

Effect of Azolla Dietary Supplementation on Productive Performance and Blood Biochemical Parameters of Awassi Lambs

Nawar Bahaa abduljabbar

Department of Animal Production, College of Agriculture, University of Tikrit

Corresponding author's email: nawar.a014@tu.edu.iq

ABSTRACT

This study evaluated the impact of supplementing Awassi lamb diets with Azolla (*Azolla pinnata*) on productive performance and blood biochemical indicators. Nine lambs, 3 to 4 months old and averaging 32-33 kg in were assigned to three dietary treatments: a control group, and two groups supplemented with 2 and 3% Azolla (on a body weight basis). During the feeding period of 56 days, all lamb groups were reared with complete veterinary care at similar conditions, lamb body weight and feed intake were recorded, while weight gain was calculated. At the end of feeding period, blood samples were collected and serum was separated for determining total protein (TP), albumin (AL), globulin (GL), and AL/GL ratio as well as activity of aspartate (AST) and alanine (ALT) transaminase. Results revealed that feeding both Azolla diets (2 and 3%) insignificantly increased lamb body weight to 37.8 and 37.6 kg, weekly weight gain to 0.61 and 0.62 kg, daily feed intake to 0.68 and 0.67 kg as compared to 37.1, 0.35, and 0.64 kg in control group, respectively. Also, 2 and 3% Azolla increased serum TP to 6.87 and 6.85 g/dl, albumin to 3.54 and 3.95 g/dl, and AL/GL ratio to 1.1 and 1.3 compared to control (5.54 g/dl, 3.21 g/dl, and 0.96, respectively), without significant differences ($P>0.05$) among groups. However, GL concentration was 3.33, 3.33, and 3.00 g/dl in control, 2% Azolla, and 3% Azolla, respectively. Both Azolla levels decreased ($P<0.05$) serum AST activity (47.4 and 39.5 vs. 65.4 U/l) and only 3% Azolla decreased ($P<0.05$) serum ALT activity to 11.45 U/l vs. 15.14 U/l) compared with control. Based on the foregoing results, the dietary supplementation of Azolla in the diet of lambs slightly improved growth rate, in terms increasing body weight, weight gain, serum TP and AL concentration, and reducing the activity of serum of AST and ALT. The present results concluded that Azolla can be used in the diet without adversely effects on growth performance and can improve protein metabolism and health status (liver function) of Awassi lambs.

Keywords: *Azolla*, *Awassi lambs*, *blood biochemistry*, *performance*, *sustainable feeding*.

INTRODUCTION

The rising costs of conventional livestock feed have driven the search for sustainable and economical alternatives. Using untraditional feed ingredients is a strategy to solve the animal protein shortage [1]. In many countries, there are urgent needs for non-conventional supplementation and feeds especially those suffer from the problem of lack of rangeland [2].

Azolla pinnata is an aquatic ferns and small leafed floating plants [3], and one of the non-conventional feeds that grows on stagnated water, canals, ponds, and plastic tubs which requires the presence of water all the time under sunlight or shade [4]. Azolla, a fast-growing aquatic fern, has emerged as a promising protein-rich feed supplement due to its high nutritional content and rapid

biomass production. It contains 25–35% crude protein, essential amino acids, vitamins (notably vitamin A and B12), and trace minerals (calcium, magnesium, potassium and iron). *Azolla* has an ability to fix atmospheric nitrogen through symbiosis with *Anabaena azollae* contributes to reduced dependence on synthetic nitrogen sources [5]. *Azolla* has rapid growth and high feeding value and it was used as nontraditional feed in animal and poultry production [6]. *Azolla* contains 23-37% crude protein, 10% carbohydrates, on dry matter basis [7]. Moreover, *Azolla* contains probiotics and biopolymers [8].

Azolla has demonstrated potential in poultry [1], aquaculture, dairy cattle, and small ruminants by enhancing feed efficiency and reducing feed costs. Its digestibility and palatability make it suitable even for selective grazers like sheep [9]. Studies have also highlighted the role of *Azolla* in mitigating enteric methane emissions [10], making it valuable for climate-smart livestock production. *Azolla* has immune

stimulating effect due to its high carotene content [11]. The chemical analysis of *Azolla* included 32.83% CP, 13.6% CF, 1.17 EE, 3.89 ash [12].

Azolla can be fed to sheep, cows, goats, and poultry as an alternative to green fodder and as protein supplement because of its palatability and abundant production within a short period [13]. In Iraq and other arid regions, where feed shortages and climatic stressors pose ongoing challenges, the adoption of local aquatic resources like *Azolla* could revolutionize livestock feeding strategies. Despite promising findings in poultry and fish, limited research exists on its effect in sheep, particularly on native breeds like Awassi. Awassi lambs, known for their adaptability and meat quality, are a cornerstone of Iraqi sheep production [14].

The current study was designed to assess the impact of dietary *Azolla* supplementation on productive traits and blood biochemical indicators in Awassi lambs under local feeding conditions.

MATERIALS AND METHODS

This study was conducted in the fields of the College of Agriculture, Tikrit University, for two months, from 1/3 to 1/5/2023

Animals

Total of 9 Awassi lambs having 3-4 months of age and weighing 32-33 kg were randomly divided to three similar groups (3 in each). The lambs were examined by a veterinarian to ensure they were free of

disease and received veterinary care throughout the trial period. The animals were grazed twice a week for 1-2 hours each grazing day in the area adjacent to the field throughout the experiment.

Veterinary care

Lambs in the rearing field were subcutaneously injected with Telmecare (1 ml) and Tylosin (1 ml) every two days, and 2 ml of Oxyterocyclin for three days (Jordanian origin) to prevent infections in general, and pneumonia in particular. Lambs

were given a 10 ml dose of Levamisole (Jordanian origin), to be repeated after 60 days, to prevent intestinal, lung, and liver worms. The doses were given in the morning before feeding, and feed was provided one hour later, according to instructions. Lambs

were given a 2 ml subcutaneous injection of Ivermectin (Belgian origin) to prevent internal and external parasites. Lambs were given a 1 ml subcutaneous injection of

Chauvoei (American origin) to prevent Clostridium difficile disease. Finally, Lambs were given 1 ml subcutaneous injection of an American origin product to prevent pox.

Feeding system and experimental design

In the station, lambs were fed concentrate feed mixture (15% CP and 9% CF) in mash form and chopped wheat straw (150 g/lamb/day), as a roughage source for the lambs (The control ration), The concentrate feed mixture contained 60% black barley, 30% wheat bran, 9% yellow corn, 0.5% salt, and 0.5% limestone. The daily amount of feed was given to the animal in the form of two equal meals daily at eight in the morning and four in the afternoon for the duration of the experiment. The feeding trial lasted 56 days.

Three different feed rations were used as experimental treatments and distributed

among the groups. Three experimental groups were used in this study, lambs in the 1st group were fed the control ration, while those in the 2nd and 3rd group were fed the control ration supplemented with 2 and 3% Azolla. The lambs were gradually fed the experimental rations for 10 days as a preparatory period before starting the actual experiment. The amount of feed given to the lambs were weekly adjusted according to the animal's live body weight. Azolla plants were purchased from a private Azolla production field in the Zaaferaniya area of Baghdad Governorate and dried without direct sunlight.

Experimental procedures

The lambs were weighed weekly and periodically throughout the experiment after fasting the animals for 12 hours before providing feed and water in the morning, using an electronic scale until the end of the

experiment to record the animals' weights. The amount of feed consumed by each lamb was recorded, then total weight gains of lambs was calculated.

Analytical procedures

Azolla were dried (65°C for 24 h), ground and milled (1 mm), and analyzed. The chemical composite for crude protein (CP), crude fiber (CF), and ether extract (EE) as well as cell wall contents extract for

neutral detergent fiber (NDF), acid detergent fiber (ADF) and NFE were determined according to methods of AOAC [15]. Chemical composition and cell wall content of Azolla are presented in Table 1.

Table 1. Chemical composition and cell wall contents of *Azolla pinnata* on dry basis.

%	Item
Nutrient	
22.48	Crude protein
4.50	Ether extract
14.70	Crud fiber
Cell wall content	
40.97	NFE

54.85	Neutral detergent fiber (NDF)
36.57	Acid detergent fiber (ADF)
24.05	Acid detergent lignin (ADL)

Blood sampling

At the end of the experiment, blood samples were collected from all experimental animals after fasting for 12 hours from the jugular vein in the neck region. Approximately 5 ml of blood was collected using a disposable syringe. The blood was placed in plastic tubes and left for one hour at laboratory temperature, then centrifuged at 3000 rpm for 20 minutes to separate the serum from the other components. The serum was then stored in sealed tubes and transferred to the laboratory to conduct the biochemical and enzymatic tests required in the experiment (the tests were carried out in an external laboratory).

Statistical analysis

Data due to Azolla feeding to growing goats were statistically analyzed by one-way ANOVA using procedure of General Linear Model [18] according to the following model:

In blood serum, concentration of total protein and albumin was determined by the biuret method using ready-made analytical kit (Biomaghreb, France) and spectrophotometer [16], however, globulin concentration was calculated by subtracting albumin from total proteins.

Activity of aspartate transaminase (AST), and alanine transaminase in blood serum of lambs was measured using the ready-made assay kit manufactured by the British company RANDOX and a spectrophotometer according to [17].

$Y_{ij} = \mu + T_i + e_{ij}$ Where: μ = Mean, T_i = Effect of Azolla and e_{ij} = error. Duncan’s multiple range test [19] was used to compare between means of the control and treatment groups.

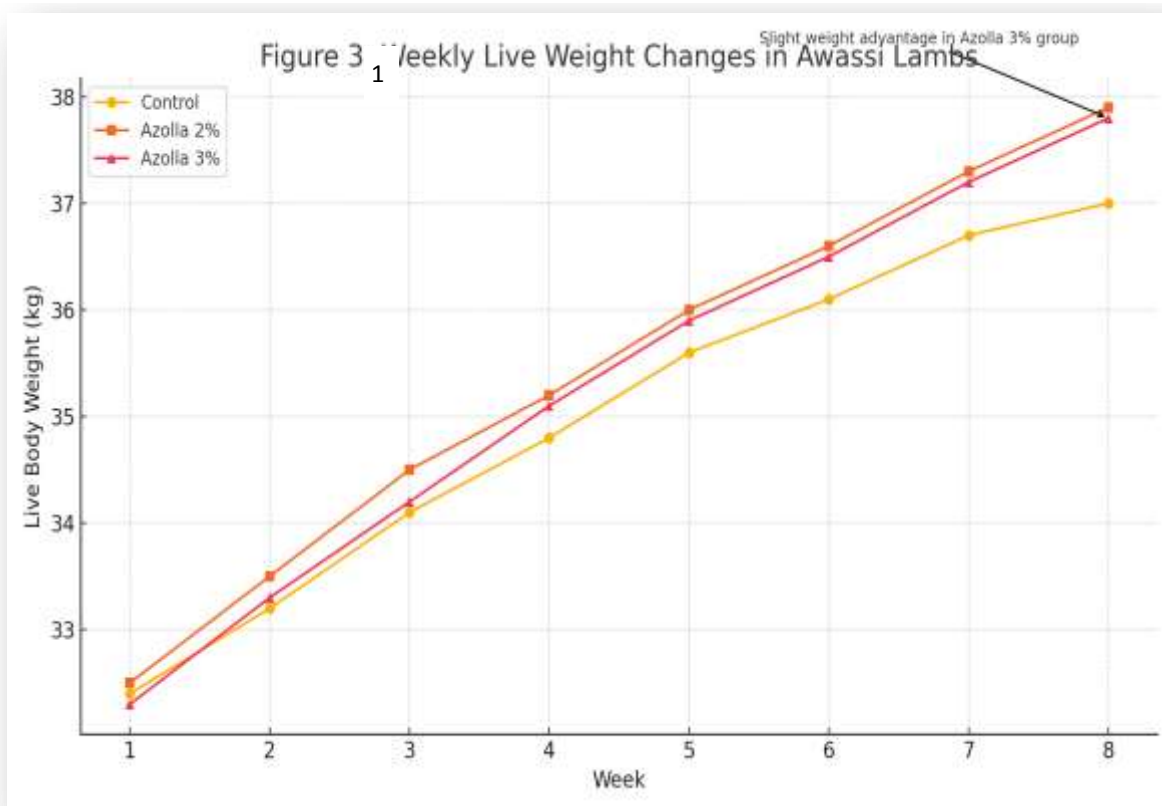
RESULTS

Growth performance parameters

Live body weight

Results illustrated in Fig. 1 showed the weekly progression in live body weight of Awassi lambs over an 8-week feeding period. Lambs in all groups showed continued increase in body weight by advancing feeding week. Notably, lambs fed

the diet supplemented with 2 or 3% Azolla displayed slightly higher weights than the those fed the control diet during the last three weeks from the feeding period, but the differences were not statistically significant ($P>0.05$).



Weight gain

The averages weekly weight gains of lambs in the experimental groups are presented in Fig. 2. Average weekly weight gain was slightly improved in both Azolla groups as compared to control group,

particularly in the early and later weeks of feeding period. Lambs in both Azolla groups showed nearly similar gains during most feeding weeks.



Feed intake

Figure 3 shows the average daily feed intake of lambs in the experimental groups during the feeding period. Feed consumption was slightly higher in both Azolla groups, being the highest for lambs fed 2% Azolla,

but the differences were not significant. These indicating good palatability of the Azolla diets.

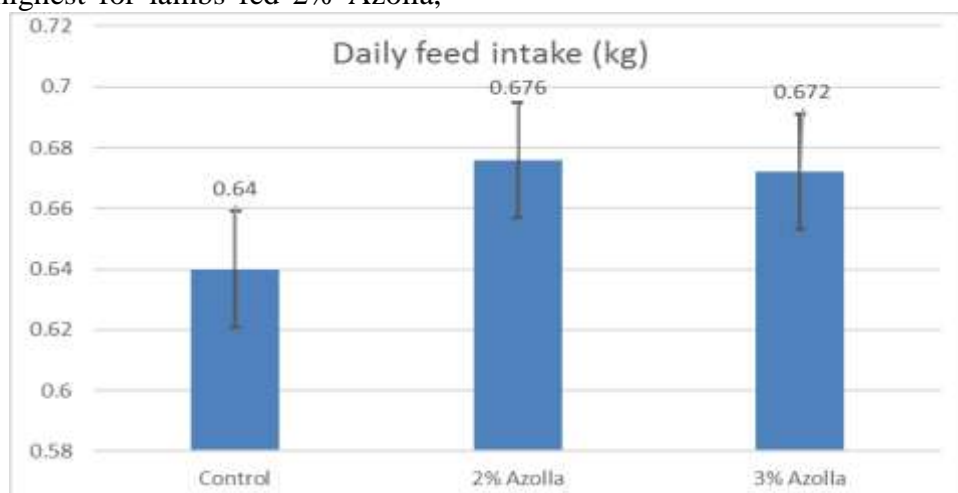


Fig. 3. Average daily feed intake \pm SEM of lambs in the experimental groups during the feeding period.

Blood parameters

Serum total protein

Fig. 4 displays total protein concentration in blood serum of lambs in the experimental groups. Concentration of serum total protein was not affected significantly by

Azolla treatment, although values of serum total protein increased by increasing Azolla level in the diet of lambs.

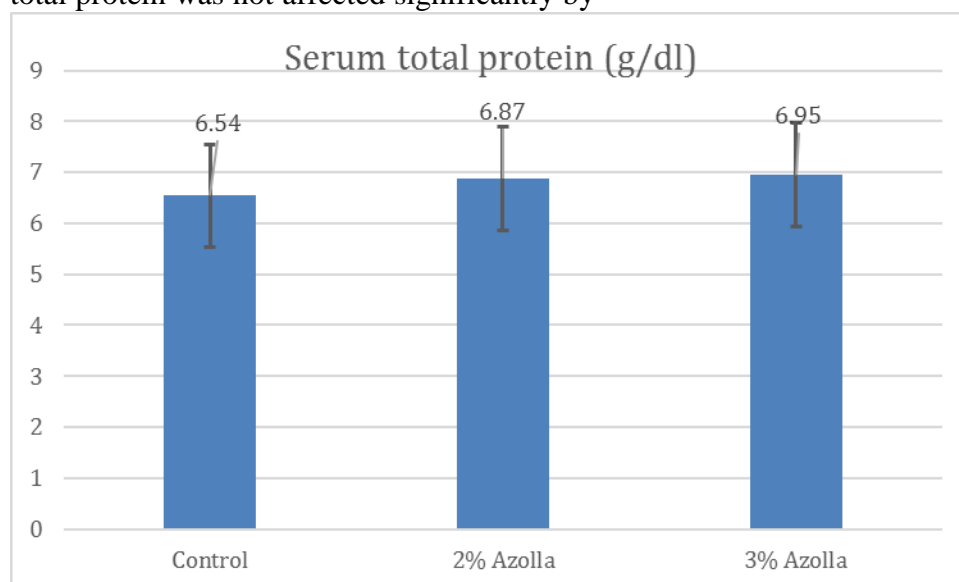


Fig. 4. Concentration of total protein \pm SEM in serum of lambs in the experimental groups at the end of the feeding period.

Serum albumin

Fig. 5 shows concentration of albumin in blood serum of lambs in the experimental groups. Also, the differences in serum albumin between both Azolla groups and control one were not significant, but

values of serum albumin increased in association with increasing values of total proteins as affected by Azolla level in the diet of lambs.

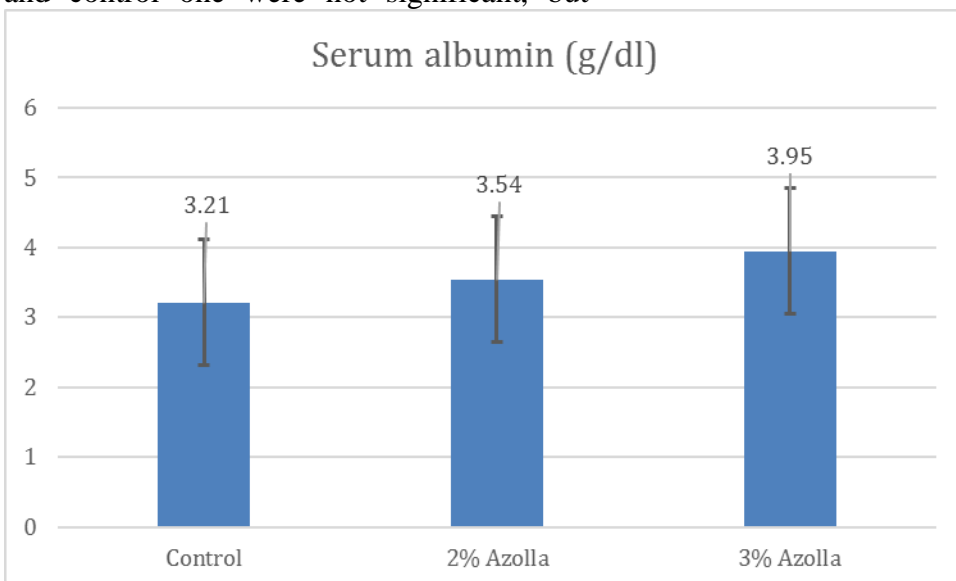


Fig. 5. Concentration of albumin \pm SEM in serum of lambs in the experimental groups at the end of the feeding period.

Serum globulin

The differences in serum globulin between the experimental groups were not significant, but values of serum globulin

slightly decreased by increasing Azolla level more than 2% in lambs fed 3% Azolla (Fig. 6).

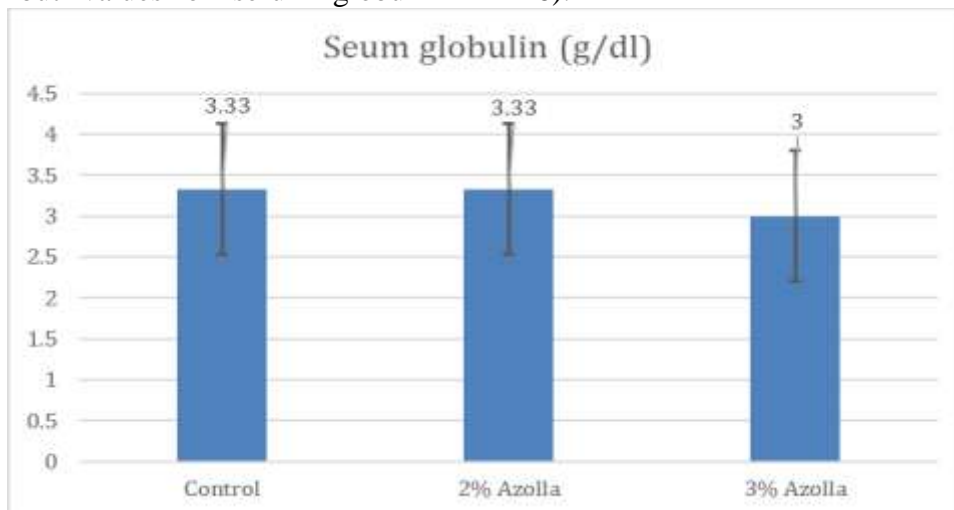


Fig. 6. Concentration of globulin \pm SEM in serum of lambs in the experimental groups at the end of the feeding period.

Albumin/globulin ratio

Albumin/globulin ratio in blood serum of lambs was not affected by Azolla treatment as presented in Fig. 7, showing slight

increase by increasing Azolla level in the diet of lambs.

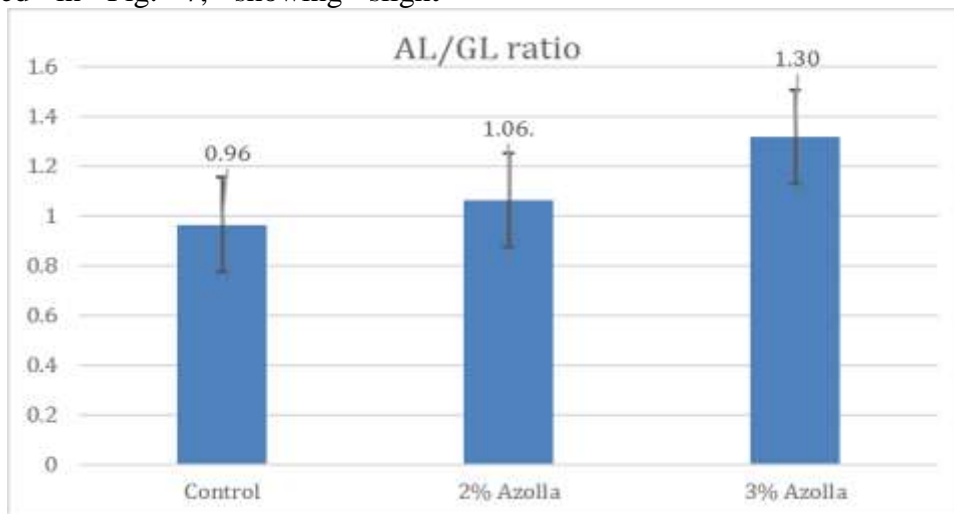


Fig. 7. Albumin/globulin ratio \pm SEM in serum of lambs in the experimental groups at the end of the feeding period.

Liver function

Activity of serum AST

Activity of AST in blood serum of lambs in the experimental groups (Fig. 8) was affected significantly by Azolla treatment, being

lower ($P < 0.05$) in both Azolla groups than in control one.

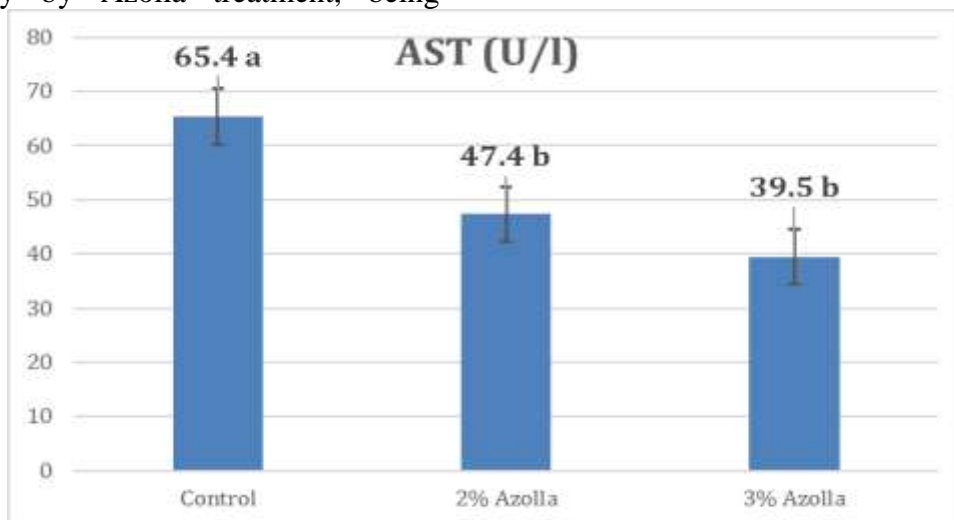


Fig. 8. Activity of AST \pm SEM in serum of lambs in the experimental groups at the end of the feeding period. (Column with different letters are significant at $P < 0.05$)

Activity of serum ALT

Activity of ALT in blood serum of lambs in the experimental groups (Fig. 9) was also affected significantly by Azolla treatment, being lower ($P<0.05$) only in

lambs fed 3% Azolla than in those fed the control diet. However, lambs fed 2% Azolla did not differ significantly from that in control or 3% Azolla group.

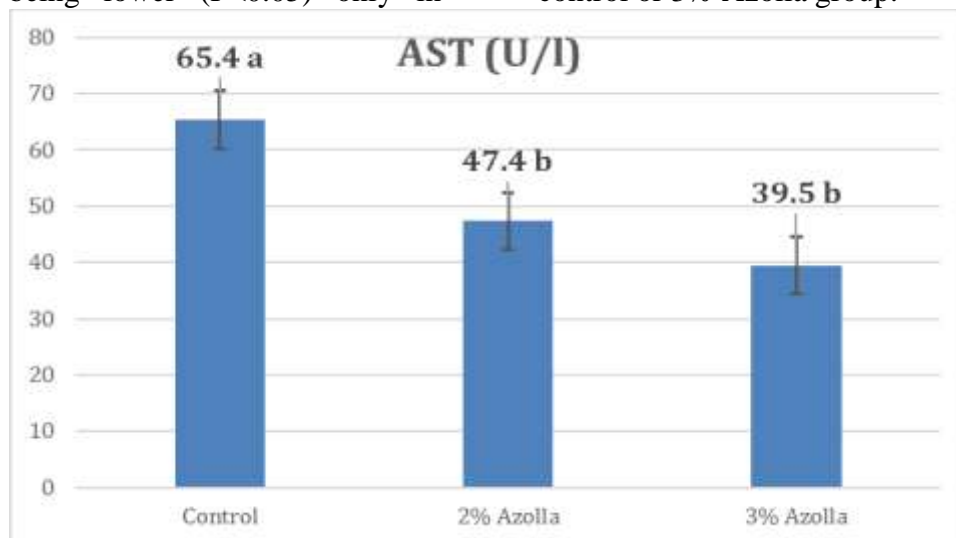


Fig. 9. Activity of ALT ±SEM in serum of lambs in the experimental groups at the end of the feeding period. (Column with different litters are significant at $P<0.0$)

AST/ALT ratio

Average of AST/ALT ratio in blood serum of lambs in the experimental groups (Fig. 10) decreased in both Azolla groups, being the lowest in 3% Azolla-group.

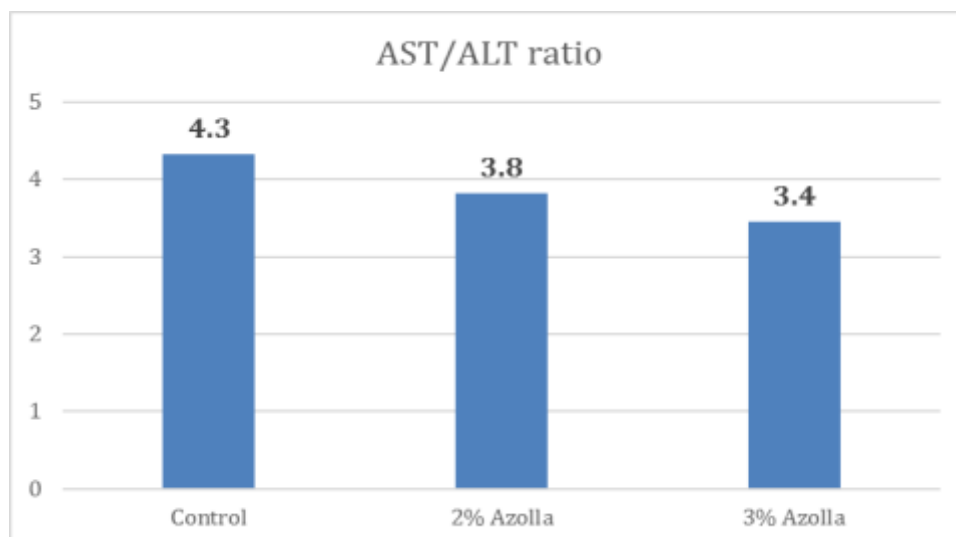


Fig. 10. Average of AST/ALT ratio in serum of lambs in the experimental groups at the end of the feeding period.

DISCUSSION

The present study to evaluate the impact of adding two levels of Azolla in the diet on on productive performance and blood biochemical parameters of Awassi lambs under local feeding conditions. In our study, the chemical analysis of Azolla included 22.8% CP, 14.7% CF, and 4.5% EE. In comparable with these results, [12] found that Azolla contained higher content of CP (32.83%), and lower content of CF (13.6%) and EE (1.17). Various results on Azolla chemical composition were reported by [20]. The differences in chemical composition of Azolla was mainly related to location of culture, type of Azolla, and chemical analysis methods.

Our results confirmed minor live body weight and gains for lambs fed Azolla-diets during a feeding period of 4 weeks without significant differences between Azolla groups and control one in body weight and weight gain of Awassi lambs. On the other hand, feed consumption was slightly higher in both Azolla groups, being the highest for lambs fed 2% Azolla, but the differences were not significant. These indicating good palatability of the Azolla diets. These results may be due to the low level of Azolla used in our study (2 and 3%), higher age of animals, or/and the small number of animals within the experimental groups. In other studies, significant improvement in body weight gain and feed conversion was reported in goats fed 20.0% Azolla-diet [12]. In chickens, Azolla had significant effect on feed consumption and body weight [21], showed the highest body weight gains in the broilers that fed 7.5% Azolla-diet [22], improved body weight gains and feed consumption of chickens that fed 5% Azolla-diet [23], and increased body weight gain of chicks fed died contained 5% Azolla [24]. Moreover, several authors

confirmed that feeding Azolla diets had positive impact on productive performances of growing sheep, Osmanabadi goat kids, Sirohi male kids, and Haryana heifers [25]. The impact of Azolla was due to that found that Azolla improves the digestibility of DM, OM, CP, CF, and EE as well as nutritive [12]. Also, the reported improvement could be due to high protein content, b-carotene and minerals in Azolla. It is well known that carotenoids, bioactive compounds, can efficiently improve body growth and health. Moreover, feeding Azolla provides amino acids and pro-vitamins, B12, and minerals such as Ca, K, P, Fe, Mg, and Cu [4], positively affect feed efficiency and growth performance in different animal species [26].

At the end of feeding period we found that the concentration of total protein, albumin, and globulin as well as albumin/globulin ratio in blood serum of lambs improved in Azolla groups as compared to control group, but the group differences were not significant, but the obtained values are within the normal range in small ruminants [27]. In mammalian species, blood metabolites are an indicators of body's health. Our results indicated slight improvement of protein metabolism and health status of lambs as affected by feeding on Azolla diets. On the other hand, several reports indicated positive impacts of Azolla on protein metabolism due to improving nutrient digestibility, antioxidant activity, and regulating metabolic pathways [4]. In this context, β -carotene and vitamin A in Azolla have antioxidant properties that might help in immune health [11].

Activity of hepatic enzymes (AST and ALT) is a reliable marker of liver function in ruminant animals [28], and the activity of AST and ALT in serum of lambs were altered due to Azolla dietary

supplementation in our study. In this respect, AST activity was reduced ($P < 0.05$) by both Azolla levels, while ALT activity decreased significantly ($P < 0.05$) by 3% Azolla and insignificantly ($P > 0.05$) by 2% Azolla, reflecting lower AST/ALT ratio in both Azolla groups as compared to the control group. The significant enhanced activities of hepatic enzymes (AST and ALT) in Azolla groups are in association with the tendency of increasing ($P > 0.05$) in serum concentration of albumin, globulin and consequently total protein concentration as recorded in our study. In agreement with our results, [12] a significant reduction in liver

enzymes in goats fed 10 or 20% Azolla diets indicating their probable protective roles against liver dysfunction. Generally, Azolla components results in a significant improvement in the liver function leading to increase in growth performance, protein metabolism, and health status of Awassi lambs. In a previous study, [28] mentioned that feeding Awassi lambs on diets supplemented with Azolla had no negative effects on carcass quality. Also, [29] found beneficial impacts on productive performances and body health in growing sheep with dietary supplementation of Azolla.

CONCLUSION

Based on the foregoing results, the dietary supplementation of Azolla in the diet of lambs slightly improved growth rate, in terms increasing body weight, weight gain, serum TP and AL concentration, and reducing the activity of serum of AST and

ALT. Azolla can be added to the diet of sheep without adversely effects on growth performance and can improve protein metabolism and health status (liver function) of Awassi lambs.

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