



Clinical, Hemato-Biochemical and Oxidative Stress Biomarkers Alterations in Dairy Heifers with Tongue-Rolling

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Abstract

THE current study aimed to determine the alterations in clinical, hemato-biochemical and oxidative stress biomarkers in dairy heifers performing tongue-rolling disorder. Seventeen stereotypic (Tongue rolling group) and 12 healthy (control group) Holstein-Frisian heifers aged 12–22 months were selected. Blood and serum samples were gathered from all animals for determination of hematological, biochemical as well as oxidative stress parameters. Tongue-rolling animals showed the characteristic stereotypic actions of tongue-playing that were never seen in controls. Rectal temperature, respiratory rate, heart rate and rumen contractions were similar in tongue-rolling and control groups. Anemia was found in tongue-rolling affected animals. Moreover, total leucocytic count and neutrophilic percentage were significantly ($P < 0.001$) decreased in affected animals. Blood serum levels of glucose, triglycerides, albumin, urea, creatinine as well as AST and ALT showed non-significant ($P > 0.05$) change between both studied groups. Cholesterol, HDL-C, total protein and globulin levels were significantly decreased ($P < 0.05$) in affected heifers. Blood serum minerals and electrolytes showed non-significant ($P > 0.05$) difference in the levels of phosphorus, Mg, Zn, iron, Copper, Na and K between both groups. Calcium levels were significantly decreased ($P < 0.05$) in affected group. SOD activities were significantly ($P < 0.05$) reduced in tongue-rolling group while, a non-significant ($P > 0.05$) increased activities of MDA were recorded in the same group. The results indicated that tongue-rolling affected animals experienced anaemia, reduced energy and oxidative stress. Those factors could be implicated in the pathophysiology of tongue-rolling problem in dairy animals.

Keywords: Tongue-rolling, dairy Heifers, clinical, hemato-biochemical, oxidative stress.

Introduction

Opening the mouth, rolling the tongue, flicking it outside the mouth, and making numerous, fast motions of different intensities, directions (rolling, twisting), and frequencies are all considered symptoms of tongue playing disorder [1-3].

There are times when the tongue-playing indication manifests as biting and licking of various surfaces, mainly made of metal and wood [1, 4]. According to [1], tongue-playing is moving the tongue side to side outside the mouth or rolling it inside. Additionally, the tongue usually protrudes again during this episode after rolling backward to the pharyngeal area inside the open mouth and wrapping around a fictitious tuft of grass while the head is upright [5].

As many as 8% of the population [6] or more than 10% [1] may be afflicted by the illness, accounting for 13.33% of cattle [7]. Six months to

six years old are among the many animal ages that are affected [1-3]. Heifers between the ages of 13 and 25 months exhibited the most tongue-playing [8]. Although the exact cause of the condition is still unknown, a number of studies have indicated that stressors related to feeding and management, genetics, mimicking instincts, and specific trace element deficiencies may contribute to the pathophysiology of the condition [9] and [4].

Deficits in particular trace elements, such as cobalt (Co), manganese (Mn), and copper (Cu), may be part of its pathogenesis. According to [10], the body generally maintains what is referred to as the "oxidative balance," or balance, between the antioxidant defence system and the creation of free oxygen radicals, which lead to lipid peroxidation. When the balance between oxidants and antioxidants is disturbed, it is referred to as oxidative stress [11, 12].

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This study was conducted to determine hematological, serum biochemical alterations and the potential occurrence of oxidative stress in cattle performing tongue-playing disorder in an attempt to understand the possible causes of this disorder in dairy cattle.

Material and Methods

Animals and experimental design

The current study was handled after being approved from Local Ethics Committee (BSU-IACUC, Approval number: 022-328). The study was operated in Beni-Suef province, Egypt. Seventeen stereotypic heifers (group A= Tongue rolling group) were selected from a dairy farm and 12 healthy heifers (group B= non-tongue rolling group) were selected from another dairy farm as controls. All heifers from both farms were Holstein-Frisian breed, aged 12–22 months. Stereotypic heifers were raised in indoor tie stall system and fed mainly on roughages (hay and straw) at infrequent intervals. The control heifers were raised in free-stall barns and fed Total Mixed Rations (TMR) three Times a day, ad Libitum. The TMR diet was formulated according to the [13]. In group (A), tongue rolling issue was diagnosed based on the clinical symptoms presented by stereotypic heifers. The affected heifers displayed tongue rolling for a period ranged from 10 to 40 minutes. Some animals were observed to perform continuous tongue-rolling, that resulted in reduced feed prehension and retarded growth. In group (B) all selected heifers didn't show any form of tongue playing.

Clinical examination and sample collection

Clinical results were documented after the study heifers underwent a thorough clinical examination [14]. By using a clean, dry needle to puncture the jugular vein of each cow, paired blood samples were collected and placed into both plain and EDTA tubes. Within two hours of the blood sample collection, hematological analyses were performed. The blood serum in the second tube was harvested and maintained at -20°C until its biochemical parameters were analyzed. The blood serum was clear and had not been hemolyzed.

Hematological examination

Hematocrit, hemoglobin content, RBC indices, and erythrocytic count, total leucocytic and differential leucocytic counts, were all determined using whole blood samples. as described by [15].

Biochemical analyses

The following parameters were estimated using commercially available chemical kits according to manufacturer instructions: total proteins, albumin, triglycerides, cholesterol (total, high- and low-density cholesterol), glucose, urea, creatinine, ALT,

AST, calcium, inorganic phosphorus, magnesium, zinc, Iron, Copper, sodium, and potassium.

Lipid peroxidation and antioxidant status evaluation

Super oxide dismutase (SOD) and malondialdehyde (MDA) levels were measured using commercial test kits from Bio-Diagnostic Company-Egypt. As advised by the manufacturer, these indications were manually examined using a spectrophotometer.

Statistical analysis

Using SPSS software, a statistical analysis of the acquired results was conducted. The t-test was used to assess the statistical differences between the two groups. Statistics were deemed significant if $P < 0.05$.

Results

The affected animals (tongue-rolling group) showed stereotypic actions of tongue-playing which included erecting heads, sticking the tongue out of the mouth for brief or extended periods of time, and making strange tongue motions like rolling, twisting, swinging, and rolling. The affected heifers displayed those abnormal tongue movements for 5 to 25 min. Certain animals showed persistent tongue-playing over extended periods of time, which led to a decrease in feed intake and ultimately resulted in emaciation and stunted growth.

Rectal temperature was (38.72 ± 0.34 °C vs. 38.78 ± 0.18 °C in tongue-rolling and control groups respectively), respiratory and heart rates recorded (25.40 ± 2.15 vs. 25.60 ± 2.18 cycle/min and 58.60 ± 2.24 vs. 57.20 ± 2.64 beat/min in tongue-rolling and control groups respectively),

Table (1), provides an overview of the hemogram results. The animals that were influenced by tongue rolling had significantly ($P < 0.001$) decreased RBC counts, hemoglobin contents, and hematocrit percentages. In contrast to control animals, MCV increased significantly ($P < 0.05$) whereas MCHC did not alter significantly in the same animals. These findings revealed that the stereotypic heifers were suffered from macrocytic normochromic anemia. The affected heifers showed a substantial ($P < 0.001$) decrease in both the neutrophilic percentage and total leucocytic count, while the lymphocyte percentage showed a significant ($P < 0.001$) increase in the same group.

The results of blood serum biochemical parameters are shown in table (2). There was no significant change ($P > 0.05$) in the blood serum levels of glucose, triglycerides, LDL-C, VLDL, albumin, urea, creatinine, AST, and ALT between the affected and control groups. In the affected heifers, there was a significant decrease ($P < 0.05$) in the levels of cholesterol, HDL-C, total protein, and globulin, however, there was a substantial increase ($P < 0.05$) in the levels of urea in the same group.

The blood serum mineral and electrolyte results (Table 3) revealed a non-significant ($P>0.05$) variation in the values of iron, copper, phosphorus, magnesium, zinc, and potassium between the two groups. The impacted group's calcium levels were considerably lower ($P<0.05$).

Table (4) shows that whereas MDA activities were non-significantly ($P>0.05$) higher in the tongue-rolling affected animals, SOD activities were considerably ($P<0.05$) lower in that group.

Discussion

The clinical manifestation of tongue-rolling disorder in cattle is aberrant tongue motions, and it is reported in many nations worldwide, particularly in high-yielding breeds.

In this investigation, animals with tongue-rolling disorder displayed typical tongue-playing behaviors, such as tilted heads, prolonged tongue outstretched from the mouth, and unusual tongue motions. Heifers that were impacted showed indications such as contortion, swinging, rolling, and circular tongue movements. Such findings are in accordance with that observed by [16].

The reports demonstrated the multifaceted nature of the causes of pathological tongue-playing in cattle [17]. Tongue-rolling appears to result from the inhibition of important oral activities like as mastication, suckling, and gripping grass. Furthermore, it's thought that dietary deficits may cause tongue-rolling [18, 19]. Furthermore, tethering after grazing has been shown to increase aberrant oral behavior levels [5]. [20, 21] discovered that compared to cows kept in groups, tethered cows bit more fixtures. [22] found that one of the primary reasons of the oral stereotypies in cattle that were detected was the length and frequency of eating. In cattle raised intensively, the state of "expectation of feeding" arises when there are significant gaps between feedings [23]. Because the animals go longer between meals, boredom might arise [24]. Dairy bulls and heifers kept in cages exhibit tongue-playing behaviors as a reaction to prolonged stress or lack of stimulation [25]. Some authors [8] concluded that the absence of environmental stimulation and food scarcity were the root causes of cattle's abnormal tongue-rolling behavior. As the variety of environmental triggers grows, tongue playing disorder becomes less common, but it may also suppress other oral physiological behaviors including grazing, rumination, and sucking [26]. In cattle, the various forms of hyperactivity of the tongue such as rolling, licking etc. are most commonly observed because of tongue prehensility in this species, e.g. strong inclination for grabbing things with the tongue due to the nature of feeding [24].

Hemogram evaluation is crucial for the diagnosis of hematological abnormalities as well as for the monitoring, diagnosis, and prognosis of a number of

systemic diseases [27]. In the current study RBCs count, hemoglobin content and hematocrit percentage were significantly ($P<0.001$) reduced in tongue-rolling affected animals. These results indicated that, tongue-rolling affected animals experienced anemia which could be attributed to deficiency of essential nutrients. Conditions that can cause low hematocrit percentage include deficiency of vitamins or minerals, liver cirrhosis and malignancies [28]. Reduced hemoglobin content suggests a deficiency in minerals, amino acids, vitamins (particularly B12), and/or vitamins [28]. Furthermore, a nutritional deficiency or inadequate intestinal absorption of iron, which is necessary for the synthesis of hemoglobin, may result in decreased hemoglobin concentration [29].

Regarding the results of leucogram, it was found that total leucocytic count as well as neutrophilic percentage were significantly ($P<0.001$) reduced in affected heifers when compared to control ones. On the other hand, [9] have found insignificant difference in the hematological findings between both control and tongue-rolling affected cattle. Leucopenia with neutropenia could be attributed to copper deficiency in stereotypic heifers enrolled in this study [30].

Lymphocytes percentage was highly significantly ($P<0.001$) increased in the affected group in comparison with the control group. As pathologic lymphocytosis is uncommon in ruminants, the considerable neutropenia observed in this study may have contributed to the relative occurrence of lymphocytosis [31].

The measurement of blood glucose and cholesterol levels in cattle could be utilized to determine their energy status [32]. Measurement of blood glucose levels can be a useful diagnostic tool for determining a cattle's nutritional status [32]. Low feed intake may result in lower cholesterol and blood sugar levels. Reduced levels of propionate from diet are linked to conditions of undernutrition, which in turn reduces the synthesis of glucose [33]. In our study, glucose level was insignificantly decreased in affected animals than the control group. Concerning cholesterol levels, the reduction was significant ($P<0.05$) in the same group.

In the current study, there were significant hypoproteinemia and hypoglobulinemia in affected heifers whereas the albumin levels were non-significantly decreased in the same animals when compared to controls. In the body, proteins serve certain purposes. Measurements of blood urea nitrogen, creatinine, total protein, and albumin can be used to determine the status of proteins [32]. Due to their low blood sensitivity, albumin and total protein are valuable diagnostic tools for determining an animal's nutritional state [32]. A highly sensitive and early nutritional indicator of protein status is serum albumin level [34] because of its brief turnover (16

days). Protein deficiencies reduce immunity, which may make an animal more susceptible to disease [35].

In the current study, urea, creatinine, AST and ALT levels were insignificantly changed between animal groups in accordance with the investigation that made by [8].

Significant hypocalcemia was shown in tongue-rolling group as compared to controls. This may be due to feeding affected heifers disturbed ration. meanwhile no significant difference was recorded in phosphorus and magnesium levels in both studied groups. Similar results were obtained by [8].

The cu level of the affected and control animals did not differ statistically significantly in the current investigation. Nevertheless, the affected animal's levels were quantitatively lower than the controls. Such results are consistent with that obtained by [15, 36]. The health and productivity of cattle greatly depend on trace minerals. Copper is essential for the growth of bone, hair, skin, heart, and nervous system tissues. Furthermore, it functions as an additional co-factor for numerous enzymes, including lysyl oxidase, cytochrome oxidase, tyrosinase, and superoxide dismutase [9]. Previous studies made by [9, 37] suggested that the origin of tongue-rolling condition may be related to a lack in cu.

Iron is a component that is essential to several metabolic processes in all living things [38]. In the current study, no significant difference was recorded in Fe levels between both groups, but the level in tongue-rolling group was lower than that of the controls. Similar results were obtained by [36].

Even though the serum's iron concentration is normal, hemoglobin synthesis is inefficient when there is a copper shortage. It is currently unclear how anemia arises in cases of copper deficiency [39]. Because Cu is a component of proteins involved in hematopoiesis, like ceruloplasmin and hephaestin, it is required to maintain Fe homeostasis [40]. The oxidation of Fe²⁺ to Fe³⁺ is catalyzed by ceruloplasmin, allowing iron to bind to transferrin and be transported in the plasma [40]. For the purpose of erythropoiesis, copper is required for the mobilization of iron from the liver and its transportation to the bone marrow. Iron is stored in the liver when there is a copper shortage, which lowers iron's bioavailability [40].

Zinc is a crucial component for ruminants. Numerous metalloenzymes, including alkaline phosphatase, carbonic anhydrase, alcohol dehydrogenase, carboxy peptidase, DNA, and RNA polymerase, depend on it for their structure and functionality [41]. In this study, no difference was recorded in Zn levels between both groups. Similarly, [9, 36, 42] recorded no significant difference in Zn levels between tongue-playing and control cattle.

Animals need sodium and chloride to survive. Sodium is the primary cation that controls blood pH and makes up over 93% of the basic mineral components in blood serum [43]. Additionally, it is essential for the transmission of nerve impulses and the rhythmic maintenance of heart activity. Approximately two thirds of all acidic ions in blood are chloride, which is the main anion. Additionally, it is necessary to preserve the blood's acid-base balance [28]. In the current study no significant difference was recorded in sodium and chloride levels in both animal groups.

Overproduction of free radicals may weaken the body's antioxidant defenses and eventually lead to oxidative stress [44]. Oxidative stress is known to cause cellular damage and altered functioning in cells [42]. MDA levels may serve as a marker for oxidative stress and an indicator of membrane deterioration in cells [42]. In the present study, SOD activities were significantly ($P < 0.05$) reduced in tongue-rolling affected animals and a non-significant ($P > 0.05$) increased activities of MDA were recorded in the same group. These results could indicate the existence of oxidative stress in tongue-rolling affected animals. Such results are in accordance with that observed by [42].

Conclusion

Tongue rolling is considered a new abnormal condition mainly apparent cattle farm. The exact etiology of this disorder is not well understood however, genetics, feeding practices (undernourishment – infrequent feed intervals), management and certain trace minerals deficiency could be involved in the development of this problem. Based on the results of this study, it was found that tongue-rolling affected animals experienced anemia, reduced energy and oxidative stress. Those factors could be implicated in the pathophysiology of tongue-rolling problem in dairy animals.

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Declaration of Conflict of Interest

Regarding the research, writing, and/or publication of this article, the author(s) have stated that they have no potential conflicts of interest.

Ethical of approval

The current study was handled after being approved from Local Ethics Committee (BSU-IACUC, Approval number: 022-328).

TABLE 1. Hemogram in affected and control groups (Mean±SD):

| Parameter | Tongue R | Control | P-value |
|----------------------------|------------|------------|------------|
| RBCs (10 ⁶ /ul) | 5.38±0.28 | 7.63±0.63 | <0.001** |
| HB (g/dl) | 8.14±0.73 | 10.64±1.02 | <0.001** |
| PCV (%) | 24.43±2.19 | 32.41±3.15 | <0.001** |
| MCV (fl) | 49.16±5.11 | 42.80±5.92 | 0.039* |
| MCH (pg) | 16.39±1.71 | 14.05±2.00 | 0.026* |
| MCHC (%) | 33.21±0.32 | 32.83±1.22 | 0.494 (NS) |
| TLC (10 ³ /ul) | 6.65±0.61 | 9.33±1.07 | 0.001* |
| Neutrophils (%) | 27.25±6.62 | 38.17±2.86 | 0.001* |
| Lymphocytes (%) | 65.60±6.50 | 54.33±2.34 | <0.001** |
| Eosinophils (%) | 3.20±1.47 | 3.17±2.04 | 0.965 (NS) |
| Monocytes (%) | 3.95±1.05 | 4.33±0.52 | 0.401(NS) |

*Affected and control cattle significantly different at $p<0.05$. **Significant at $p<0.001$. NS= Non-significant, RBCs= Red blood cells, Hb=Hemoglobin, PCV=Packed cell volume, MCV=Mean corpuscular volume, MCH=Mean corpuscular haemoglobin, MCHC=Mean corpuscular hemoglobin concentration, TLC=Total leukocyte count.

TABLE 2. Blood serum biochemical parameters in affected and healthy cattle (Mean±SD):

| Parameter | Tongue R | Control | P-value |
|---------------------|-------------|-------------|------------|
| Glucose | 66.87±10.92 | 72.66±9.32 | 0.263 (NS) |
| Triglycerides | 14.16±2.91 | 13.60±2.33 | 0.671 (NS) |
| Cholesterol | 81.37±11.85 | 109.34±9.74 | 0.006* |
| HDL-C | 59.14±8.56 | 75.32±7.07 | 0.038* |
| LDL-C | 28.92±7.93 | 25.24±8.51 | 0.546 (NS) |
| VLDL | 2.83±0.58 | 2.72±0.46 | 0.667 (NS) |
| Total protein(g/dl) | 5.12±1.81 | 7.40±0.71 | 0.020* |
| Albumin (g/dl) | 3.01±0.65 | 3.78±0.37 | 0.254 (NS) |
| Globulin (g/dl) | 2.30±0.64 | 3.62±0.68 | 0.020* |
| A/G ratio | 1.48±0.28 | 1.04±0.27 | 0.006* |
| Urea (mg/dl) | 4.35±1.41 | 4.76±1.28 | 0.266 (NS) |
| Creatinine (mg/dl) | 1.27±0.31 | 1.08±0.29 | 0.696 (NS) |
| AST | 62.38±4.38 | 61.90±5.41 | 0.822 (NS) |
| ALT | 25.67±5.21 | 25.19±3.16 | 0.834 (NS) |

*Affected and healthy cattle significantly different at $p<0.05$. **Significant at $p<0.001$. NS= Non-significant.

TABLE 3. Blood serum minerals and electrolytes in affected and healthy cattle (Mean±SD):

| Parameter | Tongue R | Control | P-value |
|-------------------------|--------------|-------------|------------|
| Ca (mg/dl) | 10.34±1.71 | 11.77±0.86 | 0.039* |
| P _{in} (mg/dl) | 5.67±0.57 | 5.89±0.38 | 0.692 (NS) |
| Mg (mg/dl) | 1.61±0.31 | 1.69±0.12 | 0.090 (NS) |
| Zn (umol/l) | 9.49±2.14 | 9.29±1.87 | 0.962 (NS) |
| Iron (umol/l) | 14.14±2.95 | 16.58±3.97 | 0.462 (NS) |
| Copper (umol/l) | 14.77±5.86 | 17.02±6.72 | 0.786 (NS) |
| Na (mEq/l) | 121.57±11.38 | 125.26±8.04 | 0.358 (NS) |
| K (mmol/l) | 4.04±1.38 | 4.02±0.48 | 0.975 (NS) |

*Affected and healthy cattle significantly different at $p<0.05$. **Significant at $p<0.001$. NS= Non-significant.

TABLE 4. Blood serum oxidative biomarkers in affected and healthy cattle (Mean±SD):

| Parameter | Tongue R | Control | P-value |
|---------------|--------------|-------------|------------|
| SOD (U/ml) | 174.43±13.75 | 199.26±0.64 | 0.009* |
| MDA (nmol/ml) | 2.18±0.31 | 1.77±0.86 | 0.064 (NS) |

*Affected and healthy cattle significantly different at $p < 0.05$. **Significant at $p < 0.001$. NS= Non-significant

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التغيرات الإكلينيكية والكيميائية الحيوية الدموية والإجهاد التأكسدي في العجلات الحلابية المصابة باضطراب لف اللسان

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الملخص

هدفت الدراسة الحالية إلى تحديد التغيرات في المؤشرات السريرية والكيميائية الحيوية الدموية والإجهاد التأكسدي في العجلات الحلابية المصابة باضطراب لف اللسان. تم اختيار سبعة عشر عجلة نمطية (مجموعة لف اللسان) وأثني عشر عجلة سليمة (مجموعة التحكم) من سلالة هولشتاين فريزيان تتراوح أعمارها بين 12 و22 شهرًا. تم جمع عينات الدم والمصل من جميع الحيوانات لتحديد معايير الدم والكيمياء الحيوية وكذلك الإجهاد التأكسدي. أظهرت الحيوانات التي تتدحرج باللسان الأفعال النمطية المميزة للعب باللسان والتي لم تُشاهد أبدًا في الضوابط. كانت درجة حرارة المستقيم ومعدل التنفس ومعدل ضربات القلب وانقباضات الكرش متشابهة في مجموعات لف اللسان والضوابط. تم العثور على فقر الدم في الحيوانات المصابة بلف اللسان. علاوة على ذلك، انخفض إجمالي عدد الكريات البيضاء ونسبة النتروفيل بشكل ملحوظ ($P < 0.001$) في الحيوانات المصابة. أظهرت مستويات مصل الدم من الجلوكوز والدهون الثلاثية والألبومين واليوريا والكرياتينين وكذلك AST و ALT تغيرًا غير مهم ($P > 0.05$) بين المجموعتين المدروستين. انخفضت مستويات الكوليسترول و HDL-C والبروتين الكلي والجلوبيولين بشكل ملحوظ ($P > 0.05$) في العجول المصابة. أظهرت معادن مصل الدم والإلكتروليتات فرقًا غير معنوي ($P > 0.05$) في مستويات الفسفور والمغنيسيوم والزنك والحديد والنحاس والصوديوم والبوتاسيوم بين المجموعتين. انخفضت مستويات الكالسيوم بشكل ملحوظ في المجموعة المصابة ($P > 0.05$). انخفضت أنشطة SOD بشكل ملحوظ ($P < 0.05$) في مجموعة لف اللسان بينما تم تسجيل زيادة غير معنوية ($P > 0.05$) في أنشطة MDA في نفس المجموعة. أشارت النتائج إلى أن الحيوانات المصابة بلف اللسان عانت من فقر الدم وانخفاض الطاقة والإجهاد التأكسدي. يمكن أن تكون هذه العوامل متورطة في الفسيولوجيا المرضية لمشكلة لف اللسان في الحيوانات الألبان.

الكلمات الدالة: لف اللسان، العجلات الحلابية، السريرية، الكيمياء الحيوية الدموية، الإجهاد التأكسدي.