SERUM SIALIC ACID, MALONDIALDEHYDE, RETINOL, ZINC, AND COPPER CONCENTRATIONS IN DAIRY COWS WITH LAMENESS

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Abstract

Concentrations of serum sialic acid, malondialdehyde (MDA), retinol, zinc, and copper were measured in 52 lactating Holstein cows. Out of them, 15 were controls, 21 mildly lame, 7 moderate lame, and 9 severely lame. The animals were housed in free-stall barns with a slurry surface and were not released for pasture feeding. Compared to control animals, the mean serum total sialic acid concentrations in animals with severe lameness, but not in those with mild and moderate lameness, were increased significantly (P<0.01). The levels of MDA were higher in severely lame animals than in controls as well as in mildly and moderately lame animals and the differences were statistically significant (P<0.001). Contrary to the elevated serum concentrations of sialic acid and MDA in severely lame cows, the mean serum retinol value of these animals showed a remarkable decline (P<0.05). As for serum zinc and copper concentrations, there were statistically no significant alterations between the groups. In conclusion, results of the present study indicate that the concentrations of serum total sialic acid, MDA and retinol are associated with a degree of the lameness.

Key words: dairy cows, lameness, sialic acid, malondialdehyde, retinol, minerals.

Lameness in dairy cows, is second in terms of its detrimental effect on herd productivity, and is only preceded by mastitis (3, 6, 14). As well as productivity, lameness severely compromises the welfare of affected animals and is probably the single most common cause of distress in dairy cattle (28). Sole ulcer, white line disease, interdigital dermatitis, sole abscess, and digital dermatitis are main causes of lameness (20). Regardless of the cause of lameness, early detection and prompt treatment minimises losses, improves outcome, and reduces animal suffering (20).

Sialic acid is a terminal component of the non-reducing end of carbohydrate chains of glycoproteins and glycolipids (21). High serum sialic acid level is important factor for certain diseases. To date, no data has been published about the possible association between serum sialic acid content and lameness.

Oxidative stress may play an important role in the pathogenesis of some diseases (30). Malondialdehyde (MDA) is one of the well-known secondary products of lipid peroxidation after exposure to reactive oxygen species and free radicals, and it may be used to evaluate oxidative damage by measuring serum levels of thiobarbituric acid reactive substances (7, 10).

Functions of retinol include regulation of cell division and differentiation, reproduction and foetal development, immunity, bone development, and maintenance of normal epithelial tissues (16). Previous investigations have shown an association between dietary retinol levels and the incidence of leg problems in birds (9). To our knowledge however, there is no data reported whether there is any relationship between lameness and serum retinol levels in cows.

Trace elements may play an important role in lameness. It is a well-known fact that they have roles in the production of horn tissue and in the maintenance of epithelial and connective tissues (27). Zinc and copper are essential trace elements that have received the most attention. Zinc is present in more than 300 metalloenzymes and is involved in DNA replication, RNA and protein synthesis, carbohydrate and lipid metabolism, gene expression, and appetite regulation (1, 8, 19). The importance of Cu has been recognised for over 70 years, with the early discovery that the element was necessary for normal haemoglobin synthesis in young rabbits and rats. Since that time, the importance of Cu for normal growth, production, and reproductive performance has been established. The biological role of Cu is exerted through a number of Cu-containing proteins including ceruloplasmin and superoxide dismutase (2, 18). When Cu is inadequate in animals, physiological and metabolic functions related to Cu-enzymes may be impaired.
The incidence of lameness has been steadily increasing over the past 20 years (13, 29), possibly due to the increased production, genetic disturbances, and changes in management practices. In modern husbandry, animals are kept in bigger groups and this complicates the observation of individual animals. Lameness is frequently undetected until the animal can no longer stand or walk. In the present study, we intended to find out whether there is any relationship between serum concentrations of total sialic acid, thiobarbituric acid reactive substances, retinol, zinc, as well as copper and lameness.

Material and Methods

Animals. Fifty-two lactating Holstein cows, out of them 15 controls (showing no lameness, locomotion scored as 1), 21 mildly lame (locomotion scored as 2), seven moderately lame (locomotion scored as 3) and nine severely lame (locomotion scored as 4-5), were enrolled in this study. The locomotion scoring was performed according to the system developed by Sprecher (24).

The herd was housed in free-stall barns and fed a concentrate ration (7-9 kg per cow) and conserved forage (grass and maize silage, 15-20 kg per cow). Hay was provided ad libitum. There was no regular hoof-trimming programme and the animals were exposed to slurry surface, particularly in the winter period. The cows were milked two times daily in milking parlours.

Biochemical analysis. Blood was collected from the jugular vein into serum tubes. Blood samples were allowed to stand for 2 h at room temperature to allow proper clotting. The samples were then centrifuged at 5 000 rpm for 10 min and the serum samples were stored at -20°C until analyses.

Serum total sialic acid concentrations were measured as described by Sydow (26). Briefly, 400 µl of serum were treated with 3 ml of 5% perchloric acid for 5 min at 100°C and centrifuged at 1 400 g for 4 min. The supernatant (2 ml) was mixed with 400 µl of Ehrlich’s reagent (5 g p-dimethylaminobenzaldehyde/50 ml HCl /50ml distilled water). After incubation at 100°C for 15 min, 2 ml of distilled water were added to the sample and a spectrophotometer (Shimadzu, UV-1601) was used to read the optical density at 525 nm. A standard curve was obtained using known quantities of fresh N-acetylneuraminic acid (Sigma, A-0812) dissolved in water. Plasma lipid peroxidation was determined using the procedure described by Yoshiko (31), in which MDA, an end product of fatty acid peroxidation, reacts with TBA to form a coloured complex with a maximum absorbance at 532 nm. Serum retinol concentrations were determined spectrophotometrically as described previously (25). Serum zinc and copper concentrations were measured using commercial available kits (Randox Laboratories, UK) with a spectrophotometer (Shimadzu, UV-1601). The analyses were carried out according to the manufacturer’s instructions.

The statistical analysis of the differences between groups was determined with ANOVA and significance of differences with Duncan’s test. Differences were considered statistically significant when P<0.05 against control group. All the values were presented as mean ± SEM.

Results

As shown in Table 1, compared to control animals, the mean serum total sialic acid concentrations of animals with severe lameness increased statistically significantly (P<0.01). Serum concentrations of sialic acid in mildly lame and moderately lame cows, however, showed a moderate increase and it remained statistically non-significant (P>0.05).

Serum MDA levels were positively related with a degree of lameness. An increase in serum levels of MDA in mildly and moderately lame cows remained insignificant (P<0.05). However, the values of MDA in severely lame cows showed a significant (P<0.001) elevation.

As for serum retinol concentrations, animals with severe lameness showed a significant (P<0.05) decline in retinol concentrations. On the other hand, a moderate decline (P>0.05) was observed in animals with mild and moderate lameness.

With reference to serum zinc and copper concentrations, there were no statistically significant alterations. Though an increase was observed in copper concentrations in animals with severe lameness, this increase remained statistically non-significant (P>0.05).

<table>
<thead>
<tr>
<th>Score of lameness</th>
<th>1 (Control)</th>
<th>2</th>
<th>3</th>
<th>4-5</th>
<th>P</th>
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<tr>
<td>n=15</td>
<td>21</td>
<td>7</td>
<td></td>
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<td>Total sialic acid (µg/mL)</td>
<td>606.09 ± 32.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>728.90 ± 45.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>735.84 ± 26.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>873.47 ± 27.85&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Malondialdehyde (µmol/L)</td>
<td>24.78 ± 0.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.72 ± 1.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.43 ± 0.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.43 ± 1.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.001</td>
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<tr>
<td>Retinol (µg/dL)</td>
<td>38.97 ± 3.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.24 ± 2.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>34.71 ± 2.68&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>27.16 ± 1.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.05</td>
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<tr>
<td>Zinc (µg/dL)</td>
<td>126.51 ± 11.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>127.17 ± 8.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>118.15 ± 16.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>127.34 ± 15.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&gt;0.05</td>
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<tr>
<td>Copper (µg/dL)</td>
<td>227.75 ± 34.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>221.17 ± 25.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>221.28 ± 27.78&lt;sup&gt;b&lt;/sup&gt;</td>
<td>263.39 ± 28.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&gt;0.05</td>
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<sup>± SEM, a,b Different letters in the same line indicate statistically significant differences.</sup>
Discussion

Bovine lameness represents a major health problem for the dairy industry. The incidence of lameness has been steadily increasing over the past 20 years (13). In problem herds, where the incidence is high, lameness accounts for tremendous economic losses (14). It is realistically impossible to achieve no lameness in a herd. However, the definition of lameness in cattle is fraught with difficulty, even among specialists. Currently, cows can be “locomotion scored”. Observing of lameness in cattle and scoring abnormalities in locomotion are subjective. To improve the objectivity of lameness, for the first time, we intended to find out whether the detection of serum total sialic acid values could be used as an indicator of lameness. Results of the present study showed that compared to control animals, the mean serum total sialic acid values in severely lame, but not in mildly and moderately lame cows, increased significantly. This result indicates that there is a relationship between serum values of sialic acid and lameness in cows. However, from this statement it cannot be concluded that the detection of serum total sialic acid value has a predictive value for lameness in cows, in particular for mild and moderate lameness. On the other hand, it should not be ruled out that studies with larger sample sizes, including treatment opportunity, would likely provide estimates that are more precise and may help to clarify this issue.

Lipid peroxidation is a well-established mechanism of cellular injury and is used as an indicator of oxidative stress in cells and tissues (15). Lipid peroxides derived from polyunsaturated fatty acids are unstable and can decompose to form a complex series of compounds. Among these, reactive carbonyl compound is the most abundant MDA. As such, the measurement of MDA is widely used as an indicator of lipid peroxidation (11). There is no data available in the literature on the effects of lameness on lipid peroxidation. In this study, levels of MDA were significantly higher in severely lame animals than mild and moderately lame animals. As a first report, these results indicate that severe lameness, but neither mild nor moderate one, induces oxidative stress in cows.

The correct nutrition of the dairy cow is critical for good hoof health and formation of tough and resilient claw horn, capable of withstanding environmental challenges. Retinol is important in the maintenance of epithelial tissue and cell replication (16). It is thought to play a role in both epithelial tissue repair and integrity and immune function. In this study, the lowest value of retinol was in the same group where we observed the highest levels of MDA. The lowest retinol concentration in severely lame cows may be linked to the utilisation of other antioxidants as a result of increased oxidative stress in these animals. Therefore, supplementation of retinol to the ration of dairy cows may assist in the reduction of lameness.

The nutritional requirements for trace elements are small; however, these nutrients play very important roles in the metabolism. Serum zinc and copper concentrations detected in the present study were higher than previous studies (12, 23). Furthermore, contrary to the previous reports (4, 5, 17) about beneficial effects of zinc and copper in reducing oxidative stress, in this study, there was no association between serum MDA levels and zinc as well as copper concentrations. Zinc deficiency is associated with impaired retinol metabolism (19). Low plasma retinol values were reported in Zn-deficient cattle (22). Contrary to these statements made by Sharma et al. (22) and Puschner et al. (19), we could not find any relationship between serum zinc levels and retinol concentrations.

In conclusion, alterations in concentrations of serum total sialic acid, thiobarbituric acid reactive substances, and retinol, indicate that lameness is associated with the serum concentrations of these compounds. Furthermore, it is assumed that the addition of retinol to the ration of cows may reduce the incidence of lameness in herds. Further studies, especially with treatment opportunity and larger sample sizes need to be conducted to elucidate the role of sialic acid in lameness as well as to clarify the issue whether the measurement of serum sialic acid could be used as an indicator of lameness.

References