*International Journal of Bio-resource and Stress Management* https://ojs.pphouse.org/index.php/IJBSM

Crossref

*IJBSM November* 2025, 16(11): 01-09 **Article** AR6560

**Research Article** *Stress Management*

DOI: HTTPS://DOI.ORG/10.23910/1.2025.6560

# Effect of Exogenous Enzyme Supplementation on Haemato-biochemical Parameters and Antioxidant Status in Dual-type Laying Hens Fed Wheat Bran-based Diets

**Aditya Raj[1], Kaushalendra Kumar[1], Sanjay Kumar[1], Pankaj Kumar Singh[1], Ravi Ranjan Kumar Sinha[2], Dharmendra Kumar[1] and Sankhanath Koley[1]**

[1]Dept. of Animal Nutrition, [2]Dept. of Livestock Production and Management, Bihar Veterinary College, Bihar Animal Sciences University, Patna, Bihar (800 014), India

Open Access

**Corresponding** drkaushalbvc@gmail.com

0000-0003-0549-6896

## ABSTRACT

The experiment was conducted during March to August months in the year 2024 at Poultry Research and Training Centre, Bihar Animal Sciences University, Patna, India to evaluate the influence of phytase and xylanase supplementation on haemato-biochemical parameters and antioxidant status in Vanaraja laying hens. A total of 280 hens were randomly allocated to seven dietary groups, each consisting of 40 birds (five replicates of eight hens), study aimed to evaluate the effects of dietary supplementation of phytase, xylanase, and their combinations on haemato-biochemical parameters and serum antioxidant status in dual-purpose laying hens (Vanaraja) fed a wheat bran-based diet under farm conditions. The dietary treatments included: $T_1$ (control, basal ration), $T_2$ (basal ration+400FTU $kg^{-1}$ phytase), $T_3$ (basal ration+1200FTU $kg^{-1}$ phytase), $T_4$ (basal ration+1000XU $kg^{-1}$ xylanase), $T_5$ (basal ration+3000XU $kg^{-1}$ xylanase), $T_6$ (basal ration+400FTU $kg^{-1}$ phytase+1000XU $kg^{-1}$ xylanase), and $T_7$ (basal ration+1200FTU $kg^{-1}$ phytase+3000XU $kg^{-1}$ xylanase). Birds received a fixed amount of feed and ad libitum access to water throughout the 24-week trial. Haematological parameters remained unaffected across all treatments. Serum biochemical indices, including total protein, albumin, AST, ALT, and ALP, showed no significant differences ($p$>0.05). Serum mineral concentrations were generally comparable, except for calcium and phosphorus levels, which significantly increased ($p$<0.05) in enzyme-supplemented groups in a dose-dependent manner. Antioxidant indices were unaffected; however, GSH levels were significantly elevated ($p$<0.05) in certain enzyme-treated groups compared to the control. In conclusion, supplementation with phytase and xylanase, alone or in combination, proved beneficial without adverse effects on blood metabolites. However, higher enzyme inclusion beyond the optimal level did not confer additional benefits and may not be warranted in Vanaraja laying hens.

**KEYWORDS:** Antioxidants, haemato-biochemical, phytase, Vanaraja laying hen, xylanase

***Citation* (VANCOUVER):** Raj et al., Effect of Exogenous Enzyme Supplementation on Haemato-biochemical Parameters and Antioxidant Status in Dual-type Laying Hens Fed Wheat Bran-based Diets. *International Journal of Bio-resource and Stress Management,* 2025; 16(11), 01-09. HTTPS://DOI.ORG/10.23910/1.2025.6560.



***Data Availability Statement:*** Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

***Conflict of interests:*** The authors have declared that no conflict of interest exists.

RECEIVED on 15th July 2025 RECEIVED in revised form on 17th October 2025 ACCEPTED in final form on 02nd November 2025 PUBLISHED on 15th November 2025

## 1. INTRODUCTION

Poultry production is one of the fastest-growing sectors within agriculture, playing a vital role in ensuring nutritional security, particularly in developing countries, by addressing protein and calorie deficiencies in the human population. Feed constitutes approximately 60-70% of the total cost of poultry production, with cereals and vegetable protein sources forming the bulk of poultry rations. However, about 30% of the composition of these plant-based feedstuffs includes non-starch polysaccharides (NSPs) (Baker et al., 2021; Papadopoulos et al., 2022), and nearly 70% of the total phosphorus in grains and their byproducts exists in the form of phytate (Vieira et al., 2016). Due to their simple monogastric digestive system, chickens are unable to efficiently digest complex compounds such as NSPs and phytate-bound phosphorus (Moghaddam et al., 2025). To enhance nutrient availability, exogenous enzymes like phytase and xylanase are commonly supplemented in poultry diets (Selle et al., 2009; Kayan et al., 2025). Phytase facilitates the hydrolysis of phytic acid, thereby releasing phosphorus, minerals, amino acids, proteins, and starch. Xylanase, on the other hand, breaks β-1,4-xylan bonds in plant cell walls, liberating encapsulated nutrients such as starch, lipids, and proteins, thus improving energy availability (Cowieson, 2005; Nguyen et al., 2021). These enzymatic actions help mitigate the anti-nutritional effects of phytate and NSPs, potentially improving gut health and nutrient absorption (Nguyen et al., 2022). While phytase alone may not significantly impact intestinal morphology (Pirgozliev et al., 2008), its combination with xylanase may have synergistic benefits (Huang et al., 2024). Xylanase improves phytase access to its substrate by degrading NSPs, and phytase, in turn, enhances xylanase efficacy by freeing nutrients otherwise bound to phytate (Schramm et al., 2017).

Beyond income generation, backyard poultry farming plays a significant role in alleviating malnutrition among rural populations by providing a readily available source of high-quality animal protein (Eltahan et al., 2023). It also serves as a powerful tool for the socio-economic empowerment of rural women (Besbes et al., 2012). Despite relatively low productivity compared to commercial systems, backyard poultry contributes approximately 30–40% of total egg production in India (Panda et al., 2008). Among the dual-purpose breeds developed for backyard systems, Vanaraja, introduced by the Directorate of Poultry Research (DPR), Hyderabad, has shown excellent adaptability to rural environments. These birds exhibit commendable growth performance, moderate egg-laying capacity, and greater resistance to several common poultry diseases, making them highly suitable for low-input village-level poultry farming.

The search for sustainable alternatives to conventional poultry feed ingredients is gaining momentum worldwide, driven by increasing demand and feed resource constraints (El-Sabrout et al., 2023). Wheat bran (WB), a byproduct of the milling industry, has emerged as a promising candidate due to its high dietary fiber content and beneficial effects on gut health. In addition to fiber, WB contains substantial amounts of protein, starch, minerals, and bioactive compounds, with global production estimated at approximately 150 mt annually (Wanzenbock et al., 2017). Rising competition between food and feed crops has further incentivized the use of agro-industrial by-products like WB in animal nutrition (Kraler et al., 2015). However, the application of WB in poultry diets remains limited due to its high fiber content and associated anti-nutritional effects (Idan et al., 2023). To address these limitations, supplementation with exogenous enzymes such as phytase and xylanase may enhance the digestibility and nutrient bioavailability of WB-based diets. While individual effects of these enzymes are relatively well-studied, limited research has investigated their combined use in wheat bran-enriched diets for laying hens. We hypothesized that the inclusion of phytase and xylanase would improve the nutritional utilization of WB, as reflected in the serum biochemical and antioxidant profiles of laying hens-potentially supporting its partial replacement of conventional feed ingredients like corn and reducing overall production costs. Therefore, this study aimed to evaluate the effects of phytase and xylanase supplementation on haemato-biochemical parameters and antioxidant status in Vanaraja laying hens fed wheat bran-based diets under farm conditions.

## 2. MATERIALS AND METHODS

This animal experiment was conducted following the ethical standards and guidelines approved by the Institutional Animal Ethics Committee (IAEC) of Bihar Animal Sciences University, India (Approval No. IAEC/BVC/2024/23).

### *2.1. Experimental materials*

For the experiment, the exogenous enzymes phytase (ADPHOS™) and xylanase (BG-XYLAN™) were procured from Advanced Bio-Agro Tech Ltd. (ABTL), Pune, India.

### *2.2. Feeding, management, dietary treatment and laboratory analysis*

The experiment was conducted over a 24-week period, from March to August, 2024, at the Poultry Research and Training Centre, Bihar Animal Sciences University, Patna, India (25.5984° N latitude, 85.0840° E longitude). A total of 280 Vanaraja laying hens (22 weeks old) were individually weighed and randomly assigned to seven treatment groups,

with 40 birds per group (five replicates of eight birds each). The birds were fed iso-nutritive diets formulated as follows: $T_1$ (control), $T_2$ (400FTU $kg^{-1}$ phytase), $T_3$ (1200FTU $kg^{-1}$ phytase), $T_4$ (1000XU $kg^{-1}$ xylanase), $T_5$ (3000XU $kg^{-1}$ xylanase), $T_6$ (400FTU $kg^{-1}$ phytase+1000XU $kg^{-1}$ xylanase), and $T_7$ (1200FTU $kg^{-1}$ phytase+3000XU $kg^{-1}$ xylanase). Birds were reared on a deep litter system using dried sawdust at a depth of 3–4 inches and were provided with proper lighting and ventilation throughout the study. All standard management practices, including a recommended vaccination schedule, were strictly followed. A fixed quantity of feed was offered daily, along with ad libitum access to clean drinking water. Feed ingredients and compounded diets were analysed for their proximate composition using standard procedures outlined by Anonymous (1995). Parameters measured included dry matter (DM; ID 930.15), organic matter (OM), total ash and acid-insoluble ash (ID 942.05), crude protein (CP; N×6.25, ID 954.01), ether extract (EE; ID 920.39), crude fibre, and nitrogen-free extract (NFE). Calcium and phosphorus contents were determined using the modified method of Talapatra et al. (1940). Feed formulations were prepared according to the Bureau of Indian Standards (Anonymous, 2007) guidelines, based on analysed crude protein values and standard metabolizable energy requirements. Experimental diets were re-analysed in the laboratory to confirm nutrient composition. The major ingredients used in the formulation included yellow maize, soybean meal, wheat bran, de-oiled rice bran, soybean oil, common salt, calcite powder, mineral mixture, and feed additives (Tables 1 and 2).

### *2.3. Haemato-biochemical and antioxidant profiles*

At the end of the 24-week trial, blood samples were collected from thirty-five birds, with five birds randomly selected from each treatment group. Blood was drawn from the wing vein using sterile disposable syringes and collected into two sets of vials - one containing EDTA as an anticoagulant and the other without anticoagulant. Samples collected in EDTA vials were used immediately for haematological analyses. Haemoglobin (Hb) concentration was determined using the cyanmethemoglobin method (Drabkin and Austin, 1932), while packed cell volume (PCV) was estimated using the micro-haematocrit method (Campbell, 1995). Total erythrocyte count (TEC) and total leukocyte count (TLC) were measured using a Neubauer haemocytometer following the method of Natt and Herrick (1952). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated using standard formulae based on Hb, PCV, and TEC values. Blood samples collected in non-anticoagulant vials were allowed to clot and then centrifuged at 1500 rpm for 15 minutes to separate the serum. The serum was analysed for biochemical parameters, including total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), calcium, phosphorus, magnesium, sodium, potassium, chloride, copper, and zinc. These were estimated using commercial colorimetric kits and a UV-visible double beam spectrophotometer (Model 2205, Systronics, India). Oxidative stress markers were also assessed. Catalase and superoxide dismutase (SOD) activities were determined according to the methods of Cohen et al. (1970) and Madesh and Balasubramanian (1997), respectively. Serum concentrations of malondialdehyde (MDA) and reduced glutathione (GSH) were measured following the procedures described by Suleiman et al. (1996) and Lin et al. (1988), respectively.

### *2.4. Statistical analysis*

All data were statistically analyzed using the Statistical Package for the Social Sciences (SPSS, version 20.0; SPSS Inc., 2011). A generalized linear model (GLM) analysis of variance (ANOVA) was employed to compare differences among the treatment groups. When significant effects were observed, Duncan's multiple range test was used for post-hoc comparisons to determine specific group differences. The statistical procedures followed were following the methodology outlined by Snedecor and Cochran (1994).

## 3. RESULTS AND DISCUSSION

The haematological and serum biochemical parameters, including haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total protein, albumin, globulin, albumin-to-globulin (A:G) ratio, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) in the experimental birds are presented

Table 1: Chemical composition of feed ingredients used in experiment (% on DM basis)

| Ingredients | DM | OM | CP | EE | CF | TA | AIA | NFE | Ca | P |
|---|---|---|---|---|---|---|---|---|---|---|
| Yellow maize | 91.18 | 97.11 | 9.79 | 4.19 | 2.26 | 2.89 | 1.16 | 80.87 | 0.11 | 0.43 |
| Soyabean meal | 91.66 | 94.02 | 46.04 | 0.46 | 5.61 | 5.98 | 1.19 | 41.91 | 0.36 | 0.64 |
| Wheat bran | 90.53 | 95.54 | 14.19 | 2.41 | 10.47 | 4.46 | 1.14 | 68.47 | 0.39 | 1.13 |
| De-oiled rice bran | 92.24 | 93.43 | 13.11 | 1.09 | 13.65 | 6.57 | 4.86 | 65.58 | 0.09 | 0.87 |

Table 2: Percentage composition of different experimental diets

| Ingredients | Layer phase-I |
|---|---|
| Yellow maize | 33.00 |
| Soya bean meal | 24.00 |
| Wheat bran | 20.00 |
| De-oiled rice bran | 9.00 |
| Vegetable oil | 3.00 |
| Common salt | 0.40 |
| Di-calcium phosphate | 1.00 |
| Calcite powder | 8.00 |
| DL-Methionine | 0.20 |
| Lysine | 0.40 |
| Mineral mixture* | 0.50 |
| Premix* | 0.50 |
| Total | 100 |
| Calculated value | |
| CP (%) | 18.10 |
| ME (kcal $kg^{-1}$) | 2565.00 |
| CF (%) | 8.36 |
| Ca (%) | 3.15 |
| Av. P (%) | 0.64 |
| Lysine (%) | 0.73 |
| Methionine (%) | 0.34 |

*Composition of mineral mixture and premix: Vitamin A (7,00,000 I.U.); Vitamin $D_3$ (70,000 I.U.); Vitamin E (250 mg); Nicotinamide (1000 mg); Vitamin $B_1$ (2 mg); Vitamin $B_2$ (4 mg); Niacin (60 mg); Pantothenic acid (10 mg); Cyanocobalamin (10 µg); Choline (500 mg); Cobalt (150 mg); Copper (1200 mg); Iodine (325 mg); Iron (1500 mg); Potassium (100 mg); Magnesium (6000 mg); Manganese (1500 mg); Selenium (10 mg); Sodium (5.9 mg); Sulfur (0.72%); Zinc (9600 mg); Calcium (25.5%) and Phosphorus (12.75%)

in Table 3. Average haemoglobin and PCV values ranged from 9.65 to 10.10 g $dl^{-1}$ and 28.98 to 30.06%, respectively, with no statistically significant differences ($p$>0.05) among the treatment groups. However, haemoglobin levels showed a moderate numerical increase in birds receiving enzyme supplementation, particularly in group $T_4$ (4.12%) and $T_5$ (4.12%), followed by $T_7$ (4.02%), $T_3$ (2.02%), and $T_6$ (1.96%) compared to the control. TEC and TLC values ranged from 3.13 to 3.49×$10^6$ $\mu l^{-1}$ and 11.37 to 12.54×$10^3$ $\mu L^{-1}$, respectively, and were comparable across all groups. Similarly, MCV, MCH, and MCHC values varied from 83.91 to 94.19 FL, 27.85 to 31.55 pg., and 33.07 to 34.28 g $dL^{-1}$, respectively, with no significant differences observed among the treatments. The serum biochemical parameters-total protein (3.26–3.78 g $dl^{-1}$), albumin (1.73–1.82 g $dl^{-1}$), globulin (1.53–1.72 g $dl^{-1}$), A:G ratio (1.08–1.13), AST (164.38–190.84U $l^{-1}$), ALT (22.02–25.86U $l^{-1}$), and ALP (14.77–16.94U $l^{-1}$) were also found to be statistically non-significant ($p$>0.05) among the treatment groups when compared to the control, indicating no adverse effect of enzyme supplementation on these parameters.

The serum mineral profile of the experimental Vanaraja laying hens, including calcium, phosphorus, calcium-to-phosphorus ratio (Ca:P), magnesium, sodium, potassium, chloride, copper, and zinc, is presented in Table 4. The average serum calcium and phosphorus concentrations ranged from 9.59 to 11.23 mg $dl^{-1}$ and 6.14 to 7.14 mg $dl^{-1}$, respectively. A significant effect of enzyme supplementation ($p$<0.05) was observed on both calcium and phosphorus levels. The highest increases were recorded in group $T_7$, with calcium and phosphorus levels elevated by 17.10% and 16.32%, respectively, compared to the control. This was followed by $T_6$ (15.64%, 14.04%), $T_5$ (13.45%, 11.76%), $T_4$ (10.22%, 8.34%), $T_3$ (8.24%, 7.53%), and $T_2$ (3.75%, 4.59%), respectively. Other serum minerals including Ca:P ratio (1.55–1.60), magnesium (3.92–4.26 mmol $l^{-1}$), sodium (129.79–137.15 mmol $l^{-1}$), chloride (109.10–118.32mmol $l^{-1}$), copper (0.31–0.34 ppm), and zinc (2.33–2.46 ppm) showed no significant differences ($p$>0.05) among treatment groups. However, serum potassium levels displayed a near-significant difference ($p$=0.052), with numerically higher values observed in $T_4$ (18.70%), $T_2$ (7.16%), and $T_3$ (3.02%) compared to the control group. These findings suggest that enzyme supplementation, particularly in higher combinations, can enhance calcium and phosphorus absorption, while exerting minimal effects on other serum mineral concentrations.

The serum antioxidant profile of Vanaraja laying hens, including lipid peroxidation (LPO), superoxide dismutase (SOD), reduced glutathione (GSH), lactate dehydrogenase (LDH), catalase, and total antioxidant capacity (T-AOC), is presented in Table 4. The average values recorded across treatment groups were as follows: LPO (1.68–2.61 nM MDA $ml^{-1}$), SOD (197.49–248.55 U $l^{-1}$), GSH (0.40–0.50 mM $ml^{-1}$), LDH (425.68–519.56 U $l^{-1}$), catalase (21.56–35.02 U $ml^{-1}$), and T-AOC (8.49–9.48 mM $ml^{-1}$). Overall, the effect of enzyme supplementation on most serum antioxidant parameters was statistically non-significant ($p$>0.05), indicating comparable antioxidant activity across treatment groups. However, GSH levels showed a significant increase ($p$<0.05), with the highest elevation observed in group $T_6$ (26.13%) compared to the control, followed by $T_7$ (18.09%), $T_3$ (18.09%), $T_4$ (15.08%), $T_5$ (10.55%), and $T_2$ (9.05%). Although the LPO levels did not differ significantly among

Table 3: Effects of different levels of phytase and xylanase supplementation on haemato-biochemical indices in Vanaraja laying hens

| Attributes | $T_1$ | $T_2$ | $T_3$ | $T_4$ |
|---|---|---|---|---|
| Haematology | 91.18 | 97.11 | 9.79 | 4.19 |
| Haemoglobin (g $dl^{-1}$) | 9.70±0.25 | 9.65±0.19 | 9.90±0.37 | 10.10±0.31 |
| Packed cell volume (%) | 29.33±0.48 | 29.08±0.55 | 28.98±0.80 | 29.47±0.81 |
| Total erythrocyte count (x$10^6$ µl) | 3.13±0.14 | 3.49±0.18 | 3.15±0.17 | 3.23±0.16 |
| Total leucocyte count (x$10^3$ µL) | 11.37±0.61 | 11.84±0.54 | 12.54±0.81 | 11.78±0.55 |
| Mean corpuscular volume (FL) | 94.19±3.13 | 83.91±3.57 | 92.53±2.54 | 91.84±3.38 |
| Mean corpuscular hemoglobin (pg) | 31.10±0.78 | 27.85±1.18 | 31.55±0.69 | 31.44±0.92 |
| Mean corpuscular hemoglobin concentration (g $dl^{-1}$) | 33.07±0.42 | 33.19±0.09 | 34.13±0.53 | 34.28±0.41 |
| Serum biochemistry | | | | |
| Total protein (g $dl^{-1}$) | 3.26±0.08 | 3.47±0.02 | 3.56±0.31 | 3.71±0.13 |
| Albumin (g $dl^{-1}$) | 1.73±0.10 | 1.74±0.07 | 1.75±0.08 | 1.76±0.12 |
| Globulin (g $dl^{-1}$) | 1.53±0.03 | 1.54±0.03 | 1.56±0.07 | 1.57±0.03 |
| Albumin: Globulin ratio | 1.13±0.08 | 1.13±0.05 | 1.12±0.03 | 1.12±0.07 |
| Aspartate aminotransferase (U $l^{-1}$) | 187.84±3.03 | 179.82±0.87 | 164.38±2.70 | 183.22±13.73 |
| Alanine aminotransferase (U $l^{-1}$) | 24.28±1.50 | 25.86±0.15 | 25.22±0.58 | 25.42±3.46 |
| Alkaline phosphatase (U $l^{-1}$) | 15.44±1.24 | 15.02±1.20 | 14.77±1.20 | 16.94±1.71 |

| Attributes | $T_5$ | $T_6$ | $T_7$ | SEm± | *p*-value |
|---|---|---|---|---|---|
| Haematology | 2.26 | 2.89 | 1.16 | 80.87 | 0.11 |
| Haemoglobin (g $dl^{-1}$) | 10.10±0.17 | 9.89±0.28 | 10.09±0.17 | 0.37 | 0.779 |
| Packed cell volume (%) | 30.06±0.47 | 29.08±0.63 | 29.73±0.56 | 0.89 | 0.878 |
| Total erythrocyte count (x$10^6$ µl) | 3.43±0.08 | 3.31±0.16 | 3.35±0.13 | 0.21 | 0.545 |
| Total leucocyte count (x$10^3$ µl) | 11.75±0.69 | 12.00±0.55 | 12.12±0.48 | 0.87 | 0.902 |
| Mean corpuscular volume (FL) | 87.74±2.52 | 88.42±3.27 | 89.26±2.75 | 4.31 | 0.294 |
| Mean corpuscular hemoglobin (pg) | 29.47±0.79 | 30.04±1.06 | 30.31±1.00 | 1.32 | 0.105 |
| Mean corpuscular hemoglobin concentration (g $dl^{-1}$) | 33.59±0.14 | 33.98±0.30 | 33.95±0.34 | 0.49 | 0.135 |
| Serum biochemistry | | | | | |
| Total protein (g $dl^{-1}$) | 3.49±0.13 | 3.78±0.23 | 3.74±0.08 | 0.24 | 0.328 |
| Albumin (g $dl^{-1}$) | 1.78±0.03 | 1.80±0.07 | 1.82±0.04 | 0.11 | 0.977 |
| Globulin (g $dl^{-1}$) | 1.60±0.08 | 1.62±0.09 | 1.72±0.11 | 0.09 | 0.539 |
| Albumin: Globulin ratio | 1.12±0.04 | 1.12±0.03 | 1.08±0.08 | 0.08 | 0.955 |
| Aspartate aminotransferase (U $l^{-1}$) | 190.74±11.36 | 186.34±21.12 | 190.84±6.45 | 15.33 | 0.632 |
| Alanine aminotransferase (U $l^{-1}$) | 24.58±2.17 | 25.32±3.43 | 22.02±2.04 | 3.17 | 0.917 |
| Alkaline phosphatase (U $l^{-1}$) | 16.23±1.65 | 15.77±1.85 | 15.80±2.47 | 2.37 | 0.976 |

groups ($p$=0.104), a numerical reduction was observed in enzyme-supplemented birds. The $T_4$ group exhibited the greatest decrease in LPO activity (35.76%), followed by $T_5$ (33.38%), $T_6$ (25.88%), $T_3$ (18.68%), $T_7$ (15.31%), and $T_2$ (3.52%) in comparison to the control ($T_1$). These findings suggest that supplementation of phytase and xylanase, particularly in combination, may exert beneficial effects on oxidative stress biomarkers, as indicated by elevated GSH levels and reduced lipid peroxidation, despite the lack of statistical significance in most parameters.

Fijabio et al. (2018) reported that graded levels of phytase supplementation (0, 250, 500, 750, and 1000FTU $kg^{-1}$) had no significant effect on the haemato-biochemical profiles of poultry. Similarly, Martínez et al. (2015) observed

Table 4: Effects of different levels of phytase and xylanase supplementation on serum minerals and antioxidant profiles of Vanaraja laying hens

| Attributes | $T_1$ | $T_2$ | $T_3$ | $T_4$ |
|---|---|---|---|---|
| Serum mineral profiles | | | | |
| Calcium (mg $dl^{-1}$) | $9.59^{a}$±0.22 | $9.95^{ab}$±0.29 | $10.38^{abc}$±0.12 | $10.57^{bc}$±0.32 |
| Phosphorus (mg $dl^{-1}$) | $6.14^{a}$±0.15 | $6.42^{ab}$±0.16 | $6.60^{abc}$±0.08 | $6.65^{abc}$±0.26 |
| Ca:P ratio | 1.57±0.05 | 1.55±0.07 | 1.57±0.01 | 1.60±0.06 |
| Magnesium (mmol $l^{-1}$) | 3.98±0.23 | 4.14±0.21 | 4.26±0.11 | 3.92±0.18 |
| Sodium (mmol $l^{-1}$) | 137.00±6.06 | 135.22±3.92 | 135.06±5.51 | 129.79±4.29 |
| Potassium (mmol $l^{-1}$) | $3.97^{a}$±0.13 | $4.25^{ab}$±0.10 | $4.09^{a}$±0.25 | $4.71^{b}$±0.05 |
| Chloride (mmol $l^{-1}$) | 114.62±3.20 | 109.10±3.09 | 115.81±2.22 | 118.32±3.54 |
| Copper (ppm) | 0.32±0.03 | 0.34±0.03 | 0.34±0.04 | 0.33±0.02 |
| Zinc (ppm) | 2.34±0.08 | 2.36±0.12 | 2.41±0.06 | 2.46±0.07 |
| Serum antioxidant profiles | | | | |
| Lipid peroxidation (nM MDA $ml^{-1}$) | 2.61±0.29 | 2.52±0.37 | 2.12±0.36 | 1.68±0.22 |
| Super oxide dismutase (U $l^{-1}$) | 211.14±27.20 | 236.74±8.24 | 212.91±17.33 | 216.88±22.78 |
| Reduced glutathione (mM $ml^{-1}$) | $0.40^{a}$±0.03 | $0.43^{ab}$±0.02 | $0.47^{bc}$±0.01 | $0.46^{bc}$±0.02 |
| Lactate dehydrogenase (U $l^{-1}$) | 425.68±23.61 | 461.88±41.12 | 528.75±31.95 | 485.70±40.02 |
| Catalase (U $ml^{-1}$) | 21.56±3.61 | 31.15±2.31 | 32.45±2.83 | 30.80±3.37 |
| Total antioxidant capacity (mM $ml^{-1}$) | 8.49±0.38 | 9.06±0.32 | 9.42±0.50 | 9.23±0.34 |

| Attributes | $T_5$ | $T_6$ | $T_7$ | SEm± | *p*-value |
|---|---|---|---|---|---|
| Serum mineral profiles | | | | | |
| Calcium (mg $dl^{-1}$) | 10.88c±0.37 | 11.09c±0.36 | 11.23c±0.31 | 0.42 | 0.004 |
| Phosphorus (mg $dl^{-1}$) | 6.86bc±0.21 | 7.00c±0.12 | 7.14c±0.21 | 0.25 | 0.008 |
| Ca:P ratio | 1.59±0.06 | 1.59±0.05 | 1.58±0.05 | 0.07 | 0.998 |
| Magnesium (mmol $l^{-1}$) | 3.95±0.11 | 4.05±0.28 | 4.03±0.29 | 0.30 | 0.929 |
| Sodium (mmol $l^{-1}$) | 132.75±3.52 | 137.15±4.63 | 135.54±3.94 | 6.55 | 0.925 |
| Potassium (mmol $l^{-1}$) | 3.92a±0.28 | 3.97a±0.23 | 3.89a±0.14 | 0.26 | 0.052 |
| Chloride (mmol $l^{-1}$) | 116.73±3.72 | 116.71±4.06 | 111.06±3.02 | 4.68 | 0.433 |
| Copper (ppm) | 0.31±0.01 | 0.33±0.03 | 0.33±0.04 | 0.04 | 0.996 |
| Zinc (ppm) | 2.39±0.24 | 2.35±0.30 | 2.33±0.18 | 0.24 | 0.998 |
| Serum antioxidant profiles | | | | | |
| Lipid peroxidation (nM MDA $ml^{-1}$) | 1.74±0.20 | 1.94±0.14 | 2.21±0.08 | 0.36 | 0.104 |
| Super oxide dismutase (U $l^{-1}$) | 248.55±7.45 | 220.99±8.81 | 197.49±19.44 | 24.71 | 0.477 |
| Reduced glutathione (mM $ml^{-1}$) | 0.44ab±0.01 | 0.50c±0.01 | 0.47bc±0.02 | 0.03 | 0.021 |
| Lactate dehydrogenase (U $l^{-1}$) | 519.56±35.17 | 489.85±35.41 | 466.25±47.65 | 52.46 | 0.504 |
| Catalase (U/ml) | 35.02±3.89 | 31.79±3.86 | 33.54±3.59 | 4.80 | 0.169 |
| Total antioxidant capacity (mM $ml^{-1}$) | 9.25±0.34 | 9.48±0.21 | 9.35±0.56 | 0.56 | 0.639 |

[a,b,c]Values with different superscripts in a row differ significantly ($p<0.05$; $p<0.01$)

that the inclusion of 150 g $kg^{-1}$ wheat bran in pullet diets during the developmental phase did not significantly alter serum calcium, phosphorus, haematocrit, or haemoglobin levels ($p>0.05$). In contrast, Musapuor et al. (2005) found that phytase supplementation at 500 and 1000FTU $kg^{-1}$ reduced plasma alkaline phosphatase (ALP) levels. Lim

et al. (2020) investigated the effects of varying available phosphorus (AP) levels (0.20%, 0.25%, and 0.30%) with or without phytase supplementation in 40-week-old laying hens. While they observed no consistent effects on serum albumin, total protein, ALT, or AST, a significant linear decrease ($p$<0.05) in serum ALP was noted with phytase, along with a numerical increase in serum calcium and phosphorus levels, suggesting enhanced mineral utilization and improved egg production potential. Attia et al. (2020) demonstrated that supplementing broilers with multienzymes (0%, 0.1%, and 0.2%) under both standard and low-density diets significantly reduced ALT, AST, and malondialdehyde (MDA) levels while increasing packed cell volume and haemoglobin concentrations. The present study aligns well with these findings. Silversides et al. (2006) observed no significant changes in serum calcium, phosphorus, or magnesium levels with phytase (0–700 U $kg^{-1}$) and xylanase (0 or 2000 U $kg^{-1}$) supplementation in diets containing either adequate or reduced phosphorus. Conversely, Hassanien and Sanaa (2011) reported significant improvements in serum calcium, phosphorus, and magnesium with increasing phytase levels (500–1000FTU $kg^{-1}$), while serum potassium remained unaffected. In agreement with these previous studies, the current findings revealed that supplementation of phytase and xylanase-either individually or in combination-enhanced serum calcium and phosphorus concentrations in laying hens. The highest levels were observed in the group receiving the higher combination of both enzymes. This improvement is likely attributed to the enzymatic hydrolysis of phytate and non-starch polysaccharides, which enhances the release and absorption of bound minerals, thereby improving overall mineral bioavailability in the diet.

## 4. CONCLUSION

The most haematological, serum biochemical, and antioxidant parameters were not influenced by enzyme supplementation. However, a significant improvement in serum calcium and phosphorus concentrations was noted in hens receiving phytase (1200FTU $kg^{-1}$) and xylanase (3000XU $kg^{-1}$) together in a wheat bran-based diet.

## 5. ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to the Vice Chancellor of Bihar Animal Sciences University, Patna, India, for providing the essential facilities and financial support to carry out this experiment.

## 6. REFERENCES

Anonymous, 1995. AOAC., 1995. Methods of Analysis. 16th edn, Vol. 1. Association of Official Analytical Chemists, Washington, DC. Available at https://www.cabidigitallibrary.org/doi/full/10.5555/19951414840. Accessed date: 21/08/2025

Anonymous, 2007. BIS., 2007. Indian standard, poultry feeds specifications, IS-1374:2007. Bureau of Indian Standards. 9, Bahadur Shah Zafar Marg, Manak Bhawan, New Delhi, India. Available at: https://law.resource.org/pub/in/bis/S06/is.1374.2007.pdf . Access date: 21/08/2025.

Attia, Y.A., Al-Khalaifah, H., Abd El-Hamid, H.S., Al-Harthi, M.A., El-shafey, A.A., 2020. Effect of different levels of multienzymes on immune response, blood hematology and biochemistry, antioxidants status and organs histology of broiler chicks fed standard and low-density diets. Frontiers in Veterinary Science 6, 510. https://doi.org/10.3389/fvets.2019.00510.

Baker, J.T., Duarte, M.E., Holanda, D.M., Kim, S.W., 2021. Friend or foe? Impacts of dietary xylans, xylooligosaccharides, and xylanases on intestinal health and growth performance of monogastric animals. Animals 11(3), 609. https://doi.org/10.3390/ani11030609.

Besbes, B., Thieme, O., Rota, A., Gueye, E.F., Alders, R.G., 2012. Technology and programmes for sustainable improvement of village poultry production. In V. Sandilands and P.M. Hocking, eds. Alternative systems for poultry: Health, welfare and productivity, 110–127. Wallingford, CAB International.

Campbell, W.T., 1995. Avian haematology and cytology. Iowa State University Press, Ames, Iowa, 32. https://lib.ugent.be/catalog/rug01:000162992.

Cohen, G., Dembiec, D., Marcus, J., 1970. Measurement of catalase activity in tissue extracts. Analytical Biochemistry 34(1), 30–38. https://doi.org/10.1016/0003-2697(70)90083-7.

Cowieson, A.J., 2005. Factors that affect the nutritional value of maize for broilers. Animal Feed Science Technology 119, 293–305. http://dx.doi.org/10.1016/j.anifeedsci.2004.12.017.

Drabkin, D.L., Austin, J.H., 1932. Spectrophotometric constants for common haemoglobin derivatives in human, dog, and rabbit blood. Journal of Biological Chemistry 98(2), 719–733. https://doi.org/10.1016/S0021-9258(18)76122-X.

El-Sabrout, K., Khalifah, A., Mishra, B., 2023. Application of botanical products as nutraceutical feed additives for improving poultry health and production. Veterinary World 16(2), 369–379. https://doi.org/10.14202/vetworld.2023.369-379.

Eltahan, H.M., Cho, S., Rana, M.M., Saleh, A.A., Elkomy, A.E., Wadaan, M.A.M., Alagawany, M., Kim, I.H., Eltahan, H.M., 2023. Dietary exogenous phytase improve egg quality, reproductive hormones, and

prolongs the lifetime of the aging Hy-Line brown laying hens fed nonphytate phosphorus. Poultry Science 102(9), 102895. https://doi.org/10.1016/j.psj.2023.102895.

Fijabio, O.E., Fakayode, T.O., Eniola, O.M., Omojola, A.B., 2018. Performance, blood profile and microbial characteristics of broiler chicken fed phytase supplemented diets. Nigerian Journal of Animal Production 45(2), 129–136. https://doi.org/10.51791/njap.v45i2.504.

Hassanien, H.H.M., Sanaa, H.M.E., 2011. Comparison difference levels of phytase enzyme supplementation on laying hen performance, egg quality and some blood parameters. Asian Journal of Poultry Science 5(2), 77–85. http://dx.doi.org/10.3923/ajpsaj.2011.77.85.

Huang, Q., Yi, W., Fan, J., Chen, R., Ma, X., Chen, Z., Wu, W., Qian, L., 2024. Effects supplementation of novel multi-enzyme on laying performance, egg quality, and intestinal health and digestive function of laying hens. Poultry Science 103(12), 104461. https://doi.org/10.1016/j.psj.2024.104461.

Idan, F., Paulk, C.B., Pokoo-Aikins, A., Stark, C.R., 2023. Growth performance, intestinal morphometry, and blood serum parameters of broiler chickens fed diets containing increasing levels of wheat bran with or without exogenous multi-enzyme supplementation during the grower and finisher phases. Livestock Science 275, 105296. https://doi.org/10.1016/j.livsci.2023.105296.

Kayan, A., Prasongsook, S., Poeikhampha, T., 2025. Diet supplementation with phytase and xylanase on laying performance, egg quality, apparent metabolizable energy, and phosphorous use in laying hens. Veterinary World 18(1), 155–161. https://doi.org/10.14202/vetworld.2025.155-161.

Kraler, M., Schedle, K., Schwarz, C., Domig, K.J., Pichler, M., Oppeneder, A., Wetscherek, W., Pruckler, M., Pignitter, M., Pirker, K.F., Somoza, V., Heine, D., Kneifel, W., 2015. Fermented and extruded wheat bran in piglet diets: Impact on performance, intestinal morphology, microbial metabolites in chyme and blood lipid radicals. Archive of Animal Nutrition 69(5), 378–398. https://doi.org/10.1080/1745039x.2015.1075671.

Lim, I.K.C., Rana, M., Kang, H.K., Ryu, K.S., 2020. Effects of dietary available phosphorus levels and phytase supplementation on performance, egg quality and serum biochemical parameters of hy-line brown laying hens from 40 to 60 weeks of age. Korean Journal of Poultry Science 47(4), 255–265. http://dx.doi.org/10.5536/KJPS.2020.47.4.255.

Lin, H.M., Dillard, C.J., Tappel, A.L., 1988. Plasma SH and GSH measurement. Methods in Enzymology 233, 380–382.

Madesh, M., Balasubramanian, K.A., 1997. A microtiter plate assay for superoxide using MTT reduction method. Indian Journal of Biochemistry and Biophysics 34(6), 535–9. PMID: 9594436.

Martínez, Y., Carrion, Y., Rodríguez, R., Valdivie, M., Olmo, C., Betancur, C., Liu, G., Al-Dhabi, N.A., Duraipandiyan, V., 2015. Growth performance, organ weights and some blood parameters of replacement laying pullets fed with increasing levels of wheat bran. Brazilian Journal of Poultry Science 17(3), 347–354. http://dx.doi.org/10.1590/1516-635X1703347-354.

Moghaddam, P.P., Ebrahimnezhad, Y., Maheri-Sis, N., Shahryar, H.A., Aghajanzadeh-Golshani, A., Gorbani, A., 2025. The effects of using a Rovabio® Plus multi-enzyme on production and incubation performance, blood parameters and duodenum morphology of broiler breeders at age of 45–60 weeks. Veterinary Research Forum 16(4), 245–252. PMID: 40400732.

Musapuor, A., Pourreza, J., Samie, A., Shahrbabak, H.M., 2005. The effects of phytase and different level of dietary calcium and phosphorus on phytate phosphorus utilization in laying hens. International Journal of Poultry Science 4(8), 560–562. http://dx.doi.org/10.3923/ijps.2005.560.562.

Natt, M.P., Herrick, C.A., 1952. A new blood diluent for counting the erythrocytes and leucocytes of the chicken. Poultry Science 31(4), 735–738. https://doi.org/10.3382/ps.0310735.

Nguyen, H.T., Wu, S.B., Bedford, M.R., Nguyen, X.H., Morgan, N.K., 2022. Dietary soluble non-starch polysaccharide level and xylanase influence the gastrointestinal environment and nutrient utilisation in laying hens. British Poultry Science 63(3), 340–350. https://doi.org/10.1080/00071668.2021.2003754.

Nguyen, X.H., Nguyen, H.T., Morgan, N.K., 2021. Dietary soluble non-starch polysaccharide level and xylanase supplementation influence performance, egg quality and nutrient utilization in laying hens fed wheat-based diets. Animal Nutrition 7(2), 512–520. https://doi.org/10.1016/j.aninu.2020.05.012.

Panda, B.K., Padhi, M.K., Sahoo, S.K., 2008. Proceedings of national seminar held at PDP, Hyderabad, 35–40. Google Scholar.

Papadopoulos, G.A., Lioliopoulou, S., Ordoudi, S.A., Giannenas, I., Hoeck, V.V., Morisset, D., Arsenos, G., Fortomaris, P., Mantzouridou, F.T., 2022. Xylanase supplementation in wheat-based diets of laying hens affects the egg yolk color, carotenoid and fatty acid profiles. Foods 11(15), 2209. https://doi.org/10.3390/foods11152209.

Pirgozliev, V., Oduguwa, O., Acamovic, T., Bedford, M., 2008. Effects of dietary phytase on performance

and nutrient metabolism in chickens. British Poultry Science 49, 144–154. https://doi.org/10.1080/00071660801961447.

Schramm, V.G., Durau, J.F, Barrilli, L.N.E., Sorbara, J.O.B., Cowieson, A.J., Félix, A.P., Maiorka, A., 2017. Interaction between xylanase and phytase on the digestibility of corn and a corn/soy diet for broiler chickens. Poultry Science 97(5), 1204–1211. https://doi.org/10.3382/ps/pew356.

Selle, P.H., Cowieson, A.J., Ravindran, V., 2009. Consequences of calcium interactions with phytate and phytase for poultry and pigs. Livestock Science 124(1–3), 126–141. https://doi.org/10.1016/j.livsci.2009.01.006.

Silversides, F.G., Scott, T.A., Korver, D.R., Afsharmanesh, M., Hruby, M., 2006. A study on the interaction of xylanase and phytase enzymes in wheat-based diets fed to commercial white and brown egg laying hens. Poultry Science 85(2), 297–305. https://doi.org/10.1093/ps/85.2.297.

Snedecor, G.W., Cochran, W.G., 1994. Statistical methods. 9th edn. The Iowa State University Press, Ames, Iowa. SPSS, 2011. Statistical Packages for Social Sciences Version 20.0. SPSS, Chicago, IL, USA. https://www.ibm.com/support/pages/downloading-ibm-spss-statistics-20.

Suleiman, S.A., Ali, M.E., Zaki, Z.M., El-Malik, E.M., Nasr, M.A., 1996. Lipid peroxidation and human sperm motility: protective role of vitamin E. Journal of Andrology 17(5), 530–537. PMID: 8957697.

Talapatra, S.K., Roy, S.C., Sen, K.C., 1940. The analysis of mineral constituents in biological materials. I. Estimation of phosphorus, calcium, magnesium, sodium and potassium in food stuffs. Indian Journal of Veterinary Science and Animal Husbandry 10, 243–58. https://www.cabdirect.org/cabdirect/abstract/19401403292.

Vieira, B.S., Barbosa, S.A.P.V., Tavares, J.M.N., Beloli, L.G.C., De Mello Silva, G.M., Neto, H.R.L., Junior, J.G.C., Correa, G.S.S., 2016. Phytase and protease supplementation for laying hens in peak egg production. Semina: Ciencias Agrarias 37, 4285–4294. http://dx.doi.org/10.5433/1679-0359.2016v37n6p4285.

Wanzenbock, E., Apprich, S., Tirpanalan, O., Zitz, U., Kracher, D., Schedle, K., Kneifel, W., 2017. Wheat bran biodegradation by edible Pleurotus fungi-A sustainable perspective for food and feed. Lebensmittel-Wissenschaft & Technologie 86, 123–131. http://dx.doi.org/10.1016/j.lwt.2017.07.051.