Research Article

Effects of Feeding *Azolla pinnata* on Growth Performance, Nutrient Digestibility and Blood Metabolites of Growing Ardi Goats in Subtropics

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Abstract | *Azolla pinnata* has received recent attention as non-conventional feed for ruminant animals. The effects of *Azolla pinnata* on nutrient digestibility, growth performance and blood metabolites of growing Ardi goats were explored. Twenty one growing Ardi goats, 27.0 ± 0.40 kg body weight and aged 4.0 - 5.0 months, were randomly allocated to three equal groups (seven/group) as *Azolla pinnata* (10.0 and 20.0 % daily feed) and control groups. The trial lasted 12 weeks. Feed intake, feed efficiency, and body weight gain were determined. In addition, nutrients' digestibility were determined. Blood samples were collected for determination of plasma proteins, glucose, liver enzymes and minerals parameters. The results indicated that *Azolla pinnata* contain protein (32.83%), crude fiber (13.6%), ether extract (1.17%) and ash (3.89%). Upon feeding, replacement diet with 20.0% *Azolla pinnata* caused significant increase in feed efficiency and body weight gain. In addition, the plasma metabolites (total protein, globulin, and urea) and minerals (calcium, phosphorus, magnesium and potassium) were improved due to 20.0% *Azolla pinnata* feeding. In conclusion, *Azolla pinnata* contain high protein compounds and other components might be helpful in modulating body weight gain and plasma metabolites if fed to growing goats in subtropics.

Keywords | *Azolla pinnata*, Growth, Digestibility, Plasma, Metabolite

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INTRODUCTION

Animal production in the world is essential for the development of the 3rd world countries and prosperity of their economics. Therefore, supplying livestock with their nutrient requirements is important for the continuity of animal productivity, maintain animal health and consequently finding an economic source necessary for the developing of the country. Non-conventional supplement and feed has become an urgent need in many countries, especially those suffer from the problem of lack of rangeland (Mohammed et al., 2021; Mohammed, 2022; Al-Mufarji and Mohammed, 2022; Al-Mufarji et al., 2022a, b, c; Al-Masruri et al., 2022). Saudi Arabia is one of those countries that suffer from the scarcity of feed. The Council of Ministers in Saudi Arabia prevented the cultivation of green fodder on 2018 in order to conserve groundwater resources. This decision created challenges to animal production projects as well as animal breeders in order to supply the feed required for the animal. Therefore, finding non-conventional feed that can fulfill the nutrient requirement of animal will ensure the continuity of livestock production projects as well as their prosperity.

One of the non-conventional feeds that grows on stagnated water, canals, ponds, plastic tubs is *Azolla pinnata* (Adake, 2015; Ahmed et al., 2016; Roy et al., 2016; Sharma et al., 2021; El-Naggar and El-Mesery, 2022), which requires the presence of water all the time under sunlight or shade of trees. *Azolla pinnata* is an aquatic ferns and small leafed floating plants, grows in the tropics, subtropics, and warm temperate regions of Africa, Asia, and America (Masoodi and Khan, 2012). The rapid growth and high nutritional value of the plant has made many animal breeders and farmers interested in planting it as well as it is using as non-traditional feed for livestock, poultry and fish (Saad et al., 2021; Varun et al., 2021). Crude protein and carbohydrates percent in *Azolla pinnata* are around 23-27% and 10%, respectively, on a dry weight basis (Cherryl et al., 2014; Kathirvelan et al., 2015). Pillai et al. (2002) reported that *Azolla pinnata* contains probiotics and biopolymers. The content of vitamins in *Azolla pinnata* is high particularly vitamin A and B12 (Mathur et al., 2013). In addition, *Azolla pinnata* contains good amounts of essential minerals including calcium, magnesium, potassium and iron. The advantages of the *Azolla pinnata* plant are ease of cultivation, low water requirements, high productivity, and high nutritional value (Prabina and Kumar, 2010). Lumpkin (1984) showed that *Azolla pinnata* can be fed to sheep, cows, goats, and poultry. *Azolla pinnata* is one of the good choices that can be fed to livestock as an alternative to green fodder and as protein supplement because of its palatability and abundant production within a short period. Thus, the goal of this research is to evaluate the nutritional effect of feeding *Azolla pinnata* to growing Ardi goats as protein supplement on the nutrient digestibility, growth performance, and blood metabolites as well.

MATERIALS AND METHODS

The procedures of the current study were approved by the Ethical Committee of the deanship of scientific research, King Faisal University [Ref. No. KFU-REC-2022-JUN-EA001020]. This study was conducted in the Station of Experimental Research of King Faisal University for 3 months from August to October 2022. The weather in Saudi Arabia during this period is characterized by high temperature (38.0 ˚C) and relative humidity (40.0%).

Azolla pinnata cultivation and analysis

Azolla pinnata was obtained from Jeddah and was transported to Agriculture and Training Station at King Faisal University. *Azolla pinnata* were dried (65˚C for 24 h), ground and milled (1 mm). The composite samples were dried for 3h at 105˚C. Chemical analyses of crude protein (CP), crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF) and ash were determined according to AOAC (2005) procedures.

Animal management and experimental design

Twenty-one growing Ardi goats, 27.0±0.40 kg body weight and aged 4.0-5.0 months, were randomly distributed to three equal groups (seven/group) as control and two *A. pinnata* groups (10.0 and 20.0 % *A. pinnata* daily). The trial lasted 12 weeks. The growing goats were kept in a metabolic pen at a stocking rate of 2.0 m2/goat. The goats were kept free inside the pen. The goats were daily fed 50% alfalfa hay and 50% concentrate for control group and basal concentrate diet replaced with 10.0 and 20.0% *A. pinnata*. The feeding levels of *A. pinnata* were chosen according to the previous studies (Majhi et al., 2006; Saad et al., 2021; El-Naggar and El-Mesery, 2022). The control and *A. pinnata* diets were offered daily at 8 a.m. after mixing alfalfa hay and the concentrate feed carefully to ensure the consistency of the diet. The experimental rations were offered to growing goats and the orts were calculated as 1.0% (Mohammed et al., 2021). The growing goats were weighted biweekly prior to morning feeding from the beginning of the trial until the end.

Digestibility trial

Nine goats were used to evaluate the digestibility of nutrients. They were transferred to metabolic crates provided with facilities for collection of feces before starting of the trial where they were adapted to those crates for seven days followed by the collection period lasting 7 days. The animals were divided into three groups; control and azolla groups (10.0 and 20.0%). The goats were fed once daily at 8 a.m. while the amounts of feed offered and orts were recorded every day to determine feed intake. Feed and orts for each goat were sampled daily and approximately 200 g was composted until the end of the collection period for estimation of chemical composition and nutrient intake. Daily fecal voided during 24 hours were collected at 7:00 AM and weighed. A representative sample of feces approximately 10-15% were collected daily from each animal, dried in a forced air oven at 60˚C for 48 h, ground to passed through a 1 mm screen to determine the dry matter percentage. The remainder of the fecal samples were carefully mixed, saved in plastic containers, and then kept in the freezer at -4˚C for further chemical analysis. The digestion coefficients for the experimental diets were calculated for dry matter, organic matter, ether extract, crude protein, crude fiber, neutral detergent fiber and acid detergent fiber as a difference between nutrient in feed and nutrient in feces divided by nutrient in feed multiplied by 100.

Blood samples collection and analyses

Four blood samples (four animals/group) were collected monthly from the two *Azolla pinnata* and control groups. The collected blood samples were centrifuged for obtaining blood plasma, which biochemically analyzed through

chemistry analyzer apparatus (Skyla VB1). The resulting parameters of blood plasma include total protein, albumin, blood urea nitrogen, glucose, liver enzymes and minerals.

Statistical analysis

Values of chemical composition of *Azolla pinnata* are presented as mean. Values of digestibility coefficient, growth performance, plasma metabolites and minerals due to *Azolla pinnata* feeding to growing goats were statistically analyzed using procedure of General Linear Model (SAS, 2006) according to the following model: Yij = μ + Ti+ eij Where: μ = Mean, Ti = Effect of *Azolla pinnata* and Eij= Standard error. Duncan's multiple range test (Duncan, 1955) was used to compare between means of the control and the two *Azolla pinnata* feeding groups.

RESULTS AND DISCUSSION

The present study shown *Azolla pinnata* chemical composition and its feeding effects on growth performance, digestibility coefficient and plasma parameters of goats in subtropics (Tables 1-5). *Azolla pinnata* contain protein (32.83%), crude fiber (13.6%), ether extract (1.17) and ash (3.89%) as indicated in other studies (Ahirwar and Leela, 2012; Mathur et al., 2013; Bhatt et al., 2020) (Figure 1). This composition of *Azolla pinnata* is of great importance for body growth, nutrient digestibility, and health.

Table 1: Ingredients of the experimental diets.

Parameters	Treatments		
	10.0% Azolla	20.0% Azolla	Control
Alfalfa hay	50	50	50
Azolla	10	20	0.00
Wheat bran	6.1	1.9	14.7
Barley	30.7	24.8	30.7
Soybean meal	0.00	0.00	0.75
Dicalcium phosphate	1.0	1.0	1.0
Limestone	2.0	2.0	2.0
Salt	0.61	0.60	0.61
Vit and Min Premix	0.10	0.10	0.10

Digestibility coefficient and body weight gain

The results of digestibility coefficient and body weight gain due to *Azolla pinnata* feeding are shown in Tables 3 and 4. The dry matter, organic matter (crude protein, crude fiber, ether extract) digestibility and nutritive value were highest ($P < 0.05$) in 20.0% *Azolla pinnata* group followed by control and 10.0% *Azolla pinnata*, respectively (Table 3). The same trend was obtained of body weight gain and feed conversion (Table 4). The significant effects of *Azolla pinnata* on productive performances and body health were confirmed in several studies (Adake, 2015; Ahmed et al., 2016; Roy et al., 2016; Sharma et al., 2021). The significant improvement in nutrient digestibility and nutritive values could be attributed to high protein content, b-carotene and minerals in *Azolla pinnata*. Carotenoids, bioactive compounds, could be efficiently transferred to the body and improve body growth and health. In addition, the feeding of green *Azolla pinnata* provides amino acids specially lysine, pro-vitamins (Hossiny et al., 2008), B12 (Leterme et al., 2010) and minerals (calcium, potassium, phosphorus, ferrous, magnesium, copper) (El-Naggar and El-Mesery, 2022). Such aforementioned components of *Azolla pinnata* were positively affected feed efficiently and growth performance in different animal species (Das et al., 2017).

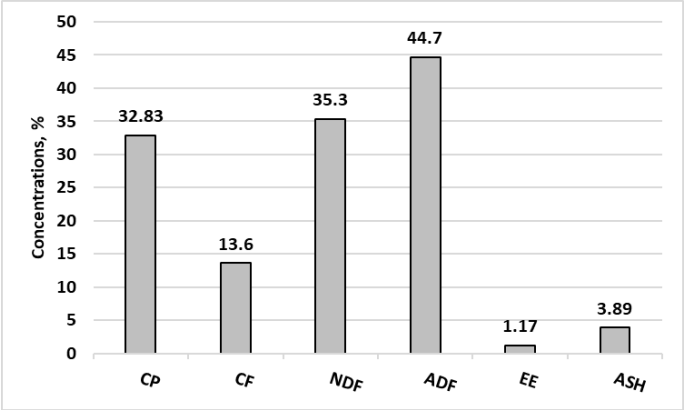

Figure 1: Chemical composition of *Azolla pinnata* on dry basis. CP, crude protein; CF, crude fiber; NDF, neutral detergent fiber; ADF, acid detergent fiber; EE, ether extract.

Table 2: Chemical composition of the experimental diets (dry basis).

Parameters	Treatments		
	10.0% Azolla	20.0% Azolla	Control
Dry matter	88.9	88.98	88.79
Crude protein	14.83	15.63	14.58
Total digestible nutrients	62.47	60.68	63.48
Fat	2.38	2.39	2.46
Ash	7.67	9.12	6.52
Crude fiber	19.62	20.07	19.52
Neutral detergent fiber	54.21	55.92	53.91
Acid detergent fiber	36.36	39.08	34.29
Calcium	1.74	1.77	1.73
Phosphorus	0.57	0.62	0.56

Plasma biochemistry profiles

Plasma of the two *A. pinnata* and control groups are shown in Table 4. The obtained plasma values fell within the normal range for healthy growing goats (Mohammed and Kassab, 2015; Kholif et al., 2016, 2019). The results

indicated improvement of plasma metabolites and minerals values (total protein, globulin and urea; P < 0.05), liver enzymes (P > 0.05) due to feeding 20.0% *A. pinnata* if compared to 10.0% *A. pinnata* or control diet. Plasma metabolites and minerals are an indicative of body's health in mammalian species. The positive effects of *A. pinnata* on plasma metabolites and minerals values might be due to several factors including nutrient digestibility, antioxidative properties, and regulating pathways involved in the metabolism (Radhakrishnan et al., 2014; Das et al., 2017; El-Naggar and El-Mesery, 2022). The green *A. pinnata* contains beta-carotene and antioxidant vitamin (A) with antioxidant activity that might help in immune health. Of note, in the present study, the positive changes in plasma total protein (g/dl) and urea nitrogen (mg/dl) values were obtained by *Azolla pinnata* feeding (20.0%) to growing goat kids. This improvement could be attributed to the significant increase of feed conversion to body weight gain (Table 3) as in other studies (El-Naggar and El-Mesery, 2022). The improvement activity of hepatic function is suggested when A. pinnata was fed, which resulted in higher concentration of total proteins as recorded in the present study. Measurements of hepatic enzymes (AST, GGT, and ALP) are considered as a reliable indicator of liver function in ruminant animals (Liu et al., 2012; Noro et al., 2013), and the liver enzymes' values were unchanged due to *A. pinnata* supplementation in the diets. The lowered values (P > 0.05) of liver enzymes with *A. pinnata* feeding, indicating their probable protective roles against liver dysfunction.

The calcium, phosphorus, magnesium and potassium values were higher (P < 0.05) in *A. pinnata* groups (10.0 and 20.0%) when compared to control group as indicated in several studies (Bhatt et al., 2020). The aforementioned minerals provide an indication of the animals' health and they are important for animals' production (Nozad et al., 2012). Herein, the aforementioned minerals values were in the normal range and reflected the adequate of mineralsof *A. pinnata* and control diets (Goff, 2008).

Table 3: Digestibility coefficient of the experimental diets upon feeding to Ardi growing goats.

Parameters	Treatments		
	10.0% Azolla	**20.0% Azolla**	**Control**
Dry matter	76.62^{c} ± 0.58	84.76^{a} ±0.92	81.62^{b} ± 0.50
Organic matter	78.55^{c} ± 0.53	85.69^{a} ±0.89	82.72^{b} ± 0.47
Crude Protein	82.25 ± 1.32	85.10±0.92	84.38 ± 0.43
Crude fiber	82.40^{b} ± 2.92	88.96^{a} ±0.87	85.83^{ab} ± 0.39
Neutral detergent fiber	77.76^{c} ± 0.65	85.41^{a} ±0.63	87.79^{b} ± 0.34
Acid detergent fiber	83.68^{b} ± 2.20	90.68^{a} ±0.52	84.95^{ab} ± 2.04
Ether extract	86.09 ± 0.32	86.16±2.12	85.02 ± 0.41
Nitrogen free extract	53.60 ± 12.73	76.62±8.87	68.57 ± 1.06
Ash	49.16^{c} ± 1.16	75.41^{a} ±1.25	68.36^{b} ± 0.87
Nutritive values (%)			
Digestible crude protein	12.24^{b} ± 0.72	13.30^{a} ±0.41	12.51^{b} ± 0.07
Total digestible nutrients	71.01^{b} ± 0.06	78.67^{a} ±0.95	78.99^{a} ± 0.49

^{a,b,c} Values with different superscripts between groups significantly differ at P < 0.05.

Table 4: Effects of feeding *Azolla pinnata* (10.0 and 20.0%) on body weight gain and feed efficiency of Ardi growing goats in subtropics.

Parameters	Treatments		
	10.0% Azolla	**20.0% Azolla**	**Control**
Initial body weight (Kg)	27.31±0 .31	27.10 ± 0.39	26.6 ± 0.49
Final body weight (Kg)	33.01±0.34^{b}	34.86±0.45^{a}	32.83±0.67^{b}
Total body gain (kg)	5.70 ±0.25^{b}	7.76±0.13^{a}	6.23±0.38^{b}
DMI (kg)	1.18±0.15	1.18 ± 0.21	1.17±0.14
ADG (gm)	67.84±2.43^{b}	92.34±2.03^{a}	73.62±1.89^{b}
FCR	18.73±0.51^{c}	12.89±0.41^{a}	16.58±0.48^{b}
DMI (% BW)	3.91±0.05^{a}	3.74 ±0.08^{b}	3.89±0.06^{a}

^{a,b,c} Values with different superscripts between groups significantly differ at P < 0.05. DMI dry matter intake, AGD average daily gain, FCR feed conversion ratio.

Table 5: Effects of feeding *Azolla pinnata* (10.0 and 20.0%) on plasma biochemistry of Ardi growing goats in subtropics.

Parameters	Treatments		
	10.0% Azolla	20.0% Azolla	Control
Total protein, g/dl	7.06±0.28[b]	7.50±0.37[a]	6.86±0.24[c]
Albumin, g/dl	3.20±0.16	3.23±0.18	3.23±0.12
Globulin, g/dl	3.86±0.13[b]	4.30±0.21[a]	3.63±0.23[c]
Blood urea nitrogen, mg/dl	19.00±0.42	18.8±0.10	19.63±0.12
Glucose, mg/dl	60.66±3.91	61.66±3.23	60.33±3.75
Alkaline phosphatase, U/L	101.3±2.25	104.3±2.88	105.3±2.45
Aspartate aminotransferase, U/L	54.66±1.88	56.6±2.52	55.33±2.34
Gamma-glutamyl transferase, U/L	41.33±2.1	38.66±1.45	42.66±1.66
Creatine Phosphokinase, U/L	106.0±4.55	102.6±2.93	111.6±5.7
Calcium, mg/dl	9.3±0.31[b]	9.63±0.51[a]	9.26±0.18[b]
Sodium, mmol/L	132.0±1.0	130.6±1.45	135.3±1.20
Chloride, mmol/L	102.3±2.07	103.0±2.30	102.3±3.28
Phosphorus, mg/dl	5.60±0.05[b]	6.9±0.70[a]	4.7±0.55[c]
Magnesium, mg/dl	0.93±0.06[a]	0.91±0.15[a]	0.78±0.10[b]
Potassium, mmol/L	5.43±0.14[b]	5.86±0.15[a]	5.00±0.05[c]

The increase in aforementioned minerals in *A. pinnata* groups could be attributed to their presence in *A. pinnata* (Bhatt et al., 2020). Collectively, *A. pinnata* components leads to a significant improvement in the functions of the digestive tract and liver leading to increase in feed utilization, body weight gain, and blood metabolites of Ardi growing goat in subtropics.

CONCLUSIONS AND RECOMMENDATIONS

The potential properties of *Azolla pinnata* as a protein source have been presented in the current study through chemical analysis. Furthermore, this study indicated that 10.0 and 20.0% *Azolla pinnata* daily feeding to goats resulted in improvement of growth performance and digestibility coefficient through modulating blood metabolites, liver enzymes and minerals. This could be owing to the bioactive compounds of *Azolla pinnata* as promising protectors of oxidative stress and inflammation processes. Moreover, *in vitro* and *in vivo* studies could be recommended on *Azolla pinnata* extract to authenticate their possible applications on body functions and metabolism.

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NOVELTY STATEMENT

Azolla pinnata supplementation to growing Ardi goats modulates body weight gain and plasma metabolites in subtropics

Conflict of interest

The authors have declared no conflict of interest.

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