Bulgarian Journal of Veterinary Medicine, 2019, **22**, Suppl. 1, 40–49
ISSN 1311-1477 (print); ISSN 1313-3543 (online)

EFFECTS OF PHYTOGENIC INGREDIENTS ON HAEMATOLOGICAL AND MORPHOLOGICAL PARAMETERS IN STRESS- EXPOSED MALE RATS

E. KOSELI, N. SEYIDOGLU & C. AYDIN

[1]Bursa Uludag University, Faculty of Veterinary Medicine, Department of Physiology, Nilufer, Bursa, Turkey; [2]Tekirdag Namik Kemal University, Faculty of Veterinary Medicine, Department of Physiology, Suleymanpasa, Tekirdag, Turkey

Summary

Koseli, E., N. Seyidoglu & C. Aydin, 2019. Effects of phytogenic ingredients on haematological and morphological parameters in stress-exposed male rats. *Bulg. J. Vet. Med.*, **22**, Suppl. 1, 40–49.

The world feeding strategies of animals have been changed due to the alterations in the ecosystem over the past century. Existing animal feeding practices cause stress that influences growth, production, disease susceptibility, and animal welfare. Agreeing to this issue, the use of complex vitamins, minerals, herbaceous plants, and herbal extracts are important for regulating homeostasis, animal production and health. The purpose of the present study was to provide an overview of the effects of Livervital (MIAVIT GmbH, Essen, Germany) which includes colin, carnitine, vitamin E, melatonin and some herbal extracts on stress conditions and describe its possible approach of action. For this purpose, forty-eight, adult, male Sprague-Dawley rats were randomly separated into four groups; Control (C), Stress (S), Livervital (Li) and Livervital+Stress (LiS). Group C and S served as control groups. Livervital was applied to Li and LiS groups by adding into water (2 mL/L) 5 days a week for 28 days. All rats were stressed by prolonged light phase conditions (18 h light: 6 h dark) for 14 days. Additional chronic stresses, hosting alone and in crowded environments, were applied to LiS and S groups. There was a significant decrease of leukocytes, erythrocytes, haematocrit, lymphocytes ($P<0.05$) and increase of haemoglobin, monocytes, neutrophils, N:L ratio, MCH, and MCHC values in group S when compared to group C ($P<0.05$). Meanwhile, there was a significant decrease of haemoglobin, monocytes, MCH and MCHC, on the other hand, a significant increase of leukocytes, erythrocytes, haematocrit and lymphocytes of Livervital-supplemented rats compared to those in stressed control rats. Besides that, some of the morphological parameters did not vary between groups. In conclusion, this study showed that supplementation of Livervital to the diet at 2 mL/L had protective effects on growth and haematological parameters under mixed stress conditions.

Key words: feed additives, hematology, morphology, phytogenic ingredients, stress

INTRODUCTION

Stress is defined as the response of the organism within physiological or behavioural conditions. As a sound expression of stress, fight or flight defense mechanism associated with hypothalamic-pituitary-adrenocortical (HPA) and sympathetic-adrenomedullary system (SAM) are taking place. Besides biological factors, environmental and management stressors are known to affect livestock and its production (Ranjan *et al.,* 2019). Crowded environment causes a high temperature and humidity, which is an important problem in animals especially for rats, ruminants, and poultry (Peng *et al.,* 1989; Ramin, 1995; Keeling *et al.,* 2003; Maraherens, 2003). As a result, a decrease occurred during growth, reproductive performance and milk yield while there was an increase in infertility, faulty eggs, and embryonic death (Simone *et al.,* 2004; Faith & Hessler, 2006; Sejan *et al*. 2010; Shinde & Sejian, 2013). Several studies tried to clarify and solve the effect of high temperature and crowded environment on poultry and ruminants due to the variability of the world's ecosystem that changes day by day (Berman, 2004; Rivington *et al.,* 2009; IPCC, 2013).

Firstly, it was determined by Thaxton *et al*. (1968) that high temperature affects homeostasis in chickens. Suzuki *et al*. (1989) reported that stress changed the intestinal microflora and, thereby, growth was depressed. On the other hand, stress reduced the physiological changes as body weight, weight increase, and haematological parameters as well as lowered infertility and reproductive function in ruminants (Mader *et al.,* 1997; Sing *et al.,* 2011). Nevertheless, some researchers identified the haematological changes such as lower white blood cells and higher neutrophil/lymphocyte (heterophil/lymphocyte) ratio (N/L or H/L), which is a stress indicator, under stress conditions in animals (Heller *et al.*, 1979; Mogenet & Youbicier-Simo, 1998; Price *et al.*, 2003; Lynch *et al.,* 2010). O'Loughlin *et al.* (2010) reported that the neutrophils and N/L ratio increased during weaning, which is a stress factor of calves. In a poultry study, it was detected that there was a decrease in lymphocytes which leads to increased H/L ratio, although heterophils were increased in chickens when they were exposed to heat stress during the summer (Zulfiki *et al.*, 2003). Also, Dereli & Fidan (2010) determined that the H/L ratio was a more reliable benchmark than serum cortisol value for stress conditions in poultry. Some researchers reported that the cage density was also a stress factor, and it affected body weight, egg production, shell quality, meat and milk quality (Keer-Keer *et al*., 1996; Feddes *et al.,* 2002; Steinhardt, 2002; Villarroel *et al.,* 2003; Zulkifli *et al*., 2004). They also reported that social stress or crowded environment caused stress due to the increase in energy expenditure.

There is a potential increase in the usage of natural additives to protect and improve the growth performances of animals (Sengezer & Gungor, 2008, Terré *et al.,* 2007; Grand et al., 2013; Uyeno *et al.,* 2015). It was reviewed that probiotics, prebiotics, antioxidants, and vitamin complexes may be utilised for this purpose (Ortuno *et al.,* 2001; Botsoglu *et al.,* 2004; Quezada-Mendoza *et al.,* 2011). However, some active additives like vitamin E, vitamin B_{12}, methionine, betaine, and choline may be healthier since they are in a complex variety. In this study, we examined the effects of Livervital (MIAVIT GmbH, Essen, Germany), which includes colin, carnitine, vitamin E, melatonin and some herbal extracts, on growth, body mass index and some haematological parameters in rats which were exposed to mixed stress models such as crowded environment and hosting alone under long lightening period. The study also aimed to clarify the effects of this additive on animal and scientific fields.

MATERIAL AND METHODS

Animals

The experimental protocols were in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the Animal Care and Use Committee of Bursa Uludag University. The study was carried out with the permission of University Animal Experimentation Local Ethics Committee (Approval No: 2018-07/01).

The rat model was selected due to the availability of the stress conditions for both humans and animals. Besides, rats have a rapid metabolic rate, which is appropriate to produce the results in a short time.

Forty-eight healthy, male Sprague-Dawley rats (age around 7–8 weeks and average body weight 150–200 g) were included in this study which randomly allocated to 4 experimental groups: Control (C – basal diet), Stress (S – basal diet), Livervital (2 mL/l/day) (Li), Livervital (2 mL/l/day) and Stress (LiS).

Experimental procedure

The animals were housed under standard laboratory conditions (22 ± 1.0 C; 55 ± 10% humidity). Rats fed with a standard chow diet. Rats were acclimatized for a week before the experimentation began. The experimental trial was conducted for 28 days. Livervital was commercially produced by MIAVIT (GmbH, Essen, Germany) and was given at 2 mL/l/daily by adding to water five days a week (Monday, Tuesday, Thursday, Friday, Saturday).

The standard lightning procedure (12 h light:12 h dark) for laboratory animals was applied during the first two weeks. For the last two weeks, all animals were exposed to light:dark cycle, which was 18 h light: 6 h dark as modified by researchers (Gancarczyk *et al.,* 2004; Kus *et al.,* 2004; Seyidoglu *et al.,* 2019). Lis and S groups were exposed to two additional stresses, and neither feed nor water was given during this stress applications:

- *Hosting alone stress:* The rat was left alone for 30 mins on Monday, Wednesday, Friday and Sunday of the 4th week of the trail. This stress was formed in a separate cage, which four sides and ground covered with white paper.
- *Crowded environment stress:* Social overcrowding stress was formed by leaving six rats in a cage, which was for three rats, for 30 minutes. This stress was studied on Tuesday, Thursday, and Saturday of the 5th week of trial.

Samples and analyses

Blood was collected by the puncture of the heart under isoflurane anaesthesia from overnight-fasted rats at the end of the trial. The haematocrit, haemoglobin, erythrocytes, leukocytes, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and leukocyte types (neutrophils, lymphocytes, and monocytes) were determined by using an automatic blood counter device (VetScan HM5, ABAXIS). Also, neutrophils:lymphocytes (N:L) ratio, which is one of the physiological welfare and stress markers, was recorded.

Some morphological parameters such as body weight, height, length, waist circumference, body mass index (BMI) and waist circumference:length ratio was determined to identify the effect of Livervital on growth.

Statistical analysis

Statistical analyses performed with SPSS (Version 17.0). Data were examined 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 all parameters to examine the difference between groups. Tukey's test (Dowdy & Wearden, 1981) evaluated whether the difference between groups was provided to be significant (P<0.05). On the other hand, in non-homogeneous groups, differences between means were analysed by Kruskal Wallis and Mann Whitney U tests. Also, variance analysis for repetitive measurements was run using repeated measures ANOVA (Dawson & Trapp, 2001).

RESULTS

The haematological parameters of feed on phytogenic ingredients in the stress-exposed male rats are shown in Table 1. The number of leukocytes, erythrocytes, haematocrit and lymphocytes decreased (P<0.05) in stressed rats. In contrast haemoglobin value, monocytes and neutrophils, MCH, MCHC (P<0.05) and N:L ratio were elevated. Feeding Livervital to rats elevated leukocytes, erythrocytes, haematocrit and lymphocytes compared to those in the stress group. Meanwhile, haemoglobin concentration, monocytes, MCH and MCHC decreased in the group LiS compared to group S one. There was a significant decrease of haemoglobin, monocytes, MCH and MCHC and significant increase of leukocytes, erythrocytes, haematocrit, lymphocytes of Livervital-supplemented rats compared to that of stressed control rats. In the study, there were no statistical between-group differences in morphological parameters as body weight, body mass index, waist circumference and waist circumference: length ratio.

Table 1. Some hematological parameters in control and experimental groups. Data are given as mean±standard error, n=48

Haematological parameters	Groups			
	Control (C)	Stress (S)	Livervital (Li)	Stress+ Livervital (LiS)
Leukocytes (10^3/mm^3)	6.53±0.41	4.97±0.30^a	7.38±0.42	6.33±0.37^b
Erythrocytes(10^6/mm^3)	7.42±0.09	6.77±0.06^a	6.91±0.19	7.55±0.13^b
Haemoglobin(g/dl)	12.86±0.21	13.71±0.14^a	12.75±0.16	13.25±0.16^b
Haematocrit (%)	42.19±0.54	37.91±0.83^a	38.88±1.29	43.43±0.58^b
Lymphocytes (%)	74.51±1.73	66.53±0.62^a	74.61±0.51	70.46±1.42^b
Monocytes (%)	5.12±0.37	9.98±1.04^a	4.52±0.52	4.76±0.56^b
Neutrophils (%)	20.54±0.98	24.66±0.65^a	21.60±0.59	22.16±1.14
N: L ratio	0.29±0.02	0.35±0.01^a	0.29±0.01	0.40±0.02
MCV (fl)	57.45±0.59	58.50±0.65	56.09±0.58	57.67±0.74
MCH (pg)	18.30±0.17	19.93±0.24^a	19.27±0.41	18.26±0.34^b
MCHC (g/dl)	32.01±0.25	34.07±0.32^a	34.57±0.84	31.67±0.51^b

* Different superscripts a, b show differences: (a) P<0.05, group S versus group C; (b) P<0.05, group LiS versus group S.

Table 2. Some morphological parameters in control and experimental groups. Data are given as mean±standard error, n=48

Morphological parameters	Groups			
	Control (C)	Stress (S)	Livervital (Li)	Stress+ Livervital (LiS)
Weight (g)				
Initial	217.00±14.95	228.58±18.29	223.09±10.78	224.17±10.70
Final	311.09±11.28	312.08±15.13	310.00±10.41	312.25±9.74
Body mass index (kg/m^2)				
Initial	0.16±0.004	0.16±0.005	0.16±0.005	0.16±0.004
Final	0.17±0.004	0.18±0.004	0.18±0.003	0.18±0.003
Body length (cm)				
Initial	36.77±0.84	37.71±0.91	37.14±0.67	37.50±0.56
Final	42.36±0.54	41.92±0.61	41.45±0.53	41.63±0.46
Waist circumference (cm)				
Initial	12.55±0.35	13.00±0.33	12.98±0.30	13.04±0.25
Final	14.32±0.21	14.25±0.29	14.41±0.26	14.29±0.21
Waist circumference /length (cm)				
Initial	0.34±0.005	0.34±0.005	0.35±0.004	0.34±0.003
Final	0.34±0.006	0.34±0.005	0.35±0.004	0.34±0.004

DISCUSSION

In this study, we assessed the association between feeding phytogenic ingredients and different stress models in the rats. We observed a protective effect on growth and haematological parameters during stress conditions. Additionally, we detected a complex relationship between stress and phytogenic ingredients.

Stress responses have been linked to physiological conditions, especially growth and haematological parameters. It was reported that stress from either crowding or loneliness caused a decrease in growth and some blood parameters in animals (Altan *et al.,* 2000; Zulkifli *et al*., 2003; Simone *et al.,* 2004; Berman, 2004; Eid *et al.,* 2010; Singh *et al.,* 2011). In the present study, although there were no differences in growth performances, significant changes were found in haematological parameters such as leukocytes, erythrocytes, haemoglobin, haematocrit, N:L ratio, types of leukocytes (lymphocytes, neutrophil, monocytes), MCH and MCHC. Importantly, the N:L ratio was increased in stress group compared to control one (p:0,016), but decreased in LiS group compared to group S (P>0.05). This situation may be explained by the stress effects on cellular populations alterations, which reversed rapidly (Dhabhar *et al.,* 1995). Nevertheless, it can also be said that the negative effects of this stress on cellular metabolism may be regulated and improved with the important additive, Livervital, due to its rich contents. Similarly to our results about stress markers, O'Loughlin *et al.* (2011)

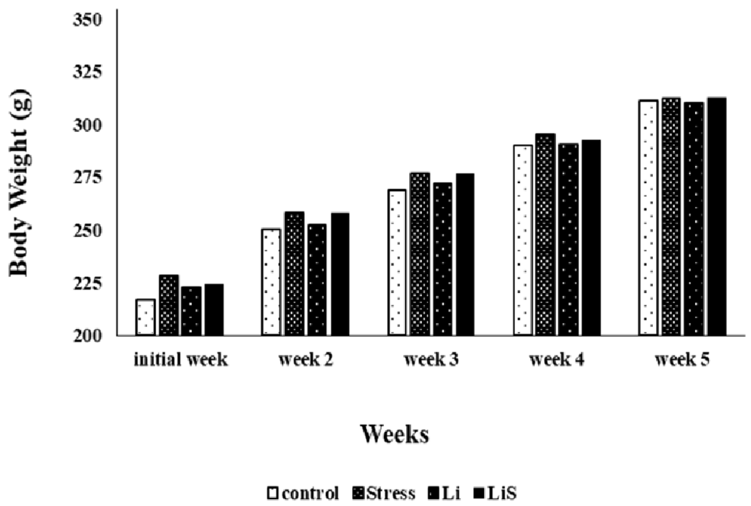

Fig. 1. Weekly body weight in in control and experimental groups. Groups – C: Control, S: Stress, Li: Livervital, LiS: Stress-Livervital. All data are expressed as means ± SEM.

evaluated the erythrocytes, haemoglobin, haematocrit, MCH, MCHC in weaning stress. They reported that erythrocytes, haematocrit, and leukocytes were decreased and neutrophils, N:L ratio and MCHC were increased although hemoglobin was not affected.

Attempts to reduce management related stressors such as housing, crowded, high temperature, handling and weaning on animal health were reported (Knowles *et al.,* 1995; Altan *et al.,* 2000; Feddes *et al.,* 2002; Arthington *et al.*, 2003; Zulkifli *et al*., 2003). Additionally, either in ruminant or in poultry, the growth, immunity and animal yields were affected negatively if the animals are exposed to these stressors. Also, it was reported that physiological changes in animals caused a decrease in weight parameters and growth rates (Arthington *et al.,* 2003). Sartorelli *et al.* (1992) and Knowles *et al.* (1995) indicated that weight gain and haematocrit are decreased by transport stress in ruminants. Also, it was clarified that this stress might be correlated with crowded, heat and cold stress (Ramin, 1995; Ali-Gholi *et al.,* 2007). On the other hand, Zulkifli *et al.* (2003) determined that haemogram parameters, especially increasing N/L (H/L) ratio is an important value for the stress response of chickens. As well, some researchers found that N/L (H/L) ratio moderated life-threatening situations (Maxwell *et al.,* 1992; Maxwell, 1993).

In conclusion, the management of stress can be regulated with feeding strategies. Nowadays, food additives are more important for livestock. All these supplements include at least one important component such as vitamins, minerals, biotins, essential oils and herbal extracts (Sengezer & Gungor, 2008; Quezada-Mendoza *et al.,* 2011; Grand *et al.,* 2013;). Livervital (MIAVIT GmbH, Essen, Germany), one of the important mixture for livestock additives, would be used for both poultry and ruminants. This additive has several ingredients, especially herbal extracts, vitamins, and colin, with effect on physiology, homeostasis, and health. In the present study, it was found that Livervital has positive effects on animal physiology under stress conditions. It can be said that Livervital had a higher value for the welfare of health and was effective to reduce the effect of stress.

ACKNOWLEDGEMENT

This work received the financial support from MIAVIT GmbH, Essen, Germany. The funders had no role in study design, data collection, analysis, decision to publish, or preparation of the manuscript.

REFERENCES

Ali-Gholi, R., A. R. Siamak & M. Daryoush, 2007. Influence of the short-term road transport stress on blood parameters in cows. *Medycyna weterynaryjna*, **63**, 1311–1315.

Altan, Ö., A. Altan, M. Çabuk& H. Bayraktar, 2000. Effects of heat stress on some blood parameters in broilers. *Turkish Journal of Veterinary and Animal Sciences,* **24**, 145–148.

Arthington, J. D., S. D. Eichert, W. E. Kunkle & F. G. Martin, 2003. Effect of transportation and commingling on the acute-phase protein response, growth and feed intake of newly weaned beef calves. *Journal of Animal Science,* **81**, 1120–1125.

Berman, A., 2004. Tissue and external insulation estimates and their effects on prediction of energy requirements and of heat stress. *Journal of Dairy Science,* **87**, 1400–1412.

Botsoglou, N. A., E. Christaki, P. Florou-Paneri, I. Giannenas, G. Papageorgiou & A. B. Spais, 2004. The effect of a mixture of herbal essential oils or α-tocopheryl acetate on performance parameters and oxidation of body lipid in broilers. *South African Journal of Animal Science*, **34**, 52–61.

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

Dereli Fidan, E., 2010. Denizli tavuklarında yetiÇtirme parametreleri, parametreler arası fenotipikkorelasyonlar ile kafes pozisyonu ve yoğunluğunun yumurtlama döneminde stresalgılama ve performansa etkisi. Ph.D. thesis,University of Adnan Menderes, Aydın, Turkey.

Dhabhar, F. S., A. H. Miller, B. S. McEwen & R. L. Spencer, 1995. Effects of stress on immune cell distribution. Dynamics and hormonal mechanisms. *The Journal of Immunology,* **154**, 5511–5527.

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

Eid, F., E. G. E. Helal & N. M. Taha, 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.

Faith, R. E. & J. R. Hessler, 2006. Housing and environment. In: *The Laboratory Rat,* eds S. H. Weisbroth & C. L. Franklin, Academic Press, pp. 314–315.

Feddes, J. J., E. J. Emmanuel & M. J.Zuidhoft, 2002. Broiler performance, body weight variance, feed and water intake, and carcass quality at different stocking densities. *Poultry Science*, **81**, 774–779.

Gancarczyk, M., A. Paziewska-Hejmej, S. Carreau, Z. Tabarowski & B. Bilińska, 2004. Dose- and photoperiod-dependent effects of 17beta-estradiol and the anti-estrogen ICI 182,780 on testicular structure, acceleration of spermatogenesis, and aromatase immunoexpression in immature bank voles. *Acta Histochemica*, **106**, 269–278.

Grand, E., F. Respondek, C. Martineau, J. Detilleux, & G. Bertrand. 2013. Effects of short-chain fructooligosaccharides on growth performance of preruminant veal calves. *Journal of Dairy Science,* **96**, 1094–1101.

Heller, E. D., D. B. Nathan & M. Perek. 1979. Short heat stress as an immunostimulant in chicks. *Avian Pathology*, **8**, 195–203.

IPCC (Intergovernmental Panel on Climate Change), 2013. Climate Change : The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, eds. Stocker, T. F., D. Qin, G. K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex & P.M. Midgley. Cambridge University Press, Cambridge, https://www.ipcc.ch/site/assets/uploads/2017/09/WG1AR5_Frontmatter_FINAL.pdf (26 June 2019, date last accessed).

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

Keer-Keer, S., B. O. Hughes, P. M. Hocking & R. B. Jones, 1996. Behavioural comparison of layer and broiler fowl: Measuring fear responses. *Applied Animal Behaviour Science*, **49**, 321-333.

Knowles, T. G., S. N. Brown, P. D. Warriss, A. J. Philips, S. K. Dolan, P. Hunt , J. E. Ford, J. E. Edwards & P. E. Watkines, 1995. Effects on sheep of transport by road for up to 24 hours. *The Veterinary Record*, **136**, 431–438.

Kus, I., A. Songur, C. Ozogul, A. Kavaklı, I. Zararsız & M. Sarsılmaz, 2004. Effects of photoperiod on the ultrastructure of Leyding cells ın rat. *Archives of Andrology,* **50**, 193– 200.

Lynch, E. M., B. Earley, M. McGee & S. Doyle, 2010. Effect of post-weaning management practices on physiological and immunological responses of weaned beef calves. *Irish Journal of Agricultural and Food Research*, **50**, 161–174.

Mader, T. L., J. B. Gaughan, & B. A. Young, 1997. Feedlot diet roughage level for Hereford cattle exposed to excessive heat load. *Professional Animal Scientist*, **15**, 53–62.

Maraherens M., I. Von-Richthofen, S. Schmeiduch & J. Hartung, 2003. Special problems of long distance road transports of cattle. *Deutsche Tierärztliche Wochenschrift,* **110**, 120–125.

Maxwell, M. H., G. M. Robertson, M. A. Mitchell & A. J. Carlisle, 1992a. The fine structure of broiler chicken blood cells, with particularreference to basophils, after severe heat stress. *Comparative Haematology International*, **2**, 190–200.

Maxwell, M. H., 1993. Avian blood leucocyte responses to stress. *World's Poultry Science Journal*, **49**, 34–43.

Mogenet, L. Y. & B. J. Youbicier-Simo, 1998. Determination of reliable biochemical parameters of heat stress, and application to the evaluation of medications: Example of erythromycin E. In: Proceedings of 10th European Poultry Conference, Israel, pp. 538–541.

O'Loughlin, A., M. McGee, S. M. Waters, S. Doyle & B. Earley, 2011. Examination of the bovine leukocyteenvironment using immunogenetic biomarkers toassess immunocompetence following exposure toweaning stress. *BMC Veterinary Research*, **7**, 45.

Ortuno, J., M. A. Esteban & A. Cuesta & J. Meseguer, 2001. Effect of oral administration of high vitamin C and E dosages on the gilthead seabream (*Sparus aurata* L.) innate immune system. *Veterinary Immunology and Immunopathology*, **79**, 167–180.

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

Price, E. O., J. E. Harris, R. E. Borgwardt, M. L. Sween & J. M. Connor, 2003. Fenceline contact of beef calves with their dams at weaning reduces the negative effects of separation on behavior and growth rate. *Journal of Animal Science*, **81**, 116–121.

Quezada-Mendoza, V. C., A. J. Heinrichs & C. M. Jones, 2011. The effects of a prebiotic supplement (Prebio Support) on fecal and salivary iga in neonatal dairy calves. *Livestock Science,***142**, 222–228.

Ramin, A. G., 1995. Physiological response tests and blood profiles in replacement dairy heifers and their relationship to growth rates and health parameters. Thesis, The University of Queensland, pp. 23–33.

Ranjan, A., S. Ranjana, D. Indu, A. Rahim & S. Tiwari, 2019. Effect of heat stress on poultry production and their managemental approaches. *International Journal of Current Microbiology and Applied Science,* **8**, 1548–1555.

Rivington, M., K. B. Matthews, K. Buchan, D. Miller & G. Russell, 2009. Investigating climate change impacts and adaptation options using integrated assessment methods. *Aspects of Applied Biology*, **93**, 85–92.

Sartorelli, P., S. Dominoni & F. Agens, 1992. Influence of duration of simulated transport on plasma stress markers in the calf. *Zentralblatt für Veterinärmedizin. Reihe A,* **39**, 401–403.

Sengezer, E. & T. Güngör, 2008. Essential oils and their effects on animals. *Journal of Lalahan Livestock Research Instute,* **48**, 101–110.

Sejian, V., V. P. Maurya & S. M. K. Naqvi, 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**, 252–258.

Seyidoglu, N., R. Gurbanli, E. Koseli, F. Cengiz & C. Aydin, 2019. The effects of *Spirulina* (Arthrospira) *platensis* on morphological and hematological parameters evoked by social stress in male rats. *Journal of Istanbul Veterinary Sciences,* **3**, 21–27.

Shinde, A. K. & V. Sejian, 2013. Sheep husbandry under changing climate scenario in India: An overview. *Indian Journal of Animal Science,* **83**, 998–1008.

Simone, M., A. C. Holt, B. John, A. D. Gaughan, L. Terry & B. Madder, 2004. Feeding Strategies for grain fed cattle in a hot environment. *Australian Journal of Agricultural Research,* **55**, 719–725.

Singh, S. V., R. C. Upadhyay Ashutosh, O. K. Hooda & M. M. Vaidya, 2011. Climate change: impacts on reproductive pattern of cattle and buffaloes. *Wayamba Journal of Animal Science,* **8**, 199–208.

Steinhardt, M., 2002. Reactions of young cattle from a suckler herd to short transport by road repeated investigations before and after permanent separation of young cattle from their dams. Plasma, cortisol, biochemical, hematological variables, minerals and heart rate. *Deutsche Tierärztliche Wochenschrift ,***109**, 239–245.

Suzuki, K., Y. Kodama & T. Mitsuoka, 1989. Stress and ıntestinal flora. *Bifidobacteria and Microflora,* **8**, 23–38.

Terré, M., M. A. Calvo, C. Adelantado, A. Kocher & A. Bach, 2007. Effects of mannan oligosaccharides on performance and microorganism fecal counts of calves following an enhanced-growth feeding program. *Animal Feed Science and Technology,* **137**, 115–125.

Thaxton, J. P., H. A. Olanrewaju, S. L. Branton & W. B. Roush, 2005. Stocking density effects on male broilers grown to 1.8 kilograms of body weight. *Poultry Science*, **708**, 344–351.

Uyeno, Y., S. Shigemori & T. Shimosato, 2015. Effect of probiotics/prebiotics on cattle health and productivity. *Microbes and Environments,* **30**, 126–132.

Villarroel, M., G. Maria, C. Sanudo, S. Garcia, G. Chacon & G. Senbet, 2003. Effect of commercial transport in Spain on cattle welfare and meat quality. *Deutsche Tierärztliche Wochenschrift,* **110**, 105–107.

Zulkifli, I., P. K. Liew, D. A. Israf, A. R. Omar

& M. Hair-Bejo, 2003. Effect of early age feed restriction and heat conditioning on heterophil/lymphocyte ratios, heat shock protein 70 expression and body temperature of heat-stressed broiler chickens. *Journal of Thermal Biology,* **28**, 217–222.

Zulkifli, I. & A. Sti Nor Azah, 2004. Fear and stress reactions, and the performance of commercial broiler chickens subjected to regular pleasant and unpleasant contacts with human being. *Applied Animal Behaviour Science*, **88**, 77–87.

Correspondence:

Eda Koseli
Bursa Uludag University,
Faculty of Veterinary
Department of Physiology
Gorukle Campus,
Nilufer, Bursa, Turkey
611647001@ogr.uludag.edu.tr