RESEARCH ARTICLE

Effect of *Azolla microphylla* Incorporated Rations on Feed Intake and Nutrient Utilization of Punganur Bulls

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Abstract

The present study was carried out to evaluate the effect of incorporation of fresh *Azolla microphylla* as replacement (0, 10, 20 and 30%) of crude protein content of concentrate mixture on feed intake, nutrient utilization and cost economics of Punganur bulls. In a 4 x 4 Latin square design, four mature Punganur bulls were fed with four different treatments, *i.e.,* T_1 ration (control) comprised of super napier (*Cenchrus purpureus*) *ad libitum* and concentrate mixture with 18% crude protein provided @ 0.5% of the body weight, while other groups T_2, T_3 and T_4 were fed with *ad libitum* super napier along with the fresh *Azolla microphylla* to replace 10, 20 and 30% crude protein in concentrate mixture, respectively. A significant ($p<0.01$) improvement was observed in the digestibility (%) of dry matter, organic matter, crude protein, ether extract, crude fibre, acid detergent fibre, hemicellulose and nitrogen free extract ($p<0.05$) and was in the order of $T_4 > T_3 > T_2 > T_1$, while digestibility (%) of neutral detergent fibre and cellulose did not differ among the treatments. The DCP intake (kg/d) was highest ($p<0.01$) in T_4 followed by T_3, T_2 and was lowest in T_1. The DM and TDN intake (kg/d) during the experimental period did not differ significantly. It can be concluded that *Azolla microphylla* can replace up to 30% of the total protein in the concentrate mixture of mature Punganur bulls economically without any adverse effects.

Key words: *Azolla microphylla*, Cost economics, Nutrient digestibility, Plane of nutrition, Punganur bulls.

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Introduction

Nowadays, the conventional protein supplements are becoming more expensive with decreased availability and there appears to be an impending need to evolve an appropriate alternative feeds to concentrates for ruminant feeding, which led to identify wonderful plant *Azolla microphylla*, which is most economical and efficient feed supplement for livestock (Jondhale, 2018). *Azolla* is one of the nature's protein-rich wonder plants, rich in essential amino acids, protein (19-30%), growth promoter intermediaries, vitamins and minerals (Korsa *et al.,* 2024). Owing to its high protein and low lignin content, Azolla can be easily digested by livestock and therefore can be replaced partially with the concentrate for livestock feeding (Panda *et al.,* 2019; Singh *et al.,* 2022).

Punganur cattle are being reared solely on grazing in the rural areas (Narendranath, 1993) which is insufficient to meet their nutrient requirements and thus limiting the genetic potentiality of the breeding animals. Maintaining germplasm is crucial in order to conserve the indigenous cattle breeds with special features. The maintenance of these cattle is very economical, because of their smaller body size. Further scientific feeding will help the small and marginal farmers to minimize the maintenance cost on feeding. Since the information on feed intake and nutrient digestibility of Punganur breeding bulls is not available in the past literature, an attempt was made to investigate the feed intake, nutrient utilization of the ration containing green forage with optimum level of concentrate mixture and the effect of incorporation of *Azolla microphylla* as a protein replacement in the concentrate mixture.

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Materials and Methods

The present research work was carried out on Punganur bulls at Cattle Farm, Livestock Research Station, Palamaner, Chittoor, Andhra Pradesh, India, following approval of the Institutional Ethics Committee of college, vide approval No. 281/go/ReBi/S/2000/CPCSEA/ CVSc/TPTY/027/Animal nutrition/2021 dated 24.08.2021.

Selection of Animals and Design of Experiment

Four mature Punganur bulls aged 8.00±0.58 years, weighing 186.25 ± 3.07 kg were assigned randomly to four dietary treatments (T_1, T_2, T_3 and T_4) consisting of chopped green fodder and concentrate mixture, supplemented with *Azolla microphylla* in a 4 X4 LSD switch over design. The rations were formulated to meet the requirements as per ICAR (2013). The T_1 ration (control) comprised of super napier (*Cenchrus purpureus*) *ad libitum* and concentrate mixture with 18% CP (maize grain - 49, DORB - 11, soybean meal - 37, MM - 2 and salt - 1) provided @ 0.5% of the body weight. The T_2, T_3 and T_4 rations were contained with the fresh *Azolla microphylla* so as to replace 10, 20 and 30% crude protein in concentrate mixture, respectively.

A digestion trial comprising of 21 days of preliminary period and seven days collection period was conducted on the experimental bulls. The bulls were weighed at the beginning and end of each feeding trial on two consecutive days before offering feed and water and the average body weight was calculated to observe the body weight changes. During the digestion trial, the amount of feed offered, feed refusals and faeces voided was recorded. Representative samples of the feeds, *i.e.,* roughage, concentrate and *Azolla microphylla* and faeces were collected on daily basis and pooled animal-wise. The samples were dried and ground to pass through 2 mm mesh screen and stored in plastic bottles till further analysis. The samples were analyzed for proximate constituents (AOAC, 2005) and fibre fractions (Van Soest *et al.,* 1991).

Statistical Analysis

Data were analysed as per the standard statistical procedures (Snedecor and Cochran, 1994). Means were subjected for Duncan's multiple range test to know the significance of the treatments at $p<0.05$.

Results and Discussion

The composition of super napier (*Cenchrus purpureus*) green fodder, concentrate mixture (18% CP) and *Azolla microphylla* fed to Punganur bulls during the trial is presented in Table 1. Chemical analysis indicated that *Azolla microphylla* was rich in protein (25.25%) and total ash (25.36%) content. Similar observations were reported by Arvindraj (2012), Khare *et al.* (2014) and Sharma *et al.* (2015). During the experimental period, the body weights of animals increased non-significantly (Table 2), which indicated that the experimental rations could be able to meet the nutritional requirements of the maintenance (ICAR, 2013) and the animals were in positive energy balance.

Table 2: Body weight changes of Punganur bulls during the experimental period

Treatment	Initial body weight (kg)^{NS}	Final body weight (kg)^{NS}	Body weight changes (kg)^{NS}
T1	186.25 ± 3.07	186.25 ± 3.20	0.00 ± 0.58
T2	186.25 ± 3.20	187.00 ± 3.31	(+) 0.75 ± 0.25
T3	187.00 ± 3.32	188.50 ± 3.43	(+) 1.50 ± 0.29
T4	188.50 ± 3.42	189.50 ± 4.25	(+) 1.00 ± 1.08

^{NS} Values in the column do not differ significantly ($p>0.05$).

The digestibility co-efficients (%) of dry matter (DM), crude protein (CP), organic matter (OM), ether extract (EE), nitrogen free extract (NFE), crude fibre (CF) and acid detergent fibre (ADF) were significantly ($p<0.01$) increased with *Azolla microphylla* incorporation in the mixture of concentrate as compared to the control, while digestibility coefficients of neutral detergent fibre (NDF) and hemicellulose did not reveal significant differences among the treatments (Table 3). Indira (2007) studied the effect of replacing 50% of groundnut cake nitrogen in concentrate mixture with sun dried *Azolla pinnata* and reported significantly ($p<0.01$) increased digestibility co-efficient of all nutrients in treatment group as compared to control in male buffalo calves. On the contrary, Kumar *et al.* (2012) reported non-significant decrease in the digestibility co-efficient of all nutrients when sun dried *Azolla* meal replaced 25% of total nitrogen in concentrate

Table 1: Chemical composition (% DMB) of concentrate mixture, Super napier green fodder and *Azolla microphylla* fed to Punganur bulls

Parameter (%)	Concentrate mixture	Super napier	*Azolla microphylla*
Dry matter	90.50 ± 0.47	20.48 ± 0.27	5.34 ± 0.33
Organic matter	89.33 ± 0.21	90.07 ± 0.36	74.62 ± 0.36
Crude protein	17.73 ± 0.08	5.20 ± 0.11	25.33 ± 0.74
Crude fibre	9.63 ± 0.18	31.26 ± 0.37	10.39 ± 0.29
Ether extract	1.58 ± 0.02	2.12 ± 0.05	3.29 ± 0.15
Total ash	10.67 ± 0.21	9.93 ± 0.36	25.36 ± 0.36
Acid insoluble ash	2.39 ± 0.08	4.35 ± 0.07	3.55 ± 0.28
Nitrogen free extract	60.40 ± 0.23	51.48 ± 0.45	35.50 ± 0.87
Neutral detergent fibre	35.84 ± 0.60	69.87 ± 0.29	52.86 ± 0.45
Acid detergent fibre	12.02 ± 0.15	34.22 ± 0.43	39.28 ± 0.90
Hemicellulose	23.82 ± 0.52	35.65 ± 0.19	13.58 ± 0.95
Cellulose	8.28 ± 0.24	19.95 ± 0.76	23.51 ± 0.39
Acid detergent lignin	2.07 ± 0.04	12.53 ± 0.30	10.48 ± 0.20

Each value is mean of eight observations.

mixture offered to buffalo bulls. The differences observed regarding the digestibility coefficients in the reports by different authors might be attributed to the differences in the composition of rations and the different animal species involved in the experiments.

The data on nutrient utilization of Punganur bulls fed rations containing *Azolla microphylla* is presented in Table 4. The daily dry matter intake (DMI) calculated in terms of kg/day, % b. wt. and as g/kg $W^{0.75}$ was comparable among the different treatments. Inclusion of *Azolla microphylla* in the concentrate mixture had no deleterious effect on DMI, which indicate that its incorporation had not affected the palatability of concentrate and hence feed intake. Similar effects were observed by Kumar *et al.* (2012) in buffalo bulls and Khare *et al.* (2016) in crossbred female calves. The DMI as % b.wt. of the Punganur bulls in the present study met the daily requirement as suggested by ICAR (2013).

The CPI in terms of g/day or as g/kg $W^{0.75}$ was increased significantly ($p<0.01$) and it was in the order of T4 > T3 > T2 > T1. The CP intake (g/kg $W^{0.75}$) of Punganur bulls fed rations containing *Azolla microphylla* of 30% CP replacement (T_4) in concentrate mixture satisfied the CP requirement for maintenance (5.21 g/kg $W^{0.75}$) as suggested by Nagi Reddy (2021). Hence it is confirmed that, the CP % level in the present rations resulted in neither loss nor gain in body weight of Punganur bulls.

The DCP (%) of the experimental rations as kg/day and g/kg$W^{0.75}$ was significantly ($p<0.01$) higher in T_3 or T_4 as compared to T_1 or T_2. The increment might be attributed to the increased digestibility of crude protein among the treatments. The increased digestibility of CP in the present study might be due to the high degradability value of the protein, as evidenced by higher B1 (soluble protein) and B2 (insoluble but degradable protein) content of the *Azolla* as reported by Parashuramulu *et al.* (2013). The results were in agreement with Wadhwani *et al.* (2010), who reported significant ($p<0.05$) higher protein intake (kg/day) with the inclusion of *Azolla* as replacement of groundnut cake at 10 and 20% level. The differences observed in the present study regarding CPI and DCPI (%) in the experimental rations can be attributed to the higher digestibility of the protein in the rations containing *Azolla microphylla* as evidenced by the CP digestibilities (Table 3).

There was no significant difference in TDN (%) of the experimental rations and TDNI when expressed as kg/day or g/kg$W^{0.75}$ among the experimental animals. In the present study there was a significant difference in the digestibility coefficient of different nutrients (Table 3). But the DMI found to be non-significant (Table 4). Hence the TDN% and TDNI

Table 3: Effect of feeding experimental rations on apparent nutrient digestibility (%) in Punganur bulls

Parameter	T_1	T_2	T_3	T_4
Dry matter**	56.67 ± 0.52^{a}	57.45 ± 0.41^{ab}	59.04 ± 0.78^{b}	62.67 ± 1.00^{c}
Organic matter**	69.36 ± 1.50^{a}	72.05 ± 0.65^{ab}	74.21 ± 0.50^{b}	76.08 ± 0.09^{c}
Crude protein**	56.68 ± 0.73^{a}	58.15 ± 1.43^{a}	62.92 ± 0.79^{b}	63.84 ± 1.27^{b}
Crude fibre**	67.61 ± 1.03^{a}	68.52 ± 1.09^{ab}	70.79 ± 0.45^{bc}	72.41 ± 0.44^{c}
Ether extract**	69.22 ± 0.33^{a}	71.24 ± 0.53^{b}	72.16 ± 0.58^{b}	74.40 ± 0.44^{c}
Nitrogen free extract*	69.04 ± 0.83^{a}	71.68 ± 1.43^{ab}	72.26 ± 0.99^{ab}	74.65 ± 0.99^{c}
Neutral detergent fibre	65.79 ± 1.55	69.06 ± 3.79	71.17 ± 2.63	73.85 ± 3.93
Acid detergent fibre**	64.74 ± 0.98^{a}	67.79 ± 0.83^{ab}	69.51 ± 1.88^{bc}	72.05 ± 0.56^{c}
Hemicellulose	70.27 ± 3.96	72.78 ± 2.17	74.16 ± 0.48	76.40 ± 0.76

^{abc}Values in a row bearing different superscripts differ significantly *($p<0.05$); **($p<0.01$).

Table 4: Effect of feeding experimental rations on plane of nutrition in Punganur bulls

Parameter	T_1	T_2	T_3	T_4
DMI (Kg/d)	4.42 ± 0.09	4.47 ± 0.11	4.42 ± 0.07	4.55 ± 0.05
DMI (% B.Wt.)	2.37 ± 0.04	2.40 ± 0.08	2.37 ± 0.05	2.42 ± 0.03
DMI (g/Kg $W^{0.75}$)	87.63 ± 1.31	88.73 ± 2.62	87.48 ± 1.53	89.49 ± 0.88
CPI (g/day)**	223.50 ± 4.24^{a}	228.53 ± 3.99^{a}	253.11 ± 7.46^{b}	265.69 ± 11.58^{b}
CPI (g/Kg $W^{0.75}$)**	4.43 ± 0.12^{a}	4.54 ± 0.04^{a}	5.01 ± 0.12^{b}	5.22 ± 0.20^{b}
DCP (%)**	2.88 ± 0.10^{a}	2.99 ± 0.15^{a}	3.61 ± 0.13^{b}	3.72 ± 0.09^{b}
DCPI (Kg/d)**	0.12 ± 0.00^{a}	0.13 ± 0.09^{a}	0.15 ± 0.09^{b}	0.17 ± 0.09^{b}
DCPI (g/Kg $W^{0.75}$)**	2.52 ± 0.09^{a}	2.64 ± 0.09^{a}	3.15 ± 0.09^{b}	3.32 ± 0.09^{b}
TDN (%)	61.61 ± 0.09	61.62 ± 0.09	62.19 ± 0.09	64.16 ± 0.09
TDNI (Kg/d)	2.72 ± 0.09	2.76 ± 0.09	2.75 ± 0.09	2.92 ± 0.09
TDNI (g/Kg $W^{0.75}$)	54.02 ± 0.09	54.82 ± 0.09	54.50 ± 0.09	57.46 ± 0.09

^{abc}Values in a row bearing different superscripts differ significantly **($p<0.01$).

either g/day or g/kgW$^{0.75}$ indicated non-significantly higher values, which was in agreement with Kumar *et al.* (2012). However, Arvindraj *et al.* (2017) reported non-significant difference in TDN intake with 10% inclusion of *Azolla microphylla* replacing concentrate mixture to the crossbred male calves.

The cost of super napier green forage, concentrate mixture and *Azolla microphylla* were considered up to arrive at the cost of feed per animal per day, which was arrived as Rs. 108.76, 108.28, 107.93 and 107.17 in T_1, T_2, T_3 and T_4, respectively. The effect of inclusion of fresh *Azolla microphylla*, as replacement of CP in the concentrate mixture at 10, 20 and 30% level resulted in the reduction in the cost of feeding of Punganur bulls by Rs. 0.48, 0.53 and 1.59/- per animal per day, respectively.

Conclusion

On the basis of results obtained in the present study, it is inferred that *Azolla microphylla* can replace up to 30% of the total protein in the concentrate mixture of mature Punganur bulls without any adverse effect on feed intake, nutrient utilization and can reduce the cost of feeding.

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