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Metabolic Health and Reproductive Performance of Crossbred Cows Fed Varying Levels of Conjugated Linoleic Acid (CLA) during Transition Period

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ABSTRACT

Conjugated linoleic acid (CLA) has been shown to influence a range of biological processes. The current study was aimed at evaluating the effect of CLA supplementation during the transition period on blood metabolites and reproductive performance in crossbred cows. Multiparous crossbred cows (n = 30) were allotted to three treatment groups: The control group (CG) was supplemented with 50 g of bypass fat top dressed on a basal diet; the CLA-25 group was supplemented with 25 g of CLA and 25 g of bypass fat top dressed on a basal diet; and the CLA-50 group was supplemented with 50 g of CLA top dressed on a basal diet. Supplementation started -21d from calving and continued until 60 days in milk (DIM). Blood samples were drawn from cows by puncturing the jugular vein at different intervals during the feeding trial (-21d prepartum, -10 d prepartum, 10 d postpartum, and 60 d postpartum) and analysed for blood metabolites. Post-calving concentrations of non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHBA), and insulin-like growth factor-I (IGF-I) were also analysed. Reproductive parameters like days to first oestrous and days to pregnancy were also recorded. No adverse effect of CLA supplementation on blood metabolites was observed. A positive effect of CLA supplementation on the levels of IGF-I was observed where CLA-supplemented animals had higher values for post-calving IGF-I concentrations. Moreover, CLA supplementation had a significant effect on reproductive parameters where CLA supplemented cows had lower days to first oestrus and days to pregnancy as compared to cows in the control group.

KEYWORDS: Conjugated linoleic acid, Crossbred cows, Metabolic health, Reproductive performance.

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INTRODUCTION

Over the past three decades, milk production in dairy cows has improved significantly due to genetic improvement. Because the conception rate in dairy heifers has stayed higher and the metabolic demands of higher production may be related to the decline in reproductive performance in cows, increased output has been linked to lower conception rates.Dairy cows are vulnerable during the transition phase, which lasts for three weeks before and after calving (Grummer, 1995). Intake of feed by dairy cows decreases roughly by one-third in the final three weeks before calving, with a notable decline observed in the final week before parturition (Hayirli et al., 2002). This is mostly caused by an increase in circulating oestrogen levels and a decreased ability of the rumen to expand as a result of the larger foetus. High yielding cows are more prone to reproductive problems as the nutrient demands for lactation exceed the nutrient intake during the first phase of lactation, resulting in a negative energy balance (NEB). The associated negative energy balance increases the risk of poor reproductive performance in the postpartum dairy cow, which is associated with a delay in the onset of the oestrous cycle postpartum, low conception rates, and a high rate of early embryonic deaths (Lucy, 2001). Delays in the onset of normal ovarian activity, thus limiting the number of oestrous cycles before breeding, may account for the observed decrease in fertility.

Therefore, feeding during the transition period is crucial to lowering the likelihood of a negative energy balance. Fat supplements have been used to boost the energy density of the diet in an effort to reduce negative energy balance (Staples and Thatcher, 2006). It's interesting to note that feeding lipid supplements rich in polyunsaturated fatty acids to animals boosted their reproductive success in some experiments, but the outcomes varied (Santos et al., 2008). However, it's plausible that some unsaturated fatty acids, if they can get past the rumen's alterations and get absorbed from the small intestine, could enhance reproductive performance by either directly affecting reproductive organs or indirectly doing so through the endocrine system (Staples and Thatcher, 2006). The effects of CLA supplementation on milk production, fertility, and the reduction of negative energy balance in dairy cattle have been studied(Bernal-Santos et al.,2003). Only a few studies on crossbred cows exist, while the majority of CLA research has been conducted on Holstein cows. Therefore, this study was undertaken with the aim of evaluating the impact of CLA on Blood Metabolites and Reproductive Performance in Crossbred Cows during Transition Period.

MATERIALS AND METHODS

Animals, management and treatments

The study was carried out at the Department of Animal Nutrition, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India, in 2019–20. On the basis of their body weight and previous milk yield, thirty healthy crossbred cows (n = 30) with a -30 to -35 day prepartum and 2 to 5 parity were selected and divided into three groups (control, CLA-25, and CLA-50) of ten animals each. All the animals were fed as per NRC (2001) feeding standards. After 10–12 days of adaptation period, feeding trial was started at -21 days prepartum and continued until 60 days postpartum. The same basal diet of corn silage, concentrate, and wheat straw was given to all of the animals. 50 g of bypass fat were added to the basal diet of the control group. 25 g of CLA (Rumen protected CLA, Lutrell Pure, BASF) and 25 g of bypass fat were given to the CLA-25 group. 50 g of rumen protected CLA were supplemented to the CLA-50 group. The chemical composition of the basal diet is given in Table 1. The level of CLA supplementation was decided on the basis of studies of previous trials conducted by several researchers. Each animal was individually tethered with cotton rope and kept in a shed with good ventilation. During an experiment, the animals received the appropriate amount of food and clean water as needed. The animals were kept healthy and disease-free by regular vaccination and deworming as per schedule. The animals and shed were cleaned regularly and thoroughly washed with water to maintain hygiene.

Blood metabolites

Blood samples were collected before feeding and watering of cows at 07:00 h in heparinized vacutainer tubes (BD Franklin, USA) at -21d prepartum, -10 d prepartum, +10 d postpartum, and +60 d postpartum days from calving. Blood samples were drawn from cows by puncturing the jugular vein. The plasma was separated from packed erythrocytes by centrifugation. Centrifugation was done at 3000 rpm for 30 minutes, and plasma samples were stored at -20 °C in different tubes for further analysis of blood metabolites like glucose, cholesterol, triglyceride, total protein, serum glutamate pyruvate transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), creatinine, NEFA, IGF-I, and BHBA.

The various biochemical indices glucose, cholesterol, triglyceride, total protein, SGPT, SGOT, and creatinine in plasma samples were analysed on a semi-automatic biochemical analyzer using Erba Manheim kits (Transasia Bio-Medical Ltd., India) as per the manufacturer's diagnostic protocols. NEFA, BHBA, and IGF-I estimation (sandwich ELISA) was done by using ELISA kits (SinoGeneClon Biotech Co., Ltd.) as per the manufacturer's protocols.

Reproductive parameters

Reproductive parameters like days to first oestrous and days to pregnancy were recorded with the help of expert Veterinarians stationed at the dairy farm.

Statistical analysis

Data was analysed by one-way ANOVA, as described by Snedecor and Cochran (1994), by using SPSS (2012) version 21. The differences in means were tested by Duncan's multiple range test (Duncan, 1955) at 5 % level of significance (P d" 0.05).

RESULTS AND DISCUSSION

Blood metabolites

Concentrations of different blood metabolites are presented in tables 2 and 3. No effect of CLA supplementation on plasma glucose, cholesterol, triglycerides, total protein, SGPT, SGOT, or creatinine was observed. The lack of dietary effect on plasma glucose concentrations is in agreement with previous studies (Bernal-Santos et al., 2003; Moore et al., 2004; Castañeda-Gutiérrez et al., 2005; Kay et al., 2006; Sigl et al., 2010; Hutchinson et al., 2011; Galamb et al., 2017) that investigated the effect of CLA supplementation on plasma glucose concentrations. Medeiros et al.(2010) reported no treatment effects on plasma cholesterol, or glucose with CLA supplementation. Roodbari et al. (2016) reported no effect of CLA supplementation on plasma triglycerides, SGOT, SGPT, or insulin. Contradictory results were reported in a study done by Esposito et al.(2013), where higher plasma concentrations of cholesterol and LDL were observed in CLA-supplemented cows ($P < 0.05$). In the current study, cholesterol levels did not vary between the groups due to the supplementation of bypass fat to achieve equivalent energy diets.

Table 1. Chemical composition of basal diet

Nutrient	Silage	Concentrate	Wheat Straw
CP (%)	8.37	23.5	5.16
Ash (%)	6.95	9.08	9.30
NDF (%)	48.9	22.1	77.2
ADF (%)	25.2	9.15	51.9
ADL (%)	2.38	2.56	6.95
EE (%)	3.11	5.68	1.38
OM (%)	93.0	90.9	90.7
DM (%)	40.1	90.1	89.8

Table 2. Effect of CLA supplementation on blood metabolites

Parameters	Control (50g bypass fat)	CLA-25 (25g CLA +25g bypass fat)	CLA-50 (50g CLA)
	-21d Pre calving		
Glucose (mg/dl)	50.7 ± 2.29	51.4 ± 1.93	54.5 ± 2.18
Cholesterol (mg/dl)	78.0 ± 7.76	76.8 ± 11.04	78.0 ± 9.48
Triglyceride (mg/dl)	19.7 ± 2.69	16.8 ± 2.84	22.0 ± 4.95
Creatinine (mg/dl)	1.31 ± 0.05	1.26 ± 0.06	1.12 ± 0.11
SGOT (iu/l)	57.1 ± 4.58	64.0 ± 4.63	64.1 ± 2.54
Total protein (g/dl)	6.02 ± 0.23	5.78 ± 0.27	6.26 ± 0.30
SGPT (iu/l)	23.7 ± 2.80	22.0 ± 2.05	25.9 ± 2.18
	-10d Pre calving		
Glucose (mg/dl)	54.2 ± 2.49	48.3 ± 2.23	49.0 ± 2.89
Cholesterol (mg/dl)	68.2 ± 11.34	73.0 ± 7.39	67.4 ± 4.68
Triglyceride (mg/dl)	23.7 ± 5.02	21.0 ± 10.03	19.1 ± 3.38
Creatinine (mg/dl)	1.42 ± 0.11	1.29 ± 0.01	1.06 ± 0.16
SGOT (iu/l)	43.0 ± 7.76	52.3 ± 2.71	49.4 ± 1.58
Total protein (g/dl)	5.26 ± 0.53	5.98 ± 0.10	5.41 ± 0.68
SGPT (iu/l)	21.4 ± 3.42	20.9 ± 4.29	19.0 ± 3.93
	10 d Post calving		
Glucose (mg/dl)	51.7 ± 2.18	49.1 ± 4.66	46.8 ± 3.62
Cholesterol (mg/dl)	82.4 ± 13.34	79.2 ± 11.28	85.9 ± 16.79
Triglyceride (mg/dl)	21.5 ± 0.52	22.6 ± 2.35	19.1 ± 3.55
Creatinine (mg/dl)	1.14 ± 0.03	1.21 ± 0.07	1.06 ± 0.10
SGOT (iu/l)	70.2 ± 4.22	72.2 ± 10.70	69.9 ± 3.35
Total protein (g/dl)	5.72 ± 0.16	5.08 ± 0.16	5.70 ± 0.25
SGPT (iu/l)	20.6 ± 2.40	24.4 ± 0.54	18.7 ± 2.78
	60 d Post calving		
Glucose (mg/dl)	47.2 ± 1.07	50.4 ± 1.00	48.7 ± 2.15
Cholesterol (mg/dl)	173 ± 13.13	172 ± 26.81	171 ± 10.41
Triglyceride (mg/dl)	24.0 ± 0.73	22.6 ± 0.92	21.0 ± 0.73
Creatinine (mg/dl)	5.24 ± 0.21	5.75 ± 0.97	4.82 ± 0.48
SGOT (iu/l)	64.5 ± 11.03	62.3 ± 3.91	66.0 ± 1.37
Total protein (g/dl)	6.30 ± 0.12	6.24 ± 0.13	6.37 ± 0.27
SGPT (iu/l)	30.1 ± 4.10	32.6 ± 3.83	31.7 ± 1.45

*The varying superscripts in a row vary significantly (P<0.05)

Table 3. Post calving concentrations of NEFA, BHBA and IGF-I.

Post calving concentration	Control (50g by pass fat)	CLA-25 (25g CLA+25g bypass fat)	CLA-50 (50g CLA)
NEFA (µmol/L)	162 ± 21.90	166 ± 36.87	146 ± 13.74
BHBA (mmol/l)	0.37 ± 0.01	0.35 ± 0.01	0.34 ± 0.01
IGF-I (ng/ml)	39.7 ± 1.03[a]	47.7 ± 3.28[b]	48.0 ± 1.97[b]

*The varying superscripts in a row vary significantly (P<0.05)

Supplementation of CLA during the transition period had a controversial effect on plasma BHBA and NEFA concentrations. Castañeda-Gutiérrez et al. (2005), Odens et al. (2007), and Medeiros et al. (2010) reported lower levels of plasma NEFA concentrations, and Baumgard et al.(2000) reported a slight increase (13%) in plasma NEFA levels during CLA supplementation. But in the current study, no effect of CLA supplementation on NEFA concentration was observed. The lack of a dietary effect (P > 0.05) on plasma concentrations of NEFA was in agreement with the previous studies (Bernal-Santos et al.,2003; Moore et al., 2004; Castañeda-Gutiérrez et al., 2007; Kay et al., 2007; Sigl et al., 2010; Hutchinson et al., 2011; Hotger et al., 2013) that investigated the effect of CLA supplementation on NEFA levels.

Oliveira et al. (2018) reported lower serum BHBA levels in CLA-supplemented cows as compared to control, but in the current study, no effect (P > 0.05) of CLA supplementation on BHBA was observed. The lack of dietary effect (P > 0.05) on plasma concentrations of BHBA is in agreement with the previous studies (Kay et al., 2007; Sigl et al., 2010; Hutchinson et al., 2011; and Hotger et al., 2013) that investigated the effect of CLA supplementation on plasma BHBA concentrations. NEFA and BHBA levels indicate levels of fat mobilisation from stores, so similar NEFA and BHBA levels suggest similar mobilisation as the diets were equivalent in energy levels.

During the first week of postpartum, to cope with increasing demands for nutrients and energy for lactation, mobilisation of fat stores occurs, which results in an increase in NEFA levels. Complete oxidation of NEFA results in energy yield, but incomplete oxidation results in a higher number of BHBA that may lead to metabolic disorders like ketosis if they go past the threshold values reported by Ospina et al. (2010). Ospina et al. (2010) reported that a postpartum serum NEFA concentration e" 0.57 mEq/L was associated with the risk of developing displaced abomasum, metritis, or retained placenta during the first 30 DIM. Ospina et al. (2010) also reported that BHBA concentrations of e"1.0 mmol/L from days 3 to 14 postpartumare associated with an increased risk of clinical ketosis as well as metritis. As the NEFA and BHBA concentrations were lower than the threshold values reported by Ospina et al.(2010), the animals were healthy and had improved negative energy balance. Lower NEFA values indicated lesser mobilisation of fat stores during the critical NEB period. A significant effect (P<0.05) of CLA supplementation was observed, where CLA supplemented cows tend to have higher plasma IGF-I concentrations than animals in the control group. The results of the current study were in agreement with Baumgard et al. (2000), where infusion of a cis-9, trans-11 CLA supplement resulted in an increase (17%) in plasma IGF-I concentrations. Castañeda-Gutiérrez et al. (2007), and Esposito et al. (2013) also reported increased plasma concentrations of IGF-I in CLA supplemented cows. Taylor et al. (2004) reported that multiparous cows having a high level of IGF-I concentration before calving had high conception rates, and multiparous cows with levels of 25 ng/ml of IGF-I during the week of calving were more likely to conceive (11 times more) than cows having less than 25 ng/ml.

Reproductive performance

In the current study, days to first ovulation were reduced in the CLA-supplemented group, as reported by Castañeda-Gutierrez et al. (2005), where the median days to first ovulation were numerically lower for cows supplemented with CLA. The CLA-supplemented cows tended to have lower days to pregnancy as compared to the control group. Castañeda-Gutierrez et al. (2005) reported a trend for the proportion of cows pregnant before 126 and 185 days in milking with the highest value observed for the CLA supplemented cows. Medeiros et al. (2010) and Bernal-Santos et al. (2003) also observed a trend for fewer days to first ovulation and a reduction in the number of days open for cows supplemented with CLA. De Veth et al.(2009) reported lower median days to conception in CLA supplemented cows. The mechanism through which CLA improves reproduction may involve higher concentrations of circulating IGF-I. Insulin-like growth factor-1 acts synergistically with gonadotropins to promote early postpartum ovarian follicular growth and ovulation in dairy cows (Lucy et al. 1992).

CONCLUSION

Lower plasma NEFA and BHBA concentrations indicated lesser mobilisation of fats from stores. Plasma NEFA and BHBA concentrations were lower than threshold values, indicating animals were healthy and in improved negative energy balance. A significant effect ($P < 0.05$) of CLA supplementation on IGF-I was observed, where CLA supplemented cows tend to have higher plasma IGF-I concentrations than animals in the control group, which correlates with higher conception rates. In the current study, days to first ovulation and days to pregnancy were reduced in the CLA supplemented group. From the results, it can be concluded that CLA supplementation positively influenced the metabolic and reproductive health of the cows.

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