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Effect of enzyme-supplemented *Azolla pinnata* in laying hen diets on some biochemical and physiological traits

Alaa Hashim AlMoula[1*], Sarmad Hashim Almaula[2], Nawaf Ghazi.Al-Tamee[3], Khalid Hadi Mustafa[4]

[1]*Department of Chemistry, Faculty of Education for Pure Sciences, University of Kirkuk, Kirkuk, Iraq*
[2,3,4]*Department of Animal Production, Faculty of Agriculture and Forestry, University of Mosul, Mosul, Iraq*
[*]*Email: alaaalmola@uokirkuk.edu.iq*

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ABSTRACT

Enzyme-supplemented Azolla pinnata is essential for increasing the nutritional value of chicken feeds. The aim of this study was to evaluate the effects of adding different quantities of enzyme-fortified azolla to the feed ration on the lipid profile, blood profile, and liver and kidney functions of laying hens. Four groups of 120 46-week-old white Leghorn laying chickens were randomly selected. While the control group received no azolla, the experimental groups received feed containing 15%, 20%, and 25% of dried enzyme-fortified Azolla pinnata (DA+E). The blood profile, lipid profile, kidney function, and liver were measured by drawing blood and serum. Our results showed that 15% and 20% Azolla significantly decreased ($P<0.05$) the levels of ALT and ALP, respectively, when compared to the control; 20% Azolla significantly improved the levels of total proteins and globulin, which had the highest significant values ($P<0.05$); uric acid levels significantly decreased ($P<0.05$) in all treated groups when compared to the control; and 15% Azolla significantly increased ($P<0.05$) Hb concentration. In conclusion, enzyme-fortified Azolla represents a step toward improved laying hen health and reduced feed costs. Thus, it might be suggested as an effective, sustainable feed additive that can be produced locally for laying hens.

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Introduction

For many nations, chicken is the major source of animal protein, as its flesh and eggs supply critical ingredients that are important for human health (Mishra et al., 2016). Since the market wants more chicken products, the industrial sector aims to enhance both the quantity and quality of poultry products via better animal health care (Riaz et al., 2022). Improving poultry's biological performance demands optimal nutrition (Chichilichi et al., 2015). By identifying new nutrients and adding supplements to current feed that promote chicken nutrient absorption from their food, scientists have devised a variety of strategies to enhance feed quality (Adil et al., 2022). Because it comprises high-quality protein, important amino acids like methionine and lysine, vitamins A, B, and C, and minerals like iron, calcium, and phosphorus, azolla is a complete nutritional source (Alagawany et al., 2024). Azolla and extra enzymes boost nutrient absorption in chickens, increasing the production of eggs and meat (Tayade et al., 2024). Enzymes boost the nutritional value of Azolla feed, as reported by Swain et al. (2020). By breaking plant bonds, enzymes boost the production of animal feed by enhancing the animal's capacity to absorb nutrients (Alebachew et al., 2024). Compared to standard feed techniques, the addition of enzymes to Azolla feed boosts feed efficiency, resulting in quicker growth rates (Seid, 2023). Shukla et al. (2018) revealed that adding Azolla to chicken diets boosted immunity and nutrition. According to Joysowal et al. (2018), Azolla is a low-cost, non-traditional feed that may be employed for poultry. According to Shambhvi et al. (2021), enzyme-enhanced Azolla delivers highly effective results for chicken farming operations. Azolla with enzymes provides an economical option for animal feed imports, according to research by Mishra et al. (2016). By employing sustainable agricultural practices, producers may minimize their expenditures. The research by Xu et al. (2023) pinpoints the specific nutrients that boost production outcomes and product quality standards. According to Chen et al. (2023), the approach boosts earning potential by helping farmers develop better product specifications.

When combined with enzymes, azolla may be added to chicken feed, as shown by Abd El-Kareem et al. (2025). Kishore (2024) showed that adding Azolla to chicken feed increases feed efficiency since birds naturally choose meals rich in protein and essential nutrients. Enzyme-treated Azolla improves fowl digestion of complex plant elements, resulting in better nutritional absorption, according to an experimental investigation by Abdissa et al. (2023). Alebachew et al. (2024) claim that using Azolla as animal feed lowers farmers' overall agricultural costs by lowering their need

for imported feed from other countries. Al-Jabari et al. (2024) found that Japanese quail that ate Azolla with a greater calcium content after enzyme treatment showed better egg yield, stronger eggshells, and quicker growth rates. Anuar et al. (2022) claim that enzyme treatment increases the availability of Azolla nutrients for animal consumption by improving their digestion in chickens. Based on the aforementioned, this study examined the effects of enzyme-fortified Azolla pinnata on liver and kidney functions, lipid profiles, and blood profiles in order to support laying hens by incorporating it into their diets in a sustainable, local, and economically viable manner.

Materials and methods

Ethics statement

The Animal Unit, Department of Animal Production, Faculty of Agriculture and Forestry, University of Mosul, Iraq, was the site of the experiments. According to their document NO. UM.VET.2023.159, dated October 1, 2023, the Animal Ethics Committee of the College of Veterinary Medicine, University of Mosul, Iraq, has established a policy for the use of laying hens.

Preparation of dried *Azolla pinnata*

The Animal Unit, College of Agriculture and Forestry, Department of Animal Production, provided an Azolla pinnata culture unit. After maturing (7 days), green Azolla was harvested and collected. The harvested Azolla was washed and dried at room temperature for 2-3 days in a well-ventilated, non-wet, and well-spreading place prior to administration to birds' rations. Table 1 shows the chemical composition of dried Azolla pinnata.

Animals

120 white leghorn laying hens at 46 weeks of age. The birds were raised in two equal-sized pin chambers (2.5 m2) on each side of a locked passageway. Every room had a door of its own. The environmental conditions required for production and rearing were all present. The daily lighting plan for this investigation included eight hours of darkness and sixteen hours of light.

Experimental design

After the birds were raised for a two-week preparatory phase, the study began at 46 weeks of age and continued for eight weeks. Birds were randomly allocated to four treatments, each consisting of three repetitions with ten hens per replicate, taking into consideration the constancy of bird weights in each replicate. The experiment included four groups. The first group, referred to as the

control group, had a meal that included no dry Azolla pinnata. In the second treatment, dried enzyme-fortified Azolla pinnata (DA+E) made up 15% of the feed components. In the third treatment, 20% of the feed ingredients were dried enzyme-fortified Azolla pinnata (DA+E). In the fourth treatment, 25% of the total feed components are dried enzyme-fortified Azolla pinnata (DA+E).

Table 1. Chemical composition of dried Azolla pinnata (Balaji et al., 2009)

Ingredients	(%)
Moisture	10.30
Crude Protein	24.50
Metabolizable Energy (kcal/kg)	1870
Crude Fiber	14.90
Ether Extract	3.70
Ash	17.00
Phosphorus	0.44
Calcium	2.14

Treatment rations

The meals were prepared with the nutritional therapy used in this study in mind. Dried Azolla pinnata was combined with the other feed ingredients in the proportions specified for each treatment. The meal was thoroughly blended and mashed before being formed into pellets. Feed was manually provided using cylindrical plastic feeders that were suspended from the ceiling at the birds' back level using metal wires in order to minimize feed loss. During the study, automated hanging feeders were used to ration the birds' food at a daily rate of 105g per chicken. According to the requirements of the research, four diets with a metabolic energy of around 2,700 kcal/kg and a crude protein content of about 17% were created. The enzyme combination (Kemin Industries, Scott Avenue, Des Moines, Iowa, USA) was added to all experimental settings in order to improve the utilization of the nutrients present in the feed materials. Enzymes were incorporated into the diets using a stepwise premixing procedure to ensure homogeneity. The predetermined quantity of the multi-enzyme product was accurately weighed using a sensitive electronic balance and initially mixed with a small portion of feed. This mixture was then progressively blended with increasing

amounts of feed until the final batch was obtained, thus guaranteeing uniform enzyme distribution in the diet. Table 2 displays the content of various diets.

Table 2. Chemical composition of our trial's treatment meals

Feed Ingredients	control	15% DA+E	20%DA+E	25%DA+E
Corn	43.25	40	38	36
Wheat	11	11	10.5	10.25
Soybean (44%)	23	17	15	13
Wheat Bran	9	3.75	3	2
DA+E	Zero	15	20	25
Oil	2	2.5	3	3.5
Limestone	9	8	7.75	7.5
Premix (25%)*	2.5	2.5	2.5	2.5
Salt	0.225	0.225	0.225	0.225
Enzyme Mix**	0.025	0.025	0.025	0.025
Total	100	100	100	100
Calculated chemical analysis				
Protein Content	17.1	17.0	17.0	.170
Energy Level	2701	2707	2705	2708
Lysine	0.878	0.751	0.711	0.670
Methionine	0.470	0.436	0.425	0.413
Calcium	3.382	3.318	3.327	3.337
Available Phosphorus	0.267	0.253	0.258	0.261
Crude Fiber	3.882	5.048	5.511	5.954

* Premix ingredients: Crude protein 25%, crude fat 2.10%, crude fiber 0.40%, crude ash 52.00%, lysine 8.03%, methionine 10.82%, methionine + cysteine 10.88%, metabolizable energy 4000 (kcal/kg), vitamin A 2500 IU, vitamin D3 500 IU, vitamin E 1 mg, ascorbic acid of vitamin C 5 mg, dicalcium phosphate 80 mg, sodium chloride 70 mg, magnesium sulfate 20 mg, zinc sulfate 2 mg, ferrous sulfate 1.5 mg, sodium propionate 5 mg, sodium selenate 1000 µg, potassium iodide 15 µg, cobalt chloride 10 µg, manganese sulfate 750 µg, calcium carbonate 1 gram.** Enzyme mixture ingredients: beta-Xylanase 3500 U/g, alpha-Amylase 400 U/g, beta-Glucanase 18,000 U/g.

Biochemical and hematological analysis

At the end of the eight-week experiment, blood was randomly extracted from the brachial vein in the laying hens' wings. Commercial kits from Biolabo, Maizy, France, were used to measure albumin, total proteins, AST, ALT, and ALP. After separating the serum from the blood by centrifuging it at 3000 rpm for 15 minutes, the blood samples were put in sealed plastic tubes

devoid of EDTA anticoagulant for biochemical testing, glucose, and enzyme estimation within 24 hours after collection. Albumin and total protein were measured according to Doumas et al. (1972) and Tietz (1995), respectively. AST and ALT were tested using the IFCC reference methods (Bergmeyer et al., 1986a, 1986b), and uric acid and ALP were evaluated using Burtis et al. (1999). Glucose was measured using the Trinder technique (1969), total cholesterol was calculated by Allain et al. (1974), triglycerides were estimated by Fossati and Prencipe (1982), HDL cholesterol was estimated by Wu (2006), and LDL-C and VLDL-C were calculated using the following formulas (Friedewald et al., 1972):

LDL-C = Total cholesterol – [T.G./5 + HDL- C]

VLDL-C = T.G./5

Creatinine was tested in compliance with Labbé et al. (1996). The hematological tubes contain anticoagulants. Packed cell volume (PCV), erythrocyte concentration (RBC), hemoglobin (Hb) and leucocyte concentration (WBC), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) were all measured using the Horiba ABX Micros ES 60 Hematology Analyzer (Montpellier Grabels, France).

Statistical analysis

The research data were statistically evaluated using statistical analysis software with a completely randomized design (CRD) (SAS Institute Inc., 2003). Duncan's (1955) multiple range test was used to evaluate the significance of mean differences. When $p < 0.05$, treatment differences were deemed significant.

Results

When enzyme-fortified Azolla (DA+E) is given to laying hens' diets at ratios of 15%, 20%, and 25%, Table 3 demonstrates a substantial influence on several liver function indices. Azolla therapy has an impact on liver enzymes. In comparison to the control, the second treatment (15% DA+E) had the lowest alanine aminotransferase (ALT) value (0.23 ± 0.05), whereas the third treatment (0.89 ± 0.29) and fourth treatment (0.79 ± 0.04) had the highest values ($P<0.05$). The levels of aspartate aminotransferase (AST) varied from 199.75 ± 6.34 to 229.25 ± 11.84, and the treatment groups and the control group did not vary significantly. This implies that the indication is rather stable and unaffected by various therapies. Alkaline phosphatase (ALP) levels differed

considerably (P < 0.05) across treatments when compared to the control. When 20% DA+E was added, ALP levels dropped considerably (P < 0.05), measuring 708.25 ± 6.94. The greatest value was obtained by treatment four (824.00 ± 57.67), with treatment two coming in second (792.75 ± 20.21). Azolla treatments did not significantly affect glucose levels when compared to the control; measurements stayed within a narrow range of 229.63 ± 6.33 to 245.13 ± 4.58. Azolla supplementation had a significant (P<0.05) effect on total protein levels; the third group (20%DA+E) had the greatest value (6.07 ± 0.39) compared to the control group (5.77± 0.18). The second and fourth results, on the other hand, were much lower than the control (5.17 ± 0.10 and 5.33 ± 0.06, respectively). Serum albumin concentration levels ranged from 1.63 ± 0.08 to 1.80 ± 0.04, and there were no significant changes between the treatment groups (P < 0.05) when compared with the control. On the other hand, globulin levels were substantially lower (P<0.05) in the second treatment (3.37±0.06) and fourth treatment (3.57±0.09) compared to the control (3.95 ± 0.09), and significantly higher (P<0.05) in the third treatment (20% azolla) (4.43 ± 0.43).

Table 3. Impact of *Azolla pinnata* (DA+E) supplemented with enzymes on liver functions

No.	Attributes	control	15% DA+E	20% DA+E	25% DA+E
1	ALT UlL	0.38±0.11 bc	0.23 ± 0.05 c	0.89 ± 0.29 a	0.79 ± 0.04 ab
2	AST UlL	227.75±10.91 a	201.75 ± 11.58 a	229.25 ± 11.84 a	199.75 ± 6.34 a
3	ALP UlL	735.75 ± 6.51 ab	792.75 ± 20.21 ab	708.25 ± 6.94 b	824.00 ± 57.67 a
4	Glucose mg\dl	243.25 ± 3.75 a	245.13 ± 4.58 a	229.63 ± 6.33 a	236.25 ± 3.88 a
5	Total Protein	5.77 ± 0.18 ab	5.17 ± 0.10 b	6.07 ± 0.39 a	5.33 ± 0.06 b
6	Albumin gldl	1.82 ± 0.11 a	1.80 ± 0.04 a	1.63 ± 0.08 a	1.77 ± 0.06 a
7	Globulin gldl	3.95 ± 0.09 ab	3.37 ± 0.06 b	4.43 ± 0.43 a	3.57 ± 0.09 b

The values reflect the four groups' mean ± SE. The values in a row indicated by distinct small letters are significantly different from the control (P 0<0.05).

Table 4 did not reveal any significant changes in the lipid profile, which comprises total cholesterol, triglycerides, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and very-high-density lipoprotein (VLDL). The fact that all of these values remained within normal limits and were distributed similarly across the different treatments indicates that lipid metabolism was unaffected in this experiment**.**

Table 4. Impact of *Azolla pinnata* (DA+E) supplemented with enzymes on the lipid profile

No.	Attributes	control	15% DA+E	20% DA+E	25% DA+E
1	Cholesterol mgldl	137.00 ± 9.93 a	115.75 ± 4.55 a	130.25 ± 0.63 a	131.00 ± 8.26 a
2	Triglyceride mgldl	840.25 ± 40.56 a	847.75 ± 38.17 a	840.75 ± 31.39 a	825.00 ± 44.31 a
3	HDL mgldl	37.00 ± 2.08 a	36.75 ± 2.17 a	30.00 ± 5.58 a	36.50 ± 3.80 a
4	LDL mgldl	68.00 ± 4.64 a	90.75 ± 8.82 a	67.75 ± 3.90 a	70.75 ± 20.43 a
5	VLDL mgldl	168.00 ± 8.01 a	169.75 ± 7.60 a	168.00 ± 6.34 a	165.25 ±8.87 a

The values reflect the four groups' mean ± SE. The values in a row indicated by distinct small letters are not significantly different from the control (P 0<0.05).

Table 5, an additional set of renal function indicators, shows that all treatments had significantly lower changes (P<0.05) in uric acid concentration when compared to the control, particularly in the fourth and third treatments (3.37 ± 0.06 and 3.47 ± 0.13, respectively).In comparison to the control, the serum creatinine concentration was substantially lower (P<0.05) in the second treatment (0.31 ± 0.004) and marginally different (P<0.05) in the fourth and third treatments (0.33 ± 0.002 and 0.33 ± 0.004, respectively). These numbers were within the typical range.

Table 5. Impact of enzyme-fortified *Azolla pinnata* (DA+E) on renal functions

No.	Attributes	Control	15%DA+E	20% DA+E	25%DA+E
1	Uric acid mgldl	4.67 ± 0.22 a	3.93 ± 0.25 b	3.47 ± 0.13 b	3.37 ± 0.06 b
2	Creatinine mgldl	0.32 ± 0.004 ab	0.31 ± 0.004 b	0.33 ± 0.004 a	0.33 ± 0.002 a

The values reflect the four groups mean ± SE. The values in a row indicated by distinct small letters are significantly different from the control (P 0<0.05).

Table 6 displayed a collection of hematological parameters, some of which fluctuated while others remained within the normal range and did not exhibit any statistically significant changes. Red blood cell (RBC) counts varied between 2.29 ± 0.07 and 2.61 ± 0.17 106/µl, and there were no significant differences (P<0.05) between treatments and the control. This implies that the formation of red blood cells was unaffected by the various therapies. The white blood cell (WBC) count, which ranged from 9.88 ± 2.13 to 10.65 ± 1.20 103/µl, did not differ substantially from the control group. Hemoglobin concentrations in the treatment groups varied substantially (P<0.05). When compared to the control, the second treatment (15%DA+E) had the highest HB value (10.20

± 0.62), followed by the third treatment (20%DA+E) (9.00 ± 0.08). However, values significantly decreased in treatment 4 (8.83 ± 0.08). With values ranging from 29.43 ± 0.09 to 31.18 ± 1.39, the packed cell volume (PCV) of the second, third, and fourth treatments did not differ substantially from the control. This implies that the quantity of red blood cells in blood is relatively constant. When comparing mean corpuscular volume (MCV) to the control group, the fourth treatment had the lowest value (125.00 ± 0.41 μm^3); the second and third treatments (128.00 ± 0.41 and 128.67 ± 1.03, respectively) showed no significant differences ($P<0.05$), but they stayed in a similar range. In comparison to the control, the mean corpuscular hemoglobin concentration (MCHC), which ranged from 30.47 ± 0.27 to 30.77 ± 1.20 g/dL, demonstrated a steady hemoglobin concentration within red blood cells and did not substantially alter between treatments**.**

Table 6. Azolla's enzyme-fortified effect (DA+E) on the blood profile

No.	Attributes	Control	15%DA+E	20%DA+E	25%DA+E
1	**RBC x 10^6/µl**	2.57 ± 0.14 a	2.61 ± 0.17 a	2.30 ± 0.02 a	2.29 ± 0.07 a
2	**WBC x 10^3/µl**	10.18 ± 1.35 a	9.88 ± 2.13 a	10.65 ± 1.20 a	10.00 ± 0.92 a
3	**HB g/dl**	10.00 ± 0.18 ab	10.20 ± 0.62 a	9.00 ± 0.08 bc	8.83 ± 0.08 c
4	**PCV %**	29.80 ± 0.15 a	31.18 ± 1.39 a	29.43 ± 0.09 a	30.80 ± 0.36 a
5	**MCV µm^3**	128.13 ± 0.37 a	128.00 ± 0.41 a	128.67 ± 1.03 a	125.00 ± 0.41 b
6	**MCHC g/dl**	30.77 ± 0.68 a	30.73 ± 0.35 a	30.47 ± 0.27 a	30.77 ± 1.20 a

The data represent the mean ± SE of four groups; in a row is significantly different from the control ($P <0.05$) in HB, but the other not.

Discussion

The effects of enzyme-fortified Azolla on liver enzyme levels, such as ALT, AST, and ALP, differed across all treatment groups, according to our findings in Table 3. ALP levels were lower in the third group (20% DA+E) than in the control, while ALT levels were lower in the second treatment (15% DA+E) than in the control. These findings are in line with Kamel and Hamed's (2021) discovery that 4%, 8%, and 12% of azolla reduced ALT. This suggests that the effects of

enzyme-fortified Azolla on the liver could not be detrimental, which may indicate a physiological reaction linked to bone or liver function by (15% and 20% DA+E). Carotenoids, alkaloids, phenolic acids, flavonoids, and tannins are among the phenolic compounds found in azolla that have been shown to have antioxidant qualities and protect the liver from oxidation (Shaaban et al., 2020). Azolla is rich in vitamins that are essential for strong bones, muscles, and overall health, such as calcium, potassium, phosphorus, iron, magnesium, and copper (El-Naggar & El-Mesery, 2022). Additionally, the Azolla-treated group was equivalent to the control group and within the normal range for broiler chickens, according to Sharma et al. (2018). Our results are at odds with those of Arram et al. (2023), who used azolla in chicken broiler feed at varying ratios of 5%, 10%, 15%, and 20% and found no appreciable effect on the levels of liver enzymes (AST, ALT, and ALP) when compared to the control group and within the birds' normal ranges. Even at 20% levels, Azolla supplementation had no hepatotoxic effects in layer hens, according to Ara et al. (2018).
The results show that blood glucose levels did not change after any of the treatments, indicating that enzyme-fortified Azolla had no impact. When different azolla percentages (4%, 8%, 12%, and 16%) were added to the diets of Japanese quail, Sherif et al. (2022) saw comparable results. Azolla diets have no appreciable impact on glucose metabolism, according to Belagoune et al. (2024) and Mishra et al. (2016). The present study's findings, however, are at odds with those of Al-Jabari et al. (2024), who discovered that quail meals containing 4% Azolla significantly affected glucose levels. Furthermore, Japanese quails given 4%, 8%, and 12% azolla had high blood glucose levels, whereas birds fed 16% azolla had the lowest values, according to Sherif et al. (2022). When compared to the control, the third treatment (20% DA+E) had greater levels of both total protein and globulin. A bird may have better meal intake and utilization if its blood contains more total protein. Similar results were reported by Kamel and Hamed (2021), who found that the group of broiler chicks fed 8% dry azolla had the highest albumin and total protein levels. Total plasma proteins, a typical measure used to evaluate avian body condition, suggest that azolla-fortified chicken may have improved protein absorption efficiency (Sinha et al., 2018). These findings were consistent with those of Al-Rekabi et al. (2020), who noted that broiler chickens fed Azolla had a significantly higher total protein content than the control group. On the other hand, Thavasi Alagan et al. (2020) found a non-significant change in albumin across various dietary regimens, suggesting a decreased capacity of chickens to effectively absorb protein. Furthermore, Japanese quails given 4% and 8% azolla showed no discernible variation in serum albumin, according to Sherif et al.

(2022). The addition of enzyme-fortified Azolla did not significantly affect body lipid balance, as seen in Table 4, where blood cholesterol and triglyceride levels were generally stable across treatments (Ismail et al., 2023). According to Arram et al. (2023), adding azolla to the feed of chicken broilers in different ratios—5%, 10%, 15%, and 20%—did not significantly affect the levels of triglycerides, cholesterol, HDL, and VLDL, which were also constant in all treatment groups. This demonstrates stable cardiovascular function in hens and shows that enzyme-fortified Azolla does not negatively affect blood levels of HDL or LDL cholesterol (Alagawany et al., 2024). Furthermore, feeding Narmadanidhi birds dried azolla up to 10% had no effect on cholesterol, according to Yadav et al. (2022). LDL, HDL, and VLDL were not significantly impacted by the addition of Azolla at 15, 30, and 45% in broiler feed (Al-Shwilly, 2022). In contrast to our findings, Rieta and Cabaral (2018) reported that broilers fed 100% Azolla had lower blood cholesterol than broilers fed 50% Azolla. Similarly, Sherif et al. (2022) reported that birds fed 4%, 8%, 12%, and 16% Azolla had significantly lower cholesterol.

Table 5 shows that the uric acid levels of all treatment groups were significantly ($P<0.05$) lower than those of the control group, indicating either increased protein metabolism or improved nitrogenous waste removal. According to Kamel and Hamed's (2021) results, birds given Azolla at 4%, 8%, and 12% had lower uric acid levels than control birds. When compared to the control, creatinine, another indicator of protein metabolism, remained constant throughout all treatments in our investigation, indicating that the treatments may have an effect on reducing the synthesis or accumulation of this end product of purine metabolism. Mishra et al. (2016) found that all treatment groups of Chabro birds fed 5%, 7.5%, and 10% azolla had creatinine readings within the normal physiological range, which is in line with our findings. Other research indicates that feeding with enzyme-fortified Azolla may significantly ($P<0.05$) enhance the biochemical parameters of laying hens. Refaey et al. (2023) discovered that fish given 10%, 20%, 30%, and 40% azolla had greater levels of uric acid than the control, but Shambhvi et al. (2021) observed no significant change in the levels of creatinine or uric acid in birds fed azolla. As all of the data were similar, Table 6 shows that there were no appreciable differences in the red blood cell count across the different treatments (2.29 ± 0.07 to 2.61 ± 0.17). This is consistent with previous research that found grass-fortified diets such as Azolla do not substantially influence red blood cell counts when supplemented with 10%, 20%, 30%, and 40% Azolla (Refaey et al., 2023). On the other hand, RBC significantly increased in birds fed a diet supplemented with 5% Azolla (Thavasi Alagan et

al., 2020). Over the course of therapy, white blood cell counts remained constant, ranging from 9.88 ± 2.13 to 10.65 ± 1.20, with no appreciable changes (Refaey et al., 2023). These results demonstrate that the addition of enzyme-fortified Azolla did not significantly affect the chickens' immune system response and corroborate those of El-Deeb et al. (2021), who discovered that most hematological characteristics in rabbits were unaffected by the Azolla-fortified diets. Related research discovered that the addition of nutrient-rich plant components marginally enhanced the health of the chicken immune system, but the white blood cell count did not change considerably (Seleiman et al., 2022).

Our results showed that, in comparison to the control, the second treatment (15% DA+E) had the highest hemoglobin level (10.20 ± 0.62), while the fourth treatment had the lowest (8.83 ± 0.08). These results imply that upping the dose of Azolla may have a greater impact on improving oxygen transport capacity and blood hemoglobin levels. Kishore's (2024) results showing that birds given 2.5%, 5%, 7.5%, and 10% Azolla had increased HB are in line with this. Hemoglobin levels may have improved due to increased absorption of essential elements like iron from Azolla (Keser et al., 2025). According to earlier studies, Azolla has significant concentrations of minerals including iron, which might increase hemoglobin levels (Chen et al., 2023). Additionally, Yadav et al. (2023) discovered that 5% and 10% Azolla in bird meals resulted in a large and substantial concentration of Hb. Kumar et al. (2018) reported no differences in Hb levels in broiler chicks treated with DA at 2.5, 5, 7.5, and 10%, which was in contrast to these findings. With a little fluctuation but no appreciable changes, the percentages of PCV remained constant throughout all treatments. The range of values was 29.43 ± 0.09 to 31.18 ± 1.39. When birds were fed 2.5% azolla, Kishore (2024) found no significant changes in PCV, but there was a notable increase at 5%. The PCV of the birds fed a meal enriched with 5% Azolla shows prior increases, according to Thavasi Alagan et al. (2020). This result is in line with recent research showing that the proportion of blood cells is not much impacted by the addition of plant components or nutrient-rich dietary supplements. 5% and 10% azolla did not affect the PCV level in Narmadanidhi birds, according to Alagawany et al. (2024) and Yadav et al. (2022). The mean corpuscular volume (MCV) was highest in treatment three (20% DA+E) (128.67 ± 1.03), followed by treatment four (125.00 ± 0.41). This indicates that adding enzyme-fortified Azolla may cause a slight increase in red blood cell volume, which might indicate improved blood metabolism and a rise in the production of red blood cells. These changes in MCV may be related to improvements in the absorption of nutrients like iron and

proteins that affect the formation of red blood cells (Kumar et al., 2015). In contrast, Yadav et al. (2022) found that 5% and 10% azolla had no effect on the MCV level in Narmadanidhi birds. When compared to the control, MCHC values for all treatments varied from 30.47 ± 0.27 to 30.77 ± 1.20, indicating that the addition of enzyme-fortified Azolla had no appreciable impact on the concentration of erythrocyte hemoglobin. According to studies showing that feeding birds diets fortified with natural ingredients typically has no effect on the concentration of corpuscular hemoglobin when the birds are fed azolla at 5% and 10%, this result suggests a stable hemoglobin composition within red blood cells (Yadav et al., 2023; Gupta et al., 2018). Our findings are in line with those of Anitha et al. (2016), who discovered that adding Azolla to rabbits' diets had no appreciable impact on many hematological indicators, such as MCV and MCHC. It may be concluded that adding enzyme-fortified Azolla to feed has a positive effect on a number of blood parameters, including hemoglobin and MCV. However, no discernible changes in MCHC, hematocrit percentage, or red and white blood cell counts were seen in the research. These results are consistent with previous studies showing that the addition of nutrient-rich ingredients such as Azolla may improve some biochemical metrics without significantly altering others (Keser et al., 2025).

Conclusion

HB, MCV, total proteins, ALT, ALP, creatinine, and uric acid were all improved by enzyme-fortified azolla at 15% and 20%; other markers remained at normal levels with no appreciable change. This improvement in some biochemical and hematological indicators is expected to lead to increased output and overall poultry health by enzyme-fortified Azolla in the meals of laying hens. Future studies on its effects on egg production and growth rate would be helpful.

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Conflict of interest

The writers attest that they have no declared conflicts of interest.

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