9.74%, and 9.59%, respectively. This indicates a treatment-specific response rather than a generalized synergistic effect. A significant PHY × NSP interaction was also observed for eggshell breaking strength (P = 0.041), whereas interactions were not significant for egg weight, Haugh unit, eggshell surface area, eggshell weight per unit area, eggshell thickness, or yolk height (P = 0.367), 0.287, 0.266, 0.212, 0.126, and 0.984, respectively).

Table 5 presents the effects of PHY and NSP supplementation on yolk color and instrumental color parameters. Neither PHY supplementation nor NSP supplementation significantly affected Roche yolk color, yolk brightness (L*), redness (a*), or yellowness (b*). For PHY, the corresponding P-values were 0.773, 0.855, 0.717, and 0.142, respectively, whereas for NSP they were 0.574, 0.958, 0.972, and 0.183, respectively. Likewise, no significant PHY × NSP interaction was observed for Roche color, L*, a*, or b* values (P = 0.429, 0.353, 0.589, and 0.863, respectively).

Table 6 shows the effects of dietary PHY and NSP supplementation

Table 4
Effects of dietary phytase (PHY) and non-starch polysaccharide (NSP)-degrading enzyme supplementation, alone and in combination, on egg weight, internal egg quality, eggshell characteristics, and eggshell breaking strength in laying hens.

Treatments	Egg weight (g)	Haugh Unit	Eggshell ratio (%)	Eggshell surface area (cm^2)	Eggshell per unit weight (mg/ cm^2)	Eggshell thickness (μm)	Egg yolk height (mm)	Eggshell breaking strength (N)
PHY Enzyme								
0	68.20 ±0.62	59.03 ±0.97	9.58±0.07	78.24±0.50	12.05±0.08	0.37±0.00[b]	4.68±0.09	34.93±1.30[b]
450	66.86 ±0.62	58.55 ±0.97	9.68±0.07	77.13±0.50	11.98±0.08	0.37±0.00[b]	4.50±0.09	39.80±1.12[a]
900	66.58 ±0.62	61.33 ±0.97	9.78±0.07	76.89±0.50	11.90±0.08	0.38±0.00[a]	4.73±0.09	42.47±1.21[a]
P	0.151	0.101	0.110	0.137	0.429	0.029*	0.179	0.001**
NSP								
-	66.95 ±0.51	60.51 ±0.79	9.70±0.05	77.21±0.41	11.96±0.07	0.37±0.00	4.70±0.07	38.64±0.93
+	67.49 ±0.51	58.76 ±0.79	9.66±0.05	77.63±0.41	11.98±0.07	0.37±0.00	4.58±0.07	39.49±1.04
P	0.453	0.121	0.688	0.464	0.840	0.867	0.235	0.542
Interactions								
0PHY×-NSP	68.41 ±0.88	59.81 ±1.37	9.50±0.09[a-f]	78.42±0.71	12.12±0.11	0.37±0.00	4.76±0.13	35.47±1.69[a-fa-f]
0PHY×+NSP	67.99 ±0.88	58.24 ±1.37	9.66±0.09[a-f]	78.06±0.71	11.97±0.11	0.37±0.00	4.61±0.13	34.39±1.98[a-f]
450PHY×-NSP	65.89 ±0.88	60.55 ±1.37	9.62±0.09[a-f]	76.35±0.71	12.10±0.11	0.37±0.00	4.56±0.13	36.72±1.61[a-f]
450PHY×+NSP	67.84 ±0.88	56.54 ±1.37	9.74±0.09[a-f]	77.92±0.71	11.86±0.11	0.37±0.00	4.45±0.13	42.88±1.55[a-f]
900PHY×-NSP	66.54 ±0.88	61.16 ±1.37	9.96±0.09[a-f]	76.86±0.71	11.67±0.11	0.38±0.00	4.79±0.13	43.71±1.55[a-f]
900PHY×+NSP	66.62 ±0.88	61.49 ±1.37	9.59±0.09[a-f]	76.92±0.71	12.12±0.11	0.38±0.00	4.67±0.13	41.22±1.86[a-f]
P	0.367	0.287	0.010*	0.266	0.212	0.126	0.984	0.041

[a-f] Values with different superscript in a column differ significantly at $P < 0.05$.

Table 5
Effects of dietary phytase (PHY) and non-starch polysaccharide (NSP)-degrading enzyme supplementation, alone and in combination, on yolk color and colourimetric parameters of eggs from laying hens.

Treatments	Roche color	yolk brightness (L*)	yolk redness (a*)	egg yolk yellowness (b*)
PHY Enzyme	7.82 ±0.15	56.40±0.22	1.22±0.08	31.52±0.22
0	7.68 ±0.15	56.27±0.22	1.15±0.08	30.89±0.22
450	7.71 ±0.15	56.43±0.22	1.23±0.08	31.27±0.22
900	0.773	0.855	0.717	0.142
P				
NSP	7.79 ±0.12	56.37±0.18	1.20±0.06	31.40±0.18
-	7.69 ±0.12	56.36±0.18	1.20±0.06	31.05±0.18
+	0.574	0.958	0.972	0.183
P				
Interactions				
0PHY×-NSP	7.71 ±0.21	56.22±0.31	1.28±0.11	31.62±0.32
0PHY×+NSP	7.93 ±0.21	56.57±0.31	1.17±0.11	31.42±0.32
450PHY×-NSP	7.79 ±0.21	56.20±0.31	1.15±0.11	31.04±0.32
450PHY×+NSP	7.57 ±0.21	56.34±0.31	1.15±0.11	30.74±0.32
900PHY×-NSP	7.86 ±0.21	56.70±0.31	1.18±0.11	31.54±0.32
900PHY×+NSP	7.57 ±0.21	56.17±0.31	1.28±0.11	31.00±0.32
P	0.429	0.353	0.589	0.863

on serum biochemical parameters. PHY supplementation did not significantly affect triglycerides, cholesterol, creatinine, phosphorus, AST, ALT, or ALP (P = 0.294, 0.184, 0.626, 0.134, 0.994, 0.907, and 0.090, respectively). NSP supplementation significantly affected ALP (P = 0.004 and 0.021, respectively), with ALP higher in the NSP-supplemented group. No significant NSP effects were detected for triglycerides, cholesterol, creatinine, phosphorus, AST, or ALT (P = 0.263, 0.375, 0.433, 0.441, 0.688, and 0.099, respectively). A significant PHY × NSP interaction was observed for creatinine (P = 0.002), whereas interactions for glucose, triglycerides, cholesterol, phosphorus, AST, ALT, and ALP were not significant (P = 0.796, 0.835, 0.843, 0.532, 0.499, 0.178, and 0.276, respectively). For creatinine, the interaction means showed that the 0 PHY + NSP and 450 PHY + NSP combinations differed from the 0 PHY + NSP and 450 PHY + NSP treatment combinations according to the reported superscripts.

Table 7 presents fecal viscosity measured on days 84 and 112. Neither PHY supplementation nor NSP supplementation significantly affected fecal viscosity on day 84 (P = 0.811 and 0.713, respectively) or day 112 (P = 0.858 and 0.856, respectively). The PHY × NSP interaction was also not significant on either day, with P = 0.832 on day 84 and P = 0.073 on day 112. Thus, fecal viscosity remained statistically comparable among the dietary treatments throughout the measured periods.

Discussion

The present study evaluated the individual and combined effects of dietary PHY and NSP-degrading enzyme complex in laying hens. Overall, the results indicate that supplementation had limited effects on productive performance and most egg-quality traits, whereas PHY produced clear improvements in selected eggshell characteristics and NSP

Table 7
Effects of dietary phytase (PHY) and non-starch polysaccharide (NSP)-degrading enzyme supplementation, alone and in combination, on fecal viscosity of laying hens at days 84 and 112.

Treatments	Day 84	Day 112
PHY Enzyme		
0	10.63±2.83	8.46±2.41
450	8.11±2.83	9.27±2.41
900	8.75±2.83	10.35±2.41
P	0.811	0.858
NSP		
-	9.78±2.31	9.10±1.97
+	8.55±2.31	9.62±1.97
P	0.713	0.856
Interactions		
0PHY×-NSP	11.25±4.01	3.75±3.41
0PHY×+NSP	10.00±4.01	13.16±3.41
450PHY×-NSP	7.46±4.01	9.17±3.41
450PHY×+NSP	8.75±4.01	9.38±3.41
900PHY×-NSP	10.63±4.01	14.38±3.41
900PHY×+NSP	11.25±4.01	3.75±3.41
p	0.832	0.073

PHY: phytase, NSP: non starch polysaccharide,0PHY: no phytase addition, 450PHY: 450 unit PHY contain, 900PHY:900 unit PHY contain, [a,b,c]: Values with different superscript in a column differ significantly at $P < 0.05$.

Table 6
Effects of dietary phytase (PHY) and non-starch polysaccharide (NSP)-degrading enzyme supplementation, alone and in combination, on serum biochemical parameters of laying hens.

Treatments	Glucose (mg/dL)	Triglyceride (mg/dL)	Cholesterol (mg/dL)	Creatinine (mg/dL)	Phosphorus (mg/dL)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
PHY Enzyme								
0	225.30±3.74[ab]	1201.00±121.88	103.52±8.76	0.10±0.01	5.95±0.48	171.90±5.26	2.30±0.37	378.60±54.22
450	216.80±3.74[b]	936.10±121.88	79.90±8.76	0.09±0.01	6.45±0.48	172.70±5.26	2.10±0.37	300.80±54.22
900	230.30±3.74[a]	998.90±121.88	91.28±8.76	0.10±0.01	7.35±0.48	172.50±5.26	2.10±0.37	477.50±54.22
P	0.053	0.294	0.184	0.626	0.134	0.994	0.907	0.090
NSP								
-	231.00±3.06[a]	964.73±99.52	86.99±7.15	0.10±0.01	6.36±0.39	173.60±4.29	2.53±0.30	308.40±44.27[b]
+	217.27±3.06[b]	1125.93±99.52	96.14±7.15	0.10±0.01	6.80±0.39	171.13±4.29	1.80±0.30	462.87±44.27[a]
P	0.004*	0.263	0.375	0.433	0.441	0.688	0.099	0.021*
Interactions								
0PHY×-NSP	230.80±5.30	1165.20±172.37	96.96±12.39	0.11±0.01[a,b,c]	6.17±0.68	175.60±7.44	2.60±0.52	229.20±76.67
0PHY×+NSP	219.80±5.30	1236.80±172.37	110.08±12.39	0.09±0.01[a,b,c]	5.72±0.68	168.20±7.44	2.00±0.52	528.00±76.67
450PHY×-NSP	223.00±5.30	867.60±172.37	73.12±12.39	0.08±0.01[a,b,c]	5.96±0.68	168.80±7.44	3.00±0.52	269.00±76.67
450PHY×+NSP	210.60±5.30	1004.60±172.37	86.68±12.39	0.11±0.01[a,b,c]	6.94±0.68	176.60±7.44	1.20±0.52	332.60±76.67
900PHY×-NSP	239.20±5.30	861.40±172.37	90.90±12.39	0.11±0.01[a,b,c]	6.96±0.68	176.40±7.44	2.00±0.52	427.00±76.67
900PHY×+NSP	221.40±5.30	1136.40±172.37	91.66±12.39	0.08±0.01[a,b,c]	7.74±0.68	168.60±7.44	2.20±0.52	528.00±76.67
P	0.796	0.835	0.843	0.002*	0.532	0.499	0.178	0.276

PHY: phytase, NSP: non starch polysaccharide, 0PHY: no phytase addition, 450PHY: 450 unit PHY contain, 900PHY:900 unit PHY contain.
[a,b,c] : Values with different superscript in a column differ significantly at $P < 0.05$.

supplementation influenced selected serum biochemical indices. The absence of broad performance responses may be related to the relatively adequate nutrient supply of the basal diet, particularly available phosphorus (AP), calcium, energy, and amino acids, which may have reduced the scope for further improvement through exogenous enzyme supplementation.

In the present study, neither PHY nor NSP supplementation significantly affected initial BW, final BW, BW change, or percentage BW change. Similarly, no significant PHY × NSP interactions were detected. The comparable initial BW among treatments indicates appropriate allocation of hens and provides a sound basis for interpreting subsequent treatment responses. The absence of differences in final BW and BW change suggests that neither enzyme supplementation substantially altered overall nutrient utilization sufficiently to affect body tissue deposition during the experimental period.

These findings are consistent with the broader literature showing that phytase responses in laying hens are highly dependent on the availability of phytate phosphorus, dietary AP, calcium concentration, phytase dose, and the nutritional adequacy of the basal diet (Medeiros-Ventura et al., 2023). Phytase hydrolyzes phytate and releases phosphorus while also reducing phytate-mediated binding of calcium, zinc, amino acids, and digestive enzymes (Pirzado et al., 2024). Consequently, its benefits are generally more pronounced when phytate-bound nutrients or available phosphorus are limiting. Previous work has similarly demonstrated that phytase may have little additional effect when dietary phosphorus is already sufficient, whereas clear responses occur under phosphorus-deficient conditions (Boling et al., 2000; Yu et al., 2023).

The lack of a significant effect of the NSP-degrading enzyme complex on body weight may reflect the substrate-dependent nature of these enzymes. Although xylanase, β-glucanase, cellulase, and hemicellulase can degrade different components of plant cell walls, their efficacy depends on the type, concentration, solubility, and accessibility of NSP in the diet (Morgan et al., 2022). NSP-degrading enzymes are expected to be particularly useful when soluble NSP increases digesta viscosity or when cell-wall encapsulation restricts nutrient accessibility (Bedford, 2022). Wheat and barley were included in the basal diet and provided potential substrates for the enzyme complex. However, the presence of these cereal ingredients does not necessarily indicate that the diet contained sufficient soluble NSP or generated sufficient intestinal viscosity to constrain nutrient utilization. Because total and soluble NSP concentrations were not determined, the magnitude of the dietary NSP challenge cannot be established. Thus, the absence of a significant body-weight response may indicate that NSP-related constraints were limited under the conditions of this experiment, although this explanation remains speculative.

In the current study, PHY supplementation did not significantly alter feed intake, FCR, egg production, egg weight, or egg mass. NSP supplementation similarly failed to significantly affect these performance traits, although FCR showed a numerical tendency toward a difference between NSP treatments ($P = 0.059$). Importantly, there was no significant PHY × NSP interaction for any performance variable. The absence of a production response to PHY agrees with studies in which phytase did not improve performance when phosphorus supply was adequate (Yu et al., 2023). A meta-analysis of laying-hen studies demonstrated that responses to phytase and non-phytate phosphorus depend strongly on dietary phosphorus concentration, and performance benefits are not necessarily observed when nutrient supply is already adequate (Ahmadi and Rodehutscord, 2012). Similarly, earlier experiments reported that phytase could maintain production under low-phosphorus conditions but produced limited additional benefits when phosphorus was sufficient (Jin et al., 2026). Therefore, the present lack of response in egg production, egg mass, FCR, and feed intake may indicate that the basal diet supplied sufficient available nutrients to support normal laying performance.

The numerical similarity among PHY treatments also suggests that increasing PHY from 450 to 900 FTU/kg did not produce a dose-dependent improvement in productivity. This is important because phytase efficacy is not necessarily linear with increasing enzyme dose; once a substantial proportion of available phytate has been hydrolyzed, additional enzyme may provide diminishing returns. Moreover, phytase effects can be modified by dietary calcium and phosphorus levels (Lim et al., 2003).

The absence of a significant response to NSP enzymes is likewise consistent with the concept that their efficacy is substrate-dependent. Xylanase and related enzymes can decrease intestinal viscosity and improve nutrient utilization, but measurable responses are more likely in diets containing substantial amounts of soluble NSP. In laying hens, increased soluble NSP has been associated with greater intestinal viscosity and a tendency toward reduced egg production, whereas xylanase can reduce intestinal viscosity and modify nutrient flow (Nguyen et al., 2022). Although the inclusion of wheat and barley provided potential substrates for the enzyme complex, the actual total and soluble NSP concentrations of the experimental diets were not measured. Consequently, it is unclear whether the basal diet imposed a sufficiently strong NSP-related constraint to produce measurable improvements in nutrient utilization or egg production. The absence of a significant response should therefore not be interpreted as evidence that the enzyme complex was intrinsically ineffective. Rather, the response may have been limited by the characteristics of the basal diet, the accessibility of its NSP substrates, or the relatively low contribution of NSP-related constraints to overall nutrient utilization. These possibilities cannot be distinguished in the present study because dietary NSP concentrations and intestinal nutrient-digestion processes were not directly characterized.

PHY supplementation significantly increased eggshell thickness ($P = 0.029$) and eggshell breaking strength ($P = 0.001$), whereas egg weight, Haugh unit, eggshell ratio, eggshell surface area, eggshell weight per unit area, and yolk height were unaffected. In contrast, NSP supplementation did not significantly influence any of the measured egg-quality variables.

The improvement in eggshell thickness and breaking strength with PHY supplementation may have biological significance, particularly given the magnitude of the difference in breaking strength. Phytate can bind phosphorus and interact with mineral cations, including calcium, thereby reducing their availability. Phytase hydrolyzes phytate and can increase the availability and retention of phosphorus and other minerals (Hatten et al., 2001). Because eggshell formation requires substantial calcium metabolism, improved mineral availability may support shell mineralization and mechanical strength. Nevertheless, calcium retention, shell mineral composition, and mineral deposition were not measured directly in the present study; therefore, this proposed mechanism remains a plausible explanation rather than a demonstrated causal pathway.

The present findings are particularly consistent with recent evidence. A study concluded that phytase supplementation improved eggshell thickness and breaking strength, particularly when phosphorus availability was limiting (De Lima et al., 2026). Similarly, Salehi et al. (2025) investigated phytase supplementation (300 FTU/kg) in Hy-Line W-36 laying hens aged 60–70 weeks, alongside calcium carbonate particle size and midnight feeding. The authors reported that phytase supplementation significantly affected eggshell percentage, feed conversion ratio, and tibial ash, calcium, and phosphorus concentrations. These findings support the importance of phytase in mineral utilization and eggshell and bone quality in laying hens. However, the outcomes differed between studies: Salehi et al. (2025) reported effects on eggshell percentage, whereas phytase supplementation in the present study significantly improved eggshell thickness and breaking strength without significantly affecting eggshell ratio or eggshell weight per unit area. These differences may reflect variation in basal dietary phosphorus and calcium supply, phytase dose, hen age, and other dietary management factors. Earlier research also reported improved eggshell characteristics and mineral retention following phytase supplementation, although

responses varied according to dietary calcium and phosphorus concentrations. Thus, the present improvement in shell strength and thickness supports the proposed role of phytase in improving mineral utilization rather than stimulating general productive performance.

Interestingly, the response was more apparent for eggshell thickness and breaking strength than eggshell ratio or eggshell weight per unit area. This suggests that phytase may have improved the structural organization or mineralization of the shell without necessarily increasing the total proportion of shell relative to egg weight (Hervo et al., 2023). Eggshell mechanical strength is influenced not only by shell quantity but also by thickness, mineral deposition, crystal structure, and organization of the shell layers (Rodríguez-Navarro et al., 2024). Therefore, an improvement in breaking strength without changes in several gross shell measurements is physiologically plausible.

The absence of a significant effect of NSP supplementation on eggshell traits indicates that NSP degradation did not substantially modify mineral deposition in the shell under the conditions of the present experiment. This may be because the NSP enzyme primarily improves nutrient accessibility and digesta characteristics rather than directly affecting phosphorus release from phytate (Moita and Kim, 2022). Therefore, its effects on shell mineralization may be secondary and dependent on the magnitude of improvement in mineral absorption.

The significant PHY × NSP interaction for eggshell ratio ($P = 0.010$) suggests that the effect of PHY on this trait was modified by the presence or absence of the NSP enzyme. In the absence of NSP supplementation, eggshell ratio increased numerically from 9.50% at 0 FTU/kg PHY to 9.62% at 450 FTU/kg and 9.96% at 900 FTU/kg. In contrast, with NSP supplementation, the corresponding values were 9.66%, 9.74%, and 9.59%, respectively. The interaction pattern indicates that shell ratio did not respond uniformly to increasing PHY concentrations across NSP treatments. This finding may reflect a treatment-specific response in eggshell ratio; however, the present results do not establish the underlying biological mechanism. Because the interaction was not accompanied by significant interactions for most other shell variables, this result should be interpreted cautiously rather than as evidence of a generalized synergistic effect.

In the current study, neither PHY nor NSP supplementation significantly affected Roche yolk color or the instrumental color parameters (L*, a*, and b*), and no significant PHY × NSP interactions were detected. These findings suggest that, under the dietary conditions of the present experiment, phytase-mediated improvements in phosphorus utilization and NSP-enzyme-mediated changes in carbohydrate digestion were insufficient to alter yolk pigmentation. Yolk color is primarily associated with the absorption and deposition of dietary pigments, particularly carotenoids, and carotenoid deposition can also be influenced by nutrient absorption and mineral status. In particular, the efficiency of fat absorption influences carotenoid deposition, while dietary calcium status may also affect carotenoid absorption (Vargas-Rodríguez et al., 2016). The absence of significant effects in the present study may therefore indicate that the experimental treatments did not substantially modify the availability or deposition of yolk pigments. This interpretation is consistent with evidence that phytase-related effects on yolk pigmentation may depend on interactions with other dietary factors, such as calcium and organic acids, rather than occurring independently (Vargas-Rodríguez et al., 2016).

PHY supplementation did not significantly affect triglycerides, cholesterol, creatinine, phosphorus, AST, ALT, or ALP. The lack of significant effects of PHY on serum phosphorus, despite the established role of phytase in increasing phytate phosphorus availability, may indicate that enhanced phosphorus availability was utilized to support physiological requirements rather than resulting in an appreciable increase in circulating phosphorus concentrations. Phytase supplementation has been associated with improved phosphorus availability, absorption, nutrient utilization, and bone mineralization, suggesting that changes in phosphorus utilization may occur without necessarily being reflected by marked changes in serum phosphorus concentrations (Selim et al., 2022).

The increase in ALP with NSP supplementation is potentially related to altered mineral metabolism, intestinal activity, or tissue turnover. ALP is associated with several physiological processes, including bone metabolism and intestinal function (Makris et al., 2023). However, because serum phosphorus was unaffected and eggshell traits did not differ significantly between NSP treatments, the precise biological basis for the ALP response cannot be established from the present data alone. Further measurements of calcium, bone mineralization, vitamin D metabolites, and intestinal mineral transport would be necessary to determine whether the ALP response represents a meaningful change in mineral metabolism.

A significant PHY × NSP interaction was detected for creatinine ($P = 0.002$), indicating that the effect of one enzyme depended on the inclusion of the other. The interaction pattern suggests that creatinine concentrations were not simply additive across the two enzyme factors. Because creatinine is influenced by muscle metabolism and renal clearance, however, the absence of significant PHY or NSP main effects and the lack of changes in BW suggest that this isolated interaction should be interpreted conservatively. It would be inappropriate to conclude that the enzyme combination improved renal function based solely on this result.

In the current study, neither PHY nor NSP supplementation significantly affected fecal viscosity at day 84 or day 112. Similarly, no significant PHY × NSP interaction was observed at either sampling point. The absence of a significant effect is somewhat unexpected for NSP-degrading enzymes because reduction of intestinal viscosity is one of the principal mechanisms attributed to xylanase and β-glucanase supplementation (Kim et al., 2024). However, the absence of a significant response does not necessarily demonstrate that the NSP-degrading enzyme complex lacked biological activity. One possible explanation is that the basal diet did not generate a sufficiently strong NSP challenge to produce a measurable reduction in viscosity or an improvement in productive performance. Although wheat and barley can contribute dietary NSP, their inclusion alone does not establish the concentration of soluble NSP or the viscosity-inducing potential of the complete diet. Total and soluble NSP concentrations were not measured in the present experiment, limiting our ability to determine whether the dietary substrate supply was sufficient to elicit a clear response to enzyme supplementation. Furthermore, fecal viscosity may not fully reflect viscosity within the upper gastrointestinal tract, where some of the effects of NSP-degrading enzymes on digesta properties and nutrient digestion may occur. Therefore, the lack of significant differences in fecal viscosity should be interpreted cautiously and does not exclude the possibility of other effects of the enzyme complex. Future studies should quantify total and soluble NSP in the experimental diets and, where possible, measure intestinal digesta viscosity and nutrient digestibility to establish whether the magnitude of the dietary NSP challenge influences the response to enzyme supplementation. Fecal and excreta characteristics in poultry are influenced by the physicochemical properties of intestinal digesta, particularly dietary non-starch polysaccharides (NSP), viscosity, and water availability. Increased digesta viscosity can impair nutrient digestion and alter the intestinal environment, while the amount and availability of water influence excreta moisture and consistency. Moreover, changes in the amount of undigested dietary material reaching the lower gastrointestinal tract may affect microbial activity and fermentation, thereby contributing to variation in excreta characteristics (Smits et al., 1997; Van der Hoeven-Hangoor et al., 2014).

Feed-processing conditions are an important consideration when evaluating the efficacy of exogenous enzymes in poultry diets. Iravani et al. (2024) highlighted the influence of conditioning temperature, conditioning time, moisture, and steam quality on feed quality and poultry performance. These factors are particularly relevant to pelleted diets, in which exposure to heat and moisture during conditioning may affect feed characteristics and potentially influence enzyme activity. In

the present study, the experimental diets were provided in mash form without steam conditioning or pelleting and were therefore not exposed to the elevated temperatures associated with these processing procedures. Nevertheless, enzyme activity was not measured in the finished diets or during storage; consequently, the actual recovery and stability of the supplemented phytase and NSP-degrading enzymes could not be confirmed. This limitation should be considered when interpreting the responses to enzyme supplementation.

An additional limitation of the present study is the pooling of repeated measurements of production performance and egg-quality traits into a single overall mean for each experimental unit before statistical analysis. Although this approach provided an estimate of the average response to dietary enzyme supplementation over the 112-day experimental period, it did not account for temporal variation or allow evaluation of treatment × time interactions. This is relevant in laying hens because responses to phytase and NSP-degrading enzymes may vary with age, stage of production, and changes in nutrient requirements during the laying cycle. Therefore, potential differences in treatment responses among individual sampling periods cannot be excluded. Future studies should analyze period-specific observations using repeated-measures mixed models that account for correlations among observations from the same experimental unit and evaluate treatment, time, and treatment × time effects.

Conclusion

Dietary PHY and NSP-degrading enzyme supplementation had limited effects on productive performance, yolk color, serum biochemical profile, and fecal viscosity of laying hens. However, PHY, particularly at 900 FTU/kg, improved eggshell thickness and breaking strength, while selected serum parameters responded to NSP supplementation and PHY × NSP combinations.

Ethics statement

The animal study was approved by Erciyes University's local ethics committee released the approval decision on 03.01.2024 and 24/14.

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Author Contribution Statement

Yusuf Konca: Contributed substantially to the study design and interpretation of findings; critically revised the manuscript. Asma Esse Yusuf: Contributed substantially to data acquisition and analysis; drafted and critically revised the manuscript. Qali Mohamed Yusuf: Contributed substantially to the experimental work and data acquisition; critically revised the manuscript. Muhammad Israr: Contributed substantially to data analysis and manuscript drafting and revision. Rifat Ullah Khan: Contributed substantially to the study and interpretation of findings; drafted and critically revised the manuscript. All authors: Reviewed and approved the final version of the manuscript and agree to be personally and publicly accountable for all aspects of the work, ensuring that any questions concerning its accuracy or integrity are appropriately investigated and resolved.

Disclosures

Authors declare no conflict of interest.

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Data availability

Data is available from the corresponding author upon reasonable request.

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