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Effect of supplementation of rumen protected fat on blood urea nitrogen and non-esterified fatty acid levels in postpartum crossbred cows

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Abstract
Postpartum reproductive failure due to negative energy balance is a major problem among the high producing dairy cattle, incurring huge economic loss. The present investigation estimated the outcome of dietary provision of rumen protected fat on blood urea nitrogen (BUN) and non-esterified fatty acid (NEFA) levels in crossbred cows. The trial was conducted with four experimental groups of six cows each (GI - control; GII - 200 g protected fat; GIII - 200 g protected fat daily + Ovsynch protocol; GIV - Ovsynch protocol alone). The mean BUN levels (mg/dl) in the experimental groups were 34.78±1.076, 24.12±0.99, 24.63±1.56 and 29.13±0.97, respectively in GI, GII, GIII and GIV. The BUN levels were significantly lower in those animals supplemented with protected fat (GII and GIII) compared to others. The serum NEFA level (mmol/L) which is a measure of negative energy balance in the body was significantly lower in GII and GIII compared to other two groups. The mean NEFA values (ELISA) were 0.37 ± 0.02, 0.25 ± 0.01, 0.28 ± 0.01 and 0.40±0.01 in group GI, GII, GIII and GIV, respectively. The results proved that dietary supplementation of protected fat modulates the blood biochemical parameters favourably, promoting early resumption of ovarian activities in postpartum dairy cows.

Keywords: Crossbred cows, ovsynch, bypass fat, BUN, NEFA

1. Introduction
In the global scenario, India ranks top in milk production but the reproductive parameters of dairy animals are not at par with international standards. Anoestrus and repeat breeding are the two major reproductive problems affecting 30 to 40 percent cattle and buffalo population of India. Infertility due to negative energy balance in high producing cows during the postpartum period is a major problem in the country. Hormonal induction of oestrus using Prostaglandin-F2α (PGF2α) and Gonadotropin releasing hormone (GnRH) has been found to be beneficial in enhancing reproductive efficiency in cross-bred cows in the late postpartum period under field conditions.

Supplementation of rumen protected fat in the ration of dairy animals has been reported to have great positive impact on their production and reproduction performances (Naik, 2013) [10]. Preliminary studies on the effect of dietary supplementation of rumen protected fat on production and reproductive parameters have been carried out earlier, but detailed study on the changes in blood biochemical parameters of cows supplemented with rumen protected fat compared to control group and those undergone hormonal induction of oestrus were not carried out earlier.

It is reported that higher levels of Blood Urea Nitrogen (BUN) and Non-Esterified Fatty Acids are associated with impaired reproductive efficiency in dairy cattle (Hammon et al., 2005) [8]. Though it is reported that dietary supplementation of rumen protected fat considerably improved the reproductive efficiency of dairy cattle, its effect of the blood parameters like BUN and NEFA has not been studied in detail. Hence the present study investigated the BUN and NEFA levels of high producing crossbred cows supplemented with rumen protected fat and subjected to hormonal induction of oestrus during early postpartum period.

2. Materials and methods
The experiment was carried out in 24 apparently healthy normally calved, crossbred cattle of similar age and parity with a body score of 3 to 3.5 out of 5 maintained at University Livestock Farm and Fodder Research Station, Mannuthy.

All the animals were fed as per standard feeding practices based on NRC recommendations.

The four experimental groups were consisted of randomly allotted six cows each. Animals in Group I (GI) were not given any additional dietary supplementation and kept as control.

The animals in Group II (GII) were supplemented with 200 g protected fat per day (Calcium salt of palm fatty acid, It contained crude fat -84 %, calcium 9% acid insoluble ash-4% and moisture -3%) from 5 days after calving till 90th day along with the feed ration, every morning.

The animals in Group III were fed 200 g of protected fat per day from 5th day of calving till 90th day along with daily ration. In addition, they were subjected to Ovsynch protocol as described earlier (Hagen *et al*., 2015) [7]. Briefly, 10 µg of GnRH analogue (Buserelin acetate - *Receptal, Intervet, India*) were administered intra-muscularly (i/m) on Day 45 postpartum followed by 500 µg Cloprostenol (*Pragma, Neovet, India*) i/m on day 52 postpartum. A second dose of GnRH analogue, 10 µg, i/m on Day 54 was also administered, followed by timed artificial insemination at 16 h after second dose of GnRH.

Animals in Group IV were not supplemented with rumen protected fat. But they were subjected to Ovsynch protocol on Day 45 postpartum.

All the animals in Groups I and II were inseminated during natural oestrus exhibited after Day 45 postpartum. Animals in Groups III and IV were subjected for timed AI and observed for induced oestrus. Time taken for the onset of oestrus was recorded for all the animals in the groups.

From all the animals, blood samples were collected on date of calving (Day 0) and thereafter on every 10 days up to Day 90 postpartum. The blood urea nitrogen concentrations of the experimental animals were determined by UV enzymatic method using kit supplied by Chromatest Linear Chemicals, Spain. The NEFA was estimated using 96-well serum/plasma fatty acid kit NEFA Detection 500 Point Kit (*Chongning Biospes co. Ltd*. China).

The data recorded were analyzed statistically using statistical software SPSS (*SPSS, Version 14, USA*).

3. Results and Discussion

3.1 Blood Urea Nitrogen

The serum BUN levels observed in the experimental animals during the early postpartum period from day 0 to 90 is given in Table 1. The BUN levels were lower in GII and GIII compared to group GI and GIV ($p<0.01$). The BUN levels were low in GI and GIV on the day of parturition, but it went up by day 20 and maintained at a higher level up to day 60, then started declining by day 80 and became lowest by day 90. In GII and GIII, the BUN values were higher at parturition, but thereafter showed a decreasing trend and observed to be lowest by day 90 (Fig. 1). The BUN levels were significantly lower in GII and GIII compared to GI and GIV ($P<0.001$).

Table 1: BUN levels in experimental animals during postpartum period

S. No.	Days	Group I (n=6)		Group II (n=6)		Group III (n=6)		Group IV (n=6)	
		Mean±SE	SEM	Mean±SE	SEM	Mean±SE	SEM	Mean±SE	SEM
1	0	31.48	1.77	30.48	0.70	28.37	0.88	27.88	0.80
2	10	32.86	1.66	27.87	0.95	26.39	0.77	28.50	1.03
3	20	34.34	0.76	25.55	0.81	25.90	0.86	29.17	1.04
4	30	33.99	0.52	24.73	1.16	25.39	1.03	29.99	1.04
5	40	36.28	0.95	22.38	1.14	25.07	1.09	30.35	1.26
6	50	36.26	1.20	23.25	1.10	24.56	1.29	30.70	0.96
7	60	36.84	1.00	22.58	1.26	23.44	1.37	30.54	0.97
8	70	36.41	1.16	21.86	1.05	23.18	1.37	28.67	0.81
9	80	35.44	0.90	21.69	0.77	22.40	1.36	27.97	0.89
10	90	33.82	0.79	20.74	0.96	21.50	1.50	27.46	0.82
Overall		34.78^{b}	1.07^{b}	24.12^{a}	0.99^{a}	24.63^{a}	1.15^{a}	29.13^{ab}	0.96^{ab}

*GI-control cows, GII- Cows supplemented with protected fat (BF), GIII- Cows supplemented with BF and subjected to ovsynch on day 45 PP; GIV- Cows subjected to ovsynch in day 45 PP. Values bearing different superscripts differ significantly ($p<0.001$).

Ferguson *et al*. (1988) [5] stated that the BUN had an inverse relationship with the conception rate of cows when the level increased from 10 mg per cent to 20 mg per cent. An excess BUN level may alter uterine PH and thereby reduce fertility (Buttler *et al*., 1996) [3].

Elevated level of BUN is related to increased ammonia, urea and nitrogen levels in the follicular fluids before ovulation on oestrus day and in the uterine fluid in the luteal phase of oestrus cycle in transient period cows. These higher levels of ammonia and urea nitrogen in this fluids leads to reproductive failure and toxic results on embryo (Hammon *et al.*, 2005) [8].

Shelke *et al.* (2012) [12] reported that blood urea nitrogen (mg/dl) in bypass fat fed murrah buffaloes were 23.15±1.14 which has lower than in normal buffaloes. Observed mean value of bun as 16.97(mg/dl) and 15.73 (mg/dl) in control group and bypass fat treated group respectively in crossbred cows.

Since the BUN levels have direct relationship with the dry matter intake and crude protein content of a diet, the increased BUN levels in the control animals may be resulted from the increased dry matter intake postpartum (Broderick and Clayton, 1997) [2]. The supplementation of bypass fat changed the energy metabolism and stress. BUN levels were reduced in supplemented group. This is in agreement with the previous report of Grewal *et al.* (2013) [6], where bypass fat supplementation at a level of 200 grams per day in lactating crossbred cows has reduced the blood urea nitrogen (BUN) levels.

3.2 Non-Esterified Fatty Acids (NEFA)

The NEFA levels experimental animals during the early postpartum period is presented in Table 2. Serum NEFA levels (mmol/L) in GII and GIII were significantly lower ($p<0.01$) compared to group GI and GIV.

In GI and GIV, the levels were more than 0.2 mmol/l until day 80 in GI, and to day 70 in GIV (value above 0.2 mmol/l denoted negative energy balance). In GII and GIII, the level started declining from day 10 and became lower than

0.2mmol/L by day 20. It was observed that NEFA levels below 0.2 mmol/L could be achieved by protected fat feeding negative energy balance was overruled.

The circulating concentration of NEFA is an indication of the extent of mobilization of adipose tissue (Pullen *et al*., 1989) [11]. When there is negative energy balance in the body, the NEFA concentration increases considerably (Bauman and Currie, 1980) [1]. Since the cows during the early postpartum period are usually in negative energy balance, the NEFA concentration also are supposed to be high during this period (McNamara, 1991) [9]. Excessive mobilization of fat from body reserves leads to higher concentration of NEFA, which in turn results in increased uptake by liver and causes fatty liver syndrome and ketosis (Drackley, 1999) [4]. NEFA levels (mmol/L) were found to be 104 .94±3.77 and 114.16±4.79 in control and crossbred cows supplemented with bypass fat respectively (Shelke *et al*., 2012) [12].

Table 2: Serum NEFA levels in experimental cows during post-partum period

S. No.	Days Post-Partum	NEFA (mean±SE) mmol/L							
		Group I (n=6)		Group II (n=6)		Group III (n=6)		Group IV (n=6)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1	0	0.82	0.03	0.88	0.04	0.90	0.04	0.95	0.03
2	10	0.63	0.02	0.44	0.03	0.54	0.02	0.69	0.03
3	20	0.44	0.01	0.16	0.00	0.23	0.01	0.51	0.02
4	30	0.39	0.03	0.15	0.003	0.22	0.02	0.41	0.02
5	40	0.30	0.03	0.16	0.004	0.17	0.006	0.31	0.01
6	50	0.25	0.02	0.16	0.008	0.16	0.005	0.27	0.01
7	60	0.25	0.01	0.15	0.008	0.15	0.003	0.23	0.01
8	70	0.23	0.01	0.14	0.007	0.15	0.006	0.20	0.00
9	80	0.20	0.01	0.14	0.007	0.14	0.005	0.18	0.00
10	90	0.16	0.01	0.14	0.009	0.16	0.006	0.18	0.00
Overall		0.37^{b}	0.02	0.25^{a}	0.0123	0.2862^{a}	0.0146	0.3976^{b}	0.0178

GI-control cows, GII- Cows supplemented with protected fat (BF), GIII- Cows supplemented with BF and subjected to ovsynch on day 45 PP; GIV- Cows subjected to ovsynch day 45 PP. *Values bearing different superscripts differ significantly ($p<0.001$).

The protected fat feeding helps to maintain the energy balance in the body there by reducing the levels of NEFA, as observed in group II and III in present study.

4. Conclusion

The results of present study proved in those animals supplemented with rumen protected fat, the blood urea nitrogen and non-esterified fatty acid levels were significantly lower. Hence postpartum dietary supplementation of protected fat considerably improves the reproductive efficiency of dairy cattle.

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