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Serum Cu, Mn and Zn Levels and Oxidative Stress in Cattle Performing Tongue-playing [1]

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Abstract

This study was aimed at determining serum copper (Cu), manganese (Mn) and zinc (Zn) levels and the occurrence of oxidative stress in cattle performing tongue-playing stereotypies. The study material comprised of 25 healthy cattle and 50 stereotypic cattle performing tongue-playing, which were of a varying age and sex composition. The stereotypic animals were observed to display non-physiological tongue movements either by raising their heads and stretching their tongue out at long or short time intervals, or by contorting, rolling or swinging their tongue and making circular tongue movements in their mouth. These animals were confirmed to perform tongue-playing stereotypies. 10 mL blood samples were taken from each animal included in the study, and the sera extracted were analysed for serum Cu, Mn, Zn, malondialdehyde (MDA), nitric oxide (NO), total oxidant status (TOS) and total antioxidant status (TAS) levels. While the serum Mn levels of the stereotypic animals were lower than those of the healthy animals ($P<0.001$), it was observed that the serum Cu and Zn levels of the stereotypic and healthy animals did not differ ($P>0.05$). While the MDA ($P<0.001$), NO ($P<0.001$) and TOS ($P<0.05$) levels of the stereotypic animals were found to be higher than those of the healthy animals, the TAS ($P>0.05$) levels did not differ between the two groups. In result, it was concluded that Mn deficiency has an important role in the aetiology of tongue-playing stereotypies, and that this behavioural disorder is associated with oxidative damage or inflammation. Oxidative stress in tongue playing disease is thought to be formed due to cellular damage or stress.

Keywords: *Cattle, Copper, Manganese, Zinc, Tongue-playing, Malondialdehyde, Nitric oxide Total oxidant status, Total antioxidant status*

Dil Oynatma Hastalığı Olan Sığırlarda Serum Cu, Mn, Zn Seviyeleri ve Oksidatif Stres

Öz

Bu çalışma dil oynatma hastalığı olan hayvanlarda bakır (Cu), mangan (Mn), çinko (Zn) seviyeleri ve oksidatif stres gelişip gelişmediğinin belirlenmesi amacıyla yapılmıştır. Çalışmanın materyali farklı yaş ve cinsiyetlerde 50 dil oynatma hastalığı ve 25 sağlıklı sığırdan oluşturuldu. Çalışmaya alınan hasta hayvanların başlarını yukarıya kaldırarak uzun veya kısa aralıklarla dillerini ağızlarından çıkararak kıvırma, döndürme, sallama ve halka oluşturma gibi fizyolojik olmayan dil hareketleri yaptıkları görüldü ve klinik olarak dil oynatma hastalığı tanısı konuldu. Çalışmaya dahil edilen tüm hayvanlardan 10 mL kan alınarak serumları ayrıştırıldıktan sonra serum Cu, Mn, Zn, malondialdehit (MDA), nitrik oksit (NO), total oksidan durum (TOS) ve total antioksidan durum (TAS) belirlendi. Hasta hayvanların serum Mn değerleri sağlıklı hayvanlara göre düşük olduğu ($P<0.001$), Zn ve Cu değerlerinde ise fark olmadığı ($P>0.05$) belirlendi. Sağlıklı hayvanların NO ($P<0.001$), MDA ($P<0.001$) ve TOS ($P<0.05$) değerleri sağlıklı hayvanlara göre yüksek bulunurken TAS ($P>0.05$) değerinde fark olmadığı belirlendi. Sonuç olarak dil oynatma hastalığının etiyolojisinde Mn eksikliğinin önemli rol oynadığı, oksidatif hasar veya yangı geliştiği belirlendi. Dil oynatma hastalığında meydana gelen oksidatif stresin hücresel hasar veya strese bağlı olarak şekillendiği düşünüldü.

Anahtar sözcükler: *Sığır, Bakır, Mangan, Çinko, Dil oynatma, Malondialdehit, Nitrik oksit Total oksidan durum, Total antioksidan durum*

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INTRODUCTION

Tongue-playing is a behavioural disorder characterized by the display of non-physiological tongue movements such as the rolling, contorting and swinging out of the mouth of the tongue, in the form of recurrent episodes. Observed particularly in fast-developing high-yield breeds, tongue-playing is encountered in Turkey and many other countries across the world [1].

Although the exact causes of this disorder remain unknown, reports indicate that stress-inducing feeding and management conditions, play and mimicry instincts, genetics, and the deficiency of certain trace elements such as copper (Cu), cobalt (Co) and manganese (Mn) can be involved in its aetiology [1-3]. Cu, Zinc (Zn) and Mn, which are trace minerals, are important for organism [4,5]. Although found at very low levels in the body, trace elements are essential to the continuity of life and several physiological processes, including growth, development and reproduction [1-3].

Normally, in the body, there is an equilibrium between the antioxidant defence system and the generation of lipid peroxidation-inducing free oxygen radicals, which is referred to as the oxidative balance [6-8]. The disrupt of this balance between oxidants and antioxidants in favour of the former is described as oxidative stress [9-11]. On human and animal diseases oxidative stress plays a significant role [12]. In such cases, the antioxidant defence system fails to neutralize oxidative stress, which results in cellular damage and the loss of cell functions [8]. Parameters commonly used for the assessment of oxidative stress associated with disorders and diseases include serum malondialdehyde (MDA), nitric oxide (NO) and total oxidant status (TOS) levels. On the other hand, the antioxidant status is assessed by means of the measurement of total antioxidant status (TAS) levels [13]. To our knowledge there is no report about oxidative stress and steorotypic behaviour disorders.

This study was aimed at determining serum Cu, Mn and Zn levels and the occurrence of oxidative stress in cattle performing tongue-playing stereotypies.

MATERIAL and METHODS

Animal Material

The study was conducted after receiving approval from the Kafkas University Animal Experiments Local Ethics Committee (KAU-HADYEK, Investigation code: 2017/020, No 2017/02). Fifty stereotypic (Group I) and 25 healthy (Group II) cattle, including 11 males and 39 females with an age ranging from 6 months to 7 years, all of which were raised under similar management and feeding conditions, constituted the study material. In Group I, 4 of the male animals and 5 of the female animals were of the Native Black breed, whilst the remainder were of the Simmental breed. Group II included 10 Native Black and 15 Simmental cattle of both sexes, which were 1-5 years old. The tongue-playing disorder was diagnosed on the basis of the clinical symptoms displayed by the stereotypic animals.

Collection of Samples

Ten-mL jugular blood samples were taken from the animals included in Groups I and II. These samples were centrifuged at 3000 rpm for 10 min for the extraction of sera. The serum samples were stored at -20°C until the biochemical analyses were performed.

Biochemical Analyzes

The extracted sera were used for the measurement of total NO levels with a colorimetric method based on the Griess reaction [14], the measurement of MDA levels with a method based on thiobarbituric acid (TBA) reactivity [15]. Serum TAS and TOS levels were detected using a commercial Rel Assay Diagnostics kit (Turkey) according to the manufacturer's instructions.

The serum Cu, Mn and Zn levels were measured with the aid of a Perkin Elmer AAS-800 (USA) atomic absorption analyser. Prior to the analyses, all equipment was treated with 10% HNO_3, washed with ultra-distilled water and dried. Four intermediate standard solutions were prepared from the stock solution (1.000 µg/mL). Four work standard solutions were also prepared from the stock solution. When employing atomic absorption spectrometry (AAS), a hallow cathode lamp was used for each element. Once the current strength and light path of the lamp, the energy, aspiration period, reading period and air type (air, acetylene) were adjusted, the air compressor was started. All other calibration was done such that the confidence interval of the standards was 0.99500-1.00000 and the calibration coefficient (C.V.) was 99.5% [16].

Statistical Analysis

Statistical evaluation of the results was done using SPSS® (SPSS 20, IL, USA) software. The statistical differences between the groups were evaluated with the t-test. In the statistical evaluation, $P<0.05$ was considered to be statistically significant.

RESULTS

It was observed that the animals included in Group I raised their heads, stretched their tongue out at either long or short intervals, and displayed non-physiological tongue movements, including contorting, rolling, swinging and circular movements. The animals displayed these tongue movements for a period ranging from 5 to 45 min. Some animals were reported to display continuous tongue-playing, which resulted in reduced feed consumption and retarded growth. Furthermore, on-farm observations revealed that

Table 1. *Serum Cu, Mn, Zn, NO, MDA, TAS and TOS levels of the stereotypic and healthy animals*

Parameters	Stereotypic Animals X±SE (n=50)	Control Animals X±SE (n=25)	P Value
Cu (ppb)	374.73±31.08	337.9±24.46	P>0.05
Mn (ppb)	0.17±0.01	0.69±0.04	P<0.001
Zn (ppm)	0.76±0.019	0.77±0.025	P>0.05
NO (nmol/mL)	31.85±0.76	17.78±0.83	P<0.001
MDA (µmol/L)	21.37±0.56	9.86±0.59	P<0.001
TAS (mmol Trolox Eqv/L)	1.55±0.27	1.62±0.06	P>0.05
TOS (µmol H_2O_2 Eqv/L)	660.31±10.51	596.40±22.91	P<0.05

1-2 animals in each barn displayed tongue-playing stereotypies.

The results of the biochemical analyses of the serum samples pertaining to the stereotypic and control animals are presented in *Table 1*. The serum Zn, Cu and Mn levels and the NO, MDA, TAS and TOS levels of the two groups were statistically compared. The comparison of the two groups for trace elements showed that, while the serum Mn levels of the stereotypic animals were lower than those of the healthy animals (P<0.001), the serum Zn and Cu levels did not differ between the two groups (P>0.05). Furthermore, the comparison of the groups for the oxidative parameters demonstrated that, while the NO (P<0.001), MDA (P<0.001) and TOS (P<0.05) levels of the stereotypic animals were higher than those of the healthy animals, no difference was observed between the two groups for the TAS levels (P>0.05).

DISCUSSION

Tongue-playing, a common behavioural disorder encountered in Turkey and many other countries across the world, is clinically characterized by non-physiological tongue movements, including contorting, rolling, swinging and circular movements, observed in the form of recurrent episodes. This disorder is generally more common in fast-developing high-yield cattle breeds [1,2]. In the present study, in agreement with the clinical symptoms previously described for the disorder, the stereotypic animals were observed to raise their heads, stretch their tongue out at either long or short time intervals, and contort, roll, swing or circulate their tongue. It was informed that this disorder resulted in reduced feed consumption and retarded growth. While the majority of the stereotypic animals were of the fast-developing Simmental breed, a few Native Black cattle were also observed to display tongue-playing stereotypies.

The exact cause of tongue-playing remains unknown, yet it is suggested that it could be a habitual disorder related to the play and mimicry instincts of animals [1-3] On the other hand, in this study, as only 1 or 2 cases were observed among animals raised in the same barn, the play and mimicry instincts were considered not to be involved in the aetiology of the disorder.

Of trace elements, Cu is known to play a significant role in skin, hair, bone, nervous tissue and myocardial development, as well as in reproduction, growth, hair pigmentation, the immune system, and the oxidation system of tissues [17]. Furthermore, Cu serves as a co-factor of several enzymes, including cytochrome c oxidase, ceruloplasmin, lysyl oxidase, peptidylglycine alpha-monooxygenase, tyrosinase, superoxide dismutase and dopamine beta-mono-oxygenase, and also acts in cell respiration [1,18-20]. Also, it has been reported that Cu deficiency is involved in the aetiology of tongue-playing [1,2]. On the contrary, in the present study, it was determined that the difference between the serum Cu levels of the stereotypic and control animals was statistically insignificant (P>0.05). Accordingly, it was concluded that Cu had no role in the aetiology of tongue-playing.

Manganese is a trace element, which is particularly important for the maturation of the bone tissue matrix as well as for bone structure development in young animals. Mn acts as the activator of several enzymes, including kinases, decarboxylases and hydrases, and is also a component of metalloenzymes. It also contributes to lipid and carbohydrate metabolism, cell functions and the building of the cell membrane. Mn is also known to influence brain functions and the immune system [21-24]. Furthermore, Mn deficiency is reported to play an important role in the aetiology of tongue-playing [1-3]. In agreement with these reports, the present study demonstrated that the serum Mn levels of the stereotypic animals were lower than those of the control animals (P<0.001), thus, it was concluded that Mn deficiency was involved in the aetiology of tongue-playing.

Another trace element, Zn is essential to the body. It is not only found in the structure of several major metalloenzymes, including carbonic anhydrase, alkaline phosphatase, RNA and DNA polymerase, and alcohol dehydrogenase, but is also involved in the functions of these enzymes [24,25]. Therefore, the deficiency of this trace element adversely affects the functions of many internal organs and body systems. A previous study carried out in

cattle performing tongue-playing stereotypies showed that there was no difference between the serum Zn levels of the stereotypic and healthy animals [17]. Similarly, in the present study, it was determined that Zn was not involved in the aetiology of tongue-playing.

Excess production of reactive oxygen species causes oxidative stress [26]. In the event of such an imbalance, the antioxidant system fails to prevent oxidative stress, and cell damage occurs, which hinders cell functions [7,8,13,27]. Plasma MDA level can be used as an indicator of degenerations in cellular membranes which can be used as indicators of oxidative stress [13,28,29]. Two of the most common parameters used for the assessment of oxidative stress associated with diseases and disorders are serum MDA and TOS levels. The most common parameter measured to determine the antioxidant status is TAS [13]. In the present study, the comparison of the serum TAS levels of the stereotypic animals with those of the control animals revealed no statistically significant difference to exist ($P>0.05$), whilst the serum MDA ($P<0.001$) and TOS ($P<0.05$) levels of the stereotypic animals having been found to be higher than those of the healthy animals demonstrated the presence of oxidative stress in the animals displaying tongue-playing stereotypies.

Nitric oxide is the end product of the conversion of arginine into citrulline by nitric oxide synthase, an NADPH-dependent enzyme [30]. NO, which contains an unpaired electron, is a very strong free radical and causes nitrosative damage to many biomolecules. Furthermore, NO also shows effect as a strong vasodilator. In addition, NO also has neurotransmitter, immunomodulatory, and cytotoxic effects, the last being exhibited against substances harmful to the body [31]. The excessive production of NO is claimed to cause neural defects and to affect impulse transmission [32]. At the same time, NO is a free radical and is involved in several physiological and pathological processes. Bacterial endotoxins, protozoa and bacterial antigens lead to NO production by macrophages and show cytotoxic effect. A similar mechanism is also valid for tumour cells. Therefore, NO is considered to be a component of non-specific immunity [30,33-35]. In the present study, the serum NO levels of the stereotypic animals were found to be higher than those of the healthy animals ($P<0.001$). This suggested that the tongue-playing disorder could be associated with the development of inflammation.

In result, it was determined that Mn deficiency has an important role in the aetiology of tongue-playing. Furthermore, the serum TAS levels of the two groups not differing from each other, and the MDA and TOS levels of the stereotypic animals having been determined to be higher than those of the healthy animals demonstrated oxidative stress to have developed in the animals performing tongue-playing stereotypies. On the other hand, the serum NO levels of the stereotypic animals having been observed to be higher than those of the healthy animals suggested inflammation to have also developed in the animals performing tongue-playing stereotypies. On the basis of these results, it was ascertained that tongue-playing was associated with oxidative stress and inflammation. It is considered that further research is required to elucidate the underlying reasons of the cellular damage and stress associated with tongue-playing.

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