RESEARCH ARTICLE

Effect of Calcium Propionate Supplementation on Ruman Fermentation and Blood Biochemical Profile in Dairy Cattle

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Abstract

This study was aimed to explore the effects of supplementation of different rates of calcium propionate on nutrient utilization, rumen fermentation pattern and blood biochemical profile of mid lactating dairy cattle. The dairy cows were fed with a common diet of *ad libitum* APBN-1 green fodder, concentrate pellet feed and paddy straw. Twenty-four lactating dairy cows (4th to 6th month of lactation) were randomly divided in to four treatment groups each of six cows. The groups were designated as T1 (control) common diet without calcium propionate supplementation, whereas the treatment groups T2, T3 and T4 were supplemented with calcium propionate at the rate of 60 g, 70 g and 80 g/animal/day in addition to the common diet. Feeding trial was carried out for a period of 40 days. The supplementation of calcium propionate to the dairy cattle had no major changes in the rumen pH within the treatment. In the present study, there was no significant difference in mean pH values among the dietary treatments, time/hours after feeding and diet x hour interaction. The mean TVFA (mEq/L) was lowest at 0 h and highest ($p<0.05$) at 3 h of SRL collection, and then showed a decreasing trend ($p<0.05$) from 6 h to 12-h post-feeding in all groups. The mean NH_3-N (mg/100 mL SRL) was higher ($p<0.05$) at 3 h and thereafter showed a reducing trend from 6 to 12 h intervals. The serum glucose and calcium values were found to be significantly ($p<0.05$) higher among treatment groups when compared with control group, whereas the BUN levels did not vary much. It was concluded that supplementation of calcium propionate to dairy cows showed a significant increase in the ruminal TVFA, NH3-N, and total nitrogen as well as serum glucose and calcium concentrations by 3rd hour of feeding and then either persists at almost same level till 6th hour or gradually declined till 12 h of sampling.

Key words: BUN, Calcium, Calcium propionate, NH_3-N, Rumen pH, Serum glucose, TVFA.

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Introduction

In India more than 65 % of the human population depends on agriculture and its allied sectors like dairy. Dairying is the most common livestock livelihood for the rural masses as it has become a supporting income. During transition period, cows lose weight due to lowered dry matter intake, and especially in early lactation dairy cows go through a period of negative energy balance directly affecting the production parameters for want of enough feed supply and altered metabolism. In this period, dairy cows are more susceptible to metabolic disorders like ketosis, milk fever (calcium tetany), which are reported as a risk factors for limiting milk production and are associated with the increasing incidence of dystocia, uterine prolapse, retained placenta, metritis, displaced abomasum etc (Serrenho *et al.,* 2021). During negative energy balance there is lipid mobilisation from the adipose tissue leading to release of non-esterified fatty acids (NEFA) which in turn produce acetyl-coA converting in to ketone bodies without entering TCA cycle (Ceciliani *et al.,* 2018), that may cause fatty live syndrome. It is hypothesised that adequate amounts of propionate in the diet could contribute to the synthesis of sufficient quantities of oxalo-acetate which prevents fat accumulation in the liver (Kennedy *et al*., 2020).

Calcium propionate can be hydrolysed into Ca^{2+} and propionic acid in rumen and it has been used for preventing or treating hypocalcemia and ketosis in early lactating dairy cows (Zhang *et al.,* 2020). Studies on dairy cows in early lactation indicate that calcium propionate was beneficial in alleviating hypocalcemia, improved energy status (Liu *et al.,* 2010) and increased milk yield (Martins *et al.,* 2019). Calcium propionate supplementation improved the rumen fermentation and the ruminal bacterial diversity but it had little impact on the major ruminal bacterial

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community composition of dairy cows in early lactation (Zhang *et al.,* 2020). It is to be noted that many factors like cud chewing, rumination, clean and fresh water for moist rumen environment, effective fibre for more salivation affect the rumen health. Therefore, this study was carried out to explore the effects of supplementation of different levels of calcium propionate on rumen fermentation pattern and blood biochemical profile in the mid lactating dairy cattle.

Materials and Methods

All the experimental procedures were approved by the Institutional Animal Ethics Committee at the College of Veterinary Science, Tirupati (Andhra Pradesh, India). A total of 24 healthy mid-lactating crossbred dairy cows (HF × Jersey, Jersey × Sahiwal) were randomly assigned into four groups with six cows in each group having isometric average body weight (399 to 405 kg) and daily milk yield (4.33 to 4.72 kg). The diet of experimental animals included *ad libitum* APBN-1 green fodder, concentrate pellet feed and paddy straw. The diets were supplemented with/without Calcium propionate (The Nipro Technologies Limited, Haryana). Group T1 was fed common diet without supplement and served as Control, while groups T2, T3 and T4 were supplemented with 60, 70 and 80 g calcium propionate/animal/day, respectively. The cows were adapted for stall feeding conditions, dewormed and vaccinated against Foot and Mouth, and LSD before the commencement of the study. The calcium propionate was mixed with concentrate feed an hour before feeding for the supplemented groups in solubilized form for effective mixing, and was fed once in a day (afternoon) throughout the forty days trial period.

Rumen liquor was collected from each animal for 3 consecutive days after the 7 days collection period of digestibility trial with help of suction pump at 0, 3, 6, 9 and 12 h post-feeding of calcium propionate mixed concentrate feed. The samples were strained with four layers of muslin cloth and the strained rumen liquor (SRL) was used to estimate ammonia nitrogen content by Micro-Kjeldahl method. Total nitrogen (mg/100 mL SRL) was estimated by Kjeltech digestion system and total volatile fatty acids (TVFA) were titrated against standard alkali in the presence of indicator (Barnett and Reid, 1956). Rumen pH was determined immediately by using digital pH meter (Thermo Orion, Model 420A+, USA).

Blood samples were collected at the final stages of experimental period for estimation of serum metabolites. Serum glucose was estimated by glucose oxidase method, and serum urea by urease/UV method using a commercial kits (Biosystems, Spain) on automated A15 biochemical analyzer (Biosystems, Spain). Serum calcium was estimated using Arsenazo-III method (Biosystems, Spain).

The data were analysed in completely randomized design (CRD) using software version 23.0; SPSS, 2015) through generalized linear model and the treatment means were ranked using Duncan's multiple range test with significance at $p<0.05$ according to Snedecor and Cochran (1980).

Results and Discussion

Rumen Fermentation Pattern

The mean ± SE values of pH, TVFA, ammonia nitrogen and total nitrogen obtained at different time intervals in strained rumen liquor (SRL) of dairy cattle supplemented with or without calcium propionate are presented in Table 1.

Rumen pH: Ruminal pH is an important indicator of proper rumen fermentation and health (Zhang *et al.,* 2018). In the present study, there was no significant difference in mean pH values among the dietary treatments, time/hours after feeding and diet x hour interaction in spite of increased level of TVFA. Lee-Rangel *et al.* (2012) and Zhang *et al*. (2020) also reported similar results that rumen pH was not affected by calcium propionate supplementation probably for weakly alkaline property of calcium propionate in aqueous solution.

Total Volatile Fatty Acids: The mean TVFA concentrations of SRL in lactating dairy cattle were found to be significantly ($p<0.05$) higher and gradually increased with increase in the level of calcium propionate in the diet at all intervals over non-supplemented control group T1, and it also increased linearly up to 3 h post-feeding beyond which declining trend was observed till 12th hour in all treatments (Table 1). The higher TVFA concentration in the calcium propionate supplemented groups is indicative of superior microbial fermentation and digestion of the dietary components in the rumen. The higher TVFA concentration could be attributed to the improved ruminal bacterial community composition in the cows fed calcium propionate diets (Yao *et al.,* 2017).

Ammonia Nitrogen and Total Nitrogen: The mean ammonia nitrogen concentration of lactating dairy cattle fed with three levels of calcium propionate in the diet did not differ significantly, and all the three treatments had significantly ($p<0.05$) higher NH_3-N concentration at 0, 3 and 6 h post-feeding compared to control (T_1) and thereafter it did not differ between treatments. The higher levels of TVFA, NH_3-N and total nitrogen at 0 h in all treatment groups over control could be the latent effect of daily calcium propionate supplementation during and before digestion trial, and that perhaps persisted along with fresh daily supplements at 3, 6 and/or 12 h in these groups. Calcium propionate, being a source of readily fermentable energy, promotes microbial growth and activity in the rumen. This increased microbial activity leads to enhanced nitrogen metabolism, resulting in the release of ammonia nitrogen (Zhang *et al.,* 2022). The ammonia nitrogen concentration was lowest before feeding, which increased at 3 h, persisted at higher level till 9th h, and then declined in most treatment groups. The total nitrogen concentrations followed the same trend as of NH_3-N in the present study (Table 1).

NH_3-N concentration is the balance between dietary protein degradation and microbial utilization. Since the protein digestibility was increased and this could be a

possible reason for increased NH_3-N concentration in the rumen. The results agree with Jiao *et al*. (2021) and Liu *et al*. (2009) who found that the ruminal CP degradability was linearly and quadratically increased with greater calcium propionate supplementation in finishing steers. Unnawong *et al*. (2021) also reported that increased CP degradation resulted in a higher ruminal NH_3-N concentration. The increased total nitrogen levels in the rumen are primarily a result of the interplay between degradable nitrogen content and the presence of nitrogen-utilizing bacteria (Reddy *et al.,* 2019). In this context, the dynamics of the N-utilizing bacteria are further shaped by alterations in the diet, specifically through the supplementation of calcium propionate (Cao *et al.,* 2020).

Serum Metabolites

Serum glucose levels were significantly ($p<0.05$) higher in all the treatment groups compared to control. Serum glucose levels were increased linearly as the level of calcium propionate increased in the ration. However, the serum urea nitrogen (SUN) levels among the treatments did not differ significantly, though numerically higher values were observed in calcium propionate supplemented groups. Serum calcium level among the treatment groups ranged from 7.36 to 9.06 and was significantly ($P<0.05$) higher in all the calcium propionate supplemented groups compared to control.

As the level of calcium propionate increased serum metabolites like glucose, urea and serum calcium were found to be higher. Increased serum glucose concentration in the present study agreed with the results reported by Abdel *et al.* (2016) in primiparous Egyptian cows. Liu *et al.* (2010) also reported that supplementation of calcium propionate improved the energy status which is indicated by the higher blood glucose. The present findings showed that the serum calcium levels were increased for calcium propionate supplemented groups and as the dose increased the serum calcium levels also tended to increase during early lactation. Peralta *et al*. (2011) reported that drenching of calcium propionate had no significant effect on milk production and milk components, but increased plasma calcium concentration. However, factors like hypophagic effect due to excess dietary calcium propionate supplementation in the

Table 1: Effect of calcium propionate supplementation on rumen parameters in lactating dairy cattle (Mean ± SE)

Rumen parameters	0 Hour	3rd Hour	6th Hour	9th Hour	12th Hour
pH					
T1	6.19 ± 0.022	6.18 ± 0.028	6.23 ± 0.014	6.18 ± 0.009	6.20 ± 0.022
T2	6.21 ± 0.059	6.12 ±0.063	6.16 ± 0.015	6.21 ± 0.009	6.18 ± 0.023
T3	6.18 ± 0.043	6.15 ± 0.030	6.19 ± 0.024	6.22 ± 0.012	6.14 ± 0.047
T4	6.25 ± 0.079	6.16 ± 0.060	6.22 ± 0.022	6.20 ± 0.015	6.19 ± 0.019
TVFA					
T1	56.45^{Aa} ± 0.14	100.12^{Ae}± 0.25	83.42^{Ad}±0.14	81.20^{Ac}±0.23	74.30^{Ab}±0.15
T2	67.65^{Ba} ±0.26	103.14^{Be}±0.35	91.42^{Bd}± 0.37	86.42^{Bc}±0.33	78.87^{Cb}±0.46
T3	69.24^{Ca}±0.27	101.67^{Be}±0.46	93.84^{Cd}±0.58	88.72^{Cc}±0.55	76.87^{Bb}±0.50
T4	73.10^{Da} ± 0.29	103.81^{Be}±0.41	95.42^{Dd}±0.25	92.42^{Dc}±0.30	79.62^{Cb}±0.40
Ammonia Nitrogen					
T1	8.24^{Aa}±0.04	12.86^{Ac} ± 0.50	11.26^{Ab}±0.37	13.96^{c}±0.47	10.94^{b}±0.33
T2	10.74^{Ba} ±0.27	14.79^{Bc} ±0.48	13.86^{Bbc} ± 0.34	13.46^{bc} ±0.35	12.46^{b}±0.48
T3	11.21^{Ba}±0.33	13.62^{ABbc}±0.33	14.32^{Bc} ±0.29	14.08^{c} ± 0.42	12.42^{ab}±0.48
T4	11.06^{Ba}±0.24	14.64^{Bb}±0.44	13.14^{Bb}±0.66	14.14^{b}± 0.45	11.24^{a}±0.36
Total Nitrogen					
T1	63.85^{Aa}±0.58	90.42^{Ad}±0.22	87.64^{Ac}±0.46	76.14^{Ab}±0.37	62.3^{Aa}±0.54
T2	65.98^{Ba}±0.32	93.64^{Bd}±0.60	91.32^{Bc}±0.33	76.25^{Ab}±0.47	64.97^{Ba}±0.52
T3	66.12^{Ba}±0.52	94.08^{Bc}±0.54	93.13^{BCc}±0.63	77.84^{ABb}±0.43	64.64^{Ba}±0.43
T4	66.37^{Bb}±0.56	94.98^{Bd}±0.70	94.02^{Cd}±0.57	78.02^{Bc}±0.45	62.2^{Aa}±0.46

Mean values bearing different superscripts within the row (a,b,c,d,e) and column (A,B,C,D) differ significantly ($P<0.05$) at different time intervals, and between the treatments at particular time intervals.

Table 2: Effect of calcium propionate supplementation on serum glucose, urea and calcium levels in the lactating dairy cows

Parameter	T_1	T_2	T_3	T_4
Glucose* (mg/dL)	48.16^{a} ± 0.41	54.63^{b} ± 0.43	55.12^{b} ± 0.48	56.42^{c} ± 1.05
BUN (mg/dL)	13.14 ± 0.40	13. 22 ± 0.33	13.64 ± 0.33	14.38 ± 0.36
Calcium* (mg/dL)	7.36^{a} ± 0.23	8.72^{b} ± 0.39	8.93^{b} ± 0.34	9.06^{b} ± 0.26

Mean values bearing different superscripts (a,b,c) within the row differ significantly *($p<0.05$).

early lactation shows negative impact on the milk yield (NRC, 2001; Stocks and Allen, 2013). Contrary to our present results, Goff (2008) and Serrenho *et al*. (2021) did not report a positive correlation between calcium propionate supplementation and serum calcium levels.

Conclusion

The finding of the present study revealed that, the addition of calcium propionate in the dairy cattle diet exhibited a beneficial influence on serum glucose and calcium levels, while BUN concentration remained unaffected. Furthermore, it resulted in improvements in ruminal total volatile fatty acids, ammonia nitrogen, and total nitrogen concentrations. These collective findings indicate that the supplementation of calcium propionate can enhance rumen function, accompanied by favourable changes in serum parameters.

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