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Effect of feeding of *Morinda citrifolia* fruit juice on the biophysical parameters of healthy as well as mastitis-affected cow milk

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The present study was conducted to assess the effect of *Morinda citrifolia* fruit juice on milk characteristics of healthy as well as mastitis-affected dairy cow. A total of 25 adult dairy cows were selected out of which 13 were healthy and 12 were found affected with sub-clinical mastitis. *M. citrifolia* fruit juice was fed to dairy cow at the rate of 100 ml/day/animal orally. The average pH of the normal milk and mastitis-affected milk was 6.60 ± 0.12 and 7.2 ± 0.17, respectively. The feeding of *M. citrifolia* juice significantly ($p < 0.05$) lowered the pH of mastitis-affected milk to 6.54 ± 0.07. The EC of mastitis-affected milk lowered significantly ($p < 0.05$) after feeding of *M. citrifolia* fruit juice. The total bacterial count in the mastitis milk also decreased significantly ($p < 0.05$) from $5.15 \pm 0.03 \times 10^8$ to $2.54 \pm 0.03 \times 10^8$ cfu/ml after feeding of *M. citrifolia* juice. Morinda feeding decreased the total protein concentration in the mastitis milk significantly ($p < 0.05$) to 21.13 ± 0.93 μg/ml. It could be concluded that the feeding of *M. citrifolia* fruit juice to dairy cows showed improvement in the quality and biophysical parameters of milk of mastitis-infected dairy animals.

Keywords: mastitis; *Morinda citrifolia*; milk; biophysical parameters

Introduction

Livestock in general and dairying in particular play a vital role in the Indian economy and in the socio-economic development of the country. These sectors also play a significant role in supplementing family incomes and generating gainful employment in the rural sector, particularly, among the landless labourers, small and marginal farmers and women, besides providing cheap nutritional food to millions of people. According to the National Sample Survey Organization's survey (NSS 61st round) 5.5% of the workforce in the country was engaged in Animal Husbandry sector in 2004–2005.

Mastitis is a multietiological complex disease, which is defined as inflammation of parenchyma of mammary glands and is characterised by physical, chemical and usually bacteriological changes in milk and pathological changes in glandular tissues (Radostits et al. 2000). The disease is the outcome of a complex interaction between host (cows, buffaloes, etc.), causative agents (microorganisms) and environment (Muhammad and Firyal 2008). Mastitis continues to be among the costliest diseases to the dairy industry, and annual economic losses attributed to this disease were approximately 2 billion dollars in the USA, 526 million dollars in India and 35 billion dollars worldwide, in which sub-clinical mastitis is responsible for approximately 70% of these economic losses (Varshney and Naresh 2004; Annapoorani et al. 2007).

Over 100 different microorganisms have been reported as a cause of intra-mammary infection (IMI) in dairy cows. The vast majority of IMI and those of significant economical importance are caused by species *of staphylococci, streptococci* and the Gram-negative organisms.

Clinical and sub-clinical cases of mastitis are routinely treated with antimicrobials. Widespread use of antibiotics for the treatment of this disease has a potential to cause contamination of milk, which has become a subject of public concern. The use of antimicrobials over long periods has triggered the development of multidrug resistant strains, which has resulted in the use of increasing doses of antimicrobials, causing the danger of increasing amounts of drug residues in milk, a potential biohazard (Dhanabalan et al. 2008).

Medicinal plants have been used for ages in developing countries as alternative treatment to health problems. India has a diverse flora and a rich tradition in the use of medicinal plants for treatment of diseases of human as well as animals. Medicinal herbs are natural and safe approaches, and are receiving attention in treatment of animals from an increasing number of veterinarians. *Morinda citrifolia* commonly known as Great Morinda was mentioned

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in Ayurvedic texts well over 1000 years ago as Ashyuka, which means longevity, and was used as a balancing agent. It is a small evergreen tree in the coffee family of Rubiaceae. The tree grows up vigorously from India to Malaysia, through Fiji and Eastern Polynesia. The original home of the plant is Polynesia, Micronesia and the Hawaiian Islands, where it is known as Noni, is now distributed throughout the tropics. Almost all the parts of the plant are being used for its medicinal and nutraceutical properties. There is a great demand for its fruit juice in alternative medicine for different kind of illness. Very little research has been conducted on its effect on animals and poultry. The present study was conducted to assess the effect of *M. citrifolia* fruit juice on milk biophysical parameters of normal as well as mastitis-affected dairy cows.

Materials and methods

Preparation of morinda juice

Fresh ripened fruits of *M. citrifolia* were collected from the Horticulture farm of Central Agricultural Research Institute, Port Blair, India. The fruits were washed and packed into polythene bags and kept at room temperature for fermentation for 8–10 days. The juice was then sieved and collected in fresh container and kept at room temperature for further use.

Experimental animals

A total of 25 adult dairy cattle were selected and screened for mastitis by employing California Mastitis Test (CMT) (Schalm et al. 1971), changes in milk pH, electrical conductivity (EC) of milk and total microbial load in milk. Based on the mastitis test, 13 animals were found healthy and 12 animals with sub-clinical mastitis. Both the group of animals was fed with 100 ml morinda fruit juice daily/animal orally for a period of 1 month. The parameters namely, daily milk yield, milk pH, milk EC, total bacterial load in milk and total milk protein concentration were observed over a period of 2 months. The animals were fed with morinda juice for 1 month and data of 2 months post-feeding were collected and analysed.

Statistical analysis

Data were analysed with the SAS Software Release 8.2 (SAS Inst., Inc., Cray, NC, USA) with the Proc GLM and Proc CORR procedures. The differences between treatments were analysed using a one-way analysis of variance. Differences with a confidence level of 0.05 or less were considered to be significant.

Table 1. Effect of *Morinda* on pH of milk (Mean ± SEM).

Group	Pre-feeding	Post-feeding
Healthy cow	6.60 ± 0.12	6.58 ± 0.02
Sub-clinical mastitis	7.2 ± 0.17	6.54 ± 0.07*

*Significantly differ at $p < 0.05$.

Results

Screening of dairy animals for detection of mastitis

A total of 25 dairy cows were screened for detection of mastitis. The incidence of mastitis was confirmed by the change in pH towards higher side, increase in the conductivity and positive result with the CMT. Out of the 25 cows, 12 animals were detected with sub-clinical mastitis. The pattern of the mastitis in the different teat of the cow suggested that the incidence was high in the right rear teat compared to other teat.

Effect of M. citrifolia *on milk pH*

The average pH of healthy cow milk was 6.60 ± 0.12 (Table 1). No significant difference was observed in the pH of cow's milk with respect to different teat of the cow. The average pH of mastitis-affected milk was 7.2 ± 0.17 (Table 1). The feeding of *M. citrifolia* juice lowered the pH of mastitis-affected milk significantly to 6.54 ± 0.07 (Table 1).

Effect on EC

In the present study, the average EC of the normal milk was found to be $4.17 \pm 0.21 \times 10^{-5}$ mS/cm (Table 2). In the mastitis-affected milk the value increased to $5.97 \pm 0.81 \times 10^{-5}$ mS/cm (Table 2). Although there was no significant change in normal milk conductivity after feeding of morinda, in the mastitis-affected cow the milk conductivity was lowered significantly to $4.77 \pm 0.14 \times 10^{-5}$ mS/cm after feeding of *M. citrifolia* juice (Table 2).

Effect on microbial load

The total bacterial count in the normal milk and mastitis-affected milk was $1.58 \pm 0.13 \times 10^{8}$ and $5.15 \pm 0.03 \times 10^{8}$ cfu/ml, respectively (Table 3). The microbial load in the mastitis-affected right teat was found more than the other teat. After feeding of

Table 2. Effect of *Morinda* on EC (mho) of milk.

Group	Pre-feeding	Post-feeding
Healthy cow	$4.17 \pm 0.21 \times 10^{-5}$	$4.16 \pm 0.01 \times 10^{-5}$
Sub-clinical mastitis	$5.97 \pm 0.81 \times 10^{-5}$	$4.77 \pm 0.142 \times 10^{-5}$*

*Significantly differ at $p < 0.05$.

Table 3. Effect of *Morinda* on microbial load of milk.

Group	Pre-feeding	Post-feeding
Healthy cow	$1.58 \pm 0.13 \times 10^8$	$1.50 \pm 0.07 \times 10^8$
Sub-clinical mastitis	$5.15 \pm 0.03 \times 10^8$*	$2.54 \pm 0.03 \times 10^8$*

*Significantly differ at $p < 0.05$.

morinda the total bacterial count in the mastitis-affected milk decreased significantly to $2.54 \pm 0.03 \times 10^8$ cfu/ml of milk (Table 3).

Effect on total protein concentration

The total protein concentration in the normal cow's milk and mastitis-affected cow's milk was 21.40 ± 1.66 and 41.83 ± 6.56 μg/ml, respectively (Table 4). The morinda feeding decreased the protein concentration in the mastitis milk significantly to 21.13 ± 0.93 μg/ml (Table 4). In case of healthy animals no significant change in milk total protein concentration was found.

Effect feeding of **M. citrifolia** *on milk yield*

No significant changes were observed in terms of the total daily milk yield of both the group of animals (Table 5). However, in the mastitis-affected cow the milk yield was found to be improved.

Discussion

Mastitis is an important mammary gland infection that is usually caused by bacteria. If untreated, it constitutes a serious problem in dairy herds with considerable economic losses, mainly due to poor milk quantity and quality (Seegers et al. 2003) as well as treatment cost (Beck et al. 1992). Several bacterial pathogens were reported to cause mastitis such as *Staphylococcus aureus* which was recorded as one of the most important etiologic agents in mastitis in dairy animals (Mork et al. 2005). The effects of mastitis on milk yield and milk quality in cows have been reported by several workers (Hortet and Seegers 1998; Seegers et al. 2003; Gröhn et al. 2004; Wilson et al. 2008; Tripaldi et al. 2010). Somatic cell count (SCC) is a measure that is widely used to assess mammary health (Smith 2002). In fact only milk from a healthy udder produces milk of a physiologically

Table 4. Effect of *Morinda* on total protein concentration (μg/ml) of milk.

Group	Pre-feeding	Post-feeding
Healthy cow	21.40 ± 1.66	20.80 ± 1.13
Sub-clinical mastitis	41.83 ± 6.56	21.13 ± 0.93*

*Significantly differ at $p < 0.05$.

Table 5. Effect of *Morinda* on milk yield.

Group	Pre-feeding	Post-feeding
Healthy cow	7.56 ± 0.15	7.46 ± 0.19
Sub-clinical mastitis	4.38 ± 0.75	4.48 ± 0.16

normal composition (Hamann 2002). Mastitis reduces milk yield and alters milk composition. The magnitude of these changes in individual cows varies with the severity and duration of the infection and the causative microorganisms. Mastitis is almost always caused by bacteria. These microorganisms produce toxins that can directly damage milk-producing tissue of the mammary gland, and the presence of bacteria initiates inflammation within the mammary tissue in an attempt to eliminate the invading microorganisms. The inflammation contributes to decreased milk production and is primarily responsible for the compositional changes observed in milk from infected quarters and cows. In general, compositional changes involve an increase in blood components present in milk and a decrease in normal milk constituents.

In the present study, the pH of mastitis-affected milk was higher than the normal milk (Table 1), which is consistent with the results of previous reports (Sena and Sahmani 2001; Vijayalakshmi et al. 2001; Bruckmaier et al. 2004). Batavani et al. (2007) also reported a similar increase in pH of milk infected with sub-clinical mastitis. Sodium and chloride increases in high SCC milk due to increased passage of these minerals from blood into milk. Potassium, normally the predominant mineral in milk, declines due to its passage out of milk to lymph between damaged secretary cells. Most of the calcium in milk is associated with casein, and disruption of casein synthesis results in reduced calcium levels in milk from mastitis cows. These alterations in mineral content affect the pH and conductivity of milk. In our present study, feeding of *M. citrifolia* significantly decreased the pH of mastitis-affected milk (Table 1).

EC measures the ability of a solution to conduct an electric current between two electrodes. EC of milk is a measure of the resistance of milk to an electric current; conductivity is the reciprocal of the resistance. The concentration of anions and cations, with Na^+, K^+ and Cl^- as the most important, determines the EC of milk. Mastitis milk has a higher EC than normal milk (Norberg et al. 2004). Typical EC of milk from an uninfected cow varies between 4.0 and 5.5 mS/cm at 25°C. During infections with mastitis, the milk concentration of lactose and K^+ are decreased and concentrations of Na^+ and Cl^- are increased because of increased blood capillary permeability, the destruction of tight junctions and the destruction of action ion-pumping systems (Kitchen

et al. 1980). The EC of the milk changes due to change in the ionic balance of the milk. EC of milk has been introduced as an indicator trait for mastitis over the last decade (Hamann and Zecconi 1998). The cow which suffers from mastitis, the concentration of Na^+ and Cl^- in the milk increases, which leads to increased EC of milk from the infected quarter (Kitchen et al. 1980). A cow suffering from mastitis may not always show an increased EC of milk from the infected quarter, but within-milking variation in EC of milk from an infected quarter may be larger than variation in EC of milk from healthy quarters. Possible reasons for this are physical changes in mastitis milk, which may affect milk flow. In case of mastitis EC will vary in the range of 5.5–8.0 mS. In the present study also it was found that the EC of mastitis-affected milk ($5.97 \pm 0.81 \times 10^{-5}$) was higher than the EC of healthy milk ($4.17 \pm 0.21 \times 10^{-5}$) (Table 2). Feeding of *M. citrifolia* fruit juice significantly decreased the EC of mastitis-affected milk (Table 2) indicating the possibility of treatment of mastitis by this medicinal plant.

In the present study, total protein concentration of mastitis-affected milk was higher than the normal milk (Table 4), which is consistent with the results of previous reports (Singh and Ganguli 1975). Although there may be little change in the total protein content as a result of sub-clinical mastitis, there are marked and significant changes in the types of proteins present such as casein. Casein content of milk with a high SCC is reduced, but lower quality whey proteins increase in concentration, resulting in similar total protein content. The lower quality whey proteins are blood serum proteins such as serum albumin, immunoglobulin and transferrin, which increase in milk as a result of the destruction of membranes that normally prevent blood serum proteins from entering into milk. After feeding of *M. citrifolia* to the mastitis-affected cows, a significant decrease in total protein concentration in milk was observed (Table 4). Zhang et al. (2010) reported that the Chinese herbal formula Shen Qi Yi Mu Tang could improve immunity in post-partun cows.

Conclusion

It could be concluded that the feeding of *M. citrifolia* fruit juice to dairy cows showed improvement in the quality and biophysical parameters of milk of mastitis-infected dairy animals and could be used as herbal medicine for the treatment of mastitis.

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