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Effect of Ascorbic Acid on Milk Lead and Cadmium Level on Subclinical and Clinical Cases of Mastitis

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Mastitis is a global problem in dairy cows because of its high morbidity rate and adverse effects on quality, quantity and processing properties of milk. The annual incidence of subclinical and clinical mastitis in India was reported as 43.9 and 6.4 per cent, respectively in dairy cows (Singh and Singh 1994). Mastitis is associated with compositional changes in milk (Tallomy and Randolph 1970). Significantly higher concentration of trace minerals viz. zinc, copper and cobalt (Naresh et al. 2001, Banga et al. 1989) and toxic metal such as lead (Naresh et al. 1999) have been noted in milk of dairy cows affected with mastitis. The increased milk lead level beyond permissible limit possesses a serious health hazard and has public health significance (Namihira et al. 1993).

MATERIALS AND METHODS

California mastitis test (CMT) and somatic cell count (SCC) were used to diagnose subclinical mastitis (Doxey 1983). Mastitis was classified as subclinical and clinical based on established parameters (Radostits et al. 1994). Thirty-six cows suffering with mastitis (18 clinical and 18 subclinical) were randomly selected for the study. Further, the cases of clinical and subclinical mastitis were divided into two groups. One group of clinical mastitis (n=12) was treated with ascorbic acid @ 25 mg/kg, subcutaneously for 5 consecutive days along with antimicrobial intramammary infusion (standard dose) for 3-5 days while the other group of clinical mastitis (n=6) was treated only with antimicrobial intramammary infusion (standard dose) for 3-5 days. Animals diagnosed to suffer from clinical mastitis were treated with antimicrobial therapy on ethical ground. However, one group of subclinical mastitis (n=12) was treated with ascorbic acid only and other group (n=6) was not given any treatment. A separate group of healthy lactating animals were also included in the experiment to serve as untreated control.

Approximately five ml each of milk and blood samples were collected in nitric acid washed vials (heparinised for blood) before treatment with ascorbic acid and thereafter on days 6 and 15 of the study and samples were digested (AOAC 1984). Five ml of milk/blood sample was placed in a digestion tube after mixing with 5 ml of concentrated nitric acid and kept for over night at room temperature.

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Samples were heated with the help of digestion assembly. When the volume reduced to 1 ml, the double acid mixture (nitric acid 4 parts and perchloric acid 1 part) was added to each digestion tube and further digested till the volume was reduced to 1 ml and the colour became transparent. Five ml of distilled water was digested in same way along with each batch of 25 samples to serve as control for digestion process. The digested samples were diluted with distilled water and final volume was made to 10 ml. The concentration of lead and cadmium was estimated in diluted samples by using atomic absorption spectrophotometer (AAS 4129, ECIL, Hyderabad, India) as per the methods laid down in operation manual of the instrument. Analytical quality was maintained by repeated analysis of reference samples. Working standards were prepared freshly from stock standards (Sigma Company, USA) for calibration.

The data were analyzed using student T test (Snedecor and Cocharan 1967).

RESULTS AND DISCUSSION

The lead and cadmium concentrations in milk and blood of ascorbic acid treated and untreated animals are shown in table 1 and 2. In ascorbic acid treated animals with clinical mastitis, the milk lead level increased significantly on days 6 and 15. A significant increase in milk Pb level was also recorded in subclinical mastitis after treatment with ascorbic acid on days 6 and 15 post treatment. No significant changes were noticed during the study in excretory pattern of Pb in milk of non ascorbic acid treated cows irrespective of clinical status of mammary gland. The milk Cd concentration remained unchanged throughout study.

Table 1. Lead and cadmium (Mean±S.E.) levels (ppm) in milk of dairy cows before and after ascorbic acid treatment

Metal	Post treatment day	Clinical mastitis		Subclinical mastitis	
		Non ascorbic acid treated	Ascorbic acid treated	Non ascorbic acid treated	Ascorbic acid treated
Lead	0	0.33±0.03	0.32±0.04	0.35±0.05	0.31±0.03
	6	0.28±0.03	0.45±0.05*[a]	0.32±0.03	0.48±0.03*[a]
	15	0.26±0.05	0.51±0.03*[a]	0.33±0.03	0.47±0.12*
Cadmium	0	0.12±0.01	0.15±0.01	0.11±0.02	0.13±0.01
	6	0.14±0.02	0.13±0.01	0.14±0.01	0.13±0.01
	15	0.13±0.01	0.14±0.01	0.15±0.02	0.12±0.01

* Significantly ($P<0.05$) different from day 0

a Values differ significantly ($P<0.05$) ascorbic acid treated and untreated controls

Blood Pb and Cd concentration is presented in table 2. There was no significant alteration in blood Pb concentration in ascorbic acid treated animals with clinical mastitis. While blood lead level was significantly ($P<0.05$) low in clinical mastitis cows after ascorbic acid treatment than untreated on day 6. The blood Pb level was significantly ($P<0.05$) lower in ascorbic acid treated cows with subclinical mastitis on day 15. In untreated subclinical mastitic cows the blood Pb level decreased non-significantly on days 6 and 15 of the study. The blood Cd profile did not show any change in ascorbic acid treated and untreated cows.

Table 2. Lead and cadmium (Mean±S.E.) levels (ppm) in blood of dairy cows before and after ascorbic acid treatment

Metal	Post treatment day	Clinical mastitis		Subclinical mastitis	
		Non ascorbic acid treated	Ascorbic acid treated	Non ascorbic acid treated	Ascorbic acid treated
Lead	0	0.50±0.02	0.40±0.06	0.62±0.05	0.53±0.06
	6	0.57±0.08	0.37±0.04[a]	0.50±0.06	0.40±0.05
	15	0.50±0.09	0.40±0.05	0.50±0.06	0.39±0.04*
Cadmium	0	0.08±0.02	0.08±0.01	0.10±0.01	0.07±0.01
	6	0.09±0.02	0.08±0.01	0.08±0.01	0.08±0.01
	15	0.06±0.01	0.08±0.01	0.09±0.01	0.07±0.01

* Significantly ($P<0.05$) different from day 0
a Values differ significantly ($P<0.05$) ascorbic acid treated and untreated controls

The Pb and Cd levels in milk and blood of healthy cows remained totally unchanged in study period. The milk lead values were 0.23±0.02, 0.27±0.02 and 0.24±0.05 ppm on days 0, 6 and 15 respectively. The Blood lead values were 0.44±0.03, 0.47±0.04 and 0.39±0.07 ppm on days 0, 6 and 15, respectively. While the milk and blood cadmium values were like the treated and untreated mastitic animals.

Ascorbic acid plays an important role in distribution and excretion of trace minerals and toxic metals (Lewin, 1974). Hughes (1974) reported that ascorbic acid is a diffusible biological reductant when present in the appropriate concentration, and contributes to the maintenance of the integrity of SH group of many proteins. The SH group of proteins is mainly responsible for metal interactions or bindings. L-ascorbic acid is a strong antioxidant and may extent its protective effects by chelating the metal or by precipitating free radicals and

removing them from the system (Tajmir- Riahi 1991). In a human survey, the serum ascorbic acid concentration was inversely associated with the prevalence of elevated blood lead concentrations (Houston and Johnson 2000). Our study revealed that inflammatory udder condition enhanced the excretion of lead in milk (Naresh et al. 1999) and the present study showed significantly ($P<0.05$) higher milk lead concentration after treatment with ascorbic acid. Our findings are in agreement with Flora and Tandon (1986) who reported that vitamin C alone or in combination with thiamine hydrchloride was capable of alleviating the inhibition of delta-aminolevulinic acid dehydratose activity in acute Pb poisoning. Ascorbic acid also reported to reduce the levels of Pb in blood, liver, and kidney during Pb exposure in rats. The ascorbic acid acted as detoxifying agent by forming poorly ionized, but soluble compound with Pb (Pillemer 1940). However, in the present study, significant reduction in blood lead level was recorded in cows with subclinical mastitis after treatment with ascorbic acid.

The blood Cd levels remained extremely low even with high dietary Cd and intravenously injected Cd disappears rapidly from the blood (Miller et al. 1968, Neathery et al. 1974) and the secretion of Cd in to the bovine milk is very little (Neathery et al. 1974, Miller et al. 1967, Miller 1973). The mechanism by which the cow selectively keeps most of the Cd out of milk has not been determined. However, a large Cd binding protein, isolated from the mammary gland of lactating rats may have an important role in limiting Cd transfer to milk (Lucis et al. 1972). However, similar binding proteins have not been identified in the mammary gland of dairy cows. The low affinity of blood for Cd and restricted excretion of Cd in milk by mammary gland could be responsible for no alteration in Cd concentration either in milk or blood. There are sufficient literary evidences that ascorbic acid affects the Cd accumulation, mobilization and excretion at higher doses in a longer duration in animals (Hughes 1974, Kapl et al. 1994; Rothe et al. 1994, Seemuller 1992). However, in present study, the dose rate of (25 mg/kg) and the duration of 5 days could be too low to influence the Cd metabolism as compared to earlier reports (Kapl et al. 1994, Rothe et al. 1994).

It is concluded from the present investigation that the ascorbic acid administration either alone in cases of subclinical mastitis or in combination with antimicrobial therapy in clinical mastitis enhanced the milk lead level thus posing higher threat for human consumption of such milk particularly in areas of lead pollution. Our findings suggest that milk of ascorbic treated dairy cows for mastitis should not be used for human consumption if toxic metal profile is already at upper side in normal milk. Further investigations are warranted urgently to evaluate the duration of toxic metal mobilization in milk after ascorbic acid treatment in women and dairy cows.

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