



CLINICAL DIAGNOSIS AND THERAPEUTIC STUDY OF PICA IN IRAQI LOCAL COWS

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Abstract

The present study conducted in the Salah Aldin Governorate in Iraq, in six months from 1/9/2019 to 1/3/2020, which designed to investigation hematological and some biochemical parameters with the treatment in Iraqi local cows suffering from pica, conducted on (14) cows diseased suffer from pica and (10) cows healthy without pica considered as a control group. Clinical signs appeared in diseased cows were 14 cows suffer from depraved appetite, 8 cows in each of agalactia and pale mucus membrane, 6 c emaciation, 4 cows in each of the decreased in appetite and hair losing, 3 cows in each of the jaundice and diarrhea appeared on 3 cows, and the study showed of 8 cows were infected by gastrointestinal parasite. After therapy of these cases the study appeared all cows responses to therapy after 2 weeks, except 2 cows only remained to suffering from pica. And revealed a non significantly ($P \leq 0.01$) in temperature, pulse and respiratory rates in cows suffers from pica, while showed decreased significantly ($P \leq 0.05$) in ruminal movement in pica cows but returned to normal after therapy. The values of RBCs, PCV and Hb a significant decreased ($P \leq 0.05$) in cows were suffering from pica, but returned after therapy to the normal values, while the WBCs values showed a significant increase ($P \leq 0.05$) in pica cows but returned to normal after therapy. In biochemical results the study showed decreased significantly ($P \leq 0.05$) in serum concentrations of phosphor, Iron, copper, and glucose in cows were suffering from pica but returns to normal concentrations after 2 weeks from therapy, and increased significantly ($P \leq 0.05$) in bilirubin, AST, ALT and ALP enzymes and returned to normal values after 2 weeks from therapy, while the calcium, creatinine, total protein, albumin, sodium, potassium and chloride showed a non significant difference ($P \leq 0.05$) in cows suffer from pica comparative with healthy cows.

Keywords : Pica, Depraved appetite, Biochemical changes, Therapeutic, Iraqi local cows.

Introduction

Pica is a depraved or abnormal appetite, is known as the ingestion of non-nutritive materials. It was ranged from drinking, licking, eating and to their consumption those undigested substances (Constable *et al.*, 2017). It is usually associated with animals that chew or eat wood, fences, trees, buildings, dirt, bones, or other inanimate objects not usually considered feedstuffs. (Radostits, 2007; Smith, 2015). licking walls, dirt and other animal skins, chewing plastic materials, osteophagia bones, eating geophagia, brush, coprophagia, wool, carcasses and animal sucking it self and drink urine. (Firyal, 2007), there are another pica forms such as osteophagia, the chewing of bones, infantophagia, the eating of young; coprophagia the eating of feces (Constable *et al.*, 2017).

Pica or deviated appetite always relate to food deficiency such as the deficiency of Proteins, fibers and some elements, such as phosphorous, magnesium, calcium, sodium, potassium, chlorine, cobalt, copper, iron, and zinc, in addition to other causes such as chronic abdominal pain and nervous system disorders such as ketosis and it has been associated with parasitism (Firyal, 2007; Smith, 2015).

Many researchers pointed to the importance of proteins, elements and salts of the body, as the protein is an important substance of food particles and its level decreases in cases of food deficiency and cases of bleeding, kidney diseases, liver diseases that lead to its lack of composition and in cases of lactation (Denniston *et al.*, 2007). The importance of the phosphorous component in many body functions, it is necessary to produce milk and build the skeleton and its deficiency leads to a lack of appetite and pica and poor growth (Aytekin and Kalinbacak, 2008; Begum *et al.*, 2010). phosphorous deficiency in the body may be primary or

secondary, as the primary deficiency arises from the lack of the presence of the phosphorus in the dietary feeds or secondary deficiency, the animal is due to large amounts of calcium, as is the case in animals that feed on green diets for a long period of time, since green fodder material is one of the nutrients rich in calcium, as there is interference and disturbance in the natural proportions between calcium and phosphorus blood the imbalance in the proportions leads to a state of appetite deviation (Haskell and Anttila, 2001; Constable *et al.*, 2017). the elements of sodium, potassium and chloride have important role in regulating fluids in the body, and in the acid-base balance with many metabolic processes and maintaining osmotic pressure in the body, deficiency occurs due to food deficiency and other diseases such as persistent diarrhea, dehydration, and kidney disease (Thrall, 2004). The aim of this study was to diagnosis the main causes of pica in cows and give treatment, beside that compare haematological and biochemical parameters concentrations of depraved appetite in Iraqi local cows with healthy cows, additionally, investigated of presence parasite infestations as well.

Materials and Methods

The area of the present study conducted in the Salah Aldin Governorate in Iraq, during 6 months from 1/9/2019 to 1/3/2020. The study was designed to investigate the diagnosis, hematological, some biochemical parameters and the treatment of Iraqi local cows suffering from pica.

Animals: The study conducted on (24) cows in age ranged between (3-8) years, body Condition Score between (2: emaciated - 4: obese) the animals had been fed on a little concentrated diet with hay and grass. Were (14) cows diseased suffer from pica and (10) cows healthy without pica clinically normal considered as a control group.

Clinical examination: Clinical examination of all cows put under investigation was done according to the method described by (Kelly, 1984; Constable, 2017), Including temperature, respiration, pulse rate, and ruminal movement. And the cows were suffer from pica appeared signs as biting or ingesting indigestible materials varies from licking hair, earth to actual eating of wood, paper, plastic and nylons, some cows loss of body conditions, decrease in the body weight.

Treatment protocol: The treatment protocol for the diseases cows was designated as follows:

- 1- Monosodium dihydrogen phosphate (NaH_2PO_4), 30 gm in 300 ml D.W./cow I.V. in the first day and repeated orally in the 2nd and 3rd days, at adose (300 gm in 500 ml D.W./cow).
- 2- Ferritas (Fe, Folic acid) 1.8% of ferrous sulfate, 5 ml daily for 3 days by I.M.
- 3- Copper sulfate at 10 gm/cow orally, weekly for 2 weeks.
- 4- Glucose (molas) at 100 ml/cow daily for 5 days, orally.
- 5- Levozan® (levamisole & oxylozanide) (7.5 mg/kg) once time, orally (Horner *et al.*, 2004; constable *et al.*, 2017).

Blood samples: The blood samples collected from all study cows according to (Pugh, 2000), from the jugular vein in an amount (10 ml). two sets blood samples were obtained from each animal, the first set of samples were collected (2.5 ml) put on labeled test tube containing (EDTA) for examination, hemoglobin concentration (Hb), Red blood cell count (RBCs), packed cell volume (PCV) and Total Leukocyte Counts (WBCs). The second one (7.5 ml) in a test tube without anticoagulant to serum isolation for a purpose the of

biochemical values examination, they centrifuged at 3000 rpm for 15 minutes to get appropriate separation of serum. After separated of the serum, then stored in aliquots at -20°C until it examined.

Hematological examination: includes Red blood cell count (RBCs $\times 10^6/100 \text{ ml}$), packed cell volume (PCV%), hemoglobin concentration (Hb g/l), WBCs count ($\times 10^3/100\text{ml}$), were determined according to (Coles, 1986).

Biochemical examination: Biochemical analysis determination of serum concentrations of phosphorus, calcium, iron, copper, sodium (Na), Potassium, Chloride glucose, urea, Creatinine, Bilirubin, Total proteins, aspartate aminotransferase (AST), Albumin, Alanine aminotransferase (ALT) Alkaline phosphatase (ALP), were carried out by using commercial test kits supplied by (Assel S.R.L., Italy) by using the atomic absorption spectrophotometer according to the method described by (Khayam-Bashi, 1977; Ogawa, 1984).

Parasitological examination: Collected the fresh fecal samples directly in sterile plastic bags from the rectum of each cow, then transferred to the laboratory, then stored at 4 C till examination of parasites. We have examined samples in the collection day. Eggs of parasite examined using saturated sugar solution flotation technique. (Rinaldi *et al.*, 2011).

Statistical analysis: Data were analyzed using the packaged SPSS program for windows version 10.01. presented as (Mean \pm SE). Differences between groups were determined by one way analysis of variance (ANOVA). The significance level was set at $P \leq 0.05$. (SPSS, 2000).

Results

Table 1 : Clinical signs in Cows with Pica before and after treatment

Parameters	Groups Parameters				Cows with Pica (n: 14)			
	Before treatment		%	After treatment		%		
Pica	14		100		2		14.29	
Agalactia	8		71.43		No		14.29	
Pale mucus membrane	8		57.14		2		14.29	
Emaciation	6		42.86		4		28.57	
Decrease appetite	4		28.57		No		No	
Hair losing	4		28.57		No		No	
Jaundice	3		21.43		No		No	
Diarrhea	3		21.43		No		No	
GIT parasite infection	8		57.14		No		No	

Table 2 : Clinical signs parameters (Mean \pm SE) in Healthy and Cows with Pica.

Parameters	Groups Parameters		Healthy Cows (n:10)		Cows with Pica (n: 14)	
			Befor treatment		After treatment	
Temperature °C			38.39 ± 0.09^a		38.28 ± 0.04^a	38.30 ± 0.06^a
Pulse rate/min			69.50 ± 1.18^a		67.25 ± 1.76^a	66.12 ± 2.05^a
Respiratory rate/min			23.60 ± 0.52^a		25.62 ± 0.92^a	25.44 ± 1.16^a
Ruminal movement/2 min.			3.30 ± 0.18^a		2.50 ± 0.12^b	3.22 ± 0.17^a

Different letters revealed a differences significantly at ($P \leq 0.05$) between the values.

Table 3 : Hematological Examination parameters (Mean \pm SE) in in Healthy and Cows with Pica.

Parameters	Groups Parameters		Healthy Cows (n:10)		Cows with Pica (n:14)	
			Befor treatment		After treatment	
RBC ($\times 10^6/\mu\text{l}$)			6.94 ± 0.69^a		6.04 ± 1.28^b	6.72 ± 1.02^a
Hb (g/ dl)			11.50 ± 0.97^a		9.41 ± 1.56^b	10.66 ± 1.23^a
PCV (g/ dl)			36.43 ± 1.52^a		31.62 ± 2.32^b	34.62 ± 2.78^{ab}
WBCs ($\times 10^3/\mu\text{l}$)			7.33 ± 2.18^b		8.22 ± 3.12^a	8.04 ± 4.12^a

Different letters revealed a differences significantly at ($P \leq 0.05$) between the values.

Table 4 : Biochemical examination (Mean \pm SE) in Healthy and Cows with Pica.

Parameters	Groups Parameters	Healthy Cows (n:10)	Cows with Pica (n:14)	
			Before treatment	After treatment
P (mg/dl)		6.12 \pm 0.68 ^a	3.45 \pm 0.89 ^b	5.84 \pm 0.31 ^a
Ca (mg/dl)		9.55 \pm 0.91 ^a	9.12 \pm 0.69 ^a	9.35 \pm 0.91 ^a
Fe (μ g/dl)		104.30 \pm 2.95 ^a	84.4 \pm 4.48 ^b	110.24 \pm 3.05 ^a
Cu (μ g/dl)		94.10 \pm 3.74 ^a	78.62 \pm 4.07 ^b	96.25 \pm 3.74 ^a
Na (mmol/L)		146.46 \pm 5.09 ^a	143.6 \pm 6.65 ^a	142.9 \pm 6.32 ^a
K (mmol/L)		5.36 \pm 0.39 ^a	5.26 \pm 0.45 ^a	5.34 \pm 0.52 ^a
Cl (mmol/L)		105.60 \pm 2.10 ^a	101.25 \pm 4.63 ^a	103.60 \pm 3.16 ^a
Glucose (mg/dl)		66.90 \pm 2.46 ^a	57.50 \pm 3.49 ^b	65.63 \pm 4.06 ^a
Urea (mg/dl)		38.30 \pm 2.97 ^a	35.62 \pm 3.65 ^a	37.30 \pm 1.97 ^a
Creatinine (mg/dl)		1.39 \pm 0.43 ^a	1.54 \pm 0.10 ^a	1.50 \pm 0.13 ^a
Bilirubin (mg/dl)		0.46 \pm 0.13 ^b	0.94 \pm 0.25 ^a	0.50 \pm 0.03 ^b
Total protein (mg/dl)		7.36 \pm 0.45 ^a	6.94 \pm 0.68 ^a	6.98 \pm 0.04 ^a
Albumin (g/dl)		3.64 \pm 0.32 ^a	3.47 \pm 0.42 ^a	3.53 \pm 0.23 ^a
ALT (IU/L)		23.40 \pm 1.53 ^b	35.75 \pm 3.71 ^a	25.30 \pm 3.33 ^b
AST (IU/L)		82.9 \pm 3.80 ^b	96.8 \pm 5.29 ^a	85.9 \pm 4.60 ^b
ALP (IU/L)		85.50 \pm 3.43 ^c	104.8 \pm 6.72 ^a	93.50 \pm 4.36 ^b

Different letters revealed a differences significantly at ($P \leq 0.05$) between the values.

Clinical Examination

Clinical signs that appear in pica cows in (Table 1), showed pica syndrome on 14 cows represented by biting or ingesting undigested materials varies from licking (hair, earth and wall) to actual eating (wood, paper, fabrics, plastic and nylons), 8 cows appeared in each one agalactia and pale mucus membrane, 6 cows emaciation, 4 cows in each of the decreased in appetite and hair losing, 3 cows in each of the jaundice and diarrhea appeared on 3 cows, and the study showed of 8 cows were infected by gastrointestinal parasite. After therapy of these cases the study appeared all cows responses to therapy after 2 weeks, except 2 cows only remained to suffering from Pica and 4 cows remained to suffering from emaciation. Other clinical signs results in (Table 2), showed a non significant difference ($P \leq 0.01$) in temperature, pulse and respiratory rates in cows suffers from pica compared with healthy cows. But showed a significant decrease ($P \leq 0.05$) in ruminal movement in pica cows but returned to normal after therapy in compared with healthy.

Hematological examination

Hematological study results in (Table 3), showed RBCs, PCV and Hb values were a significant decreased ($P \leq 0.05$) in cows were suffer from pica, but returned after therapy to the normal values after 2 weeks compared with healthy cows. While the WBCs values exhibited a significant increase ($P \leq 0.05$) in pica cows comparative with healthy but returned to normal after therapy.

Biochemical examination

Biochemical study results in (Table 4), showed a significantly decreased ($P \leq 0.05$) in means serum concentrations of phosphor, Iron, copper, and glucose in cows were suffering from pica but returns to normal concentrations after 2 weeks from therapy comparative with the healthy cows. And the study showed a significant increase ($P \leq 0.05$) in bilirubin, ALT, AST and ALP enzymes and returns to normal values after 2 weeks from therapy comparative with the healthy cows. While the calcium, creatinine, total protein, albumin, sodium, potassium and

chloride showed a non- significant difference ($P \leq 0.05$) in cows suffer from pica comparative with healthy cows.

Discussion

Pica is an important signs in different animals and numerous etiologies speculated predisposing to problem development (Meyer and Lohes, 2002). This problem in cows is a general issue in the world, particularly the mineral inadequacies furthermore, lopsided characteristics for dairy cows (Garg *et al.*, 2013).

The clinical study results showed the different signs that appeared on cows that suffered from pica (represented by eating undigested materials) were responsible by pale mucus membranes, emaciation, decreased appetite, hair losing, jaundice, diarrhea and normal of parameters of temperature, pulse and respiratory rates with decreased in ruminal movement these signs agreement with the signs indicated by Constable *et al.* (2017), the pica is clinical sign appeared due to one of the numerous causes and the other signs appear with the pica depending on the causes and reflects on the body, Our results in the current study showed that the feces contain parasites in animals, may play an important role in the etiology of pica (Smith, 2015). In this study, the most clinical findings are aberration of feeding habit as biting or ingesting undigested materials that are generally unaccustomed for a cows like wood, sticks, paper, plastic continuous licking of hair, manager, walls, floors and other equipment in nearby (Blood and Radostits, 1989; Davenport *et al.*, 1990; Aytekin and Kalinbacak, 2008). The decreased in ruminal movement and decreased in appetite appeared in the study in pica cows suggest that due to accumulation undigested material in the rumen and the deficiency of some elements responsible for ruminal movement, appetite and weakness (Aytekin *et al.*, 2010). The present study appeared after therapy of these cases the all cows responses to therapy after 2 weeks from treatment, except 2 cows only remained to suffering from pica, may be due to other causes lead to pica in cows, There are numerous reasons of pica including lack in certain proteins, α -amino-acids, vitamins, unequal dietary calcium-phosphorus ratio and phosphorus deficiency (Aytekin and Kalinbacak, 2008).

Hematological studies results showed the RBCs, PCV and Hb values were a significant decreased

($P \leq 0.05$) in cows suffer from pica, and the current study revealed these values returns to the normal values may be due to injection of Ferritas in the therapy of pica cows, suggest that due to therapy by Nikvand *et al.* (2018) detected that low concentration of serum iron occur with low ferritin serum and may with long-term iron deficiency nutrition, that lead to pica occurs and decreased of some hematological indices, or may be due to failure of intestinal absorption or dietary deficiency that inadequate or limited intake of iron, leading to effect the hemoglobin formation. Parasited infestation consider one of pica etiology (Smith, 2015). The present results revealed that the present of parasites in the feces of animals may raised the risk of pica and causes loss of blood resulting to direct deficiency of iron that cause anemia.

Biochemical results revealed a significantly decrease in means serum concentrations of phosphor, Iron, copper, and glucose in cows were suffering from pica, but returns to normal concentrations after 2 weeks from therapy compared with the healthy cows. Nikvand *et al.* (2018) who detected that low concentration of iron occur with low ferritin serum may be related to long-term deficiency of nutritional iron, which lead to a pica. Aytekin and Kalinbacak (2008) recorded a significant low of mineral concentrations copper and phosphorus in cows exhibiting pica, our results showed that the mean levels of serum phosphorus for the cows suffered from pica were not less than the control. The study by Haris *et al.* (1995), mentioned the copper deficiency could lead to lower concentrations of iron due to its transporting role of iron in the circulating blood. Predominating, the pica considered a nutritional disorder of farm animals and cattle, but the etiology is controversial. Roles imbalance or deficiency of some nutritional elements are discussed (Nikvand *et al.* 2018). Copper acts as transporting iron across membranes (Rosen *et al.*, 1995; Elshahawy and Aly, 2016). The low iron level might result from copper deficiency (Haris *et al.*, 1995). Aytekin and Kalinbacak, (2008) reported a significant decrease of phosphorus and copper mineral concentrations in the sera of calves having earth eating behavior. Decreased glucose level may attribute to the high demand for energy (Kaneko *et al.*, 1997), Phosphorus deficiency and concomitant Ze and Fe deficiency (Ellis and Schnoes, 2005).

And the study showed a significant increases in bilirubin, AST, ALT and ALP enzymes and returns to normal values after 2 weeks from therapy , there is increase in blood urea which act on catabolizing of the protein in the muscles when large qualities of body reserves are mobilized. This is accordance with condition score of the body and body weight of the cattle (Pambu-Gollah *et al.*, 2000; Allaam *et al.*, 2014). With regard to liver function tests, AST and ALT showed significant increase during 3-2 weeks pre-partum. The higher concentrations of AST in dairy cattle are associated with fatty liver syndrome and ketosis signs (Iriadam, 2007). The activity of ALT was increased, the highest of this enzyme levels may reflect ketosis, due to lower intake of dry matter or glucose and nutritional deficiency (García *et al.*, 2011). The increasing bone metabolism or high metabolic rate due to absence of feed additives mineral of dairy herds may elevated ALP activity (Soch *et al.*, 2008). While the calcium, creatinine, total protein, albumin, sodium, potassium and

chloride showed a non- significant differences ($P \leq 0.05$) in cows suffer from pica compared to healthy cows. Which are in agreed with Zhou *et al.* (2009); Li *et al.* (2014) and Nikvand *et al.* (2018). This results agree with reported of Akgül *et al.* (2000) recorded that concentrations of sodium, calcium and chloride in sheep with pica were not changed from the healthy sheep. In conclusion, an investigation of 14 cows suffering from pica is described, the main abnormality founded was a phosphor, iron, copper and glucose deficiency and parasitism. Treatment of this deficiencies and parasitism followed by disappearance of pica after 2 weeks.

References

- Akgül, Y.; Agaoglu, Z.T.; Kaya, A. and Salin, T. (2000). The relationship between the syndromes of wool eating and alopecia in Akkaraman and Morkaraman sheep fed corn silage and blood changes (haematological, biochemical and trace elements). Israel J. Vet. Med. 56(1): 23–37.
- Allaam, M.A.; Hassan, Y.H.; El-Ebissy, I. and Assar, A.M. (2014). The effect of physiological status on metabolic profile in Egyptian Zarabi does. Minufiya Vet. J., 8(1): 189-195.
- Aytekin, I.; Onmaz, A.C.; Kalinbacak, A.; Aypak, S.U. and Alp, H. (2010). Circulating mineral element concentrations in Sakiz crossbred lambs with pica disorder. Revue Med. Vet., 161(7): 332–335.
- Aytekin, I. and Kalinbacak, A. (2008). The levels of calcium, phosphor, magnesium, copper, zinc and iron in calves eating soil in the region of Afyon. Atatürk Univ. J. Vet. Sci., 3: 34-42.
- Begum, I.; Azim, A.; Akhter, S.; Anjum, M. and Afzal, M. (2010). Mineral Dynamics of Blood and Milk Buffaloes Fed on Calcium and Phosphorus Supplementation. Pak. Vet. J., 30(20): 105-109.
- Blood, D.C. and Radostits, O.M. (1989). Veterinary Medicine. A Textbook of the diseases of cattle, sheep, pigs, goats and horses, 7th Edition. Baillière Tindall London, 180: 1152-1184.
- Constable, P.D.; Hinchcliff, K.W.; Done, S.H. and Grunberg, W. (2017). General systemic state, In: Veterinary Medicine, 11th ed., W.B. Saunders, London, 88-89.
- Davenport, G.M.; Boling, J.A. and Gay, N. (1990). Bioavailability of magnesium in beef cattle fed magnesium oxide or magnesium hydroxide. J. Anim. Sci., 68: 3765-3772.
- Denniston, K.J.; Topping, J.J. and Creat, R.L. (2007). Gerenal organic and biochemistry. 5th ed. McGraw Hill, Boston, Toronto, 599.
- Ellis, C.R. and Schnoes, C.J. (2005). Eating disorder: Pica. Last updated. Medicine. Com. Inc, 19.
- Elshahawy, I.I. and Aly, M.A. (2016). Some studies on deviated appetite (pica) in cattle. AJVS, 51(1): 97-101.
- Firyal, S. (2007). Extension article: Pica (depraved appetite; allotriophagia) in domestic animals and man. Pakistan Vet. J., 27(4): 208-210.
- García, A.; Cardoso, F.C.; Thedy, D.X. and González, F.H. (2011). Metabolic evaluation of dairy cows submitted to three different strategies to decrease the effects of negative energy balance in early postpartum. Pesquisa Veterinária Brasileira, 31(1): 11-17.
- Garg, M.R.; Sherasia, P.L.; Bhanderi, B.M.; Phondba, B.T.; Shelke, S.K. and Makkar, H.P. (2013). Effects of feeding nutritionally balanced rations on animal productivity, feed conversion efficiency, feed nitrogen

- use efficiency, rumen microbial protein supply, parasitic load, immunity and enteric methane emissions of milking animals under field conditions. *Animal Feed Science and Technology*, 179: 24-35.
- Haris, Z.L.; Takahashi, Y.; Miyajima, H.; Serizawa, M.; Mac-Gillivray, R.T.A. and Gitlin, J.D. (1995). Aceruloplasminemia: molecular characterization of a novel disorder of iron metabolism. *Proc. Natl. Acad. Sci.*, 92: 2539-2543.
- Haskell, S. and Anttila, T. (2001). *Small Ruminant Clinical Diagnosis and Therapy*. 1st ed. Scott puplish.
- Horner, S. and Staufenbiel, R. (2004). The influence of different therapeutic substances applicable for phosphate substitution on the concentration of phosphorus in the blood. *Int. Care Med.*, 29:1273-8.
- Iriadam, M. (2007). Variation in certain haematological and biochemical parameters during the peri-partum period in Kilis does. *Small Rum Res.*, 73: 55-47.
- Kaneko, J.J.; Harvey, J.W. and Bruss, M.L. (1997). *Clinical biochemistry of domestic animals*, 5th ed., Academic Press, Inc, California, USA.
- Kelly, W.R. (1984). *Veterinary Clinical Diagnosis*. 3rd Ed., William Clows Ltd., London.
- Khayam-Bashi, H.; Tsan, Z. and Vern, W. (1977). *Clinical Chem.*, 23(2): 289-291.
- Li, H.; Wang, K.; Lang, L.; Lan, Y.; Hou, Z.; Zhang, L.; Zhu, W.; Yang Q. and Wang, J. (2014). Study the use of urea molasses multi-nutrient block on pica symptom of cattle. *J Anim Vet Adv.* 13(3):152-158.
- Meyer, H. and Lohse, K. (2002). Ca and P supply of ruminants in the 19th and beginning of 20th century in Middle Europe. *Dtsch. Tierarztl. Wochenschr.*, 109(1): 34-37.
- Nikvand, A.A.; Rashnavadi, M. and Tabandeh, M.R. (2018). A study of pica in cattle in Iran. *J Vet Behav.*, 23: 15-18.
- Ogawa, E.; Kobayashi, K.; Yoshiura, N. and Mukai, J. (1997). Hemolytic anaemia and red blood cell metabolic disorder attributable to low phosphorus intake in cows. *Am. J. Vet. Res.*, 50: 388-392.
- Pambu-Gollah, R.; Cronje, P.B. and Casey, N.H. (2000). An evolution of the use of blood metabolite concentrations as indicators of nutritional status in free-ranging indigenous goats. *South Afr. J. Anim. Sci.*, 30: 115-120.
- Pugh, D.G. (2002). *A text Book of Sheep and Goat Medicine*. 1st edition. W.B. Saunders, USA., 0-7216-9052-7211.
- Rinaldi, L.; Coles, G.C.; Maurelli, M.P.; Musella, V. and Cringoli, G. (2011). Calibration and diagnostic accuracy of simple flotation, McMaster and Flotac for parasite egg counts in sheep. *Vet Parasitol*, 177: 345-352.
- Rosen, A.C.; Rosen, H.R.; Huber, K.; Bauer, K.; Ausch, C.; Redlich, K.; Klein, M.J. and Moroz, C. (1995). Correlation of placental isoferitin with birth weight and time point of first contractions. *Gynecol Obstet Invest*, 39: 11-14.
- Smith, B.P. (2015). *Large animal internal medicine*, 5th ed., St Louis, MO: Mosby, Elsevier.
- Šoch, M.; Písek, L.; Kroupová , P.; Šilhavá, M. and Šťastná, J. (2008). Activity of alkaline phosphatase in cattle blood plasma according to stage of pregnancy. *Slovak J Anim Sci.*, 41(1):39-41. ISSN: 1335-3686
- SPSS (2000). *Sample Power statistics*, SPSS 11.5, Syntax reference guide for SPSS Base. SPSS Inc., 233 South Wacker Drive, Chicago.
- Thrall, M. (2004). *Veterinary hematology and clinical chemistry*. Lippincott Williams and Wilkins, 329-355.
- Zhou, L.; Long, R.; Pu, X.Y.; Juan, Q.J. and Zhang, W.W. (2009). Studies of a naturally occurring sulfur-induced copper deficiency in Przewalski's gazelles. *Can Vet J.*, 50(12): 1269-1272.