T.R.
ERCIYES UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF AGRICULTURAL SCIENCES AND TECHNOLOGIES

EFFECTS OF USING PHYTASE ENZYME WITH DIFFERENT ENZYME COMBINATIONS ON PERFORMANCE AND EGG QUALITY CHARACTERISTICS IN LAYING HENS

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Master's Thesis

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KAYSERİ

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November 2024
KAYSERİ

COMPLIANCE WITH SCIENTIFIC ETHICS

I declare that this thesis titled "**Effects of using Phytase Enzyme with Different Enzyme Combinations on Performance and Egg Quality Characteristics in Laying Hens''** is my own original work and has not been presented for an award of a degree or diploma in any other University or institution.

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SUITABILITY FOR INSTRUCTION GUIDE

The Master's thesis entitled "**Effects of Using Phytase Enzyme with Different Enzyme Combinations on Performance and Egg Quality Characteristics in Laying Hens**" was prepared by Erciyes University Graduate Thesis Proposal and Thesis Writing Directive.

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ÖZET

Bu çalışmada, düşük kullanılabilir fosfor (DP) içeren diyetlerde fitaz (FT) ile nişasta olmayan polisakkarit (NOP) enzimlerinin yumurtacı tavuklarda performans, yumurta kalitesi, kan bileşenleri ve dışkı viskozitesi üzerine etkileri araştırılmıştır. Toplam 98 adet 28. haftalık yaştaki yumurta tavukları (Hy-line kahverengi) 6 muamele grubuna 7 tekerrürlü ayrılmıştır. Muamele grupları şu şekildedir; 1: Düşük P diyeti %0,28 (DP), 2: DP+NSP enzimleri ilavesi, 3: DP+ fitaz enzimi 450 U/kg (450FT), 4: DP+fitaz enzimi 990 U/kg (900FT), 5: DP+NSP+450FT ve 6: DP+NSP+900FT. Yeme FT enzim ilavesi yumurtacı tavukların canlı ağırlık (CA), yemden yararlanma katsayısı (YDK), yumurta verimini (YV), yumurta ağırlığını (YA), yumurta kütlesini (YK), Haugh unit (HU), yumurta kabuğu ağırlığını (YKA), yumurta kabuğu yüzey alanını (YYA), albümin yüksekliğini (Hmm), yumurta sarısı kırmızılığı (a*) ve dışkı viskozite değerini önemli drecede etkilememiştir (P>0,05). Ancak rasyonlara 450 ve 900 FT enzim ilavesinin yem tüketimi (YT), yumurta kabuğu oranı (YKO), birim yumurta kabuğu ağırlığı, yumurta sarısı parlaklığı (L*) ve sarılık (b*) değerlerini bazı dönemlerde önemli derecede etkilemiş, ancak araştırmanın genelinde önemli bir etki yaratmamıştır. Yemde 900FT grubunda yumurta kabuğu kalınlığı (YKK), 2, 3, 4 ve 7. dönemlerde ve genel olarak 450FT enzim grubunda daha yüksek bulunmuştur (P<0,05). Yumurta kabuğu kırılma direnci (KKD), 900 FT enzim ilavesiyle artmıştır. Kan glikoz seviyesi, 900 FT grubunda, 450 FT grubundan daha yüksek bulunmuştur (P<0,05). Yeme NSP enzimleri ilavesi CA, YA, YYA, YKA, Hmm, Roche renk skalası, L* ve b* ile dışkı viskozitesi, YT, YDK, YK, YKK, a*, b* ve KKD değerlerini genel olarak etkilememiştir. NSP enzim ilavesi serum glikozunu artırdı ve ALP konsantrasyonlarını azalttı (P<0,05). Yemde FT ve NSP enzim kombinasyonları parametrelerin çoğunu etkilemedi, ancak YT, HU, yumurta kabuk oranını, b*, YKD ve serum kreatini etkilemiştir.

Anahtar Kelimeler: yumurta tavuğu, nişasta olmayan polisakkarit enzimi, fitaz, fosfor

EFFECTS OF USING PHYTASE ENZYME WITH DIFFERENT ENZYME COMBINATIONS ON PERFORMANCE AND EGG QUALITY CHARACTERISTICS IN LAYING HENS

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ABSTRACT

This study investigated the effect of phytase (PHY) with non-starch polysaccharide (NSP) enzyme addition to low available phosphorus (AP) diets on performance and egg quality, blood components, and feces viscosity in laying hens. A total of 98 laying hens of week 28 (Hy-line brown) were divided into 6 treatment groups with 7 replicates. The treatment groups were as follows; 1: Low AP diet 0.28% (LP), 2: LP+NSP enzyme, 3: low phosphorus+ P450 (LP450PHY), 4: LP+900 PHY, 5: LP+NSP+450 PHY and 6: LP+NSP+900 PHY. The PHY enzyme additions did not affect body weight, FCR, egg production (EP), egg weight (EW), egg mass (EM), HU, eggshell weight (ESW), eggshell surface area (ESA), albumen height (Hmm), egg yolk redness (a*) and feces viscosity value of laying hens ($P>0.05$). However, the effects of 450 and 900 PHY enzyme addition to laying hen diets on feed intake (FI), egg yield, eggshell ratio (ESR), eggshell per unit weight, and egg yolk lightness (L*), and yellowness (b*) values were found to be significant in some periods but did not significantly affect in the overall research period. Egg shell thickness (EST) in the 900 PHY group was higher 450PHY enzyme group in the periods 2, 3, 4, and 7 period and overall period ($P<0.05$). Eggshell breaking strength (SBS) increased with 900 PHY enzyme addition. Blood glucose level in the 900 PHY group was higher than the 450 PHY group ($P<0.05$). The addition of NSP enzyme enzymes in general did not affect BW, EW, ESW, ESA, Hmm, Roche color scale, L*, and b*, and feces viscosity, FI, FCR, EM, EST, a*, b*, and SBS. NSP enzyme addition increased serum glucose and decreased ALP concentrations ($P<0.05$). PHY and NSP enzyme combinations did not affect most of the parameters, however, FI, HU, egg hell traits, b*, SBS, and serum creatinine were affected.

Keywords: laying hens, non-starch polysaccharides, phytase, phosphorus

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ABBREVIATIONS

a*:	Egg yolk redness value
b*:	Egg yolk yellowness value
L*:	Egg yolk brightness value
HU:	Haugh unit
HMM:	Albumin height
CP:	Crude protein
DM:	Dry matter
AP:	Available phosphorous
NPP:	Non-phytate phosphorous
NSP:	Non starch polysaccharides
NP:	Normal phosphorus
LP:	Low phosphorus
C:	Control
PHY:	Phytase enzyme
FTY:	Fitz enzyme
BW:	Body weight
FI:	Feed intake
EP:	Egg production
FCR:	Feed conversion ratio
RCS:	Roche color scale values
EW:	Egg weight
EM:	Egg Mass
EY%	Egg yield percentage
ESW:	Eggshell weight
ESW%	Eggshell weight percentage
EST:	Eggshell thickness
ESR:	Eggshell ratio
ESA:	Eggshell surface area

ESUW:	Eggshell per unit weight
EF:	Egg force
ESB:	Egg shell-breaking strength
FV:	Feces viscosity
BP:	Blood parameters
SE:	Standard error
P:	Level of significance

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INTRODUCTION

Feed sources that compete with humans, such as wheat, corn, barley, soybean meal, and oil sources are used to nourish poultry. Using these resources efficiently is extremely important, both economically and in terms of reducing the amount of waste produced. Nutritionists focus on optimizing production by improving digestibility, accessibility of nutrients, and utilization of product for each part of feeds. These demands take into consideration total consumption of nutrients, preservation, and excretion. Monogastric animals have lower bioavailability of some nutrients due to lacking of some enzyme production in the body and a simple gastrointestinal system compared to ruminant animals.

Phosphorus (P) is one of the most important essential minerals in forming bones and teeth and maintaining mineral balance in the body phosphate compounds, the composition of phospholipids, and high-energy compounds such as ATP (Aksoy et al., 1981; Bello et al., 2020) and it has a crucial role in a variety of physiological processes in the body (Yang et al., 2024). Phosphorus in plant-based feed nutrients due to the existence of phytate which is a larger part of total P content. Phytate, also known as phytic acid, is an inedible nutrient and anti-nutritional component (Adeola and Cowieson, 2011). The total P in plant sources is bioavailable 1/3 part and utilized by poultry, however, 2/3 is excreted with feces. The phytase enzyme required to break down this bound P and utilize it in the body is not produced by the animal body. For this reason, in recent years, biotechnologically produced phytase enzymes have been used in poultry feeds and the utilization rate of phytin P in the diet is increasing (Rizwanuddin et al., 2023). Although there is sufficient total P in plant-origin feeds, poultry cannot benefit from the P in those sources, so inorganic P sources such as di-calcium phosphate, mono-calcium phosphate or organic P sources such as bone and meat-bone meal should be added to the diets. However, when these P are added to diets it may cause an increase in the diet cost. Furthermore, some

studies have discovered that phytase improves laying hen's utilization of phytate (Li et al., 2022; Iqbal et al., 2023; Yu et al., 2024). The P requirement of laying hens studied in many researches, but a new meta-analysis showed that a low concentration of P for laying hens and daily requirement of non-phytate p has been reduced to 2.2 g/kg feed (Rodehutscord et al., 2023). Long-term studies on laying hens discovered that 0.12 percent of Non-phytate P (NPN) was not enough to maintain the performance of egg production (Van Der Klis et al., 1997).

Many enzymes play a vital role in the digestion of carbohydrates, proteins, and fats in the body. The enzymes that break down nutrients must be secreted by the animal itself or by microorganisms living in the digestive system. Animals meet their energy requirements primarily from the carbohydrates found in the grains in the feed. Sugars found in the feed are divided into 3 groups: simple sugars, storage polysaccharides and cell wall polysaccharides. Cell wall polysaccharides are also known as non-starch polysaccharides (NSPs). NSPs are quite complex compounds in terms of physical and chemical structure. NSPs include cellulose, hemicellulose, pectins, and oligosaccharides (Sethy et al., 2015).

Non-starch polysaccharides (NSP) are principally non-α-glucan polysaccharides of the plant cell wall. They are a heterogeneous group of polysaccharides with varying degrees of water solubility, size, and structure. The water-insoluble fiber fraction include cellulose, xylanase, galactomannans, xyloglucans, and lignin, while the water-soluble fibers are the pectin's, arabinogalactans, arabinoxylans, and β-(1, 3) (1, 4)-D-glucanase (β-glucose) (Rodica et al., 2010). Considering it may improve the high molecular weight and digesta viscosity, the NSPs portion is especially concerning. this leads to increased instances of adhesive feces and low-quality waste, as well as negative consequences on the process of digestion and absorption (Munyaka et al., 2016).

Dietary enzymes are now widely used in laying hens to increase the efficiency of non-starch polysaccharides, gut microbiota, and nutritional digestibility (Slominski, 2011). It has been shown that giving poultry grains with high NSPs increases the viscosity of their gastrointestinal tracts, which impacts nutrient absorption, and reduces poultry productivity and digestion (Van Der Klis et al., 1993). In studies conducted on phytase enzyme, not only the phosphorus level in the feed was addressed, but also the utilization

rate of other nutrients was evaluated, and it was shown that significant productivity increases and economic benefits could be achieved. (Roque et al., 2023).

This study investigated the effect of phytase (PHY) with non-starch polysaccharide (NSP) in low available P (AP) diets on performance and egg quality, blood components, and feces viscosity in laying hens.

CHAPTER 1

GENERAL INFORMATION

Phosphorus is one of the essential elements for chickens. It is the second most needed mineral after calcium and increases the cost of the diet significantly (Konca and Bahtiyarca, 2002). Its 2/3 of the total found in plant-origin feeds is in the bound form of phytin P and cannot be used by poultry. The phytase enzyme required to break the phytin-phosphorus bond is produced by microorganisms. Since poultry do not produce phytase enzyme in their bodies, they cannot release bound phosphorus. To solve this problem, biotechnologically produced phytase enzymes have started to be used as feed additives in recent years. Thanks to phytase enzymes produced as feed additives, animals can produce satisfactory yields with lower P levels in the ration (Konca and Cufadar, 2004). NSP degrading enzymes i.e. β-glucanase α-galactosidase, xylanase, pectinase, and β-mannanase are commonly added to chicken diets as feed supplementation (Silversides and Bedford, 1999). It has been demonstrated the ability to improve the utilization of fibrous feed elements in poultry diets by reducing intestinal viscosity and enhancing nutrient-digestibility and performance (Józefiak et al. 2010; Mendes et al.,2013; Kiarie et al., 2014).

On the other hand, excessive P in feeds excreted through feces causes a significant environmental pollution problem. It blends with groundwater, impairing the quality of drinking water, and causes significant eutrophication when mixed with streams and lake waters (Ngatia and Taylor, 2019).

Oil seed and cereal grains contain an adequate quantity of P; although up to eight percent of that P is found as a phytic acid (Boling et al., 2000).

Monogastric animals, particularly poultry, have relatively limited access to the P present in the molecule. Through the formation of indigestible complexes, phytase decreases the

availability of important minerals like manganese, zinc, K, Ca, and proteins by binding with them (Nelson, 1967).

Phosphorus is necessary for laying chickens to produce eggs, repair tissue metabolites such as nucleotides, and phospholipids, and maintain the structure of skeletal (Mongin and Sauveur, 1979).

1.1. Importance of Phytase Enzyme and Phosphorus on Laying Hens

Calcium and P are required for strong bones and eggshells in animals and they must be obtained enough amount to the animals (Bello et al., 2020).

İn a study, hens fed 0,17% available P and the addition of Phytase (0, 300, or 1500 FTU/kg) and xylanase (0, 12 000 BXU/kg). The addition of 300 or 1500 FTU Phytase increased egg product, egg mass, and FCR. Phytase and xylanase inclusion to the laying hens diet did not affect egg weight average, egg weight, feed intake, yolk weight, Haugh unit, yolk color, eggshell weight, shell thickness, ileal ph., caeca ph., and shell strength, but xylanase addition with 300 FTU/kg my improve FCR, However, the absence of phytase, xylanase reduced dry matter and Ca digestibility (Taylor et al. 2018).

Another study was carried out on Hy-Line 21-week-olds were randomly assigned to one of ten diets with five levels of Nonphytate phosphorus (0.1 to 0.5% NPP) and two levels of phytase (0 and 300 U/kg feed). Feeding 0.1% NPP without supplemental phytase reduced egg production (hen-housed) by 8.1% over the entire study and 29.6% over the last 4 weeks, compared to other diets without supplemental phytase. Similarly, feed intake in hens fed 0.1% NPP without phytase declined 5.8% over 17 weeks and 13.0% over the last four weeks. Egg production and feed consumption remained constant despite the absence of phytase (Gordon and Roland, 1997).

Eltahan et al. (2023), noted that adding (1,000 FYT phytase/ kg to elder laying hen diets containing 0.20, 25, and 0.30% NP) and only improved hen daily egg production and EST (80^{th} week) 0.25 and 0.30 % AP +1000 FYT group but not 0.20+1000 PHY enzyme addition. The feed intake, egg weight, egg mass, FCR, albumen height, Haugh unit, yolk, and shell color were unaffected by the AP and PHY enzyme addition. Supplementation of 1000 FYT to the diet containing 0.25, and 0.30% of NPP increases the amount of serum albumin, HDL, P concentration, and FSH hormone in plasma. Researchers concluded that

PHY enzyme addition may increase the laying phase by promoting reproductive hormones in the elder laying hens.

Çabuk et al. (2004) tested 54-week-old Nick-Brown hens given a diet containing 3.0 or 4.5 g AP/kg without phytase, 4.5 g AP/kg, and 3.0 g aP/kg with and without phytase. Phytase supplementation to the control diet (4.5 g aP/kg) and the low 3.0 g aP/kg diet improved hen-day egg production. The addition of phytase to the control diet and low P diet increased daily feed consumption. However, eggshell weight, thickness, and strength did not change by AP and PHY enzyme addition.

The diet composition included two phytase levels (0 or 1000 FTU/kg diet) and three P sources (calcium and sodium phosphate, micro-granulated dicalcium phosphate, and triple super phosphate). Beyond the post-peak phase, triple super phosphate lowered eggshell quality and bird performance. The amount of P supplementation may have been reduced when phytase was added (Casartelli et al., 2005). Boling et al. (2000) fed hens with a corn-SBM basal diet with 0.10% AP, 3.8% Ca, and 17% CP were used and added with 0, 100, 200, 250, or 300 U of phytase/kg, 0.05% inorganic P (0.15% AP), and a positive control diet containing 0.45% AP. During the 20-28 week age, there were no significant variations in performance traits. By 28 wk of age, the 0.10% AP diet, with no supplementary phytase or P, resulted in considerably decreased egg production and body weight compared with all other dietary treatments. Feed consumption, FCR, and egg production were all reduced in hens given the 0.10% AP diet by 32 weeks of age. Hens on 0.15% AP, 0.45% AP, and 0.10% AP + phytase diets had mean daily AP intakes of 155, 474, and 103 mg, respectively.

Skřivan et al. (2018) examined diets with two calcium levels (35 or 42 g/kg) and three 6-phytase Opti Phos additions (0, 300, and 1500 FTU/kg), with a constant nonphytate P level (1.8 g/kg). A significant interaction between Ph and Ca was found in hen-day egg production. The diet with 35 g/kg Ca and 1500 FTU/kg Ph produced fewer hen-day eggs (84.1%) compared to other treatments (91.5-88.6%). Overdosing phytase negatively affected egg mass production and FCR. Both FYT additions reduced Haugh units. Higher Ca content improved shell thickness and breaking strength, while Ph addition increased shell percentage and breaking strength.

An experiment tested a new phytase in laying hens. Trial 1 used a 0.09% non-phytate P diet with 187.5 or 375 FYT phytase/kg feed. Trials 2 and 3 used a 0.1% NPP positive control and NC plus 187.5 FYT phytase/kg feed. In Trial 1, increasing phytase improved P digestibility linearly and quadratically. In Trial 2, phytase increased egg production, egg weight, and FCR while reducing broken eggs. In Trial 3, phytase significantly boosted egg production, egg weight, and feed intake, and reduced broken eggs. Phytase also increased bone Ca and P percentage and weight. No significant difference was found between the positive control and phytase treatment in Trials 2 and 3 (Zhai et al., 2022).

A previous study (Kim et al., 2017) assessed the effect of superdosing phytase on laying hens' performance and egg quality. Phytase was added to the control diet at 10,000 (SD10), 20,000 (SD20), or 30,000 (SD30) FTU/kg. The SD20 group produced more eggs than the PC, NC, and SD10 groups. There was no difference in daily egg production between the SD20 and SD30 groups. The SD30 group had higher egg production than the PC group, but no difference was found between the NC and SD10 groups. Egg mass, egg weight, FCR, and feed intake were unaffected by dietary treatments. Egg quality (yolk color, shell strength, shell color, and haugh unit) was not influenced by dietary supplements.

Abudabos (2012) studied the effects of feed-grade exogenous phytase enzyme on P excretion and utilization in laying hens. Hens were fed three levels of accessible P (0.2%, 0.3%, and 0.4%) with three amounts of phytase (0, 300, and 600 FTU/kg). The study found significant variation in total and water-soluble P excretion with 300 phytase units. Phytate P utilization was influenced by intestinal phytase activity and varied with age.

Another study explains the effect of phytase on production parameters and P deposition in laying hens. Hens were randomly assigned to one of five treatments, each with ten replicates (1) positive control (0.25% AP); (2) negative control 0.12% AP); (3) NC + 0.5% lactic acid; (4) NC + 450 FTU/kg Phytase; and (5) NC + 0.5% LA + 450 FTU/kg Phytase. Low amounts of AP (0.12%) had a negative impact on production metrics when compared to the 0.25% AP diet. LA alone increased only egg laying, egg mass, and feed intake. When PHY was added, the production parameters improved and were the same as the 0.25% AP diet. Furthermore, P, Ca, and Zn deposition in bone marrow increased, as increased the levels of iron in the yolk. When compared to diets with the same level of

AP, phytase supplementation reduced P excretion. There was no difference in performance between using Phytase alone or in combination with LA (Martinez Rojas et al., 2018).

Three levels of the phytase enzyme, two levels of AP, and two amounts of calcium were used in the experiment design. 3.7 And 4.0% Ca levels were kept constant during the experiment. Up to 55 weeks of age, the AP levels for the high and low treatments were 0.2 and 0.4%, respectively; after that, they were lowered to 0.11 and 0.22%. There were 0, 250, and 500 phytase units (FTU)/kg of feed for the enzyme phytase. The level of AP was probably adequate for the greatest result before Week 55. After this period, although feeding at lower AP levels resulted in decreased BW and egg production; However, enzyme supplementation compensated for inadequate AP. High AP and the highest amounts of phytase had a negative influence on BW, egg weight, and the FCR during this period. The Ca/AP ratio was significant; shell quality was most important with high or low amounts of both. With high Ca levels, enzyme supplementation was received for low AP levels and overreached for high AP levels. Low Ca levels decreased these effects (Scott et al., 1999).

In a long-term feeding trial of laying hens, Gao et al. (2013) observed the influence of a novel transgenic corn-derived phytase and two other commercial microbial phytase enzymes. A positive control diet is adequate in P (0.32% NPP), and a negative control diet is inadequate in P 0.10% NPP). The addition of transgenic corn-derived phytase PA, or PB enhanced laying productivity and egg quality considerably and behaved similarly in hens given the PC diet. Hens given each phytase source showed higher apparent P digestibility, tibia ash, and bone-breaking strength than NC-fed hens. As the phytase level grew from 500 to 5000 FTU/kg of diet. The results of this study show that adding transgenic corn-derived phytase to a P-deficient diet increases laying performance, egg quality, ileal P utilization, and bone mineralization, and that transgenic corn-derived phytase is as effective as two commercial microbial phytases when added to P-deficient diets for laying hens.

Another study found that supplementing low-protein diets with phytase improved laying hen performance, egg quality features, and blood biochemical parameters. Diets containing three levels of crude protein (CP), 150, 138, and 126 g/kg) and phytase 0 and

300 FTU/kg were designed and fed to hens in six repetitions. Reducing dietary crude protein decreased egg mass, egg production, and feed intake while increasing FCR. The dietary treatment had no notable impact on the yolk index, egg index, yolk color, egg weight, and shell thickness similar to the control group, decreasing dietary CP caused a greatly raised Haugh unit. In the second period of egg sampling, the Haugh unit extremely increased. Except for the heterophil count, there was no correlation between CP levels and phytase on blood parameters. The heterophil-to-lymphocyte ratio and phytase in lymphocytes are linked strongly with protein levels (Kashani et al., 2014).

Englmaierová et al. (2015) discovered the impact of different amounts of phytase 0, 150, 250, and 350 phytase units (FTU)/kg; F) generated by Aspergillus niger in feed of hens' diet reduced the amount of NPP (1.8 and 2.1 g/kg; NPP) on the hen performance, digestibility of calcium and P in the ileum, egg quality and representation of bacterial species in the ileum and caecum. A diet containing 2.1 g/kg of NPP, and 350 FTU/kg resulted in considerably lower egg weight and production. The addition of phytase at 350 FTU/kg to the diet containing 1.8 g/kg of Non-phytate P improved the shell quality to a level equal to eggs from hens fed a diet containing only 2.1 g/kg of NPP. The addition of phytase (350 FTU/kg) to the mixed feed containing 1.8 g/kg of NPP enhanced calcium and P digestibility in the ileum by 6.1% and 7.4%, respectively, while the increases were not statistically significant. Furthermore, compared to hens fed a diet without PHY enzyme addition, the prevalence of Lactobacillus spp. was higher in the ileum and caecum of hens fed the enriched diet.

Another research of laying Japanese quails fed a diet containing 0, 200, 400, or 600 phytase activity units, found that the dietary treatment of phytase increased egg quality and productivity performance, there was no difference in feed intake, egg production, egg weight, albumin weight, shell weight, specific gravity, and albumin percentage. The percent of P usage for egg mass increased from 0.13% of the available mineral in the feed to 463 FTU/kg supplementation (Lima et al., 2011).

A total of 192 white leghorn laying hens at 30 wk of age were fed different amounts of P 0, 500, and 1000 FTU/kg, 2.275, 3.25 % Ca, and 0.17 or 0.25% AP, Dietary phytase enhances feed consumption, tibia ash %, tibia ash weight, P digestibility, and FCR but had no beneficial effect on eggshell characteristics. However, adding phytase also causes

a lower excreta phosphorous percentage. Feed consumption was reduced when dietary available P was reduced (Musapuor et al., 2005).

Corn-soybean meal diets containing 0.35, 0.25, 0.15, or 0.10% NPP (NPP) were provided to hens. The addition of phytase A and B at 0.25, 0.15, and 0.10% at 250 to 300 units of phytase (FTU)/kg feed resulted in significant effects on various production parameters, including feed intake, feed conversion, and egg mass. The results showed that supplementing hens with phytase A or B did not affect feed intake when compared to the basal diet at 0.10% NPP. The feed conversion of birds fed 0.10% NPP without phytase was the least efficient. There were no statistically significant differences in egg production, egg weight, specific gravity, Haugh units, wet shell percentages, or dry yolk percentages (Jalal and Scheideler, 2001).

Bahtiyarca and Konca, (2017) noted that the quail was fed a diet added with different amounts of 0.33%, and 0.15% AP supplemented with 0, 500, 1000, 1500 or 2000 0f phytase units per kg of feed enhanced egg mass, feed intake, daily egg yield, and fat-free tibia weight. Also, PHY addition did not affect FCR, tibia ash percentage, body weight gain, abnormal egg %, eggshell parameters, and carcass weight, in groups fed with 1500 U of phytase their performance and bone variables were near and greater to the control group in some characteristics. During the last three days of the investigation, phytase levels were affected by reducing the eggshell weight of the collected eggs. The quails fed with 1500 and 2000 units of phytase breaking strength and shell thickness were notably decreased compared to the control group. However, the groups supplemented with phytase their manure ash% was considerably diminished compared to the control group.

A total of 960 ISA-brown laying hens at the age of 21-41wk were supplemented (3.0 0r 4.0%) of Ca, and (0.15 or 0.25%) of NPP and two amounts of microbial phytase (0 or 300 U/kg) on laying hens' diet. In the 2nd period, Phytase and nonphytate additions improved EP, and high level of non-phytate and low level of calcium enhanced FI. The addition of 0.25% of NPP improved FCR in week 10, and also increased fiber accessibility, retention, and excretion of P and iron. but reduced the amount of soft and broken shells in the 2nd period. During the first 10 wk., the inclusion of 0.15% NPP increased shell thickness and ESG but reduced HU. Hens that supplemented 3.0%of Ca diminished EST, eggshell-strength, during week 10 low Ca improved HU. The addition of 300 U of phytase

enzyme reduced the level of softshell and broken eggs but raised P retention caused by P excretion and egg yield (Lim et al., 2003).

A previous study by Um and Paik. (1999) reported that supplemented corn-soybean diet (0% or 1.4%,0.7%, of tricalcium phosphate), 500U/kg of microbial phytase was given a laying hens, the findings showed that adding 1.4% with phytase had a greater EP, feed intake which did not differ from one to another, there was a significant different in EW value between the groups. EW compared to the other groups, although the dietary consisting TCP with phytase had a greater feed intake than the other, there was no different in FCR value among the groups. According to the control group EST and specific gravity of the eggs were the greatest. And there was no difference in egg percentage, broken egg, and eggshell- strength between the groups. However, there was no difference in HU value between the treatment groups. Phytase enzyme addition groups had higher retention of P, magnesium, iron Zinc, and calcium. The control had the greatest excretion of these ingredients, P excretion was notably reduced in 0% or 0.7% TCP with 500U phytase groups. Finally, the addition of microbial phytase to the corn-soybean diet increases egg yield and decreases the level of tricalcium phosphate in the feed with no impact on egg quality and egg yield. Additionally, a significant decrease in P excretion was observed.

1.2. Importance of Non-starch Polysaccharide (NSP) Content in Poultry Nutrition

NSPs have been acknowledged as anti-nutritive due to their effect on digesta viscosity and their uses as a nutritive mixer in poultry diets (Morgan et al., 2021; Nguyen et al., 2021a). More lately, however, there has been an increase in interest in their ability to support gut health, promote gastrointestinal function, and serve as an energy source partly due to their prebiotic features, although recent improvements in NSP measurement techniques have enhanced accuracy for evaluating the dietary fiber content of feed ingredients and diets. The NSP varies substantially in composition, both among different feed ingredients and among batches of the same ingredient. Consequently, NSP displays variable characteristics in final feeds. NSP have traditionally been recognized as anti-nutritive, through their impact on digesta viscosity and as a nutrient diluent in poultry rations (Nguyen et al., 2021a).

It is commonly known that consuming hens feed ingredients containing non-starch polysaccharides (NSP), such as β-D glucanase, and arabinoxylans has a negative impact on nutrient absorption (Hartini and Choct, 2010; Kim et al., 2022).

β-d-Glucan (β-glucan) is an unbranched, water-soluble polysaccharide found in the endosperm and aleurone cell walls of higher plants and consists of both β-1,3- and β-1,4-linked d-glucose. It is mainly found in oats and barley, with smaller amounts in other cereals like corn, rice, rye, wheat, and sorghum. β-Glucanase enzymes that break down β-1,3 and β-1,4 glycoside linkages in β-glucans and they can be found in plants, fungi, animals, and bacteria. In practice, it can be produced via fermentation with various fungi and bacteria (e.g., *Saccharomyces cerevisia*) (Choi and Kim, 2023).

Beta-glucans in feed additives in poultry, have immunomodulation effects, which include upregulating cytokines and other immune cells to combat diseases. Additionally, beta-glucans can enhance the intestinal villi and crypts, leading to improved growth performance (Purnamasari et al., 2022).

Brufau et al. (1994) two experiments conducted to compare the performance of Leghorn hens fed a barley-based diet containing different levels of a Trichoderma viride enzyme supplement with those fed a corn-based diet. The addition of enzymes did not significantly affect EP, feed consumption, FCR, and EW of laying hens but improved egg size in the first experiment. In the 2nd study, water consumption was not significantly affected, but the ratio of water to feed consumption was significantly reduced with enzyme supplementation. So, researchers concluded that barley can replace corn in layer diets, and adding the enzyme to barley-based diets improves egg size and reduces water consumption relative to feed intake in young laying hens.

Habte.Michael. Habte.Tsion and Kumar, (2018) direct-fed cellulases as monocomponent additives are generally not very successful in improving Monogastric animals' performance, but in combination with other β-glucanase, they are said to have positive effects on the zoo technical parameters of both species as well as in ruminants.

Numerous investigations have demonstrated that NSP enzymes enhance the activities of gastrointestinal enzymes, improve the capacity for ingestion and digestion develop intestinal microbes, and contribute to animal growth. Additionally, NSP enzymes have

the potential to enhance the nutritional consumption of plant-based diets for both people and animals as well as by-products derived from those diets. However, the acceptance of the NSP enzymatic technology used by companies needs an important evaluation to fulfill the global demand for nutritional supplements (Tsion and Kumar, 2018).

The consumer group that adheres to non-viscous diets has contributed the majority of the increase in the usage of NSP enzymes in the last few years. The feed sector has accessed a diverse range of products. these include one-component enzymes, individual enzymes, mixes of many activities, and known as "complex 'enzymes that exhibit various uninterested actions in a single product (Aftab and Bedford, 2018).

de Lima et al. (2019) evaluated the effects of the enzymes endo-1,4-β-xylanase, endo-1,3(4)-β-glucanase, and 6-phytase on light layer hens. Five treatments were evaluated, including a positive control and two negative controls with a decrease in dietary nutrition by 100% and 125%. The result showed significant feed intake, egg production, egg mass, and feed conversion with enzyme supplementation. However, specific gravity, eggshell weight, thickness, and yolk color were unchanged, while the proportions of albumen and yolk were affected.

Polat. (2019) examined the impact of the dietary addition of multi-enzymes in a variety of grain-based diets on laying hen production performance and egg quality attributes. The control group had a basal diet with no supplements, whereas the treatment groups received a basal diet with 0.1% Enzyme-A (xylanase, ß glucanase, cellulose, - α amilase, and protease) and 0.05% Enzyme-B (xylanase, ß-glucanase). Throughout the trial, productivity and egg quality metrics were monitored weekly. Dietary multi-enzyme supplementation significantly altered the shape index, yolk index, and yolk color. Furthermore, adding two types of multi-enzymes to the diet did not affect egg specific gravity, EST, eggshell rate, albumen index, or Haugh units. The addition of multi-enzyme did not affect the performance parameters.

Mathlouthi et al. (2003) reported the effect of Quatrazyme HP, an enzyme preparation containing xylanase in two experiments with laying hens on wheat - barley or maize / soybean-based diet with or without 20 mg of Quatrazyme HP, which supplied 560 and 2800 IU of xylanase and b-glucanase/kg diet, hens fed enzyme supplementation increase FCRs. In the wheat/barley diet group, enzyme-fed hens gained weight, while those on

an unadded diet lost 103 g. The enzyme preparation did not affect egg weight, mass, and egg production. Enzyme addition reduced the viscosity grains, particularly in wheat, maize, soybean, and barley.

Nguyen et al. (2021) assessed how xylanase supplementation and the amount of soluble non-starch polysaccharides (SNSP) in the diet affected the laying hen production performance. The diets were designed with either 13.3 or 10.8 g/kg high or low NSP content, and they were supplemented with 0 or 12,000 BXU/kg exogenous xylanase. The results indicate that birds given a high sNSP diet without xylanase had lower egg production than those fed any other treatment, while birds fed a low sNSP diet with 12,000 BXU/kg xylanase gained more weight than those fed a high sNSP diet with 12,000 BXU/kg xylanase. Feed intake was decreased by xylanase supplementation from 29 to 32 weeks of age and enhanced yolk color. Supplementation with xylanase also increases the protein digestibility of birds fed a low sNSP diet was lower than the birds fed a high sNSP diet. Also, hens at 28 and 32 weeks of age fed high sNSP had increased shell reflectivity, and hens-fed treatments without xylanase supplementation decreased shell-breaking strength. However, haugh unite and albumen were diminished throughout the trial in all periods.

A total of 150 laying hens aged 40 weeks with phytase 675 IU/kg or interaction with NSP enzymes (cellulose 400, β-D glucanase,3200, and xylanase,6000 IU/KG). shows that laying hens fed a diet added with NSP and Phytase enzymes did not influence egg mass, EW, albumin index, haugh unit, EST, shell weight, feed intake, FCR, BW, intestinal parts, and gut morphology. The diet including PHY and NSP enzymes increased egg production. NSP enzymes addition groups diminished viscosity, intestinal PH, and E. coli (Narasimha et al., 2013).

Hartini and Choct (2010) discovered that adding millrun, wheat, barley-based, and barley enzymes to a laying hen's diet, shows that adding enzymes on barely diets decreases ceacel weight, jejunal digesta viscosity, and waste moisture but improves ileal digestion dry matter, and body weight. Hens that are fed with a diet containing wheat and barley increase viscosity. Higher viscosity led to decreased excreta moisture and DM digestion. Wheat inclusion had no significant effect on FI, intestinal digestibility, egg production, and FCR. Hens fed barley diminished caecal weight and live weight.

A study showed that adding 2500 U of xylanase and 250 U of beta-glucanase/kg, corn, or wheat to the male Ross broiler diet. Demonstrated that hens fed wheat had a greater weight gain, NDF, and reduced digestibility compared to the hens fed corn diet. Hens-fed enzymes increased FCR and body weight but decreased digesta jejunal viscosity to a high amount in the wheat diet than the corn-based diet. hens supplemented wheat-based dietary with xylanase and β-glucose had a greater starch digestibility than the chicks given the same diet without enzyme., However, feed intake was the same throughout out treatment groups (Munyaka et al., 2016).

Chen et al. (2021) reported that laying ducks fed 0%,15%,30%,45%, or 60% of barley with or without 1.5g/kg b-1,3-1,4-glucanase 15,00kg. indicated that the addition of barley to the diet had no impact on FCR, EM, FI, and egg yield. While adding glucanase increased the FCR, EP. The inclusion of glucanase enhances FI, amylase, and the activity of chymotrypsin in jejunal mucosa. Also had no impact of the activity of jejunal mucosa border enzymes. Supplementation of barley improved the amount of glucan in the duodenal digesta, however adding glucose to the diet containing barley decreased the level of glucan in the digesta, overall volatile fatty acid, and the amount of acetic acid in cecal composition. Both barley and beta-glucanase did not affect egg quality parameters except yolk color which led to lowered with improving barley addition.

In another study (Mourão et al., 2006), a total of 864 ISA brown laying hens aged 40 to 52 weeks were given a diet consisting of rye 19.6% and 15.1% of rye and alfalfa, with a commercial mixture of β- β-glucanase and β-1,4-xylanases showed that alfalfa diet decreases EM and weight gain. The supplementation of polysaccharides did not affect body weight, feed consumption, egg production, egg mass, egg weight. Dietary alfalfa inclusion improves yellowness value but does not decrease the cholesterol level in the egg yolk.

Enzymes that degrade NSP include decreased fecal moisture content, and ileal viscosity raised caecal butyrate ratio, and increased final BW in turkeys (Zduńczyk et al., 2020).

Yoruk and Bolat (2003) noted that feeding laying hens a diet adding 60% or 30% corn +30% barley, 0.05% of enzymes xylanase, amylase, and beta-glucanase were added individually to the groups given 50% barley. There are no significant variations found in feed intake, BW, EP, FCR, EST, HU, and EST during the experiment.

In another study, Yıldız et al. (2021) a total of 96 Japanese quail at 10 weeks of age were fed a diet supplemented with different levels of multi enzymes 100, 500,1000,1500, or 2000 mg/kg to control diet with and without enzymes. The multi enzymes did not affect feed consumption, FCR, EM, EW, BWG, and egg yield in the study but EST affected. No significant impact was observed on internal off-egg traits. The addition of 1000 mg/kg multi-enzyme to the hens' diet enhanced shell thickness. Also, serum parameters had no impact on cholesterol, glucose, and creatinine, but serum AST was improved at the 1000 mg/kg level. Dietary 2000 mg/kg multi-enzyme inclusion enhanced the level of Ca, globulin, total protein, P, and albumin.

Two experiments were conducted in laying hens and fed 20, 25, 30, or 35% dried cassava pulp with 0.10 and 0.15% of mixed enzymes (glucanase, xylanase, and cellulose). The addition of cassava pulp at 20 to 35% with mixed enzymes did not affect dry matter digestibility, however, reduced organic matter digestibility and nitrogen retention with dried cassava from 30 to 35% in dietary. Nutrient digestibility and retention were comparable for 0.10 and 0.15% of enzymes. In the second study, a total of 336 layers were fed a diet containing 20 to 30% of dried cassava pulp with 0.10 or 0.15% enzyme, shows that adding up 30% of dried cassava pulp with enzyme had an impact on feed consumption, EW, EM, egg yield, egg quality, FCR, and protein ratio (Khempaka et al., 2018).

Olgun et al. (2018) reported that laying hens given various amounts of wheat and two concentrations of enzyme (β-glucanase 100.000U/kg, xylanase 1500.00U/kg, cellulose 1000.000U/kg, α-amylase 160.000U/kg), The wheat-based diet had a negative impact on EST, egg mass, EW, FCR, egg yield, P and Ca levels in the tibia. However, the negative impact of wheat-based diets on tibia mineralization in laying hens was avoided by adding enzymes to the feed. The addition of enzymes was shown to improve the biomechanical quality of the tibia and plasma mineral concentration, while wheat-based feed seemed to lower these contents. The inclusion of enzymes in the diet leads to a reduction in the eggshell value.

Abdollahi et al. (2021)found that adding a diet consisting of corn-soybean with 0,3 or 6% wheat bran and sugar beet pulp including or not 100mg of xylanase/kg, birds given feed consisting of any amount of sugar-beet pulp decreased egg production, while the inclusion

of the enzyme enhanced egg yield. 6% of sugar beet pulp in addition to the basal diet had lowered EM. Except for the 3% wheat-bran feed, all fiber inclusion feeds had greatly lowered ADFI and improved enzyme supplementation. FCR, EW, egg quality, carcass, and serum quality were not affected by dietary additions, groups fed with 6% sugar beet pulp had higher ileum weight and 3% of SBP inclusion enhanced ileal dry matter digestibility. Also, diets containing 3%wheat-bran increased villus surface area, villus height to crypt depth ratio, and jejunal villus height. Enzyme additions boosted villus surface area, organic matter, protein digestibility, and dry matter. However, a diet consisting of 3% sugar beet and 3% WB with enzyme inclusion decreased cecal ph.

Kubiś et al. (2019) reported that enzyme inclusion to yellow lupine seeds diet vs. soybean and protease diets decreased the amount of AP to 50% and digestible amino acids to 5%. There was no significant difference in egg yield between the groups during the experiment. Throughout the trial, hens fed a protein source expressed the same FI and FCR. Hens fed with yellow lupine meal diets increase tibia ash amounts compared to phytase and soybean-adding groups. The addition of enzymes also increases EST, furthermore, hens fed with YLM addition developed a strong eggshell. The inclusion of phytase enhanced eggshell strengths, additionally enzyme inclusion effect on shell elastic deformation. During phase one the eggs of hens fed with phytase protease showed greater durability against elastic deformation. However, there was an interaction between the experimental parameters in the shell-breaking force.

Both NOP and FTY enzymes have been used in industry for a long time. Previously, enzymes used individually are now used as a cocktail enzyme feed additive by combining several of them. However, there are fewer studies on whether there is an interaction between these enzymes. Therefore, in this study, the effects of using FTY enzyme and NOP enzymes together to dissolve phytate P in laying hen diets were evaluated.

CHAPTER 2

METHOD AND MATERIAL

2.1. Animals and Experimental Design

The animal study was approved by Erciyes University's local ethics committee and released the approval decision on 03.01.2024 and 24/14. This study was conducted at Erciyes University Agricultural Research and Application Center (ERÜTAM).

A total of 98 laying hens (Hy-line brown) 28-weeks-old were used in the study. The laying hens were distributed in 6 experimental groups with 7 replicates and two laying hens in each cage. In the study conventional laying hen cages (cage dimensions 42- 40- 46) were used with front feeders (each cage space 42 cm) and two nipple drinkers inside of cages. The feeders were divided by wood to avoid consumption of the next cage's feeds.

Individual body weight (BW) was determined on the initial and last day of the experiment. Body weight gain (BWG) was calculated as the difference between the last and first BW of hens. The feed intake was determined at 14-day intervals on a group basis. Feed conversion ratio (FCR) was determined at 14-day intervals in group bases by using egg yield (%) and egg mass. Also, the eggs were collected to evaluate egg production, egg quality, and antioxidant activity at 14-day intervals. Blood samples were taken from each hen at the end of the experiment to determine the chemical component of blood.

2.2. Feed Materials and Enzyme Preparation

The individual feed materials (corn, soybean, barley, wheat, sunflower meal, etc, as indicated in Table 2.1.) required for the ration used in the study were purchased by a local feed manufacturing company. A layer hen ration that would meet the needs of the animals was computed and prepared as specified in the catalog. This prepared ration was divided

into 6 experimental groups, and phytase or NSP enzymes were mixed homogeneously according to their groups.

In the experiment, the feeds containing similar nutrient compositions with 0.28 % available phosphorus AP for laying hens were used. The AP content was adjusted to a lower AP (LP) than the laying hen (Hy-line brown) recommendation level (0.38% AP) in catalog. The feed composition is given in Table 2.1.

A commercial phytase enzyme (PHY) 450-unit phytase (FTU)/kg was added to the feed and a commercial non-starch polysaccharide (NSP) enzymes mixture (xylanase 145000 U/kg, β-glucanase 30000 U/kg, cellulose 64000 U/kg, and hemicellulose 120000 U/kg) was added to feeds.

Diets were as follows: 1) Control, low P (LP, 0.20 AP), 2) 3) LP+non starch polysaccharide (NSP) enzyme, 4) LP+450-unit phytase (PHY450), 5) LP+900 unit phytase (900 PHY), 6) LP+NSP+P450, and 7) LP+NSP+P900.

Fresh feeds were made every 28 days to prevent oxidation, microbial degradation, and a decrease in enzyme activity of the feeds. The experimental feed for each sub-group was filled with plastic cups and stored with a lid closed in a shaded area.

2.3. Animal Care and Nutrition

During the study, chickens were housed in egg-laying cages (dimensions 42x40x46 cm) with 3 floors and 54 cages of 2 hens on each cage made of normal wire. While being placed in the cage compartments, the treatment subgroups were distributed over the different regions of cages. Partitions are placed between feeders to prevent animals from consuming feed from the other side sections. In addition to that were applied with the electric lighting method 16 hours of lightning (between 05.00 am - 9.00 pm), and 8 hours of darkness, (between 9.00 pm - 05.00 am). Laying hens were placed in cages and fed for 16 weeks (112 days). After 10 days of adaptation with study rations, every two weeks were taken as a period.

Throughout the research, the data collection process was carried out every 14 days. The internal and external quality characteristics of 112 eggs, one egg from each hen, were sampled. Also, individual feed consumption was calculated at the end of each 14 days

according to animal numbers in a cage. Food and water were always available for laying hens. Nipple drinkers were used to fulfill the chickens' water requirements.

Table 2.1. Composition of experimental diets

Feed materials	0.28 %AP	0.28 %AP	0.28 %AP
	Quantity	Quantity	Quantity
Corn	100	100	100
Barley	250	250	250
Wheat	250	250	250
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
Mono calcium 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
NSP enzymes*	0.00	0.0 and 1.00*	0.0 and 1.00*
Total, kg	1000	1000	1000
Analyzed chemical composition*			
Dry matter, %	93,48	93,48	93,48
Crude Protein, %	17.00	17.00	17.00
Crude fat, %	5.74	5.74	5.74
Crude ash	11,60	11,24	11,77
ADF	0.66	0.66	0.66
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
Metabolic Energy Kcal ME/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, 20.0mg; Vitamin K, 3.0mg; Vitamin B1 (thiamin), 3.0mg; Vitamin B2 (riboflavin) 3.0 mg; Vitamin B6, 5.0mg; Vitamin B12, 0.15mg; Vitamin C, 50mg; niacin, 20.0mg; biotin, 0.05mg; calcium d-pantothenate, 6 mg; folic acid, 0.75mg; choline chloride, 150mg; manganese, 80.0 mg; iron, 60.0 mg; zinc, 60.0 mg; copper, 5.0 mg; iodine, 1.0mg; cobalt, 0.2mg; 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 450000 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).

2.4. Determination of Body Weight, Feed Consumption, and Feed Efficiency

Individual body weight (BW) of hens were determined by the precise balance (±1 g sensitivity) at the beginning and last day of the study and distributed similar BW, so, the BW difference was minimized. Body weight change was calculated using live weight values measured at the beginning and end of the study.

Feed consumptions were calculated by subtracting the weight of feeds left from feeds given based on subgroups for 2 weeks. Considering the number of dead animals in the calculation of the average feed consumption, the average daily feed consumption per animal was obtained by dividing the amount of feed consumed between two weighing by the number of days of feeding and the number of animals belonging to that group. Also, the feed conversion ratio (FCR) was determined by proportioning periodic feed consumption and egg mass. The FCR was calculated every 2 weeks.

2.5. Determination of Egg Yield, Egg Weight, and Internal Quality Characteristics

During the 14th, 28th, 42nd, 56th, 70th, 84th, 98th, and 112th days of the study total of 98 eggs (14 eggs from each group) were taken to determine egg external and internal quality traits. The egg weight and internal characteristics (albumin height, Haugh unit (HU), Roche color scale was determined by using Egg Analyzer® (Orka Food Tech. USA). Also, egg yolk color L* (brightness), a* (redness), and b* (yellowness) color measurements were made with a Minolta CR-400 (Minolto Co, USA) colorimeter (Figure 2.1 and 2.2).

2.6. Determination of Eggshell Properties and Breaking Strength

The broken eggshells (14 eggshells from each group) were washed with tap water and dried overnight at 70 °C in the oven. Then the eggshell weights (g) were determined by weighing on a precision scale sensitive to 0.1g. Eggshell percentage (%) was obtained by dividing the eggshell ratio: (eggshell weight/egg weight) × 100. Egg surface area (cm^2) was calculated with a formula reported by Carter (1975). Eggshell surface area was calculated by the following formula: Eggshell surface area (ESSA) = 3.9782 x egg weight $^{0.7056}$. Eggshell weight per unit area (mg/cm2) = eggshell weight/egg surface area.

Shell thickness (mm) was measured with the help of a digital micrometer sensitive to 0.01 mm in the sharp, blunt, and middle parts of the broken eggshells and then the shell thickness value was determined by taking the arithmetic mean of these three measurements.



Figure 2.1. Determination of egg internal traits



Figure 2.2. Determination of egg yolk color

The eggshell-breaking strength of the eggs was determined by the Instron universal testing machine (model 4301 Instron Ltd. UK) on d 28, 56, and 84. Uncracked eggs were allocated vertically to the machine probe 3 dimensions with a 1 cm distance two point analyses were done at room temperature with a constant speed of 10 mm/min. both compression and penetration tests were performed each on seven single eggs per sample, using a 100 N load cell. A 35 mm diameter plate was used as a compression device, while an 8 mm diameter probe was used as a puncture element. After breaking tests, the broken shells were cleaned, and thickness, including cuticle and membrane, was measured at the equator, sharp and blunt points by using a 550-501 digital micrometer (NSK, Japan). The results of thickness measurements were calculated for these three determinations per egg.

2.7. Feed Chemical Analysis

To determine the chemical composition of feed samples the feeds were ground 0,5 mm sieve and dried before analyses. Dry matter (DM), Feed's crude protein (CP), and crude ash (CA), analyses were performed according to AOAC (2013). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured using the Ankom 2000 automated fiber analyzer from Ankom Technology, USA.

2.7.1. Evaluation of Feed Dry Matter (DM)

The feed samples were dried at 70°C for 72 hours in an oven (Nüve, KD 400, Ankara, Türkiye) to stabilize their weight. The dry matter ratio of the feed samples was calculated by measuring the weight difference between the initial wet weight and the weight after drying.

2.7.2. Evaluation of Crude Protein (CP)

The nitrogen (N) content feed was measured using a Velp protein analyzer (Nitrogen NDA702, Italy). The nitrogen value was multiplied by the coefficient of 6.25 and the crude protein (CP) content was calculated. Samples, with an average weight of 0.400 mg, were prepared on specific aluminum sheets before being placed in the analyzer.

2.7.3. Evaluation of Crude Ash

A dried and ground 1 g sample of feed was weighed and burned for 5 hours at 550 °C in ash furnace. After cooling, the samples were reweighed again, and the percentage of weight difference between the two weights was calculated to measure the amount of raw ash present in the ash furnace (Protherm, RLF, 120/6, Türkiye).

2.8. Analysis of Feces Viscosity

The feces viscosity was measured by feces samples by viscosity meter (DV3T Rheometer, Brookfield, Middleboro, MA, USA). The chickens' manure was collected immediately after defecation utilizing nylons laid under the cages as wide as the cage and accumulated in the nylons. Then, the manure was stored in the refrigerator at +4 C until analyzed. On the day of analysis, the manure was removed, then 5 g was put into a 10 ml glass tube and 5 g of pure water was added as a solvent. Then, the tubes were

mixed in a vibrator for 4 min. After the manure and water were thoroughly dissolved, the tubes were placed in a centrifuge and rotated at 5000 rpm for 4 min to separate the water and solid parts. To ensure that the viscometer chamber remained at the same temperature, the laboratory-type water bath temperature was set to 41 C°, and water circulation was provided with a motor. After, 1 ml was taken from the water in the samples were removed from the centrifuge and the viscosity values were determined with spindles at 10 rpm speed.



Figure 2.3. Determination of feces viscosity

2.9. Analysis of Tibia and Feces Ash

Feces and bones were dried in the oven for 48 h 72 °C then samples were weighed and put in the ash furnace at 550 °C for 5 h. After cooling at room temperature in the desiccator the ash samples were re-weighed, and their ash content was calculated.

2.10. Determination of Blood Component

At the end of the experiment (112 d), 10 cc of blood was taken under the wing from a randomly selected laying hen from each subgroup was centrifuged and its serum was separated and stored in Eppendorf tubes at -80 ° C until it was analyzed. Serums were taken out from the freezer on the day of analysis were thawed and serum glucose, creatinine, triglyceride cholesterol, alanine aspartate aminotransferase (AST), alkaline

phosphatase (ALP), phosphorous and alanine aminotransferase (ALT) concentrations were determined in the auto analyzer with commercial kits for each parameter.

2.11. Statistical Analyses

Since the study was set up and carried out in a completely randomized design, variance analysis was performed using the multivariate ANOVA procedure in the SPSS statistics program Version 22. Duncan tests were used to determine differences between averages, $P < 0.05$ level was taken into consideration for the level of significance. All data were expressed as Means± standard errors.

CHAPTER 3

RESULTS

This study determined the effects of using phytase (PHY) enzyme with non-starch polysaccharide (NSP) enzyme combinations on performance, egg internal and external quality, feces viscosity, and bone and blood parameters in laying hens. The data were obtained and presented below.

3.1. Effect of Phytase (PHY) and Non-starch Polysaccharide (NSP) Enzymes in Low Phosphorus Diets on Performance Traits of Laying Hens

The effect of PHY and NSP enzymes addition in low phosphorus diets on final body weight (BW) and BW change of laying hens is shown in Table 3.1. The PHY enzyme and NSP enzymes addition and their combinations in low phosphorus diets did not affect final body weight, body weight gains, and changes in laying hens ($P>0.05$).

Effects of PHY and NSP enzymes in addition to low P diets on feed intake (FI) of laying hens are shown in Table 3.2. Adding 450PHY and 900 PHY enzyme additions caused a decrease in FI of hens compared to the C group ($P>0.05$), however, there were no significant differences in terms of FI in other periods and overall period. The addition of NSP enzymes to hen diets did not affect the FI of hens in all periods and overall periods except period 8. The addition of NSP enzymes increased the FI of hens compared to non-added group ($P<0.05$). The PHY and NSP enzyme interaction did not affect the FI of hens in most periods and overall periods except periods 6 and 8. In the 6th period, 0 PHY×+NSP, 450PHY×+NSP, and 900 PHY×-NSP groups FI were higher than 900 PHY×+ NSP group ($P<0.05$). In the 8th period, 450PHY×+NSP and 900 PHY×+NSP groups' FI were lower than those of 0PHY×+NSP, 450PHY×-NSP and 900 PHY×+NSP groups ($P>0.01$).

Table 3.1. Effect of phytase and non-starch polysaccharide enzymes in low phosphorus diets on body weight (BW) of laying hens

Treatments	Initial BW	Final BW	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
+	1987.62.01±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, P: probability, *:$p<0.05$.

Table 3.2. Effects of phytase and non-starch polysaccharide enzymes in low phosphorus diets on feed intake (FI) of laying hens

	Periods								
Treatments	1	2	3	4	5	6	7	8	Overall
PHY Enzyme									
0	113.47±1.60	119.32±1.32[a]	118.08±1.13	118.87±0.91	118.18±1.14	118.94±0.65	115.05±1.39	119.20±0.51	117.44±0.82
450	114.48±1.60	113.50±1.32[c]	116.53±1.13	116.88±0.91	116.66±1.14	118.89±0.65	115.15±1.39	118.02±0.51	115.35±0.82
900	112.71±1.60	115.23±1.32[b]	117.58±1.13	118.81±0.91	118.05±1.14	118.35±0.65	116.85±1.39	118.63±0.51	116.08±0.82
P	0.739	0.011*	0.615	0.229	0.584	0.784	0.591	0.271	0.205
NSP									
-	113.10±1.31	116.70±1.08	118.03±0.92	118.39±0.75	117.61±0.93	118.62±0.53	115.59±1.13	118.01±0.41[b]	116.56±0.67
+	114.01±1.31	115.33±1.08	116.77±0.92	117.98±0.75	117.64±0.93	118.83±0.53	115.78±1.13	119.22±0.41[a]	116.02±0.67
P	0.629	0.374	0.339	0.699	0.980	0.776		0.044*	0.575
Interactions									
0PHY×-NSP	113.00±2.27	119.11±1.87	116.32±1.60	118.25±1.29	117.70±1.61	118.71±0.93[ab]	112.43±1.96	118.55±0.72[ab]	116.67±1.16
0PHY×+NSP	113.94±2.27	119.52±1.87	119.85±1.60	119.49±1.29	118.65±1.61	119.16±0.93[a]	117.66±1.96	119.85±0.72[a]	118.20±1.16
450PHY×-NSP	114.43±2.27	114.37±1.87	115.34±1.60	117.14±1.29	116.54±1.61	117.98±0.93[ab]	116.58±1.96	119.77±0.72[a]	115.32±1.16
450PHY×+NSP	114.53±2.27	112.63±1.87	117.72±1.60	116.61±1.29	116.77±1.61	119.79±0.93[a]	113.73±1.96	116.28±0.72[b]	115.37±1.16
900PHY×-NSP	114.59±2.27	112.50±1.87	118.64±1.60	118.55±1.29	118.69±1.61	119.81±0.93[a]	118.32±1.96	119.36±0.72[ab]	116.07±1.16
900PHY×+NSP	110.84±2.27	117.95±1.87	116.52±1.60	119.07±1.29	117.41±1.61	116.90±0.93[b]	115.39±1.96	117.90±0.72[b]	116.10±1.16
P	0.550	0.157	0.187	0.789	0.782	0.043*	0.071	0.007**	0.761

[a,b,c]: Values with different superscript in a column differ significantly, P: probability, *:P<0.05, **:P<0.01.

Effects of PHY and NSP enzymes in low phosphorus diets on the FCR (FCR) of laying hens are presented in Table 3.3. The addition of the PHY enzyme did not affect the FCR of hens in all periods and the overall period ($P>0.05$). The NSP enzymes addition did not affect hens' FCR in most periods and overall periods, however, the added group FCR value was higher than the non-added group at period 8 ($P<0.01$). The PHY enzyme and NSP enzymes interaction did not affect of FCR values of laying hens in all experimental periods ($P>0.05$).

Table 3.3. Effects of phytase and non-starch polysaccharide enzymes (NSP) in low phosphorus diets on feed conversion ratio (FCR) of laying hens

	Periods								
Treatments	1	2	3	4	5	6	7	8	Overall
PHY Enzyme									
0	1.68±0.04	1.76±0.03	1.74±0.03	1.76±0.04	1.77±0.03	1.78±0.04	1.80±0.03	2.05±0.08	1.79±0.02
450	1.71±0.04	1.69±0.03	1.72±0.03	1.80±0.04	1.79±0.03	1.82±0.04	1.83±0.03	2.16±0.08	1.81±0.02
900	1.72±0.04	1.72±0.03	1.76±0.03	1.81±0.04	1.80±0.03	1.88±0.04	1.88±0.03	2.22±0.08	1.85±0.02
P	0.777	0.228	0.430	0.535	0.720	0.187	0.223	0.356	0.143
NSP									
-	1.70±0.03	1.74±0.02	1.74±0.02	1.79±0.03	1.78±0.03	1.81±0.03	1.82±0.02	1.98±0.07[b]	1.80±0.02
+	1.71±0.03	1.71±0.02	1.73±0.02	1.78±0.03	1.79±0.03	1.84±0.03	1.85±0.02	2.30±0.07[a]	1.84±0.02
P	0.776	0.407	0.673	0.845	0.670	0.455	0.464	0.002**	0.059
Interactions									
0PHY×-NSP	1.65±0.05	1.71±0.04	1.69±0.04	1.72±0.05	1.75±0.04	1.79±0.05	1.76±0.04	2.13±0.12	1.78±0.03
0PHY×+NSP	1.71±0.05	1.81±0.04	1.78±0.04	1.80±0.05	1.78±0.04	1.78±0.05	1.84±0.04	1.98±0.12	1.81±0.03
450PHY×-NSP	1.76±0.05	1.74±0.04	1.73±0.04	1.84±0.05	1.79±0.04	1.81±0.05	1.88±0.04	2.29±0.12	1.86±0.03
450PHY×-NSP	1.66±0.05	1.64±0.04	1.70±0.04	1.75±0.05	1.79±0.04	1.83±0.05	1.78±0.04	2.02±0.12	1.77±0.03
900PHY×-NSP	1.72±0.05	1.68±0.04	1.77±0.04	1.79±0.05	1.84±0.04	1.93±0.05	1.90±0.04	2.50±0.12	1.89±0.03
900PHY×+NSP	1.72±0.05	1.76±0.04	1.76±0.04	1.83±0.05	1.77±0.04	1.83±0.05	1.85±0.04	1.94±0.12	1.81±0.03
P	0.317	0.035	0.240	0.221	0.559	0.468	0.107	0.213	0.079

[a,b,c]: Values with different superscripts in a column differ significantly, P: probability, *:P<0.05, **:P<0.01

3.2. Effect of Phytase (PHY) and Non-starch Polysaccharide (NSP) Enzymes in Low Phosphorus Diets on Egg Production, Yield, Egg Weight and Mass of Laying Hens

Effects of PHY and NSP enzymes and low phosphorus addition diets on egg production and egg yield (%) of laying hens are given in Table 3.4 and Table 3.5. respectively. There were only significant values in period 7 ($P<0.05$) in egg production and egg yield, however, there was no significant difference between PHY enzyme addition groups in other periods and the overall period. In period 7, the PHY900 added group's egg production and egg yield values were lower than C and PHY 450 groups. The NSP enzymes addition decreased EP value in period 8 compared to the NSP enzymes non-added group ($P>0.01$) however, the PHY enzyme and NSP enzymes interactions did not influence EP values of laying hens in all periods and overall period ($P>0.05$).

The NSP inclusion did not affect of hens' EY% in all periods and overall periods except periods 2 and 8. In period 2, NSP enzymes addition increased EY% compared to the non-added group ($P<0.05$) but in contrast to this result NSP enzymes added group's EY value was lower than non-added groups in period 8 ($P<0.01$). However, the PHY enzyme and NSP enzymes interactions did not affect EY% value in all periods and overall period ($P>0.05$).

Table 3.4. Effects of phytase and non-starch polysaccharide enzymes in low phosphorus diets on egg production (EP) of laying hens

	Periods								
Treatments	1	2	3	4	5	6	7	8	Overall
PHY Enzyme									
0	27.64±0.20	27.93±0.04	27.93±0.04	27.21±0.26	27.43±0.19	27.36±0.30	26.93±0.28[a]	24.93±0.76	27.21±0.16
450	27.64±0.20	28.00±0.04	28.00±0.04	27.29±0.26	27.71±0.19	27.64±0.30	26.79±0.28[a]	24.07±0.76	27.29±0.16
900	27.57±0.20	28.00±0.04	28.00±0.04	27.43±0.26	27.43±0.19	26.57±0.30	26.64±0.28[b]	24.21±0.76	27.00±0.16
P	0.143	0.958	0.378	0.378	0.843	0.495	0.047	0.772	0.697
NSP									
-	27.48±0.16	27.95±0.03	27.95±0.03	27.33±0.21	27.52±0.16	27.24±0.25	26.90±0.23	25.76±0.62[a]	27.29±0.13
+	27.76±0.16	28.00±0.03	28.00±0.03	27.29±0.21	27.52±0.16	27.14±0.25	26.67±0.23	23.05±0.62[b]	27.05±0.13
P	0.222	0.324	0.324	0.876	1.000	0.788	0.466	0.004**	0.200
Interactions									
0PHY×-NSP	27.86±0.28	28.00±0.06	28.00±0.06	27.29±0.37	27.43±0.28	27.43±0.43	26.57±0.40	24.00±1.08	27.14±0.22
0PHY×NSP	27.43±0.28	27.86±0.06	27.86±0.06	27.14±0.37	27.43±0.28	27.29±0.43	27.29±0.40	25.86±1.08	27.29±0.22
450PHY×-NSP	27.57±0.28	28.00±0.06	28.00±0.06	26.86±0.37	27.86±0.28	27.86±0.43	26.57±0.40	23.29±1.08	27.14±0.22
450PHY×+NSP	27.71±0.28	28.00±0.06	28.00±0.06	27.71±0.37	27.57±0.28	27.43±0.43	27.00±0.40	24.86±1.08	27.43±0.22
900PHY×+NSP	27.86±0.28	28.00±0.06	28.00±0.06	27.71±0.37	27.29±0.28	26.14±0.43	26.86±0.40	21.86±1.08	26.86±0.22
900PHY×+NSP	27.29±0.28	28.00±0.06	28.00±0.06	27.14±0.37	27.57±0.28	27.00±0.43	26.43±0.40	26.57±1.08	27.14±0.22
P	0.416	0.378	0.378	0.158	0.589	0.303	0.334	0.284	0.934

[a,b,c]: Values with different superscripts in a column differ significantly, P: probability, *:P<0.05, **:P<0.01

Table 3.5. Effects of phytase and non-starch polysaccharide enzymes (NSP) in low phosphorus diets on egg yield (EY %) of laying hens

	Periods								
Treatments	1	2	3	4	5	6	7	8	Overall
PHY Enzyme									
0	98.73±0.71	97.45±0.79	99.75±0.15	97.20±0.94	97.96±0.70	97.71±1.09[a]	96.18±1.00	89.03±2.72	96.75±0.45
450	98.73±0.71	98.47±0.79	100.00±0.15	97.45±0.94	98.98±0.70	98.73±1.09[a]	95.66±1.00	85.97±2.72	96.75±0.45
900	98.47±0.71	99.49±0.79	100.00±0.15	97.96±0.94	97.96±0.70	94.90±1.09[b]	95.15±1.00	86.48±2.72	96.30±0.45
P	0.958	0.202	0.378	0.843	0.495	0.047*	0.772	0.697	0.723
NSP									
-	98.13±0.58	97.45±0.64[b]	99.83±0.12	97.62±0.77	98.30±0.57	97.28±0.89	96.09±0.82	92.01±2.22[a]	97.09±0.37
+	99.15±0.58	99.49±0.64[a]	100.00±0.12	97.45±0.77	98.30±0.57	96.94±0.89	95.24±0.82	82.31±2.22[b]	96.11±0.37
P	0.222	0.031*	0.324	0.876	1.000	0.787	0.466	0.004**	0.069
Interactions									
0PHY×-NSP	99.49±1.01	100.00±1.12	100.00±0.21	97.45±1.33	97.96±0.98	97.96±1.54	94.90±1.41	85.72±3.84	96.68±0.64
0PHY×NSP	97.96±1.01	94.90±1.12	99.49±0.21	96.94±1.33	97.96±0.98	97.45±1.54	97.45±1.41	92.35±3.84	96.81±0.64
450PHY×-NSP	98.47±1.01	98.47±1.12	100.00±0.21	95.92±1.33	99.49±0.98	99.49±1.54	94.90±1.41	83.17±3.84	96.24±0.64
450PHY×+NSP	98.98±1.01	98.47±1.12	100.00±0.21	98.98±1.33	98.47±0.98	97.96±1.54	96.43±1.41	88.78±3.84	97.26±0.64
900PHY×-NSP	99.49±1.01	100.00±1.12	100.00±0.21	98.98±1.33	97.45±0.98	93.37±1.54	95.92±1.41	78.06±3.84	95.41±0.64
900PHY×+NSP	97.45±1.01	98.98±1.12	100.00±0.21	96.94±1.33	98.47±0.98	96.43±1.54	94.39±1.41	94.90±3.84	97.19±0.64
P	0.416	0.066	0.378	0.158	0.589	0.303	0.334	0.284	0.438

[a,b,c]: Values with different superscript in a column differ significantly, P: probability, *:P<0.05, **:P<0.01

Effects of PHY and NSP enzymes in addition to low P diets EW of laying hens are given in Table 3.6. There was no significant difference in egg weight between PHY and NSP enzymes addition groups in all periods and overall period. Also, PHY and NSP enzymes addition interactions did not affect EW values in all periods and overall periods ($P>0.05$).

Effects of PHY and NSP enzymes in addition to low P diets EM of laying hens are shown in Table 3.7. There was no significant difference period in terms of EM between PHY enzyme addition groups in all periods and the overall ($P>0.05$). NSP enzymes addition increased EM value in the 8^{th} period compared to the non-adding group ($P<0.01$) and there was no significant in other periods. However, the PHY and NSP interactions did not influence the EM value during the experimental periods ($P>0.05$).

Table 3.6. Effects of phytase and non-starch polysaccharide enzymes in low phosphorus diets on egg weight (EW) of laying hens.

	Periods								
Treatments	1	2	3	4	5	6	7	8	Overall
PHY Enzyme									
0	68.71±1.04	69.81±0.94	68.30±0.95	69.87±1.01	68.37±1.11	68.34±0.95	66.56±0.84	65.66±0.85	68.20±0.62
450	68.28±1.04	68.27±0.94	67.93±0.95	67.10±1.01	66.07±1.11	66.38±0.95	65.78±0.84	65.00±0.85	66.86±0.62
900	66.97±1.04	67.54±0.94	66.80±0.95	67.18±1.01	67.30±1.11	66.92±0.95	65.70±0.84	64.14±0.85	66.58±0.62
P	0.472	0.225	0.510	0.095	0.347	0.328	0.724	0.449	0.151
NSP									
-	67.66±0.85	68.00±0.77	67.38±0.77	68.15±0.83	66.93±0.91	66.88±0.78	65.92±0.68	64.58±0.69	66.95±0.51
+	68.32±0.85	69.08±0.77	67.98±0.77	67.96±0.83	67.56±0.91	67.55±0.78	66.11±0.68	65.29±0.69	67.49±0.51
P	0.582	0.324	0.586	0.871	0.624	0.544	0.846	0.472	0.453
Interactions									
0PHY×-NSP	68.92±1.47	69.74±1.33	68.66±1.34	70.75±1.43	68.57±1.57	67.79±1.35	67.43±1.18	65.46±1.20	68.41±0.88
0PHY×+NSP	68.51±1.47	69.88±1.33	67.94±1.34	68.99±1.43	68.16±1.57	68.90±1.35	65.70±1.18	65.86±1.20	67.99±0.88
450PHY×-NSP	66.54±1.47	66.87±1.33	66.15±1.34	66.51±1.43	65.52±1.57	65.59±1.35	65.31±1.18	64.46±1.20	65.89±0.88
450PHY×+NSP	70.02±1.47	69.66±1.33	69.71±1.34	67.70±1.43	66.61±1.57	67.16±1.35	66.25±1.18	65.54±1.20	67.84±0.88
900PHY×-NSP	67.51±1.47	67.39±1.33	67.33±1.34	67.19±1.43	66.70±1.57	67.26±1.35	65.03±1.18	63.81±1.20	66.54±0.88
900PHY×+NSP	66.44±1.47	67.69±1.33	66.28±1.34	67.18±1.43	67.91±1.57	66.59±1.35	66.38±1.18	64.46±1.20	66.62±0.88
P	0.254	0.537	0.165	0.586	0.849	0.681	0.373	0.959	0.367

[a,b,c]: Values with different superscript in a column differ significantly, P: probability, *:P<0.05, **:P<0.01

Table 3.7. Effects of phytase and non-starch polysaccharide enzymes in low phosphorus diets on egg mass (EM) of laying hens

	Periods								
Treatments	1	2	3	4	5	6	7	8	Overall
PHY Enzyme									
0	67.85±1.23	67.99±1.06	68.14±1.01	67.93±1.27	66.95±1.15	66.75±1.17	64.03±1.01	58.42±1.94	66.01±0.82
450	67.42±1.23	67.22±1.06	67.93±1.01	65.40±1.27	65.37±1.15	65.46±1.17	62.93±1.01	55.81±1.94	64.69±0.82
900	65.95±1.23	67.18±1.06	66.80±1.01	65.79±1.27	65.96±1.15	63.51±1.17	62.46±1.01	55.58±1.94	64.15±0.82
P	0.525	0.831	0.609	0.330	0.621	0.158	0.539	0.523	0.270
NSP									
-	67.05±1.00	67.26±0.87	67.87±0.83	66.35±1.04	66.39±0.94	65.65±0.96	63.51±0.83	60.02±1.59[a]	65.51±0.67
+	67.10±1.00	67.66±0.87	67.38±0.83	66.40±1.04	65.79±0.94	64.83±0.96	62.76±0.83	53.19±1.59[b]	64.39±0.67
P	0.967	0.747	0.680	0.971	0.657	0.547	0.528	0.004**	0.242
Interactions									
0PHY×-NSP	68.57±1.73	69.74±1.50	68.66±1.43	68.92±1.80	67.16±1.63	66.38±1.66	64.03±1.44	56.09±2.75	66.19±1.16
0PHY×+NSP	67.12±1.73	66.24±1.50	67.61±1.43	66.94±1.80	66.74±1.63	67.12±1.66	64.03±1.44	60.75±2.75	65.82±1.16
450PHY×-NSP	65.53±1.73	65.85±1.50	66.15±1.43	63.78±1.80	65.18±1.63	65.26±1.66	61.99±1.44	53.51±2.75	63.41±1.16
450PHY×+NSP	69.32±1.73	68.58±1.50	69.71±1.43	67.03±1.80	65.55±1.63	65.67±1.66	63.86±1.44	58.10±2.75	65.98±1.16
900PHY×-NSP	67.21±1.73	67.39±1.50	67.33±1.43	66.50±1.80	65.04±1.63	62.85±1.66	62.27±1.44	49.97±2.75	63.57±1.16
900PHY×+NSP	64.70±1.73	66.96±1.50	66.28±1.43	65.07±1.80	66.88±1.63	64.17±1.66	62.65±1.44	61.20±2.75	64.74±1.16
P	0.165	0.130	0.192	0.293	0.782	0.962	0.789	0.391	0.451

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, P: probability, *:P<0.05, **:P<0.01

3.3. Effect of Phytase (PHY) and Non-starch Polysaccharide (NSP) Enzymes in Low Phosphorus Diets on Haugh Unit and Eggshell Properties of Laying Hens

Effects of PHY and NSP enzymes in addition to low P diets HU of laying hen's diet are given in Table 3.8 There was no significant difference among PHY enzyme addition groups in all periods and overall periods ($P>0.05$). NSP enzymes addition did not affect of hen's HU in all periods and overall periods except period 3. In the 3rd period, NSP enzymes addition decreased HU value compared to the non-adding group ($P<0.05$). During period 7, 450PHY×-NSP interaction had greater HU values than those of the 0PHY×-NSP, 450PHY×+NSP, 900PHY×-NSP and 900PHY×+NSP groups ($P<0.01$).

Effects of PHY and NSP enzymes addition to low P diets on ESW of laying hens are shown in Table 3.9. There was no significant difference in ESW value among PHY enzyme-added groups throughout the experimental periods ($P>0.05$). Also, NSP enzymes addition did not affect the ESW value of the laying hens in all periods and overall period ($P<0.05$). The PHY and NSP enzymes interactions did not affect the ESW of hens in all groups and periods except period 1 and the overall period. In the 1st period, the 450PHY×+NSP enzymes addition group had higher ESW value than those of 0PHY×-NSP, 0PHY+NSP, 450PHY×-NSP, 900PHY×-NSP and 900PHY×+NSP groups ($P<0.01$). In overall period Phytase and NSP enzymes interactions, the 450PHY×+NSP and 900PHY×-NSP were higher ESW values than those of 0PHY×-NSP, 450PHY×-NSP, and 900PHY×+NSP groups ($P<0.01$).

Table 3.8. Effects of phytase and non-starch polysaccharide enzymes in low phosphorus diets on Haugh unit (HU) of laying hens

	Periods								
Treatments	1	2	3	4	5	6	7	8	Overall
PHY Enzyme									
0	65.94±2.08	61.49±3.06	60.80±2.98	58.50±3.70	56.90±2.53	61.37±2.45	52.43±2.76	54.71±2.53	59.03±0.97
450	64.31±2.08	58.89±3.06	58.30±2.98	55.73±3.70	56.42±2.53	60.03±2.45	59.92±2.76	54.68±2.53	58.55±0.97
900	69.75±2.08	62.24±3.06	61.23±2.98	59.48±3.70	61.96±2.53	61.91±2.45	56.38±2.76	57.58±2.53	61.33±0.97
P	0.171	0.720	0.754	0.760	0.236	0.854	0.165	0.649	0.101
NSP									
-	68.22±1.70	63.69±2.50	64.08±2.43^{a}	54.91±3.02	60.12±2.07	62.55±2.00	54.74±2.25	55.63±2.06	60.51±0.79
+	65.12±1.70	58.06±2.50	56.14±2.43^{b}	60.89±3.02	56.73±2.07	59.66±2.00	57.75±2.25	55.68±2.06	58.76±0.79
P	0.200	0.115	0.024*	0.166	0.249	0.308	0.347	0.986	0.121
Interactions									
0PHY×-NSP	69.31±2.94	64.18±4.33	66.56±4.21	54.75±5.24	60.41±3.58	61.10±3.46	44.88±3.90^{d}	57.25±3.57	59.81±1.37
0PHY×+NSP	62.58±2.94	58.81±4.33	55.04±4.21	62.26±5.24	53.38±3.58	61.64±3.46	59.99±3.90^{ab}	52.17±3.57	58.24±1.37
450PHY×-NSP	63.05±2.94	60.81±4.33	66.04±4.21	58.26±5.24	58.92±3.58	62.78±3.46	62.12±3.90^{a}	52.19±3.57	60.55±1.37
450PHY×+NSP	65.57±2.94	56.96±4.33	50.55±4.21	53.20±5.24	53.91±3.58	57.27±3.46	57.72±3.90^{b}	57.16±3.57	56.54±1.37
900PHY×-NSP	72.30±2.94	66.07±4.33	59.64±4.21	51.73±5.24	61.04±3.58	63.78±3.46	57.21±3.90^{b}	57.44±3.57	61.16±1.37
900PHY×+NSP	67.20±2.94	58.41±4.33	6,2.83±4.21	67.22±5.24	62.89±3.58	60.05±3.46	55.55±3.90^{bc}	57.71±3.57	61.49±1.37
P	0.250	0.906	0.071	0.148	0.433	0.669	0.030*	0.376	0.287

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, P: probability, *:P<0.05, **:P<0.01

Table 3.9. Effects of phytase and non-starch polysaccharide enzymes in low phosphorus diets on eggshell weight (ESW) of laying hens

	Periods								
Treatments	1	2	3	4	5	6	7	8	Overall
PHY Enzyme									
0	6.64±0.11	6.73±0.10	6.60±0.11	6.70±0.11	6.50±0.10	6.40±0.10	6.39±0.10	6.27±0.11	6.53±0.07
450	6.68±0.11	6.64±0.10	6.53±0.11	6.59±0.11	6.36±0.10	6.45±0.10	6.21±0.10	6.31±0.11	6.47±0.07
900	6.63±0.11	6.59±0.10	6.75±0.11	6.52±0.11	6.45±0.10	6.35±0.10	6.33±0.10	6.34±0.11	6.50±0.07
P	0.941	0.620	0.342	0.493	0.587	0.786	0.427	0.912	0.813
NSP									
-	6.65±0.09	6.59±0.08	6.56±0.09	6.62±0.09	6.38±0.08	6.40±0.08	6.39±0.08	6.27±0.09	6.49±0.06
+	6.66±0.09	6.71±0.08	6.70±0.09	6.58±0.09	6.49±0.08	6.40±0.08	6.24±0.08	6.34±0.09	6.51±0.06
P	0.925	0.307	0.257	0.700	0.339	0.952	0.203	0.568	0.735
Interactions									
0PHY×-NSP	6.66±0.15^{c}	6.63±0.15	6.38±0.15	6.76±0.15	6.41±0.14	6.41±0.14	6.45±0.14	6.26±0.16	6.51±0.09^{b}
0PHY×+NSP	6.62±0.15^{d}	6.83±0.15	6.82±0.15	6.64±0.15	6.59±0.14	6.38±0.14	6.34±0.14	6.29±0.16	6.56±0.09^{ab}
450PHY×-NSP	6.47±0.15^{e}	6.44±0.15	6.41±0.15	6.48±0.15	6.19±0.14	6.34±0.14	6.19±0.14	6.14±0.16	6.34±0.09^{c}
450PHY×+NSP	6.89±0.15^{a}	6.83±0.15	6.64±0.15	6.69±0.15	6.54±0.14	6.56±0.14	6.24±0.14	6.48±0.16	6.60±0.09^{a}
900PHY×-NSP	6.81±0.15^{b}	6.70±0.15	6.87±0.15	6.64±0.15	6.55±0.14	6.45±0.14	6.51±0.14	6.41±0.16	6.61±0.09^{a}
900PHY×+NSP	6.46±0.15^{e}	6.48±0.15	6.63±0.15	6.40±0.15	6.35±0.14	6.25±0.14	6.15±0.14	6.26±0.16	6.39±0.09^{c}
P	0.048*	0.106	0.079	0.307	0.127	0.355	0.338	0.292	0.041*

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, P: probability, *:P<0.05, **:P<0.01

Effects of PHY and NSP enzymes addition to low P diets on eggshell ratio (%) of laying hens are shown in Table 3.10. There was no significant difference between PHY enzyme addition groups in all periods and overall periods except period 3 and overall. In the 3rd period, the 0PHY and 450PHY enzyme group's addition had lower eggshell ratio values than those in the 900PHY group ($P<0.05$). Phytase and NSP enzymes interactions did not affect the eggshell ratio of hens except in periods 3, 5, and overall. In period 3, and overall period 900PHY×-NSP group's ESW% value was higher than those in 0PHY×-NSP, 0PHY+NSP, 450PHY×-NSP, 450PHY×+NSP 900PHY×+NSP. In the 5th period, 450PHY×+NSP and 900PHY×+NSP group's ESW% values were higher than other groups ($P<0.05$).

Effects of PHY and NSP enzymes addition to low P diets eggshell surface area of laying hens are shown in Table 3.11. Phytase enzyme inclusion did not affect ESA value in all periods and overall periods ($P<0.05$). However, NSP and PHY enzyme interactions had no significant change on laying hen's ESA in all periods and overall period ($P<0.05$).

Table 3.10. Effects of phytase and non-starch polysaccharide enzymes in low phosphorus diets on eggshell ratio (%) of laying hens

	Periods								
Treatments	1	2	3	4	5	6	7	8	Overall
PHY Enzyme									
0	9.66±0.08	9.68±0.13	9.68±0.13^{b}	9.61±0.13	9.52±0.11	9.36±0.13	9.63±0.14	9.57±0.16	9.58±0.07
450	9.78±0.08	9.74±0.13	9.62±0.13^{b}	9.83±0.13	9.61±0.11	9.73±0.13	9.46±0.14	9.70±0.16	9.68±0.07
900	9.90±0.08	9.76±0.13	10.12±0.13^{a}	9.73±0.13	9.64±0.11	9.52±0.13	9.66±0.14	9.93±0.16	9.78±0.07
P	0.096	0.891	0.011*	0.485	0.710	0.135	0.543	0.271	0.110
NSP									
-	9.82±0.07	9.72±0.11	9.75±0.10	9.73±0.10	9.55±0.09	9.60±0.11	9.71±0.11	9.74±0.13	9.70±0.05
+	9.74±0.07	9.73±0.11	9.86±0.10	9.72±0.10	9.63±0.09	9.48±0.11	9.46±0.11	9.72±0.13	9.66±0.05
P	0.352	0.963	0.424	0.923	0.532	0.425	0.123	0.937	0.688
Interactions									
0PHY×-NSP	9.66±0.11	9.54±0.19	9.31±0.18^{e}	9.56±0.18	9.37±0.15^{d}	9.46±0.19	9.59±0.20	9.59±0.22	9.50±0.09^{f}
0PHY×+NSP	9.66±0.11	9.81±0.19	10.04±0.18^{b}	9.66±0.18	9.66±0.15^{b}	9.25±0.19	9.68±0.20	9.54±0.22	9.66±0.09^{c}
450PHY×-NSP	9.71±0.11	9.66±0.19	9.69±0.18^{c}	9.74±0.18	9.44±0.15^{c}	9.71±0.19	9.49±0.20	9.51±0.22	9.62±0.09^{d}
450PHY×+NSP	9.84±0.11	9.81±0.19	9.55±0.18^{d}	9.91±0.18	9.79±0.15^{a}	9.76±0.19	9.42±0.20	9.89±0.22	9.74±0.09^{b}
900PHY×-NSP	10.09±0.11	9.96±0.19	10.24±0.18^{a}	9.89±0.18	9.84±0.15^{a}	9.62±0.19	10.04±0.20	10.11±0.22	9.96±0.09^{a}
900PHY×+NSP	9.71±0.11	9.57±0.19	10.00±0.18^{b}	9.58±0.18	9.44±0.15c	9.42±0.19	9.27±0.20	9.74±0.22	9.59±0.09^{d}
P	0.073	0.197	0.013*	0.370	0.027*	0.726	0.072	0.230	0.010*

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, P: probability, *:P<0.05, **:P<0.01

Table 3.11. Effects of phytase and non-starch polysaccharide enzymes in low phosphorus diets on eggshell surface area (ESA) of laying hens

	Periods								
Treatments	1	2	3	4	5	6	7	8	Overall
PHY Enzyme									
0	78.66±0.84	79.54±0.76	78.32±0.77	79.57±0.82	78.38±0.89	78.36±0.78	76.91±0.68	76.18±0.70	78.24±0.50
450	78.28±0.84	78.29±0.76	78.01±0.77	77.33±0.82	76.50±0.89	76.74±0.78	76.27±0.68	75.64±0.70	77.13±0.50
900	77.21±0.84	77.69±0.76	77.09±0.77	77.40±0.82	77.45±0.89	77.19±0.78	76.20±0.68	74.90±0.70	76.89±0.50
P	0.454	0.221	0.505	0.095	0.333	0.319	0.717	0.436	0.137
NSP									
-	77.79±0.69	78.07±0.62	77.58±0.63	78.18±0.67	77.21±0.73	77.17±0.63	76.38±0.56	75.28±0.57	77.21±0.41
+	78.31±0.69	78.94±0.62	78.04±0.63	78.02±0.67	77.67±0.73	77.69±0.63	76.54±0.56	75.87±0.57	77.63±0.41
P	0.589	0.328	0.607	0.862	0.655	0.563	0.840	0.464	0.464
Interactions									
0PHY×-NSP	78.82±1.19	79.48±1.08	78.62±1.09	80.29±1.16	78.56±1.26	77.92±1.10	77.61±0.96	76.01±0.99	78.42±0.71
0PHY×+NSP	78.49±1.19	79.59±1.08	78.01±1.09	78.85±1.16	78.19±1.26	78.79±1.10	76.21±0.96	76.34±0.99	78.06±0.71
450PHY×-NSP	76.90±1.19	77.16±1.08	76.59±1.09	76.87±1.16	76.07±1.26	76.12±1.10	75.88±0.96	75.20±0.99	76.35±0.71
450PHY×+NSP	79.67±1.19	79.42±1.08	79.43±1.09	77.80±1.16	76.93±1.26	77.36±1.10	76.66±0.96	76.07±0.99	77.92±0.71
900PHY×-NSP	77.63±1.19	77.57±1.08	77.52±1.09	77.40±1.16	77.01±1.26	77.46±1.10	75.65±0.96	74.62±0.99	76.86±0.71
900PHY×+NSP	76.78±1.19	77.80±1.08	76.66±1.09	77.41±1.16	77.90±1.26	76.91±1.10	76.75±0.96	75.19±0.99	76.92±0.71
P	0.266	0.538	0.170	0.589	0.848	0.692	0.374	0.963	0.266

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, P: probability, *:P<0.05, **:P<0.01

Effects of PHY and NSP enzymes addition to low P diets on eggshell per unit weight (ESUW) are shown in Table 3.12. The PHY enzyme addition did not affect groups in all periods and overall period except period 2. Control and 450PHY had significantly increased on laying hen's ESUW value in the 2nd period (P<0.05. The NSP enzymes addition did not affect ESUW values in all periods and overall period (P>0.05). In the 2nd period 0PHY×-NSP and 900PHY×+NSP group's ESUW were higher than those of the group (P<0.001). In period 5, 900PHY+SP added groups ESU was higher than all groups; Also, 0PHY×-NSP, 0PHY+NSP, 450PHY×-NSP, ESUW was higher than 450PHY×+NSP and 900PHY-NSP groups (P<0.05). In period 6, 0PHY×+NSP group ESUW was higher than all groups except 900PHY+NSP group and, 0PHY×-NSP was higher than 450PHY×-NSP, 450PHY×+NSP and 900PHY×-NSP groups (P<0.05).

Table 3.12. Effects of phytase and non-starch polysaccharide enzymes in low phosphorus diets on eggshell per unit weight (mg) of laying hens

	Periods								
Treatments	1	2	3	4	5	6	7	8	Overall
PHY Enzyme									
0	11.90±0.10	11.91±0.16	11.93±0.15^{a}	11.96±0.15	12.10±0.13	12.31±0.17	12.09±0.17	12.21±0.20	12.05±0.08
450	11.74±0.10	11.83±0.16	12.00±0.15^{a}	11.78±0.15	12.06±0.13	11.94±0.17	12.31±0.17	12.11±0.20	11.98±0.08
900	11.69±0.10	11.83±0.16	11.46±0.15^{b}	11.92±0.15	12.04±0.13	12.21±0.17	12.11±0.17	11.88±0.20	11.90±0.08
P	0.332	0.923	0.026*	0.669	0.947	0.272	0.600	0.473	0.429
NSP									
-	11.74±0.08	11.90±0.13	11.89±0.12	11.83±0.12	12.13±0.11	12.09±0.14	12.00±0.14	12.08±0.16	11.96±0.07
+	11.81±0.08	11.82±0.13	11.71±0.12	11.94±0.12	12.00±0.11	12.21±0.14	12.34±0.14	12.06±0.16	11.98±0.07
P	0.537	0.666	0.316	0.549	0.388	0.527	0.087	0.917	0.840
Interactions									
0PHY×-NSP	11.88±0.15	12.07±0.22^{a}	12.36±0.21	11.92±0.21^{b}	12.29±0.18	12.18±0.24^{b}	12.06±0.24	12.19±0.28	12.12±0.11
0PHY×+NSP	11.91±0.15	11.74±0.22^{c}	11.49±0.21	11.99±0.21^{b}	11.91±0.18	12.44±0.24^{a}	12.11±0.24	12.24±0.28	11.97±0.11
450PHY×-NSP	11.90±0.15	11.99±0.22^{b}	11.96±0.21	11.89±0.21^{b}	12.32±0.18	12.03±0.24^{c}	12.29±0.24	12.39±0.28	12.10±0.11
450PHY×+NSP	11.59±0.15	11.66±0.22^{f}	12.05±0.21	11.66±0.21^{c}	11.81±0.18	11.84±0.24^{d}	12.34±0.24	11.84±0.28	11.86±0.11
900PHY×-NSP	11.44±0.15	11.62±0.22^{f}	11.34±0.21	11.69±0.21^{c}	11.79±0.18	12.06±0.24^{c}	11.66±0.24	11.66±0.28	11.67±0.11
900PHY×+NSP	11.94±0.15	12.04±0.22^{a}	11.59±0.21	12.15±0.21^{a}	12.29±0.18	12.36±0.24^{ab}	12.57±0.24	12.09±0.28	12.12±0.11
P	0.369	0.024*	0.156	0.019*	0.278	0.016*	0.534	0.120	0.212

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, P: probability, *:P<0.05, **:P<0.01

Effects of PHY and NSP enzymes addition to low P diets on EST of laying hens are given in Table 3.13. The PHY900 enzyme addition had significantly increased EST value in 3, 4, 8, and overall period compared to 0 and 450 PYH added groups ($P<0.05$). However, in period 2, in the C group EST was higher than pHY450 and PHY900 added groups ($P<0.05$). In period 7, NSP enzymes addition decreased the EST value ($P<0.05$). In the 4^{th} period, 0PHY×-NSP and 900PHY×-NSP had higher EST values than 0PHY+NSP and 450PHY×-NSP groups ($P<0.01$).

Table 3.13. Effects of phytase and non-starch polysaccharide enzymes in low phosphorus diets on eggshell thickness (EST, μm) of laying hens

	Periods								
Treatments	1	2	3	4	5	6	7	8	Overall
PHY Enzyme									
0	0.38±0.00	0.39±0.00^{a}	0.38±0.00^{b}	0.38±0.00^{ab}	0.36±0.00	0.36±0.00	0.36±0.01	0.37±0.01^{b}	0.37±0.00^{b}
450	0.38±0.00	0.38±0.00^{b}	0.38±0.00^{b}	0.37±0.00^{b}	0.37±0.00	0.36±0.00	0.36±0.01	0.37±0.01^{b}	0.37±0.00^{b}
900	0.38±0.00	0.38±0.00^{b}	0.40±0.00^{a}	0.39±0.00^{a}	0.37±0.00	0.36±0.00	0.37±0.01	0.38±0.01^{a}	0.38±0.00^{a}
P	0.874	0.015*	0.001**	0.015*	0.313	0.473	0.766	0.039*	0.029*
NSP									
-	0.38±0.00	0.39±0.00	0.39±0.00	0.38±0.00	0.36±0.00	0.36±0.00	0.37±0.01^{a}	0.37±0.00	0.37±0.00
+	0.38±0.00	0.38±0.00	0.39±0.00	0.38±0.00	0.37±0.00	0.36±0.00	0.36±0.01^{b}	0.38±0.00	0.37±0.00
P	0.512	0.196	0.441	0.964	0.070	0.857	0.018*	0.175	0.867
Interactions									
0PHY×-NSP	0.37±0.01	0.39±0.00	0.38±0.01	0.39±0.01^{a}	0.36±0.01	0.36±0.01	0.38±0.01	0.36±0.01	0.37±0.00
0PHY×+NSP	0.38±0.01	0.39±0.00	0.39±0.01	0.37±0.01^{b}	0.36±0.01	0.35±0.01	0.35±0.01	0.38±0.01	0.37±0.00
450PHY×-NSP	0.38±0.01	0.38±0.00	0.38±0.01	0.36±0.01^{c}	0.36±0.01	0.35±0.01	0.36±0.01	0.36±0.01	0.37±0.00
450PHY×+NSP	0.38±0.01	0.38±0.00	0.38±0.01	0.38±0.01^{ab}	0.38±0.01	0.37±0.01	0.37±0.01	0.37±0.01	0.37±0.00
900PHY×-NSP	0.38±0.01	0.39±0.00	0.40±0.01	0.39±0.01^{a}	0.37±0.01	0.37±0.01	0.38±0.01	0.39±0.01	0.38±0.00
900PHY×+NSP	0.38±0.01	0.38±0.00	0.40±0.01	0.38±0.01^{ab}	0.37±0.01	0.36±0.01	0.36±0.01	0.38±0.01	0.38±0.00
P	0.582	0.485	0.854	0.025*	0.581	0.369	0.078	0.462	0.126

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, P: probability, *:P<0.05, **:P<0.01

3.4. Effect of Phytase (PHY) and Non-Starch Polysaccharide (NSP) Enzymes in Low Phosphorus Diets on Internal Egg Quality Traits of Laying Hens

Table 3.14. shows the effects of PHY and NSP enzymes in addition to low P diets on Hmm of laying hens. The PHY and NSP enzyme's addition

There was no significant difference in Hmm between all periods and overall periods in terms of egg albumen height ($P>0.05$).

Effects of PHY and NSP enzymes addition to low P diets on RCS of laying hens are given in Table 3.15. There are no differences between the Enzyme addition groups in all periods and overall periods except periods 6 and 7. In the 6^{th} period, control and 450 PHY group's RCS values were higher than 900PHY group ($P>0.01$) and in period 7, RCS value of C group was higher than 450PHY enzyme added group ($P<0.05$). Addition of NSP enzymes and PHY$^{\times}$NSP enzymes interactions did effect of RCS values of eggs during the experimental period ($P>0.05$).

Table 3.14. Effects of phytase and non-starch polysaccharide enzymes in low phosphorus diets on egg yolk height (Hmm, mm) of laying hens

	Periods								
Treatments	1	2	3	4	5	6	7	8	Overall
PHY Enzyme									
0	5.21±0.20	4.90±0.23	4.90±0.23	4.72±0.26	4.40±0.20	4.86±0.19	4.28±0.19	4.07±0.18	4.68±0.09
450	5.11±0.20	4.61±0.23	4.61±0.23	4.38±0.26	4.26±0.20	4.51±0.19	4.44±0.19	4.01±0.18	4.50±0.09
900	5.42±0.20	4.84±0.23	4.84±0.23	4.69±0.26	4.76±0.20	4.73±0.19	4.31±0.19	4.17±0.18	4.73±0.09
P	0.552	0.647	0.647	0.577	0.192	0.439	0.820	0.814	0.179
NSP									
-	5.44±0.17	4.92±0.19	4.92±0.19	4.41±0.21	4.56±0.16	4.82±0.16	4.35±0.16	4.09±0.15	4.70±0.07
+	5.05±0.17	4.65±0.19	4.65±0.19	4.78±0.21	4.39±0.16	4.57±0.16	4.34±0.16	4.07±0.15	4.58±0.07
P	0.097	0.311	0.311	0.210	0.446	0.277	0.983	0.927	0.235
Interactions									
0PHY×-NSP	5.53±0.29	5.09±0.33	5.09±0.33	4.52±0.36	4.60±0.28	4.86±0.27	4.08±0.27	4.24±0.25	4.76±0.13
0PHY×+NSP	4.89±0.29	4.72±0.33	4.72±0.33	4.91±0.36	4.19±0.28	4.85±0.27	4.48±0.27	3.90±0.25	4.61±0.13
450PHY×-NSP	5.00±0.29	4.61±0.33	4.61±0.33	4.50±0.36	4.40±0.28	4.70±0.27	4.61±0.27	3.89±0.25	4.56±0.13
450PHY×+NSP	5.22±0.29	4.61±0.33	4.61±0.33	4.25±0.36	4.13±0.28	4.31±0.27	4.27±0.27	4.14±0.25	4.45±0.13
900PHY×-NSP	5.80±0.29	5.06±0.33	5.06±0.33	4.20±0.36	4.68±0.28	4.89±0.27	4.35±0.27	4.16±0.25	4.79±0.13
900PHY×+NSP	5.04±0.29	4.61±0.33	4.61±0.33	5.17±0.36	4.84±0.28	4.56±0.27	4.27±0.27	4.19±0.25	4.67±0.13
P	0.181	0.775	0.775	0.243	0.573	0.764	0.388	0.510	0.984

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, P: probability, *:P<0.05, **:P<0.01

Table 3.15. Effects of phytase and non-starch polysaccharide enzymes in low phosphorus diets diets on Roche Color Scale (RCS) of laying hens

	Periods								
Treatments	1	2	3	4	5	6	7	8	Overall
PHY Enzyme									
0	8.86±0.35	7.54±0.38	8.21±0.36	8.57±0.33	7.54±0.35	5.93±0.30[a]	8.07±0.41[a]	7.50±0.39	7.82±0.15
450	8.57±0.35	8.29±0.38	8.46±0.36	8.68±0.33	7.25±0.35	5.75±0.30[a]	6.46±0.41[b]	7.43±0.39	7.68±0.15
900	8.61±0.35	8.00±0.38	8.25±0.36	8.75±0.33	7.46±0.35	4.82±0.30[b]	7.54±0.41[ab]	7.46±0.39	7.71±0.15
P	0.823	0.381	0.871	0.928	0.836	0.022*	0.022*	0.991	0.773
NSP									
-	8.62±0.29	8.36±0.31	8.55±0.30	8.83±0.27	7.05±0.29	5.19±0.24	7.41±0.33	7.60±0.32	7.79±0.12
+	8.74±0.29	7.52±0.31	8.07±0.30	8.50±0.27	7.79±0.29	5.81±0.24	7.31±0.33	7.33±0.32	7.69±0.12
P	0.771	0.063	0.261	0.381	0.072	0.075	0.841	0.558	0.574
Interactions									
0PHY×-NSP	9.07±0.50	8.07±0.54	8.07±0.52	8.50±0.46	7.57±0.50	5.36±0.42	7.57±0.58	7.29±0.55	7.71±0.21
0PHY×+NSP	8.64±0.50	7.00±0.54	8.36±0.52	8.64±0.46	7.50±0.50	6.50±0.42	8.57±0.58	7.71±0.55	7.93±0.21
450PHY×-NSP	8.57±0.50	8.29±0.54	8.79±0.52	8.86±0.46	6.71±0.50	5.43±0.42	7.14±0.58	7.50±0.55	7.79±0.21
450PHY×+NSP	8.57±0.50	8.29±0.54	8.14±0.52	8.50±0.46	7.79±0.50	6.07±0.42	5.79±0.58	7.36±0.55	7.57±0.21
900PHY×-NSP	8.21±0.50	8.71±0.54	8.79±0.52	9.14±0.46	6.86±0.50	4.79±0.42	7.50±0.58	8.00±0.55	7.86±0.21
900PHY×+NSP	9.00±0.50	7.29±0.54	7.71±0.52	8.36±0.46	8.07±0.50	4.86±0.42	7.57±0.58	6.93±0.55	7.57±0.21
P	0.470	0.394	0.408	0.607	0.369	0.447	0.129	0.387	0.429

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, P: probability, *:P<0.05, **:P<0.01

Table 3.16. shows the effects of PHY and NSP enzymes in addition to low P diets on egg yolk brightness. There are no significant differences between groups in all periods and overall periods except periods 3 and 7. Control had a greater L* value in the 3rd period than that of the 450 PHY enzyme-added group (P>0.05) and in period 7, the control group's L* value was lower than the 900PHY enzyme-added group (P<0.05). NSP enzymes addition did not affect the L* value of laying hens (P>0.05). Also, addition of NSP×PHY enzyme interactions did not affect of egg's L* value during the trial (P>0.05).

Effects of PHY and NSP enzymes addition to low P on egg yolk redness of laying hens are given in Table 3.17. PHY enzyme addition groups did not affect the laying hens a* value (P>0.05). In the 2nd period NSP enzymes addition group's a* value was lower than those of the group (P<0.05). However, there was no significant difference between NSP×PHY enzyme interactions during the experimental period (P>0.05)

Table 3.16. Effects of phytase and non-starch polysaccharide enzymes in low phosphorus diets on egg yolk brightness (L*) of laying hens

	Periods								
Treatments	1	2	3	4	5	6	7	8	Overall
PHY enzyme effect									
0	55.13±0.37	56.53±0.39	56.54±0.49[a]	55.72±0.36	57.01±0.35	56.97±0.37	56.27±0.40[b]	57.03±0.54	56.40±0.22
450	55.53±0.37	55.70±0.39	54.74±0.49[b]	56.33±0.36	57.23±0.35	57.25±0.37	56.75±0.40[ab]	56.63±0.54	56.27±0.22
900	55.37±0.37	55.29±0.39	56.11±0.4[ab]	56.01±0.36	57.99±0.35	57.36±0.37	57.77±0.40[a]	55.60±0.54	56.43±0.22
P	0.739	0.078	0.029*	0.494	0.121	0.756	0.029*	0.163	0.855
NSP effect									
-	55.37±0.30	55.78±0.32	56.01±0.40	55.95±0.29	57.23±0.29	57.37±0.31	56.58±0.33	56.70±0.44	56.37±0.18
+	55.32±0.30	55.89±0.32	55.58±0.40	56.08±0.29	57.59±0.29	57.02±0.31	57.27±0.33	56.14±0.44	56.36±0.18
P	0.919	0.807	0.446	0.752	0.366	0.414	0.139	0.367	0.958
Interactions									
0PHY×-NSP	54.95±0.52	56.46±0.55	56.76±0.69	55.79±0.51	56.89±0.49	56.88±0.53	55.76±0.56	56.30±0.77	56.22±0.31
0PHY×+NSP	55.31±0.52	56.60±0.55	56.31±0.69	55.64±0.51	57.12±0.49	57.06±0.53	56.77±0.56	57.77±0.77	56.57±0.31
450PHY×-NSP	55.57±0.52	55.22±0.55	54.73±0.69	55.90±0.51	56.57±0.49	57.37±0.53	56.48±0.56	57.76±0.77	56.20±0.31
450PHY×+NSP	55.49±0.52	56.17±0.55	54.75±0.69	56.76±0.51	57.90±0.49	57.13±0.53	57.02±0.56	55.49±0.77	56.34±0.31
900PHY×-NSP	55.58±0.52	55.66±0.55	56.54±0.69	56.16±0.51	58.21±0.49	57.86±0.53	57.51±0.56	56.05±0.77	56.70±0.31
900PHY×+NSP	55.17±0.52	54.91±0.55	55.68±0.69	55.85±0.51	57.76±0.49	56.85±0.53	58.03±0.56	55.15±0.77	56.17±0.31
P	0.761	0.310	0.813	0.464	0.196	0.525	0.887	0.052	0.353

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, P: probability, *:P<0.05, **:P<0.01

Table 3.17. Effects of phytase and non-starch polysaccharide enzymes in low phosphorus diets on egg yolk redness (a*) of laying hens

	Periods								
Treatments	1	2	3	4	5	6	7	8	Overall
PHY Enzyme									
0	1.80±0.17	1.20±0.18	1.19±0.14	1.57±0.14	1.04±0.14	0.72±0.15	1.29±0.16	0.98±0.18	1.22±0.08
450	1.59±0.17	1.41±0.18	1.12±0.14	1.83±0.14	0.91±0.14	0.60±0.15	0.76±0.16	1.01±0.18	1.15±0.08
900	1.91±0.17	1.13±0.18	1.29±0.14	2.03±0.14	0.88±0.14	0.39±0.15	1.19±0.16	1.03±0.18	1.23±0.08
P	0.412	0.529	0.677	0.081	0.656	0.328	0.053	0.985	0.717
NSP									
-	1.70±0.14	1.48±0.15[a]	1.09±0.12	1.88±0.12	0.92±0.11	0.41±0.13	1.11±0.13	1.05±0.14	1.20±0.06
+	1.83±0.14	1.02±0.15[b]	1.31±0.12	1.75±0.12	0.96±0.11	0.73±0.13	1.04±0.13	0.96±0.14	1.20±0.06
P	0.510	0.034*	0.183	0.430	0.796	0.077	0.724	0.666	0.972
Interactions									
0PHY×-NSP	1.84±0.25	1.58±0.26	1.15±0.20	1.65±0.20	0.98±0.19	0.42±0.22	1.45±0.23	1.17±0.25	1.28±0.11
0PHY×+NSP	1.76±0.25	0.82±0.26	1.23±0.20	1.48±0.20	1.11±0.19	1.02±0.22	1.13±0.23	0.80±0.25	1.17±0.11
450PHY×-NSP	1.56±0.25	1.55±0.26	0.97±0.20	1.87±0.20	0.90±0.19	0.44±0.22	0.85±0.23	1.12±0.25	1.15±0.11
450PHY×+NSP	1.62±0.25	1.28±0.26	1.26±0.20	1.79±0.20	0.91±0.19	0.76±0.22	0.66±0.23	0.90±0.25	1.15±0.11
900PHY×-NSP	1.70±0.25	1.30±0.26	1.15±0.20	2.11±0.20	0.89±0.19	0.38±0.22	1.03±0.23	0.87±0.25	1.18±0.11
900PHY×+NSP	2.12±0.25	0.97±0.26	1.43±0.20	1.96±0.20	0.87±0.19	0.41±0.22	1.34±0.23	1.19±0.25	1.28±0.11
P	0.575	0.582	0.838	0.975	0.924	0.438	0.363	0.356	0.589

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, P: probability, *:P<0.05, **:P<0.01

Effects of PHY and NSP enzymes addition to low P on egg yolk yellowness (b*) of egg yolk are given in Table 3.18. The egg yolk b* values was significant in period 2, 3 and 4 but not significant the other periods. In the period 2, control groups b* value was higher than those of the PHY 450 and 900 added groups ($P<0.05$). The 450 PHY group's b* value was lower than other groups and in period 4 ($P<0.01$), 450 PHY group's b* value was higher than other groups ($P<0.05$). In period 2, NSP enzymes addition decreased the b* value compared to the non-added group ($P<0.05$). In period 5, the 900PHY×+NSP group b* value was higher than other groups and the 0PHY+NSP groups b* value was lower than all of the groups ($P<0.01$).

Effects of PHY and NSP enzymes addition to low P on eggshell-breaking strength of laying hens are given in Table 3.19. In the 28 days and overall period, 450PHY and 900PHY enzyme added group's eggshell-breaking strength was higher than 0PHY (non-added PHY) group ($P<0.01$). The NSP enzymes addition did not effect of ESB of eggshells. When PHY and NSP interactions were examined, ESB values in 450PHY+NSP and 900PHY-NSP groups were found to be significantly higher than other groups on day 28. In addition, 900 PHY+NSP enzymes supplementation was found to be significantly higher ESB than in other groups except 450PHY+NSP and 900PHY-NSP groups. The lowest ESB value belonged to 0PHY+NSP group. In the overall period, ESB values of 450PHY+NSP, 900PHY-NSP and 900PHY+NSP enzymes groups were found to be statistically significantly higher than other groups ($P<0.05$).

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Table 3.18. Effects of phytase and non-starch polysaccharide enzymes in low phosphorus diets diets on egg yolk yellowness (b*) of laying hens

Treatments	Periods								
	1	2	3	4	5	6	7	8	Overall
PHY Enzyme									
0	32.93±0.57	32.48±0.54^{a}	29.82±0.57^{a}	30.89±0.43^{b}	31.26±0.42	30.37±0.50	32.30±0.56	32.10±0.57	31.52±0.22
450	32.59±0.57	30.35±0.54^{b}	27.43±0.57^{b}	32.44±0.43^{a}	31.46±0.42	30.12±0.50	30.49±0.56	32.23±0.57	30.89±0.22
900	32.66±0.57	30.87±0.54^{b}	30.96±0.57^{a}	31.11±0.43^{b}	32.02±0.42	29.97±0.50	31.82±0.56	30.79±0.57	31.27±0.22
P	0.904	0.017*	0.001**	0.026*	0.411	0.850	0.065	0.150	0.142
NSP									
-	32.75±0.47	31.88±0.44^{a}	29.23±0.46	31.79±0.35	31.61±0.34	30.13±0.41	31.66±0.46	32.15±0.47	31.40±0.18
+	32.70±0.47	30.59±0.44^{b}	29.58±0.46	31.17±0.35	31.55±0.34	30.18±0.41	31.42±0.46	31.26±0.47	31.05±0.18
P	0.943	0.040*	0.599	0.213	0.902	0.939	0.704	0.183	0.183
Interactions									
0PHY×-NSP	32.80±0.81	32.39±0.76^{d}	30.20±0.80	31.63±0.61	31.49±0.59^{c}	30.28±0.71	32.17±0.79	31.98±0.81	31.62±0.32
0PHY×+NSP	33.06±0.81	32.56±0.76^{c}	29.44±0.80	30.15±0.61	31.02±0.59^{f}	30.45±0.71	32.43±0.79	32.22±0.81	31.42±0.32
450PHY×-NSP	32.18±0.81	32.33±0.76^{d}	26.92±0.80	31.95±0.61	30.21±0.59^{d}	29.96±0.71	31.51±0.79	33.27±0.81	31.04±0.32
450PHY×+NSP	32.99±0.81	28.38±0.76^{d}	27.95±0.80	32.92±0.61	32.71±0.59^{b}	30.28±0.71	29.48±0.79	31.20±0.81	30.74±0.32
900PHY×-NSP	33.27±0.81	30.93±0.76^{a}	30.58±0.80	31.79±0.61	33.13±0.59^{e}	30.15±0.71	31.30±0.79	31.21±0.81	31.54±0.32
900PHY×+NSP	32.05±0.81	30.81±0.76^{b}	31.34±0.80	30.43±0.61	30.92±0.59^{a}	29.79±0.71	32.34±0.79	30.37±0.81	31.00±0.32
P	0.433	0.013*	0.486	0.080	0.001**	0.882	0.133	0.365	0.863

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, P: probability, *:P<0.05, **:P<0.01.

3.5. Effect of Phytase (PHY) and Non-starch Polysaccharide (NSP) Enzymes in Low Phosphorus Diets on Eggshell Breaking Strength of Laying Hens

Table 3.19. Effects of phytase and non-starch polysaccharide enzymes in low phosphorus diets on eggshell breaking strength (nt, ESB) of laying hens

Treatments	Day 28	Day 56	Day 84	Overall
PHY Enzyme				
0	33.35±2.28^{b}	36.30±2.20	35.13±2.45	34.93±1.30^{b}
450	44.24±1.96^{a}	42.62±1.89	32.54±2.11	39.80±1.12^{a}
900	46.47±2.13^{a}	41.32±2.05	39.62±2.29	42.47±1.21^{a}
P	0.001**	0.089	0.083	0.001**
NSP				
-	42.32±1.64	39.31±1.58	34.28±1.76	38.64±0.93
+	40.39±1.83	40.85±1.76	37.24±1.97	39.49±1.04
P	0.436	0.517	0.268	0.542
Interactions				
0PHY×-NSP	38.88±2.96^{c}	36.57±2.85	32.96±3.19	35.47±1.69^{bc}
0PHY×+NSP	29.82±3.47^{d}	36.03±3.35	37.30±3.74	34.39±1.98^{c}
450PHY×-NSP	38.87±2.83^{c}	41.50±2.73	29.80±3.05	36.72±1.61^{b}
450PHY×+NSP	49.61±2.72^{a}	43.73±2.62	35.29±2.93	42.88±1.55^{a}
900PHY×-NSP	51.20±2.72^{a}	39.85±2.62	40.09±2.93	43.71±1.55^{a}
900PHY×+NSP	41.74±3.27^{b}	42.79±3.15	39.14±3.52	41.22±1.86^{a}
P	0.001**	0.831	0.562	0.023

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, P: probability, *:P<0.05, **:P<0.01

Table 3.20. Effects of phytase and non-starch polysaccharide enzymes in low phosphorus diets on eggshell breaking strength (nt, ESB) of laying hens

3.6. Effect of Phytase (PHY) and Non-starch Polysaccharide (NSP) Enzymes in Low Phosphorus Diets on Feces Viscosity of Laying Hens

Table 3.20 shows the effects of PHY and NSP enzymes addition to LP diets on the feces viscosity (FV) of laying hens. The dietary treatment did not affect viscosity in all periods during the experiment (P>0.05).

Table 3.20. Effects of phytase and non-starch polysaccharide enzymes in low phosphorus diets on feces viscosity (FV) of laying hens

Treatments	Day 56	Day 84
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, P: probability, *:P<0.05, **:P<0.01

3.7. Effect of Phytase (PHY) and Non-starch Polysaccharide (NSP) Enzymes in Low Phosphorus Diets on Blood Parameters of Laying Hens

Effects of PHY and NSP enzymes addition to low P on blood parameters of laying hens are given in Table 3.21. There were no significant differences between the treatment groups except glucose in blood serum (triglyceride, cholesterol, creatinine, phosphorous, AST, ALT, and ALP) concentrations. The 450PHY enzyme additions caused a decrease in glucose level compared to the PHY900 group (<0.05). The NSP enzyme addition decreased serum glucose (P<0.01) while increasing ALP content (P<0.05). The serum

triglyceride, cholesterol, creatinine, phosphorus, AST, and ALT concentrations were not affected by the NSP enzymes addition. However, 0PHY×-NSP, 450PHY×+NSP, and 900PHY×-NSP interactions had higher creatinine levels compared to the other groups ($P<0.01$)

Table 3.21. Effects of phytase and non-starch enzymes polysaccharide enzymes in low phosphorus diets on blood parameters (BP) 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}	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^{b}	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^{bc}	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}	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}	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^{bc}	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, P: probability, *:P<0.05, **:P<0.0

3.8. Effect of Phytase (PHY) and Non-starch Polysaccharide (NSP) Enzymes in Low Phosphorus Diets on Some Bone Traits of Laying Hens

Effects of PHY and NSP enzymes addition to low P on some bone parameters of laying hens are given in Table 3.23. Phytase supplementation significantly increased the ash content of bones compared to the control group (P<0.05). Also, 450 FTU/kg phytase supplementation to the diet significantly increased bone-breaking strength compared to the control group (P<0.05), however, 900 FTU phytase enzyme supplementation was found to be similar to the control and 450 FTU phytase supplemented groups and were not statistically different.

Table 3.22. Effects of phytase enzyme addition in low phosphorus diets on bone traits

Treatments	Bone ash, %	Bone-breaking strength, nt/kg
PHY Enzyme		
0	41.04±0.40^{b}	146.92±17.24^{b}
450	47.16±1.09^{a}	186.90±11.09^{a}
900	47.30±1.50^{a}	150.55±7.24^{ab}
P	0.013*	0.024*

PHY: phytase, NSP: non-starch polysaccharide, 0PHY: no phytase addition, 450PHY: 450 unit PHY contains, 900PHY:900 unit PHY contains, ^{a,b}: Values with different superscript in a column differ significantly, P: probability, *:P<0.05.

CHAPTER 4

DISCUSSION

The current study was carried out to determine the effects of the use of phytase and non-starch polysaccharides enzymes (phytase, non-starch polysaccharides) alone and their combinations in diets on the performance and egg quality, bone and blood traits of laying hens. Calcium and phosphorus must be included in the rations of laying hens in a balanced and sufficient manner. Phosphorus can only be obtained from raw rock phosphate sources found in limited regions or can also be obtained from the bones and shells of some animals. However, the use of bones by all animals is limited and some countries do not process them for feed. In addition, these feeds, which are P sources, are expensive. Therefore, making the best use of phosphorus sources can provide economic returns. In addition, excessive disposal of phosphorus into the environment with manure causes environmental pollution and impairs its quality by mixing with water. Therefore, in addition to calculating phosphorus well and providing it to animals without impairing animal health and production, some studies should be carried out to increase the bioavailability of phosphorus in plants (Rodehutscord et al., 2023). The newest of these is increasing the utilization of phosphorus with microbial enzymes that are not secreted in the body of animals. On the other hand, since non-starch polysaccharides, which are not digested by monogastric animals, and they cause energy loss and have negative effects such as diarrhea, sticky feces, and deterioration of litter quality (Struți et al., 2023). NSP degrading enzymes are among the most effective biotechnological products used in the digestion of non-starch polysaccharides.

4.1. Effects of Phytase and Non-starch Polysaccharide Enzymes in Low Phosphorus Diets on Performance Traits

The NSP and PHY enzyme addition in low phosphorus diets did not affect final BW, BWG, and changes in laying hens. However, PHY and NSP enzymes interactions affected the BW change % of hens and 0PHY×-NSP, 450PHY×-NSP and 900PHY×-NSP group's BW change % were lower than the other groups. In case of insufficient phosphorus in animal feeds, their appetites decrease, therefore, both live weight loss and productivity decreases occur in animals. As it is known, phosphorus is found in the synthesis of high-energy compounds such as ATP, in the structure of bones, teeth, egg shells, membranes surrounding the brain and phospholipids, and in case of its deficiency, serious damages occur especially in animal metabolism. At the same time, P should be present in the ration together with Ca and vitamin D in a certain amount and at the same time (Okuyan and Filya, 1997). While calcium sources are more abundant in nature than phosphorus sources, phosphorus sources are more insufficient. Therefore, phosphorus is one of the most expensive minerals that increases the cost of feeds (Rizwanuddin et al., 2023). In this study, laying hens were studied with 0.28% AP amount by decreasing the 0.38% AP amount stated in their feed catalogs, and enzymes were used in their feeds to reduce the possible negative effects of this, but the animals remained sufficiently productive with this phosphorus amount and there was no loss of live weight.

Polat, (2019) reported that the addition of 0.1% and 0.5% enzyme-A (xylanase, ß glucanase, α-amylase, and protease enzyme B xylanase, ß-glucanase, and Taylor et al. (2018) reported that phytase and xylanase or phytase +xylanase (300, or 1500 FTU/kg 12 000 BXU/kg) inclusion and Skřivan et al. (2018) noted that 35 Ca + 0, 300 or, 15000 FTU or 42 g/kg + 0, 300, or 1500 FTU additions had no significant difference in body weight of laying hens. In contrast to those results, Narasimha et al. (2013) 675 IU/kg, 400 cellulase, 3200 β-D glucanase, and xylanase, 6000 IU/kg and Çabuk et al. (2004) reported that 4.5 g AP with 300 FTU/kg to the increased body weight gain in hens.

There was no significant difference in terms of feed intake among PHY enzyme addition groups in all periods and overall, except period 2. The feed intake of the C group was higher than other groups, and the 900 PHY enzyme addition group's FI was higher than

that of the 450 PHY enzyme group. NSP enzymes addition to diets did not affect FI of hens in all periods and overall period except period 8. The addition of NSP enzymes increased the FI of hens compared to on added group. The Phytase and NSP enzymes interaction did not affect the FI of hens in most of the periods and overall period. Previous studies reported that 150 FTU/kg, 250 FTU/kg, and 350 FTU/kg, or 1.8 g/kg and 2.1 g/kg NPP, Jalal and Scheideler (2001) 0.35, 0.25, 0.15, or 0.10% NPP, and 0.25, 0.15, or 0.10% at 250 to 300 FTU/kg, Taylor et al. (2018) phytase and xylanase or phytase *xylanase (300, or 1500 FTU/kg 12 000 BXU/kg, and Narasimha et al., (2013) Phytase, NSP (675 IU/kg FTU, 400 cellulase, 3200 β-D glucanase, and xylanase 6000 IU/kg) additions had no effect on feed intake of laying hens. However, Çabuk et al. (2004) 4.5g, 3.0gAP/kg And 300 unit FTU/kg supplementations increased feed intake of laying hens. Also, Zhai et al., (2022) reported that the 0.1% NPP + 187.5 FYT/kg addition increased FI of hens compared to the 0.09% NPP+ 187.5 or 375 FYT/kg fed hens.

In the current experiment, the PHY enzyme addition did not affect hens' feed FCR in different experimental periods and overall periods. The NSP enzymes addition did not effect of hens' FCR in different experimental periods and overall periods except the 8th period. NSP enzymes addition increased FCR values in period 8 compared to the added group. The phytase enzyme and NSP enzymes interaction did not effect of FCR values of laying hens in all experimental periods and overall periods. Similar results were found by Kim et al., (2017) 10,000, 20,000, or 30,000 FTU/kg, Bahtiyarca, and Konca, (2017) 0.33%, and 0.15% AP, 500,1000, 1500, or 2000 FTU/g, and Narasimha et al., (2013) (675IU/kg FTU, 400cellulase, 3200 β-D glucanase, and xylanase, 6000IU/KG) supplementation had no effect on FCR of laying hens. Additionally, other experiments by Yoruk and Bolat, (2003) revealed that the corn, barley, enzyme **(**xylanase, amylase, and beta-glucanase) (60% or 30% corn +30% barley, or 0.05% enzymes), and Polat, (2019) Enzyme-A(xylanase, ß glucanase, α-amylase, and protease, xylanase, ß-glucanase (0.1%, 0.05%) inclusions did not effect on FCR of laying hens. Although, Taylor et al. (2018) found that the phytase*xylanase interactions increased FCR compared to single phytase and xylanase-fed groups.

In this study, dietary treatment did not affect egg weight, Hmm, eggshell surface area, egg mass HU. In a similar study, it was reported that addition of 0.38% C, 0.20, 0.25, and 0.30% NPP1,000 FYT /kg Eltahan et al. (2023), 0.1%, and 0.05% NSP enzymes (Polat,

2019) and 0, 150, 250, and 350 FTU/kg,1.8 and 2.1 g/kg NPP, (Englmaierová et al., 2015) addition did not effect egg weight, HMM, ESA and EM of laying hens. Furthermore, Yoruk and Bolat, (2003)60% or 30% corn +30% barley, 0.05% enzyme, and Casartelli et al.,(2005) 0 or 1000 FTU/kg had no impact on previous parameters. On the other hand, resources produced in countries without importing from local sources are important both economically and in terms of the efficiency of domestic resources. In a recent study conducted with local enzymes, reducing the available phosphorus (YP) rate in feed and adding commercial or experimental phytase did not affect performance parameters such as live weight, egg production, weight and mass, feed consumption and feed conversion ratio (Yenice et al., 2020).

4.2. Effects of Phytase and Non-starch Polysaccharide Enzymes in Low Phosphorus Diets on Egg Production and Internal Quality Traits

In this study, the treatment groups did not affect the egg production of laying hens. Similarly, Polat (2019) reported that adding NSP enzymes (0.1% and 0.05%), Jalal and Scheidelers (2001) noted 0.35, 0.25, 0.15, or 0.10% NPP, and 250 to 300 FTU/kg, and Yoruk and Bolat. (2003) also noted that 60% or 30% corn +30% barley, and 0.05% enzymes (xylanase, amylase, and beta-glucanase) additions did not influence egg production in laying hens. While Taylor et al. (2018) addition of phytase 300, or 1500 FTU/kg, and Eltahan et al. (2023) 0.20%, 0.25% NPP+1.000 FYT/kg enhance egg production in laying hens.

The current study shows no differences between the PHY and NSP enzyme addition groups in terms of egg yolk color values (Roche color scores) in most of the periods and overall periods. The PHY and NSP enzymes interactions did not affect the RCS value during the experimental period.

Similarly Taylor et al. (2018) phytase and xylanase or phytase*xylanase (300, or 1500 FTU/kg 12 000 BXU/kg, and Eltahan et al. (2023) 150,130,126g/kg CP and 0 or 300 FTU/kg inclusion in laying hens did not affect RCS. However, Polat. (2019) found that adding NSP enzymes (0.1% and 0.05%), Nguyen et al. (2021) 0 or 12.000 BXU/kg exogenous xylanase additions increase RCS values in laying hens.

In this experiment, the dietary treatments had no significant effects of egg yolk redness a* value. Similarly Polat. (2019) found that adding NSP enzymes (0.1% and 0.05%) to laying hens' diet did not influence egg yolk redness. On the other hand, Gao et al. (2013) noted that adding phytase sources (TCDP, PA, or BP) increases egg yolk redness.

In this study, there was a significant difference between the PHY addition in terms of egg yolk yellowness in most periods and overall period. However, there were no permanent differences among the PHY-added doses and even there were contrast effects in different periods. The NSP enzymes addition decreased the b* value compared to the other groups. During the 2nd period, 900 PHY×-NSP interactions had higher b* values than those of all groups. 900PHY×+NSP increased b* value in the 5th period. There was no significant effect on b* values during the overall period. Other studies by Gao et al. (2013) reported that adding different sources of phytase (TCDP, PA, or PB) at 500 or 5000 FTU/kg, with 0.32%, 0.10% NPP increased egg yolk value in laying hens.

4.3. Effects of Phytase and Non-starch Polysaccharide Enzymes in Low Phosphorus Diets on Eggshell and External Quality Traits

In the current study, there was no significant difference among PHY enzyme addition groups in terms of ESW in most periods and overall periods. Also, NSP enzymes addition did not affect the ESW of the laying hen's diet in most periods and overall period. The NSP and phytase enzyme interactions did not affect the ESW of hens in all groups and periods except period 1 and the overall period. Similar findings were reported by Çabuk et al. (2004) 4.5g, 3.0gAP/kg and 300 unit FTU/kg, and Lima et al. (2011) 200, 400, or 600 FTU/kg, Narasimha et al. (2013) 675 IU/kg, 400 cellulase, 3200 β-D glucanase, and xylanase,6000 IU/kg inclusion did not affect the ESW. However, Scott et al. (1999) found that supplementing diets with 3.7% or 0.4% AP and 4.0% or 0.2% Ca increased eggshell weight. In contrast, Olgun et al. (2018) reported that 1.0%g/kg of NSP enzymes decreased ESW.

In the present study, there was no significant difference between PHY enzyme addition groups in terms of ESW% value in most periods and overall periods. NSP and phytase enzyme interactions did not affect ESW% of hens in most periods and overall periods. Polat (2019) noted that adding 0.1% and 0.05% non-starch polysaccharides did not affect eggshell weight %. Lima et al. (2011) reported that 0, 200, 400, or 600 FTU/kg inclusion

decreases ESW% in Japanese quails. On the other hand, it was found that adding 1000 FTU/kg supplementation to the diet of laying hens increased eggshell weight percentage (ESW%) (Casartelli et al., 2005).

In this study, PHY enzyme addition had significantly increased EST value in most periods and overall period. Taylor et al. (2018) demonstrated that phytase inclusion at 300, or 1500 FTU/kg enhances EST in laying hens. Also, Eltahan et al. (2023) found that the addition of 0.20%, 0.25%, and 0.30 % NPP along with 1.000 FYT/kg enhanced EST in aging laying hens. Gao et al. (2013) observed that 0.10% NPP inclusion leads to a decrease in EST of laying hens. Another study by Englmaierová et al. (2015) demonstrated that 2.1%NPP with 350 FTU/kg inclusions respectively improve EST. Furthermore, Bahtiyarca and Konca (2017) found that 1500 or 2000 FTU/g, and Olgun et al. (2018) 1.0 g/kg supplementation decreases EST.

In the current study, there was no significant difference between PHY enzyme inclusion groups in terms of shell-breaking strength in most periods and overall periods. Additionally, PHY and NSP interactions had significantly increased shell-breaking strength values compared to the C groups. Similarly, Çabuk et al. (2004) 4.5g,3.0gAP/kg, and 300 unit FTU/kg, and Skřivan et al. (2018) 35g/kg Ca and 1500/kg, and Jalal and Scheideler. (2001), 0.35, 0.25, 0.15, or 0.10% NPP, 0.25, 0.15, and 0.10% at 250 to 300 FTU/kg inclusions did not influence the eggshell-breaking strength of laying hens.

Other studies by Olgun et al. (2018) found that adding 1.0g/kg of NSP enzymes (glucanase, xylanase, cellulose, amylase) reduced eggshell-breaking strength in laying hens.

In a study conducted with a phytase source produced in Turkiye, egg quality traits such as broken-cracked egg rate, shell weight ratio, shell thickness and strength, albumen height, and Haugh unit were not significantly affected by different treatments (Yenice et al., 2020)

4.4. Effects of Phytase and Non-starch Polysaccharide Enzymes in Low Phosphorus Diets on the Feces Viscosity of Laying Hens

In this study, dietary treatment did not affect feces viscosity. Intestinal and fecal viscosity in birds is significantly affected by non-starch polysaccharides in the feed they consume. Since NSPs are not digestible, they swell by absorbing water and increase the viscosity in the intestine, also causing sticky feces. Therefore, if enzymes are added to the feed, a decrease in fecal viscosity may occur (Bollengier-Lee et al., 1998). Using feed enzymes such as xylanases may reduce the viscosity of intestinal digesta and increase nutrient digestion and improve intestinal health (Vasanthakumari et al., 2023). Lee et al., (2014) reported that the addition of enzyme combinations increased digestion and decreased viscosity. In another study (Świątkiewicz and Koreleski, 2007), it was determined that the addition of NSP enzymes to DDGS-containing diets improved digestion and P balance and decreased viscosity. Similarly, other studies reported improved digestion and decreased viscosity with enzyme addition (Oloffs et al., 1999). On the other hand, in our study, fecal viscosity was not affected by enzyme additions. In this study, during the period when feces were collected, an intense lice outbreak occurred in the animals and therefore the feed consumption of the animals decreased significantly. Therefore, it can be thought that the feces, which the animals did not consume enough feed, may have remained in the animal intestine for a long time and this did not reflect an effective situation on the viscosity.

4.5. Effects of Phytase and Non-starch Polysaccharide Enzymes in Low Phosphorus Diets on Blood Parameters

In this experiment, there were no significant differences between the treatment groups on blood parameters in terms (triglyceride, cholesterol, creatinine, phosphorous, AST, ALT, and ALP) concentrations 450 PHY enzyme additions caused a decrease in glucose level. A previous study by Eltahan et al. (2023) indicated that a diet containing 0.25% and 0.30% NPP with 1.000FYT/kg increased the plasma concentration of albumin (ALP) and phosphorous. Lee et al. (2014) determined that the use of multi-enzyme in the diet did not significantly affect blood cholesterol AST and ALT values in laying hens. (Jing et al., 2021) phytase addition to the laying diet did not affect the eggshell thickness, specific gravity, Haugh units, plasma P, and other biochemical parameters. (Hassanien and Sanaa,

2011) phytase supplementation (1000 FTU kg-1 feed) did not show any significant effect on egg quality measurements except egg weight, shell thickness, shell breaking resistance, shell ratio, and HU in laying hens. However, they concluded that phytase supplementation had a significant effect on some blood parameters of laying hens. Çalişlar, (2021) addition of beta mannanase enzyme to laying hen diets containing guar flour affected the blood triglyceride, glucose, total cholesterol, and calcium concentration, however, no significant effect on the blood total protein and P was observed.

In our study, blood values were generally not affected by enzyme supplements. If the enzymes added to the feed show significant regular activity in the digestive system, carbohydrate sources and P sources can change from bound form to free form. On the other hand, in this study, the animals were under stress due to intense lice infestation during the blood collection period, and therefore some blood values were probably not significantly affected by enzyme supplements.

4.6. Effects of Phytase and Non-starch Polysaccharide Enzymes in Low Phosphorus Diets on Bone Parameters

In this study, the addition of phytase enzyme increased bone fracture resistance and bone ash ratio. This situation can be considered as an indication that more minerals are stored in the bones of laying hens fed with phosphorus levels that are relatively lower than the actual needs of the hens. Indeed, in previous studies, bone ash and fracture resistance were some of the most evaluated parameters in phosphorus calcium levels and phytase addition. Phytase addition increased the tibia ash and calcium ratio in the tibia ash of laying hens (Al-Sharafat et al., 2009). Jing et al. (2021) phytase addition to laying hen diet did not affect the eggshell thickness, tibia bone mineral density, tibia ash percentage, plasma P and other biochemical parameters.

In animals, bones are an important storage place for calcium and phosphorus. If animals do not receive sufficient calcium and phosphorus, they can meet the necessary Ca and P sources for blood and eggshells from their bones. When Ca and P mobilization occurs from bones, bones become weak and bone ash and fracture resistance may decrease. In studies conducted on Ca and P and phytase enzyme, ash ratio and fracture resistance of bones are frequently evaluated (Ghosh et al., 2015)v. In studies where phytase enzyme is added, bone fracture resistance and bone ash sometimes increase (Kubiś et al., 2019), but

in some studies, no significant positive effect can be obtained from either phytase enzyme (Musapuor et al., 2005; Jing et al., 2021) or multi enzymes (Rama Rao et al., 2014). Sometimes, the lack of necessary response in ration Ca P and phytase enzyme additions may be due to effects originating from other minerals in the ration other than these minerals.

Conclusion and Recommendations

In this study, it was aimed to reduce the phosphorus level of laying hen feeds from 0.38% specified in the catalog to 0.28% and to measure its effects on the performance of the animals. As long as the population growth continues in the world, animal production must also increase, and with the increase in the number of animals and also due to the limited feeds, it has become a necessity to make better use of them. In recent years, with the advancement of biotechnology, it has been desired to increase the nutrient utilization rate of animals by adding produced microbial enzymes to the feed. Phosphorus is one of the most important of these nutrients, and since only one-third of the phosphorus found in feeds is utilized, the rest is completed from inorganic phosphorus sources to meet the animal's needs. Therefore, to make economic rations without paying additional costs for these feed sources and by simplifying the structure of the ration, more utilization of existing nutrients should be sought. In this study, the effects of using enzymes required for the digestion of expensive phosphorus and non-starch polysaccharides, which is an important problem in feeds, separately or together, on the performance, egg quality, egg production, and bone parameters of laying hens, as well as their effects on fecal viscosity and blood parameters were evaluated.

According to data obtained from this experiment, we can conclude that:

1. It was determined that laying hens can be fed with feeds containing lower amounts of phosphorus (0.28%) than the values reported in the catalog (0.38%).

2. With the addition of the phytase enzyme, some improvements in egg-laying performance and eggshell traits were achieved, but one of the parameters indicating phosphorus availability was significant improvements in eggshell fracture strength and bone strength parameters.

3. No significant improvement in animal performance or egg quality parameters was observed with the addition of NSP enzymes to feed.

4. No significant improvement in animal performance or other measured parameters was observed with the provision of combinations of phytase and NSP enzymes in feeds.

As a result, it was determined that the addition of phytase enzyme to diets can cause a significant improvement in shell quality and bone parameters. Therefore, phytase enzyme can be used especially in cases where the diet P levels are below 0.28% and in different feed combinations.

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