RESEARCH ARTICLE

Effect of feeding *Azolla pinnata* on growth performance, feed intake, nutrient digestibility and blood biochemical's of Hariana heifers fed on roughage based diet

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Abstract An experiment was conducted to observe the effect of substitution of concentrate mixture with *Azolla pinnata* on growth performance, feed intake, nutrient digestibility and blood biochemical's of Hariana heifers. Twelve heifers were randomly allocated into two treatment groups for 90 days feeding trial. The heifers were fed either a control diet (C) or the control diet supplemented with *Azolla pinnata* (T). The heifers of control group were fed with a basal ration comprised of jowar fodder, wheat straw and concentrate mixture according to their nutrient requirement. Concentrate mixture was replaced at 5% level (DM basis) by *Azolla pinnata* in treatment group. Dry matter intake (DMI) and body weight were recorded fortnightly and blood samples were collected at monthly intervals for analysis of total blood protein, plasma albumin, total plasma immunoglobulin, blood urea nitrogen, plasma creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST). In present study, DMI, body weight and body condition score (BCS) were not affected by dietary supplementation of *Azolla pinnata*. Replacing concentrate with *Azolla pinnata* improved (P<0.01) fortnightly weight gain, average daily gain (ADG) and feed conversion efficiency (FCE). Blood concentration of creatinine and BUN were 53% and 17% lower, respectively in *Azolla pinnata* supplemented heifers than control group. Serum ALT and AST concentration were reported higher in *Azolla pinnata* fed heifers. However, other blood parameters and digestibility of nutrient did not change due to replacement of concentrate mixture with *Azolla pinnata*. In conclusion, replacement of concentrate mixture by *Azolla pinnata* at 5% level improved the feed conversion efficiency and growth rate of Hariana heifers without any detrimental effect on nutrient intake, digestibility and blood biochemical profile.

Key words: *Azolla pinnata*, feed efficiency, growth performance, heifer, nutrient digestibility

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Introduction

Successful dairy organizations in the present world recognize importance of heifers as a future investment. Heifers are placed at high value and regarded as a managed resource. Even though, India is the largest producer of milk in the World, dairy heifers in India are the most overlooked and under-managed asset of an individual or a farm. In India, heifers are supplemented with agro-industrial byproduct with low quality basal diet like most of the tropical and subtropical countries of the world. For a marginal farmer, affording good quality concentrate is becoming more and more difficult due to high cost. Heifers are mostly fed on cereal straw, poor quality fodder and handful of concentrate ingredients. Since decades, search for cheaper source of alternate nutritious feeds for livestock particularly for dairy heifers is going on.

Azolla is a promising water fern which is having high production rate and nutritive value (Indira et al. 2009; Wagner 1997). For centuries, *Azolla* has been used as manure in paddy field (Van Hove and Lopez 1983; Yadav et al. 2014). Its use as animal feed has been studied recently (Namra et al. 2010; Dhumal et al. 2009; Murthy et al. 2013; Kumar 2008; Kumar et al. 2012; Indira et al. 2009). *Azolla* consists of various varieties viz. *Azolla pinnata, A. maxicana, A. nilotica.* Among them *Azolla pinnata* is the most common *Azolla* species in tropics and subtropics. It can easily be grown with minimum cost of investment. It grows naturally in stagnant water of river, canal, pond etc. *Azolla* contain relatively high levels of nitrogen due to its symbiosis with blue green algae *Anabaena azollae* which fixes atmospheric nitrogen (Alalade and Iyayi 2006; Raja et al. 2012; Sood et al. 2012). Thus it can spare

costly source of protein in ruminant feed i.e. oil seed cake or meal. Moreover, *Azolla* 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). Feeding trials on pigs and poultry reported improved feed conversion efficiency, average daily gain, dry matter intake and other growth performances on *Azolla* supplementation (Leterme et al. 2009; Accodji et al. 2009; Pinto-Santini et al. 2005; Basak et al. 2002; Namra et al. 2010; Dhumal et al. 2009). Limited studies on cattle and buffaloes indicated improvement in daily gain and efficiency with reduced feed cost when concentrate mixture or protein meal are partially replaced by *Azolla* (Murthy et al. 2013; Kumar 2008; Kumar et al. 2012; Indira et al. 2009). No research has been conducted on the effect of feeding *Azolla* to heifers of zebu cattle breed in India reared by a lot many poor farmers of this country. Therefore, current study was conducted to observe the effect of partial replacement of concentrate mixture with *Azolla pinnata* on growth performance, utilization of feed nutrient and blood biochemicals in heifers of Hariana breed.

Materials and Methods

This experiment was conducted in Instructional Livestock Farm Complex, Uttar Pradesh Veterinary University (DUVASU), Mathura, India. Geographically, the University is located in between 27°27’52"N and 77°42’26"E. Animal care procedures were approved and conducted under the established norms of Institutional Animal Ethics Committee which is formed as per the article number 13 for control and supervision of animal experimentation rules of Government of India.

Animals, feeding and management

Twelve Hariana heifers were individually tied with rope in well ventilated separate concrete floor pens. Deworming of all the heifers was done before the start of the experiment. Before commencements of actual trial animals were acclimatized with experimental feeds for 3 weeks period. Experimental heifers were randomly assigned into two groups (n=6) on body weight (96.0±8.29 kg) and age basis (14±1 months). The first group was assigned to the control diet (C) and the second group to the *Azolla pinnata* supplemented diet (T). The nutrient requirements of heifers were met by feeding wheat straw (particle size - 1.5 to 2.0 cm), chaffed green sorghum fodder (particle size - 2.0 to 2.5 cm) and compounded concentrate mixture (NRC, 2001). Concentrate mixture was prepared by mixing oat grain, barley grain, gram husk, mustard oil cake and mineral mixture in 25, 25, 10, 38 and 2 parts, respectively. Experimental heifers of C group were received a basal diet comprised of wheat straw, green sorghum and concentrate mixture with a proportion of 15: 35: 50. Concentrate mixture of basal diet was replaced with *Azolla pinnata* at 5% level (DM basis) in T. *Azolla pinnata* was grown at instructional livestock farm complex (ILFC), DUVASU, Mathura. It was harvested daily and fed to experimental heifers of treatment group by mixing with concentrate mixture during 1st 3weeks of acclimatization period. Once animals were adopted, *Azolla pinnata* was offered separately to the animals.

Growth study

In a three months growth trial, feeds offered to individual experimental animals and refusals left were collected daily to calculate DMI. Heifers were weighed for two consecutive days in the morning at 07:00 h before offering feed and water. The average of two days was considered as body weight for that fortnight. Average daily gain (ADG) was estimated as a difference between initial and final BW divided by total number of days in trial. Feed conversion efficiency (FCE) was calculated by body weight gain in animals by unit dry matter intake. To assess the body condition of the animal with a fairly high accuracy, a simple technique called BCS has been described. The scale of BCS was given by Anitha et al. (2005). Chart for scoring on a 1 to 5 scale at 0.5 increments was used in present study. The BCS was recorded on the day of the start of experiment and then at fortnightly interval.

Digestibility study

A digestibility trial for a period of 6 days was conducted at the end of feeding study. The diet was weighed daily and then offered to each animal with 4 day adaptation period followed by a 6 day collection period. Daily feeds offered and residue left was recorded to determine the net feed intake. Faeces voided during 24 h were recorded for 6 days. Representative samples of feed, residue and faeces were taken for estimation of chemical composition and nutrient intake. Body weight of experimental animals was recorded before and after the digestibility trial. Digestible nutrient intake and digestibility coefficient of proximate and cell wall fractions were calculated above said observations.

Chemical analysis

Feed, residue and faeces samples were dried in hot air oven to a constant weight at 80°C and ground to pass 1 mm sieve. The ground samples were stored in airtight polyethylene bags for further analysis. Samples of the feed offered, refusal left and faeces were analyzed for DM (ID no. 973.18c, AOAC 1995), crude protein (ID no. 4.2.08, AOAC 1995), ether extract (ID no. 920.85, AOAC 1995) and ash (ID no. 923.03, AOAC 1995) contents. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were assayed using alpha amylase method of Van Soest et al. (1991). The chemical composition of experimental feed is presented in Table 1.

Blood biochemical study

Blood samples were collected from all experimental animals in sterile heparinised vacutainer (Becton Drive, Franklin Lakes, NJ, USA) tubes from jugular vein puncture, posing minimum disturbance to the animal on days 0, 30, 60, and 90 before feeding and watering in morning at 07:00 h. Day '0' represented the start of experiment. Immediately after collection, blood samples were centrifuged at 1200×g for 20 min to separate the plasma. Separated plasma samples were stored in deep freeze at -20°C until further analysis. The total immunoglobulin (TIg) in the plasma samples was estimated by zinc turbidity method (McEwan et al. 1970). Total protein, plasma albumin, blood urea nitrogen (BUN), plasma creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determinedby standard experimental procedures (Sheikh 2012).

Statistical analysis

Data on DMI, body weight, fortnightly body weight gain, ADG, FCE, BCS, blood total protein, plasma albumin, plasma TIg, BUN, plasma creatinine, ALT and AST were analyzed using MIXED procedure with repeated measures of statistical software package SPSS (2012) version 19 (SPSS Inc., Chicago, IL, USA). The statistical model was used to estimate sampling period effect, treatment group and their interaction:

$$Yij = \mu + Ti + Pj + (TP)ij + \varepsilon ijk$$

Where

μ = General mean, *Ti* = Treatment effect, *Pj* = Effect due to period, *(TP)ij* = Effect due to interaction of treatment and period and εijk = Error term

Data on digestibility coefficient of different nutrients, initial and final body weight, DMI and digestible nutrient intake during digestibility trial were analysed by general linear model.

$$Yij = \mu + Ti + \varepsilon ij$$

Where

μ = General mean, *Ti* = Treatment effect and εij= Error term

Results and Discussion

Chemical composition of feed

Chemical composition of basal ration and dietary components are given in Table 1. The chemical composition of concentrate mixture, wheat straw, sorghum fodder and basal ration were in agreement with earlier reports (Mudgal et al. 2008; Vaswani et al. 2012; Amrutkar et al. 2012). Chemical analysis indicated that dried *Azolla pinnata* meal was rich in protein and total ash content (32.05% and 18.25% respectively) whereas the fibre content was found less than green or dry fodder. Similar observations on composition of *Azolla pinnata* was reported by previous workers (Alalade and Iyayi 2006; Kumar et al. 2012; Indira et al. 2009; Tamang et al. 1992; Bolka 2011; Kumar et al. 2012; Kumarasinghe et al. 2012; Cherryl et al. 2014; Veerabahu et al. 2015; Bhaskaran and Kannapan 2015; Parashuramulu et al. 2013).

Nutrient intake

Treatment did not impact on DMI during entire experimental period of 90 days (Table 2 and Table 5). Similarly, organic matter, digestible crude protein (DCP), total digestible nutrient (TDN), NDF and ADF intake was not detected as different in both the groups (Table 5). Similar dry matter intake (DMI) indicates that inclusion of Azolla had no effect on palatability. Kumar et al. (2012) also reported no effect of dried *Azolla* supplementation in concentrate mixture on DMI in buffalo bulls. On contrary, Indira et al. (2009) recorded lower DMI in buffalo calf when 50% ground nut cake (GNC) was replaced by *Azolla pinnata* meal which might be due to higher inclusion level of azolla decreasing palatability. Reddy et al. (2011) reported higher DMI in Nellore sheep when 30% of GNC in concentrate mixture was replaced by Azolla and Wadhwani et al. (2010) observed higher DMI in Marwari weaner lamb by supplementing Azolla to basal diet. In both the above experiments, total mixed rations were prepared with azolla meal. Supporting our observation, DCP and TDN intake were found similar in a study by Wadhwani et al. (2010) where Azolla was supplemented to weaner lambs.

Growth performance

Body weight and metabolic body weight ($BW^{0.75}$) were not affected by replacement of concentrate mixture with *Azolla pinnata* ($P>0.05$) but they increased with period ($P<0.001$). Trend in body weight change (Mean ± SE) of growing Hariana heifers of C and T groups is given in Fig 1. Replacement of concentrate mixture with *Azolla pinnata* improved fortnightly gain in body weight ($P=0.006$) and ADG ($P=0.005$) compared to control. FCE was also impacted ($P=0.01$) by dietary replacement of concentrate mixture with *Azolla pinnata* and 15% more FCE was recorded in *Azolla pinnata* supplemented heifers indicating efficient conversion of feed DM into body mass (Table 2). BCS was observed similar in both the groups. Interaction of treatment × period did not affect body weight and other growth parameters. The result is in agreement with Indira et al. (2009) who also reported improvement in body weight gain in buffalo calf. Improvement of ADG was also observed by Chatterjee et al. (2013) supplemented 1kg fresh Azolla per animal in crossbred male calves. Similar results were recorded in weaner Marwari lamb (Whadhwani et al. 2010) and Osmanabadi kids (Ghodake et al. 2012) when Azolla meal was supplemented in basal diet at the rate of 20 and 15 % respectively. The present report is also

Table 1. Chemical composition of experimental feed during growth trial

Feed stuff	DM%	%DM					
		OM	CP	EE	CF	NFE	NDF	ADF
Concentrate mixture	93.33	91.75	21.89	5.45	8.21	56.21	36.33	13.94
Sorghum fodder	36.23	88.75	7.95	2.05	33.57	45.18	67.67	47.34
Wheat Straw	89.96	86.13	2.48	0.45	33.33	49.87	85.67	53.94
Azolla	8.23	81.75	32.05	1.51	10.22	38.03	59.9	42.60
Basal Ration	72.5	89.25	14.03	3.59	20.25	51.96	54.33	31.63

Values given are mean, DM dry matter, OM organic matter, CP crude protein, EE ether extract, CF crude fibre, NFE nitrogen free extract, NDF neutral detergent fibre, ADF acid detergent fibre

Table 2. Body weight and growth performance parameters of Hariana heifers

Parameters	Treatment			P value		
	C	T	SEM	Treatment (T)	Period (P)	T × P
No. of animals	6	6	n.a.	n.a.	n.a.	n.a.
Days in trial	90	90	n.a.	n.a.	n.a.	n.a.
Initial body weight (kg)	96.0	96.0	4.87	1.00	n.a.	n.a.
Final body weight (kg)	144.83	150.67	6.33	0.66	n.a.	n.a.
DMI (kg)	4.51	4.20	0.12	0.08	<0.001	0.97
Overall body weight (kg)	122.26	124.52	2.82	0.59	<0.001	1.00
Overall body weight (kg $^{0.75}$)	36.59	37.14	0.64	0.56	<0.001	1.00
Fortnightly gain (kg)	8.14^{a*}	9.61^{b}	0.31	0.006	0.009	0.54
ADG (g)	542.59^{a}	643.52^{b}	20.78	0.005	0.01	0.51
FCE	0.12^{a}	0.15^{b}	0.01	0.01	<0.001	0.92
BCS	2.98	3.01	0.07	0.59	<0.001	0.90

Values given are mean. *Means within a row with different superscript are significantly different (P<0.05)
Treatment used as follows: C control, basal straw based ration; T treatment, 5% concentrate mixture was substituted with *Azolla pinnata*
SEM standard error of mean, DMI dry matter intake, ADG average daily gain, FCE feed conversion efficiency, BCS body condition score, n.a. not applicable

Table 3. Blood biochemical parameters of Hariana heifers

Parameters	Treatment			P value		
	C	T	SEM	Treatment (T)	Period (P)	T × P
Albumin(g/dl).	3.34	3.38	0.044	0.56	0.14	0.10
Total protein (g/dl)	7.57	7.07	0.18	0.12	0.06	0.34
Immunoglobulin (mg/dl)	29.60	29.55	0.02	0.18	0.03	0.07
BUN (mg/dl)	38.45^{a*}	31.64^{b}	1.12	0.02	0.35	0.93
Creatinine (mg/dl)	0.76^{a}	0.36^{b}	0.07	<0.001	0.001	<0.001
ALT (IU/L)	26.87^{a}	31.72^{b}	1.11	0.01	0.11	0.65
AST (IU/L)	68.53^{a}	78.53^{b}	2.10	0.01	0.86	0.10

Values given are mean. * Means within a row with different superscript are significantly different (P<0.05)
Treatment used as follows: C control, straw based basal ration; T treatment, 5% concentrate mixture was substituted with *Azolla pinnata*
SEM standard error of mean, ALT alanine aminotransferase, AST aspartate aminotransferase, BUN blood urea nitrogen

Table 4. Digestibility coefficient of feed dry matter and nutrient of Hariana heifers

Digestibility Coefficient	Treatment		SEM	P-value
	C	T		
DM	0.59*	0.60	0.01	0.71
OM	0.60	0.62	0.01	0.67
CP	0.56	0.61	0.01	0.17
EE	0.83	0.81	0.01	0.50
CF	0.51	0.50	0.02	0.78
NFE	0.64	0.66	0.02	0.58
NDF	0.53	0.59	0.02	0.35
ADF	0.47	0.52	0.03	0.50

*P>0.05.

Treatment used as follows: C control, straw based basal ration; T treatment, 5% concentrate mixture was substituted with *Azolla pinnata*. SEM Standard error of mean.

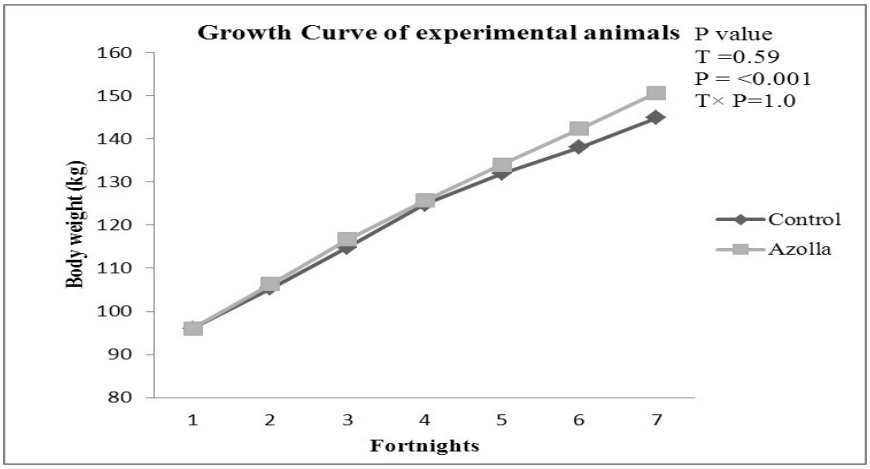
Table 5. Plane of nutrition of Hariana heifers during digestion trial

Variables	Treatment		SEM	P value
	C	T		
Initial body weight (kg)	132.83	137.25	6.17	0.74
Final body weight (kg)	136.00*	140.50	6.02	0.72
Body weight gain (kg)	3.16	3.25	0.40	0.92
Dry matter intake (kg/day)	4.60	4.49	0.25	0.84
Dry matter intake (% BW)	3.43	3.25	0.06	0.14
OM intake (kg/day)	4.10	4.01	0.23	0.85
CP intake (kg/day)	0.69	0.67	0.03	0.76
DCP intake (kg/day)	0.39	0.41	0.02	0.72
DCP intake (g/ kg $BW^{0.75}$)	9.96	10.24	0.38	0.73
TDN intake (kg/day)	2.72	2.67	0.20	0.90
TDN intake (g/ kg $BW^{0.75}$)	68.15	65.92	3.25	0.74
NDF intake (kg/day)	2.47	2.41	0.15	0.86
ADF intake (kg/day)	1.50	1.46	0.09	0.82

*P>0.05.

Treatment used as follows: C control, straw based basal ration; T treatment, 5% concentrate mixture was substituted with *Azolla pinnata.* SEM Standard error of mean

Figure 1. Growth curve of experimental animals



comparable with the report of Singh et al. (1983) worked on crossbred heifers. This may be due to efficient utilization of protein as indicated by linear increase in CP digestibility in treatment groups in our study. Decrease in blood urea concentration in treatment groups also indicate efficient utilization of AA for tissue growth, as deficiency or excess of AA than metabolic capacity of animals increases blood urea concentration (Archibeque et al. 2002). Significant increase in feed conversion efficiency was also observed by other workers (Indira et al. 2009; Chatterjee et al. 2013) in growing cattle and buffalo.

Nutrient digestibility

Experimental heifers gained 3.16 and 3.25 kg body weight during 6 days digestion trial (Table 5). No impact of treatment was found on body weight and metabolic body weight ($BW^{0.75}$) of heifers. Digestibility coefficient of crude protein was 5% higher in treatment group though the difference was non-significant ($P>0.05$). Digestibility coefficient of other nutrient was not detected as different in both the groups. Slight increase ($P>0.05$) in fiber content of the supplements might not improve nutrients supply to ruminal microbes and therefore digestibility of dietary DM and other nutrients remained unaltered (Wallie et al. 2012). No effect on average digestibility coefficients of DM, OM, CP, EE, CF, NFE, NDF and ADF with incorporation of Azolla in the concentrate mixture in present study is similar to the findings of Kumar et al. (2012) who incorporated sun dried Azolla meal in the concentrate mixture of buffalo bulls. Tamang and Samanta (1993) studied the effect of replacing standard goat diet with sun dried Azolla at 0, 10 and 20% and reported that digestibility coefficient of all nutrients except NFE, EE and cellulose were similar on all 3 diets. However, on the contrary, Indira et al. (2009) reported a significant ($P<0.01$) increase in the digestibility coefficients of all nutrients when fresh Azolla replaced 50% of groundnut cake nitrogen in concentrate mixture in male buffalo calves.

Blood biochemical

Dietary replacement of concentrate mixture with *Azolla pinnata* did not affect total blood protein, plasma albumin and plasma TIg concentration (Table 3). However, plasma TIg changed gradually with period ($P<0.05$). Treatment had an impact on creatinine concentration ($P<0.001$), blood urea nitrogen concentration ($P=0.02$) and liver enzyme (ALT & AST) activities ($P=0.01$). Plasma creatinine was found 50% lower in treatment group compared to control. BUN concentration was also found lower whereas ALT and AST activities were improved in treatment group and no interaction between treatment and period was observed (Table 3). Similar to our finding, Reddy et al. (2010) observed no significant difference among the experimental groups and diets for the total protein values in Nellore sheep fed with Azolla, but contrary to our result higher plasma albumin level was observed. Decrease in BUN and creatinine level in the treatment group may indicate more anabolic activity in animal body leading to tissue growth. However, plasma urea concentrations observed in both C and T group were within the normal range in cows (Borghese, 2005). The information on this aspect, that how feeding Azolla affect the TIg or immune status of heifers is not available, so the results can't be discussed due to lack of literature. It may be inferred from our present observation that feeding Azolla did not have any adverse effect on immune status of heifers. Though AST and ALT values were found higher in treatment, both values were within normal range as in cattle (Altug et al., 2007). Thus, the normal range of estimated blood parameters indicates no adverse effect to the animals with replacing feed of growing Hariana heifers with Azolla.

Conclusions

In conclusion, replacement of concentrate mixture with *Azolla pinnata* at 5% level in growing Hariana heifers improved gain and efficiency of animal. Moreover blood biochemicals and digestibility parameter were not adversely affected in this level of replacement and this could be used as an alternative feed resource under smallholder farming system prevalent in tropics.

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