

# COMPARATIVE STUDIES ON SELECTED ENZYMES ACTIVITIES OF CAMEL, COW AND BUFFALO MILK

Jaydeep Yoganandi<sup>2</sup>, Amit Kumar Jain<sup>1</sup>, Bhavbhuti M. Mehta<sup>1</sup>,  
K.N. Wadhvani<sup>3</sup> and K.D. Aparnathi<sup>1</sup>

<sup>1</sup>Dairy Chemistry Department, SMC College of Dairy Science, <sup>3</sup>Department of Livestock Production and Management, Veterinary College, Anand Agricultural University (AAU), Anand, Gujarat, India

<sup>2</sup>Mother Dairy, Gandhinagar, Gujarat, India

## ABSTRACT

The study was conducted to collect camel milk available nearby Anand-Kheda district and were analysed for alkaline phosphatase activity and catalase activity. The comparisons were also made with that of cow and buffalo milk samples. The average alkaline phosphatase activity in camel, cow and buffalo milk were  $21.31 \pm 1.26$   $\mu\text{g/ml}$ ,  $575.00 \pm 18.42$   $\mu\text{g/ml}$  and  $253.75 \pm 8.22$   $\mu\text{g/ml}$ , respectively. The average catalase activity in camel, cow and buffalo milk were  $6.20 \pm 0.18$  units,  $0.86 \pm 0.04$  units and  $0.62 \pm 0.04$  units, respectively. The camel milk has lower alkaline phosphatase activity but high catalase activity compared to cow and buffalo milk.

**Key words:** Alkaline phosphatase, catalase activity, camel milk

Camel milk is an important source of nutrition for the people living in the arid lands of the world. It is known for its medicinal properties, which are widely exploited for human health, as in several countries from the ex-Soviet Union (Kenzhebulat *et al*, 2000) and developing countries (Mal *et al*, 2006). In the western world, camel milk is experiencing a novel awareness in these days and even the FAO has stepped in promoting camel milk (Ramet, 2001). However, there are only a few references on camel milk, whether they concern production (Konuspayeva *et al*, 2009) or composition/enzymatic activities (Farah, 1993; Ramet, 1993). Publications dealing with the enzymatic activities of camel milk are relatively scarce and much of the information is approximate and fragmental.

Alkaline phosphatase is a native enzyme of milk. The pH of optimum activity is about 9.6. The heating of milk at a temperature of  $62^\circ\text{C}$  for 30 min will cause inactivation of alkaline phosphatase. Thus, time-temperature combination required for inactivation of alkaline phosphatase and that of the requirement for pasteurisation are coinciding well with each other. Therefore, an index to the efficiency of pasteurisation. The average alkaline phosphatase activities ranged between 15.9 and 24.3 U/l with raw milk and 5.8–10.2 U/l with pasteurised camel milk. The residual activity of alkaline phosphatase in pasteurised milk revealed that this enzyme is not suitable to verify an effective pasteurisation of camel milk (Wernery *et al*, 2006; 2008; Merin *et al*, 2005).

Catalase, indigenous milk was first recognised in 1907. It is an iron containing protein which catalyses the decomposition of hydrogen peroxide in to water and nascent oxygen. In blood, catalase is associated with leucocytes. During occurrence of mastitis in lactating animals, leucocytes count in milk increases. Therefore, catalase activity in milk can serve as a good index of mastitis. Catalase may also be involved in lipid oxidation. From review of literature, it appears that no data reported in literature regarding catalase activity of camel milk.

Camel (*Camelus dromedarius*) population in Gujarat state of India was reported to be 38,454 in 2007 (Bulletin of animal husbandry and dairy statistics 2009-2010, Government of Gujarat). However, The information on alkaline phosphatase activity and catalase activity of camel milk produce in Gujarat is not available. Therefore, there is a need to undertake systematic study to generate data. This information will be beneficial to camel owners, dairy manufacturers as well as policy makers. Hence, the present study was planned to study activities of the selected enzymes of camel (*Camelus dromedarius*) milk and its comparison was carried out with cow and buffalo milk.

## Materials and Methods

The pooled milk samples of camel milk (from Anand and Kheda district, 8 samples), cow milk (8

SEND REPRINT REQUEST TO BHAVBHUTI M. MEHTA email: bhavbhuti5@yahoo.co.in

samples) and buffalo milk (8 samples) were collected from the herd of cows and buffalos from Gopalpura village, Anand district. The samples were kept in ice and transported to the laboratory, where they stored at 4°C. Milk samples were collected in clean and dry sample bottles and kept at refrigeration temperature before its analysis. Total 24 samples (8 each) were analysed for alkaline phosphatase activity and catalase activity.

The camel, cow and buffalo milk samples for chemical analysis were prepared as per the method described in BIS Handbook (SP 18: 1981).

### Alkaline phosphatase activity

BIS Handbook (SP:18 part I -1981) procedure was used to determine Alkaline phosphatase activity for the camel, cow and buffalo milk sample.

### Method

Take 2.5 ml of buffer in a test tube and add 5 ml of buffer, keep the tube in a water bath for 5 min. at a temperature of 37°C. After the pre-incubation, add 0.5 ml of milk and mix the contents. A corresponding blank is carried out using boiled milk (inactivated enzyme) or terminating the reaction by adding 1ml of 28% TCA (Trichloro acetic acid) at zero time. Incubation is carried out for 30 min. at 37°C. Terminate the incubation by adding 1 ml of TCA to the test sample. Filter the content of the tube through filter paper.

To 5 ml of filtrate in a test tube, add 0.5 m of 14% NaOH. Centrifuge, if necessary. The colour developed is measured in a Spectrophotometer.

### Catalase activity

The method consists of incubation of 15 ml of milk taken in iodine flask (or conical flask) with 5.0 ml of 6% diluted hydrogen peroxide having 0.25 ml toluene. Corresponding blank was taken by adding boiled milk (10 min in boiling water bath). After 2 hrs, 6.6 ml of (1:1) sulphuric acid, 0.5 g of potassium iodide and 40 ml of distilled water were added in succession. After 10 min, the solution is titrated against N/10 sodium thiosulphate using starch as indicator (1 ml).

Catalase activity of milk is expressed in terms of catalase unit. One catalase unit is expressed as ml of N/10 sodium thiosulphate required for control determination less the titration value for the experimental solution per 5 ml of the enzyme solution. (Anderson and Mcwalter, 1937).

### Statistical analysis

The data obtained during investigation were subjected to statistical analysis using completely randomised design (Snedecor and Cochran, 1967).

### Result and Discussion

The collected samples of camel milk were analysed for alkaline phosphatase activity and catalase activity simultaneously cow and buffalo milk were also analysed for its comparison.

Based on source, enzymes are classified as indigenous enzymes and exogenous enzymes. Like all other foods of plant or animal origin, milk contains several indigenous enzymes when milk is secreted from udder. Amongst various indigenous enzymes present in milk, some enzymes serve as important diagnostic tools. Two most important examples are alkaline phosphatase and catalase.

### Alkaline Phosphatase

In the present investigation, alkaline phosphatase activity of camel, cow and buffalo milk was determined by using p-nitrophenylphosphate disodium salt as substrate and determining the liberated p-nitrophenol by spectrophotometric method. Total 8 replications were conducted. The data obtained for alkaline phosphatase activity of all the 3 types of milk along with their statistical analysis are presented in Table 1.

**Table 1.** Alkaline phosphatase activity of camel, cow and buffalo milk.

Type of milk	Alkaline phosphatase (µg/ml)
Camel	21.31±1.26
Cow	575.00±18.42
Buffalo	253.75±8.22
SEM	11.669
CD (0.05%)	34.319
CV %	11.65

SEM: Standard error of mean; CD: Critical difference (5% level significant); CV: Coefficient of variance

The average alkaline phosphatase activity was 21.31 µg/ ml in camel milk. Similarly, in cow and buffalo milk samples, mean value were 575 µg/ ml and 253.75 µg/ ml, respectively. Sample cow milk had the highest alkaline phosphatase activity, followed by buffalo milk and lowest alkaline phosphatase activity found in camel milk. The alkaline phosphatase activity of camel milk was significantly lower than that of the cow milk as well as buffalo milk.

Lorenzen *et al* (2011) carried the study on evaluation of indigenous enzyme activity in raw

and pasteurised camel milk. These authors reported the average alkaline phosphatase activities ranging from 15.9 to 24.3 units per liter for raw milk and 5.8 to 10.2 units per liter for pasteurised camel milk. They concluded that the residual activity of alkaline phosphatase in pasteurised milk revealed that this enzyme is not suitable to verify an effective pasteurisation of camel milk. The author opined that their result was in agreement with findings of Merin *et al* (2005) and Wernery *et al* (2006; 2008). Laxminarayana and Dastur (1968) reported alkaline phosphatase activity in cow milk was 82 units per 100 ml, whereas, in buffalo milk it was 28 units per 100 ml.

The data obtained in present study for average alkaline phosphatase activity of camel milk were very well in agreement with those reported in the literature for camel milk. However, data obtained in present study for average alkaline phosphatase activity of cow and buffalo milk were also higher than those reported in the literature for cow and buffalo milk, respectively. The overall trend for alkaline phosphatase activity of camel, cow and buffalo milk is also in agreement with that reported in the literature. The data on alkaline phosphatase activity of camel milk suggested that its application as an index for efficient pasteurisation of camel milk needs standardisation.

### Catalase activity

In the percent investigation, catalase activity of camel, cow and buffalo was determined by titration method using hydrogen peroxide as substrate. Total 8 replications were conducted. The data obtained for catalase activity of all the 3 types of milk along with their statistical analysis are presented in table 2.

**Table 2.** Catalase activity of camel, cow and buffalo milk.

Type of milk	Catalase activity (Unit)
Camel	6.20±0.18
Cow	0.86±0.04
Buffalo	0.62±0.04
SEM	0.108
CD (0.05%)	0.319
CV %	11.99

SEM: Standard error of mean; CD: Critical difference (5% level significant); CV: Coefficient of variance

The average catalase activity determined was 6.20 units in camel milk. Similarly, in cow milk average value was 0.86 units. On the other hand, average value in buffalo milk was 0.62 units. Sample camel milk had the highest catalase activity, followed by cow milk and lowest catalase activity found in

buffalo milk. The catalase activity of camel milk was significantly higher than that of the cow milk as well as that of the buffalo milk.

Kitchen *et al* (1970) conducted catalase activities studies on normal raw milk and mastitic whole milk. The author reported that the catalase activity for normal raw milk and mastitic milk was 7.5 to 36.0 and 175 units per ml, respectively.

Since, no data were reported in the literature for catalase activity of camel milk, the results obtained in the present study for the catalase activity of camel cannot be compared with the information of the literature. The results obtained for average catalase activity of cow and buffalo milk were lower than those reported in the literature for cow and buffalo milk, respectively. This may be attributed to low leucocyte count in the milk used in present study.

Amongst three types of milk studied in the present investigation for alkaline phosphatase activity, the highest alkaline phosphatase activity was found in cow milk, which was followed by buffalo milk and the lowest alkaline phosphatase activity was found in camel milk. The alkaline phosphatase activity in camel milk was significantly lower than that of the cow milk as well as that of the buffalo milk. The alkaline phosphatase activity of buffalo milk was significantly lower than that of the cow milk. The catalase activity in camel milk was significantly higher than that of the cow milk as well as that of the buffalo milk. The difference in catalase activity of cow milk and that in buffalo milk was non- significant.

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