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RESEARCH ARTICLE

Adipokine, gut and thyroid hormone responses to probiotic application in chukar partridges (*Alectoris chukar*) exposed to heat stress

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ABSTRACT

The aim of this study was to investigate the effect of *Lactobacillus reuteri* E81 (LRE) probiotic supplementation on heat stress responses in chukar partridges (*Alectoris chukar).* The birds were divided into two groups, one of which was exposed to heat stress (HS). Within each group, four subgroups, each including 64 birds, were created for the three treatment doses (200, 400 or 600 mg/kg) of LRE and the control. The experiment was started with day-old birds, kept at a temperature of 25 °C or 37 °C. After a 7-day adjustment period, the LRE supplementation lasted for 35 days. The levels of different adipokines, including visfatin (VF), adiponectin (ADP), chemerin (CHEM), as well as the concentration of plasma citrulline (CIT) and the levels of thyroid hormones (T3 and T4) and thyroid-stimulating hormone (TSH) in the blood were measured at 21 and 42 days of age. A significant correlation ($P < 0.01$) was found between LRE supplementation and the decrease in serum VF, ADP, CIT, T3 and T4 levels in partridges exposed to HS. On the other hand, no significant relationship was found between LRE supplementation and the serum CHEM and TSH levels ($P > 0.05$). We concluded that the addition of 600 mg/kg LRE is beneficial in preventing intestinal damage and inflammation provoked by HS.

KEYWORDS

adipokine, chukar partridge (*Alectoris chukar)*, heat stress, thyroid hormones, probiotic

INTRODUCTION

Heat stress (HS) is an important environmental problem due to climate change secondary to global warming and poses a threat to the poultry industry (Nyoni et al., 2019; Saleh and Al-Zghoul, 2019; Al-Sagan et al., 2020). Birds are homeothermic organisms having high metabolic activity and no sweat glands. They can tolerate humidity more than high temperatures, and clinical signs of HS develop once the environmental temperature exceeds 30 °C (Marchini et al., 2018). High environmental temperatures alter the activity of the

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neuroendocrine system in poultry. HS decreases thyroid hormone (TH) secretion because it affects thyroid function and increases the corticosterone level by stimulating the hypothalamic–pituitary–adrenal axis (Lara and Rostagno, 2013; Xu et al., 2018; Saleh and Al-Zghoul, 2019). The immunosuppressive effect of HS causes health problems ranging from growth and development retardation through metabolic problems to death in poultry (Kőrösi-Molnár et al., 2021). Moreover, secondary infections may also result in decreased performance parameters and increased morbidity and mortality rates (St-Pierre et al., 2003; Chen et al., 2013; Xu et al., 2018). Adipose tissue is an important endocrine organ that has an effect on energy metabolism through the adipokines it secretes (Medina-Gómez, 2012). Adipokines are cytokines released from adipose tissue and play a major role in various physiological processes, such as regulation of metabolism, as well as cardiovascular and neuroendocrine functions (Medina-Gómez, 2012; Wang et al., 2017). In addition, HS induces adaptive responses responsible for changes in carbohydrate and lipid metabolism (Morera et al., 2012). Visfatin (VF) is a new pro-inflammatory adipokine with a molecular weight of 52 kDa and has an insulinomimetic effects (Pilz et al., 2007; Pourvali-Talatappeh and Alipoor, 2019). It also has a protective effect in cells against the negative impact posed by inflammation and oxidative stress (Rongvaux et al., 2002). Although VF levels have been described in chickens (Diot et al., 2015), no study has investigated VF levels in chukar partridges (*Alectoris chukar*) yet. Adiponectin (ADP) is a 30-kDa multimeric protein and is an adipokine secreted mainly by white adipose tissue. ADP has a regulatory role in glucose, lipid and insulin metabolism. It also has an anti-inflammatory, oxidative stress reducing, anti-fibrotic and antioxidant effect (Tsao et al., 2003; Gradinaru et al., 2017; Barbe et al., 2019). Chemerin (CHEM) is an important adipokine with a molecular weight of 18 kDa and is responsible for regulating inflammation and adipogenesis. It ensures chemotaxis of dendritic cells and macrophages during inflammation or tissue damage. CHEM is defined as a leukocyte chemo-attractant adipokine due to its role in directing these cells to the inflammation site (Wittamer et al., 2003; Zabel et al., 2005; Goralski et al., 2019). Citrulline (CIT) is an amino acid and a product of glutamine metabolism, which can be converted into arginine (Crenn et al., 2008). It is produced in the enterocytes located in the upper and middle sections of the small intestine (Lin and Stoll, 2006; Baxter et al., 2019). The CIT in the circulating blood originates from the enterocytes of the small intestine. The CIT level of the plasma is used as a biomarker to assess the integrity of the small intestine and its function, as well as intestinal damage (Crenn et al., 2008; Gosselin et al., 2014; Baxter et al., 2019). Inflammation and loss of enterocyte mass are accompanied by reduced CIT levels in the plasma (Crenn et al., 2000, 2008).

The thyroid hormones T3 and T4 are secreted by the thyroid gland whereas thyroid-stimulating hormone (TSH) is secreted by the pituitary gland. The activity of the thyroid gland is affected by environmental temperature. T3 and T4 play an important role in the regulation of metabolism, thermogenesis and thermoregulation processes in chickens (Tao et al., 2006; Mullur et al., 2014; Sinha et al., 2018). Adipokines have a role in the regulation of thermogenesis, body weight, basal metabolic rate, and appetite by affecting the hypothalamic–pituitary–thyroid axis (Mullur et al., 2014). Any disorders in the thyroid–adipokine axis might alter the energy expenditure and cytokine networks (Ahmed, 2017), inducing oxidative stress (Sumathi et al., 2016) and cell death (Ferrante et al., 2011).

Probiotics are one of the strategies to reduce the negative impact of HS (Lara and Rostagno, 2013). Regulation of the intestinal microbiota is critical in feeding strategies. Gut performance is also an essential part of successful feeding programmes. One of the most important functions of probiotic bacteria in the intestinal system is to help the host's natural flora create a barrier for pathogens. Some probiotic strains produce hydrogen peroxide (H_2O_2), organic acid, diacetyl, biosurfactant substances, bacteriocin, and some antimicrobial agents. Thus, probiotics prevent pathogenic bacteria from adhering to intestinal epithelial cells (Gibson, 1998; Ouwehand et al., 1999; Ng et al., 2009). Recent studies have reported the regulatory effects of probiotics administration on the intestinal flora (Elabd et al., 2018). Studies supporting the use of probiotics have reported their benefits, such as an improvement in intestinal microbial balance and intestinal health (Awad et al., 2009; Mountzouris et al., 2010; Sen et al., 2012), antimicrobial activity (Pringsulaka et al., 2015), regulation (modification) of the villus-crypt structure (Al-Fataftah and Abdelqader, 2014; Incharoen et al., 2019) and stress reduction (Zhang et al., 2016).

The aim of this study was to analyse the adipokine (VF, ADP and CHEM), gut (CIT) and thyroid (T3, T3 and TSH) responses associated with various doses of probiotic supplement in chukar partridges (*Alectoris chukar*) exposed to HS.

MATERIALS AND METHODS

The study was conducted pursuant to the approval (dated 21 December 2020 and numbered 2020/15) of the Local Ethics Board for Animal Experiments of the Veterinary Control Centre Research Institute Directorate (Etlik, Ankara).

Animals and experimental design

The study included 256 day-old, male chukar partridges. It comprised an adaptation period of 7 days and a fattening period of 35 days. The birds were kept in 100 × 50 × 100 cm cages at the Poultry Unit of Bayburt University, Food, Agriculture and Livestock Application and Research Centre. From day 7 of the study, the live weights of the birds were measured, then the birds were divided into two large, non-stress (C) and stress (HS) groups, kept at 25 °C or 37 °C, respectively. Each group was further divided according to the dose of the LRE supplement (200, 400 and 600 mg//kg).

Table 1. Nutrient content and analysis (g/kg) of the basal ration

Raw material	Starter (0–14 days)	Grower (14–28 days)	Finisher (28–42 days)
Maize	52.70	54.60	58.12
Maize gluten feed	15.21	21.20	26.14
Soybean residue	26.35	18.90	10.65
Dicalcium phosphate	1.95	1.70	1.60
Calcium carbonate	1.18	1.10	1.04
Sodium chloride	0.31	0.31	0.31
Sodium bicarbonate	0.20	0.20	0.20
Salt	0.2	0.2	0.2
Methionine	0.50	0.50	0.44
Lysine	1.20	1.10	1.10
Vitamin–mineral premix[1]	0.20	0.20	0.20
Metabolisable energy (kcal/kg)	3,100	3,150	3,225
Crude protein (%)	24	22	20
Crude oil (%)	2.61	2.30	2.50
Ash (%)	5.19	4.63	3.85
Moisture (%)	13.20	13.20	13.20

The vitamin–mineral premix provided the following (per kg of diet): vitamin A, 6,000 IU; vitamin D_3, 1,000 IU; vitamin E, 15 mg/kg; vitamin K, 2 mg/kg; vitamin B_1, 3 mg; vitamin B_2, 4 mg; vitamin B_6, 4 mg; vitamin B_{10}, 0.03 mg; calcium D-pantothenate, 15 mg; folic acid, 1 mg; niacin, 25 mg; D-biotin, 0.115 mg; Mg, 80 mg/kg; I, 0.15 mg/kg; Co, 0.2 mg/kg; Cu, 5 mg/kg; Fe, 60 mg/kg; Se, 1 mg/kg; Zn, 60 mg/kg.

A homogeneous mixture was obtained by spraying the LRE dose into the feed as 200, 400 or 600 mg/kg of feed daily. The nutrient content and analysis of the basal ration are shown in Table 1. In order to evaluate feed consumption, the feeds in front of the birds were taken at the same time every day (18:00–19:00) and the new feeds were weighed and made ready for use. These prepared feeds were given to the birds *ad libitum*. The probiotics used in the study were produced in the Food Department Laboratories, Faculty of Engineering of Bayburt University (4×10^{10} CFU/g) (Bayraktar and Tekce, 2020). Nutrient analyses of the feeds used throughout the study were performed in accordance with the methods stated by the AOAC (AOAC, 2005).

Temperature, humidity and lighting

The temperature of the trial environment was 32–33 °C on days 0–2 then kept steady at 27–28 °C on days 3–7. From the 8th day, the stressed groups were continuously exposed to HS at 37 °C and 75–85% relative humidity, while the stress-free control groups were held at 25 °C and 55–60% humidity. Throughout the study, all groups were subjected to illumination (60 W) for 24 h. Chukar partridges were given fresh drinking water *ad libitum*. Heating of the cluster was provided by means of 36 ± 1 °C sensitive thermostat appliances (Newmet) connected to the central heating system for 7–42 days. Temperature and humidity values were measured daily with a digital temperature-humidity meter (TFA Dostmann, Germany). The thermometers were placed at four different points in the cages to control the temperature. The room temperature of the stress-free groups is designed to be 25 °C (Bayraktar and Tekce, 2020).

Collection of serum samples

On days 21 and 42 of the study, 8 birds were randomly selected from each group and blood samples were taken from the *vena subcutanea ulnaris*. For hormone analyses, the 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 4,100 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.

Measurement of serum VF, ADP, CHEM and CIT levels

The VF, ADP, CHEM and CIT levels were measured in blood serum samples according to the manufacturer's instructions using the ELISA technique. Absorbance values were recorded at a wavelength of 450 nm. Serum VF concentration was measured using Bird VF ELISA Kit (sensitivity 0.05 ng/ml, intra-assay CV < 8%, inter-assay CV < 10%, catalogue No. SG-82164, SinoGeneClon, China). Serum ADP concentration was measured using Bird ADP ELISA Kit (sensitivity 1.56 ng/ml, intra-assay CV < 8%, inter-assay CV < 10%; catalogue No. SG-82259, SinoGeneClon, China). Serum ADP concentration was measured using Bird CHEM ELISA Kit (sensitivity 6 ng/L, intra-assay CV < 8%, inter-assay CV < 10%; catalogue No. SG-82205, SinoGeneClon, China). Serum CIT concentration was measured using Bird CIT ELISA Kit (sensitivity 15.6 pmol/ml, intra-assay CV 8.0%, inter-assay 10.0%; catalogue No. SG-82203, SinoGeneClon, China).

Measurement of T3, T4 and TSH

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

Statistical analysis

Normal distributions of data (CIT, ADP, VF, T3, T4, and CHEM) obtained during the trial were checked. Statistical analyses of the effects of diet and temperature on hormone levels were performed using the general linear model (GLM) given below:

$$Y_{ijk} = \mu + D_i + T_j + DT_{ij} + e_{ijk}$$

(Y_{ijk} = parameter measured; μ = overall mean; D_i = diet effect; T_j = temperature effect, DT_{ij} = interaction effect; e_{ijk} = experimental error effect)

Duncan's multiple comparison tests were performed to compare differences between averages. All statistical tests

were analysed in the IBM SPSS 22.0 statistical program. All significant differences were evaluated by $P < 0.05$ level tests.

RESULTS

The results obtained for hormone parameters on days 21 and 42 showed a significant correlation between the addition of LRE to the diet and the decreased VF, ADP, CIT, T3 and T4 levels in partridges exposed to HS (Tables 2–5). The lowest levels were in the LRE 600 mg/kg dose group ($P < 0.01$). On the other hand, no significant relationship was found between LRE supplementation and the serum CHEM and TSH levels.

The highest serum VF levels were observed in the non-stress and stress control groups on day 21 (5.80 and 8.48 ng/ml) and on day 42 (6.50 and 9.25 ng/ml), respectively. Similarly, the highest serum ADP levels were observed in the non-stress and stress control groups on day 21 (6.90 and 8.45 ng/ml) and on day 42 (6.50 and 9.25 ng/ml), respectively. The highest serum CHEM levels were found in the non-stress and stress control groups on day 21 (13.48 and 17.15 ng/ml) and on day 42 (14.73 and 14.83 ng/ml), respectively. The lowest CHEM levels were 5.23 and 6.86 ng/ml on day 21 and 3.88 and 4.18 ng/ml on day 42 in the non-stress and stress groups receiving 600 mg LRE/kg. The highest serum CIT levels were 4.70 and 5.92 pmol/ml on day 21 and 5.23 and 6.86 pmol/ml on day 42 in the non-stress and stress control groups, respectively. The lowest CIT levels were 3.23 and 3.78 pmol/ml on day 21 and 3.88 and 4.18 pmol/ml in the non-stress and stress control groups receiving 600 mg LRE/kg, respectively.

Serum T3 and T4 levels in the stress and stress-free groups were found to be the lowest in the 600 mg LRE/kg group and the highest in the control group ($P < 0.01$). Similarly, although TSH levels were low in both groups, the difference was not statistically significant (Tables 3–5).

The highest serum T3 levels measured in the non-stress and stress control groups were 3.39 and 3.19 ng/ml on day 21 and 3.28 and 2.98 ng/ml on day 42, respectively. The lowest T3 levels found in the non-stress and stress control groups were 3.20 and 3.05 ng/ml on day 21 and 3.26 and 2.98 ng/ml on day 42, respectively. The highest serum T4 levels observed in the non-stress and stress control groups were 13.25 and 20.13 ng/ml on day 21 and 3.28 and 2.98 ng/ml on day 42, respectively. The lowest T4 levels measured in the non-stress and stress control groups were 3.20 and 3.05 ng/ml on day 21 and 11.65 and 17.75 ng/ml on day 42, respectively. The highest serum TSH levels observed in the non-stress and stress control groups were 2.19 and 2.37 ng/ml on day 21 and 2.25 and 2.53 ng/ml on day 42, respectively. The lowest TSH levels measured in the non-stress and stress control groups were 1.94 and 2.17 ng/ml on day 21 and 2.03 and 2.27 ng/ml on day 42, respectively.

DISCUSSION

According to the results obtained, serum VF levels in the stress and non-stress groups were reduced in the control group. The biggest decrease was detected in the LRE 600 mg/kg dose group ($P < 0.01$). Although VF has been detected in chickens, no studies have been performed on the change in serum VF levels due to the addition of LRE in HS-exposed

Table 2. Effect of *Lactobacillus reuteri* E81 (LRE) added to the ration of chukar partridges fed under stress and non-stress conditions on the levels of visfatin (VF), adiponectin (ADP), chemerin (CHEM) and citrulline (CIT) on day 21 and statistical comparisons (mean ± SEM)

		VF (ng/ml)		ADP (µg/ml)		CHEM (ng/ml)		CIT (pmol/ml)	
	n	25 °C	37 °C	25 °C	37 °C	25 °C	37 °C	25 °C	37 °C
Control	8	5.80^{d}	8.48^{a}	6.90^{c}	8.45^{a}	13.48	17.15	4.70^{c}	5.92^{a}
LRE, 200 mg/kg	8	5.28^{e}	7.70^{b}	6.30^{d}	7.43^{b}	12.15	15.75	4.15^{d}	5.45^{b}
LRE, 400 mg/kg	8	4.68^{f}	6.73^{c}	5.70^{e}	6.33^{d}	10.70	14.55	3.75^{e}	4.55^{c}
LRE, 600 mg/kg	8	4.35^{g}	6.05^{d}	4.95^{f}	5.80^{e}	9.88	13.23	3.23^{f}	3.78^{e}
SEM		0.11		0.10		0.16		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		0.00		0.51		0.00	
Main effect means, diet									
Control		7.14^{a}		7.68^{a}		15.31^{a}		5.31^{a}	
LRE, 200 mg/kg		6.49^{b}		6.86^{b}		13.95^{b}		4.80^{b}	
LRE, 400 mg/kg		5.70^{c}		6.01^{c}		12.62^{c}		4.15^{c}	
LRE, 600 mg/kg		5.20^{d}		5.38^{d}		11.55^{d}		3.50^{d}	
SEM		0.08		0.07		0.12		0.05	
Temperature									
25 °C		5.03		5.96		11.55		3.96	
37 °C		7.24		7.00		15.17		4.92	
SEM		0.06		0.05		0.08		0.04	

Means within a column showing different superscripts are significantly different ($P < 0.05$). SEM = standard error of the mean.

Table 3. Effect of *Lactobacillus reuteri* E81 (LRE) added to the ration of chukar partridges fed under stress and non-stress conditions on T3, T4 and TSH levels on day 21 and statistical comparisons (mean ± SEM)

		T3 (ng/ml)		T4 (ng/ml)		TSH (ng/ml)	
	n	25 °C	37 °C	25 °C	37 °C	25 °C	37 °C
Control	8	3.39^{a}	3.19^{c}	13.25^{e}	20.13^{a}	2.19	2.37
LRE, 200 mg/kg	8	3.38^{a}	3.14^{d}	12.25^{f}	18.93^{b}	2.11	2.29
LRE, 400 mg/kg	8	3.27^{b}	3.09^{e}	11.73^{g}	17.75^{c}	1.99	2.21
LRE, 600 mg/kg	8	3.20^{c}	3.05^{f}	11.15^{h}	17.25^{d}	1.94	2.17
SEM		0.01		0.10		0.01	
Source of variation (P values)							
Diet		0.00		0.00		0.00	
Temperature		0.00		0.00		0.00	
Temperature × Diet		0.00		0.00		0.21	
Main effect means, diet							
Control		3.29^{a}		16.69^{a}		2.28^{a}	
LRE, 200 mg/kg		3.26^{b}		15.59^{b}		2.20^{b}	
LRE, 400 mg/kg		3.18^{c}		14.74^{c}		2.10^{c}	
LRE, 600 mg/kg		3.12^{d}		14.20^{d}		2.05^{d}	
SEM		0.01		0.07		0.01	
Temperature							
25 °C		3.31		12.09		2.06	
37 °C		3.12		18.51		2.26	
SEM		0.01		0.05		0.01	

Means within a column showing different superscripts are significantly different ($P < 0.05$). SEM = standard error of the mean.

partridges. The results obtained are compatible with the findings of similar research results (Diot et al., 2015; Bayraktar and Tekce, 2020).

In cases of stress, ADP ensures that the gut microbiota reverts to its previous state by increasing insulin sensitivity and reducing hepatic and systemic pro-inflammatory responses caused by the DNA methylation signal (Lee et al., 2019). Serum ADP levels in the stress and non-stress groups decreased in the control group, and the biggest decrease was found in the LRE 600 mg/kg dose group ($P < 0.01$). This is the first study examining the effect of LRE added at different doses to the ration of partridges grown under HS on serum

Table 4. Effect of *Lactobacillus reuteri* E81 (LRE) added to the ration of chukar partridges fed under stress and non-stress conditions on the levels of visfatin (VF), adiponectin (ADP), chemerin (CHEM) and citrulline (CIT) on day 42 and statistical comparisons (mean ± SEM)

		VF (ng/ml)		ADP (µg/ml)		CHEM (ng/ml)		CIT (pmol/ml)	
	n	25 °C	37 °C	25 °C	37 °C	25 °C	37 °C	25 °C	37 °C
Control	8	6.60^{d}	9.67^{a}	6.50^{d}	9.25^{a}	14.73	16.47	5.23^{c}	6.86^{a}
LRE, 200 mg/kg	8	5.87^{c}	8.70^{b}	5.78^{e}	8.15^{b}	12.98	14.82	4.98^{d}	6.09^{b}
LRE, 400 mg/kg	8	5.17^{f}	7.40^{c}	5.10^{f}	7.17^{c}	11.60	13.73	4.30^{e}	4.75^{d}
LRE, 600 mg/kg	8	4.75^{g}	6.55^{d}	4.35^{g}	6.37^{d}	10.60	12.65	3.88^{f}	4.18^{e}
SEM		0.11		0.11		0.19		0.09	
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		0.01		0.74		0.00	
Main effect means, diet									
Control		8.14^{a}		7.87^{a}		15.60^{a}		6.04^{a}	
LRE, 200 mg/kg		7.29^{b}		6.96^{b}		13.90^{b}		5.53^{b}	
LRE, 400 mg/kg		6.29^{c}		6.14^{c}		12.66^{c}		4.53^{c}	
LRE, 600 mg/kg		5.65^{d}		5.35^{d}		11.63^{d}		4.03^{d}	
SEM		0.08		0.08		0.14		0.06	
Temperature									
25 °C		5.60		5.43		12.48		4.59	
37 °C		8.08		7.74		14.42		5.47	
SEM		0.04		0.05		0.09		0.04	

Means within a column showing different superscripts are significantly different ($P < 0.05$). SEM = standard error of the mean.

Table 5. Effect of *Lactobacillus reuteri* E81 (LRE) added to the ration of chukar partridges fed under stress and non-stress conditions on T3, T4 and TSH levels on day 42 and statistical comparisons (mean ± SEM)

		T3 (ng/ml)		T4 (ng/ml)		TSH (ng/ml)	
	n	25 °C	37 °C	25 °C	37 °C	25 °C	37 °C
Control	8	3.28^{a}	3.06^{d}	13.85^{e}	21.33^{a}	2.25	2.53
LRE, 200 mg/kg	8	3.28^{a}	3.11^{c}	12.75^{f}	19.23^{b}	2.17	2.43
LRE, 400 mg/kg	8	3.19^{b}	3.05^{d}	12.13^{g}	18.15^{c}	2.07	2.31
LRE, 600 mg/kg	8	3.26^{a}	2.98^{e}	11.65^{h}	17.75^{d}	2.03	2.27
SEM		0.01		0.10		0.01	
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		0.22	
Main effect means, diet							
Control		3.17^{b}		17.59^{a}		2.39^{a}	
LRE, 200 mg/kg		3.19^{a}		15.99^{b}		2.30^{b}	
LRE, 400 mg/kg		3.12^{c}		15.14^{c}		2.19^{c}	
LRE, 600 mg/kg		3.12^{c}		14.70^{d}		2.15^{d}	
SEM		0.01		0.07		0.01	
Temperature							
25 °C		3.25		12.59		2.13	
37 °C		3.05		19.11		2.38	
SEM		0.01		0.05		0.01	

Means within a column showing different superscripts are significantly different ($P < 0.05$). SEM = standard error of the mean.

ADP. The results obtained are consistent with the results of similar clinical studies (Hendricks et al., 2009).

Serum CHEM levels in the stress and non-stress groups decreased in the control group; no statistically significant effect was demonstrated, and the biggest decrease was found in the LRE 600 mg/kg dose group ($P > 0.05$). The results of the current study differ from the findings of other studies (Wittamer et al., 2003; Zhou et al., 2018; Bayraktar and Tekce, 2020). We think that this discrepancy may be due to differences in the dose and type of probiotics used and the duration of the stress applied.

The intestinal mucosa acts as a protective barrier for pathogenic bacteria and microbial toxins. Exposure to HS impairs intestinal integrity, thereby increasing the number of intestinal pathogens and the serum levels of inflammatory cytokines (Alhenaky et al., 2017). Therefore, protecting the intestinal barrier is strategically significant for poultry health because it affects the immune system (Yadav and Jha, 2019). CIT is synthesised only by enterocytes of the small intestine and it is an important biomarker for the assessment of intestinal damage and function (Crenn et al., 2008; Baxter et al., 2019). Probiotics are reported to have an effect on preventing inflammation by regulating the gut microbiota (Baldwin et al., 2018; Mousavi et al., 2018; Yadav and Jha, 2019). Serum CIT levels in the stress and non-stress groups decreased in the control group, and the biggest decrease was demonstrated in the LRE 600 mg/kg dose group ($P < 0.01$). In addition, according to a review of the literature, this is the first study that investigates serum CIT levels based on LRE supplementation in partridges exposed to stress. The present results are consistent with those of other studies (Baxter et al., 2019; Bayraktar and Tekce, 2020). HS decreases the production of thyroid hormones (Geraert et al., 1996). In addition, probiotics activate the thyroid gland and play a regulatory role in thermoregulatory ability and thyroid metabolism (Hosseini et al., 2013). As regards the T3 and T4 values measured on day 21 and 42 in the groups administered LRE at different doses, a decrease was found in the T3 and T4 levels in the stress and non-stress groups compared to the control group ($P < 0.01$). Serum T3 and T4 levels in the stress and non-stress groups were the lowest in the LRE 600 mg/kg group and the highest in the control group ($P < 0.01$). These results are consistent with the results of previous research studies (Hosseini et al., 2013; Bayraktar and Tekce, 2020). We believe that this was caused by changes in the activity and metabolism of the thyroid gland due to the application of LRE under HS. It has been reported that probiotics have positive effects on stress by changing the hypothalamus–pituitary–adrenal axis and neurotransmitter activity of the intestinal–brain axis (Logan and Katman, 2005; Ait-Belgnaoui et al., 2012). The gut microbiota plays an important role in health by creating an interrelationship between the brain and the gut. Probiotic microorganisms stimulate the immune system, neural pathways and the central nervous system by producing neuroactive substances acting on the intestinal–brain axis, such as gamma-amino-butyric acid and serotonin. In addition, the intestinal microbiota secretes many neurotrophins and proteins such as brain-derived neurotrophic factor, synaptophysin and PSD-95, and has been reported to have an effect on brain development and plasticity (Heijtz et al., 2011; Douglas-Escobar et al., 2013). As regards the TSH values measured on days 21 and 42 in the groups receiving different doses of LRE, there was no statistically significant effect on TSH

levels in the stress and non-stress groups ($P > 0.05$). These results are consistent with the results of the previous research studies (Hosseini et al., 2013; Bayraktar and Tekce, 2020).

In conclusion, the use of different doses of LRE supplementation proved to be effective in reducing the serum VF, ADP, CIT, T3 and T4 levels in partridges exposed to HS ($P < 0.01$). The lowest levels were found in the LRE 600 mg/kg dose group ($P < 0.01$). On the other hand, no significant relationship was found between LRE supplementation and the serum CHEM and TSH levels ($P > 0.05$) (Tables 2–5). The levels of VF and ADP increase under stress conditions. Likewise, an increase in serum CIT level is observed as a result of intestinal damage due to stress. The current experimental findings suggest that the addition of 600 mg/kg LRE may lower VF, ADP and CIT levels in partridges exposed to heat stress, and thus LRE supplementation may have a positive effect. However, it is thought that a large number of comprehensive and experimental studies should be conducted to examine the effects of HS and probiotics on adipokine levels, gut responses, and the concentrations of hormones that reflect thyroid function.

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