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EFFECTS OF SEVERAL ESSENTIAL OILS ON HAEMATOLOGICAL AND MORPHOLOGICAL PARAMETERS IN STRESS EXPOSED MALE RATS

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Summary

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In late years, aromatic plants and their extracts have received increasing attention as growth and health promoters. Essential oils possess an outstanding role in poultry nutrition as natural additives. They have various beneficial effects related to food digestion and body biological response. The purpose of the present study is to provide an overview of the effects of Miarom-L (MIAVIT GmbH, Essen, Germany) which includes several essential oils, on stress conditions and to describe their possible approach of action. For this purpose, forty-eight adult male Sprague-Dawley rats were randomly separated into four groups; Control (*C*), Stress (*S*), Miarom-L (*Mi*) and Miarom-L+Stress (*MiS*). Groups *C* and *S* served as control groups. Rats in the *Mi* and *MiS* were given Miarom-L in the water at the rate of 0.2 mL/L, three days a week for 28 days. All rats were stressed by prolonged light phase conditions (18 h light: 6 h dark) for 14 days. *S* and *MiS* groups were exposed to additional stress by hosting alone and crowded environment. There was a significant decrease of leukocytes, erythrocytes, haematocrit and lymphocytes ($P<0.05$) and increase of haemoglobin, monocytes, neutrophils, MCH and MCHC values in group S when compared to group C of rats ($P<0.05$). Meanwhile, there was a significant decrease of haemoglobin, monocytes, neutrophils, MCH, and MCHC and significant increase of leukocytes, erythrocytes, haematocrit and lymphocytes of Miarom-L supplemented rats compared to that of stressed control rats. Besides that, some of the morphological parameters did not vary both groups. In conclusion, this study showed that supplementation of Miarom L to the diet at the rate of 0.2 mL/L had protective effects on growth and haematological parameters during stress conditions.

Key words: essential oils, feed additives, haematology, morphology, rat, stress

INTRODUCTION

Stress occurs when homeostasis is threatened or perceived to be so; homeostasis is re-established by various physiological and behavioral adaptive responses. As a sound expression of stress, fight or flight defense mechanism which associated with

hypothalamic-pituitary-adrenocortical (HPA) and sympathetic-adrenomedullary system (SAM) are taking place. Besides biological factors, environmental and management stressors are known to be affected livestock and its production (Ranjan *et al.,* 2019).

The Food and Agricultural Organization (FAO) was estimated that the productions of poultry would increase its importance compared to other protein sources (Alexandratos & Bruinsma, 2012). Nutrition has a crucial role in both homeostasis and stress management in poultry farming. Weight loss, faulty eggs, lowered viability, immune depression, and gain in disease rate are the most common stress symptoms in domestic poultry. Heat stress is one of the most important environmental stressors challenging poultry production worldwide. Hosting in crowded environments, and leaving animals alone, can cause stress and may lead to physiological changes in the organism. Heat and hosting in a crowded environment may change the physiological and immunological parameters in chickens (Lara & Rostagno 2013; Gomes *et al.,* 2014). Besides these, the weather, crowding and seasonal changes also cause stress (Hoerr, 2010; QuinteiroFilho *et al*., 2010; Lay *et al*., 2011). Gomes *et al.* (2014) indicated that crowded environmental stress is resulting in a decrease in nutrient absorption, intestinal integrity, and growth performance. Heckert *et al*. (2002) also reported that weights and immune activities are decreased with the high stocking situation. In addition, leg problems and mortality were also determined in same conditions by some researchers (Estevez, 2007). In sight of these problems, it is important to create management programs to poultry belongs to nutritions and hosting.

There are some feed additives to improve animal's feed efficiency, protect the health, and increase the animal's product quality (Sengezer & Gungor 2008). In recent years, some essential oils such as wild marjoram, rosemary, clove, or cinnamon had been used as a feed additive for animals. The most important contents of these oils are arvacrol, thymol, cinnamaldehyde, ionone and eugenol which have effects on feed efficiency, intestinal health and weight gain (Platel & Srinivasan, 2000; Yildiz, 2007; Baser, 2008; Bilal *et al*., 2008). The Food and Drug Administration (FDA) was suggested that some of these essential oils are listed in Generally Recognized as Safe (GRAS) food additives (Burt, 2004). In addition, some researchers reported the antibacterial, anti-inflammatory and antioxidant properties of essential oils (Pessoa *et al.,* 2002; Alali *et al*., 2013; Bento *et al.,* 2013; Krishan & Narang, 2014).

In this study, we examined the effects of Miarom-L (MIAVIT GmbH, Essen, Germany), which includes a mixture of essential oils on growth, body mass index and some haematological parameters in rats exposed to mixed stress models such as crowded environment and hosting alone under long lightening period. Miarom-L is an important additive for improving the feed consumption and growth performance as well as supporting the health of poultry. The study also aimed to clarify the effects of this additive.

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. Also, rats have a rapid metabolic rate, which is appropriate to get the results in a short time. Forty-eight, male and healthy Sprague-Dawley rats aged about 7–8 weeks (average body weight 150–200 g) were used for the experiment. The rats were allocated to four experimental groups as follows: Control (C – basal diet), Stress (S – basal diet), Miarom-L (0.2 mL/L/day) (Mi), Miarom-L (0.2 mL/L/day) and Stress (MiS).

Experimental procedure

The animals were housed under standard laboratory conditions (22 ± 1.0 ^{o}C; 55 ± 10% humidity). Rats were fed a standard chow diet. Rats were acclimatised for a week before the experimentation began. The experimental trial was conducted for 28 days. Miarom-L was commercially produced by MIAVIT (GmbH, Essen, Germany) and it was given at 0.2 mL/L/daily by adding to water three days a week (Monday, Wednesday, Friday) for 28 days.

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 of 18 h light:6 h dark as modified by researchers (Gancarczyk *et al*., 2004; Kus *et al.* 2004; Seyidoglu *et al.,* 2019). MiS and S groups were exposed to two additional stresses, as explained below and neither feed nor water was given during the application of these stresses:

- *Hosting alone stress*: The rat was left alone for 30 mins for this stress which studied 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 over crowding stress was formed by leaving six rats in one cage, which was for three rats, for 30 min. 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 Miarom-L 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 essential oils 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, neutrophils, MCH, MCHC ($P<0.05$) and N:L ratio were elevated. Feeding rats with Miarom-L elevated leukocytes, erythrocytes, hematocrit, lymphocytes compared to Stress group. Meanwhile, haemoglobin concentration, monocytes, MCV, MCH and MCHC decreased in the MiS group compared to group S. There was a significant increase on leukocytes, erythrocytes, hematocrit, lymphocytes of Miarom-L supplemented rats compared to that of stressed control rats. In the study, there were no statistical differences in morphological parameters as body weight, body mass index, waist circumference, and waist circumference: length ratio between groups (Table 2; Fig. 1).

DISCUSSION

In this study, we assessed the association between feeding essential oils and different stress models in the rats. We observed a protective effect on growth and haematological parameters during stress condi-

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)	Miarom-L (Mi)	Stress+ Miarom-L (MiS)
Leukocytes ($10^3/mm^3$)	6.53±0.41	4.97±0.30[a]	5.42±0.48	6.18±0.33[b]
Erythrocytes($10^6/mm^3$)	7.42±0.09	6.77±0.06[a]	7.17±0.07	7.47±0.08[b]
Haemoglobin(g/dl)	12.86±0.21	13.71±0.14[a]	12.91±0.24	13.17±0.10[b]
Haematocrit (%)	42.19±0.54	37.91±0.83[a]	39.90±0.43	41.69±0.50[b]
Lymphocytes (%)	74.51±1.73	66.53±0.62[a]	74.27±1.49	73.93±1.13[b]
Monocytes (%)	5.12±0.37	9.98±1.04[a]	3.24±0.52	3.93±0.43[b]
Neutrophils (%)	20.54±0.98	24.66±0.65[a]	22.30±1.03	21.50±1.00[b]
N: L ratio	0.29±0.02	0.35±0.01[a]	0.34±0.02	0.33±0.02
MCV (fl)	57.45±0.59	58.50±0.65	55.75±0.35	56.42±0.47[b]
MCH (pg)	18.30±0.17	19.93±0.24[a]	18.54±0.18	18.11±0.23[b]
MCHC (g/dl)	32.01±0.25	34.07±0.32[a]	33.27±0.37	32.26±0.20[b]

* Different superscripts a, b show differences: (a) $P<0.05$, group S versus group C; (b) $P<0.05$, group MiS 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)	Miarom-L (Mi)	Stress+ Miarom-L (MiS)
Weight (g)				
Initial	217.00±14.95	228.58±18.29	223.75±5.48	223.75±8.87
Final	311.09±11.28	312.08±15.13	313.50±7.59	318.33±9.68
Body mass index (kg/m^2)				
Initial	0.16±0.004	0.16±0.005	0.17±0.003	0.16±0.004
Final	0.17±0.004	0.18±0.004	0.18±0.003	0.18±0.004
Body length (cm)				
Initial	36.77±0.84	37.71±0.91	36.58±0.41	37.92±0.57
Final	42.36±0.54	41.92±0.61	42.29±0.38	41.88±0.47
Waist circumference (cm)				
Initial	12.55±0.35	13.00±0.33	12.79±0.14	12.92±0.22
Final	14.32±0.21	14.25±0.29	14.79±0.13	14.67±0.18
Waist circumference /length (cm)				
Initial	0.34±0.005	0.34±0.005	0.35±0.004	0.34±0.004
Final	0.34±0.006	0.34±0.005	0.35±0.004	0.35±0.004

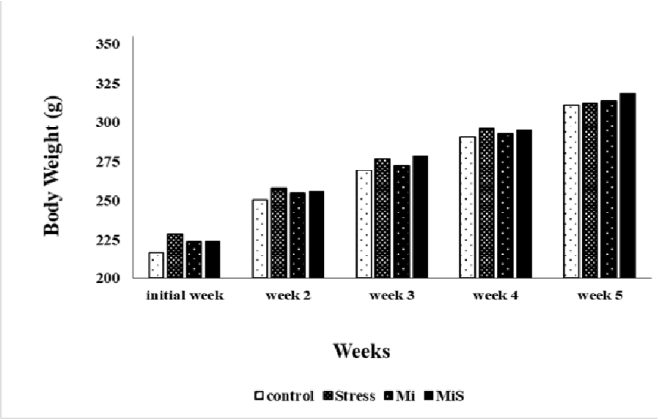

Fig. 1. Weekly body weight in in control and experimental groups. Groups – C: Control, S: Stress, Mi: Miarom-L, MiS: Stress- Miarom-L. All data are expressed as means ± SEM.

tions. Additionally, we detected a complex relationship between stress and essential oil components.

Stress occurs when animals suffer to make extreme and/or prolonged physiological and behavioral changes in order to cope with their environment. The physiological response to stress is regulated by a complex neurocircuitry that integrates and interprets stress-related and homeo-

static information. In poultry, especially heat and crowded stress have adverse effects on growth, egg production, and shell quality (Whitehead *et al.* 1998). Gomes *et al.* (2014) showed that chickens in crowded environment had mild enteritis. It was also determined that enteritis caused an increase in the intestinal permeability and thereby nutrient absorption and performance indices were decreased. Some researchers also reported that crowded environment decreased the body weight and feed conversion ratio (Thaxton *et al.,* 2005; Guardia *et al.,* 2011). Our results showed that all growth parameters were declined nonsignificantly in stressed groups as compared to non stressed and feeding with Miarom-L one.

It's well-known that neutrophil: lymphocyte ratio (N:L), as well as erythrocytes, leukocytes, haemoglobin, haematocrit, MCH and MCHC are evoked by stress (Davis *et al*., 2008; O'Loughlin *et al.,* 2011). İncreased N:L (H:L) ratio due to multiple stressors such as heat, crowded or lighting, were identified by researchers in chickens (Davis *et al.,* 2008; Redmond *et al*., 2011; Huth & Archer, 2015). Besides, there are conflicting results about either increase or decrease of haemoglobin value in a stress situation (Bedanova *et al*., 2006a; 2006b). Although neutrophils, monocytes, haemoglobin, MCH and MCHC values were increased in S group compared to controls ($P<0.05$), N:L ratio was insignificantly higher in group S compared to controls. In addition, the neutrophils, monocytes, haemoglobin, MCH, and MCHC values were decreased in MiS group compared to group S ($P<0.005$) along with significant decrease in lymphocytes, leukocytes, erythrocytes, and haematocrit values in group S compared to controls. In group MiS, lymphocytes, leukocytes, erythrocytes, and haematocrit values were increased compared to S ($P<0.05$). All these results may be due to the effects of Miarom-L additive – a mix of essential oils.

In conclusion, essential oils are complex molecules which enhance the growth due to their stimulating effect on the digestive system and enzymes (Lee *et al.,* 2004). Many studies showed that the supplementation with of essential oils improved the feed efficiency, growth performances and health (Alcicek *et al.,* 2004; Bampidis *et al.,* 2005; Cabuk *et al*., 2006; Ozek, 2011). There are various results about the effects of essential oils and their compounds which has antibacterial, immunomodulator, antioxidant or anti-inflammatory properties. It was also reported that the activity of essential oils was due to the synergistic effects between their contents and functional composition, such as phenolic compounds' important antimicrobial activity (Tongnuanchan & Benjakul, 2014). In recent years, there has been an increase in essential oils use as feed additives. Miarom-L is a mixture of essential oils which support the enzymatic digestive in intestinal mucosa and thereby increase the growth. In the present study, Miarom-L showed a potential influence on animals under stress conditions. Nevertheless, it can be said that Miarom-L may be successful for reducing the effects of stress and great for welfare and health.

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