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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(3): 427-431

www.thepharmajournal.com
Received: 04-01-2022
Accepted: 06-02-2022

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

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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 adlibitum 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 (T_1), T_1 + 30g Citric acid/animal/ day as T_2, T_1 + 60g of Citric acid/animal/day (T_3) and T_1 + 120g Citric acid/animal/day as T_4. 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, NH_3-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 T_4 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, ↓ CH_4, ↓ 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 adlibitum 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% H_2SO_4 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 T_1, T_2, T_3, T_4 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 T_4 recorded highest TVFA during all the hourly intervals.

CA supplementation had greater effect on NH_3-N (mg/100 ml SRL) in the rumen. The effect was significant at hourly intervals of SRL collection. The mean NH_3-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 NH_3-N quantity showed a decreasing trend (P<0.05). Among all the treatments T_4 recorded highest NH_3-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 T_4 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 T_4 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	1st Week	2nd Week	3rd Week	4th Week	5th Week	6th 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 (%)	T1	T2	T3	T4	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
Triglycirides**	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
Calicum*	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, T_4 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 T_2 and T_4 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 CO_2, 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 NH_3-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 NH_3-N concentration at 5 hours of post feeding. Another probable reason for an increased trend in the production of NH_3-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. T_4 group fed with higher level of Citric acid showed a hike in crude protein and NH_3-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 T_2 to T_4 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 T_4 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 pphosphorous 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

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