Effects of bypass fat on postpartum reproductive performance in dairy cattle

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Abstract: This study was conducted to determine the effects of bypass fat on postpartum reproductive performance in dairy cattle. A total of 40 advance pregnant crossbred dairy cows of 2nd to 5th parity were randomly divided into two groups, control (n=10) and treatment (n=30). The control animals were maintained on routine standard feeding schedule. The treatment animals in addition to routine standard feeding schedule, were supplemented orally daily with extra 100 g of bypass fat (Enerfat, Kemin) for about 2 weeks before expected date of calving and continued one week after calving. The level of bypass fat was then increased as per the milk production @ 10 gram per litre of milk produced until 60 days postpartum limiting to maximum of 250 gram/day. Blood samples were collected from each animal at 2 week prior to expected date of calving (-14), day 0, 10, 20 and 30 of calving, for the estimation of various blood biochemical parameters. Gross uterine involution and the interval from calving to first estrus occurrence were recorded. Bypass supplementation non-significantly reduce uterine involution time and time from calving to first postpartum observed estrus. Peripartum bypass fat supplementation significantly ($P<0.05$) increased serum glucose and total cholesterol but significantly ($P<0.05$) decreased serum (non-esterified fatty acids) NEFA level.

Keywords: Bypass fat, Cholesterol, NEFA, Serum glucose, Uterine involution

Introduction

The peak milk production, at about 5-8 weeks postpartum occurs earlier than maximum feed consumption causing cows to be in negative energy balance and to mobilize fat from adipose tissue in early lactation (Bell, 1995; Rukkwamsuk *et al.*, 1998; Miyoshi *et al.*, 2001 and Gutierrez *et al.*, 2009). Negative energy balance may also be the main cause of late uterine involution and cleansing process due to atony or hypotony of uterus (Kaczmarowski *et al.*, 2006). Recent understandings of the role of fats in metabolism open new opportunities for improving production, health, and reproduction in cattle. Inclusion of fats in the diet during peripartum period has improved reproductive performance (Thatcher *et al.*, 2006; De Veth *et al.*, 2009), improved energy balance (von Soosten *et al.*, 2012), reduced the incidence of metabolic diseases, and allowed energy density to be maintained in diets without increasing the use of rapidly fermentable carbohydrates.

Role of the bypass fat in the rations of the high producing dairy animals is very crucial for enhancing the energy density of ration (National Research Council, 2001). Feeding bypass fat to high producing lactating cows can enhance energy density of ration and energy intake in early lactation without compromising rumen cellulolytic bacterial activity (Jenkins and Palmquist, 1984). Thus, the deleterious effect of acute negative energy balance on lactation can be avoided.

Materials and methods

Experimental location and animals

The present investigation was carried out in the department of Veterinary Gynaecology and Obstetrics, CVAS, Bikaner and at a organized dairy farm in Bikaner. A total of 40 advance pregnant Holstein Friesian crossbred dairy cows of 2nd to 5th parity were utilized in the study. The animals selected for the study were diagnosed with advanced pregnancy through breeding records and gynaeco-clinical examinations.

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Management of the animals

All the pregnant cows were maintained in well ventilated hygienic sheds and were fed green fodder, hay and compounded concentrate mixture, as per standard feeding schedule. These animals were maintained under identical nutritional, hygienic and management conditions. Fresh tap water was available adlib to all the cows. All the cows were milked by milking machine twice daily at 5.30 A.M and 5.30 P.M and the amount of milk produced by each cow was recorded. The calves were weaned just after birth.

Experimental design

The experimental cows (n=40) were randomly divided into two groups, control (n=10) and treatment (n=30). The control animals were maintained on routine standard feeding schedule. The treatment animals in addition to routine standard feeding schedule were supplemented orally daily with extra 100 g of bypass fat (Enerfat, Kemin) for about 2 weeks before expected date of calving and continued one week after calving. The level of bypass fat was then increased as per the milk production @ 10 gram per litre of milk produced (Gowda *et al.*, 2013) until 60 days postpartum limiting to the maximum of 250 gram/day.

Evaluation of gross uterine involution

The evaluation of gross uterine involution was carried out daily by per-rectal palpation from day 10 postpartum until complete involution or up to day 45 postpartum whichever was earlier.

The criteria used to determine uterine involution were (1) return of the uterus to normal location in the pelvic cavity, (2) normal and approximately equal size of the uterine horns and (3) normal uterine tone and consistency (Morrow *et al.*, 1966).

Observation of first postpartum estrus

As routine management the postpartum cows were observed twice a day for the occurrence of estrus, based on vaginal discharges and behavioural signs. Further, the occurrence of estrus was confirmed by gynaeco-clinical examination of genitalia and the interval from calving to first estrus occurrence was recorded.

Blood sample collection

Blood samples were collected from each animal by jugular vein puncture in sterile vials. The serum was separated immediately after the collection by centrifugation of blood samples for 10 minutes at 3000 rpm. The serum was stored in sterilized plastic storage vials at -20°C until analyzed for various blood biochemical parameters.

Estimation of blood biochemical parameters

The parameters like blood glucose, total cholesterol, non-esterified fatty acids, calcium and phosphorus were estimated using standard procedure and assay kits procured from Coral Clinical Systems, Goa, with the help of digital spectrophotometer and Serum NEFA concentration was estimated by Elisa kit procured from Shanghai Yehua Biological Technology Co., Ltd.

Statistical analysis

Data were collected, arranged, summarized and then statistically analyzed. The data were analysed using conventional procedures as described previously (Snedecor and Cochran, 1994).

Results and discussion

Uterine involution

The mean duration taken for uterine involution following calving in control group and bypass fat supplemented cows was 23.6 ± 0.40 and 22.4 ± 0.29 days, respectively (Table 1). In the present study, the average number of days required for uterine involution post calving in treatment groups (22.4 ± 0.29) was non-significantly different than control group (23.6 ± 0.40). The findings are in close agreement with report of Khalil *et al.* (2012) and Theodore *et al.* (2016).

First estrous postpartum

The mean duration taken for exhibition of first postpartum estrus by control group and treatment group was 35.2 ± 1.25 and 33.13 ± 0.53 days, respectively (Table 1). The mean number of days taken for exhibition of first postpartum estrus was non-significantly different in control group cows than treatment group cows. The present findings are in agreement with the study of Son *et al.* (2000), McNamara *et al.* (2003), Shelke *et al.* (2012b) and Khalil *et al.* (2012).

Non-esterified fatty acids

The mean serum NEFA ($\mu\text{mol/l}$) level during peripartum periods in the control and treatment group cows are presented in table 2. The overall mean level of NEFA was significantly ($P < 0.05$) lower in treatment group than control group. The mean NEFA level varied significantly ($P < 0.05$) between the groups on the day of calving and day 10 postpartum but on other stages varied non-significantly. In control group, serum NEFA level was significantly higher at calving than peripartum level and then reached significantly lower level on day 10 postpartum and then decreased non-significantly on day 20 and 30 postpartum to reach a nadir of $274.84 \pm 3.62 \mu\text{mol/l}$. In the treatment group the trend was same but serum NEFA level was significantly decrease on day 10 and 20 postpartum and then fluctuated non-significantly to reach a nadir of $239.14 \pm 2.9 \mu\text{mol/l}$.

In the present study, the overall mean level of NEFA was significantly ($P<0.05$) lower in treatment group than control group. The results of the present study are corroborate with reports of Tyagi *et al.* (2009), Singh *et al.* (2014), Rajesh *et al.* (2014) and Yadav *et al.* (2015). In the present study, serum NEFA level was significantly higher on day of calving than prepartum level in both the groups and then subsequently decreased on postpartum intervals and reached at lowest value on day 30 postpartum. The findings were in line with the findings of Drackley (2000), Cerri *et al.* (2009), Galvao *et al.* (2010), Giuliadori *et al.* (2011), Garverick *et al.* (2013), yadav *et al.* (2015) and Theodore *et al.* (2017).

Blood glucose

The mean blood glucose level during peripartum periods in the control and treatment group cows are presented in table 3.

The mean blood glucose value spiked up ($P<0.05$) to reach an apex of 64.80 ± 1.14 mg/dl on the day of calving and reduced back to normal levels on the 10th day postpartum. The values, thereafter, were found to be fluctuating non-significantly between other postpartum intervals. A similar trend was also observed in treatment group, with the highest ($P<0.05$) mean glucose concentration of 67.63 ± 0.56 mg/dl on the day of calving then fluctuating non-significantly day 10 and 20 postpartum but mean serum glucose level on day 30 postpartum significantly differed from prepartum, at calving, day 10 and 20 postpartum level. While comparing the group-wise overall mean blood glucose concentrations of the cows, it was observed that the cows supplemented with bypass fat were having significantly ($P<0.05$) higher glucose value than the control cows (59.37 ± 0.48 vs. 57.07 ± 0.75 mg/dl). There was significantly higher ($P<0.05$) mean serum glucose level in treatment groups then control group at all the stages during both pre and postpartum period studied.

In the present study, the overall group wise mean blood glucose concentration was found significantly higher ($P<0.05$) in treatment group than control group (59.37 ± 0.48 vs. 57.07 ± 0.75 mg/dl). The results are in agreement with the findings of Waghmare *et al.* (2016) and Theodore *et al.* (2017), which can be attributed to the effect of bypass fat supplementation in the diet of treatment group. The other possible reason could be the glycolysis of sugar which might have inhibited by supplementation of bypass fat in order to improve their energy levels (Goff *et al.*, 1996; Mc Namara and Valdez, 2005, Zhang *et al.*, 2011 and Waghmare *et al.*, 2016).

Stage variations in mean glucose level within groups are in partial agreement with Yadav *et al.* (2015) and Theodore *et al.* (2017).

The significantly higher mean blood glucose concentration obtained on the day of parturition in the present study corroborated with the observations of Sato (1978), Ghosh *et al.* (1991), Hadiya (2006), Garverick *et al.* (2013), Yadav *et al.* (2015) and Theodore *et al.* (2017) as they also noted the highest blood glucose concentration at parturition. The researchers suggested that in response to physiological stimuli, glucocorticoid releases at calving and causes rise in blood glucose level due to gluconeogenesis. The fall in glucose levels after parturition could be due to high demands for lactose synthesis for milk production and insufficient gluconeogenesis (Doepel *et al.*, 2002) because energy losses could not be fully compensated by energy intakes (Yadav *et al.*, 2015). The lower fall in glucose after parturition in treatment group than control might be due to bypass fat supplementation. However, some studies reported non-significant effect on blood glucose level on bypass fat supplemented group (Fahey *et al.*, 2002; Singh *et al.*, 2014 and Wadhwa *et al.*, 2012).

Table 1 Mean time duration for 1st postpartum estrus and gross uterine involution

Parameter	Control (n=10)	Treatment (n=30)
Days to gross uterine involution.	23.6 $\pm$ 0.40	22.4 $\pm$ 0.29
1 st estrus observed postpartum (days)	35.2 $\pm$ 1.25	33.13 $\pm$ 0.53

Table 2 Serum NEFA level (μ mol/l) (mean $\pm$ SE) during peripartum period

Days pre-and postpartum	Serum NEFA (μ mol/l)	
	Control (n=10)	Treatment (n=30)
-14	313.30 $\pm$ 6.64 ^b	332.52 $\pm$ 6.26 ^b
0	567.28 $\pm$ 6.09 ^{dA}	498.34 $\pm$ 3.25 ^{dB}
10	372.42 $\pm$ 4.88 ^{aA}	340.75 $\pm$ 5.24 ^{CB}
20	281.36 $\pm$ 4.47 ^a	249.78 $\pm$ 3.14 ^a
30	274.84 $\pm$ 3.62 ^a	239.14 $\pm$ 2.9 ^a
overall	361.84 $\pm$ 15.64 ^A	332.10 $\pm$ 12.14 ^B

Note: Means having different superscripts (capital letter) within a row differ significantly ($P<0.05$).

Means having different superscripts (small letter) within a column differ significantly ($P<0.05$).

The mean blood glucose levels found in both the groups were within the normal limit (42-75 mg/dl) and concurred with the findings of Kumar (2000), Sivaraman *et al.* (2002) and Arieli *et al.* (2008).

The mean blood glucose level in the treated cows was significantly ($P<0.05$) higher (59.37 ± 0.48 mg/dl) than the control (57.07 ± 0.75 mg/dl) group. The present findings are supported by Rahbar *et al.* (2014), who opined that the supplemental fat may

Table 3 Serum blood glucose level (mg/dl) (mean $\pm$ SE) during peripartum period

Days pre-and postpartum	Blood glucose (mg/dl)	
	Control (n=10)	Treatment (n=30)
-14	56.84 $\pm$ 1.27 ^{aA}	52.61 $\pm$ 1.07 ^{aB}
0	64.80 $\pm$ 1.14 ^{cA}	67.63 $\pm$ 0.56 ^{dB}
10	54.19 $\pm$ 1.27 ^{aA}	58.56 $\pm$ 0.42 ^{bB}
20	55.08 $\pm$ 1.03 ^{aA}	58.61 $\pm$ 0.45 ^{bcB}
30	54.47 $\pm$ 1.03 ^{aA}	59.45 $\pm$ 0.42 ^{cB}
overall	57.07 $\pm$ 0.75 ^A	59.37 $\pm$ 0.48 ^B

Note: Means having different superscripts (capital letter) within a row differ significantly ($P<0.05$).

Means having different superscripts (small letter) within a column differ significantly ($P<0.05$).

Table 4 Total serum cholesterol level (mg/dl) (mean $\pm$ SE) during peripartum period

Days pre-and postpartum	Total cholesterol (mg/dl)	
	Control (n=10)	Treatment (n=30)
-14	134.97 $\pm$ 2.93 ^b	135.04 $\pm$ 1.87 ^b
0	119.67 $\pm$ 2.23 ^a	123.43 $\pm$ 1.61 ^a
10	136.67 $\pm$ 1.47 ^{bc}	142.18 $\pm$ 1.44 ^c
20	146.85 $\pm$ 1.58 ^{cdA}	158.98 $\pm$ 1.75 ^{dB}
30	157.52 $\pm$ 2.99 ^{eA}	179.83 $\pm$ 1.98 ^{eB}
overall	139.14 $\pm$ 2.06 ^A	147.89 $\pm$ 1.79 ^B

Note: Means having different superscripts (capital letter) within a row differ significantly ($P<0.05$).

Means having different superscripts (small letter) within a column differ significantly ($P<0.05$).

Table 5 Serum calcium level (mg/dl) (mean $\pm$ SE) during peripartum period

Days pre-and postpartum	Serum Calcium (mg/dl)	
	Control (n=10)	Treatment (n=30)
-14	8.86 $\pm$ 0.27	8.97 $\pm$ 0.20
0	8.62 $\pm$ 0.15	8.73 $\pm$ 0.14
10	8.76 $\pm$ 0.23	8.89 $\pm$ 0.22
20	8.87 $\pm$ 0.29	8.97 $\pm$ 0.17
30	8.94 $\pm$ 0.25	9.09 $\pm$ 0.19
overall	8.81 $\pm$ 0.11	8.93 $\pm$ 0.08

Table 6 Serum phosphorus level (mg/dl) (mean $\pm$ SE) during peripartum period

Days pre-and postpartum	Serum Phosphorus (mg/dl)	
	Control (n=10)	Treatment (n=30)
-14	5.39 $\pm$ 0.18	5.40 $\pm$ 0.11
0	5.21 $\pm$ 0.16	5.15 $\pm$ 0.10
10	5.31 $\pm$ 0.19	5.39 $\pm$ 0.08
20	5.40 $\pm$ 0.21	5.42 $\pm$ 0.09
30	5.46 $\pm$ 0.17	5.50 $\pm$ 0.12
overall	5.35 $\pm$ 0.08	5.37 $\pm$ 0.05

increase glucose production, which in turn may have a positive effect on LH release.

Total cholesterol

The mean serum total cholesterol level during peripartum periods in the control and treatment group cows are presented in table 4.

Mean serum total cholesterol level showed non-significant difference between group prepartum, at calving and on day 10 postpartum. But on day 20 and 30 postpartum, serum total cholesterol was significantly higher in treatment group than control group. While comparing the group wise overall mean serum total cholesterol concentrations. It was observed that the cows supplemented with bypass fat were having significantly ($P<0.05$) higher serum cholesterol value than the control cows (147.89 ± 1.79 vs. 139.14 ± 2.06 mg/dl).

The mean serum total cholesterol values in the cows under both the groups significantly decreased on the day of calving from prepartum level. Thereafter, the values again increased in the subsequent days postpartum to reach the highest value at day 30 postpartum in both groups. Within both groups serum total cholesterol was significantly higher ($P<0.05$) on day 30 postpartum from other all stages studied. In control group serum total cholesterol level was non-significantly higher on day 20 postpartum than day 10 postpartum, but in the treatment group the trend was significant.

In the present study, the overall mean serum total cholesterol value in bypass fat supplemented group (147.89 ± 1.79 mg/dl) was found to be significantly higher ($P<0.05$) than control group (139.14 ± 2.06 mg/dl). The results can be substantiated by the observations of Ranjan *et al.* (2012), Singh *et al.* (2014), Grewal *et al.* (2014), Waghmare *et al.* (2016). Dietary fat increases circulating concentrations of cholesterol (Staples and Thatcher, 2005 and Grummer and Carroll, 1991). However, some other previous researchers reported non-significant higher cholesterol level in fat supplemented group were Tyagi *et al.* (2010), Shelke *et al.* (2012a), Singh *et al.* (2014). While, Yadav *et al.* (2015) and Gnanasekar *et al.* (2016) observed the concentration of plasma total cholesterol was lower in bypass fat treated cows than the control cows.

The mean serum total cholesterol in cows under both the groups were found to be decreased on the day of calving from prepartum level, thereafter, with an increasing trends observed in both the groups corroborated with reports of Prakash and Tandon (1979), Saha *et al.* (1991), Guedon *et al.* (1999) and Yadav *et al.* (2015). Lactation probably also affect the level of plasma total cholesterol, which act as a fatty acid carrier in the form of cholesterol ester for milk synthesis, as a result there is gradual increase in plasma cholesterol level with advancing lactation (Theodore *et al.*, 2015).

Higher cholesterol concentration is associated with better reproductive performance in high yielding dairy cows, as it acts as a precursor of steroid hormones (Son *et al.*, 1996).

Serum calcium

The mean serum calcium level during peripartum periods in the control and treatment group cows are presented in table 5.

In the present study, mean serum calcium level was non-significantly different between the groups and within both the groups at all stages studied are in agreement with the reports of Gowda *et al.* (2014) and Waghmare *et al.* (2016). The present findings indicated that bypass fat supplementation did not have any significant impact on serum calcium level during lactation in cows. The concentration of calcium in the blood was well within the normal physiological limits (8-11 mg/dl).

Serum phosphorus

The mean serum phosphorus levels during peripartum period in the control and treatment group cows are presented in table 6. In the present study, mean serum phosphorus level was non-significantly different between the groups and within both the groups at all stages studied is corroborating with the reports of Gowda *et al.* (2014) and Sahoo *et al.* (2016). The trend of mean plasma level of inorganic phosphorus obtained from prepartum to calving then up to day 30 postpartum period are in partial agreement with the findings of Dhama *et al.*, (2005), Gowda, (2014) and Theodore *et al.*, (2017).

Conclusions

In this study peripartum bypass fat supplementation did not show any beneficial effect on the uterine involution time and the interval from calving to first estrus occurrence. It is concluded that bypass fat improved energy balance that represented through significantly lower serum NEFA and higher glucose level in bypass fat supplemented group.

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