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JIVS JOURNAL OF ISTANBUL VETERINARY SCIENCES

Effects of dietary fat source, breed and vitamin E level on lipid oxidation of sheep muscles

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Research Article

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ABSTRACT

The effects of diet and levels of dietary vitamin E on lipid oxidation were assessed in lambs in this study. Groups of Suffolk x Lleyn and Scottish Blackface male lambs were fed dietary lipid supplements containing either Megalac (C16:0), or one of two sources of n-3 PUFA: linseed which has a high content of C18:3 n-3, which had been treated with formaldehyde to aid rumen bypass and a mixture of formaldehyde treated linseed plus fish oil to provide EPA and DHA. The diets were based on dried grass had similar levels of fat (60g/kg DM). Vitamin E was included as α-tocopherol acetate at 100 and 500 mg/kg, for the low and high vitamin E diets, respectively. The six dietary treatments were: Megalac with low vitamin E, (ML); Megalac with high vitamin E, (MH); Protected linseed with low vitamin E, (LL); Protected linseed with high vitamin E, (LH); Protected linseed plus fish oil (linfish) with low vitamin E, (LFL); Protected linseed plus fish oil mixture (linfish) with high vitamin E, (LFH). At approximately half of the mature live weight for each breed, animals were slaughtered. This was on average 46 kg for the Suffolk and 36 kg for the Scottish Blackface. The meat from supplemented animals increased susceptibility to lipid oxidation in high PUFA in meat resulted from poor deposition of dietary vitamin E supplements.

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Introduction

The nutritive quality of ruminant meat and its acceptability by consumers would be greatly enhanced if the proportion of unsaturated fatty acids were high and meat were stable to oxidation. Dietary polyunsaturated fatty acids (PUFA) increase membrane unsaturation, as demonstrated in ruminants fed protected fats and consequently increases the susceptibility of meat to oxidation (Wood and Enser, 1997). In addition to the known roles of vitamin E as a nutrient serving in metabolism and reproduction, its role in product quality has been recognised. The role of supranutritional dietary levels of vitamin E for ruminants to maintain fresh beef colour and to elevate milk tocopherol which reduces lipid oxidation and resulting undesirable milk flavours, has been demonstrated (Arnold et al., 1993; Chauhan et al., 2014).

Wulf et al. (1995) reported that feeding sheep 500 mg α-tocopherol per day improved lipid stability and colour shelf life for up to four days, when the tissue levels of vitamin E were in excess of 5.5 mg/kg.

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However, one of the most important factors which alter the effectiveness of vitamin E is the dietary intake of PUFA. Consumption of n-3 fatty acids reduces vitamin E concentrations in the blood and tissues more than does n-6 PUFA (Meydani et al., 1987; Raederstorff et al., 2015).

The aim of the study was to examine the effects of supplementation of diets with n-3 PUFA, two levels of vitamin E and breed on muscle vitamin E content, lipid oxidation.

Materials and methods

48 Suffolk x Lleyn and 48 Scottish Blackface male lambs with initial live weights of 24 and 18 (± 0.3) kg, respectively, were used in this study. All diets were based on dry grass and formulated to be iso-energetic and iso-nitrogenic and to provide a similar fat level (60 g/kg) from different fat sources (Megalac, formaldehyde treated linseed and linfish). The control diet contained Megalac which is high in palmitic acid (C16:0), a saturated fatty acid. The second diet, contained formaldehyde treated whole linseed which is high in α-linolenic acid and the third diet contained a mixture of equal quantities of linseed and fish oil which supplied performed long chain n-3 PUFA, EPA and DHA. Vitamin E was included as a-tocopherol acetate (Roche Products Limited) at 100 and 500 mg/ kg, for the low and high vitamin E diets, respectively. The six dietary treatments were: Megalac with low vitamin E, (ML); Megalac with high vitamin E, (MH); Protected linseed with low vitamin E, (LL); Protected linseed with high vitamin E, (LH); Protected linseed plus fish oil with low vitamin E, (LFL); Protected linseed plus fish oil mixture with high vitamin E, (LFH). All the performance data and feed analysis procedures were published before (Demirel et al., 2004).

Lambs were individually housed in raised floor pens and gradually adapted to a mixed diet containing equal quantities on a weight basis of the three diets. After two weeks on the adaptation diet, all lambs were offered their respective dietary treatments. Feed was offered *ad libitum*. At approximately half of the mature live weight for each breed, animals were slaughtered. This was on average 46 kg for the Suffolk and 36 kg for the Scottish Blackface. From twelve hours before slaughter animals had access to water but not to feed to minimise contamination during slaughter. After slaughter, carcasses were chilled at 1^{o}C for 24 hours. Semimembranosus (SM) and Semitendinosus (ST) were cut out and vacuum packed and aged for 6 or 10 days at 1^{o}C. After 6 or 10 day of ageing, the leg steaks were repackaged in over-wrapped (OW) oxygen permeable film and 2 packs were then overpacked in a modified atmosphere (MAP) (75% O_2, 25 % CO_2). Samples were displayed under simulated retail conditions at 1000 lux illumination and 4 ± 1^{o}C for 5 days. On the last day of display SM and ST muscles were cut out and lipid oxidation determined as thiobarbituric reactivity substances (TBARS) (Vyncke, 1975).

Table 1. Diet composition

Fat source	Megalac		Linseed		Linfish	
Vitamin E Level	Low	High	Low	High	Low	High
Ingredients (g per kg)						
Dried grass	759	759	739	739	754	754
MSBP	105	105	105	105	105	105
Megalac	35	35	-	-	-	
Fish oil	-	-	-	-	15	15
Whole linseed	-	-	85	85	42	42
Soya bean meal	46	46	16	16	29	29
Molasses	25	25	25	25	25	25
Mineral and vitamin premix	20	20	20	20	20	20
Ammonium chloride	5	5	5	5	5	5
Salt	5	5	5	5	5	5
Vitamin E mg/kg	100	500	100	500	100	500

Key: MSBP Sugar beet pulp with molasses

Results and Discussion

Lipid oxidation after 6 and 10 days of ageing and 5 days of display was evaluated with the TBA-test. Statistical analysis showed that there is no significant difference between 6 and 10 days ageing of the muscles. Mean values in m.semitendinosus (ST) were 4.4 and 4.9 and in m.semimembranosus (SM) 5.5 and 5.1mg MDA/kg muscle (sed=0.30, NS) for 6 and 10 days ageing, respectively. Effects of diet, breed and vitamin E on TBA values and significance of the main effects and interaction are in Table 2.

(a) semitendinosus muscle

As a result of low vitamin E levels in muscle, TBA values was higher than acceptable value of 0.5-1.0 (Gray and Pearson, 1987) in muscles from all groups after 5 day simulated display. There was an increase in TBA numbers from Megalac to linseed and linfish. Mean values were 2.66, 4.81 and 6.56 mg MDA /kg muscle (sed= 0.38, p<0.001, Table 2) for muscles from Megalac, linseed and linfish fed lambs. There were no significant breed and vitamin E effects on TBA values. But, Scottish Blackface lambs showed a response to higher vitamin E by having lower TBA values after fed one of the diets with high levels of vitamin E (MH, LH or LFH). Mean values were 5.3 and 3.92 mg MDA /kg muscle (sed= 0.45, p<0.01, Table 2) for low and high vitamin E diets. The values were for Suffolk lambs 4.81 and 4.59 mg MDA /kg muscle (sed= 0.45, NS).

(b) semimembranosus muscle

Similar to the ST muscle, TBA values in the SM muscle were higher than acceptable values. Statistical analysis showed that the SM muscle had significantly higher values than the ST. Mean values were 4.66 vs. 5.28 mg MDA/kg muscle (sed= 0.21, p<0.01). The reason of this differences is that semimembranosus is an oxidative muscle, containing more mitochondria and therefore a higher content of long chain PUFA compared to the semitendinosus which is a more glycolytic muscle. Also oxidative muscles contain more free fatty acids than glycolytic ones (Sklan et al.,1983). The free fatty acids contain a large amount of long chain PUFA which come unambiguously from phospholipids (Zierath and Hawley, 2004). Therefore, the greater content of α-tocopherol in oxidative muscle than in white muscle may be necessary to provide protection for the increasing lipid unsaturation in the dark (oxidative) muscle. There was also a significant effect of diet on TBA values with mean values 3.96, 5.54 and 6.37 mg MDA/kg muscle (sed= 0.35, p<0.001, Table 2) for Megalac, linseed and linfish fed lamb muscles. Similarly to the ST muscle, no significant effects of breed and vitamin E on lipid oxidation were observed. As an interaction effect, Scottish Blackface lambs also showed, in SM muscle, much more response to a higher level of vitamin E and they had lower TBA values than when fed the low level of vitamin E. Mean values 5.77 vs. 4.63 mg MDA/ kg muscle (sed=0.40, p< 0.05).

In Suffolk lambs these values were similar, 5.23 vs. 5.50 mg MDA/kg muscle. In SM muscle another interaction effect was observed. In the muscle of linfish fed lambs high vitamin E decreased the lipid oxidation while linseed and Megalac fed lambs did not show a difference. In linfish fed lambs values decreased from 7.08 to 5.7 mg MDA/kg muscle (sed= 0.50, p< 0.05).

Table 2. Effects of dietary fat, breed and vitamin E on lipid oxidation (mg MDA/kg muscle) in semitendinosus and semimembranosus muscles of lamb

	Megalac				**Linseed**				**LinFish**				
	Low		High		Low		High		Low		High		
	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	SED
MDA, mg/kg (ST)	2.84	3.04	2.95	1.81	4.90	4.76	4.31	5.29	6.12	8.37	7.22	4.73	0.73
MDA mg/kg (SM)	4.95	3.43	4.77	2.70	4.79	5.77	5.04	6.55	6.01	8.28	6.71	4.70	0.71

Main effects and interactions

	Fat	Breed	Vitamin E	Fat x Breed	Fat x Vit. E	Breed x Vit. E	Fat x Breed x Vit. E
MDA mg/kg (ST)	***	ns	ns	ns	ns	**	***
MDA mg/kg (SM)	***	ns	ns	***	*	*	**

ns= not significant, *= p<0.05, **= p<0.01, ***= p<0.001, MDA: malonaldehyde, ST : m.semitendinosus SM : m. semimembranosus

Many studies have assessed the effects of added dietary fat on oxidative stability of meat. Oxidative rancidity is a major cause of meat deterioration and involves the oxidation of the unsaturated fatty acids, particularly the polyunsaturated fatty acids. Moerck and Ball (1974) showed that fatty acids with three or more double bonds are very susceptible to oxidation during refrigerated storage of meat and that the susceptibility increases with the number of double bonds in the fatty acids. The vitamin E content of muscle foods during storage influences the rate of lipid oxidation and subsequent changes in fatty acid composition. Porcine muscle with the highest concentrations of α-tocopherol per gram of fatty acids with three or more double bonds, had the lowest TBA numbers after cooking or three days of storage (Lynch et al., 1999, Yamauchi et al., 1980).

Although fresh forage has the potential to supply large amounts of vitamin E, the amount deposited in the animal may be reduced by the presence of the oxidatively unstable α-linolenic acid in the forage (Moloney et al., 2008). Furthermore, the difference in the PUFA composition of the tissues produced by the dietary α-linolenic acid from forage compared with mainly linoleic acid from grains also increases the potential for oxidation in meat hence increasing the demand for vitamin E.

In the present study, it seems that dietary n-3 stimulated lipid oxidation was not suppressed even at the high level of vitamin E supplementation because of low tissue levels (Demirel et al.,2004). Thus, the supply of vitamin E may be inadequate and/or the antioxidative function of vitamin E was inefficient in the presence of the highly unsaturated fatty acid. Farwer et al. (1994) and Kubo et al. (1997) observed similar results in rats fed on diets n-3 fatty acids. Thus, vitamin E may not protect membranes rich in n-3 fatty acids, especially those with five or six double bonds, from lipid oxidation as efficiently as membranes rich in n-6 fatty acids, as suggested by Kaasgaard et al. (1992).

In conclusion, supplementation of diet with n-3 enhanced the susceptibility of muscle to lipid oxidation concomitant with higher levels of n-3 in muscles despite the poor protection and extensive rumen biohydrogenation. In addition, the amount of vitamin E present was unable to protect membranes of these muscles from lipid oxidation even after ingestion of high levels of vitamin E.

References

Arnold, R. N., Arp, S. C., Scheller, K. K., Williams, S. N., & Schaefer, D. M. (1993). Tissue equilibration and subcellular distribution of vitamin E relative to myoglobin and lipid oxidation in displayed beef. *Journal of Animal Science, 71*(1), 105-118.

Chauhan, S. S., Celi, P., Ponnampalam, E. N., Leury, B. J. Liu, F., & Dunshea, F. R. (2014). Antioxidant dynamics in the live animal and implications for ruminant health and product (meat/milk) quality: role of vitamin E and selenium. *Animal Production Science, 54*, 1525–1536.

Demirel, G., Wachira, A. M., Sinclair, L. A., Wilkinson, R. G., Wood, J. D., & Enser, M. (2004). Effects of dietary n-3 polyunsaturated fatty acids, breeds and vitamin E on the fatty acids of lamb muscle, liver and adipose tissue. *British Journal of Nutrition, 91*(4), 551–565.

Farwer, S. R., Boer, B. C. J. D, Haddeman, E., Kivits, G. A. A., Wiersma, A., & Danse, B. H. J. C. (1994). The vitamin E nutritional status of rats fed on diets high in fish oil, linseed oil or sunflower seed oil. *British Journal of Nutrition, 72*(1), 127-145.

Gray, J. I., & Pearson, A. M. (1987). Restructured Meat and Dairy Products In: A. M. Pearson, T. R. Dutson (Ed). *Advances in meat research*, vol:3. (pp. 221). New York, US: Van Nostrand Reinhold Company.

Kaasgaard, S. G., Holmer, G., Hoy, C. E., Behrens, W. A., & Beare-Rogers, J. L. (1992). Effect of dietary linseed oil and marine oil on lipid oxidation in monkey liver in vivo and in vitro. *Lipids, 27*(10),740-745.

Kubo, K., Saito, M., Tadokoro, T., & Maekawa, A. (1997). Changes in susceptibility of tissues to lipid peroxidation after ingestion of various levels of decosahexaenoic acid and vitamin E. *British Journal of Nutrition, 78*(4), 655-669.

Lynch, M. P., Kerry, J. P., Buckley, D. J., Faustman, C., & Morrissey, P. A. (1999). Effect of dietary vitamin E supplementation on the colour and lipid stability of fresh, frozen and vacuum-packaged beef. *Meat Science, 52*(1), 95-99.

Moerck, K. E., & Ball, H. R. (1974). Lipid oxidation in mechanically deboned chicken meat. *Journal of Food Science, 39*(5), 876-879.

Meydani, S. N., Shapiro, A. C., Meydani, M., MaCauly, J. B., & Blumberg, J. B. (1987). Effect of age and dietary fat(fish oil, corn oil and coconut oil) on tocopherol status of mice. *Lipids, 22*(5), 345-350.

Moloney, A. P, Fievez, V., Martin, B, Nute, G. R., & Richardson, I. (2008). *Biodiversity and animal feed: future challenges for grassland production*. Proceedings of the 22nd General Meeting of the European Grassland Federation, Uppsala, Sweden, 9-12 June.

Raederstorff, D., Wyss, A., Calder, P. C., Weber, P., & Eggersdorfer, M. (2015). Vitamin E function and requirements in relation to PUFA. *British Journal of Nutrition, 114*(8), 1113–1122.

Sklan, D., Tenne, Z., & Budowski, P. (1983). Simultaneous lypolytic and oxidative changes in turkey meat stored at different temperatures. *Journal of Science Food and Agriculture, 34*(1), 93-99.

Vyncke, W. (1975). Evaluation of the direct thiobarbituric acid extraction method for determining oxidative rancidity in Mackerel. *Fette, Seifen, Anstrichmittel, 77*(6), 289-240.

Wood, J. D., & Enser, M. (1997). Factors influencing fatty acids in meat and the role of antioxidants in improving meat quality. *British Journal of Nutrition 78*(Suppl. 1), S49- S60.

Wulf, D. M., Morgan, J. B., Sanders, S. K., Tatum, J. D., Smith, G. C., & Williams, S. (1995). Effects of dietary supplementation of vitamin E on storage and case life properties of lamb retail cuts. *Journal of Animal Science, 73*(2), 399-405.

Yamauchi, K., Nagai, Y., & Ohashi, T. (1980). Quantitative relationship between a-tocopherol and polyunsaturated fatty acids and its connection to development of oxidative rancidity in porcine skeletal muscle. Agricultural and Biological Chemistry, 44(5),1061-1067

Zierath, J. R., & Hawley, J. A. (2004). Skeletal Muscle Fiber Type: Influence on Contractile and Metabolic Properties. *PLoS Biology, 2*(10), e337-e348.

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Determination of renal blood flow with Doppler ultrasound and the hypertension prevalence and acid-base level in dogs with chronic renal failure.

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ABSTRACT

Chronic renal failure (CRF) is an important cause of morbidity and mortality in dogs. The aim of this study is to investigate the renal Doppler measurements, blood hypertension and acid-base levels in dogs with chronic renal failure. Twenty-six dogs previously diagnosed with chronic renal failure were compared with twenty healthy dogs. A complete blood cell, biochemical profile, urinalysis, blood gasses, blood pressure were analyzed and changes in renal blood flow were measured by renal Doppler ultrasonography. The dogs with CRF had significantly higher serum blood urea nitrogen, creatinine and phosphorus concentration, significantly lower packed cell volume, and urine specific gravity than control dogs. Positive correlation was determined between serum creatinine levels and renal Doppler indices. There were significant decreases in blood pH and bicarbonate. Indirect blood pressure measurements were slightly increased in CRF dogs. Renal Doppler measurement was observed as a helpful tool in diagnosing the CRF in dogs. Identification of acidosis and hypertension may help in developing treatments that slow the rate of progression of chronic renal failure.

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Introduction

Chronic renal failure (CRF) is defined as structural and/ or functional impairment of one or both kidneys (Polzin, 2011; Bartges, 2012). Medical history and physical examination findings (eg, changes in kidney size or shape, changes in urine volume) are suggestive of kidney disease. The most commonly used serum urea and creatinine levels may be normal even when only 25% of the nephrons have normal filtration capacity. Therefore these methods may remain incapable in the early stages of renal insufficiencies.
Duplex Doppler ultrasonography, provides a real time information about the anatomy and dynamics of kidney. No absolute contraindication related to Doppler USG is reported/known (Drelich-Zbroja et al., 2018). The first study on the detection of normal values in dogs was published by Nyland et al. in 1993 (Nyland et al., 2002). When renal failure occurred, normal balance between vasoconstrictive and vasodilator factors deteriorates in time and intrarenal vasoconstriction occurs. Renal resistive index (RI) and pulsatility index (PI) measurements are used for the calculation of the resistance to tissue perfusion that

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occurs in that area (Bragato et al., 2017; Mitchell et al., 1998). They are also used for the determination of the changes in blood flow due to congestion, diuretic effects, acute and chronic renal failure and congenital dysplasia (Novellas et al., 2007). Although these alterations have been reported to be proportional to the severity and progression of chronic renal disease, there are few studies focusing on this subject in animals (Morrow et al., 1996).

Hypertension is one of the most common complication of renal failure. In recent years the prevalence of hypertension in CRF was studied by many researchers (Acierno et al., 2018; McMurphy et al., 2006). Although the direct measurement with the use of electronic transducer within the artery is accepted as the golden standard, indirect measurement with Doppler and oscillometric devices are also commonly used (McMurphy et al., 2006). Standards for the validation of indirect measurement devices in human medicine are well established. However no device has met these criteria in dogs or cats still (Acierno et al., 2018).

Metabolic acidosis is the other important complication of renal failure. It is the result of decreased renal sulfate and phosphate production, reduction of H^+ ion excretion and increased urinary bicarbonate (HCO_3) loss (Polzin et al., 2005).

The aim of this study was to investigate the effects of the disease in dogs with CRF with the help of renal Doppler measurements and blood pressure and blood gas. Thus, one of the most important causes of death in dogs, chronic renal diseases and their complications will be examined in detail.

Materials and methods

Animals: Twenty-six dogs previously diagnosed CRF and twenty healthy dogs from several breeds and ages were included into the study. All animals were privately owned and presented to our clinics. CRF was diagnosed for each patient according to the history, clinical signs, and laboratory examinations accepted typical for the disease.

Physical examination, routine blood tests (hematological and biochemical), B-mode and Doppler ultrasonography examinations were performed on all dogs on the presentation day. Total cell count (Mindray BC 2800 vet, Chine), plasma concentrations of glucose, urea, creatinine, aspartate aminotransferase (AST), Alanine aminotransferase (ALT), total proteins, albumin, calcium, phosphorus were assayed by the autoanalyzer (Tokyo Boeki TMS 1024, Tokyo, Japan). Urine specimens were obtained from dogs either by voluntary voiding or catheterization. Routine dipstick analysis were performed and all samples were underwent microscopic examinations. Urinary protein/creatinine (UPC) ratio was measured. UPC ratio was determined using routine methods.

Blood pressure: Blood pressure was measured non-invasively with the use of an ultrasonic Doppler flow monitor (Parks Medical Electronics, INC. Aloha, Oregon, USA) before all procedures. All measurements were taken from cranial tibial artery. The cuff width was approximately 40% of the limb circumference. After the hair over the artery was clipped, the cuff was inflated till the blood flow no longer was heard. The cuff was then gradually deflated. The point where the blood flow could be first detected was systolic pressure. (Acierno and Labato, 2005; Brown et al. 2007; Henik et al., 2005).

Blood gas analysis: 1 ml of blood were collected from femoral artery into a blood-gas specific syringes that included lithium heparin as anticoagulant. The samples were analyzed (Irma Trupoint blood gas analyzers, ITC, USA) immediately after the collection (Batemen, 2008).

Renal Doppler Ultrasonography: Ultrasonography and Doppler measurements (Terason 2000, Samsung, China) were performed by the same person before the treatment. A multiconvex prob with 5 or 7 MHz was used. Renal Doppler measurements were obtained from the renal, interlobar, or arcuate arteries. After hair clipping, an acoustic gel was applied to the skin. Different transducers and frequencies were used depending on animal weight. A morphometric examination was performed and renal length and width were measured on the longitudinal axis.

Color Doppler was used to visualize the intrarenal vasculature. Sample volume was positioned in the middle of the renal vessels and the insonation angle did not exceed 60° after correction (Figure 1). The Doppler examination required more than 10 minutes in most of the dogs because of the movement or tachypnea.

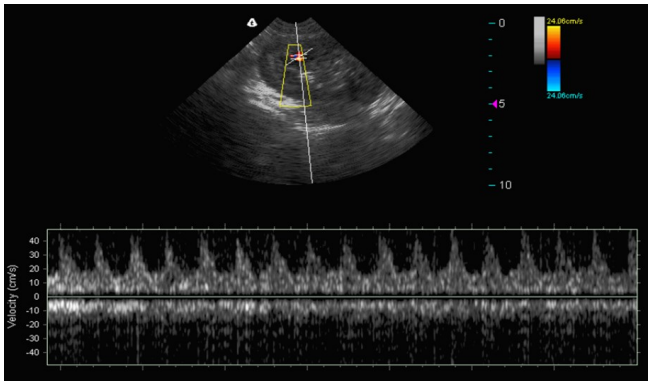

Figure 1: Doppler ultrasonography of a dog with chronic renal failure

Parameters of blood flow velocity such as systolic peak velocity (PS), end diastolic peak (ED), and time average maximum velocity (TMV), as well as hemodynamic parameters such as resistive index (RI) and systolic-diastolic ratio (S/D) were calculated automatically by the ultrasound device.

Pulsatility index (PI) was calculated manually according to on the formula below (Novellas et al., 2007). PI = (peak systolic velocity)-(end diastolic velocity) / (time average maximum velocity).

Statistical Analysis: A commercial software package (SPSS10.0) was used to analyze data. Independent samples t-test was applied to compare the changes in dogs with CRF and healthy dogs. Pearson correlation analyses were made to determine a significant correlation between UPC and renal RI and PI values. Level of significance was set at P<0 .05.

Results

A total of twenty-six dogs with previously diagnosed with chronic renal failure and twenty healthy dogs were included in our study. The majority of healthy dogs were under the age of 5, while the majority of dogs with CRF were over 10 years old. Vomiting (22), anorexia (16), polydipsia (12), polyuria (11), weakness (10), weight loss (8), diarrhea (6), reduction in the amount of urine (5), reduction in the amount of water drinking (5), wounds in the mouth (2) and halitosis (2) were revealed symptoms in dogs with CRF.

The mean RI and PI values of renal arteries were detected as 0.54 ± 0.01 and 1.3 ± 0.04 in healthy dogs, respectively. The same indices were found as 0.68 ± 0.02 and 3.4 ± 0.4 in patients with CRF, respectively (Table 1).

Among these dogs suffering from CRF, 10 of them showed increased intrarenal RI values. Additionally, 21 dogs which also include the patients with high RI values displayed PI values higher than reference limits. The mean values of intrarenal RI and PI in dogs with CRF found significantly higher than control group (P<0.001). The sensitivity and the specificity of RI were found 35% and 99%, respectively.

When the dogs with CRF evaluated in terms of blood pressure; 15 of our cases had minimal risk, 2 had low risk, 8 had moderate risk and 1 had high grade risk. Three low-grade dogs and one dog with high-grade blood pressure had grade 4 renal failure. 14% of the intermediate dogs and 40% of the minimal -grade dogs exhibited were diagnosed with third-grade renal failure. In the present study, there was no statistical significance between the systolic blood pressures of healthy dogs and dogs with CRF. When diastolic blood pressure measurements were compared between groups, no statistical significance was found (Table 2). However, when blood pressure measurement methods were compared with GLM analysis, it was determined that Doppler method was better than oscillometric method (Table 3).

Table 1: Comparison of intrarenal Doppler measurements

Parameters	Healthy dogs	Dogs with CRF
PS	13.3 ± 1.5	21.7 ± 2.2**
ED	6.2 ± 0.9	6.7 ± 1.0
SID	2.3 ± 0.2	5.2 ± 1.5
RI	0.54 ± 0.01	0.68 ± 0.02***
FV	7.2 ± 3.6	4.6 ± 1.6
TMV	5.6 ± 0.6	5.2 ± 0.8
PI	1.3 ± 0.04	3.4 ± 0.4***

** p<0.01, *** p<0.001, PS = Peak systolic velocity, ED = End diastolic velocity, SID = Systolic/diastolic velocity, RI = Resistive index, FV = Follow volume, TMV = Time average maximum velocity, PI = Pulsatility index.

Table 2: Comparison of diastolic blood pressure in healthy dogs and dogs with Chronic renal failure

Healthy dogs	Dogs with CRF
92.3 ± 3.4	89.7 ± 1

CRF = Chronic renal failure

Table 3: Comparison of Doppler and oscillometric blood pressure measurement methods between healthy and chronic renal failure dogs

	Blood pressure
Method	
Doppler	141.6 ± 2.87
Oscillometric	129.7 ± 2.87
	*
Health status	
Healthy	131.7 ± 2.96
Dogs with CRF	139.7 ± 2.79
	NS
Overall average	135.7 ± 2.03

*: p<0,01, NS = Non significant, CRF = Chronic renal failure.

In dogs with CRF, pH and total carbon dioxide (TCO_2) values were decreased, while base excess (Beb) values were statistically significant at P<0.001 level. Although serum iCa level was within normal limits, a significant decrease was observed when compared to healthy group (P<0.001). Although the serum K value was found to be within normal limits in dogs with CRF, it was statistically significant increased in dogs with CRF (P<0.01). Decrease in bicarbonate (HCO_3) and increase in partial pressure of oxygen (PO_2) were statistically significant compared to P<0.01 level. Also, a statistically significant decrease in partial pressure of carbon dioxide (PCO_2) and total haemoglobin (tHb) values was determined (P<0.05) (Table 4).

Table 4: Comparison of blood gases in healthy dogs and dogs with chronic renal failure

	Healthy Dogs	Dogs with CRF	Reference values
pCO_2	33 ± 1.3	28.4 ± 1,7*	30.8 – 42.8
PO_2	100.7 ± 6.6	129.6 ± 6**	80.9 – 103.3
Hct	41.5 ± 3,2	38.7 ± 1.3	40.3– 60.3
Na	146.9 ± 0.7	146.5 ± 2.9	150 – 165
K	4.1 ± 0.1	4.9 ± 0.2**	3.5 – 5.8
iCa	1.4 ± 0.02	1.2 ± 0.05***	1.2-1.5
HCO_3	21.1 ± 0.7	15.7 ± 1.4**	18.8 – 25.6
TCO_2	22.2 ± 0.7	15.5 ± 1.3***	22 ± 2
Beb	2.7 ± 0.4	-9.3 ± 1.3***	-2 – +2
O_2sat	96.4 ± 1.2	97.3 ± 0.7	93 – 100
tHb	15.5 ± 0.8	13.7 ± 0.4*	8 – 15

*p <0.05, **p<0.01, ***p<0.001, pCO_2 = Partial pressure of carbon dioxide , PO_2 = Partial pressure of oxygen, Hct = Hematocrit, Na= Sodium, K= Potassium, iCa = Ionized calcium TCO_2 = Total Carbon dioxide , Beb = Base excees, O_2sat= Oxygen saturation tHb = Total haemoglobin concentration.

Red Blood cell (RBC) and hemoglobin (HGB) values are known to be important in dogs with chronic renal failure (P <0.001) and normocytic-normochromic type anemia developed in 20 dogs in our study. In the animals of the CRF group, when compared with the healthy group, the mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values remained within normal range, however mean corpuscular hemoglobin concentration (MCHC) were significantly decreased in dogs with CRF (Table 5).

Blood urea and creatinine levels increased in all dogs with CRF (P<0.001). The calcium (Ca) level was found to be elevated in 3 patients however decreased

Table 5: Complete blood count in healthy dogs and dogs with chronic renal failure

Parameters	Healthy Dogs	Dogs with CRF	Reference Values
RBC (X 10^6/ µL)	6.4 ± 0.2	5.4± 0.2**	5.5-8.5
HGB (g/dL)	15.5 ± 0.6	12.3 ± 0.7**	12-18
HCT (%)	39.9 ± 1.4	35.3 ± 2	37-55
WBC (X 10^3/µL)	13.2 ± 0.7	15.8 ± 1.5	6-17
PLT (X 10^3 /µL)	282.4 ± 17.3	300.5 ± 34.8	200-500
MCV (fL)	61.8 ± 0.6	65.1 ± 1*	60-77
MCH (pg)	21.4 ± 1.4	23.2 ± 0.5	19.5-26
MCHC (%)	37.1 ± 0.7	35 ± 0.6*	32-36

** p<0.01, *** p<0.001, CRF = Chronic renal failure, RBC = Red blood cell, HGB, = hemoglobin, HCT = Hematocrit, WBC = White blood cell, MCV = Mean corpuscular volume, MCH = Mean corpuscular hemoglobin, MCHC = Mean corpuscular hemoglobin concentration.

Table 6: Serum biochemistry in healthy dogs and dogs with chronic renal failure

Parameters	Healthy Dogs	Dogs with CRF	Reference Values
Glucose (mg/dl)	129 ± 2.3	129.2 ± 5.4	60-125
Urea (mg/dl)	43.9 ± 2	310.8 ± 31*	7-27
Creatinine (mg/dl)	1.2 ± 0.03	7.7 ± 0.8*	0.4-1.8
AST (IU/L)	34.4 ± 2.5	47.3 ± 6	5-55
ALT (IU/L)	34.8 ± 5.3	50 ± 6	5-60
T. Protein (mg/dl)	6.5 ± 0.2	7.5 ± 0.6	5.1-7.8
Albumin (g/dl)	3.5 ± 0.1	2.6 ± 0.1*	2.6-4.3
Ca (mg/dl)	8.9 ± 0.2	8.6 ± 0.4	7.5-11.3
P (mg/dl)	3.8 ± 0.06	10.9 ± 1.3*	2.1-6.3

* p<0.001, CRF = Chronic renal failure, ALT = Alanine aminotransferase, AST = aspartate aminotransferase, Ca = Calcium, P = Phosphorus.

Table 7: Comparison of urea, creatinine, Resistive index and Pulsatility index values

	Creatinine	RI	PI
Urea	0.787**	0.613**	0.457**
Creatinine	-	0.432*	0.399*
RI	-	-	0.787**

*: p<0,01, **: p<0,001, RI = Resistive index, PI = Pulsatility index.

Discussion

in 6 patients. The blood phosphorus (P) level was found to be statistically higher at the level of P<0.001 compared to the control group. Albumin levels were significantly decreased at P<0.001, but this decrease was within normal limits (Table 6). CRF is one of the most important causes of morbidity and mortality in dogs (McGrotty, 2008). Clinical evaluation of renal blood flow gives important information about diagnosis, treatment and prognosis of the disease (Morrow et al., 1996). Many diagnostic methods such as physical examination, CBC and biochemical blood examination, urinalysis, radiography and ultrasonography are used in the detection of renal damage. While there are many studies in humans about renal Doppler, blood pressure and blood gases, which are helpful diagnostic methods for the diagnosis, and better prognosis of the disease, we found that there are few studies including all of them in dogs.

As the first changes in renal diseases begin with the change in blood flow, renal Doppler ultrasonography, which is rarely used in veterinary practice is one of the most important diagnostic tools (Bragato et al., 2017). Previous studies have shown many normal RI values. Although different RI intervals are specified one by one, the RI value is considered abnormal when it exceeds 0.70 (Novellas et al., 2008 ; Mitchell et al., 1998; Morrow et al., 1996). RI and PI above the upper limit indicate the presence of renal disease (Bragato et al., 2017). The mean intrarenal RI value of healthy dogs was 0.54 ± 0.01 in our study. This value is within the normal limits reported by the researchers. In dogs with CRF, the mean RI value was 0.68 ± 0.02. This value is lower than the normal value reported by Morrow et al. (1996) and Novellas et al. (2008). However, the RI value was found statistically increased at P<0.001 when compared with healthy dogs. On the other hand, in 10 cases, the RI value was above normal limits in dogs with CRF. The changes in RI values may affected by many external factors such as pressure, hypotension, digestion, and heart rate (Choi et al., 2003; Morrow et al., 1996; Szatmari et al., 2001). However, not all forms of renal failure may change the vessel resistance to the same extent. It has been observed that increased RI in glomerular diseases is rare in both human and dog studies. In addition, the elevation of intrarenal RI may occur in both tubulointerstitial and glomerular diseases, however it is not useful in differential diagnosis of these situations (Morrow et al., 1996; Rivers et al., 1997). In a study comparing RI value to histopathological findings in dogs with glomerular disease, increased RI levels were obtained in patients with interstitial nephritis or tubular degeneration (Morrow et al., 1996).

Another important parameter used in renal Doppler measurements is PI value. PI value above 1.52 is reported to be abnormal (Novellas et al., 2008, Morrow et al., 1996). In our study, we determined that the measurements in healthy dogs were within normal limits. This value was increased in dogs with CRF. In addition, compared to healthy dogs, the increase in dogs with CRF was statistically significant at P<0.001 level. Despite the more frequent use of RI value, PI is more sensitive in determination of abnormalities. Hence PI value is not affected by external factors and it is taken into account at the average rate when determining PI value (Mitchell et al., 1998, Novellas et al., 2008). Similar to the report by Novellas et al. (2008), we found that PI is more sensitive than RI. Renal PI was found increased in 21 of the dogs with CRF, whereas only 10 of them had increased renal RI in our study. High positive correlation was found between these two parameters (r = 0.787).

A positive correlation between creatinine levels and RI and PI was reported in a study on patients with CRF in human medicine (Peterson et al., 1997). In addition, Baltazar et al. (2016) and Torroja (2007), found a positive correlation between these parameters in cats and dogs. In the study of dogs managed in 1996, renal diseases were compared with the RI value and it was reported that there was no correlation between RI value and urea and creatinine (Morrow et al., 1996). Rivers et al. (1997), supported this finding in their study. In this study, a positive correlation between the RI value (r = 0.432) and PI value (r = 0.399) with creatinine was found. Similarly RI and PI positively correlated with serum urea level (respectively, r = 0.613 and r = 0.457) (Table 7).

In order to determine the availability of intrarenal RI values in CRF dogs, sensitivity and specificity were also calculated in this study. For this reason, 0.70 was selected as normal upper limit similar to the study of Morrow et al. (1996), where the specificity and the sensitivity were 36% and 96% respectively. Our results were similar to the study of Morrow et al. (1996).

Hypertension is the common sequela of renal failure (McMurphy et al., 2006; Henik et al., 2005; Uzlu and Kalınbacak, 2005). It is concluded from previous studies that 50% to 93% of canine patients with renal disease are affected from hypertension (Acierno and Labato, 2005). Similarly, 42% of dogs with CRF had high blood pressure in our study.

Kidneys, have an important role in acid-base balance regulation. Normally, daily H^+ charge is excreted in the urine by NH_3 or NH_4 or by the conversion of the phosphate into H_2PO_4. When CRF is formed, total H^+ excretion is impaired. Thus, less H+ is excreted in the kidneys and HCO_3 is not reabsorbed enough, acidosis occurs in the animal (Morais et al., 2008). In our cases, statistically significant decrease in pH value at P<0.001 level was found; which was similar to previous studies. The characteristic laboratory finding of metabolic acidosis are the reduction of the plasma bicarbonate level and the reduction of the base. In our study, HCO_3 was determined as P<0.01 and BE value was statistically lower than P<0.001. In this case, all of these show parallelism with previous studies (Bartges, 2012).

In conclusion, renal Doppler measurements are useful in the diagnosis of CRF and the monitoring of changes in blood pressure and acid-base balance, which are important complications of the disease, will help to determine the prognosis of CRF and improve effective treatments and keep the disease under long-term control.

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References

Acierno, M. J., & Labato, M. A. (2005). Hypertension in renal disease: Diagnosis and treatment. *Clinical Techiniques in Small Animal Practice, 20*(1), 23-30.

Acierno, M. J., Brown, S., Coleman, A. E., Jepson, R. E., Papich, M., Stepien, R. L., & Syme, H. M. (2018). ACVIM consensus statement: Guidelines for the identification, evaluation, and management of systemic hypertension in dogs and cats. *Journal of Veterinary Internal Medicine,32*(6), 1803-1822.

Baltazar, P. I., Moura, L. S., Pessoa, G. T., Rodrigues, R. P. S., Sanches, M. P., Diniz, A. N., Sousa, F. C. A., Guerra, P. C., Neves, W. C., Giglio, R. F., Alves, J. J. R. P., Souza, F. A. L., Braga, J. F. V., & Alves, F. R. (2016). Comparative B-mode and Doppler renal ultrasonography with histopathological findings in dogs positive for canine visceral leishmaniasis. *Microscopy Research and Technique, 79*(7), 637-645.

Bartges, J. W. (2012). Chronic kidney disease in dogs and cats. *Veterinary Clinics of North America: Small Animal Practice, 42*(4), 669-92.

Batemen, S. W. (2008). Making sense of blood gas results. *Veterinary Clinics of North America: Small Animal Practice, 38*(3), 543-557.

Bragato, N., Borges, N. C., & Fioravanti, M. C. S. (2017). B-mode and Doppler ultrasound of chronic kidney disease in dogs and cats. *Veterinary Research Communications, 41*(4), 307-315.

Brown, S., Atkins, C., Bagley, R., Carr, A., Cowgill, L., Davidson, M., Egner, B., Elliot, C., Henik, R., Labato, M., Littman, M., Polzin, D., Ross, L., Snyer, P. & Stepien, R. (2007). Guidelines for the identification, evaluation, and management of systemic hypertension in dogs and cats. *Journal of Veterinary Internal Medicine, 21*, 542-558.

Choi, H., Won, S., Chung, W., Lee, K., Chang, D., Lee, H., Eom, K., Lee, Y., & Yoon, J. (2003). Effect of intravenous mannitol upon the resistive index in complete unilateral renal obstruction in dogs. *Journal of Veterinary Internal Medicine, 17*(2), 158 -162.

Drelich-Zbroja, A., Kuczynska, M., Swiatlowski, L., Szymanska, A., Elwertowski, M., & Marianowska, A. (2018). Recommendations for ultrasonographic assessment of renal arteries. *Journal of Ultrasonography, 18*(75), 338-343.

Henik, R. A., Dolson, M. K., & Wenholz, L. J. (2005). How to obtain a blood pressure measurement. *Clinical Techniques in Small Animal Practice, 20* (3),144-150.

Novellas, R., Ruiz de Gopegui, R., & Espada, Y. (2008). Increased renal vascular resistance in dogs with hepatic disease. *The Veterinary Journal, 178*(2), 255-260.

Novellas, R., Espada, Y, & Ruiz De Gopegui, R. (2007). Doppler ultrasonographic estimation of renal and ocular resistive and pulsatility in normal dogs and cats. *Veterinary Radiology & Ultrasound, 48*(1), 69 -73.

Nyland, T. G., Mattoon, J. S., Herrgesell, E. J., & Wisner, E. R. (2002). *Small Animal Diagnostic Ultrasound 2th ed.* Philadelphia, US: W.B. Saunders.

McGrotty, Y. (2008). Diagnosis and management of chronic kidney disease in dogs and cats. *In Practice*, 30, 502-507.

McMurphy, R. M., Stoll, M. R., & Mccubrey, R. (2006). Accuracy of an oscillometric blood pressure monitor during phenylephrine-induced hypertension in dogs. American Journal of Veterinary Research, 67(9), 1541-1545.

Mitchell, S. K., Toal, R. L., Daniel, G. B., & Rohrbach, B. W. (1998). Evaluation of renal hemodynamics in awake and isoflurane-anesthetized cats with pulsed wave Doppler and quantitive renal scintigraphy. *Veterinary Radiology & Ultrasound, 39*(5),451-458.

Morais, H. A., Bach, J. F., & DiBartola, S. P. (2008). Metobolic acid-base disorders in the clinical care unit. *Veterinary Clinics of North America: Small Animal Practice, 38*(3), 559-574.

Morrow, K. L., Salman, D. M., Lappin, M. R., & Wrigley, R. (1996). Comparison of the resistive index to clinical parameters in dogs with renal disease. *Veterinary Radiology & Ultrasound, 37*(3), 193-199.

Peterson, L. J., Peterson, J. R., Talleruphuus, U., Ladefoged, S. D., Mehlsen, J. & Jensen, H. A. (1997) The pulsatility index and resistive index in renal arteries. Associations with long-term progression in chronic renal failure. *Nephrology Dialysis Transplantation, 12*(7), 1376-1380.

Polzin, D. J. (2011). Chronic kidney disease in small animals. *Veterinary Clinics of North America: Small Animal Practice, 41*(1), 15-30.

Rivers, B.J., Walter, P.A., Polzin, D.J., & King, V.L. (1997). Duplex Doppler estimation of intrarenal pourcelot resistive index in dogs and cats with renal disease. *Journal of Veterinary Internal Medicine, 11*(4), 250-260.

Szatmarı, V., Sotonyı, P., & Vörös, K. (2001). Normal duplex Doppler waveforms of major abdominal blood vessels in dogs: A review. *Veterinary Radiology & Ultrasound, 42*(2), 93-107.

Torroja, R. N. (2007). Vascular resistance determination with Doppler ultrasound in canine and feline disease. *Phd Thesis* Universidade Autônoma de Barcelona, Barcelona, Spain.

Uzlu, E., & Kalınbacak, A. (2005). Evaluation of seconder hypertension by oscillometric method in dogs with renal failure symptoms and treatment with angiotensin II receptor antagonists. *Veterinary Journal of Ankara University, 52*, 33-38.

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The bite of a rat infected with *Pseudomonas aeruginosa* in laboratory conditions: An uncommon case

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ABSTRACT

Bites of laboratory animals are treated as the bites of any other animals since the possibility of existence of pathogenic microorganisms, unfavorable for human health, in the rooms where these animals reside, is not excluded. A rare case of a laboratory rat bite, used for scientific research and previously infected with *Pseudomonas aeruginosa*, is presented here. The patient's wound was located on the forefinger of his hand and was 1 cm long and up to 0.2 cm deep. The antitetanus prophylaxis was administered in an ambulance, including antibiotic therapy with amoxicillin-clavulanate per os. There exists a need for checks and special guidelines for the handling and retention of laboratory animals. The patient has successfully remedied the wound, thanks to consistent antibiotic therapy and antitetanus prophylaxis, and possible inflammatory complications were prevented.

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Introduction

Rats successfully colonized urban ecosystems globally and since then, their bites had posed a great danger, being the gateway for the emergence of infectious diseases (Syeda, et al., 2018). Together with other rodents, they are the host reservoirs for at least 60 zoonotic diseases, and their arthropod ectoparasites are significant vectors of many pathogens (Syeda et. al., 2018; Katica et.al., 2003; Chaisiri et. Al., 2015; Han et.al., 2015).

Historically, all kinds of rats have been linked with the occurrence and spread of contagious diseases such as plague, typhus, leptospirosis and hemorrhagic fever. Numerous reports however indicate that the number of rodents' bites is undoubtedly smaller compared to that of for example dogs and cats (Bregman & Slavinskka, 2012;Morosetti et. al., 2013; Rothe et. al., 2015), despite the scary widespread population of rodents in almost all parts of the planet (Asaj, 1999).

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It is estimated that 20.000 people are bitten by rats in the United States annually. Children are the most common victims, as rats have become popular pets (Abrahamian & Goldstein, 2011). Bite injuries in children are most commonly found on the face and arms (Kalra et. al., 2006; Hirschhorn & Hodge, 1999).

Rat bite is an unusual issue of the urgent medicine; the bitten patients rarely come to tertiary medical centers and such bites usually remain neglected.

There is a large number of reports that describe the adverse impact of rat bites on human health, such as the appearance of foot ulcers in people with diabetes (Kalra et. al., 2006). The rat bites can be deadly (Yanai et. al., 1999), and are associated with fever post bite (Hudsmith et. al., 2001), tetanus (Mathiasen & Rix, 1993) and rabies (Marshall et. al., 1994 ; Kalra et. al., 2006). Rat bite fever disease, is very seriously condition caused by Streptobacillus moniliformis or Spirillum minus and when it is untreated mortality rate is 13%. The most common signs of infection are fever, vomiting, muscle pain and rush. The essential is to recognize the infection so treatment with antibiotics can start as soon as possible (Elliot, 2007).

Bites of laboratory animals are treated as the bites of any other animals, since the possibility of existence of pathogenic microorganisms, unfavorable for human health, in rooms, cages, mats, etc., where the animals reside, is not excluded. Risks to the health of researchers and laboratory staff are unavoidably higher if all the necessary zoohygienic measures within the vivarium, as well as the bio-safety measures are not fully carried out during experimental studies when, for example, toxic compounds or zoonotic agents are being used.

Protective measures taken when handling infectious material in laboratories aim at ensuring bio-safety; that is to reduce or eliminate the exposure of laboratory personnel and other persons of the outside environment to potentially dangerous causers. *Pseudomonas aeruginosa* belongs to the second bio-safety level, which implies mandatory use of personal protective equipment (glasses, masks, white coat, gloves - two pairs if necessary), good laboratory practice and the use of laboratory space corresponding to clinical and diagnostic laboratories, educational laboratories and other laboratories in which an infectious material which represents a moderate risk of transmission is present (Hukić et. al., 2010).

Because of everything above mentioned, every bite of a laboratory animal requires attention through a strict medical treatment program (National Research Council, 1997).

In the USA, out of 198 laboratories with laboratory animals, 13 reported that staff had experienced bites caused by a laboratory rodent, most common being rat (Stave et. al., 2017). Working with laboratory rats usually does not carry the risk of serious injuries. However, minor injuries can occur; most usual being bites to the fingers if the staff is not experienced and/ or if the appropriate protective measures are not applied on the rat. Bacteria *Pseudomonas aeruginosa* is the cause of serious hospital infections and significantly contributes to morbidity and mortality. It is the fifth most commonly hospitalized pathogen with mortality rate of 28 to 48%. Its clinical significance is that it can be a cause of hospital pneumonia, urinary tract infection, wound infection, bone infection and bacteremia. *Pseudomonas aeruginosa* can also cause outbreaks of nosocomial infections such as intestinal infections, skin and soft tissue infections, otitis externa (Hukić et. al., 2010). We are presenting you with a rare case of the bite of a laboratory rat infected with Pseudomonas aeruginosa, which was used for scientific research purposes.

Case

At the Urgent Medicine Clinic, a 48-year-old man asked for medical help. He was bitten in a laboratory by an adult rat, of breed Wistar, which was previously infected with an inoculum of *Pseudomonas aeruginosa* bacterium, of density of 106 CFU/ml (colony forming unit per milliliter). The design of an experimental study on the rat that caused the injury involved instilment of 0.25 ml of inoculum in the back incision in the subcutaneous tissue, and the bite happened five days after contamination time of rat.

The standard bacterial strain of (*Pseudomonas aeruginosa* ATCC® - American Type Culture Collection - 10145™) was used to prepare the inoculum. The rat that caused the bite was taken out of the cage, in which two rats from the same experimental study were located and infected with the inoculum of the same concentration.

The patient's non-sterile glove was perforated. The wound was located at the top of the forefinger of patient's left hand and was 1 cm long and up to 0.2 cm deep. The wound was thoroughly rinsed with 0.9% NaCl solution, afterwards with 3% Hydrogen Peroxide solution, and later treated with solution of povidone-iodine (Isobetadine®) (Philipsen et. al., 2006). It has been superficial abrasive wound, so it was estimated that it is contraindicated to suture the wound, and reducing the potential risk of tetanus.

The first dose of 250 units of human anti-tetanus immunoglobulin was ordinated intramuscularly (i.m.) in ambulance. Antibiotic therapy was started; amoxicillin-clavulanate *per os* (Xiclav 2 x 1000 mg) (Philipsen et. al., 2006). As part of anti-tetanus prophylaxis i.m. the second dose of 0.5 ml of tetanus toxoid was applied i.m. after 30 days and third dose of 0.5 ml of tetanus toxoid i.m. after 365 days.

During antibiotic therapy, and later during antitetanus prophylaxis, the patient reported feeling good, without any clinically manifested changes. The treatment was completed routinely and successfully.

Discussion and Conclusion

Laboratory rodents do not like changes in their environment and do not work with unknown people (Zimmerman et. al., 2015). Laboratory rats are exceptionally fast animals and if moving, they are usually trying to escape or defend themselves if they can. It is therefore extremely important to manipulate them gently, safely placing thumb and fingers around their chests, making sure that the respiratory movements do not stop. In addition, in order to achieve the utmost safety of researchers during the experiment, the use of special grips is recommended to completely fix the rat and there lays the possibility to apply the desired substance. This would reduce the risk of bite to a minimum (Katica & Delibegović, 2019).

Immediate cleansing and early disinfection is the first step in preventing infection. It is necessary to check the vaccine status of the patient to determine the risk of tetanus. If the adult has never received three doses of vaccine before, or the information is unknown, it is necessary for the patient to receive 250 units of human anti-tetanus immunoglobulin intramuscularly or 0.5 ml of tetanus toxoid in another location intramuscularly or subcutaneously, depending on manufacturer's recommendation. One month after the first dose it is necessary to administer a second dose of tetanus toxoid, and after a period of 6 to 12 months a third one too. If the person is regularly vaccinated, and the period of the injury is within five years from the last dose, no prophylaxis is required. If the period from the last dose is 5 to 10 years long, a booster dose of 0.5 ml of tetanus toxoid is to be given, and if more than 10 years have passed since the last dose, one dose of 0.5 ml of tetanus toxoid and 250 units of human anti-tetanus immunoglobulin are needed. Although there are no clear guidelines or protocols on antibiotic prophylaxis for similar injuries, on the basis of clinical presentation of injury and anamnestic data doctor ordinarius determines the need for antibiotic ordination (Chapman et. al., 2008).

It is considered that all interruptions of skin continuity pose a risk of infection development, where agents can be introduced from the skin or from the object which caused the wound. In similar situations, when it comes to animal bites, it should be borne in mind that the risk of transmission of microorganisms residing in the oral cavity of the animal is very large. When it comes to an uninfected rat, the Staphylococcus epidermidis bacterium is present in the first place in almost 50% cases, followed by *Bacillus subtilis*, diphtheroids and alpha hemolytic streptococci (Abrahamian & Goldstein, 2011; Ordog et. al., 1985). Recognized as a particular clinical entity, rat bite feveris caused by bacterium *Streptobacillus moniliformis*, and was found in less than 10% of patients suffering a bite. In Asia, the disease is known as sodoku, and is caused by *Spirillum minus* (Hagelskjaer et. al., 1998).

Treating Pseudomonas infection, especially in immunocompromised patients, is uncertain and sometimes impossible. The bacteria is sensitive to a small number of antibiotics, but can also develop resistance to those during the therapy itself (Hukić et. al., 2005).Various injuries, trauma, surgery, burns make the host susceptible to colonization of P. aeruginosa (Gužvinec et. al., 2012; Kovačić et. al., 2018). Amoxicillin + clavulanic acid is most commonly used in antibiotic prophylaxis, and in the case of patients allergic to beta-lactam antibiotics, macrolide, aminoglycoside or quinolone antibiotics are to be used. Although the case study presented faced a case of a rat experimentally infected with bacterium P. aeruginosa, it is necessary to think about other potential agents, and so ordinated was the adequate therapy.

This is the first such report in Bosnia and Herzegovina that described the rat bite, i.e. the first such case at the emergency medicine clinic, university clinical center Sarajevo, in the post-war period. Antibiotic therapy based on amoxicillin-clavulanate and consistent antitetanus prophylaxis is a key to success in treatment of a rat bite, in patients who tolerate beta-lactam antibiotics and immuno-uncompromised patients. It is a priority to ensure prompt treatment in the case of bites.

Laboratory staff must have appropriate theoretical and practical training on the protection against the potential hazards to which they are exposed and must take necessary precautionary measures and procedures for assessing exposure, where continuous education is of utmost importance. Responsible researchers should be thinking in this direction both before and during the experiment.

References

Abrahamian, F. A. & Goldstein, E. J. C. (2011). Microbiology of animal bite wound infections. *Clinical Microbiology Reviews, 24*(2), 231-246.

Asaj, A. (1999). Deratizacija u praksi. Zagreb, Croatia: *Medicinska naklada*.

Bregman, B. & Slavinskka, S. (2012). Using emergency department data to conduct dog and animal bite surveillance in New York City 2003–2006. PublicHealth Reports, 127, 195–201.

Chaisiri, S. P., Ribas, A. & Morand, S. (2015). Potentially zoonotic helminthiases of murid rodents from the Indo -Chinese peninsula: impact of habitat and the risk of human infection. *Vector-Borne and Zoonotic Diseases, 15*(1), 73–85.

Chapman, L. E., Sullivent, E. E., Grohskopf, L. A., Beltrami, E. M., Perz, J. F., Kretsinger, K. et. al. (2008). recommendations for postexposure interventions to prevent Infection with hepatitis B, virus, hepatitis C virus, or human immunodeficiency virus, and tetanus in persons wounded during bombings and similar mass-casualty events -United States. *Morbidity and Mortality Weekly Report, 57*(6), 1-19.

Elliot, P. S. (2007). Rat Bite Fever and Streptobacillus moniliformis. *Clinical Microbiology Reviews, 20*(1): 13-22.

Gužvinec, M., Butić, I., Jelić, M., Bukovski, S., Lucić, S. & Tambić-Andrašević, A. (2012). Rezistencija na antibiotike u bakterije Pseudomonas aeruginosa. *Infektološki glasnik, 32*(2), 71-80.

Hagelskjaer, L., Sorensen, I., Randers, E. (1998). Streptobacillus moniliformis infection: two cases and a literature review. *Scandinavian Journal of Infectious Diseases*, 30, 309-311.

Han, B. A., Schmidt, J. P., Bowden, S. E. & Drake, J. M. (2015). Rodent reservoirs of future zoonotic diseases. *Proceedings of the National Academy of Sciences, 112* (22), 7039–7044.

Hirschhorn, R. B. & Hodge, R. R. (1999). Identification of risk factors in rat bite incidents involving humans. *Pediatrics*, 104, e35.

Hudsmith, L., Weston. V., Szram, J. & Allison, S.(2001). Clinical picture, rat bite fever. *Lancet Infectious Diseases*, 1, 91.

Hukić, M., Dedeić-Ljubović, A., Numanović, F., Ostojić, M., Tihić, N., Petković, M., Čustović, A., Gavrankapetanović, F., Koluder, N., Čamdžić, A., Švarakić, S. (2010). Prevencija i kontrola infekcija u zdravstvenim ustanovama. *Sarajevo, Bosnia & Herzegovina: TDP*.

Hukić, M., Nurkić, M., Kandić, Z., Numanović, F., Ćemerlić-Zvizdić, A., Dedeić-Ljubović, A., et.al. (2005). Bakteriologija. *Sarajevo, Bosnia & Herzegovina: Jež.*

Kalra, B., Kalra, S., Chatley, G. & Singh, H. (2006). Rat bite as a cause of diabetic foot ulcer-a series of eight cases. *Diabetologia,* 49, 1452–1453.

Katica, M. & Delibegović, S. (2019). Laboratorijske životinje-Osnovne tehnike eksperimentalnog rada. *Sarajevo, Bosnia & Herzegovina : Dobra knjiga*.

Katica, M., Kadić, M., Šaljić, E. (2003, May 14-16). Prilog proučavanju raširenosti i biologiji komensalnih glodara kao potencijalnih izvora u prijenosu različitih bolesti.Paper presented at the 1. Simpozij poljoprivrede, veterinarstva i šumarstva. Neum, Bosnia & Herzegovina.

Kovačić, A., Tafra, D., Hrenović, J., Goić-Barišić, I. & Dumanić, T. (2018). Preživljavanje bakterije *Pseudomonas aeruginosa* u destiliranoj vodi. *Hrvatske vode, 26*(105),181-186.

Marshall, J., Sharp, E. & Barrison, I. G. (1994). Once bitten, twice shy. *British Medical Journal*, 309, 1694-1695.

Mathiasen, T. & Rix, M. (1993). Rat bite-an infant bitten by a rat. *Ugeskr Laeger*, 155, 1475–1476.

Morosetti, G., Torson, M. & Pier, C. (2013). Lesions caused by animals in the autonomous province of South Tyrol in 2010: Fact-inding for prevention. *Veterinaria Italiana*, 49, 37–50.

National Research Council. (1997). Occupational health and safety in the care and use of research animals. Washington, DC: National Academy Press.

Ordog, G. J., Balasubramanium, S. & Wasserberger, J. (1985). Rat bites: fifty cases. *Annals of Emergency Medicine*, 14, 126–130.

Philipsen, T. E. J., Molderez, C. & Gys, T. (2006). Cat and dog bites. What to do? Guidelines for the treatment of cat and dog bites in humans. *Acta Chirurgica Belgica*,106, 692-695.

Rothe, K., Tsokos, M. & Handrick, W. (2015). Animal and human bite wounds. Deutsches *Ärzteblatt International,* 112, 433–43.

Stave, G. M., Lee, E. H. & Darcey, D. J. (2017). Laboratory animal bite anaphylaxis– a national survey part 1: Case series and review of the literature. *Journal of Occupational and Environmental Medicine, 59*(8), 728–738.

Syeda, H. F., Farrah, Z., Muhammad, A., Asad, A., Qaiser, J. & Muhammad, K. (2018). Rat-bites of an epidemic proportion in Peshawar vale; a GIS based approach in risk assessment. *Environmental Monitoring and Assessment,* 190-233.

Yanai, O., Goldin, L. & Hiss, J. (1999). Fatal rat bites. *Harefuah*, 136, 611–613.

Zimmerman, K., Moore, D. M. & Smiths, S. A. (2015). Hematological assessment in pet Guinea pigs (*Cavia procellus*): Blood sample collection and blood cell identification. *Veterinary Clinic of North America: Exotic Animal Practice*, 18, 33-40.

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Examination of the carcass in terms of quality and sanitation in broiler chickens fed with marine hydrobionts

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ABSTRACT

The objective of this study was to examine the quality and sanitary characteristics after post slaughter period of the carcasses of chickens which fed with marine hydrobiont. A total of 240, 12 day-old broiler chickens from Ross 308 breed were used in the study. The chickens were divided into three groups as two experimental groups and a control group. The chickens in the control group were fed with basal diet (BD) during the experiment. In the first experimental group, 7% of the basal diet was replaced with mineral additive (MA) while in the 2nd group 7% percent protein mineral additive was added to the feed of chickens. The chicks were fed with these feeds from 21 to 42 days of age. During the study the broilers were constantly observed considering the disposal of feed consumption and their general conditions. The chickens were slaughtered at the end of the study and following slaughtering process morphologic and sanitary assessment of carcasses was carried out. In addition, external appearance, visceral organs and carcass samples of slaughtered chickens were checked during the veterinary inspection. The microbiological investigation were performed by using *Colpoda steinii* infusorium. There was no abnormal changes in organs or appearance of tissues among control and experimental groups according to the post slaughter veterinary inspection. In addition, visually examination revealed that carcasses were identical for the control group and the experimental groups. There was no difference between the control and experimental group according to microbial contamination results. As a conclusion, it is evaluated that the meat of broiler chicken in the experimental groups are safe for consuming. Therefore, it was concluded that the prepared hydrobionts could be used safely in the poultry feeding.

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Introduction

In modern conditions animal production especially, meat is one of the main sectors in agricultural of Ukraine. Accordingly, researches for new feed sources which will contribute to covering traditional raw material shortage and increasing of animal production is getting more important. In European and many other countries of the world the problem of ration enrichment in animal husbandry is solved by using marine hydrobionts as a cheap source of raw protein, vitamins, mineral and bioactive substances.

There were numerous researches conducted by Odessa veterinary and sanitary school scientists to design modern technologies allowing to utilize marine hydrobionts especially mussels (Dankevych and Rozum 2018).

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They were also investigated the effect and efficiency of those feed stuff on farm animals and poultry production (Kovbasenko and Danylova,1981; Dankevych, 2018a).

This article represents the study results (veterinary and sanitary) on using feed stuff containing marine hydrobionts (Dankevych, 2018b), protein-mineral and mineral additives in poultry meat production produced by from the waste of processed marine hydrobionts. The technology of their production was approved by two useful model patents of Ukraine No34634 MPK (2008) A23 K 1\10, A 23 K 1\175 "Method of food additives production from marine hydrobionts for the poultry." No 4200808275; No 42687 MPK (2009) A 23 K1\10, A 23 K 1\175 "Method of food additives production from marine hydrobionts for the poultry" No 4200908402 (Kovbasenko and Dronova, 2008; Kovbasenko and Karaivan, 2009).

In the course of the study the veterinary and sanitary assessment, the analysis of feed additives as well as the post slaughter veterinary and sanitary expertise of carcasses were held.

The aim of this study is to examine the quality and sanitary characteristics of chicken carcasses, fed with marine hydrobiont after post slaughter period.

Materials and methods

The experiment was conducted in a broiler-growing company. Veterinary and sanitary examinations were conducted department of Odessa State Agrarian University.

Chickens and diet: Totally 12 day old 240 Ross 308 broiler chicken were used in the study. The chickens were divided into three groups as a control and 2 experimental groups each consist of 80 chicken (forty of them were fed with a diet consist of mineral additive and the other forty fed diet consist of protein-mineral-additive). The chickens in the control group were fed with basal diet (BD) during the experiment. In the 1th experimental group (group 1), 7% of the basal diet was replaced with mineral additive or protein mineral additive, while %7 percent mineral or protein-mineral additive was added to the feed of chickens in the 2nd group. The chicks were fed with these feeds from 21 to 42 days old age. Feed consumption and general conditions of broilers were continuously checked during the study.

Experimental procedures: At the end of the study, the chickens were slaughtered (10 chicken were selected from each groups), morphologic and sanitary assessment of carcasses was carried out. In the veterinary inspection, external appearance of chicken, visceral organs and carcass samples were checked. To determine whether the feeds given to chickens had an any harmful effect, *Colpoda steinii* infusoria was used (SVMM Gudlines, 2002). The method for identifying general toxicity of animal products designed by the veterinary and sanitary examination department of Odessa State Agrarian University and approved by the State Veterinary Medicine Department in 2000 (SDV. (2003).

Preparation of Colpada steinii culture: In the first step, a matrass with dry colpoda culture and another one with growing medium were opened. Then the matrass with colpoda is mixed with 4 ml of growing medium, closed with a cotton plug and placed into a thermostat at 26-28C° for 16-24 hours. At the same time, 2ml infusoria culture transferred into 2 clean matrasses. (one of them for the test, the other one for the control).

Preparation of aqueous extract: 20g animal materials (taken from chicken meat) were transferred into a matrass and added 250 ml of distilled water. The matrass was shaken at the speed of 120 rpm for 20 minutes, afterwards the aqueous dispersion filtered using filter paper. Thus, the aqueous extract was obtained. 2 ml of this extract added to the matrass which consist of active colpoda infusoria. In addition, 2 ml of distillated water added to control matrass. Both matrasses were placed in an incubator (26-28°C) for 10 minutes. After the incubation, a drop of sample was investigated under the light microscopy. If the sample does not contain live infusoria the examination was stopped, but if lots of active infusoria were observed, the experiment was continued for 3 more hours. After 3 hours, if 80-90% of infusoria didn't die, the incubation was extended for more 16- 24 hours. At the end of the incubation period 1 drop of 5% iodine solution was added to the test and control samples to fix the infusoria. The quantity of infusoria was counted using Fuks-Rosenthal counting chamber. Toxicity was determined according to the following criteria: very toxic= death of 100% infusoria during 10 minutes, toxic = death of 100% infusoria during 3 hours; low toxic = death of less than 80-90% infusoria and 90% intensity of growth for 3 hours, non-toxic = if all infusoria are alive and the intensity of growth is same or higher than control sample.

Bacterial contamination of broilers' carcasses: Bacterial contamination was determined by washing the carcass samples obtained different part of the chickens. For this purpose, samples were taken from

the surface of the carcasses (back area), inside (abdominal cavity – serous membrane) of the body and into the femur muscles (from 0,5-1cm depth of tissue). After the samples taken coliform and salmonella species were counted according to "Compulsory minimal list of raw material, animal and plant products research." guide of the State Department of Veterinary Medicine. №87 from 18.11.2003).

Results and Discussion

According to the post slaughter veterinary and sanitary assessment of broilers' carcasses, no abnormal changes were found in the tissue or organs of control and experimental groups. Visually control group carcasses were identical with those of the study groups. According to organoleptic examination, it was found that all the carcasses were high quality and complied with the following criteria: Exterior. Dry, yellow color with a shade of pink, closed glossy beak, a little gibbous eye bulb, glossy walleye, yellow color basting and visceral fat, wet glossy serous membranes without slime, hardly wet muscles in section of pale pink color (do not leave wet traces on the paper). Consistency: Firm and elastic muscles, when pressed with a finger a small pit appears but becomes even very fast when released. Smell. Specific smell of fresh meat.

Since marine products such as mussels accumulate in heavy metals, these products can also accumulate in chicken meat when used as feed additives. therefore, it may also cause toxic effects for those consuming these meats. For this reason, samples taken from chicken carcasses were test using *Colpada steinii*. The results obtained from the *Colpada steinii* test showed that the marine hydrobionts used as feed additives in this study did not have any toxic effect on chicken's meat.

Because it is observed that infusoria has grown as in control samples in carcass samples taken from chickens. The results similar with our previous results (Dankevych, 2018c ; Dankevych, 2018d).

The results obtained from the bacterial contamination showed that there are no significant differences between control and two experimental groups. The bacterial content of the carcasses in experimental group and test groups was almost the same (Table 1). In addition, it was also observed that considering the bacterial contamination, addition of mineral or protein-mineral in chicken feeds did not change the situation.

Table 1: Bacterial contamination of the chicken carcasses

Area of the taken sampling	Total cont. (CFU per 100 ml)	E. coli (CFU per 100ml)	Salmonella (CFU per 100 ml)
	Chicken fed with protein-mineral additive diet		
Surface of carcass (Back area)			
Control	110.4 ± 4,50	1.8 ± 0.21	36.7 ± 1.27
Group 1	112.1 ± 5,42	2.4 ± 1.26	40.1 ± 2.18
Group 2	100.6 ± 3,42	2.9 ± 0.12	38.8 ± 1.19
	NS	NS	NS
Inside of carcass (A. Cavity-Serous membrane)			
Control	48.6 ± 2.64	1.2 ± 0.18	19.4 ± 0.48
Group 1	51.2 ± 3.24	2.9 ± 0.18	20.4 ± 3.24
Group 2	50.2 ± 2.17	2.9 ± 0.17	27.6 ± 2.04
	NS	NS	NS
Muscles (Femur Muscles)			
Control	-	-	-
Group 1	-	-	-
Group 2	-	-	-
	Chicken fed with mineral additive diet		
Surface of carcass (Back area)			
Control	124.5 ± 6.12	2.1 ± 0.72	41.2 ± 3.41
Group 1	118.6 ± 2.08	1.8 ± 0.36	39.6 ± 2.12
Group 2	120.2 ± 5.41	2.4 ± 0.37	20.0 ± 2.64
	NS	NS	NS
Inside of carcass (A. Cavity-Serous membrane)			
Control	52.2 ± 5.04	2.6 ± 1.21	23.2 ± 6.81
Group 1	49.2 ± 1.18	2.0 ± 0.42	21.7 ± 1.24
Group 2	50.1 ± 4.46	2.1 ± 0.62	22.4 ± 1.18
	NS	NS	NS
Muscles (Femur Muscles)			
Control	-	-	-
Group 1	-	-	-
Group 2	-	-	-

Total cont. = total contamination, CFU = colony forming unit, A. cavity = abdominal cavity, NS = non-significant. Group 1 = chicken fed basal diet, Group 1 = chicken fed 7% feed additive (7% of basal diet replaced with mineral or protein-mineral additive), Group 2 = chicken fed %7 feed additive (extra % 7 percent mineral or protein-mineral additive was added to the diet).

Conclusion

The results obtained from this study showed that the marine hydrobionts used in poultry feeds do not adversely affect the microbiological contamination and quality of the carcass. Therefore, it was thought that mineral additive or protein-mineral additives obtained from marine hydrobionts could be used safely in chicken feeds.

References

Dankevych, N. I. (2018a). Sanitary evaluation of waste from the processing of sea hydrobinotes used for the production of fees. *Scientific reports of NULES of Ukraine,4*(74). (2018.04.018). Retrieved from htpp: // dx.doi.org/10.31548/dopovidi 2018.04.018. [in Ukrainian].

Dankevych, N. (2018b). Non-waste hydrobionts management. *Journal of Istanbul veterinary Sciences, 2*(2), 61-65.

Dankevych, N. I. (2018c). The technology for producing marine hydrobiont feed additives for the poultry, their fodder value and sanitary evaluation. *Veterinary biotechnology*, *33*(2),16-24 (in Ukrainian).

Dankevych, N. I. (2018d). The impact of sea hydrobionts additives on boiler ducks. *Bulletin of Poltava state agrarian Academy*, *3*(90). [in Ukrainian].

Dankevych, N. I. & E. Y. Rozum (2018). The impact of hydrobionts additives on ducks. Agrarian Bulletin of the Black Sea Region. *Agricultural and biological sciences*,91. 75. [in Ukrainian].

Kovbasenko, V. M., & Danylov, A. A. (1981). Guidelines for processing mussels into feed additives. Guidelines. Odessa: OAI [in Ukrainian].

Kovbasenko, V. M., & Dronova, N.I. (2008). Method of producing feed supplement for poultry from marine hydrobionts. Patent of Ukraine for useful model. A23K 1/175. № 34634; declared 19.06.2008; published 11.08.2008, № 15. [in Ukrainian].

Kovbasenko, V. M., & Karaivan, N.I. (2009). Method of producing feed supplement for poultry from marine hydrobionts. Patent of Ukraine for useful model. A23K 1/10, A23K 1/175. № 42687; declared 09.04.2009; published 10.07.2009, № 13. [in Ukrainian].

Kovbasenko, V. M., & Karaivan, N. I. (2009). Method of producing feed supplement for poultry from marine hydrobionts. Patent of Ukraine for useful model. A23K 1/10, A23K 1/175. № 42687; declared 09.04.2009; published 10.07.2009, № 13. [in Ukrainian].

SDV. (2003). Mandatory minimum list of research materials, products of animal and vegetable origin, animal feed raw materials, feed, medicines and fortified al., Which should be in public veterinary laboratories. Order of the State Department of Veterinary Medicine №87 from 18.11.2003. www.document.ua.Retrievedfrom:http:// document.ua/obovjakovii-minimalnii-perelik-doslidzhensirovini-produkciy-nor8259.html[in Ukrainian].

SVMM Gudlines (2002). Method of application of culture of *Colpoda steinii* (kolpoda) dry for toxicological researches of meat and meat products from animals and poultry. Guidelines from 11.03.2002. State Department of Veterinary Medicine Ministry of Ukraine Policy. [in Ukrainian].

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The effects of *Spirulina* (*Arthrospira*) *platensis* on morphological and hematological parameters evoked by social stress in male rats

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ABSTRACT

Stress is a complex phenomenon and exposure to stress results in a series of reactions in the organism, including alterations in behaviour and various physiological changes. Role of nutrition in the maintenance of homeostatic mechanisms, including the stress, is very dense. The current study aimed to evaluate the potential effects of *Spirulina* (*Arthrospira*) *platensis* against mix stress models. For this purpose, 36 Sprague-Dawley male rats were allocated into four groups; 1. Control(C), 2. Stress(S), 3. *S.platensis* (Sp) and 4. *S. platensis* + Stress (SpS). *S. platensis* was applied to Sp and SpS groups by oral gavage (1500 mg/kg/day) for 28 days. All rats were exposed to light : dark cycle (long lightening period; 18h light : 6h dark) stress for 14 days. Also, S and SpS groups were stressed with additional mix stress by leaving in crowded environment and hosting alone under long lightening period. The animals which fed with *S. platensis*, shown significant changes in the numbers of circulating leukocytes, % of neutrophils, and the neutrophil : lymphocyte ratio. However, there were no significant differences in the morphological parameters. In conclusion, the possible preventive effect of *S. platensis* on hematological parameters was shown in a rat's stress model of social stress which was included mix stress under long lightening period.

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Introduction

Stress, depending on the type and intensity of stress, can lead to death in animals, because of adaptation problems, pathological changes and failure to cope with the new situation due to severe disorders in homeostasis (Benyo et al., 2007; Sejan et al., 2011). Many factors, such as the environmental temperature changes that exceeds the limits of thermoneutral zone, hosting in crowded environments, and leaving animals alone, which were group hosting, can cause stress and may lead physiological changes in the organism. Especially crowded hosting and high temperature may adversely affect feed intake, intestinal health and thereby growth (Meddings and Swain, 2000; Mawdsley and Rampton, 2005).

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Stress factors can influence either the intestinal flora or feed efficiency and weight gain in rats (Marin et al., 2007). Although rats can adapt to the environmental temperature between 10-30°C, homeostatic mechanisms can be forced due to changes in the environmental temperature, and thereby stress occurs. Some researchers determined that increase in environmental temperature or crowdedness and stuffiness may constitute to the decrease both in feed consumption and growth in several animals (Peng et al., 1989; Keeling et al., 2003). In addition, the light and dark periods are important throughout the day for rats to survive in the physiological limits. This situation creates the photoperiodic memory of rats. It was shown that natural bright period increases rat welfare and survival rate, however, long dark period is resulted with a low heart rate in animals (Azar et al., 2008). Besides natural lightening period of rats (12h Light: 12h Dark), long and short lightening periods have been researched for determine the animal model for human (Boon et al., 1997; Ebling 1994). Although the decrease in growth and metabolic rate of day were reported in short lightening period studies (Boon et al., 1997), it was determined that the long lightening period has the positive effect on weight (Ebling, 1994). It was also shown that increasing or decreasing lightening treatments are associated with feed consumption and body weight of rats (Shöamker and Heideman, 2002; Markova et al., 2003). Shöamker and Heideman (2002) reported that there is a decrease in body weight within the weights of heart muscle, adrenal gland and liver in rats, which had been fed with melatonin, compared to control group, under normal lightening period. Insight of literatures, to evaluate the some morphological and physiological parameters, in the present study, it was trying to modelize a stress model for future human studies. For this purpose crowded environment and hosting alone stresses were studied under long lightening period.

Exogenous vitamins such as vitamin E and vitamin C, some minerals and natural additives are considered as protective against stress. (Botsoglu et al., 2002; Sengezer and Gungor, 2008; Altiner et al., 2017). It is known that feed additives improve the digestive system of animals and enable them to capture their genetic potential in growth performance. In recent years Spirulina has a considerable place among these natural additives. Spirulina is a planktonic, spiral, blue-green algae which is also a traditional food of Mexican and African societies. *S. platensis* is widely used as a natural supplement to regulate the effects of stress in organism. It's known as an important herbal supplement due to its immunomodulator, antioxidant and protective effects. *S. platensis* has important contents such as high protein, polyphenols, phycocyanin, minerals and vitamin C (Khan et al., 2005; Seyidoglu et al., 2017). It can be digested easily due to its non cellulose structure on its cell wall, and thereby it enhances growth (Moreira et al., 2011; Seyidoglu and Galip, 2014; Seyidoglu et al., 2017a). Several studies reported the effects of Spirulina on haematological parameters and growth performances in rats (Araujo et al., 2003; Simsek et al., 2007; Promya and Chitmanat, 2011). Araujo et al. (2003) determined increased body weight gain in rats which had been added 10% Spirulina to feed. On the other hand, Simsek et al. (2007) identified that 300 mg/kg *S. platensis* increase the erythrocyte count and haemoglobin concentration in rats. In another study which have done with fishes (*Silurus glanis*), the Erythrocyte and Leukocyte counts were increased by 3% and 5% Spirulina additive (Promya and Chitmanat, 2011). Researchers specified that these effects are correlated with the stimulating effect of Spirulina on the stem cell activity of bone marrow.

In this study, we examined the effects of *S. platensis,* which is called as an alternative super food by the World Health Organization, on weight, body mass index and hematological parameters in rats exposed to a mix stress model which includes crowded environment and hosting alone, under long lightening period.

Materials and methods

Animals: The experimental protocols were approved by the Animal Care and Use Committee of Bursa Uludag University and are in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. The study was carried out with the permission of University Animal Experimentation Local Ethics Committee (Approval No: 2018-07/02). Thirty six adult, healthy, male Sprague-Dawley rats (age about 10 -12 weeks and average body weight 200-250 g) were included in this study and the animals were allocated into 4 experimental groups. The groups were Control (C -basal diet), Stress (S-basal diet), *S. platensis* (1500 mg/ kg/day) (Sp), *S. platensis* (1500 mg/kg/day) and Stress (SpS).

Experiment Set: Each cage, with four transparent sides, had 3 rats for 5 trial weeks. The first week was the adaptation period to trial. The second and third weeks are the application periods of *S. platensis* to rats.

S. platensis (Egert, Izmir-Turkey) were given 1500 mg/kg/daily by oral gavage. In the study, 3 different stress applications were mixed and applied during 4th and 5th weeks with supplementation of *S. platensis* as follow. *Light : Dark Cycle Stress (Long Lightening)*: In normal condition, the rats live in 12h light and 12h dark cycle in one day period. This stress was exposed to all rats with 18h light and 6h dark cycle during the 4th and 5th weeks of trial. *Hosting Alone Stress:* This stress was applied in a separate cage (50x50) of which 4 sides and ground covered with white paper. The rat was left alone for 30 min and was given neither food nor water during the stress application period. This stress was applied on Monday, Wednesday, Friday and Sunday of the 4th week of the trail. *Crowded Environment Stress:* This stress was applied by placing 6 rats in a cage, which is designed for 3 rats, for 30 min. Neither food nor water was given to the animals during the stress application period. This stress was applied on Tuesday, Thursday and Saturday of the 5th week of the trial.

Measurement: The effects of *S. platensis* on height, waist circumference (WC), body mass index (BMI) and waist circum/height ratio were determined at the beginning and the end of the study. Also, body weights were measured weekly. Blood samples were obtained by the puncture of the heart under isoflurane anesthesia at the end of the 5th week from overnight-fasted rats. The blood hematocrit, haemoglobin, counts of erythrocyte and leukocyte, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and types of leukocyte (neutrophil, lymphocyte and monocyte) were determined by using automatic blood counter device (VetScan HM5, ABAXIS) in laboratory of Veterinary Medicine Faculty Animal Hospital, Bursa Uludag University.

Statistical analysis: Statistical analyses were performed with SPSS (Version 17.0). Data were tested for normality distribution and variance homogeneity assumptions. All the values were grouped and the means and standard errors were calculated. One-way ANOVA was applied to the all parameters to examine the difference between groups. Differences were considered significant at P<0.05. For hematological and morphological parameters, if the differences between groups were provided to be significant (P<0.05), differences evaluated by Tukey's test (Dowdy and Wearden, 1981). On the other hand, in non-homogenous groups, differences between means were analyzed by Kruskal Wallis and following Mann Whitney U test between groups one by one. Also, the variance analysis for repetitive measurements were analyzed using a repeated measures ANOVA for weekly body weight (Dawson and Trapp, 2001).

Results

The important blood parameters which measured for stress condition such as leukocytes, neutrophils and neutrophil : lymphocyte (N/L) ratio obtained from the study are given in Figure 1-2-3. Leukocytes were decreased in S group compared to C group statistically (p:0,026). In SpS group, it was increased significantly compared to S (p:0,014). On the other hand, leukocytes were increased in Sp group compared to C (p:0,022). Neutrophils and N/L ratio were increased in S group compared to C (p:0,009 ; p: 0,002 neutrophils, N/L ratio respectively). Although there were no statistically differences, neutrophils and N/L ratio were decreased in SpS group compared to S (p>0.05). Also, N/L ratio were decreased in group Sp compared to C (p:0,030). On the other hand, there were no significant changes in other some blood parameters (Table 1; p>0.05).

In the study, there was no statistically difference among all groups for body weight weekly shown in Figure 4 (p>0.05). Nevertheless, no significant differences were found among all groups in some morphological parameters such as BMI, height, WC and waist circum./height ratio (Table 2, p>0.05).

Table 1: Some hematological parameters in control and experimental groups. (The values represent mean± standard error from n=9).

Blood Parameters	Experimental groups			
	C	**S**	**Sp**	**SpS**
RBC (10^6/mm^3)	7.43±0.14	6.99±0.06	7.29±0.17	7.08±0.20
Hb (g/dl)	12.87±0.17	13.38±0.27	13.37±0.06	12.90±0.13
HCT (%)	41.88±0.75	39.85±0.55	41.21±0.92	39.98±0.39
Lym (%)	74.33±1.27	71.61±2.16	78.05±2.88	72.06±1.86
Mon (%)	3.25±0.38	4.15±0.54	3.38±0.28	3.98±0.31
MCV (fl)	56.00±0.63	58.40±0.40	56.00±0.55	55.80±0.20
MCH (pg)	17.85±0.20	18.40±0.11	17.86±0.23	18.32±0.14
MCHC (g/d)	31.98±0.16	32.40±0.32	31.90±0.26	32.20±0.21

C = Control, **S** = Stress, **Sp** = *S. platensis*, **SpS** = Stress + *S. platensis*, **RBC** = Erythrocyte, **Hb** = Hemoglobin, **HCT** = Hematocrit, **Lym** = Lymphocyte, **Mon** = Monocyte, **MCV** = Mean corpuscular volume, **MCH** = Mean corpuscular hemoglobin, **MCHC** = Mean corpuscular hemoglobin concentration.

Table 2. Some morphological parameters in control and experimental groups. (The values represent mean ± standard error from n = 9)

	Experimenal Groups			
Morphological parameters	C	S	Sp	SpS
Initial body mass index (kg/m^2)	0.17 ± 0.01	0.17 ± 0.01	0.16 ± 0.00	0.17 ± 0.01
Final body mass index (kg/m^2)	0.19 ± 0.00	0.18 ± 0.00	0.18 ± 0.01	0.18 ± 0.01
Initial body length (cm)	38.67 ± 0.88	37.89 ± 1.08	38.78 ± 0.60	38.00 ± 0.99
Final body length (cm)	40.39 ± 0.65	41.33 ± 0.55	41.75 ± 0.33	41.17 ± 0.56
Initial waist circumference (cm)	14.00 ± 0.22	14.17 ± 0.47	13.89 ± 0.22	13.61 ± 0,47
Final waist circumference (cm)	14.39 ± 0.23	14.83 ± 0.25	15.00 ± 0.19	14.78 ± 0.32
Initial waist circum/length	0.36 ± 0.00	0.37 ± 0.00	0.36 ± 0.01	0.36 ± 0.01
Final waist circum/length	0.36 ± 0.00	0.36 ± 0.00	0.36 ± 0.01	0.36 ± 0.01

C = Control, **S** = Stress, **Sp** = *S. Platensis*, **SpS** = Stress + *S. platensis,*

Discussion

The impact of light and photoperiodicity on physiology of mammalian species is well documented. Photoperiodically sensitive animals respond to altered light regimen with changes in growth, food intake, reproductive status and behaviour. The optimal photoperiod is unknown for most species but a 12 h light: 12 h dark cycle is used for most of the laboratory animals (Harper and Lawrence, 2011). Limited studies have found the effects of the lighting period and life cycle on hematological parameters for Sprague Dawley rats. Nelson et al. (1994) reported that many rat species are sensitive to photoperiodic phase. It was also reported that although weight gains were decreased by 8 hour light stress in Harlan Sprague Dawley rats, in Brown Norway rats it was increased (Francisco et al., 2004). Poyraz (2000) reported that the growth parameters were effected by light duration in rodents. Although Warner et al. (2010) found that the shortened light for hamster is associated with low growth, Moffatt et al. (1991) determined that growth is stimulated by short light time. The other stress factor, crowded environment, is defined as animal density in a cage that stimulates the physiological, behavioral and molecular changes in organism (Benyo et al., 2007). Besides that, animals have limited physical activity, feed intake and growth in crowded environments (Armario et al., 1984). Some researchers found that crowded environment stress causes a decrease in body weight and food intake and thereby body mass index (Marin et al., 2007; Eid et al., 2010). On the other hand, it was reviewed that hosting alone is an important stress factor which has negative influences on organism such as depression, anxiety, irritability or hostility (Ernst and Cacioppo, 1999). All these instances are correlated with growth and physiology (Miller, 1998; Dantzer et al., 1999; Kiecolt-Glaser and Glaser, 2002). Some researchers reported that hosting alone stress is more stressful than other stress factors, whereas some of them observed no differences (Giralt and Armario, 1989; Gambardella et al., 1994; Sharp et al., 2002). However, in the present study, no differences were occurred on morphological parameters and weights for the stress conditions.

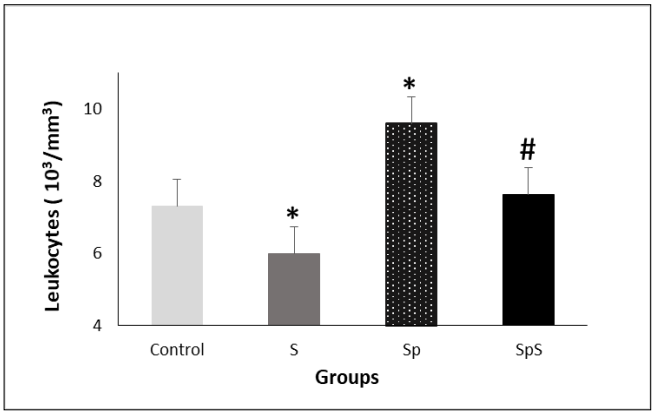

Figure 1. Leukocytes number in control and experimental groups.

S = Stress, **Sp** = *Spirulina platensis* **SpS**= Stress + *S. platensis*. All data are expressed as means ± SE. * $p < 0.05$, S versus C group ; Sp versus C group, # $p < 0.05$, SpS versus S group.

Importantly, in this study, WBC was decreased in S group although it was increased in SpS (Stress and *S. platensis*). In contrast, significant increase in WBC was observed in *S. platensis* group (Sp) when compared with control group (C). There is no significant difference on WBC between the groups Sp and S. *S. platensis* is called as a super food which has several

effects on growth, antioxidant mechanism, health and life quality (Gorber et al., 2007; Park et al., 2008; Nasirian et al., 2017; Seyidoglu et al., 2017). It's also important for growth and cell regeneration. It was reported that spirulina has an inhibitory effect on development of leucopenia and anemia induced by lead and cadmium in rats (Simsek et al., 2009).

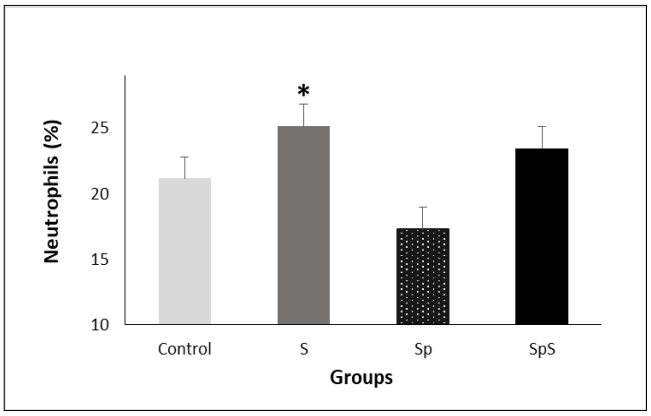

Figure 2. Neutrophil percentage in control and experimental groups.

S = Stress, **Sp** = *Spirulina platensis,* **SpS** = Stress + *S. platensis*. All data are expressed as means ± SE. * $p < 0.05$, S versus C group ; Sp versus C group, # $p < 0.05$, SpS versus S group.

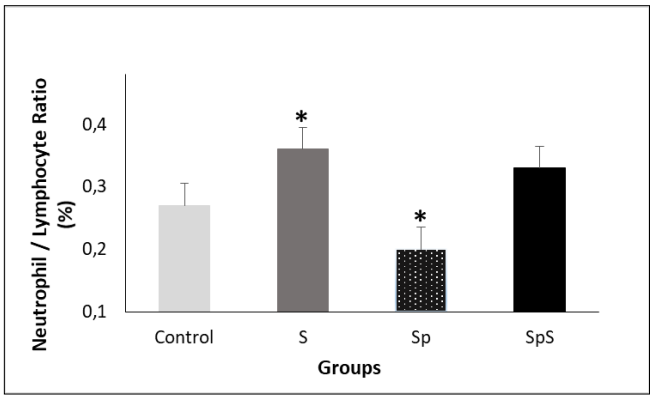

Figure 3. Neutrophil : Lymphocyte ratio in control and experimental groups.

S = Stress, **Sp** = *Spirulina platensis*, **SpS** = Stress + S. *platensis*. All data are expressed as means ± SE. * $p < 0.05$, S versus C group ; Sp versus C group, # $p < 0.05$, SpS versus S group.

Sixabela et al. (2011) determined decreased hematocrit in rats fed Spirulina due to its effect on hydration status and plasma volume. *S. platensis* known as a powerful antioxidant in herbal supplements. Its contents phycocyanin, tocopherols, beta carotene and vitamin C are in progress of growth and health (Karkos et al 2011; Abdel-Daim et al., 2013). It was observed that *S. platensis* has positive impact on interleukin and tumor necrosis factor which are responsible to cellular response in carps, and also helps to produce red and white blood cell and interferons in rats (Lisheng et al., 1991; Watanuki et al., 2006).

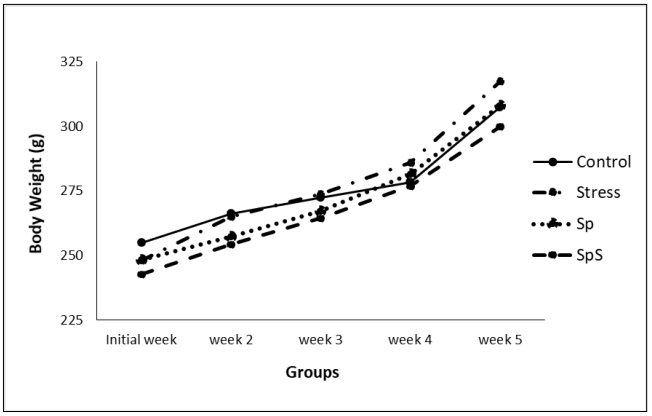

Figure 4. Weekly body weight in control and experimental groups.

Sp = *Spirulina platensis,* **SpS** = Stress + *S. platensis*. All data are expressed as means ± SE. There is no statistically difference among all groups for body weight ($p > 0.05$).

Conclusion

Stress changes the natural homeostasis of organism either growth or physiological condition. In the present study, there were no differences found in weights and hematological parameters except leukocytes, neutrophils and N/L ratio in group S compared to C. Also all parameters were in their normal reference values. This may be explained with the environmental condition, feeding procedure, the antioxidant dose and rat species. Nevertheless, N/L ratio is one of the physiological stress marker which also used to determine the stress indicator. In the present study, N/L ratio was found higher in S group compared to C, and also it was decreased with *S. platensis* feeding in group Sp, statistically. It can be said that *S. platensis* would exhibit a higher level of welfare and is effective for stress conditions.

References

Abdel-Daim, M. M., Abuzead, S. M., & Halawa, S. M. (2013). Protective role of Spirulina platensis against acutedeltamethrin-induced toxicity in rats, PLoS One, *8*(9), e72991.

Altiner, A., Atalay, H. & Bilal, T. (2017). Bir antioksidan olarak E vitamini. *Balikesir Sağlik Bilimleri Dergisi, 6*(3), 149-157.

Araujo, K. G. L., Facchınetti, A. D. & Santos, C. P. (2003). Influence of intake of Spirulina biomass on body weight and feed intake in rats. *Journal of Food Science and Technology, 23*(1), 6-9.

Armario, A., Castellanos, J. M. & Balasch, J. (1984). Effect of crowding on emotional reactivity in male rats. *Neuroendocrinology, 39*(4), 330–333.

Azar, T. A., Sharp, S. L., & Lawson, D. M. (2008). Effect of Housing Rats in Dim Light or Long Nights on Heart Rate. *Journal of the American Association for Laboratory Animal Science, 47*(4), 25-34.

Benyo, Z., Szabo, C., & Kova, A.P. (2007). Prevention of the hemorrhagic hypotension-induced hepatic arterial vasoconstriction by L-arginine and naloxone. *Shock,* 11, 342–346.

Boon, P., Visser, H., Daan, S. (1997). Effect of photoperiod on body mass and daily energy intake and energy expenditure in young rats. *Physiology & Behavior,* 62, 913 -919.

Botsoglou, N.A., Florou-Paneri, P., Christaki, E., Fletouris, D.J. & Spais, A.B. (2002). Effect of dietary oregano essential oil on performance of chickens and on iron-induced lipid oxidation of breast, thigh and abdominal fat tissues. *British Poultry Science, 43*(2), 223-230.

Dantzer, R., Wollman, E., Vitkovic, L. & Yirmiya, R. (1999). Cytokines and depression: Fortuitous or causative association?. *Molecular Psychiatry,* 4, 328-332.

Dawson, B., & Trapp, R.G. (2001). Basic & Clinical Biostatistics. 3rd ed. New York, USA: Lange Medical Books/ McGraw International Editions.

Dowdy, S. & Wearden, S. (1981). Statistics for Research. New York, USA: John Wiley & Sons Press

Ebling, F.J.P. (1994). Photoperiodic differences during development in the dwarf hamsters P. sungorus and P.campbelli. *General and Comparative Endocrinology*, 95, 475-482.

Eid, F., Helal, E. G. E., & Taha, N.M. (2010). Effect of crowding stress and /or sulpiride treatment on some physiological and histological parameters in female albino rats. The *Egyptian Journal of Hospital Medicine*, 41, 566-589.

Ernst, J. M. & Cacioppo, J. T. (1999). Lonely hearts: Psychological perspectives on loneliness. Applied and *Preventive Psychology, 8*(1), 1-22.

Francisco, N.R., Raymond, C.M., & Heideman, P.D. (2004). Short photoperiod inhibition growth inbody mass and reproduction in ACI, BUF and PVG inbred rats. *Reproduction*, 128, 857-862.

Gambardella, P., Greco, A. M., Sticchi, R., Bellotti, R. & Di Renzo G. (1994). Individual housing modulates daily rhythms of hypothalamic catecholaminergic system and circulating hormones in adult male rats. *The Journal of Biological and Medical Rhythm Research, 11*(4), 213-221.

Giralt, M. & Armario, A. (1989). Individual housing does not influence the adaptation of the pituitary–adrenal axis and other physiological variables to chronic stress in adult male rats. *Physiology & Behaviour*, 45(3), 477-481.

Gorber, S.C., Tremblay, M., Moher, D. & Gorber, B. (2007). A comparison of direct vs. self-report measures for assessing height, weight and body mass index: a systematic review. *Obesity Reviews*, 8(4), 307-26.

Harper, C. & Lawrence, C. (2011). The Laboratory Zebrafish. Florida, USA: CRC Press / Taylor & Francis Group.

Karkos, P.D., Leong, S.C., Karkos, C.D., Sivaji, N. & Assimakopoulos, D.A. (2011). Spirulina in clinical practice: Evidence-based human applications. *Evidence Based Complementary and Alternative Medicine*, 2011, article ID531053.

Keeling, L.J., Estevez, I., Newberry, R.C. & Correia, M.G. (2003). Production-related traits of layers reared in different sized flocks: the concept of problematic intermediate group sizes. *Poultry Science, 82*(9), 1393–1396.

Khan, M., Shobha, J.C., Mohan, I.K., Naidu, M.U., Sundaram, C., Singh, S., Kuppusamy, P. & Kutala, V.K. (2005). Protective effect of Spirulina against doxorubicin-induced cardiotoxicity. *Phytotherapy Research, 19*(12), 1030-1037.

Kiecolt-Glaser, J.K. & Glaser, R. (2002). Depression and immune function: Central pathways to morbidity and mortality. *Journal of Psychosomatic Research, 53*(4), 873-876.

Lisheng, L., Baojiang, G., Jihong, R., Guangquan, Q. & Botang, W. (1991). Inhibitive effect andmechanism of polysaccharide of Spirulina platensis on transplanted tumor cells in mice. *Marine Science*, 5, 33–38.

Marin, T.M., Cruz, F.C., & Planeta, C.S. (2007). Chronic restraint or variable stress differently affect the behavior , corticosteron secretion and body weight in rats. *Physiology & Behaviour*, 90(1), 29-35.

Markova, M., Adamekova, E., Kubatka, P., Bojkova, B., Ahlersova, E. & Ahlers, I. (2003). Effect of prolonged melatonin administration on metabolic parameters and organ weights in young male and female Sprague-dawley rats. Acta Veterinaria Brno, 72, 163-173.

Mawdsley, J. E. & Rampton, D. S. (2005). Psychological stress in IBD: new insights into pathogenic and therapeutic implications. Gut, 54, 1481–1491.

Meddings, J.B. & Swain, M.G. (2000). Environmental stress-induced gastrointestinal permeability is mediated by endogenous glucocorticoids in the rat. Gastroenterology, 119, 1019–1028.

Miller, A. H. (1998). Neuroendocrine and immune system interactions in stress and depression. Psychiatric Clinics of North America, 21(2), 443-463.

Moffatt, C.A., Bennett, S.A. & Nelson, R.J. (1991). Effects of photoperiod and 6-methoxy 2- benzoxazolinone on male-induced estrus in Praitie vole. Physiology & Behaviour, 49, 27-31.

Moreira, L.M., Rocha, A.S.R., Rıbeıro, C.L.G., Rodrıgues, R.S. & Soares, L.S. (2011). Nutritional evaluation of single-cell protein produced by Spirulina platensis. *African Journal of Food Science*, 5(15), 799-805.

Nasirian, F., Mesbahzadeh, B., Maleki, S.A., Mogharnasi, M. & Kor, N.M. (2017). The effects of oral supplementation ofspirulina platensis microalgae on hematologicalparameters in streptozotocin-induced diabetic rats. *American Journal of Translational Research, 9*(12), 5238-5244

Nelson, R.J., Moffatt, C.A., & Goldman, B.D. (1994). Reproductive and non-reproductiveresponsiveness to photoperiod in laboratory rats. *Journal of Pineal Research*, 17, 123.

Park, H.J., Lee, Y.J., Ryu, H.K., Kim, M.H., Chung, H.W. & Kim, W.Y. (2008). A randomized double-blind, placebo-controlled study to establish the effects of spirulina in elderly Koreans. *Annals of Nutrition and Metabolism*, 52, 322-328.

Peng, X., Lang, C.M., Drozdowicz, C.K. & Ohlsson-Wilhelm, B.M. (1989). Effect of cage population density on plasma corticosterone and peripheral lymphocyte populations of laboratory mice. *Laboratory Animals*, 23, 302–306.

Poyraz, O. (2000). Laboratuvar Hayvanları Bilimi. Ankara, Türkiye: Kardelen Yayınevi.

Promya, J. & Chitmanat, C. (2011). The effects of Spirulina platensis and Cladophora algae on the growth performance, meat quality and immunity stimulating capacity of the African sharptooth catfish (Clarias gariepinus). *International Journal of Agriculture and Biology*, 13, 77–82.

Sejian, V., Maurya, V.P., & Naqvi, S.M.K. (2011). Effect of thermal stress, restricted feeding and combined stresses (thermal stress and restricted feeding) on growth and plasma reproductive hormone levels of Malpura ewes under semiarid tropical environment. *Journal of Animal Physiology and Animal Nutrition*, 95(2), 252– 258.

Sengezer, E. & Güngör, T. (2008). Esansiyel yağların hayvanlar üzerindeki etkileri. *Lalahan Hayvan Arastırma Enstitüsü Dergisi*, 48(2), 101-110.

Seyidoglu, N. & Galip N. (2014). Effects of Saccharomyces cerevisiae and Spirulina platensis on Growth Performances and Biochemical Parameters in Rabbits. *Journal of the Faculty of Veterinary Medicine Kafkas University*, 20, 331-336.

Seyidoglu, N., Inan, S., & Aydin C. (2017). A Prominent Superfood: Spirulina platensis, In N. Shiomi, V. Waisundara (Ed). Superfood and Functional Food The Development of Superfoods and Their Roles as Medicine (pp. 1-27). Zagreb, Croita: InTech.

Seyidoglu N., Galip N., Budak F., & Uzabaci E. (2017a). The Effects Of Spirulina platensis And Saccharomyces cerevisiae On The Distribution and Cytokine Production Of CD4+ And CD8+ T-Lymphocytes In Rabbits. *Australian Journal of Veterinary Sciences*, 49, 185-190.

Sharp, J.L., Zammit, T.G., Azar, T.A. & Lawson, D.M. (2002). Stress-like responses to common procedures in male rats housed alone or with other rats. *American Association for Laboratory Animal Science, 41*(4), 8-14.

Shöamker, M.B. & Heideman, P.D. (2002). Reduced body mass, food intake and testis size in response to short photoperiod in adult F344 rats. *BMC Physiology, 2*(11).

Simsek, N., Karadenız, A. & Karaca, T. (2007). Effects of the Spirulina platensis and Panaxginseng oral supplementation on peripheral blood cells in rats. *Revue de Médecine Vétérinaire,158*(10), 483-488.

Simsek, N., Karadeniz, A., Kalkan, Y., Keles, O.N., & Unal, B. (2009). Spirulina platensis feeding inhibited the anemia and leucopenia- induced lead and camium in rats. *Journal of Hazardous Materials, 1*64, 1304-1309.

Sixabela, P.S.S, Chivandi, E., Bademhorst, M., & Erlwanger, K.H. (2011). The effects of Dietary supplemenatation with Spirulina platensis in growing rats. *Asian Journal of Animal and Veterinary Advances,* 1-9.

Warner, A., Jethwa, P.H., Whse, C.A., I'Anson, H., Brameld, J.H. & Eblind, F.J. (2010). Effect of photoperiod on daily locomotor activity, energy, expenditure and feeding behavior in a seaonal mammal. *American Journal of Physiology Regulatory Integrative and Comparative Physi*ology, 298, 1409-1416.

Watanuki, H., Ota, K., Tassakka, A.C.M.A.R., Kato, T. & Sakai, M. (2006). Immunostimulanteffects of dietary Spirulina platensis on carp, Cyprinus carpio. *Aquaculture,* 258, 157–163 .