Praca oryginalna *Original paper*

Serum mineral levels and haematobiochemical parameters in buffalo calves with allotriophagy (pica syndrome)

AKIN KOCHAN[1], AYNUR SIMSEK[1], ENES ARICA[2]

[1]Department of Internal Medicine, Faculty of Veterinary Medicine, Dicle University, Diyarbakir, Turkey
[2]Science and Technology Application and Research Center, Dicle University, Diyarbakir, Turkey

Received 15.11.2022 *Accepted 05.01.2023*

Kochan A., Simsek A., Arica E.

Serum mineral levels and haematobiochemical parameters in buffalo calves with allotriophagy (pica syndrome)

Summary

Pica is defined as a depraved or abnormal appetite characterized by licking and eating non-nutritive substances. The aim of this study was to report changes in the serum mineral levels and haematobiochemical parameters of buffalo calves with pica. The study included 30 buffalo calves exhibiting pica behaviour, such as licking stall walls or eating wool and non-food items (pica group), and 6 buffalo calves without the signs of pica behaviour (control group). The age of both groups ranged between 1 and 6 months. In the pica group, the haematological analysis revealed a decrease in red blood cells ($P < 0.001$) and red cell distribution width ($P < 0.05$) as well as an increase in the mean corpuscular volume and mean corpuscular haemoglobin ($P < 0.001$), whereas the biochemical parameters were characterized by an increase in aspartate aminotransferase ($P < 0.01$), alanine aminotransferase ($P < 0.001$) and lactate dehydrogenase ($P < 0.05$) levels and a decrease in creatinine and glucose ($P < 0.01$) levels. Furthermore, the pica group showed a significant decrease in serum manganese, iron, zinc, selenium ($P < 0.001$) and copper ($P < 0.01$) concentrations. In conclusion, it was determined that deficiencies in the levels of serum iron, copper, zinc, manganese and selenium play an important role in the aetiology of pica in buffalo calves.

Keywords: buffalo calves, iron, pica, serum mineral levels

Pica, defined as a depraved or abnormal appetite and characterized by licking and eating non-nutritive substances, is considered to be a sign of malnutrition or a behavioural disorder (3, 24). This condition is observed in humans (5, 19, 23, 30) and animals, such as dogs, cats, cattle, buffaloes, camels, sheep, horses and pigs (6-8, 11, 12, 15, 17, 21, 28, 29). The affected animals tend to lick and gnaw at almost anything they come in contact with. Aside from the tendency to eat soil and sand (geophagia), bones (osteophagia), dirty litter and even faeces (coprophagia), some animals lick stall walls and floors, and some, such as cats, pigs, sheep, goats, cows and buffaloes, eat placenta (placentophagia) (2, 7, 11, 28).

Pica is an important disease of domestic animals because of the potential complications it may produce in the gastrointestinal tract, including congestion, constipation, pain and cramps caused by indigestible objects, perforation by sharp objects, infection and diarrhoea from soil-dwelling parasites and colic (1, 2, 11).

Although the causes of pica have not been fully elucidated, deficiency of some proteins and amino acids, decrease in the body's alkaline reserve, insufficiency of certain vitamins and trace elements, imbalance of the calcium/phosphorus ratio in the diet and phosphorus deficiency play a role in its aetiology (27). In addition, diseases of the central nervous system, such as rabies, ketosis/acetonemia and lead poisoning, may also be among the causes of pica (24).

In humans, pica might be a consequence of malnutrition, undernutrition or severe starvation. Pica is directly related to the deficiency of minerals, such as zinc, phosphorus and iron (19, 23). Humans may develop pica at any stage of life, but it is a common condition in pregnancy and childhood. The prevalence of pica is highest in children aged 18 months to six years, and it is a significant cause of lead poisoning and anaemia (19).

In cats (8, 15) and dogs (6, 17, 21), pica might occur as a behavioural disorder or due to the deficiency of

minerals, such as iron, calcium or zinc, or of thiamine, niacin and some vitamins, such as C and D.

Deficiencies of macroelements (sodium and phosphate) and trace elements (especially copper, iron, zinc and selenium) have been reported as possible causes of pica in livestock (20). Decreased serum iron and copper levels in horses with pica have been shown to be involved in the disease aetiology (3, 20). Serum iron and zinc levels in lambs with pica (2) and serum zinc, iron, copper, cobalt, magnesium, selenium and phosphorus concentrations in cattle with pica (2, 10) have been reported to be low.

Although there have been numerous studies on the aetiology of pica in livestock, there have been no comprehensive studies investigating its aetiology in buffaloes. Therefore, the aim of this study was to report changes in serum mineral levels and haematobiochemical parameters in buffalo calves with pica.

Material and methods

Ethical statement. Ethical approval for this study was obtained from the University Local Ethics Committee for Animal Experiments (no. 85E-35582840-604.01.01-103178 of 08.07.2021).

A total of 47 buffalo calves, which were the animal material of this study, were evaluated in a buffalo herd of 110 animals. As a result of routine clinical examinations, 11 buffalo calves with different pathological conditions were excluded from the study. The study included 30 buffalo calves exhibiting pica behaviour (pica group), such as licking stall walls and eating wool and non-food items, and 6 buffalo calves without any signs of pica behaviour (control group). The age of both groups ranged between 1 and 6 months.

Clinical examinations. All animals were clinically examined for heart rate, respiratory rate, body temperature and skin lesions.

Sample collection. Blood samples were collected from the vena jugularis of the animals into coagulant and non-anticoagulant tubes using a sterile cannula. Fresh faecal samples were collected directly from the rectum of each calf into sterile plastic bags.

Haematological analysis. The samples were analysed with a haematology device (Mindray BC-2800Vet) immediately after being taken into coagulant tubes. The haematological parameters, including the white blood cells (WBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpus haemoglobin concentration (MCHC), red cell distribution (RDW), platelet (PLT), platelet distribution width (PDW), mean platelet volume (MPV) and platocrite (PCT) values were measured and analysed statistically.

Biochemical analysis. Serum samples were obtained by centrifuging the blood samples taken into anticoagulant tubes for 10 minutes at 1207 g after coagulation at room temperature. The serum samples were kept at –20°C until analysis. The levels of albumin (ALB), total protein (TP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatinine (CRE), glucose (GLU), phosphorus (P), calcium (Ca), magnesium (Mg), sodium (Na), potassium (K) and chlorine (Cl) in the serum samples were analysed with a biochemistry device (Fujifilm DRI-CHEM NX500).

The concentrations of manganese (Mn), iron (Fe), cobalt (Co), copper (Cu), zinc (Zn), selenium (Se) and molybdenum (Mo) in the serum samples were measured with a spectrometer device (Agilent Inductively Coupled Plasma-Mass 7700X).

Parasitological examinations. Fresh faecal samples were transferred to the laboratory, and kept at 4°C until parasitological examination. The samples were examined on the day of collection. Parasite eggs were investigated by the zinc sulphate flotation technique (26). Quantitative examination for the number of eggs per gram of faeces was not performed, because none of the buffalo calves included in the study were seriously infected. This may have been due to the regular use of anthelmintic drugs on the farm.

Statistical analysis. The study data were analyzed using the SPSS 16.0 statistical software package. The conformity of the data to the normal distribution was evaluated with the Shapiro-Wilk Test. The differences between the groups in terms of the parameters measured were compared with the Mann-Whitney U test. The values are presented as mean ± standard error of mean. $P < 0.05$ was considered statistically significant.

Results and discussion

Clinical findings. On clinical examination, it was determined that the calves in the pica group ate each other's wool, licked the stall walls, tended to consume non-food items and had growth retardation, cachexia, alopecia, skin lesions, constipation and trichobezoar.

Haematological and serum biochemical findings. Compared with the mean values of the haematological parameters of the control group, the pica group showed a decrease in WBC, RBC, HGB, HCT, RDW and MPV values, and an increase in MCV, MCH, MCHC, PLT and PDW values. The decrease in RBC ($P < 0.001$) and RDW ($P < 0.05$) levels and the increase in MCV and MCH ($P < 0.001$) levels were found to be statistically significant (Tab. 1).

The statistical comparison of biochemical parameters between the pica and control groups showed an increase in the levels of AST, ALT, LDH and TP and a decrease in those of CRE and GLU in the pica group. The increase in AST ($P < 0.001$), ALT ($P < 0.01$) and LDH ($P < 0.05$) levels and the decreases in CRE ($P < 0.01$) and GLU ($P < 0.001$) values were statistically significant (Tab. 2).

Comparison of mean serum mineral concentrations between the pica and control groups revealed no statistically significant differences in P, Ca, Mg, Na, K and Cl concentrations, but the levels of Mn, Fe, Zn, Se ($P < 0.001$) and Cu ($P < 0.01$) in the pica group were significantly decreased (Tab. 3).

Parasitological findings. Examination of faecal samples from the buffalo calves revealed only minor

Tab. 1. Haematological parameters of the pica and control groups

Parameters	Groups ($\bar{x} \pm S_{\bar{x}}$)		P palue
	Pica (n = 30)	Control (n = 6)	
WBC (10^9/L)	12.21 ± 0.56	13.85 ± 0.96	0.233^{NS}
RBC (10^9/L)	9.00 ± 0.36	13.50 ± 1.21	0.000***
HGB (g/dL)	14.81 ± 0.57	16.48 ± 0.61	0.113^{NS}
HCT (%)	41.80 ± 1.52	48.27 ± 1.66	0.029*
MCV (fL)	47.03 ± 0.89	39.57 ± 1.55	0.001***
MCH (pg)	22.11 ± 5.66	13.65 ± 0.53	0.000***
MCHC (g/dL)	35.32 ± 0.20	34.63 ± 0.48	0.172^{NS}
RDW (%)	15.73 ± 0.38	21.53 ± 1.87	0.001*
PLT (10^9/L)	409.80 ± 29.23	396.67 ± 50.06	0.918^{NS}
MPV (fL)	5.67 ± 0.12	5.90 ± 0.31	0.442^{NS}
PDW (fL)	16.38 ± 0.11	15.90 ± 0.08	0.08^{NS}
PCT (%)	0.24 ± 0.02	0.24 ± 0.04	0.918^{NS}

Explanations: $\bar{x}$ – mean; $S_{\bar{x}}$ – standard error of mean; ^{NS} – not significant; *** – P < 0.001; ** – P < 0.01; * – P < 0.05

Tab. 2. Serum biochemistry results for the pica and control groups

Parameters	Groups ($\bar{x} \pm S_{\bar{x}}$)		P value
	Pica (n = 30)	Control (n = 6)	
TP (g/dL)	6.84 ± 0.14	6.57 ± 0.09	0.548^{NS}
ALB (g/dL)	3.71 ± 0.08	3.52 ± 0.06	0.233^{NS}
AST (UI/L)	205.9 ± 8.64	147.5 ± 10.06	0.002**
ALT (UI/L)	65.43 ± 3.34	37.83 ± 3.34	0.001***
LDH (UI/L)	732.60 ± 15.04	597.67 ± 58.49	0.026*
CRE (mg/dL)	1.69 ± 0.03	1.98 ± 0.07	0.002**
GLU (mg/dL)	73.53 ± 1.73	110.50 ± 9.39	0.000***

Explanations: $\bar{x}$ – mean; $S_{\bar{x}}$ – standard error of mean; ^{NS} – not significant; *** – P < 0.001; ** – P < 0.01; * – P < 0.05

Tab. 3. Serum mineral levels of the pica and control groups

Parameters	Groups ($\bar{x} \pm S_{\bar{x}}$)		P value
	Pica (n = 30)	Control (n = 6)	
P (mg/dL)	8.76 ± 0.21	8.42 ± 0.52	0.159^{NS}
Ca (mg/dL)	10.23 ± 0.27	9.82 ± 0.63	0.467^{NS}
Mg (mg/dL)	3.04 ± 0.11	3.18 ± 0.25	0.852^{NS}
Na (mEq/L)	133.90 ± 2.62	138.50 ± 7.94	0.634^{NS}
K (mEq/L)	5.21 ± 0.15	5.63 ± 0.36	0.268^{NS}
Cl (mEq/L)	98.53 ± 2.48	103.00 ± 7.82	0.984^{NS}
Mn (µmol/L)	0.03 ± 0.01	0.06 ± 0.01	0.000***
Fe (µmol/L)	37.60 ± 1.19	51.67 ± 2.41	0.000**
Co (µmol/L)	0.01 ± 0.01	0.01 ± 0.01	0.394^{NS}
Cu (µmol/L)	11.15 ± 1.55	12.93 ± 0.95	0.008**
Zn (µmol/L)	45.17 ± 0.83	52.17 ± 0.63	0.000***
Se (µmol/L)	0.23 ± 0.01	0.33 ± 0.02	0.001***
Mo (µmol/L)	0.57 ± 0.03	0.50 ± 0.06	0.349^{NS}

Explanations: $\bar{x}$ – mean; $S_{\bar{x}}$ – standard error of mean; ^{NS} – not significant; *** – P < 0.001; ** – P < 0.01; * – P < 0.05

parasitic infestations. Since no severe infestations were found, pica was not due to the presence of parasites.

Organisms require essential nutrients and minerals to maintain their biological functions (14). Mineral deficiency can lead to loss of appetite, loss of productivity, cachexia, hair loss, colour changes and structural disorders of the skin and hair, abortion, diarrhoea, anaemia, bone disorders and pica (27). Furthermore, it has been reported that trace element deficiency plays an important role in the aetiology of pica, which causes animals to lick stall walls and eat wool, as well as other non-food items, such as bones, cloths and rags (11). In the present study, changes in the haematological and biochemical parameters and mineral levels were investigated in buffalo calves exhibiting pica behaviour.

The comparison of the mean values of the haematological parameters between the pica and the control groups of buffalo calves revealed a decrease in WBC, RBC, HBG, HCT, RDW and MPV values and an increase in MCV, MCH, MCHC, PLT and PDW values in the pica group. The decrease in RBC (P < 0.001) and RDW (P < 0.05) levels and the increase in MCV and MCH (P < 0.001) values were found to be statistically significant. In horses with pica, Aytekin et al. (3) reported a decrease in haemoglobin levels, and Li et al. (20) reported a decrease in erythrocyte and haemoglobin levels and an increase in MCV levels without statistical significance. In studies on sheep and lambs exhibiting wool-eating behaviour, significantly lower haemoglobin and haematocrit levels were found in sick animals (9, 13, 25). Onmaz et al. (25) reported a decrease in erythrocyte, haemoglobin and haematocrit values in cattle with pica, further noting that the decrease in the amount of haemoglobin was statistically significant. Mosa et al. (22) found significant reductions in the level of erythrocytes, haemoglobin and haematocrit in cattle with pica. Koted et al. (16) reported a significant decrease in the mean concentrations of erythrocytes, haemoglobin and haematocrit in camels with pica compared with those in healthy camels. Lengare et al. (18) reported a significant decrease in the mean haemoglobin concentration in buffaloes with pica, but the decrease in mean erythrocyte and haematocrit concentrations was not significant. The haematological findings of this study are in line with those of the abovementioned studies (3, 4, 10, 16, 18, 20, 22), and the decrease in haemoglobin and haematocrit levels as well as anaemia are believed to be caused by iron and zinc deficiency (8, 16).

Statistical analysis of the biochemical parameters of the pica and control groups revealed increased AST, ALT and LDH values and decreased CRE and GLU values in the pica group. The increase in AST, ALT (P < 0.001) and LDH (P < 0.05) values and the decrease in CRE (P < 0.01) and GLU (P < 0.01) values were found to be statistically significant. Previous studies (3, 4, 9, 10, 20, 25) reported increased levels of AST and

ALT and decreased levels of GLU in animals with pica. Some studies (4, 10, 20) found an increase in the serum CRE concentration, whereas others reported reduced levels of CRE (3, 9, 25). An increase in LDH levels has also been reported (1, 9, 25). The changes in serum AST, ALT, LDH and GLU levels in the present study are consistent with the findings of the other studies (3, 4, 9, 10, 20, 25). The decreased CRE level was consistent with the findings of some of the abovementioned studies (3, 9, 25), but inconsistent with the findings of some others (4, 10, 20). The increase in serum AST and ALT levels may be related to changes in adipose tissue in the liver caused by copper deficiency (4, 9).

The present study found no significant change in the amount of TP, which was consistent with the findings of other studies (10, 20, 24, 25).

There were not statistically significantly differences between the pica and control groups in mean concentrations of P, Ca, Mg, Na, K and Cl. The role of mineral substance deficiencies in pica aetiology is controversial, and there have been different reports regarding serum mineral levels in domestic animals with pica. Sahin et al. (27) reported a significant decrease in serum inorganic phosphorus levels in lambs with pica and claimed that inorganic phosphorus played an important role in the aetiology of pica, but they found no significant changes in serum magnesium and calcium levels (2). Similarly, Li et al. (20) reported a significant decrease in serum phosphorus levels in horses with pica but no significant changes in serum calcium, magnesium, sodium and potassium concentrations. Nikvand et al. (24) reported a significant decrease in serum phosphorus, potassium and chlorine levels in cattle with pica. Elshahawy and Aly (10) reported a decrease in magnesium levels as well as in phosphorus levels in cattle with pica. In parallel with the findings of our study, Onmaz et al. (25), Aytekin et al. (3) and Ebrahim (9) reported no significant changes in serum P, Ca, Mg, Na, K and Cl levels in animals with pica.

Pica is often regarded as a form of malnutrition of cattle and other livestock. It has been reported that the lack or imbalance of certain nutrients and the deficiency of trace elements plays a role in the aetiology of the disease (2, 24, 25). In the present study, serum iron, copper, zinc, manganese and selenium concentrations in buffalo calves with pica were significantly decreased compared with those in the control group. Li et al. (20) reported a significant decrease in serum copper and iron concentrations as well as in the copper/zinc ratio in horses with pica compared with the control group, but found no significant differences in serum zinc, manganese and selenium values. Aytekin et al. (3) reported a significant decrease in serum iron and copper concentrations and in the copper/zinc ratio in horses with pica compared with the control group. Koted et al. (16) reported significantly decreased serum iron concentrations in camels with pica compared with healthy controls. Sahin et al. (27) found that the mean serum copper value for lambs with pica was lower than that for healthy lambs. Icen et al. (13) reported significantly decreased serum iron, zinc and copper levels in lambs exhibiting wool-eating behaviour. Baydar et al. (4) found that serum copper, zinc, iron and manganese levels in wool-eating sheep were significantly lower. Onmaz et al. (25) reported a statistically significant decrease in serum copper, iron and selenium concentrations in feeder cattle. Elshahawy and Aly (10) found a significant reduction in serum iron, copper, cobalt and selenium levels in cattle with pica. Aytekin and Kalinbacak (2) reported a significant decrease in serum copper, iron and zinc levels in calves with pica. Mosa et al. (22) found a decrease in serum iron and copper concentrations in cows with pica. Nikvand et al. (24) suggested that a low serum iron concentration accompanied by low serum ferritin may be associated with chronic iron deficiency, which plays an important role in the development of pica in cattle. It has been reported that trace element deficiencies cause pica through their negative effects on the organism's antioxidant capacity (4). The findings of the present study are consistent with the literature (2-4, 10, 13, 16, 24, 25, 27), and serum mineral concentrations were found to be low in the animals with pica. However, our results were not similar to the findings of a study (4) that reported no significant changes in serum zinc, manganese and selenium levels in animals with pica. Such discrepancies in the results of studies on animals with pica may be due to differences in the susceptibility to certain element/mineral deficiencies, variation in breed and age of the animals, as well as differences in their nutritional requirements (24).

In conclusion, it was determined that changes in the haematological and biochemical parameters and serum mineral concentrations observed in this study were useful in the evaluation of the aetiology of pica in buffalo calves, and serum manganese, iron, copper, zinc and selenium deficiencies played an important role in the aetiology of this disease. Since deleterious effects of mineral deficiency caused by its negative impact on the antioxidant capacity of the organism lead to pica, the administration of mineral supplements and antioxidants to buffalo calves will be useful in preventing this condition.

References

1. *Akgul Y., Agaoglu Z. T., Kaya A., Sahin T.*: The relationship between the syndromes of wool eating and sheep fed corn silage and blood changes (haematological, biochemical and trace element). Isr. J. Vet. Med. 2000, 56, 12-16.
2. *Aytekin I., Kalinbacak A.*: The levels of calcium, phosphor, magnesium, copper, zinc and iron in calves eating soil in the region of Afyon. Ataturk University J. Vet. Sci. 2008, 3, 34-42.
3. *Aytekin I., Onmaz A. C., Aypak S. U., Gunes V., Kucuk O.*: Changes in serum minerals concentration, biochemical and hematological parameters in horses with pica. Biol. Trace Elem. Res. 2011, 139, 301-307.
4. *Baydar E., Ozcelik M., Gazioglu A.*: Some trace elements and serum biochemistry in sheep with fleece eating. Firat University Vet. J. H. Sci. 2015, 29, 187-190.
5. *Bhatia M. S., Kaur J.*: Pica as a culture bound syndrome. Delhi Psychiatry Journal 2014, 17, 144-147.

6. *Bradshaw J. W., Neville P. F., Sawyer D.*: Factors affecting pica in the domestic cat. Appl. Anim. Behav. Sci. 1997, 52, 373-379.
7. *Constable P. D., Hinchcliff K. W., Done S. H., Grunberg W.*: Veterinary Medicine. W. B. Saunders, London 2011, p. 88.
8. *Demontigny-Bédard I., Beauchamp G., Belanger M. C., Frank D.*: Characterization of pica and chewing behaviours in privately owned cats: a case-control study. J. Feline Med. Surg. 2016, 18, 652-657.
9. *Ebrahim Z. K.*: Clinical, haematological and biochemical studies on wool eating syndrome in sheep. Alex. J. Vet. Sci. 2015, 46, 95-99.
10. *Elshahawy I. I., Aly M. A.*: Some studies on deviated appetite (pica) in cattle. Alex. J. Vet. Sci. 2016, 51, 97-101.
11. *Firyal S.*: Extension article: Pica (depraved appetite; allotriophagia) in domestic animals and man. Pak. Vet. J. 2007, 27, 208-210.
12. *Hamed T. A. A.*: Clinical, haematological and biochemical studies of some minerals deficiency in buffalo in Basrah, Iraq. Basra J. Vet. Res. 2015, 14, 223-230.
13. *Icen H., Sekin S., Simsek A., Duz M. Z.*: Research on haematological and biochemical parameters in lambs eating each other's wool and on treatment. Firat University Vet. J. H. Sci. 2008, 22, 159-162.
14. *Karademir B., Kaya I.*: An investigation on determination of mineral deficiency in cattle in Kars province. Lalahan Livest. Res. Inst. J. 2001, 41, 21-30.
15. *Kinsman R., Casey R., Murray J.*: Owner-reported pica in domestic cats enrolled onto a birth cohort study. Animals 2021, 11, 1101-1113.
16. *Koted R., Tuteja F. C., Singh A. P., Sawal R. K., Narnaware S. D., Nath K.*: Haemato-biochemical and mineral profiles along with therapeutics of pica in camels. Vet. Pract. 2020, 21, 256-263.
17. *Kumar R., Yadav K., Jeevan K., Hemanth I., Rao S., Mayasandra L. S.*: Case report on trichophagia in dog: a rare condition. J. Entomol. Zool. Stud. 2020, 8, 1293-1294.
18. *Lengare A. S., Bhikane A. U., Ghoke S. S., Awaz K. B.*: Pica in buffaloes with special reference to its etiology and treatment. Intas Polivet. 2012, 13, 62-66.
19. *Leung A. K., Hon K. L.*: Pica: a common condition that is commonly missed – an update review. Curr. Pediatr. Rev. 2019, 15, 164-169.
20. *Li Z., Liao Q., Han Y., Deng L., Liu H.*: A study of serum mineral, antioxidant capacity, and haematobiochemical parameters in horses with pica in China. J. Vet. Behav. 2020, 37, 81-85.
21. *Masson S., Guitaut N., Medam T., Beata C.*: Link between foreign body ingestion and behavioural disorder in dogs. J. Vet. Behav. 2021, 45, 25-32.
22. *Mosa A. H., Albayati O. A. S., Hamzah K. J.*: Clinical diagnosis and therapeutic study of pica in Iraqi local cows. Plant Arch. 2020, 20, 1478-1482.
23. *Munir S., Qadir I.*: Pathophysiology and management of pica. Pharmacology online, 2010, 3, 677-681.
24. *Nikvand A. A., Rashnavadi M., Tabandeh M. R.*: A study of pica in cattle in Iran. J. Vet. Behav. 2018, 23, 15-18.
25. *Onmaz A. C., Gunes V., Cinar M., Citil M., Keles I.*: Haematobiochemical profiles, mineral concentrations and oxidative stress indicators in beef cattle with pica. Ital. J. Anim. Sci. 2019, 18, 162-167.
26. *Rinaldi L., Coles G. C., Maurelli M. P., Musella V., Cringoli G.*: Calibration and diagnostic accuracy of simple flotation, McMaster and Flotac for parasite egg counts in sheep. Vet. Parasitol. 2011, 177, 345-352.
27. *Sahin T., Cimtay I., Aksoy G.*: Investigations on some biochemical parameters in lambs with pica and in healthy lambs. Turkish J. Vet. Anim. Sci. 2011, 25, 603-606.
28. *Smith B. P.*: Large Animal Internal Medicine. Elsevier, St. Louis 2015, p. 152.
29. *Wu T., Song M., Shen X.*: Seasonal dynamics of copper deficiency in Wumeng semi-fine wool sheep. Biol. Trace Elem. Res. 2020, 197, 487-494.
30. *Young S. L.*: Pica in pregnancy: new ideas about an old condition. Annu. Rev. Nutr. 2010, 30, 403-422.

Corresponding author: Asist. Prof. Dr. Akin Kochan, Department of Internal Medicine, Faculty of Veterinary Medicine, Dicle University, Diyarbakir, Turkey; e-mail: akin.kochan@dicle.edu.tr