



ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(3): 427-431
© 2022 TPI
www.thepharmajournal.com
Received: 04-01-2022
Accepted: 06-02-2022

B Chandrika
Department of Livestock Farm
Complex, College of Veterinary
Science, Sri Venkateswara
Veterinary University,
Tirupati, Andhra Pradesh, India

MVAN Suryanarayana
Department of Livestock Farm
Complex, College of Veterinary
Science, Sri Venkateswara
Veterinary University,
Tirupati, Andhra Pradesh, India

B Devasena
Department of Livestock Farm
Complex, College of Veterinary
Science, Sri Venkateswara
Veterinary University,
Tirupati, Andhra Pradesh, India

Y Ravindra Reddy
Department of Livestock Farm
Complex, College of Veterinary
Science, Sri Venkateswara
Veterinary University,
Tirupati, Andhra Pradesh, India

Corresponding Author
B Chandrika
Department of Livestock Farm
Complex, College of Veterinary
Science, Sri Venkateswara
Veterinary University,
Tirupati, Andhra Pradesh, India

Effect of citric acid supplementation on rumen fermentation pattern, milk composition and blood biochemical profile in cross bred cattle

B Chandrika, MVAN Suryanarayana, B Devasena and Y Ravindra Reddy

Abstract

In a Completely Randomized Design Sixteen lactating cross bred cows randomly assigned to four treatments with each group containing four animals were evaluated to assess the effect of supplementation of Citric acid at different levels with concentrate pellet feed, green fodder and paddy straw on rumen fermentation pattern, milk composition and blood biochemical parameters. The dietary treatments contained ad libitum APBN-1 green fodder, concentrated pellets and paddy straw at 2.5% of body weight (basal diet). The treatments were the basal diet as control (T₁), T₁ + 30g Citric acid/animal/day as T₂, T₁ + 60g of Citric acid/animal/day (T₃) and T₁ + 120g Citric acid/animal/day as T₄. Supplementation showed a significant difference ($P < 0.05$) among rumen pH values collected at the 0, 3, 6, 9, 12 hrs. The trend was same for TVFA, NH₃-N, and N fractions which reached peak at 6 h post feeding and later followed a decreasing trend in all the treatments. The mean TVFA (mEq/L) was highest ($P < 0.05$) 6 hourly interval of SRL collection and lowest at 0 hr from 6 hourly interval. Higher milk production yield was recorded for T₄ and at 6th week of supplementation. Milk composition was significant during 4th and 5th week. A significant ($P < 0.05$) difference among the groups pertaining to serum glucose (mg/dl), BUN and triglycerides (mg/dl) and serum macro minerals was noticed. It was concluded that the citric acid supplementation can manipulate the rumen fermentation pattern with an increased volatile fatty acid production. Supplementation influenced milk production with a corresponding change in the milk composition. Citric acid also affected the serum biochemical parameters with a significant change in Calcium and Phosphorus concentrations at higher dose of supplementation. Supplementation at 120 g per day per animal could be beneficial in altering the efficient rumen fermentation and milk production.

Keywords: Citric acid, supplementation, rumen fermentation, milk yield, blood biochemical profile

Introduction

Average feed cost represents 40 to 60% of the total cost of production in dairy farms hence reducing the feed costs has become a major concern for animal nutritionists (Bozick *et al.*, 2012) [3]. Among the feed additives organic acids showed improvement in microbial growth rate, feed efficiency and milk production. The supplementation of organic acids improves the bioavailability of protein and uptake of minerals like magnesium, calcium, phosphorus and zinc (Hinton *et al.*, 1988; Thompson and Hinton, 1997). Organic acids may be a beneficial feed additive for ruminants (Martin, 1998; Castillo *et al.*, 2004) because they effect on rumen fermentation (↑ Propionate, ↓ CH₄, ↓ Lactate (Castillo *et al.*, 2004). They stabilize the rumen pH and increases the propionate which is essential for the promoting energy availability for milk production and increasing glucose and lactose synthesis.

Materials and Methods

In a Completely Randomized Design Sixteen lactating cross bred cows randomly assigned to four treatments with each group containing four animals were evaluated to assess the effect of supplementation of Citric acid at different levels with concentrate pellet feed, green fodder and paddy straw. The dietary treatments contained ad libitum APBN-1 green fodder, concentrated pellets and paddy straw at 2.5% of body weight (basal diet). The cows were adapted for stall feeding conditions, dewormed and vaccinated against Foot and Mouth disease before the commencement of the study. The animals were pre-weighed and chosen into four groups with four animals in each on the basis of their milk yield and body weight in a Completely Randomized Design maintained at Livestock Farm Complex, College of Veterinary Science, Tirupati.

A 40 days lactational trail was conducted. The product "Citric acid" used in the present study was procured from the company The Grater Scientifics Vijayawada. Animals were fed with Control diet (T1) - Basal ration (*ad libitum* Andhra Pradesh Bajra Napier -1 + concentrate pellet feed + paddy straw @ 2.5% of body weight). Experimental diet 1 (T2) - Basal ration + Citric acid @30g/day. Experimental diet (T3) – Basal ration + Citric acid @60g/day. Experimental diet (T4) – Basal ration + Citric acid @120g/day. The concentrate feed was mixed with Citric acid for an hour before feeding for the supplemented groups. All the experimental procedures were reviewed and approved by Institutional Animal Ethics Committee with a reference number 281/GO/Rebi/S/2000/CPCSEA/TPTY/024/Animal Nutrition/2021 in the College of Veterinary Science, Tirupati. Blood samples from each experimental unit was taken. The blood samples were obtained by jugular vein puncture. On the day of collection five collections were made for four animals from each group, the first one before feeding is designated as "0" hour and the other collections after feeding, at 3, 6, 9 and 12 hours for 3 succeeding days after digestion trial. Two drops of 20% H₂SO₄ for every 20 ml of SRL was added to prevent further fermentation. The animals were completely milked twice at 6:30 AM and 4:00 PM throughout the experimental period and daily milk yield was recorded. The milk samples were collected once in a week throughout the experiment and analysed for fat, SNF, protein and lactose.

Results

Citric acid supplementation on rumen fermentation parameters Citric acid supplementation had a prominent role in rumen fermentation pattern. Supplementation had a

significant difference ($P<0.05$) among the rumen pH values collected at the 0, 3, 6, 9, 12 hrs. As the quantum of Citric acid increased (Table 1) there was a tendency for a shift of pH from lower to higher side. The present study revealed that the dosage of the CA is independent of SRL collection time. The mean pH values for T₁, T₂, T₃, T₄ at (hr) 0, 3, 6, 9 and 12 hr were 5.76±0.03, 5.91±0.05, 6.07±0.03, 6.23±0.06, respectively. CA supplementation had a great effect on total volatile fatty acid production in the rumen. The effect was even significant at hourly intervals of SRL collection. The mean TVFA (*mEq/L*) has highest ($P<0.05$) 6 hourly interval of SRL collection and lowest at 0 hr from 6 hourly interval (Table 2). The TVFA quantity showed a decreasing trend ($P<0.05$). Among all the treatments T₄ recorded highest TVFA during all the hourly intervals.

CA supplementation had greater effect on NH₃-N (mg/100 ml SRL) in the rumen. The effect was significant at hourly intervals of SRL collection. The mean NH₃-N (mg/100 ml SRL) has ($P<0.05$) 6 hourly intervals of SRL collection and lowest for 0 hr from 6 hourly interval (Table 3). The NH₃-N quantity showed a decreasing trend ($P<0.05$). Among all the treatments T₄ recorded highest NH₃-N during all the hourly interval. Supplementation had more effect on total nitrogen (mg/100 ml SRL) in the rumen (Table 4). The effect was significant at hourly interval of SRL collection. The mean total Nitrogen (mg/100 ml SRL) was highest ($P<0.05$) 6 hourly intervals of SRL collection and lowest for 0 hr from 6 hourly interval (Table 4). The total nitrogen quantity showed a decreasing trend ($P<0.05$). Among all the treatments T₄ has highest total Nitrogen during all the hourly interval.

Table 1: Effect of Citric acid supplementation on rumen Ph

Time interval	T ₁	T ₂	T ₃	T ₄	Mean±SE
0	5.75	5.95	5.97	6.15	5.95±0.08
3	5.75	5.76	5.98	6.65	6.03±0.21
6	5.81	5.77	6.09	5.92	5.90±0.07
9	5.67	6.02	6.18	6.12	6.00±0.11
12	5.84	6.04	6.09	6.31	6.07±0.09
MEAN±SE	5.76 ^a ±0.03	5.91 ^{ab} ±0.05	6.07 ^{bc} ±0.03	6.23 ^c ±0.06	

^{abc} Values in the columns bearing different superscripts differ significantly * $P<0.05$ (n=4)

Table 2: Effect of citric acid supplementation on TVFA

Time interval	T ₁	T ₂	T ₃	T ₄	Mean±SE
0	54.5	68	69.55	79.1	67.78 ^a ±5.06
3	85.25	90.84	93.5	96.5	91.52 ^b ±2.39
6	103.85	100.56	102.5	113.5	105.10 ^c ±2.88
9	85.5	86.85	89.5	94.5	89.08 ^b ±1.99
12	77.5	79.9	81.35	89.4	82.03 ^b ±2.58
MEAN±SE	81.32±7.98	85.23±5.45	87.28±5.58	94.6±5.60	

^{abc} Values in the rows bearing different superscripts differ significantly * ($P<0.05$)

Table 3: Effect of Citric acid supplementation on rumen Ammonia nitrogen

Time interval	T ₁	T ₂	T ₃	T ₄	Mean±SE
0	8.14	9.42	10.41	10.92	9.72 ^a ±0.61
3	13.18	14.2	14.5	14.71	14.14 ^c ±0.33
6	14.86	15.1	14.87	16.11	15.23 ^c ±0.29
9	11.3	11.7	12.56	12.65	12.02 ^b ±0.32
12	9.85	10.13	9.98	12.65	10.65 ^{ab} ±0.66
MEAN±SE	11.46±1.18	12.11±1.10	12.46±1.00	13.40±0.90	

Table 4: Effect of citric acid supplementation on Total Nitrogen

Time interval	T ₁	T ₂	T ₃	T ₄	Mean±SE
0	62.8	64.8	65.75	65.1	64.15 ^a ±0.635
3	88.4	89.1	90.12	91.65	89.81 ^c ±0.705
6	91.3	94.5	95.8	96.1	94.45 ^d ±1.098
9	75.8	76.1	75.8	75.9	75.9 ^b ±0.07
12	64.3	64.8	64.7	68.1	63.97 ^a ±0.63
MEAN±SE	76.52±5.09	77.86±6.11	78.43±6.30	78.17±6.846	

^{abc}Values in the rows bearing different superscripts differ significantly * (P < 0.05)

Effect of Supplementation on Milk Yield and Composition
Supplementation of CA showed an increase in milk yield (lit) which was dependent on the dose of the acid. Higher production yield was recorded for T₄ and the higher yield was

depicted during 6th week of supplementation when observed from 1st week. Milk composition was significant during 4th and 5th week.

Table 5: Effect of Citric Acid Supplementation on Milk Yield WEEK WISE

Treatment	1 st Week	2 nd Week	3 rd Week	4 th Week	5 th Week	6 th Week
T ₁	40.37±3.2	40.00±3.2	41.32±3.49	40.12±3.58	40.55±3.38	41.8±2.9
T ₂	41.35±4.46	41.57±4.53	42.4±4.61	42.94±4.55	43.605±4.55	44.1150±4.5
T ₃	42.15±4.15	44.37±4.59	45.29±4.5	46.7±4.48	47.17±4.41	48.9±4.30
T ₄	44.15±4.14	46.25±4.52	47.98±4.46	48.88±4.36	49.93±4.30	51.35±4.06
MEAN±SE	42.63±1.86	43.05±2	43.85±2.04	44.56±2.08	49.93±4.30	51.55±4.06
P-VALUE	0.84	0.7	0.6	0.52	0.4	0.29

Table 6: Effect of Citric Acid Supplementation on and Composition

Component (%)	T ₁	T ₂	T ₃	T ₄	P- value
FAT**	4.66a+0.11	4.52a+0.09	4.35ab+0.07	4.325b+0.03	0.05
SNF**	8.94a+0.18	9.39ab+0.15	9.59b+0.17	9.65b+0.37	0.03
Protein***	4.295a+0.08	4.46ab+0.13	4.74bc+0.07	4.90c+0.05	0.02
TS	13.60+0.27	13.91+0.41	13.95+0.04	13.97+0.23	0.52
LACTOSE**	3.94a+0.16	4.32ab+0.211	4.37ab+0.11	4.57b+0.54	0.05

^{abc} values in a row bearing different superscripts differ significantly *** (P < 0.05)

The serum glucose (mg/dl), BUN, triglycerides (g/dl) of cattle fed with/without were for Citric acid were significantly

affected. Serum minerals (Ca, P) were also significantly affected.

Table 7: Effect of Citric Acid Supplementation on Serum Metabolites

Parameter	T ₁	T ₂	T ₃	T ₄	Mean±SE	P- Value
Glucose**	41.25 ^a ±2.59	49.5 ^{ab} ±3.32	57 ^{bc} ±3.18	64.5 ^c ±1.32	53.06±2.54	0.001
Urea**	16.8 ^a ±1.98	17.42 ^{ab} ±0.45	18.65 ^{ab} ±1.04	21.20 ^b ±0.76	18.51±0.69	0.1
Albumin	3.5±0.05	3.15±0.30	3.20±0.21	3.42±0.20	3.31±0.10	0.6
Total Protein	7.65±0.41	7.95±0.40	8.60±0.30	7.75±0.31	7.99±0.18	0.3
Globulin	4.15±0.40	4.8±0.47	5.4±0.47	4.3±0.37	4.67±0.22	0.2
Cholesterol	159±2.30	174.5±18.26	155±24.48	173.5±18.36	165.62±9.74	0.8
Triglycerides**	163 ^a ±2.08	166 ^{ab} ±0.91	169.75 ^{ab} ±1.70	181.25 ^b ±9.33	170±2.81	0.09
Creatinine	1.33±0.05	1.42±0.17	1.32±0.31	1.19±0.18	1.31±0.05	0.52
Calcium*	7.18 ^a ±2.41	8.32 ^b ±1.10	9.87 ^c ±1.28	11.81 ^c ±2.37	15.05 ^c ±1.81	0.001
Phosphorous*	3.95 ^a ±1.18	4.06 ^b ±0.64	5.9 ^c ±0.69	6.33 ^c ±1.13	7.48±0.9	0.001
AST	117.62±15.01	105±6.64	95.90±6.37	133.62±17.25	113.03±6.61	0.2
ALT	33.5±1.5	36.8±1.8	37.3±2.1	34.3±1.7	35.47±1.74	0.4

^{abc} values in a row bearing different superscripts differ significantly ** (P < 0.05)

Discussion

In the present study, T₄ fed group recorded higher TVFA and this could be the probable reason for lowered rumen pH for this treatment group. The increase in the TVFA for T₂ and T₄ may be due to an increase in ratio of Acetate to Propionate with increasing levels of Citric acid supplementation and this increase should be due to the increase in the concentration of Acetate. Similarly increase in the Total VFA concentration was due to increased Acetate concentration (Wang *et al.*, 2009). Another probable reason for increase in Acetate production may be due to its direct entry into Citric acid cycle by completely utilizing the H⁺ ions leading to decreasing the H⁺ ions concentration in rumen. It is to be reported that based

on the present results Citric acid was fermented to CO₂, methane and VFA by using unprocessed rumen contents containing a normal solid to liquid ratio along with bacteria and protozoa. It was established by Kirchgesswer and Roth (1988) [8] that supplementation of organic acids at high doses to ruminants can increase the body weight, improves feed conversion ratio and reduce colonization of pathogens in the intestines. Butyric acids and this could be the probable reason for a reduction in the rumen pH.

The NH₃-N was greatly affected with supplementation and the effect was significant (P < 0.05) at 6 hours of post feeding. These results are in agreement with those Sarwar *et al.* (2004) wherein they reported that supplementation of Acetic acid and

Fumaric acids to buffalo bulls increased NH₃-N concentration at 5 hours of post feeding. Another probable reason for an increased trend in the production of NH₃-N is that in general organic acids are anti pathogenic and stimulates the most important rumen bacteria like *Streptococcus bovis* and *Selenomonas ruminantium*.

The present results showed an increased crude protein digestibility for all the supplemented groups with a corresponding increase in rumen ammonia concentration. T₄ group fed with higher level of Citric acid showed a hike in crude protein and NH₃-N production. This clearly indicates that Citric acid improves protein utilization and Microbial protein synthesis which could be depicted in the milk production. The present results are in agreement with Amjed Ali *et al.* (2013)^[1] who reported that higher level of Organic acid supplementation results in higher microbial protein synthesis and increase in milk yield due to increase in microbial efficiency and microbial nitrogen production and less methane production in rumen (Newbold *et al.*, 2005; Sniffen *et al.*, 2006; Khampa and Wanapat, 2007)^[9, 13].

Milk constituent composition was not varied among treatment up to 3rd week but significant different were found for SNF and Lactose and Fat, SNF, Protein and Lactose during 4th and 5th week, respectively. The changes in milk constituents composition may be due to an increased milk production during last two weeks of experiment period. Since there is correlation between the concentration of milk constituents especially fat as showed a decreased value for milk (Torres-Hernandez and Hohenboken 1980). A decline in fat content with corresponding increase in milk production was also reported by Piquer *et al.* (2009)^[10] and Ebtehag *et al.* (2011)^[4] by the inclusion of whole citric fruits and organic acids (Remling *et al.* 2014)^[11]

The serum glucose concentration found to be higher for T₂ to T₄ groups and these results are agreement with Hernandez *et al.* (2011)^[5]. Since NFE rich in soluble carbohydrates more glucose would have been released into blood. This is one of the probable reasons for higher blood glucose concentration. In the present study BUN values highest for T₄ fed group. It was evident from the reports Khan *et al.* (2007) that as the feed intake and rumen degradability of the crude protein increased and higher BUN values were recorded. The present study results are in collaboration with Russel and Roussel (2007)^[12] who reported that higher BUN levels were an indication of excess rumen degradable protein. It indicated that the growth of rumen flora responsible for CP degradation would have been triggered Hernandez *et al.* (2011)^[5] and this would be probable cause for an increase in BUN values for Citric acid supplemented groups. Serum Ca and P concentration were within the range but were increases significantly ($P < 0.05$) in a linear mode. It was reported Boling *et al.* (2000)^[2] that Citric acid enhances the overall digestibility of minerals especially phosphorous and this may be justifiable reason for increased serum Ca and P levels in the present study. However, contradictory results were reported by no significant differences was reported by Yadav (2018)^[15] and Kincaid *et al.* (2008)^[7] for calcium and phosphorus

Conclusion

It was to conclude that the citric acid supplementation can manipulate the rumen fermentation pattern with an increased volatile fatty acid production contributing to higher energy Supplementation had an influence on milk production with a

corresponding change in the milk composition. Citric acid also affected the serum biochemical parameters with a significant change in Calcium and Phosphorus concentrations at higher dose of supplementation

Reference

1. Ali A, Khan, Mobashar M, Inam M, Ahmed I, Khan NA, Khan H. Effect of different levels of organic acids supplementation on feed intake, milk yield and milk composition of dairy cows during thermal stress. *Journal of Agricultural Sciences*. 2013;3:762-768.
2. Boling SD, Douglas MW, Shirley RB, Parsons CM, Koelkebeck KW. The effects of various dietary levels of phytase and available phosphorus on performance of laying hens. *Poultry Science*. 2000;79(4):535-538.
3. Bozic M, Newton J, Thraen CS, Gould BW. Mean-reversion in income over feed cost margins: Evidence and implications for managing margin risk by US dairy producers. *Journal of Dairy Science*. 2012;95(12):7417-7428.
4. Ebtehag IMAE, Hoda ME, El-Shabrawy HM. Comparing effects of organic acid (malate) and yeast culture as feed supplement on dairy cows performance. In proceedings of the 4th Scientific Conference of Animal Wealth Research in the Middle East and North Africa, Foreign Agricultural Relations (FAR). Egypt, 2011, 340-353.
5. Hernandez J, Castillo C, Méndez J, Pereira V, Vazquez P, López Alonso M *et al.* The influence of chemical form on the effects of supplementary malate on serum metabolites and enzymes in finishing bull calves *Livestock Science*. 2011;137:260-263.
6. Khampa S, Wanapat M, Wachirapakorn C, Nontaso N, Wattiaux M. Effects of urea level and sodium dlmalate in concentrate containing high cassava chip on ruminal fermentation efficiency, microbial protein synthesis in lactating dairy cows raised under tropical conditions. 2006.
7. Kincaid R. Changes in the concentration of minerals in blood of peripartum cows. In Mid-South Ruminant Nutrition Conference, 2008, 1-8.
8. Kirchgessner M, Roth FX. Energy value of organic acids in the rearing of piglets and the fattening of pigs. 1988;16:93-108.
9. Newbold CJ, Lopez N, Nelson JO, Ouda RJ, Wallace, Moss AR. Propionate precursors and other metabolic intermediates as possible alternative electron acceptors to methanogenesis in ruminal fermentation *in vitro*. *British Journal of Nutrition*. 2005;94:27-35.
10. Piquer O, Casado C, Biglia S, Fernandez C, Blas E, Pascual JJ. *In vitro* gas production kinetics of whole citrus fruits. *Journal of Animal Feed Science*. 2009;18(4):743-757
11. Remling N, Riede S, Lebzien P, Meyer U, Höltershinken M, Kersten S *et al.* Effects of fumaric acid on rumen fermentation, milk composition and metabolic parameters in lactating cows. *Journal of Animal Physiology and Animal Nutrition*. 2014;98(5):968-981.
12. Russell KE, Roussel AJ. Evaluation of the ruminant serum chemistry profile. *Veterinary Clinic of Animal Feed Practice* 2007;23:403-426.
13. Sniffen CJ, Ballard CS, Carter MP, Cotanch KW, Dann HM, Grant RJ *et al.* Effects of malic acid on microbial efficiency and metabolism in continuous culture of rumen contents and on performance of mid-lactation dairy cows.

- Animal Feed Science Technology. 2006;127:13-31.
14. Wang C, Liu Q, Meng J, Yang WZ, Yang XM, He DC, *et al.* Effects of citric acid supplementation on rumen fermentation, urinary excretion of purine derivatives and feed digestibility in steers. *Journal of Science Food and Agriculture*. 2009a;121:15-20.
 15. Yadava Santosh Kumar. Growth performance, Nutrient utilization and blood biochemicals of heifers supplemented with organic acids MS thesis, 2018.