The impact of *Azolla* (*Azolla pinnata* R.Br.) dietary supplementation on broiler chicken production performance and serum biochemical profile

El impacto de la suplementación dietética con *Azolla* (*Azolla pinnata* R.Br.) sobre el rendimiento productivo y el perfil bioquímico sérico de los pollos de engorde

O impacto da suplementação alimentar com *Azolla* (*Azolla pinnata* R.Br.) no desempenho produtivo de frangos de carne e no perfil bioquímico do soro

Asma Nour Elhouda Belagoune[1]
Saber Beghoul[1]
Samir Djemai[1]*
Kahina Hafid[2]
Douaa Amireche[1]
Amir Mansour[1]

[1]Laboratoire de Recherche de Pathologie Animale Développement des Elevages et Surveillance de la Chaine Alimentaire des Denrées Animales ou d'origine Animale (PADESCA Laboratory), Institute of Veterinary Sciences -ElKhroub, University of Constantine 1, Constantine, Algeria.
[2]BIOQUAL Laboratory, Institute of Nutrition and Food and Agri-Food Technologies, University of Constantine 1, Algeria.

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Abstract

For decades, Algeria has faced difficulties related to importing raw materials for livestock feed, such as soybeans and corn. The aim of this study was to valorize *Azolla* (*Azolla pinnata* R.Br.) as an important source of nutrients, particularly proteins, in broiler production. For this purpose, 60 broiler chicks of the Cobb 500 breed were divided into two groups, each containing three repetitions of 10 birds. The diets of the tested and control groups were the same, except for the tested group, which received *Azolla* as a supplement. The nutritional value of *Azolla* incorporated into the diet was assessed chemically. Liveweight, feed intake, feed conversion ratio, offal weight, carcass yield, and biochemical profile for both groups were observed and compared. The intake of *Azolla* had no impact on production yield ($P>0.05$); however, the best liveweight at sacrifice was obtained in the *Azolla* group, with a value of 2600.67 g, whereas the mean recorded in the control group was 2431.67 g. Regarding the weight and yield of offal and carcass, we found the same observation: the highest weight of thighs and wings was recorded in the *Azolla* group. All blood biochemical parameters examined (glucose, creatinine, cholesterol, triglyceride, high-density lipoprotein (HDL), total protein, albumin, and alanine aminotransferase (ALT)) showed no significant difference, except for aspartate aminotransferase (AST), which was found to be significantly higher in the *Azolla* group ($P<0.05$). *Azolla pinnata* R.Br., a source of unconventional protein, may therefore be an alternative to the costly poultry feed used in Algeria.

*Corresponding author:samir.djemai@umc.edu.dz

Resumen

Durante décadas, Argelia ha enfrentado dificultades relacionadas con la importación de materias primas para la alimentación del ganado, como la soja y el maíz. El objetivo de este estudio fue valorizar la *Azolla* (*Azolla pinnata* R.Br.) como una fuente importante de nutrientes, particularmente proteínas, en la producción de pollos de engorde. Para este propósito, 60 pollos de engorde de la raza Cobb 500 se dividieron en dos grupos, cada uno con tres repeticiones de 10 aves. Las dietas de los grupos evaluados y de control fueron las mismas, excepto la del grupo evaluado, que recibió *Azolla* como suplemento. Se evaluó químicamente el valor nutricional de la *Azolla* incorporada a la dieta. Se observaron y compararon el peso vivo, el consumo de alimento, el índice de conversión alimenticia, el peso de los despojos, el rendimiento del canal y el perfil bioquímico de ambos grupos. El consumo de *Azolla* no tuvo impacto en el rendimiento productivo (P>0,05), sin embargo, el mejor peso vivo al sacrificio lo obtuvo el grupo *Azolla*, con un valor de 2600,67 g, mientras que la media registrada en el grupo control fue de 2431,67 g. En cuanto al peso y rendimiento de despojos y canales, encontramos la misma observación : el mayor peso de muslos y alas se registró en el grupo *Azolla*. Todos los parámetros bioquímicos sanguíneos examinados (glucosa, creatinina, colesterol, triglicéridos, lipoproteínas de alta densidad (HDL), proteínas totales, albúmina y alanina aminotransferasa (ALT)) no mostraron diferencias significativas, excepto el aspartato aminotransferasa (AST), que resultó ser ser significativamente mayor en el grupo de *Azolla* (P<0,05). Por lo tanto, la *Azolla pinnata* R. Br., una fuente de proteínas no convencional, puede ser una alternativa al costoso alimento para aves utilizado en Argelia.

Palabras clave: suplementación con *Azolla*, dietas de pollos, sacrificio.

Resumo

Durante décadas, a Argélia enfrentou dificuldades relacionadas à importação de matérias-primas para ração animal, como soja e milho. O objetivo deste estudo foi valorizar a *Azolla* (*Azolla pinnata* R.Br.) como uma importante fonte de nutrientes, particularmente proteínas, na produção de frangos de corte. Para isso, 60 pintinhos de corte da raça Cobb 500 foram divididos em dois grupos, cada um contendo três repetições de 10 aves. As dietas dos grupos testado e controle foram as mesmas, exceto para o grupo testado, que recebeu Azolla como suplemento. O valor nutricional do *Azolla* incorporado à dieta foi avaliado quimicamente. Peso vivo, consumo de ração, conversão alimentar, peso de vísceras, rendimento de carcaça e perfil bioquímico de ambos os grupos foram observados e comparados. O consumo de *Azolla* não teve impacto no rendimento da produção (P>0,05); entretanto, o melhor peso vivo ao sacrifício foi obtido no grupo *Azolla*, com valor de 2600,67 g, enquanto a média registrada no grupo controle foi de 2431,67 g. Em relação ao peso e rendimento de miudezas e carcaça, encontramos a mesma observação: o maior peso de coxas e asas foi registrado no grupo *Azolla*. Todos os parâmetros bioquímicos sanguíneos examinados (glicose, creatinina, colesterol, triglicerídeos, lipoproteína de alta densidade (HDL), proteína total, albumina e alanina aminotransferase (ALT)) não mostraram diferença significativa, exceto para aspartato aminotransferase (AST), que foi encontrado para ser significativamente maior no grupo *Azolla* (P<0,05). A *Azolla pinnata* R. Br., uma fonte de proteína não convencional, pode, portanto, ser uma alternativa à dispendiosa ração para aves utilizada na Argélia.

Palavras-chave: suplementação com *Azolla*, dietas para frangos, sacrifício.

Introduction

Despite the development of the Algerian poultry industry, it still encounters many difficulties, the main reason was the recurrent shortage and the high cost of imported raw materials (soybean meal and maize) for broiler feeding. Therefore, additional resources should be sought. Some non-conventional food resources, such as *Azolla*, have attracted interest from some Algerian farmers. The inclusion of aquatic plants (e.g. *Azolla pinnata, Egeria densa*, *Elodea canadensis* and *Hydrilla verticillata*) at low levels in poultry feed yielded positive results, especially when included as an unconventional source of protein or as a source of pigment to eggs (Mcdowell *et al.,* 1990; Khatun and Islam, 2021). *Azolla pinnata* is an aquatic plant that can be utilized as a source of protein in animal feed; several studies have been conducted on this subject, and this, on various animal species such as poultry (Acharya *et al.,* 2015; Shukla *et al.,* 2018; Shambhvi *et al.,* 2020; Abdelatty *et al.,* 2021), fish (Datta, 2011), giant river prawn (Radhakrishnan *et al.,* 2014), and sheep (Ahmed *et al.,* 2016). Subudhi and Singh (1978) noted that fresh *Azolla* can be used to replace 20-25 % of commercial feed for chicken without any risk to health. They also stated that 100 birds would require approximately 9 kg of fresh *Azolla* per day. They also indicated that 100 birds would require approximately 9 kg of fresh *Azolla* daily to replace 20 % of commercial feed. This resulted in a decrease in the cost of poultry production.

Chickens have the ability to choose their diet, which satisfies their natural appetite, develops their digestive system, and gives them a degree of natural resistance to coccidiosis. Free choice feeding is a more natural feeding system, where each bird can choose its own nutrient balance to satisfy its own physiological needs. Gous and Swatson (2000) explained that broilers can optimize their performance by utilizing the optimal combination of protein sources whenever given the opportunity.

The objective of this study was to assess the effect of freely available fresh *Azolla pinnata* R.Br. on growth performance, blood biochemical parameters and carcass characteristics of broiler chickens.

Materials and methods

Experiment Design

60 one-day-old Cobb 500 chicks were reared together on the floor for 15 days and given *ad-libitum* the same broiler starter feed (crumbled). The birds were then randomly divided into two groups (treatments) of 30 chicks each, with three replications of ten chicks each. The grower and finisher feeds for broilers (crumbled) were provided *ad-libitum* to both the control group (C) and the *Azolla* group (A: choice-feeding group) during the grower (15-35 days of age) and finisher (35-45 days of age) phases, respectively.

The *Azolla* was given free choice (free access) to the birds in group A (choice-feeding group) when they were between 15 and 45 days old (table 1).

Table 1. Ingredients and chemical composition of broiler feed used in the study.

	Starter diet	Grower diet	Finisher diet
	Ingredient (%)		
Soybean meal (44% Crude protein)	31.50	26.00	22.75
Corn, yellow	57.25	61.30	61.60
Soybean oil	1.70	1.80	2.00
Wheat bran	5.50	7.00	10.00
Dicalcium phosphate	1.40	1.30	1.20
Monocalcium phosphates.	0.65	0.60	0.45
Vitamin mineral premix	1.00	1.00	1.00
Amino acid premix	1.00	1.00	1.00
	Chemical composition (%)		
Ether extract	4.46	4.66	4.90
Ash	5.68	5.15	4.78
Lysine	1.22	1.90	1.02
Methionine	0.63	0.61	0.59
Methionine + Cysteine	0.92	0.88	0.85
Threonine	0.78	0.71	0.67
Metabolizable energy (kcal.kg^{-1})	3,010.60	3,038.75	3,031.40
Crude protein	21.08	19.08	18.06

Data and sample collection

In order to prevent water loss, *Azolla* was cultivated in concrete basins covered with plastic sheeting (black polyethylene). Following the *Azolla* cultivating method in our region, these basins included two centimeters of soil and one centimeter of aged sheep manure.

The fresh *Azolla* was washed, dried for four to five days in the shade, and then ground into a homogeneous powder. After which, a representative sample of the flour had been analyzed for dry matter, ash, nitrogenous matter total, fat (ether extract), and crude fiber (AOAC, 1995). Weight measurements were performed every five days (in the morning, before feed distribution), after which feed conversion ratio (FCR), average daily gain (ADG), and feed intake (FI) were calculated (Abdelatty *et al*., 2021).

Nine chickens (3 from each group) were sacrificed on the 45th day of age. The carcass yield was calculated by weighing the animals' carcasses, gizzards, livers, hearts, abdominal fat, thighs, mass of breast and wings (Bhattacharyya *et al.,* 2016). All weighing was performed using an electronic scale with a 1 g accuracy (KERN CB12K1N).

Blood samples were collected at the 45th day of age (the end of the experiment) from 3 birds of each repetition (9 chickens.group) in heparinized and identified tubes. After centrifuging the tubes for 10 to 15 minutes at 2500 rpm, the plasma was collected in labeled dry tubes and kept at -20 °C until the time of the analysis. It should be mentioned that the biochemical profile was performed 24 hours after the blood samples were collected.

The following blood parameters were measured using the commercial BIOLABO kit (France; KENZA BIOLABO analyzer France): glucose, triglycerides, cholesterol, high density lipoprotein (HDL) cholesterol, total protein, albumin, creatinine, glutamate, aspartate aminotransferase (AST), and alanine aminotransferase (ALT).

Statistical analysis

The IBM SPSS Statistics 20 software was used to conduct statistical analyses. Student's t-test (for parametric data) and Mann-Whitney test (for non-parametric data) were used to compare data statistically.

Results and discussion

The chemical analysis revealed that the *Azolla* employed in this study contained: 90.3 % dry matter (DM), 24.8 % crude protein (CP), 13.25 % crude fiber (CF), 2.11 % ether extract (EE), 24.9 ash, 34.94 % Nitrogen-free extract (NFE) and 2095.66 kcal.kg^{-1} metabolizable energy (table 2).

Table 2. The chemical composition of *Azolla* used in the study.

Nutritional content*	Proportion
Dry matter (%)	90.30
Ash (%)	24.90
Crude protein (%)	24.80
Ether extract (%)	2.11
Crude fibre (%)	13.25
Nitrogen-free extract (%)	34.94
Metabolizable energy (kcal.kg^{-1})	2095.66

*Determined by analyzing dry matter

The proportions of the various chemical components present in the *Azolla* used in this study are numerically close to those found in previous studies, such as Shambhvi *et al*. (2020), who obtained the following values: 25.62 % CP, 2.11 % EE, 19.2 % Ash, and 2075.57 kcal.kg metabolizable energy. Shukla *et al*. (2018) (CP = 25.64 %, EE = 3.15 %, de Ash = 19.21 %) and Balaji *et al*. (2009) (CP=24.5 %) obtained numerically similar results.

There was no significant difference (P = 0.31) in FI between the *Azolla* group (4536 g) and the control group (3888 g). The difference in FCR between groups C (1.93) and A (2.07) was not statistically significant (P = 0.53). We found that the ADG in group A (73.02 g.day^{-1}) was numerically higher than that in the control group (67.03 g.day^{-1}); however, the difference was not statistically significant (P = 0.71) (table 3).

Acharya *et al.* (2015) also found that incorporating up to 10 % fresh *Azolla* had no effect on FI in white pekin broiler ducks. Contrary to our results, Ara *et al.* (2018) noticed that including *Azolla cristata* in the diet of laying hens at 10, 15, and 20 % resulted in a significant decrease in feed consumption, while Abdelatty *et al.* (2020) found an increase in FI in groups of chickens fed diets containing 5 and 10 % *Azolla* meal and this, compared to the control group. Shukla *et al.* (2018), found no significant difference in FCR between the growing turkeys fed *Azolla* and conventional feed and the control group fed only conventional feed. The same authors observed no significant difference in mean weekly weight gain between turkeys receiving *ad-libitum Azolla* and the control group. Mishra *et al.* (2016) reported that the introduction of 5 % to 7.5 % *Azolla* meal in chick feed significantly increased body weight gain.

Table 3. Feed intake, average daily gain, and feed conversion ratio in the two groups studied.

Parameter	Control group (C)	*Azolla* group (A)	*p*-value
FI (g)	3,887.61[a] ± 165.48	4,535.64[a] ± 180.05	0.31
ADG (g.day^{-1})	67.03[a] ± 23.66	73.02[a] ± 30.52	0.71
FCR	1.93[a] ± 0.55	2.07[a] ± 0.78	0.53

[a,b]Mean values with different superscripts within a row differed significantly (P < 0.05).
FI: Feed intake; ADG: Average Daily Gain; FCR: Feed Conversion Ratio.

The average liveweight at sacrifice and the average carcass weight in group A (2,600.67 g and 2,017.56 g respectively) were statistically higher (P < 0.05) than in group C (2,431.67 g and 1,842.22 g respectively). The carcass yield in group A (77.57 %) was significantly better (P < 0.05) than in group C (75.76 %). In this study, we found that chickens given *Azolla* had significantly higher (P < 0.05) average thighs and wings weights (529.44 g and 197.22 g respectively) than the control group (486.56 g and 182.44 g respectively). However, there was no statistically significant difference (P > 0.05) in average breast weight between groups A (691.78 g) and C (651.44 g). We also found that incorporating fresh *Azolla* to group A feed showed no statistically significant effect (P > 0.05) on the average weights of the liver (50.89 g), heart (14.33 g) and gizzard (26.67 g) compared to the control group (50.33 g, 16.11 g and 28.33 g respectively) (table 4).

Shambhvi *et al*. (2020) reported no significant difference in mean liveweight at sacrifice between the 2.5 % *Azolla* fed group (2046.25 g) and the control group (1988 g). Basak *et al*. (2002), Naghshi *et al*. (2014), and Bhattacharyya *et al*. (2016) observed very high carcass yields in broilers fed *Azolla* integrated at 5 %.

According to Naghshi *et al*. (2014), broilers fed 5 and 10 % *Azolla* showed high average thigh weights. Varadharajan *et al*. (2019) observed that incorporating 3 % *Azolla* meal in the feed improved wings weight in quail. In contrast to the results of the present study, Mishra *et al*. (2016) observed a decrease in wing weight in Chabro chickens fed 7.5 % *Azolla* compared to the control group.

Shambhvi *et al*. (2020) reported that gizzard weight and breast weight were significantly higher in chickens fed 2.5 % *Azolla pinnata* meal compared to the control group.

With the exception of AST, there was no significant difference (P > 0.05) in the serum biochemical profile parameters tested (glucose, creatinine, cholesterol, triglyceride, HDL, total protein, ALT). There was a statistically significant increase (P < 0.05) in blood AST in group A (430.78 IU.L^{-1}) compared to group C (327.33 IU.L^{-1}) (table 5).

Shukla *et al*. (2018) found no significant difference in serum concentrations of liver enzymes (AST and ALT) between *Azolla*-fed and control groups. Other studies carried out on broilers (Abdelatty *et al*., 2020) and turkeys (Shukla *et al*., 2018) reported no statistically significant impact of *Azolla* in feed on blood concentrations of liver enzymes (AST and ALT).

Conclusion

This study showed that broiler chicks fed fresh *Azolla* performed better in terms of production compared to the control group. This plant, which has a considerable amount of protein, can be used in Algeria to reduce the cost of feed for livestock animals, particularly broiler chickens, especially as it is easily available and simple to cultivate.

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Table 4. Average liveweight at sacrifice, average carcass weight and other carcass characteristics in broiler chickens at 45 day of age.

Parameter	Control group (C)	*Azolla* group (A)	*P*-value
Average liveweight at sacrifice (g)	2,431.67[a] ± 60.42	2,600.67[b] ± 36.03	0.00
Average carcass weight (g)	1,842.22[a] ± 56.40	2,017.56[b] ± 67.60	0.00
Carcass yield (%)	75.76[a] ± 1.32	77.57[b] ± 2.11	0.04
Average liver, heart, gizzard, thighs, mass of breast and wings weight (g.bird^{-1})			
Liver	50.33[a] ± 5.05	50.89[a] ± 4.46	0.89
Heart	16.11[a] ± 3.26	14.33[a] ± 2	0.18
Gizzard	28.33[a] ± 4.36	26.67[a] ± 0.88	0.34
Thighs	486.56[a] ± 47.42	529.44[b] ± 29.20	0.035
Mass of breast	651.44[a] ± 74.71	691.78[a] ± 46.49	0.188
Wings	182.44[a] ± 13.00	197.22[b] ± 12.36	0.025

[a,b]Mean values with different superscripts within a row differed significantly (p < 0.05).

Table 5. The biochemical profile of broiler chicken serum in the two study groups.

Parameter	Control group (C)	*Azolla* group (A)	*p*-value
Glucose ($g.L^{-1}$)	$2.18^{a} \pm 0.09$	$2.26^{a} \pm 0.15$	0.19
Creatinine ($mg.dl^{-1}$)	$3.79^{a} \pm 0.69$	$4.01^{a} \pm 0.31$	0.40
Cholesterol ($g.L^{-1}$)	$1.06^{a} \pm 0.11$	$1.15^{a} \pm 0.16$	0.18
Triglyceride ($g.L^{-1}$)	$0.36^{a} \pm 0.16$	$0.40^{a} \pm 0.18$	0.61
HDL ($g.L^{-1}$)	$0.56^{a} \pm 0.12$	$0.58^{a} \pm 0.14$	0.70
Total protein ($g.L^{-1}$)	$33.67^{a} \pm 4.24$	$34.22^{a} \pm 2.59$	0.74
Albumin ($g.L^{-1}$)	$15.56^{a} \pm 1.01$	$16.11^{a} \pm 1.36$	0.34
AST ($IU.L^{-1}$)	$327.33^{a} \pm 56.78$	$430.78^{b} \pm 85.58$	0.008
ALT ($IU.L^{-1}$)	$13.00^{a} \pm 6.09$	$12.75^{a} \pm 2.96$	0.918

[a,b]Mean values with different superscripts within a row differed significantly ($P<0.05$).
HDL: high-density lipoprotein; AST: aspartate aminotransferase; ALT: alanine aminotransferase.

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