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*Corresponding Author: Tegegn Dilbato
E-mail: dilbatot@gmail.com

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JIVS JOURNAL OF ISTANBUL VETERINARY SCIENCES

Capture myopathy accompanied with severe enteritis in a female lion

Case Report

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Burçak Özkan[1]*, Lora Koenhemsi[3], Kıvılcım Sönmez[2], S. Seçkin Arun[4]

1. Kadıköy Istanbul **2.** Istanbul University, Faculty of Veterinary Medicine, Department of Pathology. **3.** Istanbul University, Faculty of Veterinary Medicine, Department of Internal Medicine. **4.** Vetlab Veterinary Laboratory/Avcilar Istanbul.

ABSTRACT

A one-year-old female lion was presented with a history of lethargy, unability to stand, incoordination, unsteady, swaying gait and diarrhoea to Istanbul University Veterinary Faculty Internal Medicine Clinics. Anamnesis was followed by physical examination. The lion was immobilized with ketamine (Ketasol; Interhas) and xylazine hydrochloride combination (Rompun; Bayer, West Germany). Blood was collected from the cephalic vein for routine haematology and biochemistry analysis. The patient died during the night. A slight decrease in platelet account, a mild decrease in triglyceride level with a mild elevation in glucose and urea and a severe augmentation in aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and creatine kinase (CK) levels were observed. Pathologically, cause of death was determined as dehydration and hypovolemia due to severe hemorrhagic chronic-atrophic enteritis. Anamnesis, biochemical data and clinical findings suggested also capture myopathy (CM). In this case study it is aimed to give detailed knowledge about diseases seen in both free-ranging and captive lions and to discuss the death of this female captive lion due to severe diarrhoea and CM.

Keywords: lion, free-ranging lion, captive lion, enteritis, capture myopathy

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Introduction

Capture myopathy (CM) is a non-infectious, shock-like hyper metabolic myopathic disease triggered by stress due to pursuit, capture, restraint and transportation of animals. The disease leading to serious morbidity and mortality may also exist as secondary to other diseases or environmental situtations (Paterson, 2007; Hamidieh et al., 2011). The condition, formerly named as muscular distrophy, white muscle diseas, capture disease, cramp, overstraining disease, leg paralysis, spastic paresis, stress myopathy, transport myopathy, incipient myopathy, degenerative polymyopathy, (idiopatic) muscle necrosis is referred as "capture myopathy", "exertional myopathy" or "exertional rhabdomyolysis" recently (Paterson, 2007; Fowler, 2011; Blumstein et al., 2015). The disease shares similarities with the myodegenerative disorders of domestic cattle, sheep, horses, swine as well as exertional rhabdomyolysis in humans. CM is reported in birds, wild African ruminants, antelopes, deers, Rocky mountain goats, Rocky mountain bighorn sheeps. CM is very similar to human exertional rhabdomyolysis which is one of eight forms of rhabdomyolysis, having hundreds of underlying causes (Paterson, 2007; Hamidieh et al., 2011). Exertional rhabdomyolysis/CM, being a multifactorial disease in animals differs from other similar conditions by its pathophysiology as it influences both cardiac and skeletal muscles. Predisposing factors are listed as "species", "signalement", "nutrition" and "drugs" (Paterson, 2007). The stress of capture causes an increase in energy metabolism. This augmentation helps an animal escape,

*Corresponding Author: Burçak Özkan
E mail: burcakozkan1974@yahoo.com

but also cause a homeostatic imbalance which may damage physiological functions (Ganhao et al., 1988; Kock et al., 1987; Cattet et al., 2003). CM results from altered blood flow to the tissues and exhaustion of normal aerobic energy especially in skeletal muscle. Exhaustion of ATP in muscle cells ends by decreased rate of oxygen and nutritions delievered, an overproduction of lactic acid and failure to removal of cellular waste products. Lactic acid in the bloodstream drops the body pH; this affects cardiac output. Myoglobin released from muscle cells undergoing damage and necrosis, cause tubular necrosis in kidneys and acute renal failure. Similar process occurs also in cardiac tissue (Paterson, 2007; Fowler, 2011).

CM manifestation differs according to species, the individual, the tissue damage and the circumstances. Different classifications are explained but it is very important to remember that the disease is a continuum and animals may exhibit overlapping syndromes. The disease is defined by four syndromes cited as "capture schock syndrome" (CSS), "ataxic myoglobinuric syndrome" (AMS), "ruptured muscle syndrome" (RMS) and "delayed-peracute syndrome" (DPS). The first may occur either during capture or post-capture; death comes 1-6 hours after capture. Depression, hyperpnea, tachycardia, augmented body temperature, weak pulses are clinical symptoms. Elevations in aspartate aminotransferase (AST), lactatedehydrogenase LDH and creatine kinase (CK) exist. AMS is represented several hours or days post capture. Clinical signs are mild or severe ataxia, torticollis or myoglobinuria. AST, CK, LDH and blood urea nitrogen (BUN) elevation. RMS which doesn't manifest until 24-48 hours postcapture, is accompanied by a marked drop in hindquarters and hyperflexion of of the hock. Extreme elevations in AST, CK and LDH is noted while BUN is slightly or not elevated. In case DPS occurs, animals may seem healthy until they are disturbed; and when disturbed, they remain still and die due to ventricular fibrillation. Elevated AST, CK and LDH levels are expressed (Rose, 2005; Paterson, 2007). Liberation of huge amount of myoglobin into the bloodstream related to muscle damage causes myoglobinuric nephrosis. This myoglobin filtered by glomeruli is toxic for proximal tubules' and loop of Henle's epithelial cells. Ischemic damage and shock with acute tubular necrosis occur (Rose, 2005). The diagnosis is generally based on histologic findings (Blumstein et al., 2015).

Though treatment of CM is ineffective, cure with the use of analgesics, muscle relaxants (i.e. dantrolene), benzodiazepines, cortisone, multivitamines, hyperbaric oxygen application, sodium bicarbonate, vitamin E, selenium and fluid therapy may be performed (Paterson, 2007; Fowler, 2011). The treatment strategy is based on reverse the ongoing shock, metabolic acidose and stabilise cellular membranes (Rose, 2005).

The prevention of disease is important by minimal handling of wildlife, and the most appropriate transportation method, most appropriate anesthesic drug choice. The most important of all is understanding the behaviour and physiology of species (Paterson, 2007; Fowler, 2011; Hamidieh et al., 2011).

Differantiation from Takotsubo disease, CM which comes after metabolic acidosis due to lactic acid accumulation and typically affects muscle tissue while specifically referring to a stress-induced cardiac muscle effects only, CM in wildlife can be a model for human stress cardiomyopathy including Takotsubo/broken heart cardiomyopathy related to the surge of cathecholamines followed by left ventricular dysfunction, where emotional stress triggers heart attack-like synmptoms and sudden death in humans (Blumstein et al., 2015).

Long-living species appear to be more predisposed to CM; this is thought to be due to rapid response to autonomic response to threats. Apart from adaptations associated with reduced predation risk, sociality and larger brains are related to more complex autonomic systems. More social species have larger brains. Moreover, long-living species seem to be better adapted to avoid predation and more likely to be susceptible for CM. Thus, CM may be an unavoidable result of being social and long-living species. This comparative finding can be converted into human medicine and also be useful in understanding whether similar factors play part in similar human diseases (Blumstein et al., 2015).
Joubert and Stander (1990) report CM in an African lion severely stressed prior to immobilization in spite routine lion capture procedures involving also ketamine HCL and xylaxine HCL combination application was performed. The lion had died due to respiratory failure (Joubert and Stander, 1990).

Case Presentation

A one-year-old female lion, weighing approximately 100-150 kg, presented with a history of lethargy, incoordination, and diarrhoea for one week to Istanbul

University Internal Medicine Clinics. The lion originated by a private collection within Istanbul and previous medical history was not available. Parasite prophylaxy nor any vaccination was not performed. She was staying alone, in a room-like cage, and fed with liver, cut pieces of red meat containing bones, being or not uncooked. The lion was alert on physical examination. The patient was not anorexic. No blood was observed in the gaita.The lion was sedated with the use of ketamine (Ketasol; Interhas) andxylazine hydrochloride combination (Rompun; Bayer, West Germany) administered by hand injection for physical examination (Figure 1). Ketamine-xylazine combination

Figure-1. Lion when sedated, controlled with an examination bar; during general examination

dosage ranged between 3-10 mg/kg of ketamine and 0.3- 1-4 mg/kg of xylazine in large felids/wild lions (Currier and Russel, 1982; Melton et al., 1987; Gunkel and Lafortune, 2007; Larsson et al., 2015). The lion was fully sedated (lying down and unable to lift its head and/or consciously bite) after 15-20 minutes and, her eyes were covered with a serviette to keep calm, prevent damage and limit unnecessary excitement (Erasmus, 2008). Body temperature was between physiologic values. No mucosal colour alteration was noted. Abdominal sensitivity couldn't be evaluated since the patient was under general anestheisa. Neither abnormal heart sound nor pulmoner pathologic sound was detected.

Dermatologic inspection showed diffuse echymosis and petechiaes. 5-6 mL. of blood were collected from the cephalic vein for routine haematology and biochemistry analysis (Kaiser et al., 2014; Larsson et al. 2015). Gaita was also taken from rectum. The patient died during the night and death was not observed.

Pathologic Findings: A systemic necropsy was performed to the cub. The animal's body condition was low. Area around the tail was smudged with dark red matter. All organs in the body cavities observed congested. Foamy fluid inside the trachea detected. Liver surface was hackly and atrophy in the spleen was observed. Most remarkable finding was in the intestines. Intestinal walls were thickened, in the lumen of the organ there was a crimson red matter. Tissue samples from each organ collected, they were fixed in 10% formaldehyde overnight and embedded in paraffin. 3-4µ sections were cut and stained with hematoxylin-eosin (H&E) routinely. All sectioned were investigated under light microscope. In microscopic evaluations; distinct edema in lung with congestion determined. In some areas alveolar walls were thickened. Edema and peripheral fibrosis in liver was observed (Figure 2B). In paranchymatous cells extensive degenerative changes were detected. Apparent lymphoid atrophy and lymphoid necrosis were observed in spleen. Intestinal villi were atrophic and there was distinctive epithelial loss on their surfaces, number of the mucosal glands was diminished and there were small number of chronic inflammatory cells, erythrocytes in the lumen and between those glands (Figure 2C). In some areas the mucosa was totally atrophic without glands or villi (Figure 2D). Pathologically, cause of death was determined as dehydration and hypovolemia due to severe hemorrhagic chronic-atrophic enteritis.

Anamnesis, biochemical data and clinical findings suggested CM.

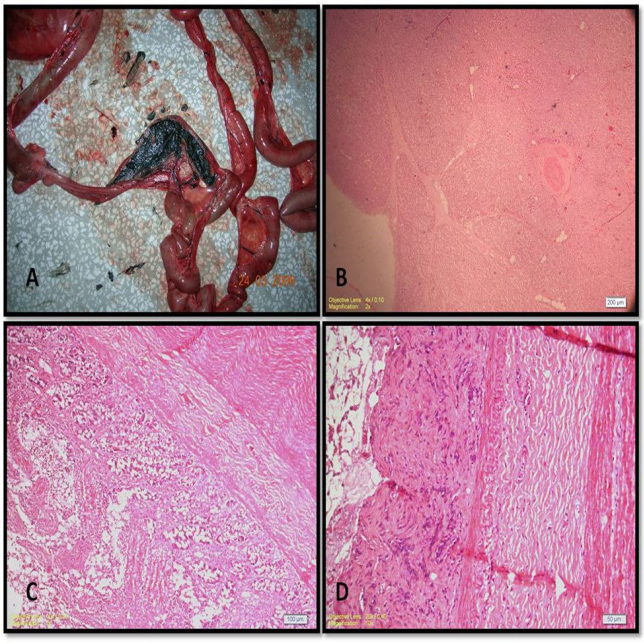

Figure-2. A. Crimson red matter in the intestines. **B**. Distinct peripheral fibrosis in liver. **C**. Atrophic villi, desquamative epithelial cell, erythrocytes, inflammatory cells in lumen and

Table 1: Reference values of haemogram resulted from various researches and measured value in this case.

Parameters	Measured value	Reference values		
		Erasmus, 2008	Maas et al., 2013	Larsson et al., 2015
RBC (1 x 10^{6}/ µl)	7.75	6.3 - 9.8	5.1 - 8.3 / 5.7 - 8.7 (males) 5.0 - 8.4 / 5.2 - 8.5 (females)	8.97
HGB (g/dl.)	11.2	5.4 - 20.5	8.9 - 14.6	14.11
HCT (%)	32	29.2 - 45.6	26.8 - 44.1	42.38
WBC (1 x 10^{3} / µl.)	16.7	-	7.2 - 25.6 / 9.7 - 28.2	9.37
PLT (1 x 10^{3}/ µl.)	246	355.41	-	-
MCV (fl)	42	43 - 50	46.6-55.9 (males) 48.0-56.7 (females)	47.70
MCH (pg)	14	9.6 - 22.4	14.8-18.5 (males) 15.5-19.1 (females)	15.84
MCHC (%)	35	-	-	33.33

Discussion

Despite no specific viral identification had been established, anamnesis, physical examination findings, and histopathology seem to be in concordance with literature.

In our case, a slight decrease in platelet count and a mild decrease in trigliseryde level were noted. On the other hand, a mild elevation in glucose (GLU) and urea accompanied a severe augmentation in AST, LDH and CK levels.

Apart from textbooks generally representing blood values of captive lions, Maas et al., explain hematologic and biochemical values for free-ranging and captive lions (Maas et al., 2013). In a study which aims to generate biochemical data, alanine aminotransferase (ALT), AST, alkaline phosphatase (ALP), albumin (ALB) calcium, creatinine (CRE), GLU, lactate dehydrogenase (LDH), phosphorus, total bilirubin (TB), total cholesterol, total protein (TP), triglyceride, urea and uric acid were estimated by standard laboratory methods and it was concluded that GLU, protein, urea and ALP varied significantly with sex and season (Behera et al., 2013).

GLU is the chief metabolic fuel for energy yield in carnivores. Its blood level depends on several factors such as type of diet and interaction of insulin, glucagon, epinephrine, thyroid, glucocorticoid, pituitary and sex hormones. The secretion of these hormones varies between season and sex. There is a positive co-relation between blood glucose level and temperature. Basal metabolic rate (BMR) is regulated by environmental temperature and sex. In summer season, significantly higher level of blood glucose in female lions is due to less utilization of glucose at low BMR and high temperature. The normal body temperature of female is more than that of male. More energy is required to maintain it in cold environment of winter season at low BMR. Here more glucose is spared for conservation of heat. Therefore female lions exhibit low blood glucose in winter season (Behera et al., 2013). Serum glucose levels were positively related to increasing group size (Melton et al., 1987). Elevation in glucose level during general anesthesia pursuing induction with ketamine as well as dose-dependent changes in glucose concentrations are reported in Bengal tigers immobilized with xylazine (Seal et al., 1987; Reilly et al. 2014). The elevation observed in this case was thought to be related to augmented metabolic rate during capture and/or ketamine use.

In lions, BUN is reported to be significantly affected by three variables; the main association is for nomadic animals to have elevated levels. BUN levels will rise within a normal range as the quality or quantity of protein ingested increases, abnormal elevation can occur if protein is catabolized during food shortage or owing to renal dysfunction. The results may also show protein catabolism following an inadequate diet (Melton et al., 1987). The elevation in this case might be due to an inadequate diet, a renal dysfunction beginningand/or CM; CM being the strongest possibility.

Intracellular liver specific serum enzymes like ALT, AST and ALP take part in cell metabolism and in different metabolic pathways. Generally their elevated

Table 1: Reference values of haemogram resulted from various researches and measured value in this case.

Parameters	Measured value	Reference values			
		Currier and Russel, 1982	Erasmus, 2008	Behara et al., 2013	Miller, 2015
Glucose (mg/dl)	191	135-154 (all)	-	77.61 ± 2.60 (M) 102.78 ± 5.52 (F)	132
Urea (mg/dl)	77	30-36.1	-	143.20 ± 5.69 (M) 133.79 ± 6.24 (F)	31
Creatinin (mg/dl)	1.3	2.46-3.07	-	1.60 ± 0.07 (M) 1.48 ± 0.09 (F)	-
AST (IU/L)	319	58-79 (wild) 40-47 (captive)	25.79	20.02 ± 2.20 (M) 18.29 ± 1.53(F)	-
ALT (IU/L	94	48-57 (all)	42.58	12.39 ± 1.35 (M) 13.09 ± 1.06(F)	32
ɣ-GT (IU/L)	4	-	1.78		-
T. Bilirubine (mg/dl)	0.3	0.2-0.3 (wild) 0.5-0.7 (captive)	-	0.56 ± 0.05 (M) 0.47 ± 0.07 (F)	0.3
LDH (IU/L)	1343	133-197 (wild) 128-153 (captive)	81.98	118.10± 14.92(M) 103.73± 11.35 (F)	-
CK (IU/L)	632	82-135 (wild) 46-78 (captive)	-	-	-
Ccholesterol (mg/dl)	189	148-185 (wild) 192-214 (captive)	-	145.23± 7.79(M) 150.32±12.8 (F)	-
Trigliserid (mg/dl)	31	-	-	85.23 ± 13.71 (M) 61.43 ± 11.67 (F)	-
T. Protein (g/dl)	6.9	7.1-7.3 (all)	-	7.86 ± 0.11 (M) 7.62 ± 0.23 (F)	7.5
Albumin (IU/L)	3.8	-	-	-	-

M: male , **F:** females

levels in serum is attributed to the alteration in architectural integrity of hepatocytes and muscle fibers (Behera et al., 2013).

ALT is found in high amounts in feline hepatocytes' cytoplasm. The enzyme enters the blood when liver cell damage occurs. In spite of indicating liver cell damage, this enzyme doesn't explain the cause or reversibilty of the damage; but only recent or ongoing hepatic cell alteration. An increase of three times may be seen as normal (Sodikoff, 2001).

AST which is a mitochondria-bound enzyme, is found in different body tissues, especially high values in liver and striated muscle. Augmented serum AST levels indicates skeletal muscle necrosis and/or hepatocellular necrosis (Sodikoff, 2001). In domestic cats, AST levels can go up to 100 IU/L. without being any pathologic cause (Currier and Russell, 1982). In lions, an elevated AST measurement in addition to BUN is evaluated as a result of tissue damage related to a disease state or perhaps trauma during capture (Melton et al., 1987). Serious elevation of AST accompanied by BUN augmentation in this case were in concordance with capture oriented trauma findings revealed in litterature, despite other BUN elevation causes must also be taken into consideration.

CK, found in high levels in the CNS and striated muscle, increases in muscle trauma, IM injection, myositis and occasionally CNS damage. Increased levels of both CK and AST mean muscle necrosis (Sodikoff, 2001). Since wild lions are subject to more strenuous physical activity before tranquilization, CK and LDH elevation is expected (Currier and Russell, 1982).

Moreover, since AST, LDH and CK levels gradually rise after muscle damage, a slight augmentation in these parameters may be related to time elapsed between tranquilizing and withdrawing blood. Another reason might be the injection procedure with the use hand-held syringe even this application causes less stress (Currier and Russell, 1982). In this case, serious elevation in AST accompanied by BUN, LDH and CK augmentation were in concordance with CM findings.

Hypotriglyseridemia is not specifically associated with any disease (Willard and Tvedten, 2012).

It is well known that this shock-like, hypermetabolic and myopathic disease may lead to mortality secondary to both other diseases and environmental factors; nutrition being one of them as a predispositive cause. The patient represented in this case had been living in inappropriate conditions for a wildlife animal. It is highlighted that stress of capture causes energy metabolism to accelerate, homeostatic and physiologic imbalance to occur. These changes end by acute renal failure which were supported by blood analysis findings in this case. It is known that death may occur 1 -6 hours or several hours after capture (Rose, 2005; Paterson, 2007; Blumstein et al., 2015).

According to what cited above, both clinical and biochemical findings seemed also to be in concordance with vitamin E deficiency strongly related to capture living and feeding conditions, in which case weakness, difficulty standing, muscle detoriation occur apart from augmentation of ALT, LDH and CPK levels (Bouts and Gasthuys, 2003).

General anesthesia influences different organ systems and may exacerbate various non-detected homeostatic imbalences. It is well known that depression of the CNS and cardiopulmonary system due to general anesthesia may end by decreased tissue perfusion, cellular metabolism and changes in the endocrine responses in cats and dogs. Despite possible physiologic changes related to general anesthesia remain unknown, similar changes may be expected during general anesthesia in lions. While no statistically significant difference was noted during anesthesia in CBC, BUN, creatinine, CK, globulins, ionized calcium, sodium, chloride, or lactate concentrations for any animal, most important clinical changes were simultaneous increase in plasma potassium and glucose concentrations with a decrease in initial plasma insulin concentration(Reilly et al., 2014). Furthermore, sudden death due to respiratory failure after ketamine HCL and xylaxine HCL combination application exists in a report (Joubert and Stander, 1990). According to the results, minimizing the duration of anesthesia, continous monitoring of clinical and biochemical changes during general anesthesia and evaluation of the effects of anesthesic drugs, drug comnbinations, a very careful evaluation of biochemical parameters and clinical situation in large wild felids is warranted (Reilly et al., 2014).

According to researchers, no significant difference exists between mortality rate of wild-born animals and captive-born animals and excess mortality of wild-born animals brought to zoo doesn't seem to be a general phenomenon. Yet, the mortality rate and situation for wild species is expected to differ between animals living in wild and captivity. In spite of researchers explaining the benefits of captive-living conditions such as veterinary care, lack of predators, regular food supply, the risk of obesity, injuries from exhibits, poor adaptation to captivity conditions and to zoo's climate as well as higher perinatal mortality rate because of inbreeding must also be highlighted. Another risk is the facilitation of infection spreading due to close quarters. Even those who argue that no difference exists between free-living and captive living, are highlighting that the error of commentary occurs when birth date or origin of animals were not recorded. Reports explain excess mortality when brought into a zoo relative to animals born and reared in a zoo, beacuse of emotional trauma and exposure to new diets (Kohler et al., 2006).

Conclusion

As a result, it is believed that the lion had severe complaints due to severe hemorrhagic chronic-atrophic enteritis which played a very important role in sudden death. On the other hand very inappropriate living conditions including environmental and nutritional factors must have been efficient in both enteritis and CM to occur. CM must have been the final cause of sudden death.

Declined population of wild fauna, extensive deforestation, destructive man made activities, nutritional deficiency, metabolic and infectious diseasesmay result in extinction of many species in near future. Environmental and genetic mutations of pathogens also adversely affect the population growth (Behera et al., 2013). To prevent animal species from extinction, augmented aid to breeding programs, exchange between institutions, greater support for veterinary care, research programs and conservation are supported. Yet the difficulty in detecting signs of diseases in captive animals remains problematic. Especially keeping individuals requiring large

territories under artifical conditions is necessary (Larsson et al., 2015).

Despite it is very important to establish serious attempts to prevent wildlife species extintions; these serious attempts should definitely performed by conscious welfare, healthcare and similar other professionals which have enough scientific knowledge to handle both living and health needs and conditions of such animals. Beyond being a medical obligation, this condition seems to be a very improtant ethical one.

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Identification of serotonergic 5HT-$_{3B}$ type receptors in broiler's small intestine

Research Article

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Indira Mujezinović [1], Muhamed Katica [2]*, Ahmed Smajlović [1], Nedžad Gradaščević [3], Dukić Behija [4]

1. University of Sarajevo, Veterinary Faculty, Department of Pharmacology, Bosnia and Herzegovina.
2. University of Sarajevo, Veterinary Faculty, Department of Pathophysiology, Bosnia and Herzegovina.
3.University of Sarajevo, Veterinary Faculty, Department of Rad obiology with Radiation Hygiene, Biophysics & Environmental Protection, Bosnia and Herzegovina.
4. University of Sarajevo, Veterinary Faculty, Department of Microbiology, Immunology and Infectious Diseases. Bosnia and Herzegovina

ABSTRACT

The study was performed in order to identify the serotonergic 5H$_{3B}$ type receptors in broiler's small intestine. Investigation were carried out on isolated smooth muscle of the circular and longitudinal layer of the broilers small intestine (strip dimension 3-4 mm x 2 cm). The muscle strips were placed in an isolated organ bath. The mechanical activity of the preparations were recorded via an isotonic force transducer coupled to a pen recorder. This was done following the addition of serotonin (nonselective 5-HT agonist), 2-Me-5HT (5-HT$_{3B}$ agonist) and Y-25130-hydrochloride (selective 5-HT$_{3B}$ antagonist). The research established a presence of serotonergic 5HT$_{3B}$ type receptors within the smooth musculature of the small intestines of broilers (COBB 500). The 5HT3B type receptors were present in smooth muscles of duodenum, jejunum and ileum, especially in longitudinal smooth muscles since this layer reacted even to low serotonin concentration (10^{-6}). Statistical tests of obtained results showed significant differences ($p< 0.001$) in responses related to muscle layers, applied concentrations and intestinal parts which were observed. In the light of these findings, we suggest that investigated substances may have considerable physiological and therapeutic implications in disturbed function of small intestine of broiler's.

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Introduction

Serotonin (enteramine; 5-hydroxytryptamine; 5-HT), a well-characterized neurotransmitter in the central and peripheral nervous system, plays a crucial role in regulating some central and peripheral function such as mood, sleep/wake cycle, thermoregulation, food intake, nociception, locomotion, sexual behavior, sleep appetite, gastrointestinal motility and secretion, blood coagulation, cardiovascular homeostasis etc. (Cirilo et al., 2011; Kato, 2013; Muminović et al., 2000).

Serotonin regulates various functions in the body through its specific serotonergic (5-HT) receptors (Darmon et al., 2015; Cirilo et al., 2011; Kato, 2013; Kaufman and Milstein, 2013; Pytliak et al., 2011). In the recent 20 years, seven distinct families of 5-HT receptors have been identified and various subtypes have been described for several of these (Darmon et al., 2015; Kaufman and Milstein, 2013; Nichols and Nichols 2008; Rang et al., 2015). At least 20 subtypes of 5-HT

***Corresponding Author:** Muhamed Katica , University of Sarajevo, Veterinary Faculty, Department of Pathophysiology, Bosnia and Herzegovina.
E mail: muhamed.katica@vfs.unsa.ba

receptors have been cloned yet (Hannon and Hoyer 2002; Berger et al., 2009).

These receptors are localized in the brain and in peripheral organs but their distribution is not homogeneous. The majority of 5-HT receptors are postsynaptic, with some exceptions, most notably 5-HT1A and 5-HT1B that are mainly presynaptic and modulate serotonin release. The signaling pathways to which these receptors are coupled are known, but it has not been possible to link direct clinical effects systematically to their stimulation. Serotonin receptors are coupled to G proteins except 5-HT3 receptors which are receptor-channels, also called ionotropic receptors, which, in the activated state, are open and permeable to sodium and potassium cations (Darmon et al., 2015; Pytliak et al., 2011; Rang et al., 2015).

Since it is known that the effect of serotonin, especially in pathological conditions, is very important, we believe that determining the types of receptors for this substance, as importantly, allow the use of their agonist or antagonists, which would undoubtedly enrich the pharmacotherapy of functional disruption of the small intestine in broilers. If we add that the disturbances of bowel function are common in broilers and if we know that mentioned dysfunction cause great loss to the poultry industry, due to the high mortality, the results of research could form the basis for the introduction of new drugs in the pharmacotherapy of this species. Thus, the aim of the present study was to try to determine the possible distribution of 5-HT3B receptors in small intestine of broilers (duodenum, jejunum and ileum).

Material and Methods

Experimental animals

The research was carried out on 20 broiler chickens (Cobb 500). Broilers had body weight between 2.1 and 2.3 kg, and of the age of up to 42 days. Broilers were obtained and transferred to the local private poultry slaughterhouse, where they were slaughtered in accordance with the regulations.

In vitro organ bath experiments

The following substances were used in the study: Kreb's bicarbonate solution (mM): NaCl 118.4; KCl 4.7; $CaCl_2$ 2.5; $MgSO_4$ 1.2; $NaHCO_3$ 25; KH_2PO_4 1.2 and glucose 11.5 (pH 7.3 - 7.4); Serotonin (3-(2-Aminoethyl)-1H-indol-5-ol) (Sigma-Aldrich, Germany); 2-Me-5HT [3-(2-aminoethyl)-2-methyl-1H- indol-5-ol hydrochloride] (5-HT3 agonist); Y-25130-hydrochloride (N-(1-Azabicyclo [2.2.2]oct-3-yl)-6-chloro-4-methyl-3-oxo-3,4-dihydro-2H-1,4-benzoxazine-8-carboxamide hydrochloride) (selective 5-HT3 antagonist) (Tocris Cookson Ltd., Bristol, UK); 2-(Acetyloxy)-N,N,N-tri-methylethanaminum chloride (a solution that the viability of the strips was tested with) (F. Hoffmann- la Roche & Co. Ltd. Basle Switzerland). All the substances used in the experiment were dissolved in distilled water. The sensitivity of the tissues to acetylcholine was tested before starting the experiments.

After the animals were sacrificed, parts of small intestine were taken; i.e. the strips of the first parts of duodenum, jejunum, ileum in length of 5 cm. They were then immersed in a cold, freshly prepared Krebs-bicarbonate solution and transferred to a laboratory. The tissue strips were taken to the procedure 20 minutes after the animal was sacrificed. Small intestines were cleansed of fatty and connective tissue, followed by a preparation of circular and longitudinal strips (2 cm in length and a width of about 3-4 mm) and placed in an isolated organ bath with a volume of 10 ml.

In order for conditions to be as close as possible, 4 strips were used simultaneously. The strips were placed in isolated organ baths having 10 ml volume, (Ugo Basile, Model 4050 Two-chamber, Italy). Such suspended strips were aerated in Kreb's bicarbonate solution with a mixture of oxygen and carbon dioxide (95 % O_2 and 5 % CO_2) at a constant temperature of 41 °C. Tissues were suspended under a resting tension of 2 g and were allowed to equilibrate for 45-60 minutes and were rinsed every fifteen minutes. Movement registration was performed on a single-channel printers of isometric transducers (Ugo Basile, Italy). Viability of the strips was verified by adding acetylcholine at a concentration of 10^{-5} M at the beginning and/or the end of the experiment.

After the acetylcholine solution was washed, tissue strips were leave to rest for 20 minutes, and then serotonin the (non-selective agonist) was added to the bath using an insulin syringe to achieve sufficient concentrations (10^{-7} -10^{-3} M). In next part of experiment was used 2-Me-5HT receptor agonist at concentration from 10^{-6} to 10^{-3}. Each concentration of serotonin and 2-Me-5HT was maintained in the bath for one minute and the other concentration was applied after washing. The solution of serotonin was then washed after one minute. The period between the individual applications

lasted for about 20 minutes. For statistical data processing, application of a single substance was repeated at least six times on different preparations obtained from different animals. The 5-HT_{3B} receptor antagonist, Y-25130 at concentration of 10^{-6} and 10^{-5} was added to the bath 3-4 minutes before the agonist.

Statistical Analysis

Basic statistical data diagnostics was conducted by using Microsoft Excel® (Microsoft Office package, Microsoft, USA). Values of standard deviations of obtained results are presented in the tables. Testing the significance of the differences among obtain results was performed by ANOVA tests, f-tests and t-tests.

Results

Results are expressed as percentages of the maximum response (expressed as 100%) produced by serotonin (10-3) on longitudinal layer of smooth muscle of broiler's duodenum, due to a fact that we obtained the best respond to the action of serotonin in this part of small intestine, so we used it as a positive control for all other intestinal parts in experiment (Table 1.; Fig. 1., 2. and 3).

Results are expressed as percentages of the maximum response (expressed as 100%) produced by selective agonist 5-HT_{-3B} type serotonergic receptor (2-Me-5HT at concentration of 10^{-7} to 10^{-3} M) on longitudinal layer of smooth muscle of broiler's duodenum and ileum, due to a fact that we obtained the best respond to the action of 2-Me-5HT in this part of small intestine, so we used it as a positive control for all other intestinal parts in experiment (Table 2.; Fig. 1.,2 and 3.).

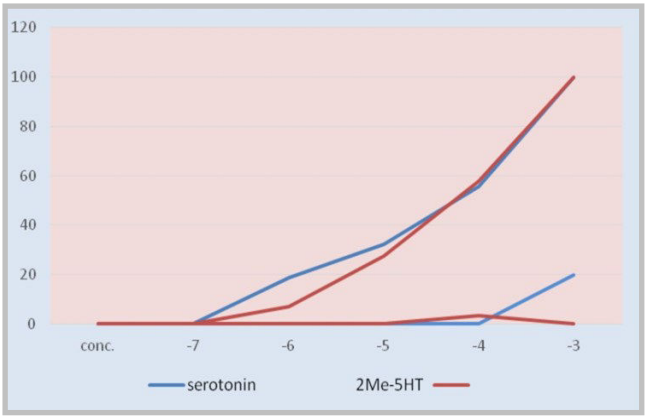

Figure 1. Effect of various concentrations of serotonin and 2-Me-5HT on circular (lower values) and longitudinal layer (higher values) of smooth muscles of broiler's duodenum.

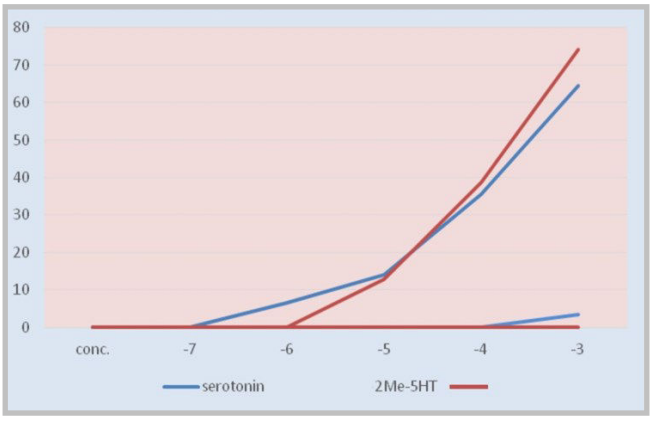

Figure 2. Effect of various concentrations of serotonin and 2-Me-5HT, on circular (lower values) and longitudinal layer (higher values) of smooth muscles of broiler's jejunum.

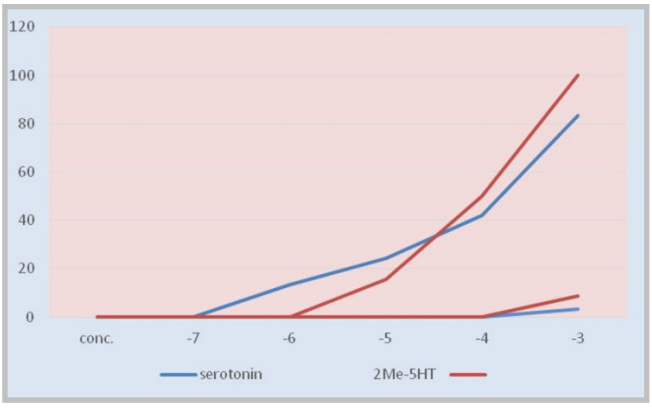

Figure 3. Effect of various concentrations of serotonin and 2-Me-5HT, on circular (lower values) and longitudinal layer (higher values) of smooth muscles of broiler's ileum.

Discussion

Approximately 90% of the total content of serotonin in the body is located in the gastrointestinal tract, i.e enterochromaffin cells isolated from the stomach and intestines (Kato, 2013; Rang et al. 2015; Riviere and Papich, 2009). It is peripheral and the most evident effect of serotonin on smooth muscles of gastrointestinal tract (Penkova and Nikolova, 2017; Rang et al., 2015). Serotonin, among other substances, causes contractions of smooth muscles of bovine rumen (Muminović et al., 2000), regulates the motility of the digestive system, enhances secretion, etc. (Rang et al., 2015). 5-HT3 receptors in the enteric nervous system regulate gut motility, secretion, and peristaltic movements and are involved in information transfer in the gastrointestinal tract (Galligan, 2002). Some authors

Table 1. Effects of different serotonin concentrations on both layers of smooth intestinal muscles of broiler.

	Duodenum				Jejunum				Ileum				
Conc.	Circ. Layer	SD	Long. Layer	SD	Circ. Layer	SD	Long. Layer	SD	Circ. Layer	SD	Long. layer	SD	P Values
10^{-7}	0	0	0		0	0	0	0	0	0	0	0	-
10^{-6}	0	0	18.9	1.394	0	0	6.6	0.261	0	0	13.3	0.200	0.001
10^{-5}	0	0	32.2	1.271	0	0	13.9	0.385	0		24.4	1.265	0.001
10^{-4}	0	0	55.6	0.635	0	0	35.5	2.15	0	0	42.2	1.289	0.001
10^{-3}	20	1.133	100	0	3.3	1.088	64.4	1.54	3.3	0.089	83.3	0.089	0.001

SD = Standard deviation, **Conc.** = Concentrations, **Circ.** = Circular , **Long.** = Longitudinal

tried to identify the 5-HT3 receptors in isolated smooth muscles from gastrointestinal tract of rats (Glatzle et al., 2002), jejunum of rats (Veeresh et al., 2009), bovine rumen (Muminović et al., 2000), human small intestine (Coleman et al., 2003), human stomach (Penkova and Nikolova, 2017), smooth intestine musculature during the turkey fattening phase (Katica, 2015) and effect of 5-HT3 receptors on gastric motility in fasted and fed dogs (Nagakura et al., 1997), but not in smooth muscles of broilers small intestines. There is a general agreement that 5-HT3 receptors in the intestinal tract are located on enteric sensory neurons and activate a cholinergic mechanism to stimulate secretion (Hendriks et al., 1989).

Receptor 5-HT3, more precisely its subtype 5-HT_{-3B} which was investigated in tunica muscularis of small intestine, is bound to membrane ionic channels, for difference to the other serotonergic receptors (Katica, 2015, Pauwels, 2003, Siegel et. al., 1999). Peripheral effects which appears after $5HT_{-3B}$ activation, are reflecting in cardio-vascular system, control of intestinal tonus and secretion as well as in contraction of bladder's smooth muscle in cat. Central effects are manifested as psichological disorders and vomiting (Hardman and Limbrid, 2001; Katica, 2015).

Table 2. Effects of different Me2-5HT concentrations on both layers of smooth intestinal muscles of broiler.

	Duodenum				Jejunum				Ileum				
Conc.	Circ. Layer	SD	Long. layer	SD	Circ. layer	SD	Long. layer	SD	Circ. layer	SD	Long. layer	SD	P Values
10^{6}	0	0	7.2	0.586	0	0	0	0	0	0	0	0	0.001
10^{5}	0	0	27.5	0.374	0	0	12.8	0.473	0	0	15.7	0.914	0.001
10^{4}	3.6	0.261	58	3.178	0	0	38.6	0.089	0	0	50	0.707	0.001
10^{3}	0	0	100	0	0	0	74.2	0.562	8.6	0.109	100	0	-

SD = Standard deviation, **Conc.** = Concentrations, **Circ.** = Circular , **Long.** = Longitudinal

Results obtained for different serotonin concentrations (nonselective agonist of serotonergic receptors) indicated on evident presence of serotonergic receptors in smooth intestinal muscles of broiler. Application of different serotonin concentrations showed significant differences in distribution of serotonergic receptors among the intestinal parts (duodenum, jejunum and ileum) as well as among the layers of tunica muscularis in broiler's small intestine ($p < 0.001$). The highest response was obtained in longitudinal

duodenal layer under the concentration 10^{-3}. The lowest values were obtained in the circular layer. Statistically significant differences among the different intestinal parts were recorded under the same serotonin concentrations (Table 1).

Results obtained by application of Me-2-5HT (selective agonist of $5HT_{-3B}$ type receptor) indicated the significant contractions in longitudinal layer of tunica muscularis in duodenum and ileum, under the concentration of 10^{-3} (Table 2). These responses were the highest and expressed by maximal percent. Effects Me-2-5HT on circular layer were lower than those obtained for longitudinal layer which were in accordance with the results obtained by serotonin application (Table 1 and Table 2). Statistically significant differences were recorded for the results obtained in different part as well as in different layers of broiler's small intestine ($p < 0.001$).

According to the study of Mujezinović et.al (2011), it could be concluded that 5H71A type receptors were present mainly in smooth muscles of the broiler broiler's small intestine, duodenum and ileum, especially in the longitudinal smooth muscle layer which reacted with contractions even to low serotonin concentration (10^{-6}). Similar reaction was not recorded in the jejunum.

Results for duodenum and jejunum obtained in this study partly corresponded with similar study of serotonergic receptors distribution, as well as with the study of 5 HT_{-3B} in tunica muscularis of small intestine during the turkey fattening phase (Katica, 2015), while the results for both muscular layers of ileum showed high accordance with the results from the same study.

The research established general a presence of serotonergic, receptors within the smooth musculature of the small intestines of broilers (COBB 500). The 5HT-3B type receptors are present in smooth muscles of duodenum, jejunum and ileum, especially in longitudinal smooth muscles since this layer reacted even to low serotonin concentration (10^{-6}). In the light of these findings, we suggest that investigated substances may have considerable physiological and pathophysiological implications in functions of small intestine of broiler's.

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Parasitary infestation in a fox

Burçak Özkan*

Kadikoy, Istanbul / **Turkey**

Case Report

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ABSTRACT

Foxes are a member of Canidae family belonging to carnivora order. There are a lot of fox species all around the world. Human population growth, anthropogenic landscape changes, ameliorated vaccine protocols, laws against hunting, the increasing interest towards wildlife and wild animals resulted in augmented fox population and human-fox interaction. These all have important biologic consequences such as prevention and treatment of zoonotic diseases and endangered species protection. Both sarcoptic mange and coccidiois are two important parasitary diseases seen in foxes. In this case, a young female fox suffering sarcoptic mange and coccidiosis is presented. Haemogram and biochemistry measurements of the patient were between normal intervals except values pointing out a slight anemia. This anemia was thought to be due to parasitary infestations. A therapy schedule including ivermectin, trimetoprim/ sulfa, vitamin-B was applied according to the literature and the treatment was judged as successful. Important conclusions gained from this report have been shared in the result of the case report.

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Introduction

Due to anthropogenic landscape alterations related to human population growth, wild terrestrial carnivores are increasingly seen in urban and peri-urban areas since these regions contains easily-accessible food sources. This highlights the augmented risk of interspecies spillover of infections and zoonotic transmission (Lempp et al., 2017).

Domestic dog is the most common carnivore of the world; which makes this species known or suspected reservoir of epidemic-related infectious pathogens of most wild carnivores. Dogs generally form large, unowned, unvaccinated, free-ranging populations worldwide. They also increase the risk of pathogen transmission since they travel long distances into wildlife habitat (Vanak et al., 2018). Interspecies infectious and parasitary disease transmission between dog and fox is very common (MacDonald, 1996; Razmjoo et al., 2013; Truyen et al., 1998).

Ectoparasites such as fleas, mite and ticks are common in carnivores (Razmjoo et al., 2013). Cruz-Vazquez et al., (2001) identified three taxa of ectoparasites in the Island fox which is endemic relative of gray fox (*U. cinereoargenteus*). These three taxa contain fleas (*Pulex irritans*), lice (*Neotrichodectes mephiditis*) and ticks (*Ixodes pacificus*) (Cruz-Vazquez et al., 2001; Emerson and Price, 1987). *Centenocephalides canis* was the most often identified flea type. Same study revealed after fleas, *R. sanguineus* tick were the most isolated ectoparasite in red foxes. Other researchers explain that although foxes can be infested with many other types or fleas and ticks they acquired from both their prey animals or from those sharing the same environment, the fox tick (*Ixodes canisuga*) and the fox flea (*Chaetopsylla globiceps*) are the most relevant types of ectoparasites identified (Forchhammer, and Asferg, 2000; Sréter et al., 2003). In a research, Mainland gray foxes are found to host at least 15 species of fleas, two species of lice and nine species of ticks (Fritzell, 1987). Their role as vectors in most of infectious disease and in flea allergy makes

*Corresponding Author: Burçak Özkan
E mail: burcakozkan1974@yahoo.com

fleas a very important disease effect to investigate; thus their biology and epidemiology are widely studied (Razmjoo, et al., 2013). Cruz-Vazquez et al. (2001) also add various researchers' reports explaining that both gray foxes and island foxes may be infected by *Pulex* spp. and *Ixodes* spp. Yet, *Suricatoecus* is more often reported than *Neotrichodectes* (Cruz-Vazquez et al., 2001; Emerson and Price, 1987).

Another important disease, sarcoptic mange caused by *Sarcoptes scabiei*, is a very contagious skin infection. Mites are transmitted between species but inter-species transmission is limited. Infectious agent is transmitted either via direct contact or indirectly via environment. After having borrowed into the epidermis, mites form tunnels in order to deposit antigenic material which later causes severe skin irritation. The infection may easily and rapidly become heavy. The disease which is often seen in foxes causes fur loss, sores from scratching and a crusty film surrounding mouth, nose and eyes. Lightly infected animals represent only short-time clinical signs whereas heavily infected ones suffer hyperkeratosis, alopecia, general wastage. An ultimate muscular catabolism occurring from host response epidermal cell destruction, accompanies these clinical findings. The inflammatory trauma may result in physical self-trauma and secondary dermatologic infections. Death may occur related to several causes, some of them being starvation and hypothermia (Soulsbury et al., 2007). Animals suffering from mange are generally hungry and search for food without being afraid of humans. Several wildlife diseases including also distemper and mange mimic rabies but in fact, they are not zoonotic (Soulsbury et al., 2007; Yery, 2018). Sarcoptic mange which represents a negative correlation with habitat quality, also negatively alters female and male reproductivity (Soulsbury et al., 2007).

While burrowing, mite ingests living cells and tissue fluids. Life cycle of *S. scabiei* is well known (Mueller, 2000). Mites leaves behind excretions and secretions which causes an irritant and allergic effect; which is pursuited by an immense amount of antigenic material released into the skin. As a result, the biggest part of sarcoptic mange pathogenesis is due to mite hypersensitivity. In spite both type 1 and type 4 hypersensitivity were explained in other species, only hypersensitivity type-1 is known to be represented in foxes (Little et al., 1998b). The worse becomes the hypersensitivity, the lesser becomes the amount of mites in the lesions. Moreover, at the same time the pruritus increases, also does the amount of the traumatic lesions. The change of non pruritic and localized lesions into generalized, urticarial and diffuse ones is the hallmark of allergic response developed by the host. In wildlife, allergic response and self-trauma related to intense pruritus are the most relevant symptoms of sarcoptic mange. Yet, some recently exposed individuals may develop non pruritic severe crusting dermatitis characterized with severe hyperkeratosis (Pence and Ueckermann, 2002).

The degree to which animals are affected, depends on animals' immunological and nutritional condition as well as parasite intensity. Infestations may even lead to death in severe situations (Kočišová, et al., 2006). Not only parasitary and/or host related factors, but also sociological ones as native husbandry being runned by people living in a geography is an important factor. Environmental contamination with ascarids also forms another important health issue for humans (Razmjoo, et al., 2013).

Thus, foxes suffering endoparasitary infestations may be a risk factor for humans either via direct contamination by contamination of soil with eggs or with indirect way by infecting intermediate hosts and cats and dogs (Vervaeke et al., 2005).

Figure 1: The fox on the first day of the treatment.

Case

Clinical examination and findings

A young female fox was brought to the clinic. She was in poor condition, she showed poor corporal status and she was slightly dehydrated (Figures 1 and 2). Only a few crustaceous, reddish, alopecic lesions, a mild pruritus and a wound were arousing attention. The lesions were localized in ventral and lumbar areas and paws. First of all, "less is best" rule highlighting

Figure 2 = The fox on the first day of the treatment.

minimal handling must be made was taken into consideration. The less possible drug administration, especially streoid use avoidance also very important (Burwell, 2018).

According to this, a quick examination was performed. As she was docile, she could be manually and easily restrained during examination procedure without anesthetic use. The body temperature (BT), heart rate (HR) and resting respiratory rate (RR) were evaluated according to reference values accepted for domestic dogs. The measured values were respectively as BT = 39.2, HR = 84/min. and RR = 26/min. All values were between normal intervals (BT = 39.1, HR =70-120/min. and RR = 18-34/min. for domestic dogs) (Khan and Line, 2005).

No life threatening situation such as head trauma, any breathing problem or another kind of trauma was detected. Then, as suggested, wound, external parasites, possible fractures and dehydration were checked. Weight was controlled; she was weighting 12 kg. Five mL. of blood was collected from V. jugularis following physical restraint. Urine was also collected. Haemogram, blood biochemistry analyses and urinalysis were performed immediately.

As feeding an animal which is dehydrated, in shock, cold or in starvation state can result in worse their situation since they will be unable to digest the food which is going to be decomposed and fermented in their GI tract, feeding didn't started until the animal was completely stabile. Moreover feeding wild species with wrong foods can cause severe GI distress. Feeding should be started when GI tract is sure to be functional. Otherwise putting food in a non functional GI tract may cause the animal to die since the food will spoil into the patient (Burwell, 2018).

The fox has been kept warm. Since a slight dehydration was present, fluid therapy started. In case the animal is stable, it is recommended to keep him/her in a warm and quiet environment. The fluid therapy consisted on lactated Ringer's solution. On the second day, as the very slight dehydration has been no more observed, fluid therapy has been stopped since she was also eating and drinking well and a slight anemia was present

In addition to all of these, attention was made in order to not to let anyone who is not vaccinated against rabies to contact patient. The fox was not encouraged to become too friendly to go back to natural habitat. It is well known that friendly wild animals are likely to be killed. First of all, they cannot survive in wildlife. Secondly, they die eventually either because they become pests or because someone may misunderstand this attitude for rabies (Burwell, 2018).

Diagnosis and Treatment

Biochemical values were evaluated both according to reference values explained for foxes that could be found in the literature and according to those accepted for domestic dogs. They were all accepted to be between reference values or close; at least not abnormal. Urinalysis didn't represent any pathology neither. Haemogram results showed only a slight anemia. Skin scrapings and gaita samples were sent to the parasitological examination. Sarcoptes scabiei in dermatologic samples and Coccidian oocystes in fecal samples were identified.

As recommended in literature, the wound was cleaned. Following the wound cleaning, ivermectin (IvomecTM; Topkim, Turkey) treatment for sarcoptic mange and trimetroprim/sulfa (BactrimTM Roche, Turkey) application for coccidiosis was organized. Sarcoptes treatment in foxes is similar to what must be adopted when domestic dogs are infested; moreover ivermectin should be added to treatment protocol (Canadian Council on Animal Care, 1984). Other researchers confirm that the avermectins, especially ivermectin is very effective in the treatment of various domestic and wild species (Arends et al., 1999). Ivermectin dosage is recommended as 0.2-0.4 mg/kg SC every 1-2 weeks for 3-4 treatments (Tilley and Smith, 2004). In this case 0.2 mg/kg. ivermectin was applied SC once a week. At the end of 4th. week, no scabies was detected in skin samples. Trimetoprim/ sulpha was dosed as 15 mg/kg PO q 12h. for 2 weeks (Tennant, 2005). Vitamin B Complex (Dodex™; Vetaş, Tukey) was added into therapy in order to control slight anemia existing. The dosage was as 0.5 ml/daily (IM) (Steneroden and Wydallis, 2014; Tennant, 2005). The therapy didn't cause any side effect. Slight anemiahadn't been noticed at the end of the second week. The therapy was judged as successful.

Table 1: Reference values of haemogram of different fox breeds resulted from various researches and measured value in this case.

		Reference values (According to various research results)				
	Measured Values	Rui et al., 2011	Mattoso et al., 2012	Korhonen and Huuki, 2014	McCue and O'Farrell, 1987	Canadian Council on Animal Care, 1984
RBC (X 10 ^{6}/µL.)	4.2	-	-	-	6.6-10.2	7.1-11.2 (RR) 7.8-9.4 (WR)
HGB (g/dL)	14.2	F = 139.7±14.5 (g/L) M= 140.3 ± 10.9 (g/L)	(both sex) 10.0-18.1	-	11.5-17.5 (S) 12.6-18.6 (W)	-
HCT (%)	30	-	(both sex) 28-53	52.0- 58.4	38.9-54.9	-
WBC (X 10 ^{3} /µL.)	8.3	-	-	-	2.0-10.4 (RR) 3.9-11.1 (WR)	4.9-12.4 (RR) 4.4-7.3 (WR)
PLT (X 10 ^{3} /µL.)	284	-	-	-	-	-
MCV (fL)		F = 53.2 ± 1.6 M = 54.4 ± 1.1	(both sex) 78.1-100	-	49.3-63.3	-
MCH (pg)	21.4	F = 15.6 ± 0.4 M = 16.3 ± 0.3	(both sex) 28.1-34.9	-	15.6-20.0 (S) 17.0-19.8 (W)	-
MCHC (%)	34.2	-	(both sex) 30.2-38.4	-	-	-

F= Female ; **M** = Male ; **RR** = Ranch raised ; **WR** = Wild raised ; **S** = Summer ; **W** = Winter

Discussion

Sarcoptic mange due to Sarcoptesscabiei has been identified and published in 104 species of domestic and wild mammals The disease is the most often diagnosed one in both domestic and wild Red foxes (*Vulpes vulpes*) which are living worldwide (Bornstein et al., 2001; Little et al., 1998a).

It has been discussed that the mite causing disease in wild and domestic animals is a single species and it represents physiological specifity across different hosts. Thus, *S. scabiei* has at the same time high specificity of host and low degree of cross infectivity. The varieties are named according to their predominant host species, such as *S. Scabiei varcanis*, *S. Scabiei varsuis*, etc. (Pence and Ueckermann, 6446). Host specifies may vary related to various strains. Though dogs may acquire sarcoptic mange from canids, it is not clear how frequently domestic canids cause infection in foxes and other species. Standing in contrast to other species, the red foxes failed to develop immunity against mange (Little et al., 1998a). While the immune response in sarcoptic mange is poorly understood, the immune reaction seems to be cellular one (Pence and Ueckermann, 2002). Mueller (2000) explains both cellular and humoral immune responses develop.

Any pruritic dog can possibly be infected with Sarcoptes scabiei ; particularly if these pruritic lesions are localized in pinna, ventrum and elbows thus; treatment is necessary (Mueller, 2000).

Although sarcoptic mange is generally difficult to detect, examining deep skin scrapings in % 10 potassium hydrochloride is very effective when mites are still abundant in the skin. Skin scrapings generally necessity to be repeated. Detecting only one mite and/ or egg is sufficient in order to identify sarcoptic mange. Furthermore, negative results doesn't mean sarcoptic infestation doesn't exist especially in canine mange. ELISA tests for sarcoptes specific IgG may be used. According to a research, in atopic individuals, these tests have a very high predictive value despite having very low positive predictive value due to false positive results. A positive pinnal scratching reflex also generally confirms the infection (Mueller, 2000; Harvey et al., 2009). In dogs, primary lesions are erythematous papules with a grayish-yellow crust. Self-trauma may accompany severe excoriation, lichenification and patchy alopecia. Malaise, weight loss, lymphadenopathy and crusting in severe long-standing cases are also known (Harvey et al., 2009). In a study about scabies in foxes, it is explained that the commencement, the progression and the clinical signs of the lesions vary according to various species and the host's immunologic properties. As the immunologically competent host develops hypersensitivity, intensely pruritic lesions, hyperkeratosis, alopecia and dermal

Table-2 : Serum biochemistry reference values of different fox breeds obtained from different studies and serum biochemical measurements in this case

		Reference values (According to various research results)			
	Measured Values	Rui et al., 2011	Mattoso et al., 2012	Nowakowicz-Dębek et al., 2015	Korhonen and Huuki, 2014
Urea (mg/dL)	28	-	22 - 87	-	-
Creatinine (mg/dL)	1.3	-	0.5 -1.5	-	-
ALT (IU/L)	124	F = 136.3 ± 59.4	2.6 - 231.5	164.8 (FL)	171.5
		M = 168.7 ± 95.1		88.9 (B)	
AST (IU/L)	74	F = 55.9 ± 15.1	-	117.5 (FL)	49.2
Glucose (mg/dl)	82	M = 62.7 ± 14.5	-	48.9 (B)	-
Total Bilirubin (mg/dl)	0.4		-		
LDH (IU/L)	226	F = 68.08 ± 48.4	-	529.0 (FL)	-
		M = 84.4 ± 80.8		654.9 (B)	-
CK (IU/L)	114	F = 119.9 ± 77.7	-	-	84.0
	-	M = 258.9 ± 117.8	-	-	-
Total Protein (g/dL)	6.3		4.6 - 9.4	-	-
Albumin (IU/L)	33.4	-	-	-	-

F= Female, **M** = Male, **FL** = Free-Living , **B** = Breeding

inflammation develop. Finally, greatly thickened, wrinkled, hairless, discolored or grey skin is observed.

In the end, the animal becomes listless, dehydrated, emaciated and dies from the disease (Pence and Ueckermann, 2002). In the later stages of sarcoptic mange animals seek shelter; which makes difficult to help them to recover (Soulsbury et al., 2007). Death realizes within 3-4 months from first infection (Stone et al., 1974). In red foxes sarcoptic mange is not limited with skin lesions; lymphoid hyperplasia and testicular degeneration develop (Little et al., 1998a).

Ivermectin use resolves sarcoptic mange problems in foxes as it does in dogs (Arends et al., 1999; Canadian Council on Animal Care, 1984; Pence and Ueckermann, 2002). It makes also the related lesions vanish (Little et al., 1998a). The dosage is scheduled as 0.2-0.4 mg/kg. either weekly P.O. or every 14 days SC for 4-6 weeks. Ivermectin is not licensed in dogs and should be avoided in sensitive breeds (Harvey et al., 2009). Though idiosyncratic toxicities are documented in Collies and Old English Sheepdogs, other dog breeds may also be affected inversely. Gradual dose increase from 50 µg/kg on day 1 to 100 µg/kg on day 2, 150 µg/kg on day 3, on day 1 to 100 µg/kg on day 2, 150 µg/kg on day 3, 200 µg/kg on day 4 and 300 µg/kg on day 5 to identify sensitive patients is recommended before adverse reaction develops. An administration of gradual increase from 50 to 300 µg/kg within 4 days for SC scabies treatment is recommended. Lethargy, ataxia, tremors, mydriasis, coma and respiratory arrest are noted between side effects (Mueller, 2000).

Topical treatment is difficult, time consuming is not always safe. Amitraz dip application (every 7-14 days, for 4-6 weeks) and 0.25 % fipronil solution (3-6 ml./kg. every 7-21 days for 3-6 weeks in young, pregnant and nursing dogs when more potent cures are dangerous) aid to the treatment. An application of 2.5 % lime sulfur (weekly for 4-6 weeks) is also effective (Harvey et al., 2009).

Since mange causes juvenile deaths, failure to undergo spermatogenesis and female reproduction decline, population recovery during the enzootic stage is limited (Newman et al., 2002). The success to either endure infection and/or survive is affected by various factors including age, genetic predisposition, nutrition and physical status and finally environmental conditions and habitat properties which directly influences stress. According to various reports, the initial decline in population density is due to mortality increase of both juveniles and adults, juveniles being more susceptible to the disease since they experience more physical contact during their early development. Yet, direct transmission between adults is also common because adults in the same social group share the same densities. The importance of indirect transmission in urban habitat is related to the concordance of mange levels between foxes and dogs (Soulsbury et al. 2007).

In this case, the infestation wasn't heavy. Only a mild pruritus and some reddish, alopecic lesions were arousing attention. The lesions were localized in ventral and lumbar areas and paws. These findings were in concordance with reports explaining that some

recently exposed individuals may develop non pruretic severe crusting dermatitis characterized with severe hyperkeratosis even the lesions were not severe. The lesions vanished slowly and the pruritus ceased as treatment went on.Coccidia is an obligate intracellular parasite normally found in the intestines and all warm-blooded animals are infected with this agent. Coccidia of dogs and cats were accepted biologically clinically unimportant until *T. gondii* life cycle was discovered. Coccidia is now divided into several distinct genera such as *Toxoplasma*, *Neospora, Isospora* (*Cystoispora*), *Hammondia*, *Besnoitia*, *Sarcocystis*, *Cyrptosporidium* and *Cyclospora* (Dubey and Lindsay, 644[3]). Foxes are generally infested with *I. bigemina*. The signs vary from mild to bloody diarrhea, anorexia and death (Khan and Line, 2005). A case report focusing on foxes explains the first clinical symptoms seen in three weeks old fox whelps unthriftinesse, watery stool, moisted and clamped furs. Poor growth accompanied by significantly smaller body size were the other signs (Juokslahti et al., 2010). Parasitised free foxes have a higher mass/body ratio than infested ones. (Vervaeke et al., 2005). According to Skirnisson et al. (1993), food consumed by the fox influences the number and species of parasite type causing the infection.

The treatment of coccidiosis in fox consists of the procedures adopted in dogs (Khan and Line, 2005). The primal aim of the cure in *Isospora* spp. infections is to resolve diarrhoea. Supportive care including fluid therapy in order to correct dehydration is recommended. An important amount of the drugs used in coccidiosis treatment have only coccidiostatic effect. Amprolium is used in puppies despite being unapproved in dogs (25 mg/kg. PO q24h. for 3-5 days). Ponazuril, diclazuril and toltrazuril are widely chosen nowadays. Toltarzuril is known to cause the oocysts shedding to diminish, at least temporarily. A dosage of 30-60 mg. trimeptoprim/sulfamethoxazole daily for 6 days is recommended for the dogs if the animal weights more than 4 kg. Another source suggest sulfadimethoxine, trimetoprimsulfa treatment for 10-20 days. But, some sulfa drugs are known to cause GI irritation, keratoconjunctivitis sicca, cholestasis, hepatocellular necrosis and thrombocytopenia. The prognosis is good unless there is an underlying cause (Nelson and Couto, 2009; Steneroden and Wydallis, 2014).

A report explains foxes suffering the disease were cured with oral sulfadiatzine/trimetoprim dosed as 120 g per ton of semimoist feed for five days. The treatment was accepted to be satisfactory. When a second outbreak occurred, a treatment of oral toltrazuril was applied at a dose of 10 mg. supplied by oral sulfadiatzine-trimetoprim per whelp for 5 days. Recovery was recorded (Juokslahti et al., 2010). In a report about emergency stabilization of wildlife, the dosage of trimetoprim/sulfamethoxazole is scheduled as 30 mg/kg. PO q12 hrs. in mammals (Burwell, 2018; Steneroden and Wydallis, 2014).

In this case, the fox could be accepted as asymptomatic. Asymptomatic ongoing of coccidiosis is explained in older individuals. Despite the exact age of the fox in this case was unknown, she was a young one.

The only signs that could be related to the disease were poor growth, a slight dehydration and a slight anemia, which all were resolved with appropriate therapy.

Mattoso et al., (2012) explain that hematologic values they had found for crab-eating foxes were in general similar to those explained for domestic dogs. These measurements didn't differ between genders. Yet, female foxes' percentage of eosinophil was higher compared with the percentage of eosinophil in males. (Crooks et al., 2000; Rui et al., 2011).

Hemoglobin, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), WBC, band and segmented neutrophils and lymphocytes showed significant variations between sub-adults and adults. MCH and MCHC showed higher results in older individuals. This should be due to bigger hemoglobin reduction in youngers. WBC, segmented neutrophils and lymphocytes were higher in sub-adults; which was evaluated to be due to higher stress expressed by younger when physical and chemical restraint are applied (Jain, 1993; Mattoso et al., 2012).

In our case, the measured haemogram values were compared to both with reference values explained for foxes by different researchers and the reference values accepted for domestic dogs showing similar values with foxes since fully established and accepted reference values for each haemogram parameter in foxes don't yet exist. According to a such comparison, only a slight decrease in RBC and HCT values were present. Thus, the patient was accepted to be suffering a slight anemia. This was thought to be related to both sarcoptes and coccidosis infestations. I

n foxes, serum biochemical parameters such as ALT, ALP, GGT, TP and albumin levels are similar to those measured in dogs (Choi et al., 2011; Zhan et al., 1991). Nowakowicz-Debek et al. (2015) concluded ALT and AST activity as well as urea level were measured

significantly augmented in free-living foxes while bilirubin and LDH levels were found similar in farmed ones. ALP, BUN, creatinine, cholesterol and glucose levels were higher in wet season in island foxes (Rui et al., 2011; Seal et al., 1975; Smith et al., 1980). Similar to hematologic parameters, biochemical ones didn't represent any difference related to gender (Mattoso et al., 2012; Seal et al., 1975). Yet, glucose levels in male foxes were measured higher than measured in females (Crooks et al., 2000). According to these, urea, ALP, GGT, fibrinogen, calcium and phosphorus differed between sub-adults and adults. High urea levels in older animals was related to the high-protein diet they are fed with. GGT and fibrinogen levels were also higher in older animals. ALP, calcium and phosphorus were detected higher in younger foxes. These results were thought to be related to fast osteogenesis and osteoclast differentiation in younger individuals (Mattoso et al., 2012; Rui et al., 2011; Seal et al., 1975; Smith et al., 1980).

Similar to haemogram values, completely established and accepted biochemical reference values don't exist yet for the foxes either. Thus, similar to what was done when evaluating haemogram measurements, comparison with both reference values explained for foxes in literature and with those accepted for domestic dogs were realized. In dogs measured ALT value must be lower than 100 (IU/L); thus a slight increase in the patient's value seems to exist according to this. On the other hand, when reference values explained for foxes are taken into consideration, the measured value seems normal. In our case, it was concluded no pathologic situation existed since even regarding to reference values accepted for dogs, only an increase of at least three times normal indicates a liver damage (Sodikoff, 2001).

In a research, physical examination and values of urinalysis were found similar to those known for domestic dogs. On the other hand, proteinuria measurements were higher than those of them (Lees et al., 1994). The urinalysis of the fox represented in this case didn't show any pathology. to this. On the other hand, when reference values explained for foxes are taken into consideration, the measured value seems normal. In our case, it was concluded no pathologic situation existed since even regarding to reference values accepted for dogs, only an increase of at least three times normal indicates a liver damage (Sodikoff, 2001).

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Conclusion

Wild mammals play a crucial role as vectors and host for a range of bacterial, viral and parasitary diseases transmitting in a two-way manner between humans and animals. Zoonotic infectious diseases which have a strong effect on host population dynamics, are very hard to be controlled and their controversial management has also economic costs in spite of being used in resolving feral or wildlife species' troubles. Furthermore, epizoodics permits hosts and pathogens to coexist; which also makes the diseases an occasion for establishing key evolutionary and ecological main points (Soulsbury et al., 2007). Zoonotic invasions can be transmit directly through the environment, from the water or food or from close contact with other animals (Rataj et al., 2013).

Several attempts to decline the infection in foxes with the use of distributing baits containing praziquantel is reported. Most of them are known to provide substantive decline of parasitary infestations of foxes. Other baiting campaigns with various frequencies showed various results, while applications with lower frequencies didn't represented any significant reduction. The main property of FOI is to represent wide temporal and spatial variations which necessity to establish control strategies according to local FOI conditions (Lewis et al., 2014).

For those living in captivity, the length of time the animal are removed from wildlife, adjustment to captivity, quality of life in captivity, husbandry for the species' needs and proximity to other species influence the burden of infectious diseases. Contact with other individuals, humans, food, water, mites and iatrogenic introduction may also cause such diseases (Williams and Thorne, 1996).

In spite of being an important indicator for health status, neither hematologic nor biochemical values of foxes can be properly evaluated due to the lack of reference values (Courtenay et al., 2001). The importance of haemogram and biochemical parameters in order to diagnose and evaluate the prognosis of diseases is accepted; thus it is crucial and urgent to establish studies focusing on wildlife species reference physiologic values. It is also concluded that differences related to age must always be taken into consideration (Mattoso et al., 2012). In conclusion, the necessity to establish reference values for haemogram and biochemistry for wildlife species is of crucial

importance in order to detect and treat any pathology. Wildlife specialization is another necessity if the wildlife is desired to be protected and appropriate medical prevention and care is applied. According to these, this case defines sarcoptes and coccidiosis in a fox and tries to highlight the importance of a fully examination even when definitive and severe signs don't exist since the disease (s) may be ongoing asymptomatically.

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Hypothermia in newborn calves

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Süleyman Kozat*

Yuzuncu Yıl University, Faculty of Veterinary, Department of Internal Medicine, Zeve Campus Van/**Turkey**

ABSTRACT

One of the most important losses in animal husbandry is yield loss of neonatal calves. The first hour of birth and life in newborn neonatal is very important for the survival of life. According to the results of the research, it is reported that the mortality rates of the calves are higher in the beginning of spring and in the winter season. Approximately 50 to 53% of calf defects in the neonatal period occur at birth or within two days of life. The most important cause of calf's losses is the formation of hypothermia in newborns because of the temperature of the environment is much lower than body temperature of calf, the change of air temperature (cold air and strong wind), the calf wetness and the lack of thermoregulation. In this review, detailed information about causes of hypothermia, treatment and prevention of neonatal calf defects will be provided.

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Introduction

The neonatal period is one of the most critical stages in the development of farm animals because morbidity and mortality rates in this period are highest of all life stages (Hammon et al., 2012). There are lots of factors which can cause loss of life after birth in neonatal calves such as dystocia, inherited problems, infectious agents, and stressors of environment (Bellows, 1997). Surveys show that mortality in beef herds from birth to weaning ranges from 3 to 7%. Calf survivability also depends on cow feeding (Bull, 1983; Bellows, 1997; Lammoglia et al., 1999). Colostrum intake is another factor which is dividing to type and timing. These factors can dramatically influence calf life either alone or in combination with each other (Bull, 1983; Bellows 1997; Lammoglia et al., 1999; Godden, 2008). Most of newborn calf's deaths happen along the first day of life and leading causes of death during this period are dystocia which is defined slow and difficult births beside hypothermia which is known as cold stress correct and to the point care strategy beside proper treatment of the hypothermic calf can reduce the rate of mortality (Odde, 1988; Kasari, 1994; Butler et al., 2006; Nagy, 2009). Late winter and early spring are the most calves born period and as normal in these periods of year and due to exposure to sever environmental situations, cold stressed and hypothermia can occurred (Martin et al., 1975; Stott et al., 1976; Waltner-Toews et al., 1986; Holland and Odde, 1992). Newborn calves can face up with a dramatically temperature as they leave the comfortable warm zone of the uterus throughout the cold zone (Bellows, 1993). Soaked newborn calves can lost most amount of body heat due to surface area exposed is large and the vaporization of birth fluids from the respiratory tract and skin. Hypothermia in newborn calves is related with a prolonged time to standing, a holdup in suckling which may due to frostbite, elevated range of calf's death and weak soaking up of colostrum in this case hypothermia is leader percent of all neonatal calf fatality (Wiltbank et al., 1961; Bellows, 1993). Normal rectal temperature in term and preterm calves is at 38.6 - 39.4°C. Body temperature of 35 to 38°C is mildly hypothermic and

***Corresponding Author**: Süleyman Kozat. *Department of Internal Diseases, Faculty of Veterinary Medicine, University of Yuzuncu Yil, 65080 Van, Turkey.*
E mail: skozat@hotmail.com

will usually respond to tubing with warm colostrum and being placed in a sheltered or warm room (Wiltbank et al., 1961; Lammoglia et al., 1999; Godden, 2008). Calves with a temperature lower than 35 °C are considering as hypothermic and importance of urgent treatment is required (Wiltbank et al., 1961; Bellows, 1993; Howard, 1993). In premature calves known as hypothermia which is elevate fatality rate and it may be totally environmental or represent interaction diseases such as sepsis (Howard, 1993). Holding a proper environmental temperature in the operating room and delivery room is vital in limiting hypothermia. This kind of calves should be warmed again and any predisposing factors must be identified and treated (Wiltbank et al., 1961; Howard, 1993; Lammoglia et al., 1999; Godden, 2008).

Mechanisms of thermogenesis

Process of heat production neonatal calves is in a relationship with several factors: physical activity the metabolism of brown adipose tissue, heat increment of feeding, the metabolic rate of body tissues, trembling (Alexander et al., 1975; Vermorel et al., 1989; Carstens 1994).

Non-trembling thermogenesis

According to data's neonatal calves have brown adipose tissue which is amounted about 2 % of body weight (Alexander et al., 1975; Carstens, 1994). It is situated in the prescapular, inguinal, prerenal regions (Cannon and Nedergaard, 2004; Mattson, 2010). Administration of noradrenaline elevates production of heat in 30 minutes more than 70% (Carstens, 1994). In another research more than to 200% and as a result an elevate in rectal temperature of 1 or 2 °C (Alexander et al., 1975). Non-trembling thermogenesis is a responsible of a huge part of whole heat producing of neonatal calves in the cold weather. But by the aging of neonatal calf noradrenaline administration reacts less and less due to brown adipose tissue is quickly changed to white adipose tissue during the first month of life (Alexander et al., 1975; Carstens, 1994).

Trembling thermogenesis

Trembling emerges a bit after birth in calves held at 10 °C and ceases when the hair coat is approximately dry. It affects first muscles of skin and quickly skeletal muscles. In 12 h old calves lying in a 37 °C water bath, trembling begins when water degree falls to 32 °C. In despite of large between animal variations, the lower the water temperature, the more the calf trembles. Trembling is rapidly followed by an elevate in heat production changing from 30% to more than 100 % (Vermorel et al., 1989).

Physical activity

Consuming of energy corresponding to physical activity participates also elevate thermogenesis. Neonatal calf attempts to stand up, its heat production elevates by 30 to 100% and after 10 min standing, its energy consuming is also elevated by 100% and by the passing time nearly 30 minutes, heat production is elevated by 40% on average over this period (Vermorel et al., 1989).

Energy sources available for thermogenesis

Body reserves are known as available Energy sources at birth: labile proteins, lipids, hepatic and muscular glycogen. Fasting is a cause of rapid mobilization and breaking down of glycogen stores. As shown by the increase in respiratory quotient from 5% when the calf is struggling to stand up, Glucose is used for thermogenesis and for physical activity. Corticoids are responsible for high level of mobilization of protein after birth (Olson et al., 1981). In newborn held at 10°C the respiratory quotient is close to 0.80 so glucose is probably not the major energy source. Moreover, it reduces by 5 to 13% in 12 h old calves held in a water bath when water temperature drops from 37 to 30 °C (Vermorel et al., 1989). Non esterified fatty acids (NEFA) are one of plasma parameters which is increased due to body lipids mobilization. The latter are broken down in the brown and in the white adipose tissues and in the muscles. Colostrum is a nutrition which is constitutes an excellent energy source in calves for head production. It provides huge amounts of fatty acids, glucose and amino acids to the body. Rapid absorption of nutrients cause immunoglobulins emerge in the blood less than one hour after colostrum consumption (Olson et al., 1980). In 24 Friesian calves held at 10 °C, during the first and the second hour following colostrum consumption at 12 h of age thermogenesis was elevated on average by 18% and 9% (Vermorel et al., 1989). As a result, 2 kg fed of colostrum is providing energy requirement of a 40 kg neonatal calf held at 10 °C for 24 hours. So the importance of early colostrum intake for thermoregulation as it is for passive immunity is clears (Warnes et al., 1977; Vermorel et al., 1989).

Regulation of thermogenesis

Thyroid-stimulating hormone (TSH) secretion and thyroxine (T4) cause an increasing of plasma tri-iodothyronine (T3) and T4 amounts at birth and high

during the first hours of life (Davicco et al., 1982). In hypothermic neonatal calves level of cortisol, adrenaline and noradrenaline secretions are increased and up to the end of the stress Plasma levels remain so high (Olson et al., 1981; Cannon and Nedergaard, 2004). This increases process of thermogenesis considerably. On the other hand, high vitality calves shake or struggle before trembling begins, at the first sense of cold (Vermorel et al., 1989). Nervous thermoregulatory and hormonal processes (except peripheral vasoconstriction) are therefore well expanded in the neonatal calves and operative to maximum, supplied good calving conditions (Warnes et al., 1977; Davicco et al., 1982; Vermorel et al., 1989).

Pathophysiology

Thermal balance is in a relationship with direct contact, ambient air temperature, air flow with cool surface, humidity and proximity to cool objects. Neonatal calves are susceptible hypothermia due to a large surface area to volume ratio, which is even greater in low-weight neonatal calves (Butler et al., 2006; Godden, 2008)

There are several factors for hypothermia: Neonatal calves are wet with amniotic fluid which can cause evaporative heat loss. Neonatal calves are placed in contact with a cool surface or object which can cause Conductive heat loss. Heat loss due to environment containing objects of cold conditions. A flow of cooler environmental air carries heat away from the neonate which can bring about Convective heat loss. Thermolysis, thermogenesis and variations of rectal temperature

Rectal temperatures at birth is about 0.8°C above that of the cow and nearly 39.5 °C (Thompson and Clough, 1970, Rawson et al., 1989) decrease to 38.6 °C during the initial hour of life and then remain at around 38.8 °C 6 hour after (Vermorel et al., 1989). In cold exposed calves during the second day of life rectal temperatures elevate to about 39.2 °C (Webster et al., 1970). In neonatal calves held at 20 °C, thermogenesis is at its best 15 minutes after birth. And continue for 3 hour, then decline slowly by 30% from the 3 to 6 hour of life (Thompson and Clough, 1970) evaporation of amniotic fluid and drying of the coat are occurred in this period which is become more and more insulating. The process of calf thermogenesis increases in 5 day and decreases after one week of age (Roy et al., 1957). This event is in a relation with functioning of the liver, hematopoietic organs and digestive tract (Borderas et al., 2009).

Effects of climatic conditions

Climatic conditions are important in the process of thermolysis and thermogenesis and specifically on ambient temperature windy and rainy affect process of heat production and consequently energy requirement for thermogenesis. In 3 to 5 to week-old Friesian neonatal calves, respectively thermogenesis was elevated by 25%, 45% and 65% at ambient temperatures of 10 °C, 5 °C and 0 °C (Holmes and McLean, 1975).

Types of hypothermia

Hypothermia could be divided in tree stage: 1. Mild hypothermia (exposure) which is the continuous loss of body heat in a cold ambient. This type affects all classes of livestock but particularly affects young, old and thin animals via evaporation, respiration and lack of adequate hair coat, body flesh or weather protection (Laster and Gregory, 1973; Holland and Odde, 1992; Lammoglia et al., 1999). Mild hypothermia happens as the body's temperature decreases under nearly 38.6 °C for dairy calves and 37.7 °C for beef calves (Laster and Gregory, 1973; Odde, 1988; Holland and Odde, 1992; Lammoglia et al., 1999). 2. Acute to sever hypothermia (immersion) which is the quick loss of body heat due to covered hair coat in a cold ambient. Acute heat loss is often happened on during birth when the calf is born covered with birthing liquids. Other possibilities may consist being born in wet ground or snow, falling into a stream or being covered from rains followed by coldly winds (Laster and Gregory, 1973; Holland and Odde, 1992; Lammoglia et al., 1999). 3. Severe hypothermia results as decrease body temperature less than 34.5 °C which in this condition the vital organs are beginning to get cold. Below 30°C, signs of life are very difficult to detect and the calf may be mistaken for dead (Laster and Gregory, 1973; Odde, 1988; Holland and Odde, 1992; Lammoglia et al., 1999). The usage of the thermometer is crucial to diagnose the level of hypothermia. Sometimes appearance of calf does not represent hypothermia, but use of temperature show that the calf's body temperature is under normal. This is occurring in a situation such as dystocia, which lead calf to hypoxic (lack of oxygen). The calf being hypoxic, is slow to dry off, allows hypothermia to set in (Bruning -Fann and Kaneene, 1992; Lammoglia et al., 1999; Butler et al., 2006; Godden, 2008). Unrecognized, long term cold stress may leads calories to produce heat, and have negative effect on growth. Neonatal calves have a metabolic response to cooling that involves chemical (no trembling) heat production by norepinephrine which is produced by sympathetic nerve in the brown fat. This specialized tissue of the neonatal calf responds by lipolysis and emerges of fatty

oxidation or re-esterification of the fatty acids is done. Result of this process is produce of local heat which is carried to the rest of the body by supply of blood to the brown fat (Martin et al., 1975; Bruning-Fann and Kaneene, 1992; Butler et al., 2006). This process elevates the metabolic level and oxygen consumption two to three fold. Thus, in neonatal calf's with respiratory problem (e.g. asphyxia), cold stress can cause neurologic damage and tissue hypoxia and hyperglycemia may appear as a result of Activation of glycogen. Prolonged hypothermia can bring about metabolic acidosis, hypoglycemia and enhances the risk of sepsis and fatality (Martin et al., 1975; Warnes et al. , 1977; Olson et al., 1980; Vermorel et al., 1989; Bruning-Fann and Kaneene, 1992; Butler et al., 2006). Despite their compensatory mechanisms, neonatal calves, especially low birth weight calves, have finite capacity to thermoregulation and are inclined to reduce temperature level. Even before temperature decreases, cold stress occurs when heat loss requires an increase in metabolic heat production (Roy et al., 1957; Martin et al., 1975; Warnes et al., 1977; Olson et al., 1980; Vermorel et al., 1989; Bruning-Fann and Kaneene, 1992; Butler et al., 2006).

Etiology

The etiology of hypothermia in neonatal calves can divide in two sections which are described below: 1. Physiologic responses of well resistant neonatal calves (animals which respire immediately after birth, get up and suckle soon after). 2. The variations with breed and ambient situations and the thermoregulation failures of low resistant calves, mostly dystocial calves. Strong neonatal calves are able to face severe ambient conditions according to a quick elevate in thermogenesis through the process of brown adipose tissue, trembling, physical activity and colostrum fed (Alexander et al., 1975; Vermorel et al., 1989; Carstens, 1994). Conversely, low vitality calves, born under bad calving situations (dystocia, premature) are disabled by acidosis, hypoxia and hyperlactatemia and also limited mobilization of body lipids, which reduce thermogenesis. Moreover, their physical activity is reduced and they lost their standing ability, which elevates their heat losses. More than half of neonatal calf's death happens during 24 to 48 hours after birth. Fatality rates are elevated by hard birthing and sever ambient situations. But, processes of heat productions are operative at birth, consists the process of brown adipose tissue, trembling and physical ability (Martin et al., 1975). As in single calves also in twin, process of heat production is just as effectual provided that calving situations are good. Blood pH at birth was very low in dystocial calves, in eutocial calves, lactatemia was 2 or 3 times higher than, plasma T3 and T4 amounts were also decrease and mobilization of body lipids was lowered. This explain the cause of decrease in rectal temperature and also explain thermolysis in this kind of calves (Uetake, 2013). Thermoregulation process of Friesian was higher than of Charolais and Salers calves which were due to less basal metabolism rate rather from a good thermic insulation the physiological responses of calves born by caesarean parturition depend on the delay incurred during surgical removal (Laster and Gregory, 1973; Carstens et al., 1987; Uetake, 2013). The neonatal calf has face up to severe thermolysis in a situation of low body physiological resistance, principally because of occurrence of hypoxia. This is one of the reasons why hypothermia often occurs and may cause death of low resistance neonatal calves (Martin et al., 1975).

Hypothermia and environmental factors

Environment stressors like cold or wet ambient can be harsh on neonatal calves in winter and early spring and these calves are more susceptible to hypothermia (cold stress) (Martin et al., 1975). Rainfall have negative role in neonatal calf life when temperatures decrease (Azzam et al., 1993). So it is significant to fight hypothermia (cold stress) in neonatal calves as mentioned before rectal temperature is done to finding if calves are facing up with cold stress (Torell et al., 1998) when body temperature decrease under 37.5 °C consider as mild hypothermia and decrease below 34.5°C consider severe hypothermia. To fight hypothermia, the calf trembles to elevate process of thermoregulation and shunt blood from peripheral area of body to the body core.

Symptoms

Faced with a cold ambient, the body tries to resist in two ways: trembling, to elevate muscle thermoregulation, and blood flowing, to decrease heat loss by limiting blood flow from the body extremities and delivering blood flow to the body core (Torell et al., 1998). A decreasing in body temperature, impair teat seeking activity, malfunction of gastric mechanisms and lack of absorption of immunoglobulins and nutrients. Mild hypothermia happens as the body's core temperature decreases under normal (nearly 37.5 °C for beef calves). Vigorous trembling is usually come along with elevated pulse and breathing rates as a first symptom and sign. A cold nostril and pale cold hooves and ears are happened as a result of limiting of blood flow from the body's extremities. In the case of a

neonatal calves, intense trembling may follow with their disability to get up and suckle this signs can lead calves for intense hypothermic situation. Unsteady behavior, stupor and slowness are all symptoms of dummy calf, which is happens in mild hypothermia. Severe hypothermia results as the body temperature decreases less than 34.5 °C. Continues blood shunting bring about poor oxygenation of the tissues near the body surface which is responsible of appearing cold and pale nostril, ears and hooves. Decreased circulation also results in a buildup of acid metabolites (waste products) in the muscles of extremities. After the shivering stops, it is replaced by muscle rigidity. The pulse and respiration begin to slow as the body core cools to 31 °C (Azzam et al. 1993, Torell et al. 1998). As continues body temperature falling to decrease, the body continues to lead blood flow to body core and the negative aspect of this event is acid metabolic acidosis which is due to accumulation of acid metabolites in the extremities muscle of the body. Coldness of vital organs and impaired brain function are significant signs at 34.5 °C of body temperature and as decreases below 30 °C. Pupils are fixed and dilated and the pulse may be undetectable and signs of life are hard to identify (Odde, 1988). Irregular gasps for air may be the only signs of life disorders that impair thermoregulation such as sepsis, hemorrhage, cold stress due to environmental factors, problems that impair thermoregulation or a mixture of them (Torell et al., 1998). Risk factors for hypothermia include giving birth in an cold ambient area temperature below than recommended levels, maternal hypertension, cesarean, and low Apgar scores or a mixtue of them (Bruning-Fann and Kaneene, 1992; Butler et al., 2006; Godden, 2008). All these factors during the first day of life contribute to the high fatality rate of neonatal calves. Giving birth during late winter and early spring, are often cold stressed and may become hypothermic because of exposure to harsh ambient conditions (Martin et al., 1975; Waltner-Toews et al., 1986; Holland and Odde, 1992). Neonatal calves were predisposed to cold stress and made hypothermic by immersion in water at 15 to 17 °C. Cold stress delayed the start and notably declined the amount of immunoglobulins absorption up to 12 hours after first feeding colostrum. But, this wasn't impressed the net sorption of immunoglobulins. The feasible disadvantageous effect of cold stress on sorption of immunoglobulins by neonatal calves under range situations is discussed. Hence, it seems possible hypothermia has a negative effect on sorption of immunoglobulins by neonatal calves which can bring about further problems such as weak calf imperfect grows, bad body condition and easily get sick which are some of the effects of cold stress on the lack of sorption of immunoglobulins in neonatal calves (Torell et al., 1998).

Treatment

The metabolic reactions of the less strong calves and the hypotheses advanced to clarify the phenomena open a fascinating field of research with respect to medicines bound to guarantee the survival and a quick recovery of less strong calves (Lombard et al., 2007). The emergency medicine idea of the 'golden hour' can be performed to in danger neonatal calves. This term alludes to the principle of rapid intervention to avert ensuing sequelae. High hazard calves can be recognized (a) preceding birth by the anticipated probability of agony from dystocia; (b) amid birth by huge forelimbs, cyanosed muzzle and gums, swollen tongue; or (c) after birth by apnea or dyspnea, lateral recumbence, flabby musculature and poor pedal and suck reflex (Torell et al., 1998). Strategies for rewarming are changed and incorporate warm water shower, warm air or warmth lights (hot box), and warm covers.

Warming methods

Warm covers ought not to be hot to the point that they cause skin burns. Change the covers frequently to keep up a reliable temperature. As the calf turns outs to be more dynamic, it might end up plainly hard keeping the cover on the calf yet keeping after it. It is basic not to give the calf a chance to chill out after being warmed. A warming or hot cover case is another instrument you cause to warm neonatal calves. There are commercial ones accessible. Think about temperature control strategy, condition and ventilation. Some hot box concerns are the same as for warming covers (Griffen et al., 1960; Bull, 1983). The temperature ought not to be high that it can cause injures. Keep the temperature at 40.5-42.2 °C (Griffen et al., 1960; Borderas et al., 2009). Some sort of venting is important to anticipate development of carbon monoxide and dampness. Dampness development could chill the calf back off. Ventilation, for example, from a fan, is essential to guarantee thorough warming of neonatal calves (Torell et al., 1998). It can likewise avert problem areas in the warming cover box. Thermostatic management will help keep up reliable temperatures in light of the fact that warmth will cycle on and off as required. Access to clean condition is critical to keep the spread of ailment between neonatal calves. Clean out the hot box between uses to reduce the spread of disease (Lombard et al., 2007). Heating box are a potential supply of calf

diarrhea organisms and may make the spread of scours inside a group easier if consideration isn't paid to thorough cleaning and purification among calves (Torell et al., 1998). It is important support the calf to prevent drowning when utilizing a warm water shower. The water ought to be step by step warmed to 37.5 °C. At that point noticed the thermometer; the water should be changed to keep it at 37.5 °C (Griffen et al., 1960; Lombard et al., 2007). Calves should be steady before returned to the cow. Notice it intently to screen its condition, now and later on. The calf might be more prone to illness difficulties, such as scours and pneumonia subsequent to having such a hard birth (Lombard et al., 2007).

Rewarming process

Hypothermia is treated by rewarming and the neonatal calf ought be checked and treated as required for hypoglycemia, hypoxemia, and apnea. Hidden conditions, for example, drug withdrawal, sepsis or intracranial hemorrhage requires particular treatment (Butler et al., 2006).

Colostrum administration

The viability of the calf can be defined by its ability to live and grow with physical, mental energy and endurance (Murray and Leslie, 2013). Low calf viability can result from pain, injury, homeostasis, hypoxia, and inability to protect against acidosis (Besser et al., 1990; Kasari, 1994). These physiological responses may cause behavioral effects such as decreased motivation to achieve natural behaviors for survival, such as survival and postpartum colostrum absorption (Barrier et al., 2013). Not taking enough colostrum shortly after birth can affect the long-term health of the calf and put it at greater risk for disease and mortality (Bellows, 1997). To start thermogenesis in the calf, the colostrum is an excellent source of energy (6.7 Mj / kg). This supplies large amounts of glucose and amino acids and is absorbed from the intestines into the blood within one hour with possible immune globulins (Olson et al. , 1980). For a 40 kg calf, 2 kg of colostrum is an ideal source of energy for 24 hours. Colostrum thermoregulation is crucial for passive immunity (Davicco et al., 1982). It has been reported that hypothermia is usually caused by excessive heat loss due to wetness of newborn calves and heat production due to starvation, thus reducing the risk of hypothermia by taking enough colostrum in the first 2 hours after birth (Drost, 1980).

Glucose injection

Since hypothermia always accompanies hypoglycemia, glucose solutions are used for the treatment of hypothermia. For this purpose, 5-10% glucose solutions at body temperature can be used orally, subcutaneously or intraperitoneally at a dose of 750 mg / kg. It has been reported that parenteral glucose injections would be a suitable method when clinically evaluating hypothermic frostbite heat when glucose measurements are not possible (Stanko et al., 1992).

Adjustment of Conditions of marriage

Dystocia in cows (Barrier et al., 2013), which can result in loss of calf, sometimes loss of cerebrospinal fluid and often late pregnancy, and which impairs thermoregulation ability of the calf (Murray and Leslie, 2013). In many studies conducted in this area, it has been reported that cold temperatures at 0 °C barriers in the ambient temperature fail to fight cold and a significant decrease in rectal temperature is reported (Vermorel et al., 1989; Barrier et al., 2013). It is important that neonatal calves survive and are not affected by diseases such as ambient temperature, conditions of marriage and care and feeding of calves. It has been reported that the hutches used in recent years have made a significant contribution to the prevention of calf losses. The hutches have a vital emphasis in that the placement is not directed at the cold winds and the bottom is often changed to prevent wetness (Torell et al., 1998). The idea that the animal will drink less water in cold weather conditions is very common. The animals of the growers do not drink water because the water they put in front of them is mostly frozen. The increase in consumption of the initial bait, which gives birth to the newborn calves, depends on water consumption. An animal that does not drink water cannot consume food. Studies have also shown that giving cold water 3-4 times a day increases cold water consumption and thus growth (Vermorel et al., 1989; Murray and Leslie, 2013).

Prevention

Keeping up a suitable natural temperature is the most vital advance in anticipating hypothermia in neonatal calves. Untimely neonatal calves that are hypothermic when admitted to the neonatal have expanded bleakness and fatality; elevating the temperature in the giving birth and operating rooms has been found to decrease the occurrence of hypothermia. Consequently, raising room temperature just when giving birth is anticipated may enable warm misfortune to cool surfaces and convective warmth misfortune caused by wind stream, the room ought to be kept up at the prescribed temperature persistently (Bellows, 1997; Butler et al., 2006; Lombard et al., 2007). At the time of birth, neonatal calves ought to be promptly dried and

then swaddled (consisting the head) in a warm cover to counteract evaporative, conductive, and convective misfortunes. For untimely neonatal calves, placement into a polyethylene bag instantly after giving birth has been found to help keep up the calves temperature; a few clinicians do not dry the calves before placing it into the bag because the elevated moistness might be advantageous (Bellows, 1997; Butler et al., 2006; Lombard et al., 2007). As a result, neonatal calves uncovered for resuscitation or observation ought to be put under a warm box to prevent heat misfortunes. Sick neonatal calves should be kept up in a neutral warm ambient to minimize the metabolic rate. The correct incubator temperature varies relying upon the neonatal calf's birth weight and postnatal age, and humidity in the incubator. On the other hand, warming can be balanced with a servomechanism set to keep up skin temperature standard level (Bellows, 1997; Butler et al., 2006).

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Effect of daily milk production levels on plasma calcium, phosphorus and magnesium concentrations in dairy cows

Adis Mukača[1], Muhamed Katica[2]*

1.Veterinary Inspection of the Zenica-Doboj Canton, Bosnia and Herzegovina, Bosnia and Herzegovina
2. University of Sarajevo, Veterinary Faculty, Department of Pathophysiology, Bosnia and Herzegovina.

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ABSTRACT

The aim of this study was to determine the serum level of calcium (Ca), phosphorus (P) and magnesium (Mg) and hence determine the relationship and the effect of daily milk production on the mentioned essential macro elements. The metabolism and neurohumoral regulation of Ca, P and Mg are closely related, and the metabolic disorder of one of these electrolytes inevitably affects the metabolism of the other two. The study was carried out in the winter period of animal keeping and nutrition, and it included 63 Holstein-Friesian breed cows in the northern region of Bosnia and Herzegovina, aged 2-9 years in different lactation stages (1-8). The largest number of cows, eighteen of them, were in the second, third and fourth lactation stage, while nine cows were in the first lactation stage. This study was conducted on three different groups of cows corresponding to the amount of daily milk production. First was the group of lower daily milk production n=21, the second group of examined cows was the group of medium daily milk production n=23 and third was the group of higher daily milk production n=19. Ca, P and Mg were determined in blood plasma using the Beckmann spectrophotometer. By examining the obtained results and the dynamics of the tested mineral substances, we point out the different behaviour of the Ca-P relationship in correlation with the different level of daily milk produced. Although the average values of both minerals are lowered with an increase in daily milk production, the analysis of single linear regression shows that there is a negative correlation between P concentration in blood plasma and the amount of daily milk produced, while for Ca it has not been established. Although the concentration of Mg in the blood plasma increases as the daily milk production increases, the analysis of single linear regression does not show a significant interconnection of these two values. Lower average values of Ca and P concentrations in the blood of cows with higher daily milk production may be associated with increased total excretion of these minerals through milk, unlike the cows which daily produced lower amounts of milk.

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Introduction

Maintenance of dairy cows in high lactation and good health condition are top priorities of modern breeding, whilst the control of nutritional metabolic status is of great importance. The metabolic overload of highly-productive cows is most evident in late gravidity and puerperium. Considering the needs of cows in the last two months of gravidity and in early lactation, especially during the winter period, meals are often not well and rationally balanced, both in terms of nutrient selection and in terms of energy content, such as: proteins, dry matter and cellulose. During the production cycle, the organism of each cow has great demands such as labor and lactogenesis. Both processes require the change and increased metabolism of Ca.

***Corresponding Author:** Muhamed Katica , University of Sarajevo, Veterinary Faculty, Department of Pathophysiology, Bosnia and Herzegovina.
E mail: muhamed.katica@vfs.unsa.ba

After the calving, Ca is directed to the milk gland with previous bone mobilization and enhanced absorption from the digestive system (Bigras-Poulin and Tremblay, 1998) aiming to maintain the physiological level in blood and other tissues (Hays and Swenson, 1993). Concentration of Ca in cow serum during lactation is associated with changes in the concentration of P in serum (Bigras Poulin and Tremblay, 1998). Mg is being excreted in cow milk during the lactation, while the homeostasis depends on the resorption of Mg from the digestive system and the excretion from the kidneys. Therefore, any deviation in the serum concentration of Mg can be quickly detected (Herdt, 2000, Kaneko et al., 2008). These specificities can at the same time disturb the normal flow of physiological processes and influence highly-productive cows to become particularly prone to metabolic disorders.

Metabolism and neurohumoral regulation of P, Ca, and Mg traffic are closely related, and the metabolic disorder of one of these electrolytes inevitably affects the metabolism of the other two. Therefore, the pathophysiological changes associated with these three electrolytes are studied together (Jovanovic, 2012; Zivancevic - Simonovic, 2006; Katica, 2007, Mukaca, 2015).

Puerperal paresis as a serious metabolic disorder implies visible or moderate hypocalcaemia with hypophosphatemia, but the concentration of Mg may be normal, reduced or most frequently increased (Jovanovic, 2012; Hadzimusic and Krnic, 2012). Milk fever is caused by insufficient Ca absorption in the initial part of lactation. The most common cause of this disease is the imbalance in the ratio of P, Mg and Ca. Hypocalcaemia may be the major cause of hypomagnesaemia (Aladrovic et al., 2018; Mukaca, 2015; Katica, 2007). Although there is no strong correlation between clinical signs and Mg in the serum, cows which have a concentration of serum Mg lower than 0.4 mmol / L, also have a higher risk of developing pasture tetanus.

Cutuk (2010) described hematological profile of cows in postpartum phase and shortly after delivery, while Khan et. al. (2002) as well as McNamara et.al. (2003) reported on influence of quality and composition of dairy cow's food on the blood metabolite concentration. Hadzimusic and Krnic (2012) analyzed values of Ca, P and Mg in blood plasma during the summer period of breeding cows in lactation depending on reproductive cycle. However, there is not enough research on the effect of daily milk production on essential plasma minerals profile, especially during winter period.

It is obvious that the relationship between production characteristics of high dairy producing cows (daily milk production) and metabolic capacity of their organism is still very poorly researched; therefore the reason for this kind of research is highly justified. In this regard, the aim of this study was determining the levels of Ca, P i Mg in plasma, and as well determining the link between the daily milk production effect on the mentioned essential macro elements.

Material and Methods

The research was carried out at the farm of Holstein-Friesian breed cows in the northern region of Bosnia and Herzegovina placed in modern and very good zoo-technical conditions. The Radio Frequency ID (RFID) technology was used during the breeding and production in this farm, which implies that each cow owns a chip through which the animal's activities related to reproduction, lactation and history of the diseases is being monitored and recorded.

The study was carried out during the winter period of, and it included 63 cows aged 2-9 years (the largest number of cows was between 3 and 5 years old) in different lactation stages (1-8). The largest number of cows, eighteen of them, were in the second, third and fourth lactation stage, while nine cows were in the first lactation stage. Three groups of cows were formed according to the amount of daily milk produced, based on the control of the amount of daily milk production which was performed during the last fifteen days, prior to the blood sampling:

- Group 1 = Low-level of daily productivity of cows: 20 - 28 L/day of milk (n = 21).
- Group 2 = Medium-level of daily productivity of cows: 29 - 34 L/day of milk (n = 23).
- Group 3 = High level of daily productivity of cows: 35 - 52 L/day of milk (n = 19).

Examined cows (n=63) were unselectively grouped into three groups (group: 1., 2., and 3.), regardless of age or lactation stage. In the winter period, the animals were fed depending on the physiological status and reproductive-production cycle: high milk producing cows were fed with 30 kg of silage, 15 kg of haylage,10

kg of concentrated mixture containing 18-20% of proteins. Cows in dry cow period are fed with 20 kg of silage, 10 kg of haylage and 3-4 kg of concentrate with 18-20% protein. Postpartum cows had meal consisted of silage ad libitum, 2 kg of concentrate mixture, 3 kg of haylage and 0.05 kg of soybean meal. All categories were provided with cattle salt and chalk in the amount of 0.05 kg.

Blood samples were taken in the morning, after cow's morning meal and after milking, by puncturing the coccygeal vein into 5 ml heparinized vacutainers. Blood from each animal was taken into two vacutainers. The blood was then transported to the laboratory while being placed in a handy refrigerator at a temperature of + 4 ° C. Immediately upon arrival at the laboratory, the blood was centrifuged (LC 320, 3000 rpm / 10 min) in order to extract the plasma. Ca (mmol / L), P (mmol / L) and Mg (mmol / L) were determined in blood plasma using the "Beckmann DU-64 UV / VIS" spectrophotometer. For the purpose of analysis were used the commercial kits of the manufacturer "Human", Germany.

Statistical analysis

The results were statistically processed using descriptive statistics. Differences in arithmetic mean were determined by using the ANOVA test. Testing differences in mean values of parameters between individual groups of cows was performed at the significance level of $p < 0.05$. After determining the existence of statistically significant differences in the individual parameters regression analysis was used among the groups of cows (single linear regression). Based on this it is determined which blood parameters are dependent on the amount of milk produced.

Statistical analysis of the results obtained by the research was carried out using the software program SPSS 16.

Results

Concentration of Ca in blood plasma of all 63 examined cows at the farm ranged from 1.900 to 2.560 mmol / L with an average value of 2.103 ± 0.021 mmol / L (Table 1). Comparison of these values with values of 2.2-3.0 mmol / L (Meyer et al., 2003; Jovanovic et al., 1997), 2.1-2.8 mmol / L (Merck Veterinary Manual, 2003), 2.43 - 3.1 mmol / L (Radostits et al., 2000; Kaneko, 2008), 2.0-3.0 mmol / L (Jazbec, 1990), show that these are slightly lower.

Our research confirmed that cows with higher daily milk production have lower concentration levels of Ca in the blood plasma than cows with the lowest milk producing characteristics (Figure 2). However, the intergroup differences are not statistically significant. However, our results show that there is no statistically significant effect of daily milk production on the Ca concentration in blood plasma of cows ($R2 = 0.000$, $p = 0.976 > 0.05$; these indicators can be seen in Table 2 and Figure 2).

Table 1. Results of descriptive statistics for Ca, P and Mg from the blood of the entire sample.

Parameters	NVO	Mean	SE	Med.	SD	Var.	SDA	Min.	Max.
Calcium (mmol/L)	63	2.103	0.021	2.060	0.157	0.025	0.587	1.900	2.560
Magnesium (mmol/L)	63	1.197	0.031	1.185	0.246	0.061	0.315	0.720	1.720
Phosphorus (mmol/L)	63	1.850	0.052	1.795	0.402	0.161	1.018	1.230	3.080

NVO : Numberof valid observations, **SE** : Standart error, **Med**: Median, **SD**: Standart deviation, **Var**: Variance, **SDA**: Skewness-distribution asymmetry. **Min**: Minimum, **Max** : Maximum.

Table 2. Results of regression analysis for determination of Ca, P and Mg values depending on the amount of daily milk produced.

Regression parameters (sum)	Model		ANOVA		NSC (B)	
	R	R^2	F	Sig.	Constant	Production (L/day)
Calcium (mmol/L)	0.004	0.000	0.001	0.976	2.106	-9.83E-02
Magnesium (mmol/L)	0.201	0.041	2.491	0.120	0.954	0.008
Phosphorus (mmol/L)*	0.388	0.150	10.780	0.002	2.586	-0.023

NSC: Non-standardized coefficients (B), *Significantly on the level from 5% ($p < 0.05$)

According to the data of most authors, the concentration of inorganic P in the cattle blood amounts between 1.6 - 2.3 mmol / L), 1.81-1.1 mmol / L (Radostits et al., 2000; Whitaker, 2000; Kaneko, 2008), respectively 1.4-2.5 mmol / L (Merck Veterinary Manual, 2003).

In our study, the determined P concentration in the blood plasma of 63 cows amounted 1.850 ± 0.052 mmol / L within the range from 1.230 to 3.080 mmol / L (Table 1). These variations are slightly higher than the ranges indicated above. When it comes to the concentration of P in the blood plasma of cows with different amounts of daily milk production, our results show that the highest average concentration of P (2.091 ± 0.114 mmol / L) had cows with daily milk production of 20-28 L, and the lowest concentration of P have cows with the highest daily production of milk (1.715 ± 0.066; Figure 4). The P concentration of cows with the lowest daily milk production is significantly higher in comparison to cows with higher amounts of daily milk production ($p < 0.05$) (Table 2). Daily milk production is higher and the concentration of P is lower ($R^2 = 0.150$, $p = 0.002 < 0.05$) (Table 2, Figure 3 and 4).

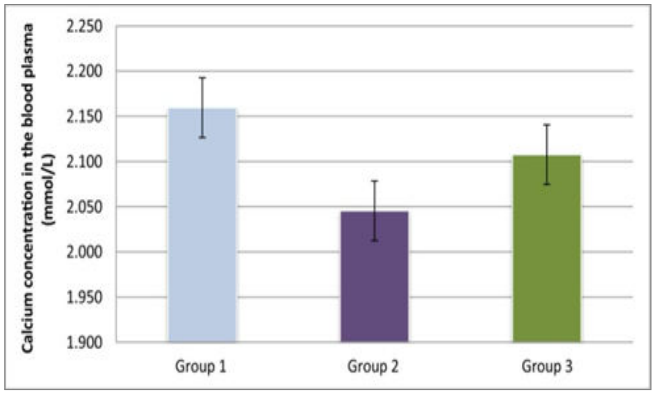

Figure 1. Ca concentration (mmol / L) in blood plasma of cows with different amounts of daily milk produced (Group 1 = 20-28 L; Group 2 = 29-34 L; Group 3 = 35-52 L of milk). All values are represented as $\overline{x} \pm S\overline{x}$

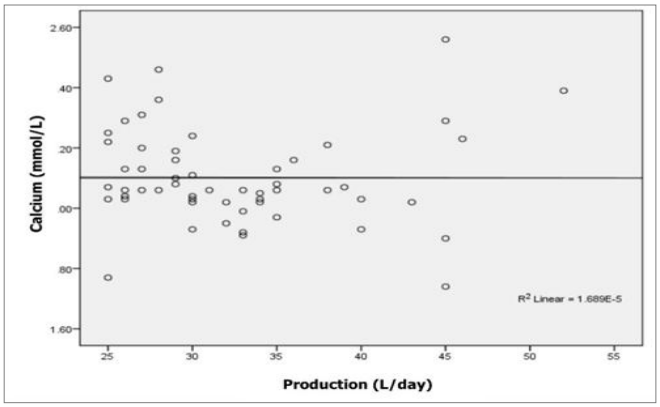

Figure 2. The dependence of Ca concentration (mmol/L) on the amount of daily milk produced.

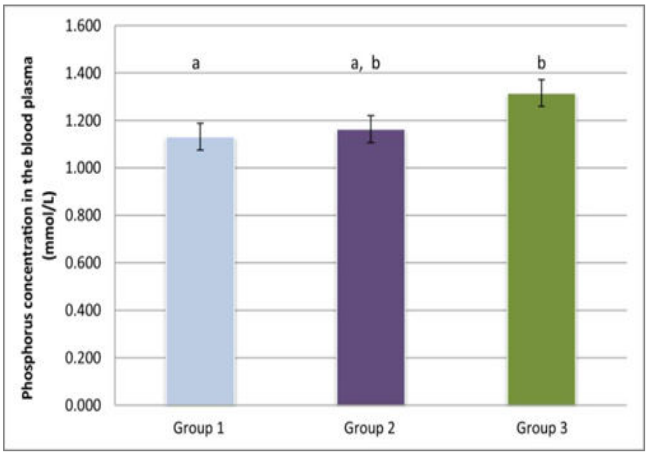

Figure 3. P concentration (mmol/L) in the cow blood plasma with different levels of produced milk (Group 1 = 20-28 L; Group 2 = 29-34 L; Group 3 = 35-52 L of milk). a, b significantly differ $p < 0.05$.

The determined concentrations of Mg in the blood plasma of the examined cows (n = 63) ranged from 0.720 to 1.720 mmol / L with an average value of 1.197 ± 0.031 (Table 1). Values of Mg concentration in our study provide slightly higher contribution than those found in the available literature, whereas the following reference intervals are given: 0.8 - 1.3 mmol / L (Whitaker et al., 2000), 0.7-1, 2 mmol / L (Merck Veterinary Manual, 2003), 0.74-0.95 mmol / L (Radostits et al., 2000; Kaneko, 2008).

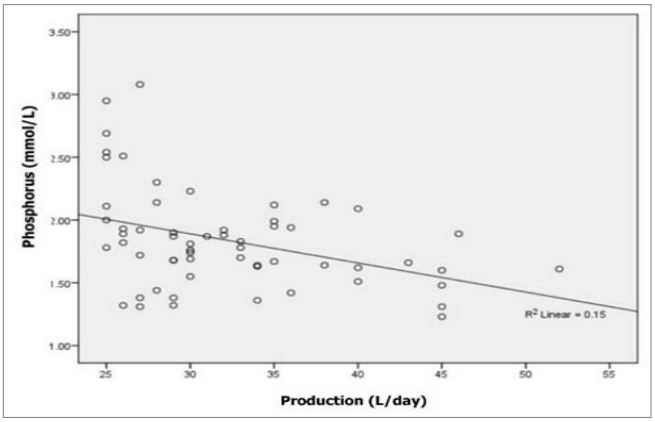

Figure 4. Dependence of P concentration (mmol / L) on the amount of daily milk produced

The lowest concentration value of Mg was found in cows with the lowest daily milk production (1.131 ± 0.063), and the highest in the blood plasma of cows with the highest daily milk production (1.315 ± 0.055), hence this difference was statistically significant ($p<0.05$, Figure 5). The analysis of single linear regression does not show a significant interconnectedness of these two values (R2 = 0.041, $p = 0.120 > 0.05$, Table 2 and Figure 6).

Discussion

In relation to the needs of cows in the last two months of gravidity and in early lactation, especially during the winter period, meals are often not well and rationally balanced in terms of nutrient selection and energy content- protein, dry matter and cellulose (Rajic et al., 1996; Jovanovic et al., 1997). Cow's supply with minerals depends exclusively on their intake through the digestive tract. Sufficient amount per meal does not assure the required amount of the organism. Mineral resorption depends on concentration of the minerals in the digestive system content, solubility, interaction between cations and anions, location of the resorption, loss through kidneys, fetus and milk, as well as the effects of a whole range of other factors, such as hormones and vitamins (Hadzimusic, 2010).

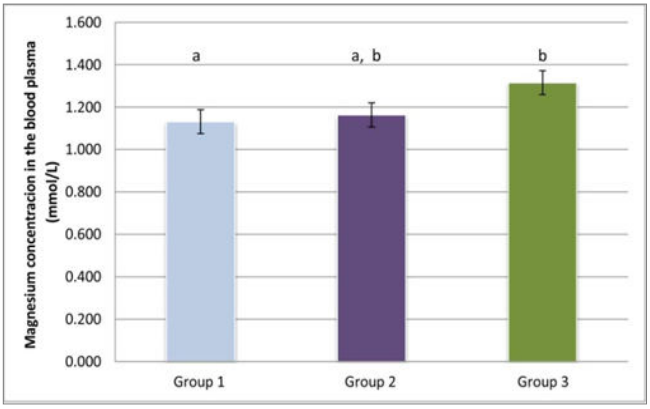

Figure 5. Mg concentration (mmol / L) in blood plasma of cows with different amounts of daily milk produced (Group 1 = 20-28 L; Group 2 = 29-34 L; Group 3 = 35-52 L of milk). a, b statistically significantly differ. P<0.05 .

Changes in the concentrations of some indicators of metabolic process show that the metabolic profile assessment cannot be strictly adhered to the physiological range from the existing literature, but should take into consideration all factors that could in any way affect the obtained results. Therefore for better assessment of the metabolic profile, it is necessary to consider all the anamnestic and clinical data concerning the pathology, nutrition, behaviour of animals, animal keeping conditions and production technology, as well as the knowledge of the physiological values of biochemical parameters of dairy cows (Bouraoui et al., 2002).

It is especially important to perform the determination of these elements in lactation. Namely, at the very beginning of milk secretion the needs of cows for Ca and P are significantly increasing because the milk contains significant amount of these two elements.

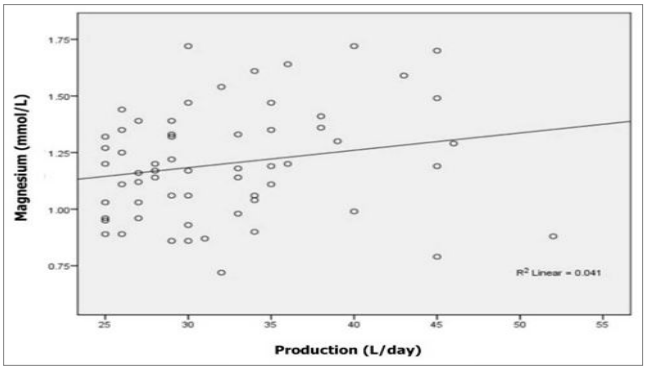

Figure 6. Dependence of Mg concentration (mmol / L) on the amount of daily milk produced.

Underwood (1969) states, that Ca concentration below 2 mmol / L can be considered as a critical value and indicates to hypocalcaemia. In our study only eight cattle had insignificant hypocalcaemia - lower than 2 mmol / L, but none showed clinical manifestations of hypocalcaemia. Olayemi et al. (2001) reported that in 39% of the examined cows was noticed a Ca concentration of less than 2 mmol / L, and Hadžimusic (2010) states that out of total 442 cows, 30.77% of cows had Ca values below 2 mmol / L. However, as examined animals did not show symptoms of hypocalcaemia, a possible reason for slightly lower calcemia of the examined cows could be related to possible errors in blood sampling and laboratory procedures. It is also possible that the lower limit of the reference values of Ca concentration is slightly lower in our geographical area. According to Cvetkovic et al. (1978), the calcemia values of recently calved cows were 1.87 mmol / L. These authors consider that high-milk producing cows normally experience the decrease in the value of calcemia, whilst later, during lactation the values stabilize and range within the physiological limits for cattle, regardless of their production characteristics.

A cow that produces 9.000 kg of milk during a single lactation meanwhile excretes 63.6 kg of minerals from its organism (Jovanovic et al., 2001). Bearing in mind that 1 L of milk contains 1.2 g of Ca means that about 10.8 kg of Ca is excreted during milk lactation (Sjaastad et al., 2003). Consequently, the increase in milk production also increases the secretion of mineral elements from the organism, which is accompanied by a possible decrease in the level of Ca in the blood.

It's obvious that phosphorus and calcium jointly participate in the bone building, these two minerals are often considered together. The effect of calcium and phosphorus ratios on the performances of ruminants is considered to be overstated. Alfaro et al. (1989) showed that the calcium and phosphorus ratios in feed in the range of 1:1 up to 7:1 resulted in the same performances. Phosphorus is indispensable for rumen's microorganisms for their growth and cellular metabolism (Mukaca, 2015; Hadzimusic and Krnic, 2012; Cutuk, 2010). The analysis of single linear regression, which determined that the concentration of P in the blood plasma and the amount of daily milk produced is in the negative correlation. Daily milk production is higher and the concentration of P is lower (Table 2, Figure 3 and 4). This finding can be explained with the fact that during lactation with higher milk production consequently higher amounts of P are being "lost", therefore cows with higher amounts of daily milk production are spending more P. Our results obtained for P showed high accordance with study of Hadzimusic and Krnic, (2012). Because, according to their research; the biggest variations in phosphorus concentrations were determined in the period around calving with the increasing need of lactic glands at the beginning of lactation.

Considering the obtained results and the dynamics of the examined mineral matter, outlined is the different behavior of the relation between Ca and P in correlation with the different amount of daily milk produced. Although the average values of both minerals are decreasing with an increase in daily milk production, however, the analysis of single linear regression indicates a negative correlation between the concentration of P in blood plasma and the amount of daily milk produced, whilst for Ca it has not been identified. A possible explanation for this occurrence might be due to increase in the amount of excreted Ca through colostrum and milk, which consequently creates a tendency towards lowering the Ca, which in return stimulates the increased secretion of the parathyroid hormone. Parathormone leads to increased mobilization of Ca and P from the bones, hence encouraging the Ca reabsorption from primary urine, and increases the elimination of P through urine.

In many respects Mg metabolism is specific in relation to other microelements. Since it is considered that the level of Mg in the blood is more a reflection of its intake through food and not necessarily its body reserves (Whitaker et al., 2000), therefore it is assumed that the animals examined in our research have received different amounts of this element through food intake. However, the literature allegations treat as hypomagnesaemia conditions in which the level of Mg is less than 0.7 mmol / L, while the symptoms of hypomagnesaemia manifest when its value drops below 0.4 mmol / L (González, 2000).

Although it is noticed that concentration of Mg in the blood plasma increases as the daily milk production increases, however, the analysis of single linear regression does not show a significant interconnectedness of these two values ($R2 = 0.041$, $p = 0.120 > 0.05$). Our results obtained for Mg were slightly different than similar study of Cutuk (2010).

Conclusion

The values of the Ca, P and Mg concentration in the blood of the examined cows varied within the physiological limits which is a result of satisfactory mineral ingestion through food and mineral-vitamin supplements, but also well-coordinated neurohumoral mechanisms which control the transport of these minerals into the organism. Lower average values of Ca and P concentrations in the blood of cows with higher daily milk production may be associated with increased total excretion of these minerals through milk, unlike the cows which daily produced lower amounts of milk. On the other side, concentration of Mg in the blood plasma increases as the daily milk production increases, may be associated. Therefore it is assumed that the animals examined in our research have received sufficient amounts of this element through food intake. In order to clarify the conditions which bring about the above indicated changes we suggest to carry out our future research under the controlled conditions, including a dry period, age of cows within the lactation stage.

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