

MINERAL ANTIOXIDANT STATUS IN SERUM AND ITS RELATIONSHIP WITH SOMATIC CELL COUNT IN CAMEL MILK

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ABSTRACT

Serum Zn, Cu, Co and Fe concentration was estimated in lactating camels having different types of mastitis. Mean serum Zn, Cu and Fe concentration varied non-significantly between negative, subclinical, non-specific and clinical groups ($P < 0.05$). However, Co concentrations in these groups were 1.78 ± 0.12 , 1.34 ± 0.18 , 1.26 ± 0.10 and $0.70 \pm 0.41 \mu\text{g/ml}$, respectively which were statistically significant among groups ($P < 0.05$).

Mean serum Zn, Cu, Co and Fe status of animals having SCC upto 2.0, 2.0-5.0, 5.0-10.0 and > 10 lacs were 1.30 ± 0.60 , 1.56 ± 0.16 , 2.42 ± 0.84 and 1.78 ± 0.12 ; 2.37 ± 0.32 , 1.35 ± 0.08 , 3.93 ± 0.46 and 1.26 ± 0.10 ; 2.45 ± 0.51 , 0.95 ± 0.14 , 2.77 ± 0.71 and 1.41 ± 0.16 ; 2.60 ± 0.95 , 1.25 ± 0.25 , 3.35 ± 1.33 and $1.35 \pm 0.29 \mu\text{g/ml}$, respectively. Here the difference in Serum Zn, Fe and Co concentration varied non-significantly among groups ($P < 0.05$). However, Cu concentration varied significantly among groups ($P < 0.05$).

Key words: Camel, mastitis, mineral anti-oxidant, milk, somatic cell

Deficiencies of antioxidant micronutrients like copper and zinc are reported to have detrimental impact on the efficient functioning of immune system (Benedich, 1993). Therefore, one of the latest control concepts in mastitis includes optimising the resistance of the mammary gland to infection either by supplementation of nutrients required for enhancement of immune mechanism of the udder or the immune status of the animal in general or both. Immune function may be enhanced not only by maintaining appropriate amounts of antioxidants, but also by the involvement of enzymes in aiding the production of energy, proteins, cells and hormones in defence mechanisms. Some of the antioxidants are those enzymes that help reduce oxidants in cells e.g. Cu, Zn superoxide dismutase, etc. According to Radostits *et al* (2000), trace elements like Cu, Se, Zn, Co, Fe, I, Mn and Mo are nutritionally essential for ruminants.

It is speculated that trace element deficiencies are wide spread, but their incidence and importance are probably underestimated because subclinical forms of deficiency can occur and go unnoticed for prolonged periods. Therefore, the present study was envisaged to find out mineral antioxidant status in

serum and to establish its relationship with somatic cell count in camel milk.

Materials and Methods

The study was conducted at an organised farm. Aseptically collected, 128 quarter milk samples from 32 apparently healthy camels were evaluated for the prevalence of mastitis. Somatic cell count (SCC) was performed as per the method of Schalm *et al* (1971), using Giemsa stain. For bacteriological examination standard procedures were followed (Brown *et al*, 1981) using 5 per cent sheep blood agar and MacConkey's lactose agar. Based on the results of cultural examination and somatic cell count, the animals were divided into four groups:

The grouping of the animals was done as per International dairy federation criteria adopted for cattle.

Group 1: Negative (Culturally -ve and $\text{SCC} < 500000$ per ml).

Group 2: Subclinical (Culturally +ve and $\text{SCC} > 500000$ per ml).

Group 3: Latent (Culturally +ve and $\text{SCC} < 500000$ per ml).

Group 4: Non-specific (Culturally -ve and $\text{SCC} > 500000$ per ml).

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Blood sample from one clinically infected animal was taken as a separate group. The samples were collected from these lactating female camels, taking sterile precautions. Blood samples were harvested by jugular venepuncture and serum was separated.

Serum (2.5 ml) was mixed with equal volume of nitric acid in Kjeldhal digestion tube. The samples were kept overnight at room temperature and then heated over digestion bench using low heat below 90° C till the volume of the sample was reduced to 0.5 ml. It was later cooled and 5.0 ml. of double acid mixture containing three parts of nitric acid and one part of 70% perchloric acid was added to it. The samples were again transferred to digestion bench for slow digestion. This procedure was repeated till white fumes emanated and the volume was reduced to 0.5 ml. The digested samples were cooled and diluted to 25 ml with distilled water. The digested samples were subjected to estimation of the minerals by atomic absorption spectrophotometer (model AAS4141 of ECIL, Hyderabad, India). Copper, Zn, Fe and Co were estimated at wavelength 324.4 nm, 212.9 nm, 248.3 nm and 240.4 nm and sensitivity was checked at 1.50 ppm, 1.00 ppm, 2.00 ppm and 2.00 ppm, respectively.

Results and Discussion

A non-significant variation among negative, sub clinical, non-specific and clinical groups ($P < 0.05$) was recorded in the mean serum Zn, Cu and Fe concentrations. However, Co concentrations recorded in these groups were 1.78 ± 0.12 , 1.34 ± 0.18 , 1.26 ± 0.10 and 0.70 ± 0.41 $\mu\text{g/ml}$, respectively which varied significantly ($P < 0.05$) among groups (Table 1). The latent type of cases were not recorded in the present study. Cultural examination of milk of clinically infected animal revealed β -haemolytic staphylococci.

Mean serum values of Zn, Co and Fe varied non-significantly among groups ($P < 0.05$). However, Cu concentration varied significantly among groups ($P < 0.05$) (Table 2).

Values reported by Damir (1998) for levels of copper, zinc and iron in camel blood plasma do not correspond with present values. Recordings are partially matching to the reports of Noro *et al* (1992) who also failed to observe any significant difference in the serum Cu levels during lactation and dry period in the healthy and affected cows. Whereas, Sanders and Sanders (1983) observed that supplementation of Cu and reduction of the sulphur content of the water resulted in improved herd health, greater reproductivity, lower calf mortality and increased milk yield in dairy herd. Xin *et al* (1991) showed that neutrophils from Cu deficient steers had significantly lower capacity to kill *S. aureus* than neutrophils from Cu supplemented animals.

The keratin lining of teat canals in susceptible quarters is thinner, less dense and detached from the epithelium compared with that in resistant quarters (McDonald, 1970), therefore, Zn supplementation decreases the exposure of teat ends to pathogens. Spain (1994) reported lower incidence of mastitis in cows receiving Zn in their ration. Incidence and severity of mastitis is high during early lactation. Such cows in early lactation develop borderline zinc deficiency, accentuated by high levels of calcium in diet (Miller, 1970). Moderate to severe blood lypozincemia during mastitis particularly acute coliform mastitis has been documented (Lohuis *et al*, 1990). Harmon and Torre (1994) also suggested a decrease in the percentage of infected quarters and a reduction in SCC at calving in cows supplemented with Zn as compared to untreated controls. Whitaker *et al* (1997) in paired dairy cows, did not observe

Table 1. Mean serum Zn, Cu, Co and Fe concentration of mastitic camels.

Type of mastitis	Number of animals	Zn conc. ($\mu\text{g/ml}$)	Cu conc. ($\mu\text{g/ml}$)	Co conc. ($\mu\text{g/ml}$)	Fe conc. ($\mu\text{g/ml}$)	Mean SCC ($\times 10^5$)
Effect				*		
Negative	5	1.30 ± 0.58	1.56 ± 0.17	1.78 ± 0.12	2.42 ± 0.83	369025 ± 209717
Sub clinical	16	2.62 ± 0.33	1.19 ± 0.10	1.34 ± 0.18	3.94 ± 0.48	1059593 ± 117235
Latent	0	-	-	-	-	-
Non-specific	11	2.12 ± 0.39	1.33 ± 0.12	1.26 ± 0.10	3.07 ± 0.56	867606 ± 141391
Clinical	1	2.20 ± 1.30	1.80 ± 0.39	0.70 ± 0.00	6.90 ± 1.86	Clumps of cells on direct microscopic examination.

*Significant ($p < 0.05$).

Table 2. Relationship between mean SCC and mean Zn, Cu, Co and Fe concentration.

SCC per ml of milk (lacs)	Number of animals	Mean ± S.E. Zn conc. (µg/ml)	Mean ± S.E. Cu conc. (µg/ml)	Mean ± S.E. Fe conc. (µg/ml)	Mean ± S.E. Co conc. (µg/ml)
Effect			*		
Upto 2.0	5	1.30 + 0.60	1.56 + 0.16	2.42 ± 0.84	1.78 ± 0.12
>2.0-5.0	18	2.37 + 0.32	1.35 + 0.08	3.93 + 0.46	1.26 ± 0.10
>5.0-10.0	7	2.45 ± 0.51	0.95 + 0.14	2.77 ± 0.71	1.41 ± 0.16
>10.0	2	2.60+ 0.95	1.25 + 0.25	3.35 + 1.33	1.35 ± 0.29

*Significant (p<0.05).

significant difference between the Zn supplemented and control groups in terms of clinical mastitis, mastitis caused by environmental organisms, new infection or recovery rate and SCC during the first 100 days of lactation.

Clinical studies are supported by experimental studies using other well known pathogenic bacteria *E.coli* which on producing the mastitis in Holstein cows resulted in a decrease in the mean serum concentration of Zn, Fe and Cu of 28, 35 and 52 per cent of prechallenge concentration, respectively (Erskine and Bartlett, 1993).

Lactating ewes were inoculated through the teat canal with *Mycoplasma mycoides var. mycoides* by Banga *et al* (1989), and on comparison with normal milk, mastitic milk showed increase in Cu and Fe and no changes in Co and Zn concentration. In udder tissues, concentration of Cu and Zn increased, whereas concentration of Fe and Co decreased.

Endotoxin mastitis induced by Lappalainen *et al* (1988) to determine the effect of endotoxin induced inflammation exerted its effect on transfer of selective elements into milk during the course of inflammation and milk Cu and Fe closely paralleled the increase of milk BSA, indicating that these elements are transferred from blood to milk during inflammation. Shang-Chang Fa *et al* (1996) analysed the trace element content in hair samples from healthy and latent mastitic dairy cows. The contents of Zn, Cu and Co in the samples from the infected cattle were lower than those of the normal cows, but the Fe, Pb and Se levels of hair from the infected and normal cows did not vary significantly.

It is concluded that mean serum Zn and Fe concentration in all the non clinical mastitic groups did not vary significantly in camels. Whereas, Cu and Co was found to have some effect either in terms of mastitis or SCC in milk.

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References

- Banga MS, Gupta PP and Singh R (1989). Changes in minerals and electrolytes in milk and udder tissues during experimental mycoplasmal mastitis in sheep. *Indian Journal of Animal Sciences* 59(5):555-557.
- Bendich A (1993). Physiological role of antioxidants in the immune system. *Journal of Dairy Science* 76:2789-2794.
- Brown RW, Barnum DA, Jasper DF, McDonald JS and Schultze WD (1981). Microbiological procedures for use in the Diagnosis of Bovine Mastitis. 2nd Edn. National Mastitis Council, Inc., 30F street, N.W. Washington, D.C.
- Damir HA (1998). Mineral deficiencies, toxicities and imbalances in the camels: a review. *Veterinary Bulletin* 68(10):1103-1119.
- Erskine RJ and Bartlett PC (1993). Serum concentrations of Cu, iron and Zn during *E. coli* -induced mastitis. *Journal of Dairy Science* 76:400-413.
- Harmon RJ and Torre PM (1994). Copper and zinc: Do they influence mastitis. Proceedings National Mastitis Council Annual Meeting. Madison, WI. pp 54.
- Lappalainen R, Kaartinen L, Veijalainen K, Kuosa PL, Sankari S, Pyorala S and Sandholm M (1988). Sequential changes of mineral and trace elements in milk during the course of endotoxin induced mastitis as analysed by particle induced-x-ray (PIXE), gamma ray emission (PICE) and ion selective electrodes. *Journal of Veterinary Medicine, B. Infectious Diseases, Immunology, Food Hygiene, Veterinary Public Health* 35:664-674.
- Lohuis JACM, Schukken YH, Verheijden JHM, Brand A and Van Miert ASJPAM (1990). Effect of severity of systemic signs during the acute phase of experimentally induced *Escherichia coli* mastitis on milk production losses. *Journal of Dairy Science* 73:333-341.
- McDonald JS (1970). Microscopic observations of teat canals from susceptible and resistant bovine mammary glands. A preliminary report. Proceedings, 6th International Conference of Cattle Disease. Philadelphia, PA. pp 97.
- Miller WJ (1970). Zinc nutrition of cattle: A Review. *Journal of Dairy Science* 53:1123-1135.

Noro A, Higuchi K, Ishida Y, Nakajima N, Saitoh T and Tomabechi T (1992). Changes in serum alpha tocopherol, selenium and related enzymes in three cows with mastitis. *Journal of the Japan Veterinary Medical Association* 45(2):84-88.

Radostits OM, Gay CC, Blood DC and Hincheliff KW (2000). Disease caused by deficiencies of mineral nutrients. In: *A Textbook of the Disease of Cattle, Sheep, Pigs, Goats and Horses (Veterinary Medicine)* 9th Ed., W.B. Saunder and Co., London, UK. pp 1480.

Sanders DE and Sanders JA (1983). Diagnosis of Cu deficiency in dairy cattle. *Modern Veterinary Practice* 64(8):613-616.

Schalm OW, Carroll EJ and Jain NC (1971). *Bovine Mastitis*, 1971. Lea and Febiger, Philadelphia, USA.

Shang-Chang Fa, Li-GuangHui, Huang-BiXia, Shang-CF and Huang-Bx (1996). Determination of trace elements of hairs of dairy cows with latent mastitis. *Chinese Journal of Veterinary Science Technology* 26(8):40-41.

Spain JN (1994). Tissue integrity: a key defense against mastitis infection. The role of zinc proteinates and theory for a mode of action. *Proceeding 8th Annual Altech European Lecture Tour* 125-132.

Whitaker DA, Eayres HF, Aitchison K and Kelly JM (1997). No effect of a dietary Zn proteinate on clinical mastitis, infection rate, recovery rate and somatic cell count in dairy cows. *Veterinary Journal* 153:197-204.

Xin Z, Waterman DF, Hemker RW and Harmon RJ (1991). Effects of copper status on neutrophil function, superoxide dismutase and copper distribution in steers. *Journal of Dairy Science* 74:3078-3085.

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