



## RESEARCH PAPER

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## Inclusion of varying levels of azolla from starter to finishing stage: potential on production performance, immunity and gut development of broiler chickens

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### Abstract

Broiler chicken production sustainability requires an alternative source of protein as one of the major sources of feed ingredients. This study evaluated the effects of incorporating varying levels of Azolla leaf meal (*Azolla pinnata*) on the production performance, immunity, and gut development of broiler chickens. A total of 60-day-old broiler chicks were randomly assigned to four dietary treatments: a control group (T<sub>0</sub>) with no Azolla, and three experimental groups supplemented with 10% (T<sub>1</sub>), 15% (T<sub>2</sub>), and 20% (T<sub>3</sub>) Azolla leaf powder. The experiment followed a Randomized Complete Block Design (RCBD) and lasted 21 days. Parameters measured included body weight gain, average feed consumption, feed conversion ratio (FCR), average feed consumption efficiency immune organ development, gut morphology, and overall liveability. Results showed that broilers in the T<sub>1</sub> group (10% Azolla) exhibited similar growth performance to the control group, indicating no negative impact on body weight, feed efficiency, or overall development. However, higher inclusion levels (15% and 20%) resulted in reduced growth performance due to increased dietary fiber, which may have affected nutrient absorption. Immunity assessment revealed increased thymus and spleen weights in T<sub>2</sub> and T<sub>3</sub>, suggesting enhanced immune function. Gut development analysis demonstrated a significant increase in the size of the crop, gizzard, and intestines in higher Azolla inclusion groups, indicating potential improvements in digestive efficiency. Overall, the findings suggest that Azolla supplementation of up to 10% is optimal for maintaining broiler growth without compromising feed efficiency. Higher inclusion levels may benefit immunity but could reduce growth rates due to excess fiber.

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## Introduction

Broiler chicken (*Gallus gallus domesticus*) is a breed of chicken raised intended for meat production. They are one of the most competitive sectors of the Philippine poultry industry, with a considerable impact on food availability and economic development. Poultry meat is becoming increasingly popular among consumers due to its low cost, high level of protein, and preference over other meats. With rising demand, broiler farming has become more prevalent and needs production efficiency and sustainability advances. Commercial production is the poultry industry's main market supply source, distinguished by backyard and commercial agricultural techniques.

In recent years, the poultry business has encountered challenges due to the increased price of feed ingredients, most notably soybean meal and corn. Food safety, environmental concerns, harmonizing welfare standards, banning nutritional antibiotics, gut health, feeding high-fiber meals, and maintaining high production efficiency are just a few of the hurdles that the chicken industry faces today. Feed costs account for almost 70% of the total cost of producing broilers, making it the most critical issue facing the poultry business. One solution to the aforementioned concerns is to use feed additives that can boost growth and/or egg production efficiency, reduce illness, and enhance feed utilization (Pirgozliev *et al.*, 2019).

To solve the issue, farmers and companies look for an alternative protein source to maintain production efficiency while reducing costs. One of these alternatives is *Azolla* because it's not only inexpensive but also high in protein as well as other necessary elements. Kundo *et al.* (2013) stated that there is constant demand for alternative local feed supplies to substitute commercial and compounded poultry to lower production costs. Incorporating *Azolla* into poultry diets has been shown to improve growth rate and feed conversion ratios (FCR) without negatively impacting meat quality (Abdelatty *et al.*, 2021).

*Azolla* (*Azolla pinnata*) is also described as a globally distributed aquatic fern with heterosporous growth (Asim *et al.*, 2022). This fern resembles duckweed or moss and appears to be floating on the water's surface. It can be utilized as a protein source in animal and poultry feed. *Azolla* has numerous pharmacological effects, including antioxidants, immunological stimulants, hepatoprotectants, phytoremediation, bioremediation, and nutritional value. *Azolla* is rich in vitamins (B12, beta-carotene, and A), biopolymers, minerals, and amino acids (Pascal *et al.*, 2010).

*Azolla* species are well-known for their substantial amount of protein, which normally ranges between 20% and 30%, as well as large levels of mineral and vitamin content. This plant has the unusual capacity to fix the nitrogen in the atmosphere, which improves its nutritional value when utilized as a feed element. The use of *Azolla* leaf meal in the diet of poultry can potentially improve the overall availability of nutrients and the ability to digest, making it an appealing alternative for broiler nutrition (Pramanik *et al.*, 2002).

This fast-growing aquatic fern has drawn interest for its possible advantages in the immune system and gastrointestinal development when added to broiler chicken diets. *Azolla* supplementation may improve intestinal shape by encouraging villus growth and boosting food absorption efficiency. It is rich in protein, vital amino acids, and bioactive compounds.

Its immunomodulatory and antioxidant qualities may also improve the bird's general health by boosting their immune system and decreasing their vulnerability to illnesses. These effects depend upon various circumstances, including digestibility, amounts of inclusion, and associations among other dietary components.

By assessing *Azolla*'s effects on immune system performance and gut development in broiler chickens, this study seeks to determine whether it is a viable feed substitute for poultry production.

## Materials and methods

### IACUC protocol

Before the conduct of the study, the proposal was submitted for approval by the Institutional Animal Care and Use Committee (IACUC) of Cebu Technological University.

### Time and location of the study

The study was conducted at P. Nellas St. Poblacion III, Carcar City, Cebu, Philippines from September 26 to October 16, 2024.

### Experimental treatment design and lay-out

The experiment was conducted using a Randomized Complete Block Design (RCBD). A total of 60 heads of day-old broiler chicken regardless of sex were used in the study. The experimental animals were grouped into four treatments (T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>) and were replicated three times with five birds per replication that were distributed randomly into 12 cages.

The following treatments were the following;

T<sub>0</sub>= control

T<sub>1</sub>= 10% Azolla Leaf Powder + 90% CF

T<sub>2</sub>= 15% Azolla Leaf Powder + 85% CF

T<sub>3</sub>= 20% Azolla Leaf Powder + 80% CF

### Preparation of rearing house

The experimental house was prepared a month before the day-old chicks were purchased. The experimental cages measuring 3m high × 4m wide were made with plastic screen sidings and plywood for flooring, provided with 2 inches of rice hulls as litter material. Black EPDM Rubber was used as the roofing. The house was constructed into three levels which consisted of four (4) rows and three (3) columns with a total of twelve (12) cages that represent the four (4) treatments and three (3) replications as shown in Table 1. Each cage was provided with feed and water troughs.

**Table 1.** Experimental lay-out

T <sub>1</sub> R <sub>3</sub>	T <sub>0</sub> R <sub>1</sub>	T <sub>2</sub> R <sub>1</sub>	T <sub>3</sub> R <sub>1</sub>
T <sub>0</sub> R <sub>2</sub>	T <sub>1</sub> R <sub>3</sub>	T <sub>0</sub> R <sub>2</sub>	T <sub>0</sub> R <sub>3</sub>
T <sub>2</sub> R <sub>2</sub>	T <sub>0</sub> R <sub>3</sub>	T <sub>2</sub> R <sub>3</sub>	T <sub>1</sub> R <sub>2</sub>

### Collection and preparation of azolla

The Azolla leaves used in the study were personally grown by the researcher located in P. Nellas, Poblacion III, Carcar City. During harvesting, the leaves were thoroughly washed with water and air dried for a maximum of four days until a maximum of 75% percent of dryness was achieved. The leaves were finely ground to powder using a meat grinder and were placed in a clean container.

### Chemical analysis of Azolla

The chemical compositions of Azolla are shown in Table 2. This is based on the study of Khurshedd *et al.* (2019). It shows that the Azolla in dry matter basis contains 90.3% of Dry matter, 22.79% Crude Protein, 3.59% of Ether Extract (EE), 15.49% of Crude Fiber, 38.67% of Nitrogen Free Extract (NFE), 19.46% of Total Ash, 2.03% of calcium and 0.48% of Phosphorus. These findings were also close with the result of Joysowal *et al.* (2018).

**Table 2.** Chemical analysis of azolla

Sl	Nutrients	Azolla (% DM)
1	Dry matter	90.3
2	Crude Protein	22.79
3	Ether Extract (EE)	3.95
4	Crude Fiber	14.39
5	Nitrogen Free Extract (NFE)	38.73
6	Total Ash	17.5
7	Calcium	2.06
8	Phosphorus	0.72

### Acquisition of experimental birds

A total of 60-day-old broiler chicks healthy and free from any defects were used in the study. The day-old chicks were acquired from a local Agrivet store and brooded for 14 days. They were placed in two cages measuring a floor space of 4x4 square feet each. Each cage had an optimal heat using electric light bulbs maintaining a temperature range of 30 to 36C throughout the brooding period. After the brooding phase, the initial weight of the chicks was documented and they were randomly assigned to their respective treatments.

### Feeds and feeding management

After the brooding period, the designated experimental treatments were administered to the

broiler chicks from the 15<sup>th</sup> day until the 35<sup>th</sup> day of the feeding trial. The experimental birds were fed based on the daily feeding guide shown in Table 3.

This guide was based on the Unifeeds Broiler Feeding Guide (<https://www.unifeeds.ph/assets/broiler-feeding-guide>).

**Table 3.** Daily feeding guide for the different treatment groups

Age (days)	Daily feed consumption (g/15 heads)	Treatments			
		T <sub>0</sub> (g)	T <sub>1</sub> (g)	T <sub>2</sub> (g)	T <sub>3</sub> (g)
15	840	840 BSC	756 BSC 84 Azolla	756 BSC 126 Azolla	756 BSC 168 Azolla
16	930	930 BSC	756 BSC 93 Azolla	756 BSC 139.5 Azolla	756 BSC 186 Azolla
17	1,080	1,080 BSC	756 BSC 108 Azolla	756 BSC 162 Azolla	756 BSC 216 Azolla
18	1,140	1,140 BSC	756 BSC 114 Azolla	756 BSC 171 Azolla	756 BSC 228 Azolla
19	1,200	1,200 BSC	756 BSC 120 Azolla	756 BSC 180 Azolla	756 BSC 240 Azolla
20	1,290	1,290 BSC	756 BSC 129 Azolla	756 BSC 193.5 Azolla	756 BSC 258 Azolla
21	1,320	1,320 BSC	756 BSC 132 Azolla	756 BSC 198 Azolla	756 BSC 264 Azolla
22	1,395	1,395 BSC	756 BSC 139.5 Azolla	756 BSC 209.25 Azolla	756 BSC 279 Azolla
23	1,455	1,455 BSC	756 BSC 145.5 Azolla	756 BSC 218.25 Azolla	756 BSC 291 Azolla
24	1,515	1,515 BSC	756 BSC 151.5 Azolla	756 BSC 227.25 Azolla	756 BSC 302 Azolla
25	1,545	1,545 BSC	756 BSC 154.5 Azolla	756 BSC 231.75 Azolla	756 BSC 309 Azolla
26	1,575	1,575 BSC	756 BSC 157.5 Azolla	756 BSC 236.25 Azolla	756 BSC 315 Azolla
27	1,605	1,605 BSC	756 BSC 160.5 Azolla	756 BSC 240.75 Azolla	756 BSC 321 Azolla
28	1,620	1,620 BSC	756 BSC 162 Azolla	756 BSC 243 Azolla	756 BSC 324 Azolla
29	1,650	1,650 BFC	756 BFC 165 Azolla	756 BC 247.5 Azolla	756 BFC 330 Azolla
30	1,680	1,680 BFC	756 BFC 168 Azolla	756 BFC 252 Azolla	756 BFC 336 Azolla
31	1,725	1,725 BFC	756 BFC 172.5 Azolla	756 BFC 258.75 Azolla	756 BFC 345 Azolla
32	1,800	1,800 BFC	756 BFC 180 Azolla	756 BFC 270 Azolla	756 BFC 360 Azolla
33	1,830	1,830 BFC	756 BFC 183 Azolla	756 BFC 274.5 Azolla	756 BFC 366 Azolla
34	1,845	1,845 BFC	756 BFC 184.5 Azolla	756 BFC 276.5 Azolla	756 BFC 369 Azolla
35	1,890	1,890 BFC	756 BFC 189 Azolla	756 BFC 283.5 Azolla	756 BFC 378 Azolla

Feeding was done twice a day, at 7:00 AM and 5:00 PM, leftover feeds were recorded every morning to calculate the levels of feed intake.

#### Sanitation

Daily inspections of the water and feed troughs were carried out in compliance with safety procedures. In addition, the removal and disposal of manure from

pens was maintained every day. To minimize contamination and the growth of parasites and other disease-causing organisms, broiler pens were kept clean and dry at all times.

After the feeding trial, the chickens were harvested. The weight of birds in every replication per treatment was recorded. After the documentation of

their final weight, they were subjected to slaughtering. To start the process, the bird was killed by hand by cutting the jugular veins and the carotid arteries at the neck. They were permitted to bleed for an amount of time. After bleeding, they were scalded in a container that contained hot water to soften their skin allowing the removal of feathers. After defeathering, the heads and legs were removed and then they were eviscerated. After the evisceration, the carcasses of the birds were opened subsequently and the entire gastrointestinal tract was aseptically removed. The organs needed to determine the gut development and immunity of the birds were weighed accurately and properly recorded.

#### *Data gathered*

##### *Growth performance parameters*

##### *Average body weight gain*

This was obtained by adding up all the individual weight gains and dividing by the number of birds in the sample group

$$\text{Average Body Weight Gain (ABWG)} = \frac{\text{sum of individual weight gains}}{\text{number of birds}}$$

##### *Average feed consumption*

This is calculated by getting the difference between the amount of feed supplied to the bird and the amount of feed that remained at the end of each feeding period and divided by the total number of birds per treatment

$$\text{Average Feed Consumption (AFC)} = \frac{\text{total feed given} - \text{total left over feeds}}{\text{total number of birds per treatment}}$$

##### *Feed conversion ratio*

The Feed Conversion Ratios of the broiler chicken were evaluated based on the total feed intake divided by the total weight gain of the broiler chicken throughout the study.

$$\text{FCR} = \frac{\text{Total feed consumed (kg)}}{\text{total weight gain (kg)}}$$

##### *Average feed conversion efficiency (%)*

This was calculated by getting the average weight gain of the birds divided by the average feed consumption.

$$\text{Average Feed Conversion Efficiency (AFCE)} = \frac{\text{average weight gain}}{\text{average feed consumption}}$$

##### *Average final weight*

This was obtained by dividing the total weight of birds at harvest from the total number of birds per treatment.

$$\text{Average Final Weight (AFW)} = \frac{\text{weight of birds at harvest}}{\text{total number of birds per treatment}}$$

##### *Weight of immune organs*

This was obtained by weighing the thymus, spleen and bursa of fabricus of the chicken individual, they were weighed accurately using the digital weighing scale and then recorded properly.

##### *Gut parameters*

Gut organs in each treatment were weighed using the digital weighing scale.

##### *Mortality rate*

This was obtained by dividing the number of dead birds by the total number of live birds.

$$\text{Mortality Rate (\%)} = \frac{\text{number of dead birds}}{\text{total number of live birds}} \times 100$$

##### *Liveability rate*

This was obtained by dividing the number of birds that are alive by the total number of animals raised during the duration of the study.

$$\text{Liveability (\%)} = \frac{\text{number of live birds}}{\text{total number of raised birds}} \times 100$$

##### *Statistical analysis*

The data was computed, tabulated, and statistically analyzed using the Analysis of Variance. Significant differences between and among treatment means were further subjected to Tukey's Honest Significant Difference (HSD) Test at a 5% level of significance.

## **Results and discussion**

### *Growth performance of broiler chicken*

Broiler chicken growth performance is characterized by rapid growth and efficient feed conversion, making them an important component in meat production.

Table 4 shows that the control group (To) and the group supplemented with 10% Azolla leaf meal (T1) performed similarly in terms of growth, as evidenced by their final weight, feed consumption, feed conversion ratio (FCR), and average body weight

acquisition. These results were in line with the findings by Subudhi and Singh (1997), who also noted a significant difference in the body weight of broiler chickens receiving lesser dosages of Azolla supplements.

**Table 4.** Effects of azolla leaf meal supplementation on the growth performance of broiler chicken

Treatments	Average body weight gain	Average feed consumption	Feed conversion ratio (FCR)	Average feed conversion efficiency	Average final weight
To–Control	392.82 a	687.33	1.75 c	57.15 a	492.76 a
T1–Azolla + 10% CF	393.02 a	687.33	1.75 c	57.18 a	493.22 a
T2–Azolla + 15% CF	364.58 b	681.03	1.87 b	53.53 b	465.66 b
T3–Azolla + 20% CF	323.06 c	677.95	2.10 a	47.65 c	423.06 c
CV%	2.48	1.27	2.52	2.49	1.98
P-value	0.0002	0.5035	0.0003	0.0004	0.0003

Means with the same letter are not significantly different at 5 % level using Tukey's Honest Significant Difference (HSD) Test.

**Table 5.** Effects of azolla leaf meal supplementation on the immunity of broiler chicken

Treatments	Bursa of fabricus (g)	Thymus (g)	Spleen (g)
To–Control	5.10	2.09 c	1.42 b
T1–Azolla + 10% CF	5.18	2.35 c	1.67 b
T2–Azolla + 15% CF	5.10	2.99 b	2.81 a
T3–Azolla + 20% CF	5.09	4.30 a	3.15 a
CV%	3.49	9.95	7.98
P-value	0.9122	0.0004	0.0001

In terms of feed consumption, the results showed that the bird's total meal intake varied numerically but did not change significantly between treatments. The study's findings are consistent with those of Parthasarathy *et al.* (2002) and Balaji *et al.* (2009), who found that dietary inclusion of Azolla powder did not affect feed consumption, but rather decreased it with increasing levels.

As the amount of Azolla supplementation increased to 15% (T2) and 20% (T3), however, feed conversion efficiency, body weight gain, and ultimate weight all decreased. As observed, the T3 group has the least average body weight gain (323.06 g) and final weight (423.06 g). It is similar to the findings of Cambel (1984) in which those who are supplemented with higher levels of Azolla obtained the lowest gain of weight.

The increased FCR values with more Azolla inclusion indicate that broilers in T2 and T3 needed more feed to acquire the same weight as the control group and

T1. This could be attributed to Azolla's high fiber content, which may impair digestion and nutrient utilization.

This pattern is in line with research by Pramanik *et al.* (2002), who found that poultry's development performance and nutritional absorption can be negatively impacted by an excessive amount of fiber in substitute protein sources like Azolla. While the Azolla meal is high in essential amino acids and crude protein, its high fiber content may cause digestive problems when consumed in excess (Dhumal *et al.*, 2009). Therefore, it would seem that a moderate supplementing level—say, 10%—is ideal for broiler growth.

#### *Immunity of broiler chicken*

Table 5 shows how Azolla supplementation affects the immunological organs, specifically the Bursa of Fabricius, thymus, and spleen. The Bursa of Fabricius, which is critical for B-cell development in chickens, remained somewhat stable throughout all

treatment groups. However, there were substantial disparities in thymus and spleen weights. The thymus, which is crucial for T-cell maturation, increased significantly in weight in both the T2 and T3 groups, with T3 having the largest weight (4.30 g). Similarly, the spleen, an important organ for immunological response and pathogen filtration, had larger weights in the T2 and T3 groups than in the control. These suggest that Azolla may have an immunostimulatory impact, which is consistent with the findings of Swain *et al.* (2022), who

highlighted Azolla's nutritional value, emphasizing its protein, vitamin, and mineral content, as well as its role in improving immune function in poultry. Furthermore, because of its antibacterial and antioxidant qualities, Azolla supplementation increased disease resistance in chicken Riaz *et al.* (2022). In addition, their research showed that Azolla improves gut health by boosting nutrient absorption and general digestion, which supports its role in boosting broiler growth and immunological function.

**Table 6.** Effects of azolla leaf meal supplementation on the gut development of broiler chicken

Treatments	Crop (g)	Heart (g)	Liver (g)	Cecum (g)	Kidneys (g)	Proventriculus (g)	Gizzard (g)	Small intestine (g)	Large intestine (g)
To-Control	7.33 d	10.70	49.46	6.31 d	8.73	7.27 c	28.33 c	53.83 c	10.75 c
T1-Azolla + 10% CF	8.62 c	10.50	50.43	7.33 c	8.80	8.17 b	31.37 c	56.12 c	12.11 b
T2-Azolla + 15% CF	10.83 b	10.84	50.75	9.13 b	8.72	8.22 b	37.34 b	68.25 b	14.57 a
T3-Azolla + 20% CF	15.39 a	10.99	51.44	11.95 a	8.70	10.47 a	47.03 a	88.69 a	15.57 a
CV%	6.13	4.09	1.30	5.87	1.12	1.41	4.36	3.59	4.22
P-value	0.0000	0.6025	0.0514	0.0000	0.6444	0.0000	0.0000	0.0000	0.0001

Means with the same letter are not significantly different at 5 % level using Tukey's Honest Significant Difference (HSD) Test

#### Gut development of broiler chicken

Gut development is important for the broiler's general wellness, digestion, and nutritional absorption. The digestive organs, which include the crop, proventriculus, small intestine, large intestine, and cecum, are in charge of breaking down food, absorbing important nutrients, and maintaining gut flora. Table 6 shows that Azolla supplementation had a major impact on gut growth, with considerable gains in the weights of specific digestive organs as Azolla inclusion increased. This section examines how Azolla supplementation affects each gastrointestinal organ and its effects on broiler chicken's performance and overall health.

With the greatest supplementation group (T3-Azolla + 20% CF) reaching 15.39 g and the control group (To) having the lowest value (7.33 g), the crop weight demonstrates an increasing tendency. This implies that increasing Azolla levels promote crop development, presumably as a result of higher fiber content, which may encourage enhanced capacity for digestion and storage. According to the study of

Parashuramulu *et al.* (2012), dietary fiber improves gizzard and crop development, which improves feed retention and chicken digestive efficiency. Similar trends can be seen in the weights of the large intestine and cecum, with T3 having the greatest values (11.95 g for the cecum and 15.57 g for the large intestine). This suggests that the lower intestine has better microbial activity and digesting efficiency because Azolla has been shown to encourage the growth of good gut bacteria (Basak *et al.*, 2002).

As supplementation rises, gizzard weight also increases dramatically; T3 recorded the highest value (47.03 g) in comparison to the control (28.33 g). Because Azolla meal has more fiber, which necessitates more grinding action, this result implies that it improves gizzard function. According to Han *et al.* (2017), broilers that consume a lot of fiber make gizzards heavier as a result of the organ compensating for the greater demands of mechanical digestion. The weight of the small intestine is also greatly impacted, with T3 (88.69 g) being much more than To (53.83 g). This implies that Azolla encourages intestine

development, which could enhance the absorption of nutrients. According to Korsá *et al.* (2024), plant-based protein sources that include bioactive substances may promote intestinal growth and improve the effectiveness of nutrient absorption.

There are few differences between treatments for the liver, heart, and kidneys, showing that Azolla has little effect on these organs. However, the proventriculus exhibits the same trend as the gizzard, with T<sub>3</sub> exhibiting the greatest value (10.47 g), implying improved digestive efficiency.

**Table 7.** Effects of Azolla leaf meal supplementation on the mortality and liveability of broiler chicken

Treatments	Mortality rate	Liveability rate
To-Control	0%	100%
T1-Azolla + 10% CF	0%	100%
T2-Azolla + 15% CF	0%	100%
T3-Azolla + 20% CF	0%	100%

Table 7 shows a 0% mortality rate and a 100% liveability rate of broiler chicken fed with different levels of Azolla during the experimental period. This means that the inclusion of Azolla from 10%-20% to the diet of broilers does not have a detrimental effect on broiler chicken. The result is the same as the study of Joysowal *et al.* (2018b); Rengma *et al.* (2019), and Basak *et al.* (2002) which showed that the inclusion of Azolla powder in the diet of broiler chicken do not have a deleterious effect on their health and overall performance.

### Conclusion

The findings indicate that Azolla supplementation at 10% is ideal for broiler growth performance since it has no negative effects on weight gain, feed efficiency, or gut health. Higher inclusion levels (15% and 20%) improve immune organ development but may reduce growth performance due to excess fiber. Future research should look at fiber reduction techniques to improve the digestibility of Azolla in poultry diets.

### Recommendations

It is recommended that poultry farmers use a 10% inclusion level of Azolla leaf meal in broiler diets to maintain optimal growth performance and feed

efficiency. Further studies should explore methods to reduce the fiber content in Azolla to improve digestibility and enhance its benefits at higher supplementation levels. Additionally, long-term research should be conducted to evaluate the economic viability and overall sustainability of Azolla as an alternative protein source in commercial poultry farming.

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