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(Original Article)

Impact of *Azolla pinnata* as a Sustainable Feed Supplement on Nutrient Digestibility, Blood Parameters, and Growth Performance in Buffalo Calves

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Abstract

Fifteen buffalo calves (145 ± 1.5 kg) for 180 days were randomly divided into three equal groups, CON, Az 12.5, and Az 25. The control group received the basal diet without supplements (CON). In the treatment groups, (Az 12.5) and (Az 25) we partially replaced crude protein in concentrate feed mixture with *Azolla pinnata* at rates of 12.5 and 25%, respectively. All diets were adjusted which contain 69% TDN and 16 % CP. Blood samples were collected during experiment and manure for digestion using the AIA. The results showed that substation protein of CFM with *Azolla* decreased (P<0.05) most nutrient digestibility and nutritive value in comparison with control group. Total protein and ALT were decreased (P<0.05) with the inclusion of *Azolla* at a rate of 25% of calves' diets when compared with the control group. The value of Albumin was higher at Az 12.5, while the value of globulin was increased with calves receiving 25% *Azolla* when compared control groups. The triglycerides concentration was lower (P< 0.05) with all *Azolla* groups than the control one. The glucose, total protein, and albumin were higher (P< 0.05) at 180 days than 1 and 120 days. However, the values of cholesterol, triglycerides, and creatinine were lower (P< 0.05) at 120 days than at 1 and 180. It could be concluded that the partial substitution of concentrate feed with *Azolla pinnata* by up to 25 % from protein of buffalo calve diets had no deleterious effects on growth performance, nutrient digestibility, and plasma constituents.

Keywords: *Azolla pinnata, Buffalo calves, Feed efficiency, Growth performance*

Introduction

The cost of feeding appears to be the major factor hindering the development of animal farming. Feeding cost alone account for about 70 to 80% of the cost of animal production and protein represents the most expensive component of the diet (Mederos *et al*., 2002). Not enough feed is available in Egypt, especially in the form of protein sources. Unconventional feed resources must be employed to close this gap without sacrificing the nutrient supply's quality. The conventional protein sources such as fish meal, soya bean meal and groundnut cake are not used by farmers as they are too expensive. Examining cheaper and alternative feed sources

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that are locally accessible is necessary given the general lack of feeds high in protein and energy and their corresponding costs.

The most cost-effective and productive feed additive for cattle is *Azolla*. Since *Azolla* has a greater protein content (19–30%) than other green fodder crops and aquatic macrophysics, as well as a relatively favorable essential amino acid composition for animal nutrition, research on promoting *Azolla* as a livestock feed have been increased, (Alalade and Iyayi 2006). *Azolla* is rich in protein, essential amino acids, vitamins, growth promoter intermediaries and minerals like calcium, phosphorus, potassium, ferrous, copper, and magnesium (Alalade and Iyayi 2006). *Azolla,* on a dry weight basis, contains 25 - 35% protein, 10-15 % minerals, 7-10 % amino acids, bio-active substances and biopolymers, and 15% of total ash (Tamang and Samanta, 1993). Meanwhile, on dry matter basis, *Azolla* contains 7% dry matter, 15.40% crude fibrer 2.80% ether extract, 20.40% total ash, 47.40% NFE, 51.90% acid detergent fiber, 67.70% neutral detergent fiber, 1.50% calcium and 0.40% phosphorus. (Tamang and Samanta, 1993).

Azolla consists of various varieties (*Azolla pinnata*, *A. maxicana*, *A. nilotica*). Among them, *Azolla pinnata* is the most common *Azolla* species in the tropics and subtropics area. It requires a little investment and is easily grown. It grows naturally in ponds, rivers, and canals with stagnant water. Due to its symbiotic relationship with the blue-green algae Anabaena, *Azolla* fixes atmospheric nitrogen, therefore, has relatively high amounts of nitrogen. (Alalade and Iyayi 2006; Raja et al., 2012).

Azolla can spare costly sources of protein in ruminant feed i.e., oil seed cake or meal. Moreover, it is an accumulator of mineral matter and contains all macro and micro elements responsible for animal growth and production (Srinivas *et al*. 2012). *Azolla* contains all essential amino acids and carotene (Mandal *et al*. 2012; Alalade and Iyayi 2006). Limited studies on cattle and buffaloes indicated the improvement in daily gain and efficiency with reduced feed cost when concentrate mixture or protein meal are partially replaced by *Azolla* (Kumar, 2008; Indira *et al*., 2009; Kumar *et al*., 2012; Murthy *et al*., 2013). No more research has been conducted on the effect of feeding *Azolla* to heifers of buffalos breed reared in Egypt.

Because of *Azollas* low lignin level and high protein content, livestock can easily digest it. When it comes to feeding animals, *Azolla* is a rich source of protein that can partially replace concentrate (Singh *et al*, 2019). (Singh *et al*., 2016) working on growth performance in crossbred calves reported an enhance in growth performance while mixing *Azolla* meal in wheat and rice straw based complete feed block. *Azolla* can be fed straight to livestock or combined with concentrates. Animal nutrition is limited by high total ash (Singh *et al*., 2016). For this reason, current study was carried out to see how *Azolla pinnata* substituted for concentrate mixture affected the buffalo calves' blood parameters, growth performance, and feed nutrient digestibility.

Material and Methods

Animals, management and diets

Animal Production Research Farm served as the experiment's site, Faculty of Agriculture, Assiut University, Assiut, Egypt. Dried *Azolla pinnata* were obtained from local suppliers from commercial markets in Zagazig city, Egypt. Fifteen buffalo calves (145 ± 1.5 kg) were randomly divided into three comparable groups (five calves each). The control group received only the basal diet and no supplements (CON). In the treatment groups, (Az 12.5) and third (Az 25) we partially replacement of crud protein in concentrate feed mixture with *Azolla pinnata* at rates of 12.5 and 25%, respectively. All the calves were fed a basal diet of 60% concentrate feed mixture (CFM) and 40% roughage (wheat straw). Nutritive values of CFM were 69% total digestible nutrients (TDN) and 16% crude protein (CP) approximately. The offered feeds were assessed to cover the maintenance and gain production requirements for each animal (NRC, 1985). Chemical composition of ingredients is shown in Table 1. Concentrate mixture was offered for each animal individually once daily at 9.00 am, while wheat straw was offered at 12.00 pm. Calves were weighed at the start of the trial and every two weeks to modify the amount of feed needed. All animals have access to clean drinking water. There was a 180-day experimental period Ingredients composition of CFM of experimental diets are presented in Table (1). The chemical analysis of the experimental diets, wheat straw and *Azolla pinnata* are shown in Table (2). The chemical analysis of feeds was analyzed using the procedures of AOAC (2012). Van Soest *et al*. (2010) methods were utilized to identify neutral detergent fiber (NDF), and acid detergent fiber (ADF).

Table 1. Ingredients composition (g/kg) of concentrate feed mixture of experimental rations.

Items	Diets[1]		
	Con	Az 12.5	Az 25
Corn	44	40	37
Wheat bran	35	33	30
Soyabean meal	17	14	11
Azolla pinnata	0	9	18
Limestone	2.2	2.2	2.2
Sodium bicarbonate	0.3	0.3	0.3
Salt	1.2	1.2	1.2
Trace mineral and vitamin premix	0.3	0.3	0.3

Diets[1]: CON group, animals received consist of concentrate mixture (CM) and wheat straw (60:40% dry matter (DM) basis); Az 12.5 group, animals fed Az 12.5 diet, and Az 25 group, animals fed Az 25 diet.
* The premix comprised (per kg) 20,000,000 IU vitamin A, 200,000 IU vitamin D3, 10,000 mg vitamin E, 10,000 mg Fe, 2500 mg Cu, 20,000 mg Mn, 100 mg Mo, 100 mg Co, 800 mg I, 20,000 mg Zn and 100 mg Se.

Table 2. Chemical analysis of the experimental diets, wheat straw and *Azolla pinnata* (% on DM basis)

Items	Con	Az 12.5	Az 25	Wheat straw	*Azolla pinnata*
DM	88.37	88.55	88.73	94.36	90.30
OM	81.36	80.45	79.55	85.69	74.50
CP	16.02	15.88	15.78	3.26	29.50
CF	5.88	6.74	7.511	37.50	15.49
EE	3.63	3.54	3.46	1.55	2.80
NDF	22.41	26.604	26.503	71.50	43.60
ADF	6.30	8.68	10.955	42.92	31.50
TDN	69.98	69.15	68.49	44.00	65.00
ME MJ	11.79	11.59	11.43	5.80	10.46

Diets[1]: CON group, animals received consist of concentrate mixture (CM) and wheat straw (60:40% dry matter (DM) basis); Az 12.5 group, animals fed Az 12.5 diet, and Az 25 group, animals fed Az 25 diet. Dry matter (DM), organic matter (OM), crude protein (CP), Crude fiber (CF), Ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF).

Digestibility trial

Nutrient digestibility was carried out using the methods of acid insoluble ash (AIA) % as a natural marker according to Van Keulen and Young (1977). The fecal samples were obtained directly from the animal rectum. The fresh feces were collected for 7 days and stored in a refrigerator. At the end of the digestibility trail, the fecal samples from each animal and diet samples were composited, dried at 60°C for 72 hours, and then ground through a 1 mm screen for subsequent chemical analysis. Digestibility of nutrients was estimated according to the following equation, the marker must be an inert compound. The following equation is used to determine the apparent digestibility of a nutrient:

$$\text{Digestibility (\%)} = 100 - (100 \times \frac{\text{marker concentration in feedstuff}}{\text{marker concentration in feces}} \times \frac{\text{nutrient concentration in feces}}{\text{nutrient concentration in feedstuff}})$$

Blood samples and analyses

Blood samples were collected from each calf jugular vein at 6 hrs. after the morning feeding in glass tubes continuing anticoagulant (EDTA) during 0, 90, and 180 days of the experimental period. Blood samples were directly centrifuged at 3000 rpm for 15 mins and the plasma was separated and stored at -20 ˚C until the chemical analyses of plasma total protein, and albumin. However, globulin concentration was obtained as the difference between the total protein and albumin concentration. Glucose, triglyceride, total cholesterol, creatinine, alanine aminotransferase (ALT) enzymes using assay kits supplied by Diamond Diagnostics, Egypt. Thyroid hormones (T3 and T4) concentrations were determined using enzyme-linked immunosorbent assay kits supplied by Biotecx, USA.

Statistical analysis

Results were statistically analyzed using SPSS statistical package 22 (SPSS Institute, Chigaco, IL, USA). Data were evaluated using General Linear Models (GLM) procedure for analysis of variance and subjected to one-way ANOVA accompanied by Duncan's multiple range tests to detect the differences among treatments. the general linear model denoted as: $Yij = \mu + Ti + eij$ Where Yij is the observed value of the dependent variable determined from a sample taken from each animal; μ is the overall mean, Ti is the treatment effect (i = 1-3), and eij is the residual random error. Probability values less than 0.05 ($P< 0.05$) were considered significant.

A repeated-measures General Linear Model (GLM) was applied to the data for blood plasma considering the sampling day as repeated measure, with fixed effects of dietary treatments (D) (Control vs. *Azolla* treatments), sampling day (S) (0, 90 and 180 days) and the interactions among them (D × S) according to the model: $Yijk = \mu + Ai + Bj + Ai*Bj + eijk$, where Yijk values of observation; μ= general mean; Ai is the effect of the diet (CON vs. *Azolla* treatments); Bj is the effect of sampling time; Ai*Bj is the interaction between the diet and sampling time; and eijk is the residual error.

Results

Chemical composition

Results revealed that the chemical composition of *Azolla* rations (Az 12.5 and Az 25) had the highest values of CF, NDF and ADF percentage, which were increased by increasing *Azolla* in the concentrate mixture (Table 2).

Effect of *Azolla* on performance on buffalo calves

The results in Table (3) demonstrated that the partial replacement CP of CFM with *Azolla* to calves' rations did not reveal any significant effect on DMI of concentrate, wheat straw, and total DM intake during the experimental period. However, the CF and ADF intake was higher ($P < 0.05$) in the group of calves that received 25% *Azolla* of CFM than the control one. The body weight gain and average daily gain were not significantly affected with partial substation CP of CFM with *Azolla*. However, the average daily gain tended to be higher by 10 and 12% of Az 12.5 and Az 25, respectively than the control group.

Feed conversion ratio in terms of g DM/ g gain, g TDN/ g gain and g DCP/ g gain did not significantly affect by dietary treatment.

Effect of *Azolla* on buffalo calves digestibility

Substation crud protein of CFM with *Azolla* decreased ($P < 0.05$) DM, OM, and CP digestibility in comparison with the control group (Table 4). Moreover, the EE and NDF digestibility were lower ($P < 0.05$) with the group receiving 12.5% *Azolla* than that receiving 25 % *Azolla* and control groups. However, CF and ADF digestibility was increased ($P < 0.05$) in the *Azolla* groups when compared with the control group. The feeding value in terms of TDN was decreased with *Azolla*

treatments when compared with the control group (64.82 and 63.16 vs. 68.33 % for Az 12.5, Az 25, and control groups, respectively)

Table 3. Effect of feeding experimental diets on performance of lambs throughout the experiment

Items	Diets[1]			P- value
	CON (n=5)	Az 12.5 (n=5)	Az 25 (n=5)	
Initial weight (kg)	146.2±1.66	145.6±1.7	145.4±1.42	0.282
Final weight (kg)	254.8±1.36	266.6±2.16	268.6±1.92	0.251
BW gain (kg)	108.6±1.53	121±1.55	123.2±1.48	0.429
Daily gain (g)	0.60±0.11	0.67±0.12	0.68±0.11	0.429
DMI of concentrate	2.92±0.18	2.97±0.23	3.00±0.17	0.320
DMI of wheat straw	1.84±0.15	1.88±0.19	1.89±0.16	0.229
Total DM intake	4.76±0.23	4.85±0.3	4.89±0.24	0.280
OM intake	4.36±0.22	4.43±0.29	4.44±0.23	0.294
CP intake	0.59±0.08	0.6±0.1	0.61±0.08	0.317
CF intake	0.93^{b}±0.1	0.99^{ab}±0.14	1.04^{a}±0.12	0.078
EE intake	0.14±0.04	0.14±0.05	0.14±0.04	0.304
NDF intake	2.14±0.16	2.31±0.21	2.31±0.17	0.125
ADF intake	1.05^{b}±0.11	1.15^{ab}±0.15	1.24^{a}±0.13	0.023
TDN intake	3.17±0.19	3.2±0.24	3.2±0.19	0.320
ME intake MJ	0.5±0.07	0.5±0.1	0.5±0.07	0.331
Feed conversion ratio g/g gain)	7.99±0.48	7.24±0.31	7.17±0.28	0.485
TDN	3.17±0.19	3.2±0.24	3.2±0.19	0.320
DCP	0.99±0.17	0.9±0.11	0.89±0.1	0.508

Diets[1]: CON group, animals received consist of concentrate mixture (CM) and wheat straw (60:40% dry matter (DM) basis); Az 12.5 group, animals fed Az 12.5 diet, and Az 25 group, animals fed Az 25 diet.
[a-b] within a row, different superscripts differ indicating significant differences among diets ($p< 0.05$).

Table 4. Digestibility of nutrients in different treatment groups

Items	Diets[1]			P- value
	Con	Az 12.5	Az 25	
DM	64.12^{a}±0.6869	57.85^{b}±1.0275	57.06^{b}±0.8819	0.022
OM	70.12^{a} ±0.6839	66.45^{b} ±0.5774	65.88^{b} ±0.8819	0.032
CP	65.21^{b} ±0.8819	69.01^{a} ±0.5774	64.45^{b} ±1.2019	0.023
CF	51.29^{c} ±0.8442	55.41^{b} ±0.3333	61.51^{a} ±0.5774	0.026
EE	72.54^{a} ±0.8671	67.54^{b} ±0.5774	72.13^{a} ±0.8819	0.042
NDF	57.14^{a} ±0.5933	50.6^{b} ±0.5774	$54.5^{a\,b}$ ±2.0817	0.032
ADF	44.1^{b} ±0.4933	47.68^{a} ±0.5774	47.62^{a} ±1.453	0.025
TDN	68.33^{a} ±0.8756	64.82^{b} ±0.3333	63.16^{b} ±1.453	0.044
DCP	10.53^{b} ± 0.25	12.14^{a} ± 0.38	11.16^{b} ± 0.25	0.026

Diets[1]: CON group, animals received consist of concentrate mixture (CM) and wheat straw (60:40% dry matter (DM) basis); Az 12.5 group, animals fed Az 12.5 diet, and Az 25 group, animals fed Az 25 diet.
[a-b] within a row, different superscripts differ indicated significant differences among diets ($p< 0.05$).
TDN, total digestible nutrients; DCP, digestible crude protein.
DMD%= [1–AIA% of feed DM/AIA% of feces DM]×100.
Y = 100–{N/M(100-DMD)}. Where: DMD, dry matter digestibility; Y, nutrient digestibility.
M, nutrient in feed, %DM; N, nutrient in feces, %DM.

Effect of *Azolla* supplementation to buffalo calves on blood metabolites

The results of blood metabolite analysis are shown in Table 5. The value of plasma glucose is not affected (P> 0.05) by dietary treatment with *Azolla* and his values are within in normal range.

The values of total protein and ALT were increased (P< 0.05) with the inclusion of *Azolla* at a rate of 25% of calves' diets when compared with the control group. However, no differences were detected between Az 12.5 and the control group or between Az 12.5 and Az 25%. The value of Albumin was higher at Az 12.5 than in the control group. Also, the value of Globulin was increased with calves receiving 25% *Azolla* when compared with that received Az25 and control groups.

The cholesterol concentration was decreased (P< 0.05) with calves that received a high level of Az 25% when compared with those that received Az 12.5%. However, the Triglycerides concentration was lower (P< 0.05) with all *Azolla* groups than that the control one. The creatinine and thyroid hormones (T3 and T4) were not significantly (P> 0.05) affected with supplement *Azolla* to calves' diets.

Concerning the effect of sampling time on blood metabolites, it could be observed that the glucose, total protein, and albumin increased with time and recorded the highest (P< 0.05) values at 180 days of the experiment when compared with 1 and 120 days (Table 5). The values of cholesterol, triglycerides, and creatinine were lower (P< 0.05) at 120 days than at 1 and 180 days. However, the thyroid hormones (T3 and T4) were decreased (P< 0.05) at 1 day of the experiment and increased with time at 120 and 180 days, but no differences were found between 120 and 180 days.

Table 5. Effect of experimental treatment on some blood Parameter concentrations

Items	Diets[1]			Sampling Time (day)			Effects		
	CON (n=5)	Az 12.5 (n=5)	Az 25 (n=5)	Day 1 (n=15)	Day 90 (n=15)	Day 180 (n=15)	Diets	Sampling Time	interactions
Glucose (mg/dl)	50.43 ±2.8566	47.97 ±1.7518	50.1 ±2.3436	44.39^{b} ±1.8869	46.41^{b} ±2.0916	57.7^{a} ±1.3022	NS	**	NS
Total protein (g/dl)	5.69^{B} ±0.1059	5.92^{AB} ±0.0937	5.98^{A} ±0.0885	5.69^{b} ±0.0892	5.81^{b} ±0.1125	6.08^{a} ±0.0712	**	**	NS
Albumin (Al) (g/dl)	2.84^{B} ±0.0667	3.07^{A} ±0.0487	2.9^{AB} ±0.0722	2.85^{b} ±0.0485	2.88^{b} ±0.0935	3.08^{a} ±0.0279	**	**	NS
Globulin (Gl) (g/dl)	2.85^{B} ±0.0751	2.85^{B} ±0.0609	3.08^{A} ±0.065	2.84 ±0.0563	2.93 ±0.0831	3 ±0.0706	**	NS	NS
ALT (U/l)	64.85^{A} ±3.1676	63.8^{AB} ±1.0989	57.93^{B} ±1.7932	60.73 ±1.1678	64.85 ±2.5825	61 ±2.7659	**	NS	NS
Cholesterol (mg/dl)	97.03^{AB} ±1.4836	101.78^{A} ±1.5324	93.82^{B} ±2.8189	100.89^{a} ±2.1097	92.27^{b} ±2.369	99.47^{a} ±1.2951	**	**	NS
Triglycerides (mg/dl)	41.33^{A} ±3.8935	30.53^{B} ±3.3202	30.54^{B} ±2.283	39.3^{a} ±2.5276	39.65^{b} ±3.493	23.45^{a} ±2.4748	**	**	NS
Creatinine (mg/dl)	1.35 ±0.0407	1.32 ±0.0346	1.35 ±0.0251	1.34^{a} ±0.0422	1.28^{b} ±0.0228	1.4^{a} ±0.0274	NS	NS	NS
T4 (nmol/L)	112.61 ±3.4553	110.15 ±1.5698	110.01 ±2.0385	101.94^{b} ±1.2778	117.81^{a} ±2.2923	113.01^{ab} ±1.5863	NS	**	**
T3 (ng/mL)	5.61 ±0.3968	5.86 ±0.1616	5.97 ±0.233	4.88^{b} ±0.2246	6.57^{a} ±0.1758	5.99^{ab} ±0.2357	NS	**	**

Diets[1]: CON group, animals received consist of concentrate mixture (CM) and wheat straw (60:40% dry matter (DM) basis); Az 12.5 group, animals fed Az 12.5 diet, and Az 25 group, animals fed Az 25 diet.
$^{A-B}$ within a row, different superscripts differ indicating significant differences among diets (p< 0.05).
$^{a-b}$ within a row, different superscripts differ indicated significant differences among sampling time (p< 0.05).

Discussion

The percentage of dry matter content of dried *Azolla* meal was 90.0. Similarly, Gupta *et al.,* (2018) observed 90 percent DM and Navnath (2015) observed 89.91 Percent. *Azolla* has higher fiber, ether extract, total ash and similar crude protein content compared to concentrate. The chemical composition of *Azolla* estimated in the present study was similar to earlier reports. In the current study, CF intake was significantly higher in the group fed on Az 25 than the intake in the control group. This analysis agreed with Indria and Ravi (2009) who recorded that, the crude fiber content of *Azolla* was higher than the other groups. Current study analyses recorded insignificant changes in the body weight of buffalo calves in all 3 groups, and this result agrees with Sankar *et al*. (2020) who reported that there was no significant difference in body weight gain. On the other hand, the results disagreed with Roy *et al*. (2016), who reported that BW was not affected by the replacement of concentrated mixture with *Azolla* at the beginning, but they increased with period.

In the present study, DMI, FCR recorded insignificant differences in all groups, these results were against (Sankar *et al*., 2020) who reported that The DMI was numerically lower in *Azolla* group. The numerically lower dry matter intake may be because of high lignin and fiber content of *Azolla* meal and decreased FCR in the *Azolla* supplemented group.

In this study, no significant differences were reported regarding EE and NDF. The analysis disagreed with Roy *et al*. (2016) who reported high level of EE, and lower level of NDF in *Azolla* group regarding others. Similarly, ADF recorded a lower level in *Azolla* group than in other groups which is in contrast to current results which showed an increase in ADF level in *Azolla* group.

No significant difference was shown in current analyses regarding TDN, and DCP values, these outcomes contrasted with Abou El-Fadel *et al*. (2020) who recorded a significant decrease in TDN, and DCP values in *Azolla* groups than the control group.

Current results disagreed with Roy *et al*. (2016), who reported that treatment did not impact DM during entire experimental period. Similarly, CP, total digestible nutrient, NDF, and ADF intake were not detected as different in all groups.

Additionally, the partial replacement CP up to 25 % of CFM with *Azolla* to calves' rations had no harmful effects on the performance, or digestibility. Feed conversion efficiency was reduced with the inclusion of *Azolla* meal (Indira et al., 2009), however, the present results were similar and corresponded with observations by Sihag *et al*. (2018) for DM intake, which showed numerically higher ADG when the CFM was replaced with *Azolla*. *Azolla*, due to its high protein content, can play an important role in accelerating the growth of animals; thus, it can be used as a growth enhancer.

The treatment groups showed low digestion levels compared with control group in the present study, which is in line with Abou El-Fadel *et al*. (2020), who

reported that when *Azolla* meal was added to testing meals, the digestibility coefficients of OM decreased.

In this study no significant difference was recorded over the 3 groups regarding the glucose level which was in line with Bhatt *et al*. (2021) who reported no significant different impact through the entire experiment groups.

In general, the partial replacement CP up to 25 % of CFM with *Azolla* to calves' rations resulted in no harmful effects to hematological and biochemical parameters. The total blood protein and its fractions are considered as a biological index reflecting productive performance and health of the animal. Significant increasing levels of total protein values, and Albumin results agree with previous findings (Hassanein *et al*., 2023, Kahn *et al*., 2010) and indicate normal ranges for the sample calves.

In the current study, ALT showed significant decrease level in Az 25 group when it was compared to the control group this result disagreed with Roy *et al*. (2016) who reported no significant difference.

Moreover, our study reported no significant change in all groups regarding the creatinine, T3, and T4 levels which disagreed (Bhatt *et al*., 2021) who observed a significant decline with ($P < 0.001$, 0.03, 0.04) respectively.

Conclusion

This study concluded that *Azolla pinnata* at the levels (12.5% to 25%) of buffalo calve diet can be utilized as a good source of nutrients (e.g., protein, trace minerals) in place of concentrate mixture without producing a negative impact on blood parameters, digestibility, or growth performance.

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تأثير الأزولا كمصـــدر علف مســـتدام على هضـــم العناصـــر الغذائية وخصـــائص الدم وأداء النمو لعجول الجاموس.

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الملخص

تمت دراسة تأثير استبدال بروتين الخليط المركز بالأزولا على أداء النمو وهضم العناصر الغذائية وبعض خصائص الدم في عجول الجاموس.

تم تقسيم عدد 15 عجل جاموس نامي عشوائيًا إلى ثلاث مجموعات متماثلة متوسط وزن (145 ± 1.5 كجم) ولمدة 180 يومًا وتم تغذيتها على أساس وزن الجسم. كانت المعاملات:

1- مجموعة CON: تغذت على خليط مركز وتبن القمح بنسبة 60:40

2- مجموعة Az 12.5: تم استبدال 12.5% من بروتين العلف المركز باستخدام الازولا

3- مجموعة Az 25: تماستبدال 25% من بروتين العلف المركز باستخدام الازولا.

كانت جميع العلائق متســـاوية في الطاقة (69% إجمالي المركبات المهضـــومة) والبروتين الخام (16% بروتين خام).

خلال فترة التجربة تمت متابعة أوزان العجول شـهرياً لتحديد المقررات الغذائية حسـب الوزن، وتم حســـاب متوســـط الزيادة اليومية. وتم تســـجيل كمية العلف المتناولة والمتبقي يومياً، وأخذت عينات الدم قبل التغذية اليومية من جميع الحيوانات عدد ثلاث مرات خلال الفترة التجريبية في الأيام 1، 90، و180. وتم فصـــل البلازما وتخزينها عند -20 °م حتى التحليل الكيميائي. وفي نهاية التجربة تم جمع عينات من الروث لإجراء تجارب الهضم بطريقة AIA (الرماد الحمضي غير القابل للذوبان).

لوحظ ارتفاع معنوي في البروتين الكلي والألبومين والجلوبيولين وانخفاض في مســـتوى الدهون الثلاثية والكوليســـترول والكيراتنين في مجموعات الأزولا المختلفة. كما لوحظ زيادة هضـــم الألياف الخام وADF في عليقة مجموعة الأزولا. ولوحظ انخفاض معدل هضـــم DM، OM، CP، EE، و NDF بشكل ملحوظ في مجموعات الأزولا المختلفة مقارنة بمجموعة الكنترول. في الخاتمة، يمكن استبدال الخليط المركز بالأزولا بيناتا بنســـب من 12.5% إلى 25% من بروتين العلف المركز كبديل لبروتين مواد العلف التقليدية دون أي تأثير ضار على أداء النمو والهضم وخصائص الدم.