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Hematobiochemical profiles, mineral concentrations and oxidative stress indicators in beef cattle with pica

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ABSTRACT

Pica, or "depraved appetite", is defined as ingestion of non-nutritive substances. Many factors influencing pica in animals have been identified , but the etiology is controversial. This study was conducted to evaluate haematological and biochemical parameters, trace element concentrations, the total antioxidant status (TAS), total oxidative status (TOS) and oxidative stress index (OSI) in beef cattle with and without pica. Ten beef cattle with pica (group I) and another ten healthy beef cattle (Group II) were used in the study. Serum TAS and TOS were measured by a colorimetric method. In group I, haemoglobin value and serum iron, copper and selenium concentrations were significantly lower than those of group II ($p < .05$). Cortisol level and the other biochemical parameters were not statistically different between the groups ($p > .05$). Compared to the healthy controls mean TAS values were significantly decreased in the group I ($p < .05$), whereas the TOS and OSI values did not differ statistically between the two groups ($p > .05$). Parasitological analysis revealed that Trichostrongylidae spp eggs were more common in group I. In conclusion, serum iron, copper and selenium deficiency with decreased antioxidant capacity may cause the symptoms of pica in beef cattle. Prophylactic use of trace mineral supplement containing iron, copper, selenium and antioxidants in beef cattle may be beneficial in preventing deviated appetite and in strengthening the antioxidant defence system.

Highlights:

- Pica or is defined as the ingestion of non-nutritive substances
- As a result of this several disorders may occur such as reticuloperitonitis traumatica, poisonings, toxications or digestive system obstructions.
- Serum iron, copper and selenium deficiency with decreased antioxidant capacity may cause the symptoms of pica disorders in beef cattle.

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Beef cattle; pica; mineral concentrations; oxidative stress

Introduction

Pica defined as a depraved or abnormal appetite and is often considered a nutritional disorder of cattle and other farm animals (Nikvand et al. 2018). Appetite disorders may be secondary effects of diseases, most of which cause a reduction in food intake, or they may be diseases themselves, such as anorexia. Many diseases cause high body temperature. So a reduction in food intake occurs, which is presumed to derive from a direct effect of the elevated temperature on the brain. Diseases involving abdominal discomfort, also depress food intake: the reduction in intake should alleviate the discomfort, particularly if the food was the source of the problem. Metabolic disorders may also cause reduction in the appetite.

Pica involves the intake of inedible or non-nutritive materials such as soil, hair, bone or faeces (Constable et al. 2017). The causes of pica in animals are not well defined. There are several factors playing roles in the etiology of pica, some of which are protein-calorie malnutrition (PCM), parasitic infestation, obesity, deficiencies of protein (kwashiorkor) and micronutrients (Devlin 1969; Smith 2015). Although mineral deficiencies of some elements such as phosphorous, sodium, copper or magnesium have been suggested as

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possible causes of sporadic forms of pica, the concomitant imbalance of various serum minerals and proteins in cattle suffering from pica have not been completely determined (Nikvand et al. 2018). Furthermore, the relationship between pica and oxidative status has not been investigated well. Therefore, the objective of this study was to compare haematological and biochemical parameters, trace element concentrations, the total antioxidant status (TAS), total oxidative status (TOS) and oxidative stress index (OSI) in beef cattle with and without pica. In addition, presence of parasite infestations was also investigated.

Materials and methods

Animals and diet

A beef cattle farmer owning 300 Angus beef cattle applied to Animal Hospital of the Erciyes University for help as some of his cattle were having coprophagia and other symptom resembled pica. The farm was visited and some of the cattle were observed to be healthy and some of them had pica. Then, the study was conducted on 10 beef cattle exhibiting pica behaviour such as coprophagia (the eating of feces) and geophagia (Group I) and 10 healthy beef cattle (Group II) showing no clinical signs of any diseases including pica. The beef cattle exhibiting pica and control animals were selected randomly from a large herd, which was imported from abroad (South America) two months ago. The mean age of beef cattle was 10.4 ± 1.5 months. All animal's live weights were between 270 and 310 kg. The animal's mean body condition score (BCS) was of 3 ± 0.5 out of 5 (1 = emaciated to 5 = obese). Accordingly, the chosen beef cattle were divided into two groups. Group (I) consisted of 10 beef cattle that showed signs of pica and Group (II) consisted of 10 healthy beef cattle, which were used as a control. In addition, pica behavior has also been observed several times a day. Some of the more common types were coprophagia (the eating of feces), geophagia and the eating of soil or sand, but hair eating and urine drinking were not observed. The beef cattle were housed in a free-stall barn and were kept in intensive environments.

All the animals were offered 9.90 kg DM diet per day. Standard daily rations given to cattle included (as is bases) 5.0 kg concentrate mix (2700 Kcal/kg ME, %14 CP), 3.3 kg ground barley, 750 gr sunflower meal, 750 gr DDGS (Distillers Dried Grains with Solubles), 2.5 kg corn silage, 2.0 kg harnup silage (carob), 2.3 kg wheat straw and 80 gr of vitamin-mineral premix. A vitamin–mineral combination added to diet as 2 kg per ton. One kilogram of vitamin–mineral combination contains vitamin A: 12.000.000 UI, vitamin D3: 2.400.000 UI, vitamin E: 50.000 mg, vitamin K3: 1.000 mg, vitamin B1: 600 mg, vitamin B2: 2.500 mg, vitamin B6: 150 mg, vitamin C: 2.000 mg; niacin, 200.000 mg; folic acid, 2.000 mg; biotin, 200 mg; choline chloride, 100.000 mg; DL-methionine: 330 mg; iron: 80.000 mg, copper: 15.000 mg, manganese: 50.000 mg, cobalt: 150 mg, zinc: 150.000 mg, iodine: 800 mg, selenium: 150 mg. The ration was provided twice a day (07:00 and 17:00) and fresh water was available all the time.

Haematological and biochemical analyses

After puncture (with a 25-gauge needle) of the medial coccygeal vein, blood samples (9 mL per tube) were collected in two tubes, one tube containing ethylenediaminetetraacetic acid for measurements of haematological parameters using the automatic haematology analyser Exigo EosVet (Boule Medical AB, Stockholm, Sweden), and the other without any anticoagulant for biochemical analysis. After clotting, the blood samples were centrifuged at 1.300 × g for 10 min to separate the serum.

All biochemical tests were determined by using commercially available kits from Randox (Randox Laboratories, Crumlin, England, UK) and serum samples were analysed with a BT 3000 Plus biochemical analyser (Biotecnica, Italy), which is commercial colorimetric assay kit that uses the spectrophotometric method. Cortisol level of each animal was analysed with a DXI 800 immunoanalyser (Beckman Coulter Inc, USA). The concentrations of trace elements (manganese, iron, copper, zinc and selenium) in all samples were determined using an inductively coupled plasma-mass spectrometer (Agilent 7500a ICP-MS series, Agilent Technologies).

TAS levels were determined in serum via a commercial kit (Rel Assay Diagnostics, Gaziantep, Turkey) (Erel 2004). The method was based measuring the antioxidative effect of the sample against the potent free radical reactions, which is initiated by the produced hydroxyl radical. The TAS levels were measured with an auto analyser (Gesan Chem 400, Italy). The results were expressed as μmol Trolox Eq/L.

TOS values were determined in serum via a commercial kit (Rel Assay Diagnostics, Gaziantep, Turkey) (Erel 2005). The colour intensity, which is related to the total amount of oxidant molecules present in the sample, was measured by auto analyser. The analyses were calibrated with hydrogen peroxide (H_2O_2) and the results were expressed in terms of micro molar

hydrogen peroxide equivalent per liter (μmol H_2O_2 Eq/L).

Oxidative stress index, an indicator of the degree of oxidative stress, was calculated using the following formula: [(TOS, μmol/l)/(TAS, (mmol Trolox Eq/l) × 100].

Parasitological examination

Fresh faecal samples were directly collected from the rectum of each cattle in sterile plastic bags, transferred to the laboratory, and kept at 4 °C until parasitological examination. The samples were examined on the day of collection. Zinc sulphate flotation technique was applied to investigate parasite eggs (Rinaldi et al. 2011).

Statistical analysis

Data were analysed with the SPSS 15.0 statistical package program (SPSS Inc, Chicago, IL, USA). An independent samples t-test was used to reveal the statistical significance between the two groups. Statistical significance was considered to be $p < 0.05$. The results were expressed as means ± standard deviations.

Results

The haematological parameters were not different significantly when the two group values compared except haemoglobin value. There was a significant decrease ($p < .05$) in haemoglobin concentration in group I compared to group II (Table 1).

There was no significant differences in biochemical parameters when two group values compared including Cortisol value ($p > .05$; Table 2).

Table 1. Hematological Parameters of the Beef Cattle with Pica (group I, $n = 10$) and Healthy Cattle (group II, $n = 10$)

Parameters	Group I	Group II	*p* value
RBC, 10^{12}/L	6.25 ± 2.25	7.47 ± 0.47	.059
Hb, g/L	10.29 ± 3.50	12.29 ± 1.07	.050
Ht, volume fraction	36.76 ± 2.90	39.33 ± 4.09	.087
MCV, fL	51.64 ± 5.07	52.58 ± 3.18	.318
MCH, fmol/cell	17.48 ± 3.17	16.47 ± 0.74	.171
MCHC, g/L	34.80 ± 8.64	31.36 ± 0.77	.140
WBC, 10^9/L	8.26 ± 1.83	8.96 ± 1.14	.168
Lymphocytes, 10^9/L	4.86 ± 1.17	5.62 ± 0.91	.070
Monocytes, 10^9/L	0.70 ± 0.21	0.76 ± 0.16	.251
Granulocytes, 10^9/L	2.55 ± 0.58	2.58 ± 0.70	.462
Thrombocytes, 10^9/L	264.00 ± 92.10	269.40 ± 128.90	.461
RDW, %	16.48 ± 2.52	15.34 ± 1.11	.109
MPV, fL	4.83 ± 0.68	4.95 ± 0.39	.315

Results are expressed as mean ± standard deviation
RBC: red blood cells; Hb: hemoglobinemia; Ht: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin quantity; MCHC: mean corpuscular hemoglobin concentration; WBC: white blood cells; RDW: red cell distribution width; MPV: mean platelet volume.

Furthermore, there was a significant decrease in serum iron, copper and selenium concentrations in group I compared to group II ($p < .05$; Table 3). There was also significant decrease ($p < .01$) in the mean TAS concentrations between two groups, whereas the TOS and OSI values did not differ statistically between the two groups ($p > .05$; Table 4).

Fecal parasitological analysis revealed the presence of parasites. Eight cattle in group I and two cattle in group II were positive with concern to Trichostrongylidae spp eggs.

Table 2. Biochemical Parameters of the Beef Cattle with Pica (group I, $n = 10$) and Healthy Cattle (group II, $n = 10$)

Parameters	Group I	Group II	*p* value
Cortisol, ug/dL	1.95 ± 1.43	1.99 ± 0.88	.488
ALT, U/L	42.11 ± 9.98	38.71 ± 7.04	.214
AST, U/L	125.55 ± 26.29	135.91 ± 24.53	.202
ALP, U/L	109.90 ± 43.94	97.63 ± 34.53	.264
GGT, U/L	23.25 ± 5.18	23.33 ± 9.63	.492
Lipase, U/L	7.30 ± 2.75	8.71 ± 2.36	.144
Total Protein, g/L	6.13 ± 0.41	6.24 ± 0.37	.288
Albumin, g/L	3.51 ± 0.23	3.38 ± 0.23	.119
Total Bilirubin, mg/dl	0.33 ± 0.16	0.34 ± 0.11	.428
Urea, mg/dl	49.40 ± 4.45	45.50 ± 12.83	.190
Creatinin, mg/dl	1.27 ± 0.20	1.29 ± 0.22	.419
BUN, mg/dl	23.06 ± 1.99	22.25 ± 4.36	.303
Triglyceride, mg/dl	17.75 ± 5.55	21.20 ± 4.92	.140
Uric Acid, mg/dl	4.34 ± 0.52	5.18 ± 1.34	.118
GLU, mmol/L	19.60 ± 7.07	20.00 ± 9.12	.459
Ca, mg/dl	11.40 ± 0.51	11.45 ± 0.87	.440
P, mg/dl	9.56 ± 0.95	9.24 ± 0.83	.233
Mg, mg/dl	2.94 ± 0.25	3.16 ± 0.52	.122
Na, mmol/L	149.33 ± 5.05	149.71 ± 5.09	.442
K, mmol/dl	4.65 ± 0.98	5.49 ± 0.75	.033
Cl, mmol/L	106.90 ± 2.85	108.75 ± 3.06	.102

Results are expressed as mean ± standard deviation.

Table 3. Serum Mineral concentrations of the Beef Cattle with Pica (group I, $n = 10$) and Healthy Beef Cattle (group II, $n = 10$)

Parameters	Group I	Group II	*p* value
Mn, ppm	0.16 ± 0.05	0.17 ± 0.25	.303
Fe, ppm	3.07 ± 0.98	5.77 ± 1.87	.001
Co, ppm	0.02 ± 0.01	0.02 ± 0.01	.290
Cu, ppm	0.09 ± 0.06	0.17 ± 0.10	.036
Zn, ppm	0.27 ± 0.22	0.25 ± 0.14	.377
Se, ppm	0.01 ± 0.01	0.03 ± 0.01	.001

Results are expressed as mean ± standard deviation.

Table 4. Serum Total Antioxidant Status (TAS), Total Oxidative Status (TOS) and Oxidative Stress index (OSI) of the Beef Cattle with Pica (group I, $n = 10$) and Healthy Cattle (group II, $n = 10$)

Parameters	Group I	Group II	*p* value
TOS, μmol H2O2 Equiv. /L	10.67 ± 1.83	11.65 ± 4.17	.337
TAS, mmol Trolox Equiv. /L	1.02 ± 0.21	0.83 ± 0.06	.012
OSI, arbitrary unit	11.75 ± 1.50	13.06 ± 4.24	.296

Results are expressed as mean ± standard deviation.

Discussion

Pica or in another word "depraved appetite" has been an important subject in various animals and different aetiologies speculated to play role in the development of the problem (Meyer and Lohes 2002; Aytekin et al. 2010, 2011). Many factors have been identified in the aetiology of the pica in animals, therefore, pica is an interesting and very complex topic for researchers. The tendency to pica has not been defined thoroughly, but under some circumstances pica may occur during expression of a learned taste aversion or during states of nutrient deficiency. Therefore, in the present study, cattle having pica symptoms were investigated to find out possible reasons of the behaviour.

When results were evaluated, the haematological parameters were similar for both the groups, except for haemoglobin concentration which was significantly lower ($p < .05$) in the pica group (Group I). This is probably due to the fact that dietary deficiency or failure of intestinal absorption may cause limited or inadequate iron intake, which is effective in the formation of haemoglobin. Furthermore, all biochemical parameters including cortisol concentrations were not different in both groups. Additionally, all values determined in the present study for haematological (apart from haemoglobin concentration) and biochemical parameters of beef cattle with and without pica were within the reference values (Turgut 2000; Kraft Dürr 2005).

Pica is often considered a nutritional disorder of cattle and other farm animals, but the aetiology is controversial. Roles for the shortage or imbalance of some nutritional elements have been debated (Nikvand et al. 2018). Thus, in the present study, serum mineral and trace element concentrations in the animals with and without pica were also investigated. With concern to this, a significant decrease in serum iron, copper and selenium concentrations in beef cattle with pica compared to a control group were determined ($p < .05$). On the other hand, macro element levels such as Ca, P and Mg were not different in both groups.

Nikvand et al. (2018) determined that low serum iron concentration accompanied by low serum ferritin might be associated with long-term nutritional iron deficiency, which then plays an important role in the occurrence of pica in cattle. Similarly, Aytekin et al. (2011) found that horses with pica had lower concentrations of serum iron and copper in relation to healthy horses. In another study performed on 15 lambs having pica symptoms; iron deficiency was found and it was related to the aetiology of pica in lambs (Aytekin et al. 2010). Similar findings were also reported for human beings (Lopez et al. 2007). Furthermore, Nikvand et al. (2018) reported that low serum iron concentrations appear to prompt chronic nutritional iron inadequacy, which increase the risk of pica in cattle due to iron deficiency. In an enzootic pica in young calves and lambs that graze on pasture rich in manganese related to iron deficiency because high soil manganese may interfere with iron absorption in digestive tract (Neser et al. 2000). In the present study, the manganese concentration in the soil is not investigated, but its serum concentration didn't change significantly.

In contrast to our results, Naci et al. (2008) investigated 30 cattle that suffered from pica and had normal amounts of calcium, copper and zinc but slightly elevated iron concentration. Although many researches (Lopez et al. 2007; Aytekin et al. 2010; 2011; Nikvand et al. 2018) with regard to low serum iron value in animals with pica are in agreement with the present study, some papers (Akgül et al. 2000; Naci et al. 2008) do not agree with each other or with us about the role of the other elements, especially iron and copper, in the occurrence of pica. To determine the main causes of pica, several studies were carried out on cattle, horses and lambs. Although Aytekin and Kalinbacak (2008) reported significant low phosphorus and copper mineral concentrations in the blood of cattle exhibiting pica, we found that the mean serum phosphorus levels for the cattle with pica was not lower than control cattle. Jain and Chopra (1994) also observed pica symptoms in calves fed on diet with insufficient phosphorus supply. In the present study, the causes of pica may be copper or other nutritional deficiencies, as well. A previously mentioned study by Haris et al. (1995), copper deficiency can lead to the low iron concentrations due to its important role in transporting iron into the blood circulation. Nikvand et al. (2018) reported that differences in aetiology of pica may be due to differences in sensitivity to particular element/mineral shortage, breed and age diversity of animals studied, and their differences in nutritional requirements. According to the results of the present study, iron and copper deficiencies may be considered as an important cause of pica, for instance, coprophagia and geophagia, in beef cattle and differences in aetiology might be related to the type of pica.

The results of the biochemical analyses in the present study showed no significant differences in serum values of total protein, albumin and urea in both groups which are in agreement with Zhou et al. (2009), Li et al. (2014) and Nikvand et al. (2018).

Parasitism may also play an important role in the etiology of pica (Smith 2015). Our results showed that the parasites found in the feces of animals with pica might increase the risk of pica and it causes blood loss leading to direct iron-deficiency related to anaemia in cattle.

In the present study, when oxidative stress parameters evaluated, TAS decreased significantly in cattle with pica. In the present study, pica symptoms in the cattle reported to be observed just after a week of transport and the symptoms are increased during two months period. Therefore, the reason could be due to transport of the animals from long distance and their accommodation in a crowded barn conditions. Such studies with concern to the effect of transport on oxidative stress has been investigated, but their role in the development of pica has not been understood well (Chirase et al. 2004; Onmaz et al. 2011; Piccione et al. 2013). In the present study, transport may affect antioxidant status of the animals negatively, so, the risk of pica may have triggered. Therefore, antioxidants should be given to the animals before transport to protect them from pica. But the exact role between imbalance of oxidative and antioxidative status in cattle with pica should be investigated in future studies with all the details.

Conclusions

In conclusion, pica is considered as a multifactorial condition, including iron, copper and selenium deficiency in addition to decreased antioxidant capacity. If so, other possible factors related to pica should be investigated in future studies as well. To protect the animals from such condition; in addition to above deficiencies, transport, parasitismus, barn conditions and antioxidant status should also be taken into consideration. Furthermore, mineral treatment and the response to the treatment may be useful in differentiating etiology. In prophylaxis, using antiparasitic agents and trace mineral supplements containing iron, copper, selenium and antioxidants in beef cattle may be beneficial in preventing deviated appetite and in strengthening the antioxidant defence system.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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