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Cholesterol, triglycerides and progesterone levels of postpartum cows supplemented with rumen bypass fat

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Abstract
Postpartum infertility due to negative energy balance is a major problem among the high producing cows of Kerala, incurring a huge economic loss. The present study aimed to evaluate the effect of supplementation of bypass fat on blood biochemical parameters and circulating hormonal levels. The trial was conducted with four experimental groups of six cows each (GI – control; GII- 200 g bypass fat; GIII – 200 g bypass fat daily + Ovsynch protocol; GIV – Ovsynch protocol alone). It was observed that the total cholesterol ($P<0.01$) levels were significantly higher and triglycerides ($P<0.01$) levels were significantly lower in those animals fed with bypass fat, compared to others. The mean circulating blood progesterone levels were observed to be significantly higher ($P<0.01$) in animals fed with bypass fat. The results proved that dietary supplementation of bypass fat modulates the blood biochemical parameters and hormonal profile of postpartum cows, making them fit for an early resumption of ovarian activities.

Keywords: crossbred cows, *ovsynch*, progesterone, NEFA, NEB

Introduction
In the global scenario, India ranks top in milk production but the reproductive parameters are not at par with global standards. Anoestrus and repeat breeding are two of the major reproductive problems affecting 30 to 40 percent of total 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 postpartum period under field conditions.

Preliminary studies on the effect of supplementation of bypass fat on production have been carried out earlier, but a detailed study on the changes in blood biochemical parameters and hormonal profile of cows supplemented with bypass fat and comparing it to a control group and those treated with hormonal induction of oestrus were not carried out earlier.

Hence the present study was aimed to evaluate some important blood biochemical parameters and hormonal profile of high producing crossbred cows supplemented with bypass fat and subjected to hormonal induction of oestrus during the postpartum period.

Materials and Methods
A total of 24 apparently healthy normally calved, crossbred cattle of similar age and parity with a body score of 3 to 3.5 out of 5 were selected from University Livestock Farm and Fodder Research Station, Mannuthy for the study.

The animals were randomly allotted to four groups of six cows each. All the animals in these four groups were fed as per standard feeding practices based on ICAR recommendations (2013). Animals in Group I were not given any supplementation and kept as control.

The animals in Group II were fed with 200 g bypass 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 compounded cattle feed, every morning.

The animals in Group III were fed 200 g of bypass fat per day from the 5th day of calving till 90th day along with compounded cattle feeds every morning. In addition, they were subjected to the *Ovsynch* protocol as described by Hagen *et al*. (2015) [6]. Briefly, 10 µg of GnRH analog (Buserelin acetate - *Receptal, Intervet, India*) were administered intramuscularly (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 analog, 10 µg, i/m on Day 54 was also administered, followed by timed artificial insemination at 16 h after the second dose of GnRH.

Animals in Group IV were not supplemented with bypass 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 observed for induced oestrus and then timed AI. Time taken for the onset of oestrus was recorded for all the animals in the groups.

Blood samples were collected from all the animals on Day 1 (calving date) and thereafter on every 10 days up to Day 90 postpartum. Blood levels of cholesterol and triglycerides were estimated using standard ELISA kits (*Euro Diagnostic Systems Pvt. Ltd*., India). Serum progesterone level also analyzed using diagnostic ELISA kit (*Pathozyme® Progesterone*, Omega Diagnostics Ltd., UK).

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

Results and Discussion

The serum cholesterol estimation results are presented in Table 1. The serum cholesterol level in GII and GIII are significantly higher compared to GI and GIV ($p<0.01$). This clearly indicates that those animals fed with cholesterol have higher levels of cholesterol in their body.

Cholesterol is the chief precursor for the biosynthesis of progesterone by the luteal cells of the ovary, which helps for the implantation of embryos and responsible for maintenance of pregnancy (Grummer and Carroll, 1988; Niswender and Nett, 1994) [5, 11]. Higher levels of blood cholesterol levels are always related to improved reproductive efficiency in high producing dairy cattle (Son *et al*., 1996) [13].

It is reported that after calving, the mean serum total cholesterol levels exhibit a linear path of increasing (Gowda, 2014) [4]. In the present study also, though the control group animal did not show any linear increase in total cholesterol levels, those animals supplemented with bypass fat have shown a linear increase in total cholesterol levels.

Previously, Grummer and Carrol (1988) [5] reported that dietary supplementation of bypass fat leads to higher levels of cholesterol in circulation. Our results are in concomitant with the earlier findings of West and Hill (1990) [15], Khorasani *et al*. (1992) [9], Fahey *et al*. (2002) [2] and Gowda (2014) [4], who found the association between blood cholesterol levels and bypass fat feeding.

Table 1: Serum cholesterol level in early postpartum cows during the study period from day 0 to 90

S. No.	Days	Cholesterol (mg/dl)							
		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	84.967	5.317	118.903	5.388	119.445	5.101	120.360	8.564
2	10	87.100	5.592	123.875	5.187	124.997	5.035	111.248	9.481
3	20	85.808	4.419	134.687	7.156	133.888	5.658	102.778	10.206
4	30	84.730	7.046	141.843	7.374	138.678	5.211	103.480	12.456
5	40	83.925	5.401	152.477	6.717	145.162	6.690	100.615	11.685
6	50	82.565	5.113	151.317	6.896	143.373	5.472	105.895	11.397
7	60	80.352	5.842	151.285	6.633	149.880	7.188	105.752	9.272
8	70	81.153	5.189	154.783	8.652	152.090	7.396	108.355	9.112
9	80	80.833	5.260	155.172	9.311	155.225	8.448	106.592	8.622
10	90	83.605	5.099	155.795	8.778	160.203	11.053	110.865	9.642
Overall		83.50^{a}	5.4278	144.01^{c}	7.21	142.29^{c}	6.725	107.59^{b}	10.04

GI-control cows, GII- Cows supplemented with bypass 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 ($p<0.001$).

Triglycerides

Triglyceride level measured at 10 days interval from 0^{th} day to 90^{th} day is given in Table 2. The levels of serum triglycerides in GII and GIII are significantly lower than that of group GI and GIV ($p<0.01$).

There existed a negative correlation between the milk yield and serum triglyceride concentrations. Highest triglyceride level was found three months after postpartum which may be attributed to the decrease in daily milk yield. During the first two to three months of lactation, cows produce milk at their heaviest which is also associated with an increase in thyroid activities which might be the cause for reduced levels of triglycerides during these months. Moreover, the loss in body condition is also maximum during this stage. During mid-lactation, cows are generally in a positive nutrient balance i.e. nutrient intake exceeds nutrient outgo. As a result, losses of body weight during heavy milking are starting to be regained at this time (Qureshi *et al*. 2016) [12].

The reduced levels of triglyceride through bypass fat feeding, as observed in the present study may be through improving the energy balance of cows in the treatment groups.

Table 2: Serum triglyceride concentrations in experimental animals

S. No.	Day	Triglyceride (mg/dl)							
		Group I		Group II		Group III		Group IV	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1	0	22.58	1.20	22.88	1.18	23.97	1.03	24.35	0.84
2	10	15.68	1.07	18.06	0.48	17.88	1.17	19.47	0.33
3	20	15.76	0.73	13.10	0.64	11.95	0.61	15.26	0.27
4	30	19.52	0.81	13.15	0.28	11.46	0.34	15.16	0.29
5	40	19.42	0.78	12.50	0.37	11.78	0.51	15.18	0.37
6	50	19.58	0.75	11.75	0.45	12.22	0.60	15.46	0.40

7	60	19.85	0.73	12.29	0.40	12.67	0.62	15.41	0.46
8	70	21.23	0.77	13.63	0.19	12.60	0.69	17.18	0.55
9	80	20.66	0.90	13.79	0.21	12.73	0.46	17.33	0.38
10	90	20.54	0.84	13.28	0.24	13.19	0.38	17.61	0.40
Overall		19.49^{b}	0.86	14.44^{a}	0.44	14.05^{a}	0.64	17.24^{b}	0.43

GI-control cows, GII- Cows supplemented with bypass 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 ($p<0.05$).

Progesterone

The serum progesterone levels measured in the experimental animals belonging to different groups are given as Table 3. It was observed that there was no significant difference between groups during the period 0 to the 90th day. But between 30 to 60th day there was a significant difference in GII and GIII compared to group GI and GIV at ($p<0.01$).

Table 3: Serum Progesterone levels in experimental animals

S. No.	Days	Progesterone (ng/ml)							
		Group I		Group II		Group III		Group IV	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1	0	0.66	0.05	0.59	0.10	0.68	0.10	0.55	0.09
2	10	0.47	0.08	0.47	0.05	0.49	0.05	0.49	0.07
3	20	0.95	0.15	0.83	0.13	1.36	0.17	0.81	0.14
4	30	1.33	0.37	2.13	0.38	2.96	0.11	1.25	0.28
5	40	0.75	0.15	2.39	0.72	0.91	0.13	2.20	0.80
6	50	1.74	0.54	1.75	0.57	4.07	0.39	1.77	0.39
7	60	1.18	0.19	2.33	0.58	2.05	0.22	1.38	0.09
8	70	2.20	0.55	4.09	0.60	4.17	0.23	3.70	0.36
9	80	1.06	0.19	3.97	1.15	3.68	0.43	2.34	0.79
10	90	2.09	0.65	4.86	0.68	4.79	0.56	3.47	0.49
Overall		1.24^{a}	0.13	2.34^{b}	0.26	2.52^{b}	0.22	1.80^{a}	0.19

GI-control cows, GII- Cows supplemented with bypass 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 ($p<0.05$).

The corpus luteum is mainly responsible for the synthesis of progesterone which is essential for the implantation of the embryo and responsible for the maintenance of pregnancy. The maternal recognition of pregnancy through interferon-π is mainly dependent on the circulating blood progesterone levels (Mann and Lamming, 2001) [10].

It is reported that severe negative energy balance leads to reduced levels of progesterone during the early postpartum period (Britt, 1992) [1]. Thus, this leads to a delay in follicular development, luteal function and disrupting the cyclic ovarian activities (Godoy *et al*., 1988) [3].

Previously, Hawkins *et al*. (1995) reported that the rise in circulating progesterone levels in cows supplemented with bypass fat is due to reduced clearance from circulation rather than increased production. Similarly, it was observed that the dietary fat profile was highly correlated to plasma progesterone levels. Tyagi *et al*. (2010) [14], and Gowda (2014) [4] had similar observations were an increase in plasma progesterone concentration following bypass fat supplementation same as reported in the present study.

Conclusion

The results of the present study proved that bypass fat feeding is a natural way of improving the energy status of lactating animals. It improvises the blood biochemical parameters and hormonal parameters favorably leading to reduced calving to conception interval and better reproductive efficiency of dairy animals.

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