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Use of Azolla the in Presence of Fiber-Degrading Enzyme Additives and its Effect onthe Productive Performance in Broiler

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A B S T R A C T

In this study, we used unsexed broiler chicks of the hybrid (Ross 308), raised in animal production fields/College of Agriculture and Forestry for the period from 7/2/2024 to 19/ 3 /2024. The chicks were subjected to the treatments under study from the first day of the experiment. The experiment included five treatments: T1 : Standard ration (without Azolla and enzyme).T2: ration contained Azolla 7% and without enzyme .T3: ration contained Azolla 7% + 0.30% enzyme.T4: ration contained Azolla 7% + 0.35% enzyme.T5: ration contained Azolla 7% + 0.40% enzyme. Measurements of production characteristics were taken weekly and statistical analysis shows that there was a significant increase in live body weight, weight gain, an improvement in feed conversion ratio, a decrease in the feed consumed In enzyme addition transactions, and no significant effect of the treatments in the relative growth rate.

This study aimed to prepare a feeder containing Azolla with the addition of enzymes as a technical management method that contributes to increasing the decomposition of fibers to increase the rate of utilization as a non-traditional protein source for locally available fodder

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Introduction

One of the most important elements of profitable continuous production is the availability of feed components that are included in the composition of the feed, which has become a competitor to traditional human foodstuffs [1], and their prices are considered part of the production cost. Recently, non-traditional feeds have been used to provide locally available protein sources, including Azolla, which was used in feeding large animals and animals with simple stomachs [3], [4], [5], [6], [7]. In addition to being considered a protein supplement in many studies as a source of the amino acids methionine, lysine, and histidine and containing vitamins A and B12 [8], [9], [10], Azolla is also characterized by its high content of phenols, flavonoids, carotenoids, and probiotics, and therefore it is considered an antioxidant and antibacterial. [11] Azolla contains a percentage of fibers, which may be a cause of nutritional problems because it is considered an anti-nutrient that affects the digestion of feed materials. This study came with the addition of enzymes as a nutritional supplement to the feed to contribute to increasing the rate of utilization and simplifying its content of fibers and nutrients and increasing its digestion coefficient and absorption efficiency, which is reflected in the benefit of food, as enzymes are

used to decompose the fibers that make up the cell wall [12], which are the digestive enzymes for non-starchy polysaccharides such as beta-glucanase, cellulase, mannanase, and xylanase, which are the most widely used in the field of poultry nutrition [13]. They are of great importance in treating the viscosity resulting from non-starchy polysaccharides inside

the digestive tract increasing their digestion coefficient and enhancing the work of internal enzymes, causing an increase in the availability of decomposed nutrients, which increases their absorption process. [31],[32] and thus increasing the rate of utilization of released nutrients, improving the feed conversion ratio, and reducing feeding costs [34].

Materials And Methods

The research was carried out in the poultry field of the Department of Animal Production at the College of Agriculture and Forestry / University of Mosul, the experiment was 42 days from 7/2/2024 to 19/3/2024, and the research aimed to study the effect of preparing the feed containing Azolla with enzymes. Fiber hydrolyzate is a nutritional management tool to increase the utilization rate of nutrients when used as a non-traditional locally available source and to know its effect on productive traits. The chickens used are a hybrid type (Ross 308) and the total number of chickens is (390) with a starting weight of 42.11 grams, The chicks were treated as follows: T1 (control): feed without additives, T2: feed containing 7% Azolla, T3: adding 0.30% enzyme to the feed containing Azolla, T4: adding 0.35% enzyme to the feed containing Azolla, T5: adding 0.40% enzyme to the feed containing Azolla. The chicks were subjected to the experimental treatments from the first day of rearing and were randomly distributed to the experiment replicates (15). Feed and water were continuously available with an incubation temperature of 35°C and continuous monitoring of the chicks' movement and vitality.

Table 1. shows the percentages of feed components.

Feed ingredients %	Ratios used %			
	Starter ration		Finisher ration	
Dried Azolla	0.0	7.0	0.0	7.0
Yellow Corn	57.72	53.1	60.91	55.12
Soybean Meal	38.85	36.34	33.69	31.4
Limestone	2.38	1.83	1.85	1.83
Table Salt	0.25	0.25	0.25	0.25
Vegetable Oil	-	0.99	2.44	3.84
Dicalcium Phosphate	0.8	0.49	0.86	0.56
Total	100	100	100	100
calculated chemical analysis				
The energy represented ilo/kg.	2800	2800	3000	3000
Protein %	22	22	20	20

* Calculated according to [14]

The type of Azolla in this study is Azolla pinnata. The Azolla was green and dried in the shade with stirring, then added to the feed when grinding the feed components to ensure its mixing with the feed. Table (2) shows the chemical composition of the Azolla used in the study.

Table 2. shows the laboratory chemical analysis of dried Azolla .

the components	percentage
Protein	29.8
Ash	15.9
Fiber	19.7
Ether Extract	1.7
Moisture	10.15

The enzymes used in the study were a powder of enzymes from a company specialized in veterinary treatments. The composition used is shown in Table (3).

Table 3. shows the enzymes used in the study.

Enzymes	Amount in powder U/g
beta-xylanase	35000
alpha-amylase	400
beta-glucanase	2350
Baciliolysin	1700

The studied production traits were measured, namely live body weight, as the chicks were weighed weekly using a scale with a sensitivity of (5 g). The weight gain was calculated, the weekly growth rate, feed consumption, feed conversion ratio, production coefficient, and production index. The Completely Randomized Design (C.R.D) was used in data analysis using SAS (2001) test significance according to [16] New Multiple tests to find significant differences between the treatments at the probabil ity level ($p \leq 0.05$).

Results and discussion

Live body weight

Table (4) shows that average live body weight of broilers in the first and second weeks did not differ significantly ($P \geq 0.05$) between treatments, while a significant increase was observed in the third week of the fifth treatment, where it reached (785.60) g compared to T I, T2 and third treatments and its broiler feed and differed with [9] where it was found that there was a decrease in the body reached(712.43, 717.14 and 722.47) g, respectively, while it did not differ significantly from the fourth treatment, which reached (754.3) g. It was also observed that the average body weight increased significantly in the fourth week in the T5 compared to the T1 and T2, and reached (1431.83, 1304.33, 1321.31) g, respectively. Also, in the fifth week, the fifth treatment outperformed the control and second treatments, and reached (2041.23, 1894.67, 1911.11) g, respectively, while we find that there is a significant increase in the sixth week for all treatments of added the enzyme to the feed containing Azolla (the third, fourth and fifth treatments) and also the increased significantly of the second treatment over the control and reached respectively (2548.33, 2552.0, 2,630.0, 2437.0) g compared to (2429.70) g. This effect did not appear in the first and second weeks, but it appeared in the last weeks.

The addition rate of 0.40% was able to contribute to raising in live body weight and increasing the rate of feed utilization (food authorization factor, Table 8) despite the decrease in the amount consumed in the same treatments and the same weeks (Table 7). The average body weight was enhanced in the sixth week by the cumulative effect of the enzyme's work as a result of improving the internal environment of the birds' intestines, which improved the rate of Azolla digestion and increased the availability of decomposed nutrients, which increased their absorption rate. The added enzymes also contributed to enhancing the work of internal enzymes as nutritional supplements, which led to improved nutrient utilization [17],[18], which was reflected in body weight.

These results agreed with the researcher [19], [20] as they found a significant increase in body weight when adding Azolla to the feed, while these results differed with the researcher [2], [23]

, [24] as they showed no effect of adding azolla to the feed of broilers.Regarding the use of enzymes, these results agreed with researchers [25], [26] stated that adding the enzyme mixture led to an increase in the final body weight of broilers compared to the control treatment, while they differed with researchers [27] who showed that when adding the enzyme, the body weight decreased significantly compared to the control. Researchers [28], [29], [30] did not find differences in adding the enzyme to broiler feed and differed with [9] where it was found that there was a decrease in the body weight of broilers at the age of 42 days by adding the enzyme to feed containing Azolla.

Weight Gain Rate

Table 5 shows no significant differences in weight gain during the first, second, and fifth weeks of age, while it was found that in the third week, there was a significant increase in the fourth and fifth treatments compared to the control treatment and amounted to (394.3, 422.23, 372.26) gm respectively, while the second and third treatments did not differ significantly with the previously mentioned studied treatments and amounted to (378.94, 371.17) gm respectively, and also in the fourth week we find that the fifth treatment increased significantly in the first, second, third and fourth treatments as it amounted to (646.23, 591.9, 604.17, 597.43, 594.87) gm respectively. It noted that in the sixth week, there was a significant increase in the enzyme addition treatments at different rates (0.30, 0.35, 0.40)% to the feed containing Azolla compared to the treatment (first and second) and reached (602.66, 597.4, 589.37, 535.7, 525.89) gm respectively. As for the weight gain during the total period of rearing (1-6) weeks, a significant increase was found in the treatments to which the enzyme was added (third, fourth, and fifth) compared to the treatments (first and second) and reached (2506.93, 2505.0, 2589.0, 2388.37, 2394.9) gm respectively.

These significant differences in this trait are a result of the significant differences in the body weight table (Table 4) and confirm the fact that the enzyme mechanism works in benefiting from Azolla by increasing digestion and absorption processes as

a result of reducing the viscosity of the food and enhancing the work of internal enzymes, which was reflected in the performance of the birds [31], [32]. These results were consistent with [2], [23] as he did not find a significant effect of adding Azolla on weight gain. They differed from [33], [20], [24] when they added Azolla to the feed, there was a significant increase in weight gain rate of broiler chickens at the age of 42 days. Regarding the addition of enzymes, these results agreed with [35] noted that when added enzyme mixture there was an insignificant increase in the rate of weight gain during 28 days of rearing, and differed with [27], [28], [36]. [9] stated that when adding the enzyme to the azolla feed there was a decrease in the average final weight gain.

Growth Rate

Table 6 shows no significant differences between the 1, 2, 3 , 4 , and fifth treatments in all weeks of rearing as well as the total rearing period from (1-6) weeks. These results agreed with the researcher [24] while these results did not agree with the researcher [37]. The reason may be due to the protein obtained from Azolla being able to give the same production performance as the feed protein in terms of growth indicators (Table 4) , (Table 5), (Table 6) and economic indicators (production index and coefficient).

Feed Consumed

Table (7) shows that there are no significant differences ($P \geq 0.05$) in the first, second, and fourth weeks between the first, second, third, fourth, and fifth treatments. As for the third week, we find that the enzyme addition treatments decreased significantly compared to the control treatment and amounted to (64.519, 552.02, 570.01) g for the third, fourth, and fifth treatments, respectively, compared to the control treatment (573.28) g, In the fifth week, the second, third, fourth, and fifth treatments decreased significantly compared to the control treatment and amounted to (1013.75, 1021.19, 1047.48, 1073.44, 1166.50) g/bird. It was found that the amount of feed consumed during the sixth week In the second treatment, it decreased compared to the control and the third treatment, and they reached (1009.71, and 1096.74) g, respectively, compared to the control treatment (1187.54) g. As a result, we find that the total amount of feed consumed for the period from (1-6) weeks decreased significantly in the second, third, fourth, and fifth treatments compared to the control treatment, as it reached (3568.4, 3974.03, 3901.24, 3968.33, 4556.36) g/bird for the total rearing period, respectively.

The reason may be that the birds in the Azolla-containing treatments consumed less feed than the control, as they aged, because the dried Azolla absorbed water, which increased the volume of food mass in the intestines while reducing the amount of feed consumed. This gave enough time for the food to pass through the intestines. With the presence of Azolla and the enzyme, the activity of beneficial bacteria increased, which enhanced the digestion and absorption processes, and the nutrients became more abundant, which increased the rate of utilization of the feed (Food Conversion Coefficient Table 7).

These results agreed with [38], [20] where a decrease in the feed consumed was observed using Azolla, and differed with [21], [22], [39] and [23]. Regarding the addition of the enzyme, these results agreed with [27], as the amount of feed consumed decreased significantly by adding the enzyme to the feed. And these results differed from [29], [26], and [30], who showed that when adding the enzyme,

Feed conversion ratio

Table (8) showed that there were no significant differences in the first and second weeks between the first, second, third, fourth, and fifth treatments, while we find that the third, fourth, and fifth treatments decreased significantly compared to the first and second treatments during the third week (1.40, 1.41, 1.39, 1.54, 1.56, 1.54, 1.56) g feed/g weight gain, and in the fourth week the conversion factor decreased significantly in the treatments (second, third, fourth, here were no significant differences in the feed conversion factor by adding the enzyme to the feed. They differed with [9], [40] that there was no significant improvement when adding the enzyme to the Azolla feed, the best feed conversion factor when raising broilers for a total period of 42 days. fifth) compared to the control treatment, and in the sixth week, the treatments (third, fourth, fifth) decreased significantly compared to the control treatment. in the sixth week and the conversion factor (1-6) weeks, all addition treatments (second, third, fourth, and fifth) decreased compared to the
first control treatment, so the values reached (1.49, 1.47, 1.51, 1.51, 1.66) g feed/g weight gain, respectively.

This may be because the enzyme contributed to the digestion and decomposition of feed materials, which led to an increase in the abundance of nutrients, which was reflected in the absorption and improved utilization of nutrients as a result of reducing the viscosity of the feed [31]and [32] and enhancing the work of internal enzymes [17], [18], which was reflected in the performance of the birds. These results agreed with the researchers [41], [26] who stated that when enzymes were added, the feed conversion factor decreased significantly. These

results did not agree with the researcher [42],[36] as the amount of feed consumed, and differed from [9], that when adding Azolla with the enzyme, there was an increase in the amount of feed consumed.

Economic Implications

Table (9) showed that the production coefficient was high in the second, third, fourth, and fifth treatments compared to the control treatment for the periods (1-5) and (1-6) weeks, reaching (32.41, 38.63, 35.34, 35.34, 29.88) g, (27.39, 33.11, 32.29, 31.18, 24.16) respectively. The production index for these treatments also increased for the period (1-5) weeks, reaching (300.02, 343.15, 328.50, 339.07) for the second, third, fourth, and fifth treatments compared to treatment (273.95) respectively. It was also noted that the enzyme addition treatments raised the production index for the period (1-6) weeks compared to the first treatment. It reached (400.05, 394.13, 397.67) respectively compared to (313.3) and this was a result of raising the performance of the weight gain trait and the average live body weight. This confirms the fact that the rate of utilization of food-degrading enzymes has contributed to raising the production performance, which raised the economic efficiency.

Conclusion

Adding enzymes to the feed containing Azolla contributed to the decomposition of fibers and increased the abundance of nutrients in the digestive tract, which increased the rate of digestion and absorption, which was reflected in
the production performance and reduced the cost of production.

Conflict of interest

The researchers pledge that there is no conflict in writing the research with the interests of publishing this article.

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Table 4. Effect of added levels of enzyme % of the ratio containing Azolla on an average weekly live body weight of broilers, g/bird.

Character	Body weight (g)					
Treatment	Weeks					
	First	Second	Third	Fourth	Fifth	Sixth
T1 Control	141.00 a ±2.28	340.17 a ± 9.61	712.43 c ±11.50	1304.33b ±40.57	1894.67b ±15.81	2429.70 b ±20.72
T2 Azolla %7	138.00 a ±0.51	338.2 a ±9.48	717.14 bc ± 5.93	1321.0 b ± 2.91	1911.11b ± 25.145	2437.0a ± 35.0
T3 Azolla7% +0.30% Enzyme	144.8 a ±0.51	351.30a ±9.47	722.47 bc ±15.93	1319.0 b ±2.91	1945.67b ±25.145	2548.33a ±35.04
T4 7%Azolla +0.35% Enzyme	149.00 a ±2.95	359.73 a ± 2.25	754.3ab ±6.43	1353.9ab ±33.36	1954.6ab ±51.25	2552.0a ±53.38
T5 %7Azolla + 0.40% enzyme	154.0 a ±1.07	363.37 a ±5.03	785.60a ±13.37	1431.83a ±26.18	2041.23a ±49.37	0 2630.6 a ± 71.72

Different letters vertically indicate the presence of significant differences (P≤0.05) between the means ± S.E

Table 5. Effect of added levels of enzyme % of the ratio containing Azolla on a weight gain of broilers g/bird.

Character	Weight gain (g) Weeks						Total weight gain
Treatment	First	Second	Third	Fourth	Fifth	Sixth	1-6 weeks
T1 Control	99.0 a ±2.28	199.17a ± 11.78	372.26b ± 2.54	591.9b ±9.11	590.34a ±24.77	535.7b ±14.93	2388.37 b ±20.73
T2 Azolla %7	95.9a ±2.5	200.2a ± 9.04	378.94ab ±19.742	604.17b ±14.25	589.80a ±2.09	525.89b ±11.50	2394.9 b ±1.83
T3 Azolla7%+ 0.30% Enzyme	102.6a ±2.50	207.3a ±9.04	371.17ab ±19.72	597.43b ±14.25	625.77a ±2.06	602.66a ±11.50	2506.93 a ±8.33
T4 Azolla 7%+ 0.35% Enzyme	107 ab ±2.95	210.73a ±1.68	394.3a ±7.21	594.87b ± 27.14	600.70a ±17.88	597.4a ±13.78	2505a ±3.39
T5 Azolla7% + 0.40% enzyme	112.4a ± 3.4	209.37a ± 5.69	422.23a ±10.5	646.23a ±16.44	609.4a ±24.48	589.37a ±23.42	2589.0 a ±71.15

Different letters vertically indicate the presence of significant differences (P≤0.05) between the means ± S.E

Table 6. Effect of added levels of enzyme to ratio containing Azolla on the growth rate of broiler

Character	Relative growth rate% Weeks						relative growth
Treatment	First	Second	Third	Fourth	Fifth	Sixth	(1-6)
T1 Control	107.7 a ±6.3	82.79 a ±9.2	70.73a ±3.7	58.70a ±4.4	36.91a ±7.13	24.74 a ±3.12	63.60a ±4.65
T2 Azolla %7	106.50 a ±5.4	84.08a ±5.3	71.81a ±5.7	59.28a ±9.3	36.49a 5.5±	24.19a 5.33±	63.72a ±7.12
T3 Azolla7%+ 0.30% Enzyme	109.73a ±5.4	83.25a ±5.3	69.13a ±5.7	58.5a ± 9.35	38.33a ±5.5	26.82a ±5.33	64.29a ±7.12
T4 Azolla 7%+ 0.35% Enzyme	112.04a ±8.0	86.79a ±7.1	70.84a ±5.9	56.88a ±11.6	36.31a ±6.04	26.51a ±4.75	64.90a ±8.13
T5 Azolla7% + 0.40% enzyme	114.93a ±11.1	80.93a ±6.43	73.50a ±9.3	58.29a ±7.13	35.09a ±11.3	27.59a ±3.15	65.06a ± 9.01

Different letters vertically indicate the presence of significant differences (P≤0.05) between the means ± S.E

Table 7. Effect of added levels of enzyme to ratio containing Azolla on the feed intake of broiler

Character	feed intake(g) Weeks						total feed consumed 1-6
Treatments	First	Second	Third	Fourth	Fifth	Sixth	weeks
T1 Control	124.68a ± 15.3	304.75a ±2.45	573.28a ±57.59	1000.31a ±35.10	1166.5a ±46.37	1187.54a ±40.52	4556.36a 92.238 ±
T2 Azolla %7	119.88a ± 3.51	268.27a ± 8.67	591.15ab ±25.11	924.38b ±41.30	1037.38b ±18.71	1009.71b ± 34.26	3568.40b 84.251±
T3 7% Azolla ± 0.30% Enzyme	115.39a ±3.38	284.41a ±1.82	519.64b ±22.17	900.00 b ±32.00	1013.75b ±12.08	1096.84b ±25.02	3974.03b 41.710±
T4 7%Azolla + 0.35% Enzyme	124.67a ±2.62	292.0a ±2.71	552.02b ±31.15	905.99b ±14.00	1021.19b ±15.36	1105.19a ±19.27	3901.24b 40.979±
T5 %7Azolla + 0.40% enzyme	126.85a ± 2.76	270.96a ±8.69	570.01b ±5.11±	990.67a ±41.30	1047.48b ±18.71	1113.91a ± 34.46	3968.33b 84.251±

Different letters vertically indicate the presence of significant differences (P≤0.05) between the means ± S.E.

Table 8. Effect of added levels of an enzyme to ratio containing Azolla on the Feed conversion ratio (g feed/g weight gain) of broiler

Character	Feed conversion ratio (g feed/g weight gain) Weeks						Feed conversion ratio 1-6 w
Treatment	First	Second	Third	Fourth	Fifth	Sixth	
T1 Control	1.21a ±0.04	1.38a ±0.03	1.54a ±0.02	1.69a ± 0.05	1.97a ± 0.08	2.21a ± 0.01	1.65a ± 0.03
T2 Azolla %7	1.25a ±0.01	1.34a ± 0.03	1.56a ±0.02	1.53b ± 0.01	1.82ab ± 0.04	1.92ab ± 0.01	1.49b ± 0.08
T3 7% Azolla ± 0.30% Enzyme	1.22a ±0.03	1.31a ±0.05	1.40b ±0.03	1.50b ± 0.04	1.62b ± 0.02	1.82b ± 0.04	1.47b ± 0.02
T4 7%Azolla+ 0.35% Enzyme	1.29a ±0.02	1.39a 0.0±	1.41b ±0.02	1.52b ± 0.01	1.70b ± 0.02	1.85b ± 0.01	1.50b ± 0.05
T5 %7Azolla + 0.40% enzyme	1.30a ±0.01	1.35a 0.027±	1.39b ±0.01	1.53b ± 0.01	1.72b ± 0.04	1.89b ± 0.01	1.51b ± 0.08

Different letters vertically indicate the presence of significant differences (P≤0.05) between the means ± S.E

Table 9. Effect of added levels of the enzyme to ratio containing Azolla on the feed consumption (g) of broiler

Indicators	1-5 weeks		1-6 weeks	
Treatment	Production index	Production factor	Production index	Production factor
T1 control	273.95	29.88	313.13	24.16
T2 %7Azolla	02.003	32.41	302.27	27.39
T3 + 7%Azolla 0.30% Enzyme	343.15	38.63	400.05	33.11
T4 Azolla 7%+ 0.35% Enzyme	328.50	35.34	394.13	32.29
T5 Azolla7% + 0.40% enzyme	339.07	35.43	397.67	31.18