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Effect of Malic Acid Supplementation on Haemato-Biochemical and Reproductive Parameters of Lactating Kankrej Cows

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ABSTRACT

This study was conducted to evaluate the effect of supplementation of malic acid on haemato-biochemical and reproductive parameters of lactating Kankrej cows. Twenty-four lactating Kankrej cows (15 days post- partum) were assigned randomly into four groups (6 animals in each) and on the basis of their standard lactation milk yield of previous lactation and parity of animals. The four treatment groups were as T_1: Basal diet, in T_2, T_3 and T_4 groups basal diet was supplemented with 30, 60 and 90 g/d of malic acid, respectively. The duration of experiment was of 120 days. The blood samples were collected at the end (on 120th day) of experimental feeding. The mean concentrations of haemoglobin, haematocrit, red and white blood cells count were comparable ($P>0.05$) among the treatment groups. The serum concentrations of total protein, albumin, globulin, urea, creatinine, cholesterol, ALT, and AST were not influenced ($P>0.05$) due to supplementation of malic acid. There was an increase ($P<0.05$) in serum glucose concentration (mg/dL) in T_2 (78.17), T_3 (79.83) and T_4 (78.67) groups as compared to the T_1 (73.67) group. In reproductive parameters, the first heat after calving, services period and number of services per conception in malic acid supplemented groups showed improvement than the control. It may be concluded that malic acid supplementation in lactating Kankrej cows had beneficial effect on reproductive performance Moreover, dietary malic acid did not have any adverse effect on haemato-biochemical parameters of Kankrej cows.

Key Words: Blood metabolites, Haematology, Kankrej, Malic acid, Reproduction.

INTRODUCTION

India has one of the twelve mega biodiversity countries in the world, is home to a large and diverse array of cattle genetic resources, with 193.46 million cattle and 53 registered native cattle breeds (Srivastava *et al,* 2019; BAHS. 2023). Among these, the Kankrej breed from Gujarat is notable for its distinct body size and characteristic "savai chal" gait (Madhavatar *et al,* 2023). During the early lactation (14 to 100 d after calving), cows achieve peak milk production, undergo weight loss, reduced feed intake and alterations in haemato-biochemical parameters. Assessment of haemato-biochemical profile is very important for monitoring the metabolism and health status of dairy animals during the lactation period (Patel *et al,* 2022; Modi *et al,* 2023). The values of haemato-biochemical parameters of cows are influenced by several factors such as breed, age, physiological status, sex, nutrition or season (Antunović *et al,* 2017). Blood biochemical parameters indicate possible metabolic disorders and disorders caused by inadequate nutrition (Rios *et al,* 2006). Dietary supplementation of organic acid in lactating cows may have beneficial influence on their metabolism and reproduction.

Organic acids act as acidifiers by favourably manipulating intestinal microbial populations and improving the immune response. Among these, malic acid is the most widely used organic acid in ruminant animals. Its molecular

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formula is $C_4H_6O_5$, and it has two stereoisomeric forms (L- and D-enantiomers), though only the L-isomer occurs naturally. In the citric acid cycle, malic acid, in the form of malate, plays an important role in energy production (Pearlin *et al,* 2020). It also alters the microbial fermentative pattern by enhancing the numbers and growth of *Selenomonas ruminantium* bacteria (Bugra *et al,* 2020), stimulating the utilization of lactate and converting it to propionic acid, which is essential for promoting energy availability in the form of glucose. It also beneficially affects reproductive parameters. Wang *et al*. (2009) supplemented malic acid to Holstein dairy cows, resulting in a substantial increase ($P<0.01$) in serum glucose levels. Similarly, El-Zaiat *et al* (2019) and Gouda *et al*. (2022) observed same findings. In contrast, Carrasco *et al* (2012) reported no effect found on glucose due to malic acid feeding in cows. For reproductive performance El-Nour *et al* (2009) reported no any significant effect found on reproductive parameter. There is scarce literature available on the effects of feeding malic acid on the haemato-biochemical and reproductive parameters of Indigenous cows. Therefore, the present study was planned to evaluate the effects of malic acid supplementation on the haemato-biochemical and reproductive parameters of lactacting Kankrej cows.

MATERIALS AND METHODS

Twenty-four lactating Kankrej cows (14 days post-partum) were assigned randomly into four groups (6 cows per group) on the basis of body weight and standard milk yield of previous lactation. All the selected animals were apparently healthy and free from diseases. The four experimental groups were as T_1: Basal diet, T_2: Basal diet + 30 g/animal/day of malic acid supplementation; T_3: Basal diet + 60 g/animal/day of malic acid supplementation and T_4: Basal diet + 90 g/animal/day of malic acid supplementation. The duration of experiment was of 120 days. The experimental animals were fed as per ICAR (2013) to the nutrient requirements. The use of animals and the experimental procedure were approved by institutional Animal Ethics Committee (approval No. VETCOLL/IAEC/2023/21/PROTOCOL-1).

The blood samples were collected at the end (on 120th day) of experimental feeding. The blood samples from external jugular vein were collected from each experimental animal in two sterilized vials one with anti-coagulant for hematological parameters and other without anti-coagulant for analysis of blood biochemical parameters. The fresh blood samples the one with anti-coagulantwere analysed for haematological parameters *viz.,* haemoglobin, haematocrit, erythrocytes, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), leucocytes, neutrophils, lymphocytes and monocytes using automatic analyser. The serum was harvested from the blood without anti-coagulant. The serum samples were analysed for glucose, total proteins, albumin, urea, creatinine, triglycerides, cholesterol, alanine amino-transferase (ALT) and aspartate aminotransferase (AST) concentrations using commercial diagnostic kits.

Reproductive parameters, such as the first heat after calving, service period and the number of services per conception, were recorded during the experimental period.

All the experimental data obtained were statistically analyzed using SPSS v.16.0 (SPSS Inc., Chicago IL) as per the standard statistical method (Snedecor and Cochran, 1994). Significant differences between means of treatments were assessed by Duncun's test, and differences between treatments were declared significant at $P<0.05$.

RESULTS AND DISCUSSION

The effect of supplementation of malic acid on haematological parameters of lactating Kankrej cows is given in Table 1. The mean haemoglobin (Hb) and haematocrit values were comparable ($P>0.05$) among the treatment groups. The obtained values of Hb and haematocrit were within normal range of 8-15 g/dL and 26-37% (Kaneko *et al,* 2008). There was no effect ($P>0.05$) on red blood cells count (erythrocytes, MCV, MCH and MCHC) due to dietary addition of malic

Table 1. Effect of supplementation of malic acid on haematological parameters of lactating Kankrej cows (n=24).

Attribute	Groups[¥]				SEM	P value
	T_1	T_2	T_3	T_4		
Haemoglobin (g/dL)	11.20	10.28	9.93	11.02	0.245	0.216
Haematocrit (%)	27.08	28.99	29.78	29.50	0.549	0.312
Erythrocytes (10^6/µL)	5.89	5.18	5.47	5.50	0.137	0.347
Leukocytes (10^3/ µL)	9.75	9.92	10.40	9.63	0.455	0.946
MCV (fL)	49.23	49.62	49.00	48.30	0.833	0.961
MCH (pg)	18.73	17.72	17.47	19.07	0.601	0.768
MCHC (g/dL)	36.62	35.02	36.62	37.68	0.760	0.694
Granulocyte (10^3/ µL)	3.78	3.85	3.72	3.38	0.207	0.877
Lymphocytes (10^3/ µL)	3.43	3.82	3.70	3.47	0.227	0.930
Monocytes (10^3/ µL)	0.65	0.60	0.68	0.65	0.045	0.940

[¥]T_1: Basal diet; T_2: Basal diet + 30 g/d of malic acid; T_3: Basal diet + 60 g/d of malic acid; T_4: Basal diet + 90 g/d of malic acid

MCV: meancorpuscular volume, MCH:mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration

acid at different supplemental doses in lactating Kankrej cows. Rate variables for red blood cells observed in this study agreed with the reference described by Jones and Allison (2007). Therefore, no inflammatory process was reported in the lactating cows during the experimental period due to malic acid supplementation. The red blood cells series reflect the animals' health and nutrition (Jones and Allison, 2007). The white blood cell counts (leucocytes, neutrophils, lymphocytes, monocytes and eosinophils) were not influenced (P>0.05) by the dietary addition of malic acid in lactating Kankrej cows. Results for all dietary treatments groups agreed with reference for cattle (Jones and Allison, 2007). The haematological parameters reported in the present study was also in line with the findings of previous studies conducted in lactating Kankrej cows (Ahuja *et al,* 2022; Pawar *et al,* 2023). The results obtained for the counts of red and white blood cells in this trial clearly showed that supplementation of malic acid at the dose rate 30, 60 and 90 g/d were safe for use in dairy cattle without compromising the health and welfare of animals.

The effect of supplementation of malic acid on blood biochemical parameters of lactating Kankrej cows is presented in Table 2. The serum concentrations of total protein (7.73, 7.47, 8.20 and 7.47 g/dL), albumin (3.80, 3.48, 3.67 and 3.62 g/dL), globulin (3.93, 3.98, 4.53 and 3.85 g/dL), urea (31.64, 34.62, 33.71 and 35.74 mg/dL), creatinine (1.14, 1.09, 1.10 and 1.08 mg/dL), triglycerides (17.83, 19.78, 17.78 and 18.23 mg /dL), cholesterol (288.93, 307.58, 329.97 and 306.60 mg /dL), ALT (47.73, 46.37, 49.93 and 39.32 U/L) and AST (70.73, 66.90, 73.73 and 71.87 U/L) were not affected (P>0.05) by the dietary supplementation of malic acid in Kankrej cows. No effect on serum concentrations of total protein, albumin and globulin in lactating Kankrej cows suggesting that feeding of malic acid unaltered protein catabolism in the muscles of cows.

Table 2. Effect of supplementation of malic acid on blood biochemical parameters of lactating Kankrej cows (n=24)

Attributes	Groups ¥				SEM	P value
	T_1	T_2	T_3	T_4		
Glucose (mg/dL)	73.67 [a]	78.17 [ab]	79.83 [b]	78.67 [b]	0.764	0.013
Total Proteins (g/dL)	7.73	7.47	8.20	7.47	0.186	0.485
Albumin (g/dL)	3.80	3.48	3.67	3.62	0.088	0.675
Globulin (g/dL)	3.93	3.98	4.53	3.85	0.130	0.238
Urea (mg/dL)	31.64	34.62	33.71	35.74	1.657	0.862
Creatinine (mg/dL)	1.14	1.09	1.10	1.08	0.022	0.748
Triglycerides (mg/dL)	17.83	19.78	17.78	18.23	1.058	0.912
Cholesterol (mg/dL)	288.93	307.58	329.97	306.60	7.480	0.297
SGPT (U/L)	47.73	46.37	49.93	39.32	2.645	0.548
SGOT (U/L)	*70.73*	*66.90*	*73.73*	*71.87*	*2.195*	*0.756*

¥T_1: Basal diet; T_2: Basal diet + 30 g/d of malic acid; T_3: Basal diet + 60 g/d of malic acid; T_4: Basal diet + 90 g/d of malic acid

[ab]Means with different superscriptsin a row differed significantly (P<0.05).
ALT: alanine aminotransferase; AST: aspartate aminotransferase

Moreover, lack of effect on liver enzymes ALT and AST due to feeding malic acid in lactating Kankrej cows indicates that supplementation did not have any adverse effect on liver function. The serum urea levels in ruminants are known to be dependent on the amount of ammonia nitrogen concentration in rumen, which is formed from deamination of proteins. No change in serum urea concentration was indication of no effect on deamination and nitrogen concentrations in rumen as well as efficient use of nitrogen (Pawar *et al,* 2019). The blood biochemical parameters reported in the present study was also in line with the findings of previous studies conducted in lactating Kankrej cows (Joshi *et al,* 2021; Pawar *et al,* 2021).

The serum glucose concentration was 73.67, 78.17, 79.83 and 78.67 mg/dL in T_1, T_2, T_3 and T_4 groups, respectively. There was a significant increase in serum glucose level in the lactating Kankrej cows supplemented with different doses of malic acid in diet. The increase in serum glucose level in malic acid supplemented groups might be due to higher digestibility of organic matter and facilitation of increased rumen propionate absorption through ruminal papillae. This absorbed propionate is then transported to the liver, where it undergoes active conversion to glucose through gluconeogenesis. Consequently, this process results in elevated glucose synthesis (El-Zaiat *et al,* 2019). El-Nour *et al* (2009) and Wang *et al* (2009) reported that serum glucose levels were significantly increased in the malic acid supplemented group than in control group. Similarly, Gouda *et al* (2022) also reported increased glucose concentration in goats fed malic acid.

The effect of supplementation of malic acid on reproductive parameters of lactating Kankrej cows is presented in Table 3. The first heat post-calving numerically occurred 2, 5 and 4 days

Table 3. Effect of supplementation of malic acid on reproductive parameters of lactating Kankrej cows (n=24)

Attributes	Groups				SEM	P value
	T_1	T_2	T_3	T_4		
First heat after calving (days)	102.67	100.50	97.33	98.17	1.492	0.611
Service period (days)	163.17	161.67	159.17	160.17	1.148	0.656
Number of services per conception	2.00	1.83	1.67	1.67	0.147	0.849

¥T_1: Basal diet; T_2: Basal diet + 30 g/d of malic acid; T_3: Basal diet + 60 g/d of malic acid; T_4: Basal diet + 90 g/d of malic acid

earlier in T_2, T_3 and T_4 groups, respectively than the control. Similarly, the service period was 2, 4 and 3 days earlier in T_2, T_3 and T_4 groups, respectively as compared to the control. Malate supplement increases in ruminal propionate. It improves ovarian sensitivity, achieved through the release of the GnRH hormone by the pituitary gland.

Moreover, the impact of elevated glucose levels extends to the regulation of insulin and its receptors, playing a pivotal role in the synthesis and release of hormones such as LH (luteinizing hormone), progesterone and estradiol. These hormonal changes influence reproductive performance (Abou-Seri and Mahmoud, 2022). In agreement with the results of present study, Abou-Seri and Mahmoud (2022) reported significant ($P<0.05$) decrease in time duration between calving to the first oestrus and calving to conception. Additionally, there was a reduction in the number of services per conception. In contrast, El-Nour *et al* (2009) reported no change in reproductive performance of buffaloes supplemented with malic acid.

CONCLUSION

Based on the results, it may be concluded that malic acid supplementation in the diet of Kankrej cows during lactation improved serum glucose and reproduction performance. Moreover, there was no adverse effect of malic acid supplementation on haemato-biochemical parameters of lactating Kankrej cows.

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