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Amelioration of reproductive problems in crossbred cattle with high blood urea nitrogen levels by *ragi* (finger millet) supplementation- A field study

P S P GUPTA[1], S SELVARAJU[2], D T PAL[3], G RAVIKIRAN[4] and J P RAVINDRA[5]

National Institute of Animal Nutrition and Physiology, Bangalore, Karnataka 560 030 India

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ABSTRACT

High blood urea nitrogen levels have been considered as one of the important causes of infertility in cattle. A field study was conducted in crossbred cattle with reproductive problems in a Harohalli village, Devanahalli (TK), Bangalore (R) District, in which 21 crossbred cattle having high blood urea nitrogen (> 19 mg%) with reproductive problems were selected. Out of these, 11 were anoestrus (5 heifers and 6 cows) and the rest were repeat breeding animals. They were supplemented with additional energy in the form of 1–2 kg *ragi* (finger millet) per day for 2–3 months. Blood samples were collected on 0 day and every fifteen days till 3 months of the experiment. They were analyzed for blood urea nitrogen (BUN), glucose, non-esterified fatty acids (NEFA), total protein, cholesterol, calcium, phosphorus, magnesium, copper, zinc and progesterone. All the supplemented animals except one animal that was infested with worms responded positively and showed a significant fall in the BUN levels. But there was no significant difference in the glucose, cholesterol, NEFA, protein and mineral levels between the pretreatment and post treatment period. The plasma progesterone levels improved from below detectable levels at pretreatment period to > 1ng/ml at post treatment period in anoestrous animals. Following *ragi* supplementation, all the anoestrous animals started cycling within 15 to 60 days and later conceived. Of the 10 repeat breeding cattle (4 heifers and 6 cows), all animals (except one heifer which was infested with worms) conceived within 3 months and later calved. Results indicated that the energy and protein balance is very important and BUN levels are to be maintained below 19 mg% for maintenance of the fertility in cattle.

Key words: Anoestrus, Blood urea nitrogen, Crossbred cattle, Repeat breeding

The protein and energy balance is critical for optimum reproductive efficiency. The blood urea nitrogen (BUN) levels are known to affect gametogenesis, fertilization and survival of the conceptus (Rhoads *et al.* 2005, Leroy *et al.* 2008). Though the problem of high BUN and milk urea nitrogen levels of more than 19 mg% known to affect reproduction in cattle is widely reported (Butler 2000, Kim *et al.* 2001, Cottrill *et al.* 2002, Melendez *et al.* 2003), studies on reducing their levels by additional energy feeding (in the form of locally available grains) are lacking. *Ragi* is widely used as energy source for dairy cattle and contains high proportion of bypass protein (68% of its crude protein) as compared to other grains (Sampath *et al.* 1999). Hence this study was conducted to know the effect of feeding additional energy in the form of locally available grain i.e. *ragi* in reducing BUN levels thereby nullifying their effects on reproduction in crossbred cattle.

Present address: [1]Professor and Head, Department of Physiology, Veterinary College, Hyderabad.[2]Scientist (SS), [5]Principal Scientist, Animal Physiology Division. [3]Scientist (SS), Animal Nutrition Division, [4]Scientist (SS), Bioenergetics and Environmental Sciences Section.

MATERIALS AND METHODS

Holstein- Friesian crossbred cattle (21) with reproductive problems (free from any infectious diseases) that were having high blood urea nitrogen (> 19 mg%) were selected from the adopted village of Harohalli, Bangalore Rural District. Out of these, 11 were anoestrus (5 heifers and 6 cows) and the rest were repeat breeding animals. They were being maintained by the local livestock farmers in thatched roof sheds under semi intensive system. They were being fed compounded livestock feed at the rate of 1 to 1.2 kg/100 kg. body weight and *ragi* straw (finger millet, *Eleusine coracana*) *ad lib*. During the experiement, they were supplemented with additional energy in the form of 1–2 kg *ragi* (finger millet) grain (in semi-ground form, approximately @ 500 g/100 kg body weight) per day for 2 months.

Once in 15 days, jugular venous samples (12–15 ml) were collected in heparinized vials within 4–7 h after morning feeding. As the suitable blood sampling time for the estimation of BUN levels was 3 to 8 h after morning feeding in cattle (Hwang *et al.* 2001), in the present study, the blood samples were collected between 4 and 7 h after feeding.

Blood urea nitrogen was estimated by enzymatic urease method using diagnostic kits. Blood plasma glucose was estimated by enzymatic, GOD-POD, end point colorimetry method using diagnostic kits. NEFA level was estimated by copper soap extraction method as modified by Shipe *et al.* (1980). Plasma total protein (Biuret method) and cholesterol (one step method of Wybenga *et al.* 1970) were also estimated using diagnostic kits. Plasma minerals i.e. calcium, magnesium, copper and zinc were estimated by atomic absorption spectrophotometer. Phosphorus was estimated by spectrophotometer method of Fiske and Subbarao (1925). Plasma progesterone was estimated by Radioimmunoassay using kits.

Statistical analysis

The difference between the various parameters recorded before the *ragi* treatment and after the *ragi* treatment was estimated by students ' t' test using statistical package of Graph Pad PRISM. Differences between the mean values were considered significant when the P values were less than 0.05.

RESULTS AND DISCUSSION

All the supplemented animals (except 1 animal that was infested with worms) responded positively and showed a significant (P<0.01) fall in the BUN levels (from 27.4±3.90 mg% to 15.9±1.30 mg%). Reproductive problems ameliorated within 15 days to 3 months time. Following *ragi* supplementation, all the anoestrous animals started cycling within 15 to 60 days and later conceived. Of the 10 repeat breeding cattle (4 heifers and 6 cows), nine conceived within 3 months and later calved. The non-respondent heifer was the one with parasitic infestation. Meendez *et al.* (2003) reported that concentrations of BUN above 19 mg/dl influenced microenvironment of the uterus and altered the pH level and prostaglandin production in endometrial cultures. The presence of urea significantly increases the secretion of $PGF_{2á}$ and PGE_2. High ammonia and urea concentrations in the blood changes intrafollicular, oviductal and uterine environments. Oocytes and embryos are highly sensitive to such changes in their microenvironment, possibly leading to a disturbed maturation, fertilization or early cleavage (Leroy *et al.* 2008).

There was no significant difference (P>0.05) in any of the parameters studied except BUN, between the pretreatment period and post treatment period (Table 1). In the anoestrous animals, wherein the progesterone levels were below detectable levels before the treatment were increased to above 1 ng/ml after the treatment indicating the initiation of luteal activity in them due to the *ragi* feeding.

The NEFA levels recorded in the present study are within the range reported earlier in cows (0.03 to 0.54 m mol/ml) by Delavaud *et al.* (2002). Since there was no significant difference either in the glucose or NEFA levels, we can

Table 1. The mean blood plasma parameters in crossbred cattle before and at the end of *ragi* feeding for a period of 2 months

	Before *ragi* feeding	2 months after *ragi* feeding
BUN (mg%)	27.4±3.90[a]	15.9±1.30[b]
Glucose (mg%)	61.45±3.81	54.00±7.42
NEFA (μ mol./ml)	0.25±0.03	0.18±7.0.02
Protein (gm%)	6.491±0.23	6.350±0.24
Cholesterol (mg%)	123.6±8.46	1213±8.32
Calcium (mg%)	10.13.±0.55	10.95±0.52
Phosphorus (mg%)	4.69±0.39	4.85±0.27
Magnesium (mg%)	2.82±0.28	3.03±0.28
Copper (ppm)	0.84±0.11	1.41±0.13
Zinc (ppm)	1.21±0.14	2.37±0.49

Values in a row with different superscripts differ significantly (P<0.05).

assume that the energy status did not changed significantly with the *ragi* treatment and the positive effect of *ragi* on reproduction may not be due to change in the energy status. This might be due to efficient utilization of protein in rumen with additional supplementation of energy in the form of *ragi*, that might have resulted in lower ammonia- nitrogen (NH_3–N) production and thus leading to lower BUN levels. The unknown beneficial factors in *ragi* are need to be probed further. Recent reports indicate that negative effect on oocyte and embryo quality is major factor in the complex pathogenesis of reproductive failure in animals with negative energy balance (Leroy *et al.* 2008).

Since the total protein was in normal range, we cannot attribute the high BUN levels during the pretreatment period to excess protein feeding. The positive effect observed by feeding *ragi* might be due to its high proportion of bypass protein (Sampath *et al.* 1999). The role of protein in reproduction of ruminants is equivocal. Diets deficient in protein have resulted in weak expression of estrus, cessation of estrus, repeat breeding etc. However, total proteins in circulation represent a balance between the biosynthesis and catabolism or mechanical loss (Ghosh *et al.* 1991). The lower level of serum proteins may cause deficiency of certain amino-acids required for the synthesis of gonadotrophins, thereby causing reproductive disturbances. Cholesterol is the precursor for steroid hormone synthesis. In the present study, there was no significant difference in the cholesterol levels also, after the treatment with *ragi*, which might indicate that the beneficial effect may not be routed through steroid synthesis.

The negative effects of BUN on reproduction was related to altered uterine environment, impaired progesterone secretion and compromised oocyte and embryo quality and development (Sinclair *et al.* 2000). There may be an indirect effect also, which is the higher energy deficit due to the expense of ammonia conversion by the liver and urea excretion (Formigoni and Trevisi 2003). In the present study, the definite positive effect of *ragi* feeding on reproductively abnormal animals with high BUN levels might have resulted due to the lowering of BUN levels. Energy supplementation in the form of *ragi* might have contributed to the energy requirement of rumen microbes, which in turn would have converted excess ammonia prevalent in the rumen, thereby leading to decrease in BUN levels. This might have restored the normal uterine environment and thus the improved conception rates in the experimental animals in the present study. There is no work reported on these aspects in any of the geographical regions of our country.

There was no significant difference ($P>0.05$) in the concentration of minerals studied between the pretreatment period and the post treatment period, which indicates that there is no interference of minerals in the positive treatment effect by *ragi* and the mineral levels were within the normal range reported earlier in different agroclimatic zones of India (Prasad *et al.* 1999, Das *et al.* 2002). In the present study, it appears that *ragi* expressed its positive effect in repeat breeding animals probably by reducing uterine pH (due to reduction of high BUN levels) that might have favoured fertilization, since it was observed in earlier studies that high BUN levels contributing to low pH leads to failure of fertilization (Meendez *et al.* 2003). The positive effect expressed by *ragi* in anoestrous animals might be due to the reduction in BUN levels thereby favouring optimum production of endocrine and paracrine substances influencing the onset of estrus (Formigoni and Trevisi 2003). In conclusion, the present study indicated that feeding of more energy to the cattle with high BUN levels (>19 mg%) would help in reducing BUN levels and thereby ameliorating reproductive problems. The present study suggests that other energy sources (maize, *jowar* etc., locally available at cheaper rates) may also be helpful for reducing BUN levels However, more detailed studies including additional parameters like insulin like growth factor, essential amino acids, estradiol and enzymes like aromatase and β hydroxy-steroid dehydrogenase will throw more light on the exact mechanism of positive action of *ragi* feeding on ameliorating reproductive problems in cattle with high BUN levels.

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