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Investigation of endocrine response of thyroid and intestinal and adipose tissues due to the addition of *Moringa oleifera* essential oil in diet for quails exposed to heat stress

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ABSTRACT - In this study, we aimed to determine the effect of *Moringa oleifera* essential oil (MOEO) supplementation to rations of Japanese quail (*Coturnix coturnix japonica*) grown under heat stress (HS) on some adipokine (visfatin, adiponectin, and chemerin), intestinal (citrulline), 3,5,3′-triiodothyronine (T3), thyroxine (T4), and thyroid-stimulation hormone (TSH) levels in the serum. For this purpose, 320 day-old male quail chicks were used. The study was conducted over 42 days, including a 7-d adaptation period and a 35-d experimental period. Eight trial groups were established, each including 10 quail: CT (control temperature, 25 °C), MOEO 200, MOEO 400, MOEO 600, HSC (HS control), SMOEO 200, SMOEO 400, and SMOEO 600. Based on the results, a dose-dependent decrease was observed on days 21 and 42 in serum adiponectin and T3 in the stress and stress-free groups compared with the control group due to MOEO supplementation. The lowest decrease was observed in the MOEO 600 mg/kg dose group. In addition, an increase in stressed groups was observed when examining serum citrulline levels, while chemerin levels did not have a statistically significant effect. While the decrease in serum visfatin, T4, and TSH levels on the 21st day did not have a statistically significant effect, a significant effect was determined on the 42nd day. The addition of MOEO at 600 mg/kg to the ration may be beneficial in preventing intestinal damage and inflammation that may occur due to HS.

Keywords: adiponectin, chemerin, citrulline, Japanese quail, thyroid hormones, visfatin

1. Introduction

Heat stress (HS) is one of the major environmental problems threatening the poultry industry due to the expected increase 0.6-2.5 °C in temperature caused by global warming, especially in the next 50 years (Xu et al., 2018; Saleh and Al-Zghoul, 2019). Because poultry do not have sweat glands,

they exhibit clinical signs of HS at ambient temperatures >30 °C (Yardibi and Türkay, 2008). Stress stimulates the hypothalamus, causing an increase in adrenocorticotropic and glucocorticoid hormone release and a decrease in thyroid hormones. Heat stress leads to serious physiological disorders including respiratory alkalosis and intestinal damage (Xu et al., 2018; Saleh and Al-Zghoul, 2019). The immunosuppressive effect of HS leads to secondary infections, decreased performance parameters, and increased morbidity and mortality; furthermore, various physiological processes, including metabolic, endocrine, and cardiovascular systems, are negatively affected (St-Pierre et al., 2003; Bayraktar and Tekce, 2018; Xu et al., 2018).

One of the most important anatomical structures in the adaptive thermogenesis mechanism of poultry is the adipose tissue (Gray et al., 2013; Song et al., 2020). Moreover, the adipose tissue is a highly active metabolic and endocrine organ (Medina-Gómez, 2012). Adipokines, secreted from the adipose tissue, are hormones that play an important role in various physiological processes such as energy balance, regulation of metabolism, and cardiovascular and neuroendocrine functions (Medina-Gómez, 2012; Wang et al., 2017).

Heat stress is a significant environmental factor that affects carbohydrate and lipid metabolism, energy regulation, endocrine system, and adipokine expression in poultry. Adipokines are involved in regulation of the process of metabolic adaptation response to HS (Estienne et al., 2019; Mellouk et al., 2018) as well as in various diseases including cancer (Dutta et al., 2019), cardiovascular diseases (Lau et al., 2017), diabetes (Mazaherioun et al., 2017), oxidative stress, and inflammation (Frühbeck et al., 2017).

We studied visfatin, adiponectin, and chemerin hormones to examine the endocrine response of the adipose tissue. Visfatin is a 52-kDa pro-inflammatory adipokine that exerts insulin-mimetic effects (Pilz et al., 2007; Bayraktar, 2018; Pourvali-Talatappeh and Alipoor, 2019). It has a protective effect on cells exposed to inflammation and oxidative stress (Rongvaux et al., 2002; Gradinaru et al., 2017). Although visfatin level has been established in chickens (Diot et al., 2015), studies reporting its level in quail are lacking.

Adiponectin is a 30-kDa adipokine secreted from white adipose tissue and is present in three different isoforms: low-, middle-, and high-molecular weight (Hendricks et al., 2009; Barbe et al., 2019; Tsao et al., 2003). Also known as a marker with insulin-sensitising, anti-inflammatory, and anti-atherogenic functions, adiponectin is one of the most abundant adipokines in the plasma (Ouchi and Walsh, 2007; Barbae et al., 2019). Chemerin, an 18-kDa adipokine, is involved in inflammation and regulation of adipogenesis. Chemerin stimulates the dendritic cells and chemotaxis of macrophages by expressing the chemokine like-receptor 1 gene during inflammation or tissue damage. Due to its ability to direct these cells to the inflammation site, chemerin is also known as a leukocyte chemoattractant adipokine (Wittamer et al., 2003; Zabel et al., 2005).

Citrulline, which can be converted into arginine, is an end product of the glutamine metabolism and is one of the biomarkers used for assessing intestinal damage (Crenn et al., 2008). This amino acid is synthesised only in the gut, proximal part of the small intestine, and enterocytes in the middle and upper parts of the intestinal villi (Lin et al., 2004; Baxter et al., 2019). Plasma citrulline is derived from the enterocytes in the small intestine; its levels decrease in case of inflammation and enterocyte mass loss (Crenn et al., 2000; Crenn et al., 2008). Therefore, plasma citrulline level is used for evaluating small intestine integrity and function as well as intestinal damage (Crenn et al., 2008; Baxter et al., 2019).

The main regulators of basal metabolic rate, thyroid hormones (TH) including thyroxine (T4) and 3,5,3'-triiodothyronine (T3), and thyroid stimulating hormone (TSH) play a role in the differentiation, growth, development, and functioning of all tissues (Mullur et al., 2014). Thyroid hormones must be within a specific range in the blood to regulate its metabolic activity and maintain body homeostasis (Sinha et al., 2018; Braun and Schweizer, 2018).

Moringa oleifera essential oil (MOEO) is one of the strategies to reduce the negative impact of HS (Onasanya et al., 2015). The regulation of the intestinal microbiota is critical in feeding strategies.

Recent studies have been reported on the regulatory effects of MOEO administration on the intestinal flora (Elabd et al., 2018). *Moringa oleifera* is a feed additive. It is a leguminous plant used by both humans and animals (Tiamiyu et al., 2016). This plant has antioxidant, anti-inflammatory, hypoglycemic, hypolipidemic, cholesterol-lowering, and hepatoprotective properties (Chumark et al., 2008; Singh et al., 2009; Leone et al., 2015; Gıdık, 2018). Its leaves have multiple antioxidants such as phenolic acids (gallic, chlorogenic, ellagic, and ferulic) and flavonoids (kaempferol, quercetin, and routin) (Siddhuraju and Becker, 2003). It is also an important source of vitamins (B-complex, C, D, and K) and alpha-carotene (Ferreira et al., 2008; Singh et al., 2009). *Moringa oleifera* prevents oxidative damage by increasing the activity of antioxidant enzymes. It also reduces the formation of free radicals by reducing the intensity of lipid peroxidation (Singh et al., 2009; Mahfuz and Piao, 2019; Hassan et al., 2016; Gouda et al., 2018; Leone et al., 2015).

This study aimed to investigate the adipokine (visfatin, adiponectin, chemerin), intestinal (citrulline), and thyroid (T3, T4, and TSH) endocrine response of different levels of MOEO (200, 400, and 600 ppm) supplementation to Japanese quail exposed to HS.

2. Material and Methods

The experiment was conducted in Bayburt, Turkey (Latitude: 40°14'59" S and 40°13'52" W). Research on quails was conducted according to the institutional committee on animal use (case no. 2020/16).

2.1. Quail and experimental design

In this study, 320 one-day-old male Japanese quail were used. Male quail were identified by a quail chick development unit officer and gender discrimination specialist team in accordance with gender discrimination techniques (Hays and Sambardo, 1927). The study was conducted over 42 days, including a 7-d adaptation period and a 35-d experimental period. The trial was conducted in groups of 10 quail, which were raised in cages measuring 100 × 50 × 100 cm in size. On the seventh day of the trial, quail were assigned to eight groups: control temperature (CT, 25 °C), MOEO-200, MOEO-400, MOEO-600, HSC (HS control), SMOEO-200, SMOEO-400, SMOEO-600), each comprising 40 quail, such that the groups included quail of equal body weight. Each group was divided into four subgroups, each comprising 10 quail. Starting from day 7 until day 42 of the trial, the stress-free groups were raised at a comfort (thermoneutral) temperature of 25 °C (CT, MOEO-200, MOEO-400, and MOEO-600) and the heat-stressed groups (HSC, SMOEO-200, SMOEO-400, and SMOEO-600) were raised at a temperature of 37 °C, which was above the thermoneutral zone.

To calculate daily feed intake, before being given to the quails, the feed was weighed, and leftovers were removed the following day. The control groups were fed a basal diet (Table 1), and the trial groups were given the basal diet supplemented with different doses of MOEO. The MOEO used in the present study was supplied from a commercial company operating in the Trabzon province. The feed provided to quails was analysed according to the official methods of analysis described by the Association of Official Analytical Chemists (AOAC, 2005) (Table 1).

2.2. Poultry temperature, humidity, and lighting

The general temperature of the Poultry section was kept constant at 32-33 °C for the first two days and at 27-28 °C for the subsequent five days, in the following periods, while the groups subjected to thermal stress were placed at 37 °C and 75-85% (relative) humidity, and the remaining groups were placed at 25 °C and 55-60% (relative) humidity. Furthermore, 24 h of lighting (60 W) was applied to all groups. Japanese quail were given fresh drinking water *ad libitum*. Trial groups are divided into stress and stress-free, starting from day 7. Heating of the cluster was provided by means of 37±1 °C sensitive thermostat appliances (Turkey) connected to the central heating system for 7-42 days. Temperature and humidity values were measured with daily digital temperature-humidity meter (TFA Dostmann, Germany) thermometers placed at four different points of the coop to control the temperature therein. The room temperature of stress-free groups was designed to be 25 °C.

Table 1 - Nutrient content (%) and chemical analysis of basal diet ration

Raw material	Quail chick feed (0-21 d)	Quail grower feed (21-42 d)
Corn	52.70	58.12
Corn gluten meal	15.21	26.14
Soybean meal (% 44 CP)	26.35	10.65
Di-calcium phosphate	1.95	1.60
Limestone	1.18	1.04
Sodium cloride	0.31	0.31
Sodyum bicarbonate	0.20	0.20
Salt	0.2	0.2
Methionine	0.50	0.44
Lysine	1.20	1.10
Vitamin-mineral premix[1]	0.20	0.20
Metabolizable energy (MJ/kg)	12.97	13.50
Crude protein (%)	24	20
Crude oil (%)	2.61	2.50
Ash (%)	5.19	3.85
Moisture (%)	13.20	13.20
Calcium (%)	1.00	0.90
Total P (%)	0.72	0.60
Total sulphur aminoacids (%)	0.71	0.71

The vitamin-mineral premix provided the following (per kg of diet): vitamin A, 6,000 IU; vitamin D3, 1,000 IU; vitamin E, 15 mg; vitamin K, 2 mg; vitamin B1, 3 mg; vitamin B2, 4 mg; vitamin B6, 4 mg; vitamin B10, 0.03 mg; calcium D-pantothenate, 15 mg; folic acid, 1 mg; niacin, 25 mg; D-biotin, 0.115 mg; Mg, 80 mg; I, 0.15 mg; Co, 0.2 mg; Cu, 5 mg; Fe, 60 mg; Se, 1 mg; Zn, 60 mg.

2.3. Collection of serum samples

On days 21 and 42 of the study, 12 quail were randomly selected from each group, and blood samples were taken from the *vena subcutenea ulnaris* of 24 quail. For hormone analyses, samples were placed in 2-mL tubes (VACUETTE® TUBE 9 mL Z Serum Clot Activator) without anticoagulant and numbered. Serum samples were obtained by centrifuging the blood samples at +4 °C and 4100 rpm for 12 min in a cooled centrifuge (NF 1200, CORE, Ankara, Turkey), placed in Eppendorf tubes, and stored at −80 °C until further laboratory analyses.

2.4. Composition of *Moringa oleifera* essential oil

Based on analysis results (ASU 64 LFGB L 07.00.40, high-performance liquid chromatography with ultra-violet absorption and fluorescence detection (HPLC-UV/FLD), the MOEO in the present study was determined to contain the aromatic compounds naphthalene (23 µg/kg), phenanthrene (8.5 µg/kg), fluorene (4.4 µg/kg), pyrene (4.1 µg/kg), fluoranthene (3.6 µg/kg), acenaphthene (1.7 µg/kg), and anthracene (0.79 µg/kg). Fat analysis results (ASU 64 LFGB L 07.00.40, HPLC-UV/FLD) revealed a total fat content of 98.7 g/100 g, saturated level of 22.2 g/100 g, monounsaturated level of 74.2 g/100 g, and polyunsaturated level of 3.2 g/100 g. *Moringa oleifera* essential oil contained vitamin A (<5 µg/100 g), vitamin D3 (<2.5 µg/100 g), and vitamin E (21 mg/100 g), and the carotenoids lutein (0.25 mg/100 g), zeaxanthin (<0.04 mg/100 g), canthaxanthin (<0.04 mg/100 g), beta-cryptoxanthin (<0.04 mg/100 g), alpha-carotene (<0.04 mg/100 g), beta-carotene (<0.04 mg/100 g), and lycopene (<0.04 mg/100 g).

2.5. Measurement of serum visfatin, adiponectin, chemerin, and citrulline levels

The minimum detectable concentrations of hormone kits used to measure visfatin, adiponectin, chemerin, and citrulline hormone levels in blood serum samples obtained from the quail

were 0.05 ng/mL, 1.56 ng/mL, and 15.6 pmol/mL, respectively, and their determinations were 0.156–10 ng/mL, 15–480 ng/mL, and 37.5–1000 pmol/mL, respectively. Species-specific bird visfatin (Visfatin, SinoGeneClon, ELISA Kit Product code: SG-82164, China), adiponectin (Adiponectin, SinoGeneClon, ELISA Kit Product code: SG-82259, China), chemerin (Chemerin, SinoGeneClon, ELISA Kit Product code: SG-82205), China), and citrulline (Citrulline, SinoGeneClon, ELISA Kit Product code: SG-82203, China) were used in ELISA. Using intra- and inter-assay coefficients of 8.0 and 10.0%, respectively, ELISA was performed following the manufacturer's instructions. Absorbance values were recorded at a wavelength of 450 nm.

2.6. Measurement of thyroid hormones

The measurements of T3 (Lot No: 1006125590), T4 (Lot No: 1006123200), and TSH (Lot No: 1006156500) in the blood serum samples obtained from the quail were performed by enzyme-linked fluorescent assay (ELFA, applied in Mini-Vidas instrument, France) using an auto analyser and compact auto immuno-assay method based on the principles of ELFA.

2.7. Statistical analysis

Normal distributions of data (visfatin, adiponectin, chemerin, citrulline, T3, T4, and TSH) obtained as a result of the trial were checked. Statistical analyses of the effects of diet and temperature on hormone levels were performed using the general linear model multivariate test given below.

$$Y_{ijk} = \mu + DI + T_j + (D + T)_{ij} + e_{ijk},$$

in which Y_{ijk} = observation, μ = mean, DI = diet effect, T_j = temperature effect, $(D + T)_{ij}$ = interaction effect, and e_{ijk} = experimental error effect. Duncan multiple comparison tests were performed to compare differences between averages. All statistical tests were analyzed in the IBM SPSS 22.0 statistical program. All significant differences were evaluated by $P<0.05$ level tests.

3. Results

A dose-dependent decrease in serum adiponectin and T3 was observed in stress and stress-free groups compared with the control group ($P<0.01$). On the other hand, while the decrease in serum visfatin, T4, and TSH levels on the 21st day did not have a statistically significant effect ($P>0.05$), a significant effect was determined on the 42nd day ($P<0.01$) (Tables 2 and 3).

In addition, an increase in serum citrulline levels ($P<0.01$) was observed in stressed groups, while chemerin levels did not have a statistically significant effect ($P>0.05$). The highest serum visfatin levels, 6.03 and 8.55 µg/mL, were found in the stress-free and stress control groups on day 21, and 6.80 and 9.95 µg/mL on day 42, respectively. The lowest serum visfatin levels, 4.43 and 6.48 µg/mL, were found in the stress-free and stress control groups on day 21, and 4.83 and 6.98 µg/mL on day 42, respectively. Similarly, the highest serum adiponectin levels, 4.53 and 7.55 ng/mL, were observed in the stress-free and stress control groups on day 21, and 3.98 and 8.33 ng/mL on day 42, respectively (Tables 4 and 5).

The lowest serum adiponectin levels, 3.45 and 5.08 ng/mL, were found in the stress-free and stress control groups on day 21, and 3.20 and 6.05 ng/mL on day 42, respectively. Furthermore, the highest chemerin levels, 13.20 and 15.32 ng/mL, were found in the stress-free and stress control groups on day 21, and 14.33 and 13.88 ng/mL on day 42, respectively (Tables 4 and 5). The lowest chemerin levels were observed in the MOEO 600 mg/kg groups. Plasma citrulline is reported to be a reliable marker of intestinal function (Crenn et al., 2000). Serum citrulline levels decreased with increased dose administration in stress groups compared with the control group, and the lowest MOEO level was detected in the 600 mg/kg group. Serum citrulline levels for quails at 21 and 42 days in the MOEO 600 mg/kg group were determined as 3.58 and 4.02 pmol/mL, respectively.

Table 2 - Effect of *Moringa oleifera* essential oil (MOEO) on T3, T4, and TSH hormone levels in quail fed under stress and nonstress on the 21st day

	N	T3 (ng/mL)		T4 (ng/mL)		TSH (ng/mL)	
		25 °C	37 °C	25 °C	37 °C	25 °C	37 °C
Control	24	3.28a	3.19b	13.33	20.00	2.17	2.37
MOEO 200 mg/kg	24	3.18bc	3.16c	12.65	18.98	2.09	2.30
MOEO 400 mg/kg	24	3.06e	3.10d	11.85	18.43	2.01	2.23
MOEO 600 mg/kg	24	2.99g	3.03f	11.35	17.53	1.94	2.17
	SEM	0.01		0.10		0.01	
Source of variation (P-values)							
Diet		0.00		0.00		0.00	
Temperature		0.33		0.00		0.00	
Temperature × diet		0.00		0.09		0.60	
Main effect of diet							
Control		3.23a		16.66a		2.27a	
MOEO 200 mg/kg		3.17b		15.81b		2.20b	
MOEO 400 mg/kg		3.08c		15.14c		2.12c	
MOEO 600 mg/kg		3.01d		14.44d		2.06d	
	SEM	0.01		0.07		0.01	
Temperature							
25 °C		3.13		12.29		2.05	
37 °C		3.12		18.73		2.27	
	SEM	0.01		0.05		0.01	

SEM - standard error of the mean.
a-g - Means within a column showing different letters are significantly different (P<0.05).

Table 3 - Effect of *Moringa oleifera* essential oil (MOEO) on T3, T4, and TSH hormone levels in quail fed under stress and nonstress on the 42nd day

	N	T3 (ng/mL)		T4 (ng/mL)		TSH (ng/mL)	
		25 °C	37 °C	25 °C	37 °C	25 °C	37 °C
Control	24	3.16a	3.06cd	14.00e	21.20a	2.23e	2.53a
MOEO 200 mg/kg	24	3.08c	3.13b	13.15f	19.28b	2.15f	2.44b
MOEO 400 mg/kg	24	2.98e	3.05d	12.33g	18.83c	2.08g	2.35c
MOEO 600 mg/kg	24	2.93f	2.96e	11.85h	18.03d	2.06g	2.28d
	SEM	0.01		0.11		0.02	
Source of variation (P-values)							
Diet		0.00		0.00		0.00	
Temperature		0.09		0.00		0.00	
Temperature × diet		0.00		0.00		0.01	
Main effect of diet							
Control		3.11a		17.60a		2.38a	
MOEO 200 mg/kg		3.11a		16.21b		2.30b	
MOEO 400 mg/kg		3.02b		15.58c		2.21c	
MOEO 600 mg/kg		2.95c		14.94d		2.17d	
	SEM	0.01		0.08		0.01	
Temperature							
25 °C		3.04		12.83		2.13	
37 °C		3.05		19.33		2.40	
	SEM	0.01		0.06		0.01	

SEM - standard error of the mean.
a-h - Means within a column showing different letters are significantly different (P<0.05).

Table 4 - Effect of *Moringa* oleifera essential oil (MOEO) on citrulline, chemerin, visfatin, and adiponectin levels in quail fed under stress and nonstress on the 21st day

	N	Citrulline (pmol/mL)		Chemerin (ng/mL)		Visfatin (µg/mL)		Adiponectin (ng/mL)	
		25 °C	37 °C	25 °C	37 °C	25 °C	37 °C	25 °C	37 °C
Control	24	3.44h	4.56a	13.20	15.32	6.03	8.55	4.53e	7.55a
MOEO 200 mg/kg	24	3.67f	4.32b	11.70	13.60	5.38	7.65	4.10f	6.85b
MOEO 400 mg/kg	24	3.91e	4.00d	10.78	12.09	4.83	6.98	3.83g	6.05c
MOEO 600 mg/kg	24	4.21c	3.58g	9.13	10.38	4.43	6.48	3.45h	5.08d
	SEM	0.02		0.25		0.10		0.07	
Source of variation (P-values)									
Diet		0.00		0.00		0.00		0.00	
Temperature		0.00		0.00		0.00		0.00	
Temperature × diet		0.00		0.26		0.11		0.00	
Main effect of diet									
Control		4.00a		14.26a		7.29a		6.04a	
MOEO 200 mg/kg		3.99ab		12.65b		6.51b		5.48b	
MOEO 400 mg/kg		3.96b		11.43c		5.90c		4.94c	
MOEO 600 mg/kg		3.89c		9.75d		5.45d		4.26d	
	SEM	0.01		0.18		0.07		0.05	
Temperature									
25 °C		3.81		11.20		5.16		3.97	
37 °C		4.11		12.85		7.41		6.38	
	SEM	0.10		0.18		0.05		0.04	

SEM - standard error of the mean.
a-h - Means within a column showing different letters are significantly different (P<0.05).

Table 5 - Effect of *Moringa oleifera* essential oil (MOEO) on citrulline, chemerin, visfatin, and adiponectin levels in quail fed under stress and nonstress on the 42nd day

	N	Citrulline (pmol/mL)		Chemerin (ng/mL)		Visfatin (µg/mL)		Adiponectin (ng/mL)	
		25 °C	37 °C	25 °C	37 °C	25 °C	37 °C	25 °C	37 °C
Control	24	3.78e	5.07a	14.33	13.88	6.80d	9.95a	3.98e	8.33a
MOEO 200 mg/kg	24	3.97d	4.43b	13.38	12.37	6.33e	8.60b	3.80e	7.65b
MOEO 400 mg/kg	24	4.23c	4.24c	11.75	10.99	5.38f	7.70c	3.53f	6.83c
MOEO 600 mg/kg	24	4.49b	4.02d	10.65	9.62	4.83g	6.98d	3.20g	6.05d
	SEM	0.03		0.23		0.12		0.08	
Source of variation (P-values)									
Diet		0.00		0.00		0.00		0.00	
Temperature		0.00		0.00		0.00		0.00	
Temperature × diet		0.00		0.57		0.00		0.00	
Main effect of diet									
Control		4.42a		14.10a		8.38a		6.15a	
MOEO 200 mg/kg		4.20b		12.87b		7.46b		5.73b	
MOEO 400 mg/kg		4.23b		11.37c		6.54c		5.18c	
MOEO 600 mg/kg		4.26b		10.14d		5.90c		4.63d	
	SEM	0.02		0.16		0.08		0.05	
Temperature									
25 °C		4.12		12.53		5.83		3.63	
37 °C		4.44		11.71		8.31		7.21	
	SEM	0.02		0.12		0.06		0.04	

SEM - standard error of the mean.
a-g - Means within a column showing different letters are significantly different (P<0.05).

4. Discussion

To investigate the response of adipose tissue in Japanese quail under HS, we examined the changes in visfatin, adiponectin, and chemerin, which are associated with the use of MOEO. Adiponectin is an anti-inflammatory marker (Lee et al., 2019). In the case of exposure to HS (Bernabucci et al., 2009; Shiloah et al. 2007; Al-Dawood, 2017) and metabolic syndrome (Metwally et al., 2017), serum adiponectin levels are increased. It reduces the hepatic and systemic pro-inflammatory responses induced by DNA methylation signalling by increasing insulin sensitivity under stress conditions and in the metabolic syndrome, thus providing intestinal microbiota restoration for the microbial ecosystem (Al-Dawood, 2017; Lee et al., 2019). The results of our study showed that HS significantly reduced the adiponectin concentration compared with the control group ($P \leq 0.01$). It is concluded that MOEO administration in all groups could have a significant effect on normalization of serum adiponectin levels with increasing doses. The lowest adiponectin levels were determined in the MOEO 600 mg/kg group. In comparison with the controls, the increase in adiponectin expression caused by HS and the decrease in adiponectin levels due to MOEO use are consistent with the literature regarding the anti-inflammatory role of the adipokines in the adaptive process against HS (Min et al., 2015). On the other hand, although the adiponectin levels in quail have been studied for the first time, the results were consistent with studies conducted in chickens (Hendricks et al., 2009; Al-Dawood, 2017) and using MOEO (Metwally et al., 2017).

Chemerin is an adipokine, known as a regulator of adipogenesis, inflammation, and glucose metabolism. It is used as an inflammatory marker due to an increase in its levels in inflammation (Roh et al., 2007; Ernst and Sinal, 2010; Tu et al., 2020). Although the results of our study were also expressed in decreased chemerin levels in all groups due to the addition of MOEO, we found that it did not have a statistically significant effect ($P > 0.05$). On the other hand, the results of our study are consistent with the reported results of Diot et al. (2015).

Visfatin is an adipokine involved in the regulation of glucose homeostasis, oxidative stress, and inflammatory response. It has a role in the regulation of processes against stress (Rongvaux et al., 2002). Depending on the increase in the MOEO dose added to the ration, a decrease in serum visfatin level occurred. While the decrease in serum visfatin level on the 42nd day had a statistically significant effect ($P < 0.01$), the decrease on the 21st day was not statistically significant ($P > 0.05$). The lowest decrease was observed in the MOEO 600 mg/kg dose group ($P < 0.01$). The results of our study are consistent with the reported results of the study by Diot et al. (2015). We hypothesize that the increase in serum visfatin level under stress and the decrease due to MOEO dose increase can give an idea about the physiological process shaped by HS and MOEO addition.

The intestinal mucosa acts as a barrier for the protection against bacteria and bacterial toxins present in the intestinal lumen. The deterioration of intestinal integrity due to HS and, consequently, the increased intestinal permeability to endotoxins leads to an increase in intestinal pathogens and serum inflammatory cytokines (Alhenaky et al., 2017). Measures to protect the intestinal barrier are important to ensure the health, immunity, and welfare of poultry. In terms of the investigation of the endocrine response of intestinal tissue, citrulline is an important biomarker for evaluating the condition and function of the small intestine because it is produced only by enterocytes in the small intestine (Baxter et al., 2019). An increase in serum citrulline level has been observed in possible intestinal damage and diseases (Crenn et al., 2008). *Moringa oleifera* essential oil is effective in preventing inflammation by regulating intestinal microbiota (Elabd et al., 2018; Gao et al., 2017; Wang et al., 2017). The results revealed that HS significantly lowered citrulline hormone ($P < 0.01$) compared with the control group, but MOEO use had a favourable and significant effect on the normalisation of citrulline levels. However, according to the literature review, this is the first study to examine the serum citrulline levels in relation to the use of MOEO in quail exposed to stress. The results of our study are consistent with those of another study (Baxter et al., 2019). In terms of the evaluation of serum citrulline level as a marker of intestinal function, we suppose that increase due to HS and decrease due to the MOEO dose may provide an idea about the adaptive process and intestinal function in the gut depending on HS and the addition of MOEO.

Heat stress in poultry leads to a decrease in thyroid hormone levels (Hosseini et al., 2013; Khalil et al., 2008). There was a significant decrease in serum T3 levels in the stress and stress-free groups at 21 and 42 days compared with the control groups ($P<0.01$). While the decrease in serum T4 and TSH levels on the 21st day did not have a statistically significant effect ($P>0.05$), a significant effect was detected on the 42nd day ($P<0.01$). These results suggest that MOEO supplementation against stress may contribute to the development of thermoregulatory ability of the thyroid gland and may play a regulatory role in thyroid metabolism (Spiers et al., 1974; Hosseini et al., 2013; Khalil et al., 2008).

5. Conclusions

This study revealed the effect of *Moringa oleifera* essential oil supplementation on visfatin, adiponectin, citrulline, chemerin, T3, T4, and TSH hormone levels in quail experimentally exposed to heat stress. According to the results of our current study, it is believed that *Moringa oleifera* essential oil can be used as an alternative product at 600 mg/kg in poultry to reduce inflammation and intestinal damage due to the harmful effects of heat stress.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: B. Bayraktar, E. Tekce, S. Bayraktar, G. Böyük, M. Genç and H. Kaya. Data curation: B. Bayraktar, E. Tekce, S. Bayraktar, G. Böyük, Ç. Takma, V. Aksakal, M. Genç, H. Kaya and U. Ülker. Formal analysis: B. Bayraktar, S. Bayraktar, G. Böyük, Ç. Takma, V. Aksakal, M. Genç, H. Kaya and U. Ülker. Funding acquisition: B. Bayraktar, S. Bayraktar, G. Böyük, Ç. Takma and V. Aksakal. Investigation: B. Bayraktar and Ç. Takma. Methodology: B. Bayraktar. Project administration: B. Bayraktar. Resources: B. Bayraktar, E. Tekce, S. Bayraktar, G. Böyük, Ç. Takma, V. Aksakal, M. Genç, H. Kaya, U. Ülker and A.B. Gürbüz. Software: B. Bayraktar, E. Tekce, G. Böyük, Ç. Takma, V. Aksakal, M. Genç, H. Kaya and A.B. Gürbüz. Supervision: B. Bayraktar, E. Tekce, G. Böyük, Ç. Takma, V. Aksakal, M. Genç, H. Kaya, U. Ülker and A.B. Gürbüz. Validation: B. Bayraktar, S. Bayraktar, Ç. Takma, U. Ülker and A.B. Gürbüz. Visualization: B. Bayraktar, S. Bayraktar, U. Ülker and A.B. Gürbüz. Writing-original draft: B. Bayraktar, S. Bayraktar and M. Genç. Writing-review & editing: B. Bayraktar, S. Bayraktar and U. Ülker.

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