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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(4): 182-185

www.thepharmajournal.com
Received: 13-02-2019
Accepted: 16-03-2019

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Effect of dietary supplementation of rumen bypass fat on postpartum energy metabolism of crossbred cows

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Abstract
Postpartum infertility due to negative energy balance is a major problem among the high producing cows of Kerala, incurring huge economic loss. The present study was aimed to evaluate the effect of supplementation of bypass fat on glucose and insulin levels of postpartum crossbred cows. 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). The mean blood glucose levels (mg/dl) during the experimental period were 45.78±1.75, 69.82±1.78, 69.45±1.53 and 45.33 ± 0.91 in GI, GII, GIII and GIV respectively. The mean blood glucose levels were significantly (P<0.01) higher in GII and GIII compared to the other two groups. It was observed that the mean serum insulin levels were significantly higher (p<0.05) in GII and III compared to the other two control groups. The mean insulin values were 7.04±0.28, 8.02 ± 0.29, 8.10 ± 0.28 and 7.00 ± 0.35 µ IU/ml respectively in GI, II, III and IV. The results of present study proved that bypass fat feeding is a natural way of improving the energy status of lactating animals, thereby enhancing the reproductive efficiency.

Keywords: Negative energy balance, Ovsynch, insulin, glucose

1. Introduction
The agricultural economy of India considerably depends upon animal husbandry activities, especially dairying, in supporting the livelihood requirement of the majority of rural population. India is the leading milk producing country in the globe and the milk production has recorded impressive growth after independence which has jumped from 17 million tonnes in 1950-51 to 155.5 MT in 2015-16. The annual growth rate of milk production in Kerala was far below than the national average with less than 2.5 percent for the last few years and contributed only 1.7 percent of total milk production of the country (Economic Review, 2016) [4]. Even though the average productivity of the crossbred cows in Kerala is high comparing to national average, the production in the state is lagging behind the actual domestic requirement. To achieve this goal, improving the reproductive efficiency of available female cattle population is of utmost importance.

The reproductive efficiency is influenced by several physiological, pathological, nutritional and environmental factors. Anoestrus and repeat breeding are two of the major reproductive problems affecting 30 to 40 per cent of total cattle and buffalo population of India (Chakurbar *et al.*, 2008) [2]. In Kerala, the incidence was reported to be even higher (61%) and this is mostly attributed to the negative energy balance in high producing cows during the postpartum period (Kutty and Ramachandran, 2003) [7]. The postpartum negative energy balance in dairy cows adversely affects the uterine involution thus leading to delay in resumption of cyclic ovarian activities.

Supplementation of rumen protected or rumen bypass fat in the ration of dairy animals has been reported to have great positive impact on their production and reproduction performances (Naik, 2013) [9]. It is reported that reproductive efficiency of dairy cows had improved by the supplementation of bypass fat, by increasing the conception rate and reducing the days open (Sklan *et al*., 1994, Naik *et al.*, 2009) [16, 10]. Though preliminary studies on the effect of supplementation of bypass fat on production has been carried out earlier, detailed studies on the changes in blood biochemical parameters and hormonal profile of cows supplemented with bypass fat and comparing this to control group and those treated with hormonal induction of oestrus were not carried out earlier. Hence the present study was aimed to evaluate the changes in blood glucose and insulin levels of postpartum crossbred cows supplemented with bypass fat.

2. 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, containing crude fat-84%, calcium-9%, acid insoluble ash-4% and moisture-3%) from 5th day of 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 5th day of calving till 90th day along with compounded cattle feed every morning. In addition, they were subjected to Ovsynch protocol as described earlier (Hagen *et al*., 2015). Briefly, 10 µg of GnRH analogue (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 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 bypass fat. But they were subjected to Ovsynch protocol from 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.

Blood samples were collected from all the animals on day 1 (calving date) and thereafter on every 10 days up to day 90 postpartum. The glucose estimation was done using commercially available kit supplied by Euro Diagnostics Systems Pvt. Ltd, Chennai, India. The assay conditions were; Wavelength - 500 nm, Hg 546 nm, Optical path - 1 cm and Temperature - 37 °C. Adjusted the instrument to zero with 1 ml reagent and 10 µl standards, and the samples 10 µl sample and 1 ml reagent. Mixed and incubated the samples for 10 min. at 37 °C and the absorbance against the blank was read within 30 min. The insulin estimation was done using RayBio® Bovine Insulin ELISA Kit (GA, USA), following the manufacturers guide lines.

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

3. Results and Discussion

3.1 Serum glucose level

Serum glucose level measured at 10 days of interval from 0th day to 90th day is given in Table 1. At calving the level of blood glucose was higher in all the groups which decreased gradually up to day 60 postpartum and there after showed an increase up to day 90 in GI and GIV. In bypass supplemented animals (GII and GIII) the values showed an increasing in trend and it was much higher than in group I and IV by day 90 (Figure 1). Blood glucose levels in GII and GIII were significantly higher (p<0.01) compared to GI and GIV.

Table 1: Postpartum serum glucose level (mg/dl) in experimental animals

Days Postpartum (PP)	G1 (n=6)	GII (n=6)	GIII (n=6)	GIV (n=6)	Overall (n=24)
	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE
0	56.66±2.19	58.57±3.06	56.72±3.32	57.79±3.34	57.43±1.42
10	36.59±2.04	56.88±1.50	56.83±2.26	45.49±2.26	48.95±2.01
20	34.80±1.52	60.90±2.10	61.96±4.73	37.96±1.74	48.90±2.94
30	31.06±1.62	68.58±5.25	65.65±4.07	37.8 ±1.19	50.79±3.81
40	36.52±3.86	66.07±3.78	68.80±3.52	40.06±1.15	52.86±3.42
50	39.90±4.28	64.13±2.32	72.11±4.51	41.35±1.16	54.37±3.32
60	52.23±6.41	68.00±3.03	75.80±3.79	46.06±0.72	60.52±3.11
70	54.19±6.34	76.71±3.33	78.88±3.07	47.85±1.04	64.40±3.37
80	59.32±3.57	93.63±5.15	80.22±3.28	49.99±0.75	70.79±3.94
90	56.49±2.32	84.76±3.13	77.52±3.65	48.90±1.01	66.92±3.32
Overall	45.78±1.75^{a}	69.82±1.78^{b}	69.45±1.53^{b}	45.33±0.91^{a}	57.59±1.09

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 in the same row differ significantly (p<0.001)

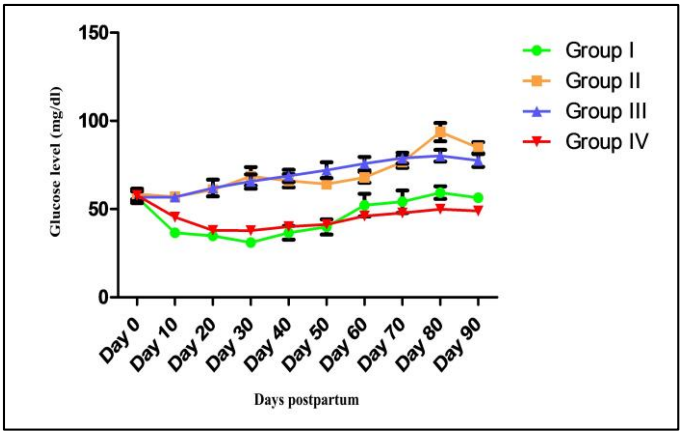

Fig 1: Postpartum changes in serum glucose level of experimental animals

In dairy animals, blood glucose level is an indication of the gluconeogenesis (synthesis from precursors) and glycogenolysis (mobilization of stored glucose). Glucose is the chief source of energy for the primary reproductive organs in the body of cattle (Rabiee *et al*., 1997) [13]. It exerts substantial effect on thecal cell steroidogenesis invitro and responsible for achieving early ovulation in postpartum cows (Stewart *et al*., 1995) [18]. Blood glucose levels modulate the hypothalamic-hypophysial-ovarian axis, there by promoting the synthesis and secretion of gonadotropins and stimulating the growth of graffian follicles (Rutter and Mann, 1987) [14].

It is well documented that a positive correlation exist between the glucose uptake and cholesterol levels, which prove that glucose is essential for cholesterol uptake in to ovarian cells (Rabiee and Lean, 2000) [12]. It is also reported that glucose is essential for the development of embryos post blastulation

(Boland *et al*., 2001) [1]. In dairy animals, under nutrition and reduced blood glucose levels usually leads to delayed onset of postpartum cyclicity (Patil and Deshpande, 1979) [11]. Previously, it was established that during the fertile phase of oestrus, usually the glucose levels were found to be elevated in normally cycling animals than anoestrus or suboestrus animals (Zaman *et al*., 1985) [22]. Similarly, a reduced concentration of blood glucose in non-fertile animals, an indication of subnormal energy status of cows is reported earlier (Sathish and Sharma, 1991) [15]. Guzel and Tanriverdi (2014) [6] observed that serum glucose level (mg/dl) were lower in repeat breeder cows (44.71±5.17) compared to fertile cows (65.00±6.27).

Waghmare *et al*. (2016) [20] reported that the dietary provision of rumen protected fact helps to maintain the serum glucose, triglyceriedes and some other minerals in optimum levels, but in animals those not supplemented with rumen protected fats glucose levels were altered adversely. So it is clear that glucose is an indispensable component for efficient reproductive performance of dairy cattle. In the present study the values of blood glucose in treated groups (GII and III) are in confirmation to the above findings. As the values in GI and GIV were significantly lower.

3.2 Serum Insulin Concentration

The serum insulin levels (µIU/ml) observed in experimental animals during the study period are given in Table 2.

Table 2: Serum insulin profile in crossbred cows from day 0 to 90 postpartum

S. No.	Days Post partum (PP)	Insulin (mean ± SE) µIU/ml							
		Group I		Group II		Group III		Group IV	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1	0	12.13	0.49	13.01	0.84	13.62	0.38	13.71	0.74
2	10	11.04	0.59	12.04	0.72	12.68	0.40	12.54	0.64
3	20	8.69	0.20	9.97	0.31	10.58	0.40	8.70	0.19
4	30	7.29	0.26	8.35	0.11	8.84	0.22	7.17	0.48
5	40	4.50	0.13	5.46	0.19	5.64	0.17	4.19	0.29
6	50	5.03	0.20	6.14	0.18	6.10	0.08	4.59	0.20
7	60	5.32	0.18	6.21	0.13	5.79	0.33	4.71	0.27
8	70	5.63	0.31	6.26	0.10	6.09	0.32	4.96	0.31
9	80	5.42	0.25	6.50	0.14	5.78	0.18	4.76	0.27
10	90	5.34	0.17	6.25	0.13	5.85	0.29	4.67	0.15
Overall		7.0419^{b}	0.2822^{b}	8.0236^{b}	0.2899^{b}	8.101^{b}	0.2808^{b}	7.0048^{a}	0.3598^{a}

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 on day 45 PP. Values bearing different superscripts differ significantly (p<0.05).

Overall levels of insulin showed a significant (P<0.05) difference animals group IV compared to other 3 groups (Fig 2).

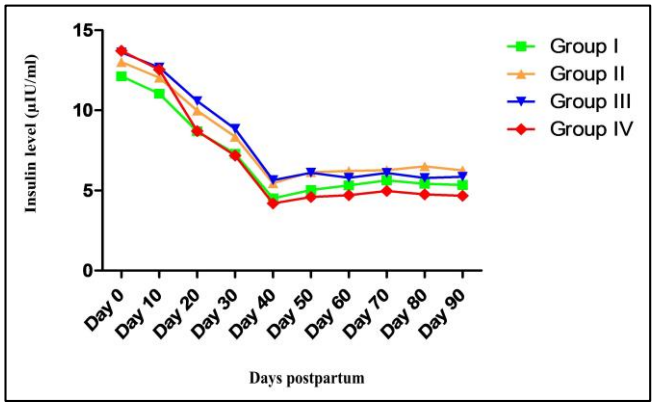

Fig 2: Postpartum changes in serum insulin level of experimental animals

It is reported that normal hypothalamo-pituitary-ovarian feedback mechanism in animals is influenced by insulin, which is responsible for the mediation of normal pituitary activities (Downing and Scaramuzzia, 1997) [3]. Metabolic hormones such as insulin highly influence the cyclic ovarian activities postpartum. Dietary supplementation of certain feed components increased plasma insulin thereby bringing congenial impact on follicular dynamics and early resumption of post-partum cyclic ovarian activities (Gong *et al*. 2002) [5] and increased the proportion of cows ovulating before 50 days postpartum from 55 to 90 per cent. The levels of Insulin exert stimulation of activities of cells in the follicles of ovary. In-vitro studies with addition of insulin to granulosa cells proved that there was significant improvement in the cellular development and synthesis of progesterone compared to that without insulin (Spicer *et al*. 1993) [17].

It was hypothesized that fat supplementation affects the glucose in circulation, thereby increasing the concentration of insulin through feedback mechanism (Tyagi *et al*., 2010) [19]. Earlier reports pointed out better blood insulin levels in cows provided with polyunsaturated fatty acids compared with non-supplemented group (Williams and Stanko, 2000) [21]. It is reported that high levels of insulin decrease hepatic availability of progesterone catabolic enzymes such as P450-2C and P450-3A (Lemley *et al*., 2008) [8] that added to serum progesterone levels. Tyagi *et al*. (2010) [19] observed insulin levels (µIU/ml) of 8.42±1.00 and 8.61± 0.83 in controls and crossbred cows treated with bypass fat respectively.

The present study supports these hypotheses, whereas Garnsworthy *et al*. (2008) and Tyagi *et al*. (2010) [19] couldn't find any rise in circulating insulin concentrations following bypass fat feeding. Hence it is prudent from the results of the present study that postpartum dietary supplementation of bypass fat in crossbred cows improves the energy metabolism considerably, thereby improving the reproductive efficiency.

Conclusion

The results of present study proved that bypass fat feeding is a natural way of improving the energy status of lactating animals. It improves the blood glucose and insulin levels favourably leading to enhanced reproductive efficiency of dairy animals.

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