



Conjugated Linoleic Acid in the Diet of Lactating Cows

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Effect of Conjugated Linoleic Acid (CLA) Supplementation on Dry Matter intake, Metabolisable Energy intake and changes in Bodyweight of Crossbred Cows during Transition Period

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ABSTRACT

The effect of CLA supplementation on dry matter intake (DMI), metabolisable energy intake (MEIntake) and bodyweight was studied in cross bred cows during the transition period. Thirty multiparous crossbred cows (n=30) were split into three groups and fed one of the following three experimental diets: 50 g of bypass fat was added as a supplement to the T1 group's basal diet, 25 g of CLA and 25 g of bypass fat were added to the T2 group's diet, and 50 g of CLA was added to the T3 group's diet. Beginning 21 days after calving, supplementation continues until 60 days in milk (DIM). The basal diet was same in all the experimental groups. Samples of the feed and residue were examined for chemical makeup. The ME values of the ration were determined using the invitro gas fermentation technique on feed samples. Body weight of cows was recorded at 14-day intervals before the morning feeding and on error-free weighing balance. DMI, ME, and nutritional intake were comparable among groups, indicating that supplementation had no significant impact. Moreover, the loss/changes of body weight in the animals during the transition period was similar between the different experimental groups suggesting the similar efficacy of bypass fat and CLA in countering negative energy balance and improving the animals' body condition scores as the animals continued to gain weight in the postpartum period.

KEYWORDS: Conjugated linoleic acid, Crossbred cows, Dry matter intake, Metabolisable Energy intake, Body weight.

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INTRODUCTION

Livestock is an integral part of India's economy and plays an important role in providing livelihood support to the rural population. Transition cow's nutrition and management has received much attention in the recent years because of its importance in the productivity and health of cows. The occurrence of health problems during the transition period is a major complicating factor for subsequent poor reproductive performance and milk production resulting in economic losses (Remppis et al., 2011). The high-yielding modern dairy cow is a product of many years of genetic selection that continually placed emphasis on milk yield through the utilization of progeny tested bulls, sexed semen

technology improved management technologies and better nutrition. So, these cows have higher metabolic demands for production of milk but there is one-third decrease in feed intake during the last three weeks prior to calving, with significant reduction observed in the final week before parturition (Hayirli et al., 2002). This is mainly due to increase in concentration of circulating estrogen and less capacity for rumen to expand because of increased foetus size. The resultant decreased dry matter intake (DMI) and inability of cow to cope with the increasing energy demands of lactation during the first phase of lactation lead to negative energy balance (NEB) and metabolic disorders like ketosis, retention of placenta occurs due to disturbed energy

balance (Bargo et al., 2002). Such cows need better energy supplements to minimize the deleterious effect of less dietary intake and body weight losses during transition period. This could be achieved to a greater extent by providing high energy diet like rumen protected or inert fat during transition and early lactation.

The use of bypass fat and CLA has been the topic of research to augment milk production for many years (Wadhwa et al., 2012). Bypass fat supplementation increases energy density of the diet which is reflected in improved BCS and productive performance of animals (Ganj Khanlou et al., 2009) and CLA also been used as a tool to reduce negative energy balance and improve milk yield and fertility in dairy cattle (Bernal-Santos et al., 2003). So, in current study we compared bypass fat and CLA at different levels during transition period and their effect on energy balance and body weight losses.

MATERIALS AND METHODS

Animals, management and treatments

After getting approval from the IAEC (Institutional Animal Ethics Committee), the study was conducted at the Department of Animal Nutrition, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India. A 90-day feeding trial was conducted in which thirty healthy cross-bred cows (n = 30) with a -30 to -35 day prepartum and 2 to 5 parity were selected and distributed into three groups (T1, T2, and T3) of ten animals each and fed one of the following three experimental diets: 50 g of bypass fat was added as a supplement to the T1 group's basal diet; 25 g of CLA and 25 g of bypass fat were added to the T2 group's diet; and 50 g of CLA was added to the T3 group's diet. All the animals were fed a basal diet comprising corn silage, concentrate, and wheat straw. The chemical composition of the basal diet is given in Table 1.

Table 1. Chemical composition of basal diet

Nutrient (%)	Silage	Concentrate	Wheat Straw
Crude Protein (CP)	8.37	23.50	5.16
Total Ash	6.95	9.08	9.30
Neutral Detergent Fibre (NDF)	48.9	22.1	77.2
Acid Detergent Fibre (ADF)	25.2	9.15	51.9
Acid Detergent Lignin (ADL)	2.38	2.56	6.95
Ether Extract (EE)	3.11	5.68	1.38
Organic Matter(OM)	93.0	90.9	90.7
Dry Matter(DM)	40.1	90.1	89.8

Rumen-protected CLA (Lutrell Pure, BASF) was supplemented, and it contained both isomers trans-10, cis-12, and cis-9, trans-11, in equal ratios. Bypass property was achieved by hydrogenation of vegetable fats. All the animals were fed according to NRC (2001) feeding standards. First, the animals were tethered individually using cotton rope and housed in a well-ventilated shed. Animals were provided *ad libitum* with clean drinking water and then made

accustomed to the experimental diets for a 10-day adaptation period. After the adaptation period, the feeding trial was started from 21 days prepartum to 60 days postpartum. All the standard management practises were followed, and regular vaccinations and deworming were done as per schedule. Good hygiene was maintained by regular and thorough cleaning of the animals and the shed.

***In vitro* gas production and ME intake**

Formulated diets were subjected to the *in vitro* gas production technique (Menke and Steingass, 1988). Ruminal fluid collected from three rumen-cannulated Murrah buffalo bulls weighing around 558 ± 6.08kg served as inoculum for the feed evaluation. The ruminal fluid was collected pre-prandially in a pre-warmed double-walled (thermo) flask (flushing with carbon dioxide gas) and blended for 3 minutes in a blender at 39 °C. The ruminal fluid, after straining through a two-layered muslin cloth, was mixed in a ratio of 1:2 with buffer solution (Menke and Steingass, 1988) to prepare the incubation medium under continuous flow of carbon dioxide at 39 °C. The TMR of about 375 mg was weighed in syringes and incubated in a water bath maintained at 39 °C after dispensing 30 mL of incubation medium for 24 h (swirled hourly). Three syringes were prepared for each of the treatments and blanks (without any substrate). The incubation was ended after a stipulated period, and the gas volume in syringes was noted, and net gas production (NGP) was calculated after blank correction. The ME value of the sole ingredient and TMR were calculated by using the following equation developed by Menke et al. (1979).

$$\text{ME (MJ/kg DM)} = 1.24 + 0.146 \text{ G} + 0.007 \text{ CP} + 0.0244 \text{ EE}$$

Where,

ME = Metabolizable energy.

G = Net gas production (ml/200 mg DM)

CP = Crude protein of sample, g/kg DM.

EE = Ether extract of sample, g/kg DM.

Feed intake, nutrient intake and body weight

The daily amount of feed required was weighed and fed to the animal twice a day. The residue (orts) left behind after feeding was weighed and recorded daily. Feed and residue samples were analysed for dry matter (DM) to calculate the amount of DMI of the animal. Feed and ort samples were subjected to proximate analysis to determine the composition and calculate the nutrient intake. The body weight of

cows was recorded for three consecutive days at 14-day intervals. Animals were weighed in the morning before feeding on an error-free weighing balance.

Digestion trial

A 5-day metabolic trial on 15 animals (5 cows per treatment) was performed during the last week of the feeding trial. The weighed quantity of TMR was offered to the animal, and the leftover was noted to calculate the dry matter intake. After an adaptation period of 2 days, samples of faeces were collected. A suitable aliquot of total faeces was sampled in containers containing 20% sulphuric acid solution (25 mL) for analysis of crude protein (CP) content. Also, a suitable amount of faeces was dried at 60 °C for 48 h in a hot air oven, and then pooled together for each animal separately. The dried faeces samples were stored after grinding for nutrient analysis to determine apparent nutrient digestibility.

Lignin was used as an internal marker for calculating digestibility during the digestion trial. The digestibility of nutrients was calculated by using the following formula:

$$\text{Digestibility (\%)} = 100 - 100 \left\{ \frac{\% \text{ marker in diet}}{\% \text{ marker in faeces}} \right\}$$

$$\text{Nutrient Digestibility (\%)} = 100 - 100 \left\{ \left(\frac{\% \text{ marker in diet}}{\% \text{ marker in faeces}} \right) \times \left(\frac{\% \text{ nutrient in faeces}}{\% \text{ nutrient in feed}} \right) \right\}$$

Chemical composition of TMR, orts and excreta

The TMR, orts, and faeces samples were analysed for DM and total ash following AOAC International (2007) methods. The nitrogen content in fresh faeces was determined by the Kjeldahl method. Total ash content was subtracted from DM content to obtain organic matter (OM) content. The forage detergent system was used to assay fibre fractions (Van Soest et al., 1991). The components obtained after subtracting acid detergent fibre (ADF) content from neutral detergent fibre (NDF) content and acid detergent lignin (ADL) content from ADF represented hemicellulose and cellulose concentrations, respectively.

Statistical analysis

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

RESULTS AND DISCUSSION

DMI and ME intake

The transition period is known to be a critical

period in a cow’s life, where cows experience a one-third decrease in feed intake during the last three weeks prior to calving, with a significant reduction observed in the final week before parturition (Hayirli et al., 2002). This is due to less space for the rumen to expand because of the increased foetus size. This was evident in our current study, where cows had decreased DMI (Table 2) in the prepartum period around 11.70–12.24 kg/d, and after calving, cows tend to have higher DMI and keep on increasing to meet the metabolic demands of milk production.

Table 2. Effect of CLA supplementation on dry matter intake (DMI) (kg/d)

Days from calving	T1 (50g FAT)	T2 (25g FAT +25g CLA)	T3 (50g CLA)
-21	11.7 ± 0.20	11.7± 0.21	11.7 ± 0.14
-10	11.2 ± 0.10 ^a	11.5 ± 0.06 ^{ab}	11.4 ± 0.06 ^{ab}
+10	12.6 ± 0.44 ^c	12.4 ± 0.42 ^{bc}	12.0 ± 0.41 ^a
+30	16.2 ± 0.20	15.9 ± 0.23	16.0 ± 0.32
+45	15.7 ± 0.12 ^{ab}	15.6 ± 0.18 ^a	15.6 ± 0.10 ^a
+60	16.7 ± 0.03	16.6 ± 0.20	16.7 ± 0.06
Overall DMI	13.2 ± 0.33	13.2 ± 0.31	13.1 ± 0.33

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

In the postpartum period, DMI was recorded at 12.05–12.61 kg/d at +10 days, 15.94–16.24 kg/d at +30 days, 15.68–15.73 kg/d at +45 days, and 16.62–16.78 kg/d at +60 days in milk. As the DMI increased, so did the ME intake, but over the period of the feeding trial, ME intake (Table 3) and DMI were similar among the different experimental groups. Animals in all the groups followed a similar trend where DMI decreased during the prepartum period and increased after calving to meet the metabolic

demands of milk production. The overall variation of DMI between different experimental groups was non-significant. So, bypass fat and CLA did not significantly affect the DMI of the crossbred cows. The results reported here are in agreement with previous studies (Perfield II et al., 2002; Bernal-Santos et al., 2003; Kay et al., 2006; Castañeda-Gutiérrez et al., 2007; Odens et al., 2007; Medeiros et al., 2010).

Nutrient intake and body weight

nutrient intake or body weight (Table 3).

CLA supplementation also had no influence on

Table 3. Effect of CLA supplementation on nutrient intake, ME intake and BW

Parameters	T1 (50g FAT)	T2 (25g FAT +25g CLA)	T3 (50g CLA)
ME Intake (MJ/d)	129 ± 3.42	128 ± 3.32	129 ± 3.42
BW (kg)	526 ± 7.09	538 ± 13.79	531 ± 9.41
Nutrient Intake			
CPI (kg/d)	1.69 ± 0.07	1.67 ± 0.07	1.67 ± 0.07
OMI (kg/d)	12.2 ± 0.29	12.2 ± 0.29	12.0 ± 0.29
NDFI (kg/d)	5.81 ± 0.14	5.79 ± 0.13	5.76 ± 0.14
ADFI (kg/d)	3.10 ± 0.08	3.07 ± 0.07	3.06 ± 0.08

Nutrient intake was similar in all the groups during prepartum but increased in postpartum to cope with the increasing nutritional demands of lactation. A decline in body weight was observed on the day of calving due to parturition, but after the calving period, animals continued to gain weight throughout the period of the study. Changes in body weight during the transition period are depicted in figure 1. The lack of effect of CLA supplementation on body weight was in agreement with previous studies on CLA supplementation (Perfield II et al., 2002; Kay et al., 2006; Castañeda-Gutiérrez et al., 2007; Medeiros et al., 2010). The loss of body weight was similar in all the groups. During the period of negative energy balance (NEB), when dietary nutrient supply

is deficient for the higher metabolic demands of lactation, animals depend on their body fat stores. To counter NEB, body fat stores are mobilised, and NEFA are oxidised to yield ATP. So similar levels of NEFA (162.61 ± 21.90 , 166.25 ± 36.87 , and 146.20 ± 13.74 $\mu\text{mol/L}$ for T1, T2, and T3, respectively, Sidhu, 2020) denote that both by-pass fat and CLA had similar efficacy in improving the negative energy balance.

Digestibility of nutrients

Average dry matter digestibility (%) was 74.05 ± 1.34 , 72.42 ± 0.75 and 73.17 ± 0.55 in groups T1, T2 and T3, respectively, which was comparable ($P > 0.05$) among the groups (Table 4).

Table 4. Effect of CLA supplementation on nutrient digestibility (%)

Digestibility (%)	T1 (50g FAT)	T2 (25g FAT +25g CLA)	T3 (50g CLA)
NDF	58.5 ± 1.60	53.5 ± 2.29	57.0 ± 1.90
ADF	51.6 ± 1.58	46.7 ± 3.32	48.6 ± 1.89
N	77.3 ± 1.26	79.1 ± 0.93	76.8 ± 2.13
EE	86.0 ± 0.35	87.8 ± 0.43	87.3 ± 1.30
DM	74.0 ± 1.34	72.4 ± 0.75	73.1 ± 0.55
OM	76.6 ± 0.88	77.3 ± 0.88	76.6 ± 1.3

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

Likewise, digestibility obtained for other nutrients like OM, N, EE, NDF and ADF were also found to be similar ($P > 0.05$) among the groups. Supplementation was done in such a way that it didn't interfere with rumen fermentation (bypass nutrients), so bypass fat and CLA did not have any significant effect on nutrient digestibility.

CONCLUSION

CLA and fat supplementation did not affect the DMI, nutrient intake, or ME intake significantly. Nutrient digestibility was also similar among the groups, as both bypass fat and CLA were fed as bypass nutrients, so they didn't interfere with the rumen fermentation processes. Moreover, the losses and changes in body weight were similar, indicating the similar efficacy of bypass fat and CLA in countering negative energy balance. So, both bypass fat and CLA can be fed to the animal to counter negative energy balance and increase the energy density of the ration. The author recommended bypass fat and CLA be fed in combination.

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