



Trace mineral status of wall and sole portions of healthy and diseased hoofs in cattle and buffaloes

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Received: 7 March 2012; Accepted: 23 December 2013

ABSTRACT

Trace mineral composition of wall and sole portions of healthy and diseased hoofs was analyzed in cattle and buffaloes from organized and unorganized dairy farms. Hoof samples were collected from 10 animals each with healthy and diseased hoofs from each of the 4 groups (cattle-organized farms, cattle-unorganized farms, buffalo-organized farms, buffalo-unorganized farms). A significant high level of zinc and copper was observed in the wall portion as compared to sole portion in both healthy and diseased hoofs in cattle and buffaloes. The manganese and iron were significantly low in wall portion as compared to sole portion in healthy as well as diseased hoofs in both cattle and buffaloes. Although not much difference in the zinc levels of healthy and diseased hoofs from cattle and buffaloes was observed but buffaloes from unorganized farms have low hoof zinc levels as compared to those of organized farms. Significantly low copper levels were observed in diseased hoofs as compared to those of healthy in dairy cattle whereas high iron levels were observed in diseased hoofs as compared to healthy ones in dairy buffaloes. It was concluded that trace minerals do have a role in healthy horn synthesis.

Key words: Copper, Manganese, Mineral, Sole, Toe, Zinc

Lameness is a major constraint to the dairy industry due to its significant effect on production and reproduction performance of dairy animals after mastitis and reproductive failures. The major determinants of lameness include management, housing and good nutrition along with cow level factors like parity, stage of lactation, body weight and genetics (Vermunt and Greenough 1994). Calcium is required for activation of epidermal transglutaminase which is necessary for keratinization as well as terminal differentiation of the epidermal cells (Mulling *et al.* 1999). Trace minerals play an important role in production and maintenance of healthy keratinized tissues (Mulling *et al.* 1999). Increasing the bioavailability of trace minerals like zinc, copper and manganese can help in improving the integrity of keratinized tissues (Ballantine *et al.* 2002). Zinc plays a key role in the formation of the structural proteins during the keratinization process (Cousins 1996). Cows fed an additional 200 mg/day of zinc from zinc methionine had fewer cases of foot rot, heel cracks, interdigital dermatitis and laminitis than cows not fed zinc methionine (Moore *et al.* 1989). Copper also activates thiol oxidase enzyme which is responsible for the formation of the disulfide bonds

between Cys residues of the keratin filaments (O'Dell 1990). Manganese also helps in minimizing the feet problems by maintaining proper leg formation (Radostits *et al.* 2003).

The present study was conducted to assess the levels of zinc, copper and manganese in the hoof tissue. Iron status is also analyzed because copper containing enzyme ceruloplasmin helps in mobilization of iron from iron stores for hemoglobin synthesis. As buffaloes seem to have harder hoofs than cows, the samples from their hoofs were separately analyzed. In fact, this is the first study on mineral status of hoofs in buffaloes.

MATERIALS AND METHODS

Hoofs from animals which have no characteristic foot lesion in any of the digit were placed in healthy group.

Diseased hoof group: Hoofs from the animals, which have any of the following foot lesions such as heel erosions, sole hemorrhages, sole ulcers, white line hemorrhages, white line fissures, toe hemorrhage, abaxial wall overgrowth, abaxial wall degeneration, underrun soles, double soles, overgrown hoofs, overgrown soles, corkscrew hoofs, slipper/slatted foot in any of the digit were placed in diseased group.

Collection of samples: Hoof trimming of animals from both organized and unorganized dairy farms was undertaken while doing the epidemiology of foot lesions. Ten animals each for healthy and diseased hoof groups were selected randomly from each of the 4 groups (cattle-organized farms, cattle-unorganized farms, buffalo-organized farms, buffalo-

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unorganized farms). To reduce variation between farms, if a sample from a healthy hoof group was taken at a particular farm, a sample from the diseased hoof group was also taken from the same farm. The hoof samples were collected from all the eight digits of the selected animal. The hoof slivers obtained from the solar surface during hoof trimming were sliced into two portions and portion outside the white line was taken as wall and inside the white line was taken as sole portion. Wall and sole portions were mixed separately, washed, dried and thoroughly grinded.

Analysis: Half a gram of each wall and sole sample was taken and kept overnight in 8 ml of distilled concentrate nitric acid in a conical flask. Next morning, samples were digested on a hot plate first at light heat and then at maximum heat. When 1–2 ml of solution was left, it was removed from the hot plate and 2 ml of perchloric acid was added and heated at maximum heat until 1 ml solution was left. The final volume was made up to 10 ml with distilled water. Minerals were estimated as per standard procedure with the help of atomic absorption spectrophotometer.

Statistical analysis: Data were analyzed using factorial in CRD with the help of GSTAT and CPCS software developed by Department of Maths and Statistics, Punjab Agricultural University, Ludhiana.

RESULTS AND DISCUSSION

Wall and sole zinc concentrations in the hoofs of cattle and buffaloes are presented in Tables 1a, b. The significant differences in the zinc concentrations of wall and sole portions of hoofs in cattle and buffaloes, respectively, were in agreement with the findings of Baggot *et al.* (1988) who concluded that wide variation in mineral composition was there in respect of site of hoof sampling. This could be further associated with differences in hardness of keratin at different sites of hoof (personal observation). It seems that keratinocytes are more tightly packed in the wall portion of hoofs as compared to sole portion. Low amount of total lipids was observed in wall/toe portion (9.03 mg/g) as compared to sole portion (15.36 mg/g) (Randhawa 2008) that could be due to less amount of intercellular cementing

substance between tightly packed keratinocytes in the wall portion. As a result, more number of keratinocytes per unit of wall tissue may be responsible for significantly more amounts of zinc, an intracellular element in the wall portion in the present study. Further, Arkins *et al.* (1986) reported highest water content in softest horn (sole) and lowest in hardest horn (toe or wall) which might be a factor for decrease in mineral content per unit of sole tissue as compared to wall. Baggot *et al.* (1988) reported low levels of zinc in claws of lame cows as compared to normal cows which was not apparent in the present study. The significant difference between the zinc levels of buffaloes on organized and unorganized farms may be due to irregular use of mineral mixture on unorganized farms. A comparatively similar concentration of zinc was observed by Kalsi (1997). The improvement in the heel erosions, sole avulsions and overall disease score of claws was observed in zinc supplemented cows in a study though there was not much increase in the levels of zinc in the hoof tissue after 5 months of supplementation of zinc methionine (Randhawa 2006). This could be a reason that we could not get much alteration in the zinc levels of healthy and diseased hoofs in the present study.

A significant decline (Table 2a, b) was observed in the overall copper levels of diseased hoofs in dairy cattle where as decline in diseased hoofs in buffaloes were non-significant. A significant wide gap was observed between wall and sole copper levels in both cattle and buffaloes. The significant decline in overall copper levels of diseased hoofs in cattle in the present study might have caused decreased strength of horn tissue due to defective cross-linking of keratin filaments. Copper is required for activation of cytochrome oxidase enzyme involved in aerobic respiration, lysyl and thiol oxidases for structural integrity of cells, ceruloplasmin essential for absorption and transport of iron for hemoglobin synthesis and superoxide dismutase which works with zinc in reducing the toxic effects of oxygen metabolites (NRC 2001). The activity of thiol oxidase is of greatest importance in the keratinizing horn cell. Copper activates thiol oxidase enzyme which is

Table 1a. Zinc status of healthy and diseased hoofs of cattle and buffaloes at organized and unorganized dairy farms (mean±SE)

Status of hoof	Part of hoof	Cattle (n=10) (Organized farms)	Cattle (n=10) (Unorganized farms)	Buffaloes (n=10) (Organized farms)	Buffaloes (n=10) (Unorganized farms)
Healthy	Wall (ppm)	151.60±5.82	150.20±8.55	158.80±5.80	146.40±5.99
	Sole (ppm)	69.80±4.37	63.60±3.68	84.20±9.09	82.00±4.14
Diseased	Wall (ppm)	145.20±3.96	138.00±5.13	147.20±3.77	146.80±5.49
	Sole (ppm)	74.00±3.11	69.80±4.07	95.40±3.41	78.00±4.01

Table 1b. Overall mean zinc levels in healthy and diseased hoofs of cattle and buffaloes

Species	Group 1		Group 2		Group 3	
	Organized	Unorganized	Healthy	Diseased	Wall	Sole
Cattle	110.15	105.40	108.80	106.75	146.25	69.30*
Buffalo	121.40	113.30*	117.85	116.85	149.80	84.90*

Table 2a. Copper status of healthy and diseased hoofs of cattle and buffaloes at organized and unorganized dairy farms (mean±SE)

Status of hoof	Part of hoof	Cattle (n=10) (Organized farms)	Cattle (n=10) (Unorganized farms)	Buffaloes (n=10) (Organized farms)	Buffaloes (n=10) (Unorganized farms)
Healthy	Wall (ppm)	6.10±0.56	5.88±0.18	5.08±0.37	5.46±0.68
	Sole (ppm)	3.46±0.38	2.78±0.28	3.42±0.18	3.53±0.18
Diseased	Wall (ppm)	5.01±0.31	5.13±0.18	4.71±0.16	5.04±0.44
	Sole (ppm)	3.21±0.31	2.64±0.17	3.68±0.35	3.58±0.34

Table 2b. Overall mean copper levels in healthy and diseased hoofs of cattle and buffaloes.

Species	Group 1		Group 2		Group 3	
	Organized	Unorganized	Healthy	Diseased	Wall	Sole
Cattle	4.45	4.11	4.56	4.00*	5.53	3.02*
Buffalo	4.23	4.41	4.37	4.26	5.08	3.55*

responsible for formation of the disulfide bonds between Cys residues of keratin filaments (O'Dell 1990). This process is essential for maintaining structural strength at the cellular level giving rigidity to the keratinized cell matrix.

Cattle suffering from a subclinical Cu deficiency are more susceptible to heel cracks, foot rot, and sole abscesses (Puls 1984). More susceptibility to these lesions could be the result of insufficient cytochrome oxidase activity, resulting in reduced respiration and oxidative phosphorylation and thereby deficient energy supplies for differentiating keratinocytes (Linder 1996). Further, in copper deficiency, reduced activity of the Cu/Zn SOD is expected to enhance the fragility of cell membranes making them more vulnerable to oxidative damage (Linder 1996). The intercellular lipids are an integral part of the cementing substance responsible for cell to cell adhesion (Mulling and Budras 1998). Any nutrient deficiency that leads to the production of inferior intercellular cementing substance or predisposes it to excessive oxidative damage may lead to production of dyskeratotic horn tissue prone to damage. Moreover, copper also protects animals from osteoporosis

of bones by maintaining proper osteoblastic activity (Radosiits *et al.* 2003) so helps in maintaining healthy leg conformation. The shifting from normal to cowhock rear leg view in lame animals might be attributed to the deficiency of copper leading to weaker and osteoporotic bones (Randhawa 2006). Nonsignificant difference in healthy and diseased hoof buffaloes might be due to the reason that buffaloes were less vulnerable to the copper deficiency than cattle being low producing animals. The significant high levels of copper in wall as compared to sole could be similarly explained as high zinc levels in the hoof wall.

A comparatively low level of manganese, although nonsignificant, was noticed in the affected animals as compared to healthy ones (Table 3a, b). Manganese minimizes feet problems by maintaining proper leg formation and by defending animals from different skeletal abnormalities by synthesis of organic matrix of the bones (McDowell 1992). As a part of galactotransferase and glycosyltransferase enzymes, manganese is required for synthesis of chondroitin sulfate side chains of proteoglycan molecules (Keen and Zidenberg-cherr 1996). These

Table 3a. Manganese status of healthy and diseased hoofs of cattle and buffaloes at organized and unorganized dairy farms (Mean±SE)

Status of hoof	Part of hoof	Cattle (n=10) (Organized farms)	Cattle (n=10) (Unorganized farms)	Buffaloes (n=10) (Organized farms)	Buffaloes (n=10) (Unorganized farms)
Healthy	Wall (ppm)	0.532±0.115	0.473±0.163	0.503±0.170	0.085±0.060
	Sole (ppm)	0.506±0.060	1.514±0.766	0.464±0.096	0.956±0.398
Diseased	Wall (ppm)	0.332±0.052	0.300±0.077	0.280±0.052	0.156±0.042
	Sole (ppm)	0.589±0.089	0.971±0.176	0.766±0.065	1.206±0.274

Table 3b. Overall mean manganese levels in healthy and diseased hoofs of cattle and buffaloes

Species	Group 1		Group 2		Group 3	
	Organized	Unorganized	Healthy	Diseased	Wall	Sole
Cattle	0.490	0.814	0.756	0.548	0.409	0.895*
Buffalo	0.505	0.601	0.502	0.603	0.257	0.848*

Table 4a. Iron status of healthy and diseased hoofs of cattle and buffaloes at organized and unorganized dairy farms (mean $\pm$ SE)

Status of hoof	Part of hoof	Cattle (n=10) (Organized farms)	Cattle (n=10) (Unorganized farms)	Buffaloes (n=10) (Organized farms)	Buffaloes (n=10) (Unorganized farms)
Healthy	Wall (ppm)	9.64 $\pm$ 1.89	7.98 $\pm$ 3.77	10.76 $\pm$ 2.70	4.45 $\pm$ 2.38
	Sole (ppm)	23.26 $\pm$ 2.35	18.38 $\pm$ 4.38	14.34 $\pm$ 2.31	17.31 $\pm$ 4.28
Diseased	Wall (ppm)	9.14 $\pm$ 0.97	8.47 $\pm$ 1.52	8.37 $\pm$ 1.66	9.68 $\pm$ 1.47
	Sole (ppm)	23.86 $\pm$ 2.62	27.43 $\pm$ 2.76	17.15 $\pm$ 1.38	29.74 $\pm$ 3.25

Table 4b. Overall mean iron levels in healthy and diseased hoofs of cattle and buffaloes

Species	Group 1		Group 2		Group 3	
	Organized	Unorganized	Healthy	Diseased	Wall	Sole
Cattle	16.48	15.57	14.81	17.23	8.81	23.23*
Buffalo	12.66	15.30	11.72	16.24*	8.32	19.64*

proteoglycans are essential building blocks for the formation of normal cartilage and bone. Animals suffering from Mn deficiency will exhibit skeletal abnormalities, crooked legs and shortening of tendons, as noted by knuckling over of feet (NRC 2001).

Manganese also plays a role in the activation of pyruvate carboxylase, an enzyme that catalyzes the carbohydrate synthesis at the first step. This process is responsible for gluconeogenesis and the production of cellular energy, an essential component in the production of quality horn tissue (Keen and Zidenberg-cherr 1996). Similar to Cu/Zn SOD, Mn plays a role in the activation of Mn superoxide dismutase (Mn SOD) and the removal of superoxide free radicals. Therefore Mn SOD may play a protective role for the lipids involved in cementing together mature keratinocytes. The affected animals may not be able to maintain their proper leg placement and in turn lead to lameness. However, hoof manganese levels in the present study were very low as compared to those reported by Sugg *et al.* (1996) and Kalsi (1997). Significantly high levels of manganese were observed in the sole portion of hoofs as compared to wall in the present study. This might be attributed to the increased blood supply to affected part usually sole which might have increased the mineral incorporation in that part.

Iron levels in wall and sole portions of hoofs of cattle and buffaloes are presented in Table 4a, b. Although, not any direct role in the hoof horn synthesis but nonsignificantly high levels in diseased hoof cattle as compared to healthy hoof cattle were observed in the present study. These findings were similar to the findings of Baggot *et al.* (1988) who reported higher mean contents of this element in the lame or diseased cows as compared to normal cows. However, hoof iron levels in the present study were very low as compared to the hoof iron levels reported by Sugg *et al.* (1996) and Kalsi (1997). Significantly high sole levels of iron as compared to wall in the present study might be attributed to the increased blood supply to the affected sole portion.

It may be concluded from the results that significantly

high levels of zinc and copper are present in wall portion of hoofs as compared to sole portion whereas manganese and iron are more in sole portion of the hoofs as compared to toe or wall portion. Further, it was observed that zinc and copper are the important trace minerals responsible for synthesis of a healthy and resilient hoof tissue. Low levels of copper and zinc in diseased hoofs in cattle in the present study indicated their importance in the prevention of lameness in dairy animals.

ACKNOWLEDGEMENTS

Authors are highly grateful to the Head, Department of Veterinary Medicine for timely services of their mineral laboratory and associated staff members.

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