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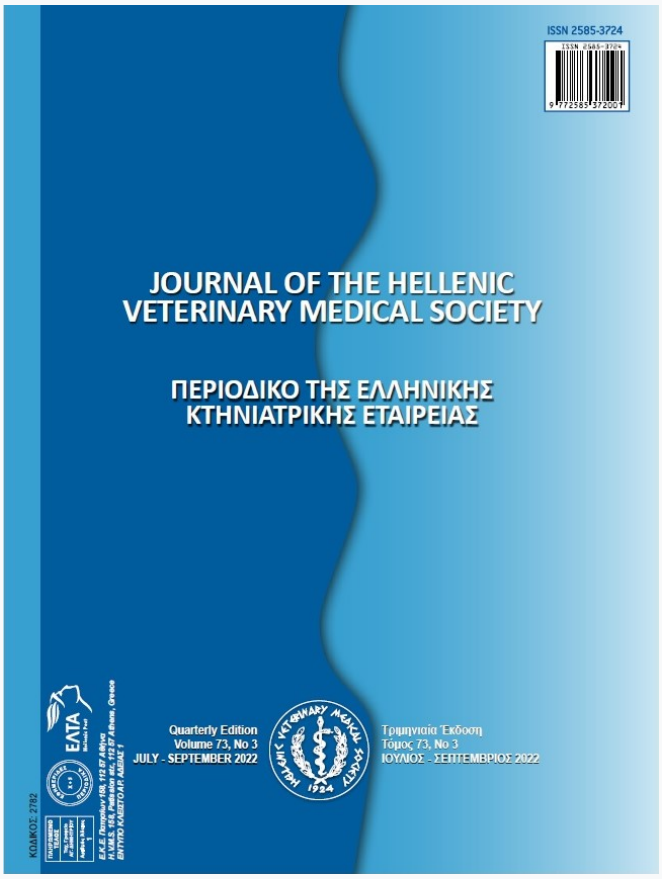

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Evaluation of total sialic acid, paraoxonase activity, and malondialdehyde in cows with subclinical paratuberculosis

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ABSTRACT: This study was aimed to evaluate total sialic acid (TSA), paraoxonase activity (PON1), malondialdehyde (MDA), and some serum biochemical parameters in cows with subclinical paratuberculosis. Paratuberculosis (PT) test was performed on 500 cows aged 2-6 years by ELISA method. Twenty-four cows diagnosed with paratuberculosis constituted the PT group and 12 healthy cows constituted the control group. According to the results, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and urea levels were found to be statistically higher ($p<0.05$) in the group with paratuberculosis compared to the control group. Iron (Fe) levels were found to be lower than the control group ($p<0.001$). Compared to the control group, there was a decrease in PON1 ($p<0.001$) activity and a significant increase in TSA ($p<0.001$) and MDA ($p=0.002$) levels in the PT group. In conclusion, in subclinical paratuberculosis infection, the long incubation period and the absence of clinical findings until advanced stages constitute a handicap. However, our findings show that the determination of lipid peroxidation (MDA), PON1 activity, and TSA concentration caused by the inflammatory and oxidative stress state caused by the infection can be used as new biomarkers for this infection.

Keywords: Paratuberculosis, Malondialdehyde, Total sialic acid, Paraoxonase activity

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INTRODUCTION

Paratuberculosis (PT) can be seen in many parts of the world. The disease is known as Johne's or Hohnesche Krankheit disease. The causative agent of the disease is *Mycobacterium avium subsp. paratuberculosis*. PT is an infectious disease that causes economic losses due to a decrease in meat and milk yield and a decrease in reproductive efficiency, as well as causing chronic enteritis. Although the disease causes severe cachexia, it is also important as a zoonosis (Makav and Gökçe, 2013; Li et al., 2017; Davis and Park, 2018; Karatay et al., 2020).Although the disease is mostly seen in older ages, it generally occurs between the ages of 2-6. The disease can also be seen in calves at an earlier age (Karakaş and Civelek, 2018). Because the incubation period of the disease is quite long, infected animals may start to spread bacteria with feces 15-18 months before clinical symptoms are observed(Mecitoğlu and Demir, 2012). In the subclinical period, variations occur in the antigen response to paratuberculosis and the immune response due to increased gamma interferon levels (Strickland et al., 2005). Paratuberculosis causes serious productivity losses in animals. In addition, its role in the etiology of Crohn's disease makes paratuberculosis important (Çetinkaya et al., 1997; Diéguez et al., 2009; Osterstock et al., 2010; Karatay et al., 2020).

Sialic acids, which are derivatives of N-acetyl neuraminic acid, are found in the structure of macromolecules and receptors as terminal carbohydrate residues of the oligosaccharide side chain of polysaccharides, glycoproteins, and mucoproteins (Varki and Varki, 2007; Uzlu et al., 2010). Determination of total sialic acid (TSA) is indicated to be a valuable marker for the diagnosis and prognosis of inflammatory diseases (Uzlu et al., 2010; Deveci et al., 2017). As a response to an inflammatory stimulus, free radicals or reactive oxygen species (ROS) are released from dendritic cells, neutrophils, and macrophages (Kostadinović et al., 2016).The produced ROS should be sustained in balance with the antioxidant system in the cell. An increase in ROS levels in cells can cause oxidative stress, which has an important role in the pathophysiology of diseases (Kara et al., 2016; Kükürt et al., 2020;Kostadinović and Lević, 2018.

Paraoxonase enzyme activity (PON1)is an antioxidant enzyme found in the serum, liver, kidney, and intestine (Kuru et al., 2020). PON1 coexists with high-density lipoprotein in plasma and plays a role in preventing oxidation of the plasma lipoproteins. Because peroxidized lipids are metabolized by this enzyme, the accumulation of lipid peroxides in both high-density lipoprotein and low-density lipoprotein is inhibited(Deveci et al., 2017). Malondialdehyde (MDA), which is the result of non-enzymatic oxidative lipid peroxide degradation, shows toxic effects by binding to nucleic acids, phospholipids and amino groups of proteins (Puvača et al., 2015). It is measured as an indicator of lipid peroxidation in oxidative stress (Cenesiz et al., 2016). MDA is a lipid peroxidation product and is a biomarker that generally indicates oxidative stress. In cases where oxidative stress increases, its level increases (Aguiar et al., 2012; Çevik et al., 2012; Giera et al., 2012).

It has been reported that there may be changes in PON1(Kuru et al., 2020), TSA, and MDA levels as a result of inflammation caused by the existing infection (Uzlu et al., 2010; Deveci et al., 2017). Although ELISA is the most commonly used method for subclinical paratuberculosis diagnosis, this method has disadvantages (Duran and Çenesiz, 2019). Because, in subclinical paratuberculosis infection, the long incubation period and the absence of clinical findings until advanced stages constitute a handicap. ELISA has a low (about 45%) sensitivity for subclinical paratuberculosis (Collins, 1996). Therefore, alternative methods are sought to increase the accuracy of the diagnosis. In this context, it was aimed to determine total sialic acid, paraoxonase activity, malondialdehyde, and some serum biochemical parameters in cows with subclinical paratuberculosis.

MATERIALS AND METHODS

Animals

The presented study was carried out after the approval of the Kafkas University Animal Experiments Local Ethics Committee (KAÜ-HADYEK/2020-099). For the study, 500 clinically healthy cows (2-6 years old, middle lactation, semi-intensive fattening, under the same care and feeding conditions, Simmental cows), whose basic physical examination findings (rectal temperature, breathing per minute, and pulse rate) were within normal limits, were selected in Kars province. ELISA test was performed from blood samples collected. Twenty-four animals with positive ELISA test constituted the subclinical paratuberculosis group and 12 animals with negative ELISA test constituted the control group.

Blood samples taken from the jugular vein were kept at room temperature for about 1 hour and then

centrifuged at 3000 rpm for 10 minutes (Hettich Rotina 380R®, Hettich, Germany) to obtain serum samples. Serum samples were stored at -20 °C until analysis.

Diagnosis of Paratuberculosis

The serum samples were tested with paratuberculosis using a commercial antibody ELISA kit (IDEXX, Montpellier SAS, France) at 450 nm wavelength by a spectrophotometer (PowerWave XS, BioTek, USA). The percentage results for the obtained OD values were calculated with the following formula:

$$\text{Result (\%)} = (OD_{samples} - OD_{negative}) / (OD_{positive} - OD_{negative})$$

According to the formula above, from the obtained results; 55% and above are considered positive, 45-55% are considered as suspected and 45% and below are considered negative.

Biochemical analysis

Serum TSA levels were measured colorimetrically using a spectrophotometer (Power Wave XS®, BioTek, USA) according to the method described by Sydow (1985), the results were presented in mg/dL. The MDA concentration was determined according to the method reported by Yoshioka et al.(1979). The resulting MDA forms a pink-coloured complex with thiobarbituric acid (TBA) and the absorbance of this solution is measured spectrophotometrically at 535 nm (PowerWawe XS®, Biotek, USA) to determine the degree of lipid peroxidation. MDA levels were calculated as µmol/L.PON1 activity was determined by measuring the absorbance of the colored composite caused by 4-nitrophenol (an enzymatic product of paraoxon-ethyl (Sigma®, London, UK) at 412 nanometers with a spectrophotometer (PowerWawe XS®, Biotek, USA) and the results were recorded as U/L(Eckerson et al., 1983). Serum Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), creatinine (CREA), UREA, total protein (TP), lactate dehydrogenase (LDH) and iron (Fe) levels were determined with a commercial kit by an automated analyzer(Mindray BS120®, Mindray Medical International Limited Istanbul, Turkey).

Statistical analysis

Biochemical parameter values in the study were given as mean ± standard deviation (SD). Since the groups (control and paratuberculosis) showed normal distribution according to the Shapiro-Wilk test, they were compared with the independent samples t-test. SPSS® (SPSS Version 18.0, Chicago, IL, USA) program was used for all statistical analyses. In terms of parameters examined, differences between groups were considered significant at the $p<0.05$ level.

RESULTS

According to the data, when being statistically compared with the control group, it was determined that there was a significant decrease ($p<0.001$) in PON1 in the PT group. However, it was determined that there was a significant increase in TSA ($p<0.001$) and MDA ($p=0.002$) levels (Table 1).

In the PT group, a significant increase was found in ALT ($p<0.001$), AST ($p=0.002$), and UREA ($p<0.001$) according to the results obtained from the analyses. A significant decrease was observed in Fe ($p<0.001$). CREA, GGT, TP and LDH concentrations were not statistically significant between groups ($p>0.05$ Table 2).

Table 1. PON activities, TSA and MDA levels of the control and PT groups.

Parameters	Groups	N	Mean value ± SD	P value
PON1 (U/L)	Control	12	41.23 ± 5.47	<0.001
	PT	24	21.83 ± 3.74	
TSA (mg/dL)	Control	12	43.38 ± 3.05	<0.001
	PT	24	58.72 ± 3.42	
MDA (µmol/L)	Control	12	3.64 ± 0.81	0.002
	PT	24	7.53 ± 0.59	

Notes: PON1: paraoxonase activity, MDA: malondialdehyde, TSA: total sialic acid, PT: paratuberculosis group, control: healthy group, N: number of animals in the group.

Table 2. Some biochemical parameters levels of the control and PT groups.

Parameters	Groups	N	Mean value ± SD	P value
ALT (U/L)	Control	12	15.73 ± 0.37	<0.001
	PT	24	25.36 ± 0.63	
AST (U/L)	Control	12	45.72 ± 1.27	0.002
	PT	24	83.61 ± 3.87	
GGT (U/L)	Control	12	13.16 ± 0.49	NS
	PT	24	14.25 ± 0.72	
CREA (mg/dL)	Control	12	1.27 ± 0.04	NS
	PT	24	0.98 ± 0.07	
UREA (mg/dL)	Control	12	283.1 ± 4.27	<0.001
	PT	24	835.2 ± 6.32	
TP (g/dL)	Control	12	7.62 ± 0.23	NS
	PT	24	8.11 ± 0.34	
LDH (U/L)	Control	12	643.58 ± 19.02	NS
	PT	24	697.71 ± 12.55	
Fe (µmol/L)	Control	12	47.38 ± 2.72	<0.001
	PT	24	21.17 ± 1.62	

Notes: NS: not significant, ALT: alanine aminotransferase, AST: aspartate aminotransferase, CREA: creatinine, TP: total protein, LDH: lactate dehydrogenase, Fe: iron, PT: paratuberculosis group, control: healthy group, N: number of animals in the group.

DISCUSSION

Reactive oxygen species (ROS) are required for the defense system against pathogenic microorganisms. Macrophages and neutrophils generate large amounts of ROS as a result of oxidation. (Kuru et al., 2018a, 2020).Free radicals, which are the most important products of antimicrobial activity in the host, are difficult to measure due to their short life and high reactivity. Therefore, methods are used by which the end products of various reactions are measured. MDA, which is an indicator of lipid peroxidation, is the most common of these (Valko et al., 2007). *Mycobacterium paratuberculosis* sustains its life in macrophages after passing the intestinal barrier. There, they come across reactive oxygen and nitrogen species in cellular defense (Ehrt and Schnappinger, 2009). An antioxidant-oxidant defense mechanism develops between the host and the bacteria. With this aim, we determined the serum MDA concentration to evaluate lipid peroxidation and observed a significant increase ($p<0.05$) when compared with the control group. These findingsare consistent with the results of the studies performed (Cenesiz et al., 2016; Qasem, 2016). The reason for its high in our study was attributed to the increase in oxidative stress associated with the disease.

Paraoxonase is an endogenous antioxidant produced by liver cells. It has protective properties against lipid peroxidation caused by free radicals on cell surfaces (Aguiar et al., 2012; Çevik et al., 2012; Giera et al., 2012; Deveci et al., 2017). In the PT group, PON1 activity was found to be lower compared to the control group. The decrease in PON1 activity suggested that it was the result of liver function loss. The increase in ALT and AST levels may indicate that the disease may cause hepatocyte destruction. The decrease in PON1 activity also suggested that it was the result of hepatocyte destruction.

Sialic acid is an acute-phase protein that forms the terminal sugar of carbohydrates in the glycoprotein structure (Karapehlivan et al., 2007). It has been suggested that sialic acid is an antioxidative agent responsible for removing oxygen from the vascular system in living organisms. It has also been reported that oxidative stress can initiate the release of sialic acid from oligosaccharides on the cell surface without sialidase activity or induction (Eguchi et al., 2005).In the presented study, it was found that TSA levels differed significantly compared to the control group. These results confirm that sialic acids are important during paratuberculosis-induced stress. It has been reported that serum levels of TSA are increased in inflammatory conditions or such conditions (Uzlu et al., 2010). In our study, it was concluded that the high TSA level of the PT group may be the result of infection-induced inflammation.

Malondialdehyde is a lipid peroxidation product and is a biological biomarker that generally indicates

oxidative stress. In cases where oxidative stress increases, its level increases (Aguiar et al., 2012; Çevik et al., 2012; Giera et al., 2012; Kuru et al., 2018b). In a study, MDA levels of the PT group were found to be statistically higher than the control group (Qasem, 2016). In bacterial infections or inflammation, oxidative stress has been reported in different studies (Akyüz et al., 2017; Kuru et al., 2018a, 2018b; Kükürt et al., 2020). It is thought that the high level of MDA in the presented study following the literature may be due to the increase in oxidative stress with infection.

In a study, it was reported that the PT group had higher ALT levels and lower AST levels compared to the control group (Abdelaal et al., 2019). ALT and AST enzyme levels may increase in liver damage (Cebra et al, 1997; Stojević et al, 2005; Akyüz and Gokce, 2021). In our study, ALT and AST levels were found to be higher than in the control group. It was thought that the reason for this might be liver function loss due to infection. The high ALT level in both studies compared to the control group was found to be consistent with the literature. The amount of UREA in the blood may vary depending on the type of protein in the diet, and kidney and liver function. UREA is converted into ammonia in the liver(Sosa et al., 2010). In the presented study, we think that the reason for high UREA in the PT group might be a malfunction in conversion to ammonia as a result of impaired liver function, and therefore its level increased. No statistical significance could be determined for other parameters in serum biochemistry.

CONCLUSION

As a result, a decrease in PON1 activity and an increase in MDA, TSA, ALT, AST, and UREA concentrations were determined with PT, which may show a hidden course despite the absence of any clinical findings. For the diagnosis of PT, alternative methods are sought to increase the accuracy of the diagnosis. In conclusion, in subclinical PT infection, the long incubation period and the absence of clinical findings until advanced stages constitute a handicap. However, our findings show that the determination of lipid peroxidation, PON1 activity, and TSA concentration caused by the inflammatory and oxidative stress state caused by the infection can be used as new biomarkers for this infection. Also, these data may cause a loss of yield by revealing the harmful effects of inflammation in the long term. Besides, we think that the data obtained from the presented study will contribute to new studies in this field.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Abdelaal AM, Elgioushy MM, Gouda SM, El-Adl MM, Hashish, EA, Elgaml SA, Ali MO and El-Gedawy AA (2019) Hemato-biochemical and molecular markers (IS900) of cattle infected with Johne's disease in Egypt. Slovenian Veterinary Research, 56, 421-431.

Aguiar CCT, Almeida AB, Arajo PVP, Abreu RNDC De, Chaves EMC, Vale OCD, MacÊdo DS, Woods DJ, Fonteles MMDF and Vasconcelos SMM (2012) Oxidative stress and epilepsy: Literature review. Oxidative Medicine and Cellular Longevity, 2012, 795259. doi:10.1155/2012/795259.

Akyüz E, Gökce G (2021). Neopterin, procalcitonin, clinical biochemistry, and hematology in calves with neonatal sepsis. Trop Anim Health Prod, 53 (3), 354.doi: 10.36483/vanvetj.1066050

Akyüz E, Kuru M, Kükürt A, Erkılıç EE, Uzlu E, Kırmızıgül AH, Gökçe G. and Karapehlivan M (2017) The determination of oxidative stress index of enteritis caused by *Escherichia coli K99* strain in simmental new-born calves, I. International Turkish Veterinary Internal Medicine Congress, Antalya, Turkey.

Cebra CK, Gerry FB, Getzy DM, Fettman MJ (1997) Hepatic lipidosis in anorectic, lactating holstein cattle: retrospective study of serum biochemical abnormalities. J. Vet. Int. Med. 4, 231-237.

Cenesiz M, Ciftci G, Dalgin D, Kilic Y, Yarim GF and Cenesiz S (2016) Evaluation of oxidant and antioxidant capacity in paratuberculosis positive cattle. Pakistan J. Zool, 48, 1603-1606.

Çetinkaya B, Erdoğan H and Morgan K (1997) Risk factors for bovine paratuberculosis II. the multiple analysis of risk ractors for bovine paratuberculosis. Turkish Journal of Veterinary and Animal Sciences, 21, 303-306.

Çevik M, Varol S, Yücel Y, Akıl E, Çelepkolu T, Arıkanoğlu A, Yüksel H and Aluçlu M (2012) Serum paraoxonase-1 activities and malondialdehyde levels in patients with epilepsy. Dicle Med J, 39, 557-560.

Collins MT (1996) Diagnosis of paratuberculosis. Vet Clin North Am Food AnimPract, 12, 357-371.

Davis WC and Park KT (2018) Progress towards control of a mycobacterial pathogen, Mycobacterium aviumsubsp. paratuberculosis, the causative agent of Johne's Disease in cattle and humans. Journal of Food Hygiene and Safety, 33, 221-228.

Deveci HA, Kuru M, Kükürt A, Makav M and Karapehlivan M (2017) Determination of serum paraoxonase activity, total sialic acid concentration, and oxidative status in cattle with clinical mastitis. International Journal of Veterinary Science, 6, 136-140.

Diéguez FJ, González AM, Menéndez S, Vilar MJ, Sanjuán ML, Yus, E and Arnaiz I (2009) Evaluation of four commercial serum ELISAs for detection of Mycobacterium avium subsp. paratuberculosis infection in dairy cows. The Veterinary Journal, 180, 231-235.

Duran U and Çenesiz S (2019) Determination of acute phase protein levels in cattle with paratuberculosis. International Journal of Advances in Science, Engineering and Technology, 7, 46-49.

Eckerson HW, Romson J, Wyte C and La Du BN (1983) The human serum paraoxonase polymorphism: identification of phenotypes by their response to salts. American Journal of Human Genetics, 35, 214-227.

Eguchi H, Ikeda Y, Ookawara T, Koyota S, Fujiwara N, Honke K, Wang PG, Taniguchi N and Suzuki K (2005) Modification of oligosaccharides by reactive oxygen species decreases sialyllewis x-mediated cell adhesion. Glycobiology, 15, 1094-1101.

Ehrt S and Schnappinger D (2009) Mycobacterial survival strategies in the phagosome: Defence against host stresses. Cellular Microbiology, 11, 1170-1178.

Giera M, Lingeman H and Niessen WMA (2012) Recent advancements in the LC- and GC-based analysis of malondialdehyde (MDA): A brief overview. Chromatographia, 75, 433-440.

Kara A, Gedikli S, Sengul E, Gelen V and Ozkanlar S (2016) Oxidative stress and autophagy. In:, R. Ahmad (ed), Free Radicals and Diseases, (InTech), London, 37-55.

Karakaş B and Civelek T (2018) Seroprevalence of subclinical paratuberculosis in dairy cattle in Kırklareli Region. Kocatepe Veterinary Journal, 11, 160-164.

Karapehlivan M, Atakisi E, Citil M, Kankavi O and Atakisi O (2007) Serum sialic acid levels in calves with pneumonia. Veterinary Research Communications, 31, 37-41.

Karatay M, Akyüz E and Gökce G (2020) Seroprevalence of paratuberculosis in cattle in Ardahan region. Kocatepe Veterinary Journal, 13, 327-331.

Kostadinović LM, Popović SJ, Puvača NM, Čabarkapa IS, Kormanjoš SM, and Lević JD (2016) Influence of *Artemisia absinthium* essential oil on antioxidative system of broilers experimentally infected with Eimeria oocysts. VeterinarskiArhiv, 86 (2), 253-264.

Kostadinović J and Lević J (2018) Effects of phytoadditives in poultry and pigs diseases. Journal of Agronomy, Technology and Engineering Management, 1(1), 1-7.

Kükürt A, Kuru M and Karapehlivan M (2020) Nitrik oksit, nitrik oksit sentaz ve dişi üreme sistemindeki rolleri. In:, C. Everekliоğlu (ed), Sağlık Bilimleri Alanında Akademik Çalışmalar - II, (GeceKitaplığı), 113-123.

Kuru M, Kükürt A, Akyüz E, Oral H, Kulaksız R and Karapehlivan M (2020) paraoxonase activities, total sialic acid concentration and lipid profile after use of controlled internal drug release (CIDR) in Gurcu Goats. International Journal of Veterinary Science, 9, 517-522.

Kuru M, Kükürt A, Oral H and Öğün M (2018a) Clinical use of progesterone andits relation to oxidative stress in ruminants. in: Sex Hormones in Neurodegenerative Processes and Diseases. (InTech), London, 13; 303-314.

Kuru M, Ögün M, Kulaksız R, Kükürt A and Oral H (2018b) Comparison of oxidative/nitrosative stress, leptin and progesterone concentrations in pregnant and nonpregnant Abaza goats synchronized with controlled internal drug release application. KafkasUniv Vet FakDerg, 24, 287-292.

Li L, Bannantine JP, Campo JJ, Randall A, Grohn YT, Katani R, Schilling M, Radzio-Basu J and Kapur V (2017) Identification of sero-reactive antigens for the early diagnosis of Johne's disease in cattle. PLoS One, 12.

Makav M and Gökçe E (2013) Seroprevalence of subclinical paratuberculosis in cattle in Kars region.Kafkas Univ. Vet. Fak. Derg, 19, 913-916.

Mecitoğlu Z and Demir G (2012) Problems in diagnosis of paratuberculosis in cattle. Uludag Univ. J. Fac. Vet. Med., 31, 19-23.

Osterstock JB, Sinha S, Seabury CM and Cohen ND (2010) Effect of classifying disease states in genetic association studies for paratuberculosis. Preventive Veterinary Medicine, 95, 41-49.

Qasem A (2016) Malondialdehyde (MDA) and glutathione peroxidase (GPx) are elevated in Crohns Disease-Associated with Mycobacterium Avium Subspecies Paratuberculosis (MAP), University of Central Florida, Masters Thesis, Orlando.

Puvača N, KostadinovićLj, Popović S, Lević J, Ljubojević D, Tufarelli V, Jovanović R, Tasić T, Ikonić P, Lukač D (2015) Proximate composition, cholesterol concentration and lipid oxidation of meat from chickens fed dietary spice addition (Allium sativum, Piper nigrum, Capsicum annuum). Animal Production Science, 56, 1920-1927.

Sosa I, Leytona L, Coreaa E and Elizondo-Salazar J (2010) The Correlation between milk and blood UREA nitrogen in high and low yielding dairy cows. in: Sustainable İmprovement of Animal Production And Health. Food and Agriculture Organization of the United Nations, Rome.

Stojević Z, Piršljin J, Milinković-Tur S, Zdelar-Tuk M, Ljubić BB (2005) Activities of AST, ALT and GGT in clinically healthy dairy cows during lactation and in the dry period. Veterinarskiarhiv, 75, 67-73.

Strickland SJ, Scott HM, Libal MC, Roussel AJ and Jordan ER (2005) Effects of seasonal climatic conditions on the diagnosis of mycobacterium avium subspecies paratuberculosis in dairy cattle. Journal of Dairy Science, 88, 2432-2440.

Sydow G (1985) A simplified quick method for determination of sialic acid in serum. BiomedicaBiochimicaActa, 44, 1721-1723.

Uzlu E, Karapehlivan M, Çitil M, Gökçe E and Erdoğan H (2010) Investigation of serum sialic acid and some biochemical parameters in calf with diarrhea symptoms. YYU VeterinerFakultesiDergisi, 21, 83-86.

Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M and Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol, 39;(1):44-84.

Varki NM and Varki A (2007) Diversity in cell surface sialic acid presentations: Implications for biology and disease. Laboratory Investigation, 87, 851-857.

Yoshioka T, Kawada K, Shimada T and Mori M (1979) Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. American Journal of Obstetrics and Gynecology, 135, 372-376.