Pathological and Biochemical Findings of the Cows with Dermatitis Fed with Excessive Molasses

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ABSTRACT

Background: In ruminant diets additives are widely used in ensiled forages to enhance the acidification. Molasses used as additive could provide fermentable substrates for lactic acid bacteria and act as a fermentation stimulant. Adding molasses to materials before ensiling could decrease pH, and increase lactic acid levels and residual water-soluble carbohydrates. These changes may cause disease in farm animals. Therefore, the objective of the present study was to investigate the biochemical and pathological changings of dairy cows which fed with excessive molasses.

Materials, Methods & Results: The blood samples were collected from 10 affected (8 Holstein and 2 Montofon cows) and 10 healthy (8 Holstein and 2 Montofon) cows (median age: 4.5). Skin biopsies were also taken from the skin of dorsal and rump area from two affected cows. Albumin, glucose, total protein, calcium, potassium, magnesium, sodium, phosphorous, uric acid levels were measured in blood serum samples. The serum glucose, sodium, calcium, magnesium and, uric acid concentrations of dermatitis group revealed significantly (P < 0.05) lower values compared to control group. But no significant differences were found in total protein, albumin, potassium, and phosphorous levels between the groups. Histopathology of the skin biopsy samples from affected skin parts revealed subacute inflammatory dermatitis and subacute chronic hyperplastic perivascular dermatitis. A significant (P < 0.05) reduction in the mean concentration of calcium, magnesium, and sodium were determined in the dermatitis group compare to the control group. Calcium concentration decreased to 9.42 ± 0.17 mmol/L in dermatitis group. The magnesium and sodium concentrations, the mean values of the dermatitis group were decreased to 2.03 ± 0.06 mEq/L and 148.30 ± 0.97 mEq/L respectively. Glucose and uric acid levels in the dermatitis group decreased significantly (P < 0.05) (53.8±3.5, 0.58±0.05) compared to the controls. No significant differences were found in albumin, inorganic phosphorus, total protein and, potassium concentrations between the groups. Histopathology of the biopsy samples taken from affected skin samples revealed subacute inflammatory dermatitis and subacute chronic hyperplastic perivascular dermatitis.

Discussion: Animals, especially dairy cows, are susceptible to sudden changes on carbohydrate ratio in their feeding regime. All dietary changes, especially to the carbohydrate-roughage ratio, should be made gradually in animals. In dairy cows feeding rations high in easily digestible carbohydrates or experimental oligofructose overload was generally associated with laminitis. In this regard, the present study constitutes the first work that explores the biochemical and pathological findings of dermatitis associated with excessive consumption of molasses in dairy cows. Significant decrease in serum calcium, magnesium and sodium, uric acid, glucose concentration in dermatitis group compared to control group. It is suggested that endotoxin plays a role in development of grain-related metabolic diseases such as fatty liver, LAD, and laminitis. Cytokines are also associated with inflammatory abomasum displacement and related liver diseases. Further studies are necessary to clarify this mechanism. In conclusion, excessive consumption of molasses may cause allergic reactions of skin of dairy cows and alter biochemical parameters.

Keywords: dairy cows, molasses, dermatitis, biochemical findings, pathological findings.
INTRODUCTION

In dermatitis the lesions on skin can be seen in many different forms. One of them is perivascular dermatitis. Perivascular dermatitis is the least specific of the patterns of inflammation which reaction locates around the blood vessels [12].

In farm animals, laminitis, rather than dermatitis, is widely studied because it is more common and could affect the productivity of animals [8]. In dairy cows most common results of carbohydrate overload are metritis, mastitis or laminitis [3,16]. Consumption of non-structural carbohydrate is also one of the laminitis inducing factors for horses [6]. Laminitis was also experimentally induced as a result of oligofructose overload [5]. Additionally, Yeruham et al. [16] described the clinical, biochemical and, radiological findings in laminitis-dermatitis syndrome in heifers caused by feeding large amounts of readily fermentable carbohydrate. However, there is not information about dermatitis in cattle associated with excessive carbohydrate intake.

The aim of the present study was to evaluate the biochemical and pathological alterations in dairy cows which were fed with excessive amount of molasses.

MATERIALS AND METHODS

Animals

Sixteen Holstein and four Montofon cows (median age: 4.5 year) in postpartum period, which were presented to the clinics of the Veterinary Faculty, University of Mehmet Akif Ersoy constituted the material of the present study. Among them, 10 exhibited dermatitis whereas the others were considered as healthy controls.

All animals in dermatitis group brought to the clinic with history of excessive consumption of molasses. In clinical examination, skin lesions were determined on dorsal and rump areas. Diagnoses were confirmed by pathologic examinations. All skin samples were free from scabies and fungal agents.

Biochemical Analysis

The blood samples (5-7 mL) were taken from jugular vein to vacuum blood collection tubes (Sterile vacuum tubes) and centrifuged at 2720 g for 10 min. The sera were separated and stored at -20°C until the analyses were performed.

Assays of albumin, glucose, total protein, calcium, potassium, magnesium, sodium, phosphorous, and uric acid levels were performed at least two measurements spectrophotometrically using an AutoAnalyzer (Gesan Chem 200) and commercial kits.

Histopathological Analysis

Full depth skin biopsies were collected from the affected areas of two cows showing dermatitis lesions. All skin biopsy materials fixed in 10 % formalin. Following fixation, samples were embedded in paraffin wax, sectioned in 5 μm (Leica, RM2125RT) and were stained with haematoxylin and eosin (HE), Periodic Acid Schiff (PAS). The prepared sections were examined microscopically (Nikon, Eclipse E600).

Statistical Analysis

Statistical analysis of biochemical parameters was carried out with MINITAB® program (Minitab Inc. USA) and the differences between the groups were analyzed with t test (P values of less than 0.05 were considered significant).

RESULTS

Clinical Biochemistry

A significant (P < 0.05) reduction in the mean concentration of calcium, magnesium, and sodium were determined in the dermatitis group compare to the control group. Calcium concentration decreased to 9.42 ± 0.17 mmol/L in dermatitis group. The magnesium and sodium concentrations, the mean values of the dermatitis group were decreased to 2.03 ± 0.06 mEq/L and 148.30 ± 0.97 mEq/L respectively (Table 1). Glucose and uric acid levels in the dermatitis group decreased significantly (P < 0.05) (53,8±3,5, 0,58±0,05) compared to the controls (Table 1).

No significant differences were found in albumin, inorganic phosphorus, total protein and, potassium concentrations between the groups.

Histopathology

Histopathology of the biopsy samples taken from affected skin samples revealed subacute inflammatory dermatitis and subacute chronic hyperplastic perivascular dermatitis.

In microscopic examination of the affected part of skin biopsy samples, perivascular dermatitis was observed in the superficial vessels of the dermal layer (Figures 1 & 2). Macrophages accompanied to lymphocytes and plasmaocytes were located around...
the superficial dermal blood vessels (Figures 1 & 2). Profound dermal blood vessels and epidermis were found to be relatively normal and rete peg formations were observed. Exudative type crust formation which consists of inflammatory cells, cellular debris and bacterial colonization was determined in the stratum corneum (Figures 5 & 6) together with parakeratotic type hyperkeratosis. A rete ridge formation was seen the epidermis (Figures 5 & 6). Vacuolar degeneration was observed in keratinocytes which located in the stratum granulosum and upper stratum spinosum of epidermal layer (Figures 5 & 6).

Fibroplasia, characterized by a significant fibrovascular proliferation was noticed (Figures 5 & 6). PAS staining was negative for fungal infestation in all samples (Figures 3, 4 & 7). In both samples, eosinophil infiltration was not observed or it was very limited, and the hair follicles were intact.

### Table 1. Biochemical parameters in blood serum of cows.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 10)</th>
<th>Dermatitis (n = 10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (g/dL)</td>
<td>3.05 ± 0.09</td>
<td>3.02 ± 0.07</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>91.3 ± 11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.8 ± 3.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.05</td>
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<tr>
<td>Sodium (mEq/L)</td>
<td>152.70 ± 1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>148.30 ± 0.97&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>8.27 ± 0.52</td>
<td>6.99 ± 0.52</td>
<td>NS</td>
</tr>
<tr>
<td>Total protein (mg/dL)</td>
<td>7.82 ± 0.28</td>
<td>7.32 ± 0.25</td>
<td>NS</td>
</tr>
<tr>
<td>Uric Acid (mg/dL)</td>
<td>1.73 ± 0.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.58 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>4.98 ± 0.24</td>
<td>4.50 ± 0.24</td>
<td>NS</td>
</tr>
<tr>
<td>Magnesium (mEq/L)</td>
<td>2.46 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.03 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>10.84 ± 0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.42 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.05</td>
</tr>
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NS: Not Significant. Different superscripts a, b in the same row indicate significant differences among group (P < 0.05).

**Figure 1.** Perivascular dermatitis characterized by mononuclear cell infiltration in the superficial veins of dermis (arrows). HE staining (Obj. 4x); [bar= 100 μm].

**Figure 2.** Lymphocytic (white arrow) plasmacytic (black arrows) inflammatory cell infiltration around vessels (Obj. 20x); [bar= 30 μm].

**Figure 3.** There were no any fungal agent, stained with PAS/nucleus stain Gill’s Hematoxylin (Obj. 10x). [bar= 50 μm].

**Figure 4.** There were no any fungal agent, stained with PAS (Obj. 10x). [bar= 50 μm].

**Figure 5.** Parakeratotic type hyperkeratosis (black vertical line) and crust formation which consist of keratin, cellular debris, inflammatory cells, and bacterial colonization. A rete ridge formation (black arrow) accompanied to acantosis in epidermis. Fibroplasia characterized by significant fibrovascular proliferation in the dermis (white vertical line). HE staining (Obj. 4x). [bar= 100 μm].

**Figure 6.** Parakeratotic type hyperkeratosis in the stratum corneum (black arrow). Vacuolar degeneration in keratinocytes located in the stratum granulosum and upper stratum spinosum of epidermal layer (white arrows). Fibroplasia, characterized by significant fibrovascular proliferation. HE staining (Obj. 10x). [bar= 50 μm].

**Figure 7.** There were no any fungal agents. PAS/Nucleus Stain, Gill’s Hematoxylin (Obj. 10x). [bar= 50 μm].
DISCUSSION

To the best of our knowledge, the association between excessive molasses consumption and dermatitis occurrence in dairy cows has not been reported. However, in addition to laminitis, Yeruham et al. [16] reported skin lesion on the leg and tail as a result of excessive carbohydrate consumption in cattle. In previous studies [5,14,15] feeding rations high in easily digestible carbohydrates or experimental oligofructose overload was generally associated with laminitis in dairy cows. In recent studies, it was generally suggested that laminitis and metabolic diseases such as ketosis, fatty liver and abomasal displacement were interrelated [1,2,7]. In this regard, the present study constitutes the first work that explores the biochemical and pathological findings of dermatitis associated with excessive consumption of molasses in dairy cows.

In the present study serum uric acid concentration (0.58 ± 0.05) significantly decreased in dermatitis group compare to control group (1.73 ± 0.45). This result was compatible with previous report of Yeruham et al. [16].

Inorganic mineral requirement is provided from dairy cow diets. Thus, the levels of minerals in cows diet effects the serum levels of these minerals. In dairy cows because of lactation, the mineral requirements of an animal increases dependent on its physiological state. The digestibility of calcium is dependent on the diet, mainly the level of dietary calcium [9]. Dietary factors, such as the amount of ammonium in the rumen, dietary starch and dietary calcium can also influence the uptake of magnesium [13]. In the present study, significant decrease in serum calcium, magnesium and sodium levels recorded in dermatitis group compared to control group are similar to previous report in cows with laminitis [16].

Carbohydrates are used as an important source of energy and the primary precursors of fat and sugar (lactose) in cows’ milk [4]. In the current study, reduction in glucose level was observed in cows with dermatitis which fed with excessive molasses could be related to energy requirement of cows for milk production.

Animals, especially dairy cows, are susceptible to sudden changes on carbohydrate ratio in their feeding regime. All dietary changes, especially to the carbohydrate-roughage ratio, should be made gradually in animals. The theory is that cows may not accustom to it, and as a result of lactic acid production, ruminal acidosis occurs. Rumen acidosis results release of histamine and other vaso-active agents. Peripheral circulatory failure may be caused by toxic vaso-active substances such as histamine, lactic acid and endotoxin [15]. Endotoxin is a component of the membrane of all Gram-negative bacteria and is suggested to play a role in development of grain-related metabolic diseases such as fatty liver, LAD, and laminitis [1,2]. Cytokines are also associated with inflammatory diseases [11], abomasum displacement and related liver diseases [7,10].

CONCLUSION

It is concluded that excessive consumption of molasses may alter clinical biochemical parameters such as glucose, sodium, calcium, magnesium and, uric acid concentrations and play a role in the development of dermatitis in dairy cows. Endotoxins play a role in development of grain-related metabolic diseases such as fatty liver, LAD, and laminitis. However, the certain role of endotoxin in these important diseases of dairy cattle has not yet been cleared. Consequently, further studies would be conduct to understand the role of endotoxins in dermatitis mechanism in dairy cows which consumed excessive amount of molasses.

REFERENCES


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