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Assessment of the serum zinc, copper, β-carotene and vitamin A and hoof zinc and copper status in different locomotion scores of dairy cattle

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Summary

Serum level of zinc, copper, β-carotene, vitamin A, and concentrations of Zn and Cu in hoof of 97 multiparous Holstein dairy cows with different locomotion scores (LSs) were evaluated. Simultaneously, Zn and Cu concentrations of close up, fresh and high yielding cattle rations were 77, 94, 75 and 31, 28, 22 ppm, respectively. Based on LSs, despite normal serum copper levels in all three groups, with increasing degree of lameness serum and hoof Cu concentrations of the severe lame group were significantly lower than healthy and moderate lame groups (P=0.0178) and (P=0.0002), respectively. Serum β-carotene and vitamin A level of the three groups were sub-optimal. Significant negative correlations were observed between LSs with serum vitamin A level (r=-0.246, P=0.0145), LSs with hoof Cu concentration (r=-0.323, P=0.0150) and serum Zn concentration and Cu (r=-0.281, P=0.032). The results suggest that the deterioration of hoof tissue may be associated with reduced copper hoof content. Our findings demonstrated that lame group was at risk of subclinical vitamin A deficiency, which could be established from the healthy stages. Possibly, excess consumption of Zn could conflict with Cu absorption and utilization. Furthermore, vitamin A supplementation may have assisted in reduction of lameness in experimental animals. In conclusion, the serum and hoof concentrations and dietary intake of copper, zinc and vitamin A can have an impact on degrees of lameness and their interactions should be considered.

Key words: Locomotion score, Zinc, Copper, Vitamin A, Cattle

Introduction

Lameness, with etiologically different risk factors, is an important cause of economic losses in dairy cattle industry. Some nutritional elements are very important in maintenance of hoof epithelial and connective tissues, of which, Zn, Cu and vitamin A deficiencies have received the most attention by investigators. Zinc and copper play key roles in keratinization by activation of many essential enzymes (Tomlinson *et al*., 2004; Griffiths *et al*., 2007; Suttle, 2010).

Zinc increases hoof consistency and rate of epithelial tissue reconstruction, and maintain cellular integrity by catalytic, structural, and regulatory functions in synthesis and maturation of the keratin. In addition, this element promotes the process of wound healing (Mozaffari and Derakhshanfar, 2007; Mohamadnia, 2008). Some biological properties of Cu are achieved through contribution in the

structure of enzymes, such as ceruloplasmin, superoxide dismutase, the latter of which has lipid protecting function (Tomlinson *et al*., 2004; Suttle, 2010). Copper activates thiol oxidase, an important enzyme responsible for the formation of disulfide bonds between cysteine residues of keratin filaments in keratinocytes (Tomlinson *et al*., 2004; Suttle, 2010). Therefore, the rigidity of keratinocytes is essentially dependent on Zn and Cu functions (Tomlinson *et al*., 2004; Suttle, 2010).

Vitamins are also important in claw health. Vitamin A plays integral roles in gene expression of keratinocytes, cell differentiation, and evolution of the structure and quality of normal keratin (Tomlinson *et al*., 2004; Radostits *et al*., 2007). β-carotene is a vitamin A precursor whose plasma levels vary largely with diet, and evaluation of both values can give better animal health estimation (Radostits *et al*., 2007).

The objective of this study was to evaluate the potential impact of serum and hoof Zn and Cu values and serum vitamin A status of post parturient period cattle on the production of normal horn tissue.

Materials and Methods

Animals and sampling

The experimental dairy herd was housed in naturally ventilated open shade straw bedded barns. According to their daily milk yielding, cows were allocated in other barns after 30 days in milk (DIM). They were walked about 100-200 meters toward the milking house three times a day. All animals were trimmed approximately at 90 to 120 DIM and drying time, routinely. All lame animals were examined, trimmed and treated individually.

From 19 February to 04 May 2011, cows with 2-4 parity, with 40-100 DIM and 40.51 ± 8.32 kg of milk production per day were classified in three different Locomotion scores (LSs) using a modified method (Sprecher *et al*., 1997). Cows with LSs 1 and 2 of Sprecher locomotion score were classified as healthy, score 3 as moderate and scores 4 and 5 as severe lame (Sprecher *et al*., 1997). Based on simple random sampling, blood samples of 97 cows [healthy=40, moderate=20, severe=37] were collected through the jugular vein using disposable plastic syringes and 14 G needles. The specimens were centrifuged at 1800 × g and sera were transferred to glass test tubes for Zn and Cu determinations and stored at -20°C. All glass tubes were acid-distilled water washed three times.

Hoof pieces of experimental animals were clipped, scraped and then rinsed with distilled water, 99.5% acetone, 96% ethanol and distilled water, separately (Nouri *et al*., 2008). All samples were dried at 60°C and milled by an electric grinder. Grinder case and blades were cleaned after each use. None of severely lame cows suffered from soft tissue damages.

Chemical analysis

Air-acetylene flame atomic absorption spectroscopy (Varian spect AA 220 atomic absorption spectrophotometer) was employed for Zn and Cu determinations. One hundred mg of total mixed ration (TMR) fed to close up, fresh and high-yielding cows based on dry matter (DM), 0.5 ml of serum (Burtis and Ashwood, 1999) and 100 mg of the hoof powder (McKenzie, 1979; Nouri *et al*., 2008) were digested in 5 ml of 3:7 nitric acid: perchloric acid solution for two separate 8 h periods at 100°C (Burtis and Ashwood, 1999). To prevent the trace elements contamination, all distilled water, washing acids and tubes were tested along with the digestion procedure.

According to previously used methods, fresh sera were analysed for β-carotene and vitamin A (Suzuki and Katoh, 1990). One ml of each serum sample was mixed with 1.0 ml of 96.5% ethanol in a test tube, followed by 3.5 ml 98.5% hexane. Tubes were shaken for 10 min and centrifuged at 800 × g for 10 min. Spectrophotometric absorbance of supernatants was measured at 453 nm and 325 nm for β-carotene and vitamin A, respectively. β-carotene and vitamin A concentrations (μg/dL) were calculated by related equations (Suzuki and Katoh, 1990).

Statistical analysis

Data normality was evaluated using Kolmogorov-Smirnov test. Transformation

of square root was used to normalize serum level of β-carotene and logarithmic transformation to normalize hoof Cu concentration and serum levels of vitamin A. Spearman's rank correlation coefficients between LSs and the studied parameters were estimated using Proc CORR of SAS software (SAS, 2004). The differences of all variables including serum Zn, Cu, β-carotene, vitamin A and hoof Zn and Cu concentrations between three different LSs were analysed using one-way analysis of variance. Duncan's multiple range tests was used for comparison of means with an error level of 0.05. Proc GLM of SAS (SAS, 2004) was employed for analysis of variance and comparison of means.

Results

Zinc and copper concentrations of TMR and their requirements, based on NRC (2001) are presented in Table 1 (NRC, 2001). The analysis of variance and comparison of mean±SD of results for serum Zn, Cu, β-carotene, vitamin A, and hoof Zn and Cu in three locomotion scores are presented in Table 2. There were significant differences between serum and hoof Cu concentration of healthy and moderate lame groups with severely lame group, (P=0.0178) and (P=0.0002), respectively. There were no significant differences between the other variables (Table 2). Spearman's correlation coefficient

Table 1: Zinc and Copper (ppm) concentrations of TMR based on dry matter (DM)

Mineral		Close up cows	Fresh cows	High yielding cows
Zn	TMR	77	94	75
	Requirement*	21	65	55
	Difference	56	29	20
Cu	TMR	31	28	22
	Requirement*	12	16	11
	Difference	19	12	11
Zn/Cu		2.48	3.36	3.41

* Requirements are referred based on NRC (2001)

Table 2: The levels of zinc, copper, β-carotene, vitamin A in serum and the concentrations of zinc and copper in hoof (mean±SD) of cows with different LSs

Parameter	Serum				Hoof	
	Zinc (µg/dL)	Copper (µg/dL)	β-carotene (µg/dL)	Vitamin A (µg/dL)	Zinc (µg/g)	Copper (µg/g)
Healthy	86.43±55.28	62.28±11.40[a]	19.83±18.12	25.81±15.52	71.03±13.78	10.34±7.62[a]
Moderate	92.92±47.92	67.73±13.58[a]	20.34±12.22	17.12±11.36	77.96±19.69	12.07±5.9[a]
Severe	95.12±52.94	55.94±12.81[b]	20.70±14.6	23.66±14.62	69.76±16.26	5.00±3.9[b]
CV*	52.32	20.53	32.78	22.82	22.94	38.55
P-value	0.8566	0.0178	0.2260	0.1985	0.2869	0.0002

[ab] Different superscript letters in the same column means a significant difference (P≤0.05). *CV: Coefficient of variation

Table 3: Spearman's correlation coefficient between parameters (p-values are presented in parenthesis)

Parameter	LSs*	Serum Zn	Serum Cu	Serum β-carotene	Serum vitamin A	Hoof Zn
Serum Zn	-0.015 (0.904)	1				
Serum Cu	-0.103 (0.443)	-0.281 (0.032)	1			
Serum β-carotene	0.221 (0.105)	-0.311 (0.020)	0.233 (0.093)	1		
Serum vitamin A	-0.246 (0.0145)	-0.332 (0.012)	0.181 (0.182)	0.287 (0.034)	1	
Hoof Zn	-0.038 (0.780)	-0.114 (0.396)	0.131 (0.343)	0.104 (0.461)	0.098 (0.483)	1
Hoof Cu	-0.323 (0.0150)	-0.151 (0.267)	0.4918 (0.0002)	0.222 (0.118)	0.020 (0.886)	0.3920 (0.0031)

* LSs: Locomotion Scores

(Table 3) demonstrated significant negative relationships between serum Zn and Cu levels (r=-0.281, P=0.032), LSs and vitamin A (r=-0.246, P=0.0145), and LSs and hoof Cu concentration (r=-0.323, P=0.0150).

Discussion

Significant interactions between trace minerals have already been reported (NRC, 2001; Tomlinson *et al*., 2004; Radostits *et al*., 2007; Suttle, 2010). Their uptake can vary due to various stages of lactation, health or stress status in dairy cattle (NRC, 2001; Tomlinson *et al*., 2004). Hence, to maintain an appropriate balance of trace minerals and maximize animal performance, proper formulation of rations is essential (NRC, 2001; Tomlinson *et al*., 2004; Suttle, 2010) and extra supplementation of micronutrients is sometimes warranted (Nocek *et al*., 2000). Despite the mentioned physiological concerns, there are some inconsistencies in the role of trace minerals on claw disorders (Nocek *et al*., 2000; Siciliano-Jones *et al*., 2008). Some researchers have observed only a tendency in improvement of hoof status (Ballantine *et al*., 2002; Kessler *et al*., 2003). Conflicting results regarding effects of minerals on incidence and indices of claw disorders, including reduction, tendency or even increasing have been reported (Drendel *et al*., 2005).

In the present study, there was no significant relationship between serum Zn concentrations and lameness (Table 2). Our result is similar to the studies that have already been performed (Drendel *et al*., 2005; Griffiths *et al*., 2007; Cope *et al*., 2009; DeFrain *et al*., 2009). Spearman's correlation coefficient demonstrated a negative relationship between serum Zn and Cu concentrations (r=-0.281, P=0.032), and numerically increasing serum levels of Zn were simultaneous with a significant decrease in serum Cu in severe lame cows (Table 2). Seyrek *et al*. (2008) demonstrated that serum Zn and Cu concentrations of healthy, mild, moderate and severe lame cows were not significantly different. Conversely, it was reported that the mean concentrations of Zn in serum of lame cattle were significantly lower than control group (57.81±3.8 vs. 85.51±6.01 µg/dL), hence a potential interaction between lameness and serum Zn concentration in dairy cows was suggested (Kilic¸ *et al*., 2007). Zinc and Cu requirements and Zn:Cu for lactating dairy cows have been estimated as 55-65, 11-16 ppm and 3:1 to 2:1, respectively (NRC, 2001). High levels of consumed Zn may subsequently decrease Cu utilization in the body (NRC, 2001; Smith *et al*., 2010; Suttle, 2010). It is speculated that the adequate amount of Zn in diet might result in optimal serum Zn levels (Ballantine *et al*., 2002), and excess amounts of this element in diet may conflict with absorption, bioavailability and utilization of copper (NRC, 2001; Smith *et al*., 2010; Suttle, 2010). Under stress conditions, the trace minerals supplementation may improve reproduction, production performances (Nocek *et al*., 2000), healing process and decrease claw lesions and lameness (Tomlinson *et al*., 2004; Mohamadnia, 2008). However, based on dietary requirements, minerals should be supplemented precisely and their interactions should also be considered. In our study, factors contributing to poor cow comfort, including excess distance from pen to parlor house, rough flooring, abrasive and unsuitably designed stalls could contribute to lameness.

Normal serum vitamin A in cattle ranges from 25 to 60 µg/dL, and to be completely safe, the optimum level is higher than 25 µg/dL. Optimum level of serum β-carotene in cattle is 150 µg/dL and varies largely with the diet (Radostits *et al*., 2007). Serum vitamin A and β-carotene levels of all groups of our study were lower than optimum ranges. Consistent with Seyrek *et al*. (2008), in our study, serum vitamin A values of moderate and severe lame cows were numerically lower than healthy group (Table 2). Spearman's correlation coefficient (Table 3) demonstrated a negative relationship between LSs and serum vitamin A (r=-0.246, P=0.0145) and a positive relationship between serum vitamin A and β-carotene (r=0.287, P=0.034). Regarding the high correlation between vitamin A and dietary β-carotene, this positive relationship is completely anticipated (Kessler *et al*., 2003; Tomlinson *et al*., 2004). In this study, the numerical decrease in vitamin A may

also be explained by considering its antioxidant role. Decreasing serum vitamin A concentration has been associated with increasing malondialdehyde (MAD) in severe lame cows. This reduction of vitamin A in severe lame cattle may be linked to the utilization of antioxidants as a result of increased oxidative stress (Seyrek *et al*., 2008).

Vitamin A intake of fresh and high-yielding cows has been formulated as 5.1 and 3.1 KIU/kg of DM, respectively, which was much lower than the recommended levels (NRC, 2001). This is another point which may emphasize the establishment of subclinical vitamin A deficiency from healthy stages. Based on our findings, healthy, moderate and lame groups were at risk of subclinical vitamin A and β-carotene deficiencies and feed supplementation could help in reduction of lameness.

Very few studies have evaluated the actual biological values of hoof Zn and Cu (Kessler *et al*., 2003; Tomlinson *et al*., 2004). Some researchers noted that the harder keratin of the hoof wall contained a greater Zn concentration than the softer keratin of the heel. It is found that concentrations of Zn in the hoof horn of lame cows were lower than healthy animals (Kessler *et al*., 2003; Tomlinson *et al*., 2004). In the present study, Zn concentrations of claw samples (Table 2) were lower than another study with 117±11 µg/g (Kessler *et al*., 2003). A significant negative correlation observed between LSs with hoof Cu concentration (r=-0.323, P=0.0150). Severe lame cattle showed significantly lower hoof Cu (P=0.0002) than normal and moderate lame cows (Table 2). Some hypotheses may interfere with these findings, indicating the need for more research to be done: 1) Again, the chronic imbalances of Zn and Cu in ration may result in decreasing the utilization of Cu at hoof epithelial cells level. 2) There was a negative association between LSs and Cu concentration of the hoof (r=-0.323, P=0.0150). On the other hand, positive correlations were detected between the levels of serum and hoof Cu (r=0.4918, P=0.0002) and the concentrations of Zn and Cu in hoof (r=0.392, P=0.0031). Surprisingly however, the LSs were not associated with serum values of Cu. According to these results, hoof lesions are likely a cause, not an effect. It is possible that softened hoof loses Cu and other constituents, as hoof Zn values were lower than other studies (Kessler *et al*., 2003; Tomlinson *et al*., 2004). 3) Hoof growth may be affected by the deprivation of Cu (Suttle, 2010); accordingly, this may justify a significant decrease of hoof Cu in severely lame cows.

According to our findings, in addition to the opposed interaction of Zn and Cu, subclinical vitamin A deficiency and some environmental risk factors might affect mineral utilization in hoof, which may lead to softening the claw.

In conclusion, although a strong relationship between serum Zn and Cu concentrations with different LSs has not been found in this study, dairy cow lameness should still be considered as a "syndrome". If a nutritional deficiency is suspected, closer attention must be paid to rations and the interrelationships between trace elements and vitamins.

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