



Augmentation of the ovarian follicular development in buffalo heifers by feeding azolla

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Supplementation of nutrient rich feed like azolla, which contains many minerals, vitamins, poly unsaturated fatty acids (PUFA) in addition to high protein content may improve the reproductive performance of buffalo. Azolla is able to fix unlinked nitrogen (N_2) directly from the atmosphere because of its endosymbiotic blue-green alga/cyanobacterium *Anabaena azollae* (Van Hove 1989). It is sometimes used as a fertilizer in botanical gardens because of nitrogen-fixing capability (Peters and Meeks 1989) and also as a feed supplement to poultry, pigs and cattle in China and Vietnam (Lumpkin and Plucknett 1982, Van Hove 1989). Lukiwati *et al.* (2008) indicated that feeding of azolla enhanced rabbit production. Studies on the effect of feeding azolla on reproductive efficiency are lacking. It was hypothesized that supplementation of azolla to buffalo heifer will have a positive effect in augmenting their reproductive efficiency. Hence, this study was taken up to know the effect of supplementation of azolla (*Azolla pinnata*) on the reproductive efficiency of buffalo heifers.

Non-descriptive buffalo heifers (2-year-old) were maintained at the Experimental Livestock Unit of our institute at Bengaluru, India under normal management conditions. Artificial ponds made of silpauline sheets of 16 ft × 12 ft were used to grow azolla. Daily 1–2 kg fresh azolla was harvested from each pit and dried.

A 3-month feeding experiment was conducted with 2 groups of buffalo heifers (control: 6 animals and treatment group (azolla fed): 6 animals). Animals in the treatment group were fed with dried azolla equivalent to 500 g freshly harvested azolla/100 kg body weight, as a feed supplement. The proximate composition of azolla (after draining away the water) was estimated. Observations related to the reproductive efficiency in buffalo heifers in terms of occurrence of estrus, follicular turnover, ovulation and expression of estrus symptoms were recorded. Blood samples were collected for the estimation of plasma biochemical parameters (calcium, phosphorus, blood urea nitrogen (BUN), glucose, non-esterified fatty acids (NEFA)

and cholesterol. Animals were subjected to ultrasound scanning for observing the cyclicity and follicular population. Observations were recorded during 1 month of pre-treatment period and during the 3 month feeding experiment period and post-experiment period of 1 month. All the biochemical parameters except progesterone were estimated using commercial kits. Progesterone was estimated using radioimmunoassay using kits. Before starting the feeding experiment, the animals were fed normal diet for 3 months for acclimatization. Observations on the number of medium (3–8 mm) and large (>8mm) follicles were recorded in the buffalo heifers, once a week. Ovarian follicles were examined by ultrasonography using an ultrasound equipment with a 7.5MHz rectal probe (linear array transducer).

The differences between the number of follicles among the 3 different periods of the experiments i.e. pre- azolla feeding period, azolla feeding period and post azolla feeding period were analyzed by ANNOVA and subsequently by Tukeys multiple comparison test. The differences between the treatment and control group of animals were analyzed by 't' test. Statistical analysis was made using statistical software. Differences between the mean values were considered significant when the P values were less than 0.05.

The dry matter, total ash, crude protein, neutral detergent fibre, acid detergent fibre and total fat content of fresh azolla were 5.65, 21.73, 19.48, 54.74, 36.78 and 3.95 %, respectively. During 1 month preceding the feeding experiment, the number of medium sized follicles in control and treatment group were 1.29 ± 0.28 and 1.25 ± 0.23 , respectively. And the corresponding numbers for large follicles were 0.29 ± 0.11 and 0.50 ± 0.15 , respectively. There was no significant difference ($P > 0.05$) between the 2 groups of animals either in medium size follicles or large follicles during the period preceding the feeding of azolla. During the 3 months of the feeding period, number of medium sized follicles in control and treatment group was 1.42 ± 0.14 and 1.28 ± 0.15 , respectively; and the corresponding numbers for large follicles were 0.12 ± 0.04 and 0.43 ± 0.07 , respectively. Only in large follicles, the numbers were significantly more ($P < 0.01$) in azolla fed buffalo heifers during the period of

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feeding azolla. Significantly more number of large follicles observed in the azolla fed group compared to unfed control group during the feeding period was not observed in the post-feeding period. During the 5 weeks of post feeding period, the numbers of medium sized follicles in control and treatment group were 2.67 ± 0.32 and 2.46 ± 0.36 , respectively. And the corresponding numbers for large follicles were 0.12 ± 0.07 and 0.17 ± 0.08 , respectively. Feeding of azolla increased the occurrence of estrus / expression of estrus symptoms / ovulations during the feeding phase of the experiment. There was no effect of feeding on the body weight of buffalo heifers.

The plasma progesterone levels validated the observations on corpus luteum appearance and external heat symptoms. The positive effect of feeding azolla on the ovarian activity could be clearly observed by the progesterone profile. During post feeding period, in 5 out of 6 animals, the progesterone profile indicated that the animals did not come to heat, though they came to heat regularly during the feeding period. It is noteworthy to mention here that both feeding period and post feeding period coincided with the low breeding season. The progesterone profile authenticated the observation of estrus symptoms and ultrasound scanning observations in the buffalo heifers.

There was no significant difference ($P > 0.05$) in any other blood plasma parameters excepting cholesterol between the 2 groups during any of the 3 phases of experiment (i.e. pre-feeding, feeding period and post feeding period). The different parameters were: BUN (range: 9.6 to 19.3 mg%), creatinine (range: 1.18 to 1.92 mg%), glucose (range: 58.6 to 89.4 mg%), protein (range: 7.4 to 10.2 g%), NEFA (range: 205 to 507 mg%), calcium (range: 7.8 to 8.6 mg%) and phosphorus (4.9 to 5.8 mg%). But cholesterol was significantly more ($P < 0.05$) in control animals than the treatment group during post feeding period (range: 35 to 103 mg%). There are no published reports on the effect of azolla on the reproduction of domestic animals. However, a study in rabbits indicated that azolla might increase libido (Lukkiwati *et al.* 2008). In a recent study in tilapia fishes, feeding of azolla improved significantly the gonado-somatic index, which indicated that azolla has a positive effect on reproduction in animals (Bag *et al.* 2011). However feeding of azolla did not affect the fatty acid profile in the fish. The positive effects of azolla on reproduction observed in the present study might be due to its high nutritive value. Paoletti *et al.* (1987) found that azolla contains 0.8–6.7% dw crude fat, with 6.1–7.7% and 12.8–26.4% total fat for polyunsaturated acids (PUFA) omega 3 and omega 6. Azolla

seems to be rich in some vitamins, notably carotenes and vitamin A (300–600 ppm dw, Leonard 1997). In the present study, the increase in the occurrence of estrus, ovulation as evidence by the observation of corpus luteum through ultrasonography and increase in the number of large follicles clearly indicated that feeding of azolla might augment the reproductive efficiency in farm animals. The observations made in the present study promises azolla as a potent feed supplement to augment reproduction in livestock, in particular buffaloes.

SUMMARY

Azolla, a freshwater fern, is rich in minerals, protein and polyunsaturated fatty acids (PUFA). Dried azolla equivalent to 500 g freshly harvested azolla / 100 kg body weight was fed daily to treatment group (6 buffalo heifers) over a 3 month feeding experiment. Another 6 animals, which were fed normal diet without azolla stood as control group. Feeding of azolla promoted the progesterone synthesis. There was more occurrence of estrus in the azolla fed animals. Also, there was a significant increase in the turnover of large ovarian follicles in these animals. The observations indicated that feeding azolla as a supplement may increase the reproductive efficiency in farm animals.

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