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Improving the nutritional value of soybean hulls using a specialized enzyme mix to diet on some biochemical blood parameters of broiler Ross308

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Abstract:

This study was conducted at the poultry farm of the Animal Production Department, College of Agriculture, Al-Muthanna University, from February 1, 2025, to March 8, 2025. 225 one-day-old broiler chicks were used. They were randomly distributed into five treatments with three replicates per treatment. The treatments were as follows: T1: Control (no additives). Treatments T2, T3, T4, and T5, an enzyme mixture (β-xylanase 2000 IU + β-glucanase 100 IU + cellulase 500 IU) was added at levels of 250, 500, 750, and 1000 gm ton of feed, respectively. The results of this study show that the use of soybean hulls enhanced with a mixture of enzymes (β-xylanase, β-glucanase, and cellulase) in broiler chicken diets, led to a significant improvement ($P \leq 0.05$) on some hematological parameters and vital signs of the broilers.

Keywords: soybean hulls, enzyme mix, blood parameters, broiler Ross308.

Introduction:

The use of enzymes as feed additives has become widespread. This is due to their ability to reduce feed costs and improve feed utilization efficiency. Enzymes are amino acid-based biocatalysts that control the rate at which a specific chemical reaction occurs in a given substrate, by improving nutrient availability, enzymes contribute to reducing the cost of feed ingredients. It is estimated that the addition of exogenous enzymes, could save the global feed industry between US$3 billion and US$5 billion annually (Abbas and Nurdianti, 2023).

Poultry rely entirely on the nutrients present in their feed, because they lack the enzyme-secreting microorganisms found in ruminants. The importance of adding exogenous enzymes to release nutrients from complexes and compounds in feed rations has been demonstrated (Pariza and Cook, 2010). Adding exogenous enzymes to poultry diets, breaks down antinutrients present in feed, which can reduce productive performance when they interfere with normal digestion. It increases the availability of nutrients trapped within the plant cell wall, which cannot be digested by naturally occurring enzymes in the small intestine. Digestive enzymes help young birds digest nutrients, due to the immaturity of their digestive system and the inadequacy of their own enzyme production (Sheppy, 2003).

Phytases, which catalyze the hydrolysis of phytate, are among the most widely used enzymes in the poultry industry. Carbohydrases follow in terms of usage. They are responsible for breaking down non-starch sugars (NSPs) found in sticky grains. These two groups of enzymes constitute approximately 90% of the enzyme market used in the poultry sector (Kong et al., 2011).

The importance of protease enzymes has increased, due to their ability to improve the digestibility of proteins and amino acids. It is worth noting that amino acids are not only the building blocks of proteins, but are also essential for the production of enzymes, hormones, neurotransmitters, and energy-providing compounds, and play a role in supporting other immune and nutritional functions. Because of the relatively low activity of protease enzymes in young birds, these enzymes have become particularly important in poultry farming (Walker *et al*., 2024).

Enzymes can be commercially produced from microbial, plant, or animal sources. However, enzyme production from plant and animal sources is limited. Plant cultivation is largely a seasonal activity, constrained by geographical areas suitable for plant growth. It is impractical to rely on these sources extensively to meet industry needs (Devi et al., 2026).

Due to the generally low concentration of enzymes in plants, it is not possible to cultivate sufficient quantities of plants to meet the demands of commercially produced enzymes. As for enzymes derived from animal sources, most of these are produced by the meat industry, an industry that already faces significant competition from other sectors (Alabi *et al*., 2019).

This study aims to improve the nutritional value of soybean hulls, using a specialized enzyme mixture for feed on some biochemical blood parameters of broiler Ross308.

Materials and methods:

This study was conducted at the poultry farm of the Animal Production Department, College of Agriculture, Al-Muthanna University, from February 1, 2025, to March 8, 2025. 225 one-day-old broiler chicks were used. They were randomly distributed into five treatments with three replicates per treatment. The treatments were as follows:

T1: (Control Treatment).

T2: The birds were fed a diet containing 250 gm of enzyme mix per ton of diet.

T3: The birds were fed a diet containing 500 gm of enzyme mix per ton of diet.

T4: The birds were fed a diet containing 750 gm of enzyme mix per ton of diet.

T5: The birds were fed a diet containing 1000 gm of enzyme mix per ton of diet.

Three specialized fiber-digesting enzymes are mixed: β-xylanase 2000 IU + β-glucanase 100 IU + cellulase 500 IU. The fiber content of all treatments is 3.5%.

Results and Discussions:

Table (1) shows the effect of using soybean hulls enhanced with an enzyme mixture on the concentration of cholesterol, LDL, HDL, triglycerides, and glucose in broiler blood serum. Regarding the concentration of cholesterol, LDL, triglycerides, and glucose, a significant increase ($P \leq 0.05$) was observed in the first treatment (control) compared to all the supplementation treatments T2, T3, T4, and T5. As for high-density lipoprotein (HDL), treatment T4 was found to be significantly superior ($P \leq 0.05$) to all treatments T2, T3, and T5, respectively, which in turn were superior to the control treatment T1.

The reason for the higher cholesterol, LDL, triglycerides, and glucose levels in the first treatment (control), which did not contain soybean hulls enhanced with the enzyme mixture, compared to the T2, T3, T4, and T5 treatment groups, because soybean hulls are high in dietary fiber, which directly reduces fat absorption and increases the viscosity of intestinal contents. The breakdown of these fibers by a mixture of enzymes (β-glucanase, xylanases, and cellulase), contributes to improved digestive efficiency and reduced viscosity of intestinal contents, thus decreasing the amount of fat absorbed into the bloodstream. This results in a decrease in the concentration of cholesterol, LDL, triglycerides, and glucose (Erdaw *et al*., 2016). The significant increase in HDL concentration in the T4 treatment may be explained by the added enzymes that break down non-starch compounds. This improves both digestion and nutrient absorption. This leads to improved fat and energy utilization. This contributes to improved lipid metabolism in the blood serum, reflected in the increased HDL concentration (Chen *et al*., 2023).

Table (1) Effect of using enzyme-enhanced soy husks on the concentration of cholesterol, LDL, HDL, triglycerides and glucose of blood serum of broiler (Means ± standard error).

Treatments	Cholesterol (mg/ 100 ml)	LDL (mg/ 100 ml)	HDL (mg/ 100 ml)	Triglycerides (mg/ 100 ml)	Glucose (mg/ 100 ml)
T1	276.82±0.43 a	246.53±0.63 a	30.29±0.19 d	146.18±0.15 a	201.16±0.62 a
T2	267.39±1.16 c	234.98±0.89 c	32.41±0.36 b	143.75±0.17 c	196.44±0.46 c
T3	263.09±0.08 d	230.03±0.12 d	33.06±0.05 b	143.23±0.20 cd	195.18±0.34 cd
T4	260.71±0.35 e	226.06±0.39 e	34.65±0.04 a	142.77±0.14 d	193.96±0.42 d
T5	271.85±0.80 b	240.56±0.74 b	31.29±0.26 c	144.77±0.30 b	198.21±0.23 b
Sig.	*	*	*	*	*

Table (2) shows the effect of using soybean hulls enhanced with an enzyme mixture on the concentration of total protein, albumin, and globulin in broiler blood serum. A significant increase (P≤0.05) in protein, albumin, and globulin concentrations was observed in treatment T4, which was significantly superior (P≤0.05) to all other treatments. T3 also outperformed treatments T2 and T5. Meanwhile, the control treatment showed a significant decrease compared to the other treatments.

The noticeable improvement is attributed to the addition of soybean hulls enhanced with an enzyme mixture (β-glucanase, xylanase, and cellulase), which led to a clear improvement in protein digestibility and increased absorption of essential amino acids compared to the control treatment. This is due to the synergistic role of these enzymes in breaking down non-starch polysaccharides (NSPs). These enzymes are important and effective additives that contribute to improving digestive efficiency and nutrient utilization in poultry diets (Velázquez-De Lucio *et al*., 2021). This effect is reflected in improved serum albumin and total protein concentrations compared to the control treatment, indicating an improvement in the birds' physiological and nutritional status.

A study by Walker *et al.* (2024) showed that introducing a mixed enzyme product into broiler diets led to improved production performance, metabolizable energy, and increased calcium and phosphorus retention. This reflects an overall improvement in metabolism and nutrient utilization. It confirms that improved digestion by enzyme mixtures positively impacts the birds' overall physiological state, including increased serum total protein and albumin. Furthermore, the observed changes in globulin levels may indicate a functional improvement in the immune response, likely resulting from improved gut health and reduced intestinal stress, along with support. Microbial balance in the digestive system.

Table (2) Effect of using soy husks enhanced with enzyme mixture on the concentration of albumin, globulin and total protein in broiler blood serum (mean ± standard error).

Treatments	Protein (gm/ 100 ml)	Albumin (gm/ 100 ml)	Globulin (gm/ 100 ml)
T1	3.84±0.00 e	2.03±0.008 e	1.80±0.008 e
T2	4.14±0.01 c	2.11±0.006 c	2.03±0.005 c
T3	4.26±0.01 b	2.18±0.008 b	2.08±0.005 b
T4	4.42±0.01 a	2.29±0.011 a	2.13±0.008 a
T5	4.02±0.02 d	2.07±0.008 d	1.94±0.014 d
Sig.	*	*	*

Table (3) shows the effect of using soybean husks enhanced with an enzyme mixture on the concentration of malondialdehyde, glutathione peroxidase, and catalase enzyme in broiler blood serum. A significant increase ($P \leq 0.05$) in malondialdehyde concentration was observed in the first treatment (control) compared to treatments T2, T3, T4, and T5. Treatment T5, in turn, outperformed treatments T2, T3, and T4. Regarding glutathione peroxidase and catalase concentrations, a significant increase ($P \leq 0.05$) was observed T4 compared to the other treatments. Treatment T3, in turn, outperformed treatments T2 and T5.

The current results indicate that the addition of soybean husks enhanced with an enzyme mixture (β-glucanase, xylanase, and cellulase) significantly affected the biomarkers of oxidative balance in broiler blood serum. Meat. Enzymatic enhancement led to a significant decrease in malondialdehyde (MDA) concentration compared to the control treatment. This indicates a reduction in lipid peroxidation and improved protection against oxidative stress in living tissues. This is consistent with studies showing that improving oxidative balance through antioxidant-enriched diets can enhance antioxidant enzyme activity and lower MDA.

GPx and CAT concentrations were found to increase with antioxidant dietary support, thus reducing the harmful oxidation of proteins and lipids. The significant increase in glutathione peroxidase (GPx) and catalase (CAT) activity in treatments T3 and T4 compared to the control treatment, reflects an improved ability of the endogenous immune-oxidative system to combat free radicals, and increased elimination of hydrogen peroxide (H_2O_2) and superoxide radicals before they cause cell damage (Delles *et al*., 2014).

The marked improvement in antioxidant enzyme activity in the added treatments is attributed to the vital role of enzyme additives in improving gastrointestinal health and nutrient utilization efficiency. This interpretation aligns with that of Moita and Kim (2022), who explained that feed enzymes such as xylanase contribute to the breakdown of non-starch sugars and reduce the viscosity of intestinal contents, thus improving nutrient absorption. This, in turn, leads to a reduction in free radical (ROS) production and enhanced oxidative balance in broiler chickens.

Table (3) Effect of using soy husks enhanced with enzyme mixture on the concentration of malondialdehyde, glutathione and catalase enzyme of broiler blood serum (mean ± standard error).

Treatments	Malondialdehyd (mg/dl)	Peroxidase (U/L)	Catalase (IU)
T1	4.91±0.03 a	33.02 ±0.06 e	45.63±0.10 e
T2	4.37±0.03 c	34.29±0.06 c	47.42±0.12 c
T3	4.17±0.04 d	34.78±0.03 b	48.17 ± 0.02 b
T4	3.83 ±0.05 e	35.14 ±0.03 a	49.09 ± 0.03 a
T5	4.65±0.05 b	33.62 ±0.08 d	46.73±0.12 d
Sig.	*	*	*

Table (4) shows the effect of using soybean husks enhanced with an enzyme mixture on some biochemical indicators of blood serum in broiler chickens. The table shows a significant increase ($P \leq 0.05$) in uric acid levels in the first treatment (control) compared to treatments T2, T3, T4, and T5. Treatment T5 outperformed treatments T2, T3, and T4. The table also shows a significant increase ($P \leq 0.05$) in ALP enzyme levels in the first treatment (control) compared to treatments T2, T3, T4, and T5. The table also shows a significant increase in treatments T2 and T5 compared to treatments T3 and T4. Regarding the ALT enzyme, control treatment significantly outperformed ($P \leq 0.05$) all other treatments.

The results of this study show that the control treatment exhibited a significant increase ($P \leq 0.05$) in uric acid concentration and the ALT and ALP enzymes compared to the treatments containing soybean hulls enhanced with a mixture of enzymes (β-glucanase, xylanase, and cellulase). This indicates a clear effect of improving the digestibility of non-starch fiber (NSP) on the metabolic status of broiler chickens. The elevated uric acid levels in the control treatment are attributed to impaired protein utilization due to the high undigested fiber content in soybean hulls. This leads to increased use of amino acids for energy production at the expense of protein synthesis. Excess nitrogen is converted to uric acid as the end product of nitrogen metabolism in birds in treatments T2, T3, T4, and T5. The use of fiber-degrading enzymes improved protein digestion and the release of nutrients trapped within plant cell walls. This was reflected in a significant decrease in uric acid concentration in treatments T2–T5, and more pronouncedly in treatment T5.

The significant increase in ALP enzyme levels in the control treatment is interpreted as an indicator of increased metabolic stress associated with impaired mineral absorption and impaired liver and intestinal function. Conversely, the decrease in ALP levels in the enzyme treatments reflects improved physiological status and digestive efficiency. This improvement is attributed to reduced viscosity of intestinal contents and enhanced absorption of calcium and phosphorus. As for the ALT enzyme, it showed a significant increase in the control treatment compared to all other treatments, indicating an increased functional burden on the liver due to the accumulation of unwanted metabolic byproducts. Conversely, its decrease in the enzyme-enriched treatments, particularly T5, suggests the role of enzymes in reducing liver stress and improving overall health (Hajati *et al*., 2009).

These results are consistent with recent studies confirming that adding NSP enzymes to poultry diets improves liver function and reduces markers of liver damage, by improving digestion and reducing the production of enterotoxins, this is reflected positively in the biochemical blood indicators of broiler chickens (Yi *et al*., 2024).

Table (4) Effect of using soy husks enhanced with enzyme mixture on some biochemical indicators of blood serum in broiler chickens (mean ± standard error).

Treatments	Uric acid (mg/dl)	ALP	ALT
T1	82.90±0.0 a	61.18±0.35 a	16.98±0.05 a
T2	77.98±0.47 c	58.90±0.04 b	15.33±0.10 c
T3	76.54±0.29 d	57.39±0.31 c	14.56±0.16 d
T4	74.21±0.55 e	55.47±0.07 d	13.95±0.04 e
T5	80.58±0.24 b	59.48±0.26 b	15.85±0.06 b
Sig.	*	*	*

Table (5) shows the effect of using soybean husks enhanced with an enzyme mixture on the calcium and phosphorus concentrations in broiler blood serum. The results in the table show that treatment T4 significantly outperformed treatments T1, T2, T3, and T5 in terms of calcium and phosphorus concentrations ($P \leq 0.05$). T3, in turn, significantly outperformed treatments T2 and T5 ($P \leq 0.05$). However, treatments T2 and T5 did not differ significantly in terms of phosphorus concentration. These results can be explained by the known digestive and absorption processes and the role of exogenous enzymes in poultry nutrition.

Mineral elements such as phosphorus and calcium are found in plant feeds in large quantities as complex compounds within plant cell walls. They are particularly bound to non-starch fibers and compounds such as phytic acid, thus reducing their digestibility for birds. The enzymes β-glucanase, xylanase, and cellulase work to break down these complex fibers (arabinoxylans and β-glucans), which form the cell walls of feed ingredients such as grains and soybean hulls. This process releases the nutrients and minerals trapped within these fibers, facilitating their access to absorption sites in the digestive tract (This is achieved by reducing the viscosity of the intestinal contents and increasing the absorption surface area). This is reflected in the improved absorption of calcium and phosphorus and the higher serum concentrations of these minerals in the T4 treatment group compared to other treatments. These results are consistent with recent studies examining the supplementation of multiple enzymes in poultry feed.

One study indicated that using an enzyme mixture including β-glucanase and xylanase, enhanced with other enzyme activities, led to improved phosphorus and calcium retention in the diet and enhanced their availability without negatively impacting bird productivity, while simultaneously reducing phosphorus excretion (Walker *et al*., 2024). Studies have shown that xylanase can produce hydrolysis products such as xylo-oligosaccharides, which act as prebiotics, promoting gut health and indirectly improving mineral absorption. This is achieved by improving the intestinal environment and reducing fiber-associated antinutrients (Walker et al., 2024). Therefore, the higher levels of these enzymes in treatment T4 likely provided optimal conditions for the breakdown of complex fibers and the more efficient release of phosphorus and calcium compared to other treatments. Meanwhile, the levels in treatment T3 were sufficient only to improve phosphorus retention compared to treatment T2.

Table (5) Effect of using soy husks enhanced with enzyme mixture on calcium and phosphorus concentration in broiler blood serum (mean ± standard error).

Treatments	Phosphorus	Calcium
T1	6.10±6.05 d	12.30±12.22 e
T2	6.37±6.32 c	12.97±12.88 c
T3	6.73±6.61 b	13.21 ±13.06 b
T4	7.12±6.97 a	13.26±13.21 a
T5	6.25±6.22 c	12.56±12.51 d
Sig.	*	*

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