

Influence of *Lactobacillus reuteri* on internal organ weight, performance and meat quality of Japanese quail (*Coturnix coturnix japonica*) under heat stress

Einfluss von *Lactobacillus reuteri* auf das Gewicht der inneren Organe, die Leistung und die Fleischqualität Japanischer Wachteln (*Coturnix coturnix japonica*) unter Hitzestress

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Abstract

In the present study, the effects of *Lactobacillus reuteri* E81 (LRE) supplemented at different doses (200, 400 and 600 mg/kg) into the diets on performance, meat quality and internal organ weights of Japanese quail (*Coturnix coturnix japonica*) were investigated under heat stress (HS) conditions (37°C). For the experiments, 448 one-day old quails were used. Seven days of training (physical exercise) and a 35-day fattening period were applied. On day 7 of the trial, 8 groups [4 under control temperature of 25°C (CT): control (C), LRE-200, LRE-400 and LRE-600 and 4 under HS: control (HSC), SLRE-200, SLRE-400 and SLRE-600] were formed, each group consisting of four replicates with 14 quail each. At the end of this study, the CT group fed 400 mg/kg LRE showed the best performance results, whereas no effect was found for groups under HS in comparison to the control group (HSC). Samples taken on the 21st and 42nd day revealed that addition of probiotics in the CT and HS groups induced no effect on weight of the internal organs ($p > 0.05$). Analysis of meat quality on the 21st day showed that LRE caused an increase in pH in both the HS and CT groups. Further, 400-mg/kg LRE supplementation induced an increase in thiobarbituric acid reactive substances (TBARS) in the group under HS. In the CT groups on day 42 no effect of LRE was found on colour parameters or on the TBARS value, except for a decrease in the pH value in the LRE supplemented groups compared with the control. In HS groups a decrease in the L* value and an increase in the TBARS value was found compared to CT groups ($p < 0.05$), but there was no effect on other colour parameters or pH value. In conclusion, in the present study supplementation of different doses of LRE E81 to quail diets had no effect on performance, internal organ weight or meat quality under HS.

Key words

Japanese quail; probiotics; performance; meat quality; meat colour; TBARS; internal organ weights

Zusammenfassung

In der vorliegenden Studie wurde der Einfluss von *Lactobacillus reuteri* E81 (LRE), verabreicht in verschiedenen Dosen (200, 400 und 600 mg/kg), die den Futtermischungen zugefügt wurden, auf die Leistung, die Fleischqualität und das Gewicht der inneren Organe Japanischer Wachteln (*Coturnix coturnix japonica*) unter Hitzestressbedingungen (37°C) untersucht. Für die Untersuchungen wurde mit 448 Wachteln, beginnend im Alter von einem Tag, zunächst ein siebentägiges Bewegungstraining durchgeführt. Darauf folgte eine 35-tägige Mastperiode. Am 7. Lebenstag wurden 8 Gruppen gebildet [4 unter Kontrolltemperatur von 25°C (CT): Kontrolle (C), LRE-200, LRE-400 und LRE-600 und 4 unter HS: Kontrolle (HSC), SLRE-200, SLRE-400 und SLRE-600], wobei für jede Gruppe vier Wiederholungen mit je 14 Wachteln durchgeführt wurden. Am Ende dieser Studie zeigte unter den CT-Gruppen die Gruppe, die mit 400 mg/kg LRE gefüttert wurde, die besten Leistungsergebnisse. Demgegenüber konnte bei den Gruppen unter HS im Vergleich zur Kontrolle (HSC) kein Effekt durch LRE-Zugabe nachgewiesen werden. Die am 21. und 42. Tag entnommenen Proben ergaben, dass ein Zusatz von Probiotika in den CT- und HS-Gruppen das Gewicht der inneren Organe nicht beeinflusste ($p > 0,05$). Am 21. Tag zeigten die Analysen zur Fleischqualität, dass LRE einen Anstieg des pH-Wertes sowohl in den HS- als auch in CT-Gruppen verursachte. Eine Supplementierung mit 400-mg/kg LRE führte zu einem Anstieg Thiobarbitursäure-reaktiver Substanzen (TBARS) in den HS-Gruppen. In den CT-Gruppen wurde am 42. Tag kein Einfluss von LRE auf Farbparameter oder den TBARS-Wert gefunden, mit der Ausnahme dass LRE-Supplementierung zur Abnahme des pH-Wertes im Vergleich zur Kontrollgruppe führte. In den HS-Gruppen konnte im Vergleich zu den CT-Gruppen eine Abnahme des L*-Wertes und eine Zunahme des TBARS-Wertes nachgewiesen werden ($p < 0,05$), aber es gab keinen Einfluss auf andere Farbparameter oder den pH-Wert. Es kann die Schlussfolgerung gezogen werden, dass in der vorliegenden Studie eine Nahrungsergänzung mit LRE E81 in unterschiedlichen Dosen bei Wachteln unter HS keinen Einfluss auf die Leistung, das Gewicht der inneren Organe oder die Fleischqualität hatte.

Stichworte

Japanische Wachtel; Probiotika; Leistung; Fleischqualität; Fleischfarbe; TBARS; Gewicht der inneren Organe

Introduction

Heat stress (HS) not only threatens the welfare of poultry (ROUSHDY et al., 2018) but is also a serious problem that negatively affects the rate of mortality and the obtained quantity and quality of products (SAHIN and KUCUK, 2003; ROUSHDY et al., 2018; SALAH et al., 2019). In addition, in the poultry industry HS has been reported to cause global economic losses ranging between 128 and 165 million dollars yearly (LARA and ROSTAGNO, 2013; NAWAB et al., 2018). During high temperatures the body develops stress to defend itself and this affects the hormone and nervous system (DEMITRACK et al., 1991; MINTON, 1994). To counteract stress, corticotropin-releasing hormone is first released from the hypothalamus, which stimulates the production of the adrenocorticotropic hormone from the pituitary gland (BUGAJSKI et al., 2004; SHIPP et al., 2015). As a result, the release of both cortisol and adrenaline increases, which is reflected in an increase in blood pressure, breathing rate and heart rate. This leads to a rise in the blood flow rate, which in turn causes a sudden rise in blood sugar, and at this stage, the body starts sweating in order to keep the body temperature constant (RHODES, 2016). As a result, there is an increasing demand for energy. This energy is activated by stress hormones (cortisol, adrenaline and noradrenaline) and neurogenic amines like hepatic adenylcyclase in the liver, which converts glycogen to glucose, to activate defence mechanisms of the body in the "alarm" phase (SCANES, 2016; AVILÉS-ESQUIVEL et al., 2018). The organism then takes measures to regulate homeostasis again. However, if the condition of the organism does not improve, it enters the next stage (the adaptation or resistance stage) (MCCARTY, 2016). As result of stress, in the final stage, if the organism cannot maintain homeostasis, the immune system is suppressed (reduction in immunoglobulin G and M). Further, cardiac and renal parameters are impaired, thymus atrophy and adrenaline hyperplasia occur, the number of lymphocytes in the blood circulation decrease and the consumption of heterophiles and basophiles increase. Finally, sudden death may occur (the exhaustion stage) (OLANREWAJU et al., 2006; MAILYAN, 2016).

To reduce the negative effects of high ambient temperatures and to meet consumer demands, there has been a trend in recent years towards research into natural products, with probiotics being one of these "alternative" products. Probiotics are defined as non-pathogenic micro-organisms (bacteria or yeast) that have beneficial effects on health of an organism when taken at appropriate concentrations (SEN et al., 2012; DE MELO PEREIRA et al., 2018).

Studies on the use of probiotics prove their benefit in terms of:

- improvements in intestinal microbial balance and intestinal health (AWAD et al., 2009; MOUNTZOURIS et al., 2010; SEN et al., 2012),
- positive effects on growth performance (SHIM et al., 2010; AL-FATAFTAH and ABDELQADER, 2014; ATTIA et al., 2018; INCHAROEN et al., 2019),
- antimicrobial activity (ATTIA et al., 2012; PRINGSULAKA et al., 2015; DE MELO PEREIRA et al., 2018),
- regulation (modification) of the villus-crypt structure (AL-FATAFTAH and ABDELQADER, 2014; INCHAROEN et al., 2019),
- anti-cholesterol activity (NGUYEN et al., 2007; COSTABILE et al., 2017),
- reduction of cardiovascular risk (COSTABILE et al., 2017),
- anti-depression and anti-anxiety activity (AKKASHEH et al., 2016),
- anti-obesity and anti-diabetic activity (HU et al., 2017; DE MELO PEREIRA et al., 2018),
- reduction of stress (ZHANG et al., 2016),
- improvement in meat quality (PELICANO et al., 2003; WATTANACHANT et al., 2004),
- reduction of aflatoxin (ATTIA et al., 2013a; ATTIA et al., 2016),
- no adverse effect on the blood parameters (ATTIA et al., 2013b) and
- anti-cancer activity (KHANI et al., 2012; DE PRISCO and MAURIELLO, 2016).

In this study, the effects of *Lactobacillus reuteri* E81 (LRE) supplemented at different doses (200, 400 and 600 mg/kg) into the diet on performance (body weight, daily weight gain, feed conversion ratio and feed intake), internal organ weights, and colour parameters and antioxidant properties of the meat of Japanese quail (*Coturnix coturnix japonica*) fed under HS conditions were investigated.

Materials and Methods

This study was conducted pursuant to the approval (dated 12.11.2019 and numbered 2019/15) of the Local Ethics Board for Animal Experiments of Directorate of Etlik Veterinary Control Center Research Institute.

Animals and experimental design

Four hundred forty-eight day-old male Japanese quail (*Coturnix coturnix japonica*) chicks constituted the animal material of the study. Throughout a 7-day acclimatisation and a 35-day fattening period, the animals were housed at the poultry unit of the Food, Agriculture and Livestock Practice and Research Centre of Bayburt University. The quail were kept in 4-storey cages, measuring 100 × 50 × 100 cm, in groups of 14 animals. on day 7 of the trial, the animals were assigned to 8 groups [4 groups kept at control temperature (CT, 25°C) and 4 groups under heat stress (HS, 37°C)], each composed of 56 animals of equal body weight. The groups were fed with diets supplemented at different LRE-doses (mg/kg) [CT: control (C, without LRE supplementation), LRE-200, LRE-400 and LRE-600, and HS: control (HSC, without LRE supplementation), SLRE-200, SLRE-400 and SLRE-600]. Each group was divided into 4 subgroups, including 14 animals per compartment. Trial groups were divided into HS and CT starting from 7 days. Heating of the cluster was provided by means of 36 ± 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 4 different points of the coop to control the temperature in the coop. The room temperature of CT groups was planned to be 25°C.

Feed

The quail feed used in the experiment (quail starter and growth feed) were prepared by a private company operating in Turkey (Erzurum). The nutrient content of this feed is shown in Table 1. After the initial weighing of the basal diets, probiotics were supplemented to the diet in appropriate doses. The respective feed mixtures were fed daily (18:00–19:00) to all experimental groups at the same time. The control groups received feed without LRE. Throughout the experiment, it was ensured that clean water was freely available to the animals. The probiotic [*Lactobacillus reuteri* E81 (LRE), 4×10^{10} CFU/g] used in this study was produced at the Food Engineering laboratories of Bayburt University and were added to the feeds daily. Nutrient analyses of the feeds used throughout the research were performed in accordance with the methods described by AOAC (AOAC, 2005).

Table 1. Nutrient content and analysed content of basal quail diets (g/kg)

Nährstoffgehalt und Analyse der Grundfuttermittel (g/kg) für Wachteln

Raw Materials	Starter feed (0–21 d)	Grower feed (22–42 d)
Maize	52.9	58.32
Maize gluten meal	15.21	26.14
Soybean meal (44% CP)	26.35	10.65
Di-kalcium phosphat	1.95	1.6
Limestone	1.18	1.04
Sodium chloride	0.31	0.31
Sodium bikarbonat	0.2	0.2
Methionine	0.5	0.44
Lysine	1.2	1.1
Vitamin- mineral premix ¹	0.2	0.2
ME (MJ/kg)	12.97	13.50
Crude protein %	24	20
Total sulphur amino acids %	0.71	0.71
Lysine (%)	1.28	1.04
Methionine (%)	0.50	0.38
Calcium (%)	1.00	0.90
Crude oil %	2.61	2.50
Ash %	5.19	3.85
Moisture %	13.20	13.20
Total phosphorus (%)	0.72	0.60

The vitamin-mineral premix provided the following (per kg of diet): vitamin A, 6 000 IU; vitamin D3, 1000 IU; vitamin E, 15 mg/kg; vitamin K 2 mg/kg; 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/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 (LARBIER and LECLERCQ, 1994)

House temperature, humidity and lighting

The general temperature of the poultry housing section was kept constant at 32–33°C for the first 2 days, at 27–28°C for the next 5 days. In the period following and until day 42 the HS groups were kept at 37°C and 75–85% relative humidity and the CT groups at 25°C and 55–60% relative humidity. Furthermore, 24 h of lighting (60 W) per day was applied to all experimental groups.

Performance parameters

Body weight (BW), daily body weight gain (DBWG) and feed intake (FI) were measured at 7, 14, 21, 28, 35 and 42 days of age. Feed conversion ratio (FCR) was calculated as total FI (g)/total BWG (g). Mortality was recorded when it occurred.

Internal organ weights

At the end of the trial, three birds from each subgroup, which equals 12 birds per experimental group and 96 male quail in total, were selected on a random basis. These animals were sacrificed by cervical dislocation at the laboratory of the Food, Agriculture and Livestock Practice and Research Centre of Bayburt University on days 21 or 42 of the trial, and their visceral organs were excised and weighed on a digital high-precision balance accurate to 0.001 g (Shimadzu BI-3200 h, Germany).

Quality and antioxidant properties of meat

Breast meat samples were taken on 21st and 42nd days from three randomly selected birds of each subgroup (12 samples from each experimental group and 96 samples in total). The analysis (meat colour values, TBARS and pH) was conducted in the Bayburt University Food Engineering Department's Meat Technology Laboratory.

Determination of pH. The pH values of the breast meat were determined after storing for 24 h at 4°C using samples weighing 10 g, mixed with 100 ml of distilled water and subjected to homogenisation using Ultra-Turrax (IKA Werk7 T 25, Germany) for 1 min. The pH value of the homogenate was measured with a pH meter (Mettler-Toledo AG, 8603 Schwerzenbach, Switzerland).

Determination of Thiobarbituric acid reactive substances (TBARS) value. Determination of TBARS value of the breast meat samples (after 24 h cold storage) was performed by the method of LEMON (1975). Samples with a weight of approximately 2 g were weighed and after addition of 12 ml trichloroacetic acid (TCA) solution [7.5% 28 TCA, 0.1% EDTA (ethylenediamine tetra acetic acid) and 0.1% propyl gallate (dissolved in 3 ml ethanol)] homogenisation was applied with Ultra Turax (IKA *Werk T25, Germany). Then the homogenates were filtered through Whatman N° 1 filter paper and 3 ml of the filtrate was transferred to a glass tube. Three ml 0.02 M thiobarbutyric acid was added to the 3 ml filtrate and it was kept in a boiling water bath for 40 minutes. After removing the samples from the water bath, they were cooled in cold water for 5 min and then centrifugated at 2000 g for 5 min (Hettich, 0003771-02-00, Germany). After centrifugation, the absorbance of the samples was determined against spectra at 530 nm using a spectrophotometer (Shimadzu Corporation, UV-1800 240V, Japan). The calculation of TBARS values was performed using absorbance values obtained with the formula below and the results were given in mg MDA/kg.

$$\text{TBARS} = ((\text{absorbance}/k (0.06) \times 2/1000) \times 6.8) \times 1000/\text{sample weight}$$

Instrumental colour evaluation. The determination of colour values of breast meat samples (L^* , a^* , b^*) were performed with a Chroma Meter (CR-400 Konica Minolta, Japan) colorimeter. Colour measurements were evaluated according to the criteria set by the International Commission on Lighting (Commission Internationale de l'Eclairage). According to these criteria; L^* ; $L^*=0$, black; $L^*=100$ white (darkness/lightness); a^* ; $+ a^*$ =red, $- a^*$ =green and b^* ; $+ b^*$ =yellow, $- b^*$ =blue indicates the intensity of the above-mentioned colours. Four measurements were obtained from every sample.

Statistical analysis

The performance parameters (BW, DBWG, FCR and Fi), internal organ weights, meat colour parameters (L^* , a^* , and b^*) and meat pH were controlled for and found to be normally distributed. The statistical analyses of the diet and temperature effects on performance parameters, internal organ weights, and meat quality were performed using the general linear model (GLM) that was given below.

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

where: Y_{ijk} = an observation, μ = overall mean, D_i = Diet effect, T_j = Temperature effect, $(D^* T)_{ij}$ =the interaction effect and e_{ijk} = experimental error.

The One Way ANOVA was used for 2 (two temperature levels) x3 (three dose levels) factorial design. The Duncan multiple comparison test was used for comparing the group means. All data are presented as the mean \pm SEM with $p < 0.05$ deemed significant. The statistical analyses were performed by using SAS software, version 9.0 (SAS Institute, Cary, NC, USA).

Results

Performance parameters

Table 2 presents the data on performance (BW, DWG, FCR and FI). The groups fed LRE at 200 and 400 mg/kg in the absence of heat stress (LRE-200 and LRE-400) showed better performance. However, it was determined that probiotic supplementation had no effect on performance in groups under HS ($p < 0.05$).

Table 2. Effects of diet, temperature and their interaction on performance in the experimental groups under control temperature (25°C) and heat stress (37°C)

Einfluss von Futter, Temperatur und deren Wechselwirkung auf die Leistung in den Versuchsgruppen unter Kontrolltemperatur(25°C)- und Hitzestress(37°C)bedingungen

	N	Body weight (g)		Average daily weight (g)		Feed intake (g)		Feed conversion ratio (g/g)	
		25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C
Control	112	185.88 ^{bc}	178.56 ^c	5.31 ^{bc}	5.10 ^c	17.36 ^{ab}	17.09 ^b	3.26	3.35
LRE 200 mg/kg	112	216.07 ^a	159.27 ^c	6.17 ^a	4.55 ^c	17.46 ^{ab}	14.12 ^d	2.83	3.12
LRE 400 mg/kg	112	227.15 ^a	161.79 ^c	6.49 ^a	4.62 ^c	16.73 ^{bc}	14.52 ^d	2.57	3.15
LRE 600 mg/kg	112	211.52 ^{ab}	167.63 ^c	6.04 ^{ab}	4.79 ^c	18.84 ^a	15.16 ^{cd}	3.12	3.17
SEM		5.72		0.16		0.37		0.11	
Main effect means diet									
Control		182.22		5.20		17.23 ^b		3.31 ^c	
LRE 200 mg/kg		187.67		5.36		15.79 ^a		2.98 ^{ab}	
LRE 400 mg/kg		194.47		5.55		15.62 ^a		2.86 ^a	
LRE 600 mg/kg		189.57		5.41		17.00 ^b		3.15 ^{bc}	
SEM		4.04		0.12		0.26		0.08	
Temperature									
25°C		210.15		6.00		17.60		2.95	
37°C		166.81		4.76		15.23		3.20	
SEM		2.85		0.08		0.18		0.05	
Source of variation (P-values)									
Diet		0.22		0.22		0.00 ^{**}		0.00 ^{**}	
Temperature		0.00 ^{**}		0.00 ^{**}		0.00 ^{**}		0.03 [*]	
Temperature × Diet		0.00 ^{**}		0.00 ^{**}		0.00 ^{**}		0.09	

Lactobacillus reuteri E81 [LRE]. Means within a column showing different superscripts are significantly different ($P < 0.05$): least significance difference test was applied to compare means. * Significant at 0.05 level, ** Significant at 0.01 level, SEM = standard error of the mean

Internal organ weights

Tables 3 and 4 present the data on the internal organs (liver, heart, gizzard, spleen and proventriculus) taken from the quail on the 21st and 42nd days of the study. In the CT and HS probiotic groups no effect of LRE was found on the weight of the internal organs and intestine pH value compared to the control group ($p > 0.05$).

Table 3. Effects of diet, temperature and their interaction on percentage of internal organ weight (g/100 g BW) on the 21st day in the experimental groups under control temperature (25°C) and heat stress (37°C)

Einfluss von Futter, Temperatur und deren Wechselwirkung auf den Anteil der Gewichte der inneren Organe (g/100 g KM) am Tag 21 in den Versuchsgruppen unter Kontrolltemperatur(25°C)- und Hitzestress(37°C)bedingungen

	N	Heart (%)		Liver (%)		Gizzard (%)		Intestinal pH	
		25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C
Control	24	1.01	0.82	5.06	2.51	4.13	2.51	6.80	6.97
LRE 200 mg/kg	24	1.22	0.99	3.97	3.66	4.92	3.72	7.02	6.91
LRE 400 mg/kg	24	1.24	0.97	3.94	3.87	4.12	3.50	6.83	6.93
LRE 600 mg/kg	24	1.23	0.81	3.90	3.50	4.15	2.63	6.98	6.86
SEM		0.13		0.50		0.41		0.07	
Main effect means diet									
Control		0.92		3.79		3.31		6.88	
LRE 200 mg/kg		1.10		3.81		4.32		6.96	
LRE 400 mg/kg		1.10		3.91		3.81		6.88	
LRE 600 mg/kg		1.03		3.70		3.39		6.91	
SEM		0.09		0.34		0.29		0.05	
Temperature									
25°C		1.18		4.22		4.33		6.91	
37°C		0.90		3.38		3.09		6.90	
SEM		0.06		0.25		0.21		0.04	
Source of variation (P-values)									
Diet		0.41		0.98		0.08		0.62	
Temperature		0.00*		0.03		0.00**		0.93	
Temperature × Diet		0.77		0.08		0.63		0.13	

Lactobacillus reuteri E81 [LRE]. Means within a column showing different superscripts are significantly different (P < 0.05): least significance difference test was applied to compare means. * Significant at 0.05 level, ** Significant at 0.01 level, SEM = standard error of the mean

Table 4. Effects of diet, temperature and their interaction on percentage of internal organ weight (g/100 g BW) on the 42nd day in the experimental groups under control temperature (25°C) and heat stress (37°C)

Einfluss von Futter, Temperatur und deren Wechselwirkung auf den Anteil der Gewichte der inneren Organe (g/100 g KM) am Tag 42 in den Versuchsgruppen unter Kontrolltemperatur(25°C)- und Hitzestress(37°C)bedingung

	N	Heart (%)		Spleen (%)		Proventriculus (%)		Liver (%)		Gizzard (%)		Intestinal pH	
		25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C
Control	24	1.06	1.02	0.09	0.08	0.68	0.56	3.50	2.84	2.28	2.39	7.91	7.75
LRE 200 mg/kg	24	1.14	0.96	0.06	0.10	0.61	0.61	3.41	3.79	2.15	2.94	7.84	7.98
LRE 400 mg/kg	24	1.15	1.14	0.09	0.09	0.61	0.69	4.03	2.97	2.59	2.85	7.94	7.99
LRE 600 mg/kg	24	1.03	0.95	0.11	0.09	0.55	0.55	3.50	3.36	2.58	2.59	8.04	7.79
SEM		0.11		0.01		0.10		0.41		0.22		0.12	
Main effect means diet													
Control		1.04		0.08		0.62		3.17		2.33		7.83	
LRE 200 mg/kg		1.05		0.08		0.61		3.60		2.55		7.91	
LRE 400 mg/kg		1.14		0.09		0.65		3.50		2.72		7.96	
LRE 600 mg/kg		0.99		0.10		0.55		3.43		2.59		7.91	
SEM		0.11		0.01		0.06		0.29		0.16		0.09	
Temperature													
25°C		1.10		0.09		0.61		3.61		2.40		7.93	
37°C		1.02		0.09		0.60		3.24		2.69		7.87	
SEM		0.06		0.01		0.04		0.21		0.11		0.06	
Source of variation (P-values)													
Diet		0.59		0.35		0.63		0.75		0.39		0.76	
Temperature		0.34		0.61		0.86		0.22		0.07		0.50	
Temperature × Diet		0.87		0.28		0.66		0.35		0.31		0.34	

Lactobacillus reuteri E81 [LRE]. Means within a column showing different superscripts are significantly different (P < 0.05): least significance difference test was applied to compare means. * Significant at 0.05 level, ** Significant at 0.01 level, SEM = standard error of the mean

Meat quality

Results of the influence of LRE on the quality of quail meat colour (L*, a* and b*), TBARS and pH values are shown in Tables 5 and 6. It was determined that the addition of different doses of LRE to the diet had no effect on meat colour parameters in either the CT or HS groups after 21 days. However, at a dose of 400 mg/kg, an increase in the pH value was found in both the CT and HS groups, whereas TBARS values increased only in the HS groups. For the samples taken on day 42, it was determined that, in comparison to the control group, in the HS groups b* values decreased depending on the dose of probiotic. However, the data were not statistically significant (p > 0.05). Furthermore, it was found that apart from the decrease in pH values compared to the control group, there was no effect on the other colour parameters and TBARS values. In the HS groups there was a significant decrease in L* values and an increase in TBARS values in comparison to the control group (p < 0.05). There was no effect on the other colour parameters or pH values.

Table 5. Effects of diet, temperature and their interaction on meat quality and colour parameters on 21st day in the experimental groups under control temperature (25°C) and heat stress (37°C)

Einfluss von Futter, Temperatur und deren Wechselwirkung auf Fleischqualität und Farbparameter am Tag 21 in den Versuchsgruppen unter Kontrolltemperatur(25°C)- und Hitzestress(37°C)bedingungen

	N	L*		a*		b*		TBARS (mg MDA/kg)		pH	
		25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C
Control	24	46.85	38.44	14.34	17.50	15.46	10.33	0.62	0.58	6.17 ^a	6.35 ^b
LRE 200 mg/kg	24	45.26	40.36	13.38	14.72	14.87	10.70	0.70	0.89	6.54 ^c	6.43 ^{bc}
LRE 400 mg/kg	24	37.42	41.24	17.09	16.31	12.83	12.91	1.18	1.41	6.37 ^b	6.11 ^a
LRE 600 mg/kg	24	38.37	36.37	15.31	14.34	15.98	9.71	1.21	0.92	6.41 ^{bc}	6.07 ^a
SEM		3.11		1.64		1.55		0.27		0.04	
Main effect means diet											
Control		42.64		15.92		12.89		0.59		6.25 ^b	
LRE 200 mg/kg		42.81		14.05		12.78		0.79		6.48 ^a	
LRE 400 mg/kg		39.33		16.69		12.87		1.29		6.24 ^b	
LRE 600 mg/kg		37.37		14.82		12.84		1.06		6.24 ^b	
SEM		2.20		1.16		1.09		0.19		0.03	
Temperature											
25°C		41.97		15.03		14.79		0.93		6.37	
37°C		39.10		15.72		10.91		0.95		6.24	
SEM		1.55		0.81		0.77		0.13		0.02	
Source of variation (P-values)											
Diet		0.26		0.41		1.00		0.09		0.00 ^{**}	
Temperature		0.21		0.56		0.00 ^{**}		0.91		0.00 ^{**}	
Temperature × Diet		0.28		0.56		0.23		0.75		0.00 ^{**}	

Lactobacillus reuteri E81 [LRE]. TBARS (Thiobarbituric Acid Reactive Substances). L *(Lightness), a * (Redness), b * (Yellowness). Means within a column showing different superscripts are significantly different (P < 0.05): least significance difference test was applied to compare means. * Significant at 0.05 level, ** Significant at 0.01 level, SEM = standard error of the mean

Table 6. Effects of diet, temperature and their interaction on meat quality and colour parameters on 42nd day in the experimental groups under control temperature (25°C) and heat stress (37°C)

Einfluss von Futter, Temperatur und deren Wechselwirkung auf Fleischqualität und Farbparameter am Tag 42 in den Versuchsgruppen unter Kontrolltemperatur(25°C)- und Hitzestress(37°C)bedingungen

	N	L*		a*		b*		TBARS (mg MDA/kg)		pH	
		25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C
Control	24	45.93	51.28	24.75	18.89	19.54	16.17	0.62 ^a	0.31 ^a	6.04 ^{def}	6.05 ^{ef}
LRE 200 mg/kg	24	47.72	49.34	20.78	18.33	17.00	15.12	0.61 ^a	2.17 ^b	5.90 ^{bc}	5.97 ^{cd}
LRE 400 mg/kg	24	46.74	43.20	22.35	17.84	17.55	12.91	1.37 ^{ab}	0.42 ^a	5.80 ^a	6.09 ^f
LRE 600 mg/kg	24	48.59	43.20	22.36	16.12	12.75	12.26	1.03 ^a	1.37 ^{ab}	5.88 ^b	5.98 ^{de}
	SEM	2.14		2.57		1.49		0.32		0.03	
Main effect means diet											
Control		48.61		21.82		17.86 ^a		0.46 ^b		6.04 ^a	
LRE 200 mg/kg		48.53		19.56		16.06 ^a		1.39 ^a		5.93 ^b	
LRE 400 mg/kg		44.97		20.09		15.23 ^{ab}		0.90 ^{ab}		5.94 ^b	
LRE 600 mg/kg		45.89		19.24		12.51 ^b		1.20 ^a		5.93 ^b	
	SEM	1.52		1.82		1.06		0.23		0.02	
Temperature											
25°C		47.25		22.56		16.71		0.91		5.90	
37°C		46.76		17.79		14.12		1.07		6.02	
	SEM	1.07		1.29		0.75		0.16		0.01	
Source of variation (P-values)											
Diet		0.26		0.76		0.02		0.05		0.00	
Temperature		0.75		0.02		0.03		0.49		0.00	
Temperature × Diet		0.09		0.88		0.55		0.01		0.00	

Lactobacillus reuteri E81 [LRE]. TBARS (Thiobarbituric Acid Reactive Substances). L *(Lightness), a *(Redness), b *(Yellowness). Means within a column showing different superscripts are significantly different (P < 0.05): least significance difference test was applied to compare means. * Significant at 0.05 level, ** Significant at 0.01 level, SEM = standard error of the mean

Discussion

Performance parameters

In the present study, the effects of incorporation of LRE into the diet of Japanese quail (*Coturnix coturnix japonica*) at different doses (200, 400 and 600 mg/kg) fed under HS on performance, internal organ weights, meat colour parameters (L^* , a^* and b^*) and their relative pH values were investigated. HS is a serious problem that can harmfully affect the welfare of poultry during hot and humid weather conditions. This problem can be seen in poultry at any age but particularly after 21 days of age. If the balance between heat production and heat loss is disturbed, the animals are under stress (ABBAS et al., 2012; SAEED et al., 2019). As a preventive measure against this problem, probiotics can be supplemented to the feed, as these additives are known to have positive effects on intestinal function and growth performance (GADDE et al., 2017a, 2017b; INCHAROEN et al., 2019). Previous studies have reported that the application of probiotics for animals under HS has a positive effect on performance (HALDAR et al., 2011; KHONYOUNG and YAMAUCHI, 2012; SOHAIL et al., 2012; AL-FATAFTAH and ABDELQADER, 2014; INCHAROEN et al., 2019). However, in contrast, other studies have reported that an application of probiotics induced no effects (ÖNOL et al., 2003; ASLI et al., 2007; ATTIA et al., 2017). In the present research, in comparison to the control group, positive results were obtained in all groups fed probiotics without HS ($p < 0.05$). The best results in terms of body weight, average daily weight, feed intake and feed conversion ratio were obtained with application of LRE at a dose of 400 mg/kg ($p < 0.05$). However, under HS no effect of LRE was found, compared to the control group, on performance in any experimental group. The results obtained in this study are in accordance with the results of a number of published studies (ASLI et al., 2007; ÖNOL et al., 2003; NIKPIRAN et al., 2013; MANAFI et al., 2016; ATTIA et al., 2017). In discordance were some other studies on similar topics (HALDAR et al., 2011; KHONYOUNG and YAMAUCHI, 2012; SOHAIL, et al. 2012; AL-FATAFTAH and ABDELQADER, 2014; INCHAROEN et al., 2019). These differences between the studies can be attributed to the differences in probiotic varieties, doses and preparation methods, as well as the probiotic's ability to survive in the gastrointestinal tract (ROSS et al., 2005; KHONYOUNG and YAMAUCHI, 2012; SEN et al., 2012).

Internal organ weights and intestinal pH

Internal organ weights have been reported to vary depending on the weight gain and age of the organism (HERNÁNDEZ-GARCÍA et al., 2015). Changes of living conditions in stressful environments cause significant changes in intestinal microflora and live weight gain (SAEED et al., 2019), and these changes also affect the organs. Probiotics are used as alternative products for reducing these negative effects (KABIR, 2009). While some previous studies on internal organ weights have reported that the addition of probiotics to feed improved weight gain compared to control groups (ASHAYERIZADEH et al., 2009; KARIMI et al., 2010; JAMSHIDPARVAR et al., 2017; AHMED et al., 2019), some other studies have reported that no effect was found (ÇINAR et al., 2009; BEHROUZ et al., 2012). After the examination of the internal organ samples taken on the 21st and 42nd day of the study, no effect was observed on the weight of the internal organs and intestinal pH value for both the HS and CT groups ($p > 0.05$). While the data obtained in this study are consistent with some literature reports (ÇINAR et al., 2009; JAMSHIDPARVAR et al., 2017), they are also in contrast to some other published results (ASHAYERIZADEH et al., 2009; KARIMI et al., 2010; BEHROUZ et al., 2012; AHMED et al., 2019). This discordance of results found in the literature can be attributed to the differences in the variety of probiotics incorporated into the diets and the doses used.

Meat quality (TBARS and meat colour)

Generally, probiotics are used to correct abnormalities caused by stress factors in the gastrointestinal tract (AKSU et al., 2005). In some studies, the use of probiotics caused an influence on the pH values of the breast meat (MAHAJAN et al., 2000; AKSU et al., 2005; ZHENG et al., 2014). Conversely, other studies reported that the addition of probiotics did not affect the pH (ZHANG et al., 2012; PARK and KIM, 2014; BAI et al., 2016; LAN et al., 2017). It was reported, for instance, that a drop in pH in the chest muscles caused by HS may be mitigated by probiotic supplementation (CRAMER et al., 2018). On the 21st day of the present experiment, it was determined that the addition of different doses of LRE in the feed increased the pH value in both treatments (HS and CT). As for the samples taken on the 42nd day, it was found that the pH decreased in the CT groups compared to its control group ($p < 0.01$). In opposite, in the groups under HS, no effect on pH was observed. These results are in accordance with other studies (MAHAJAN et al., 2000; AKSU et al., 2005; ZHANG et al., 2012; PARK and KIM, 2014; BAI et al., 2016; LAN et al., 2017; CRAMER et al., 2018).

In some studies, probiotic supplementation has been reported to reduce oxidative degradation (LODDI et al., 2000; ZHANG et al., 2012; PARK and KIM, 2014; BAI et al., 2016; JAHROMI et al., 2016; LAN et al., 2017; CRAMER et al., 2018; KHAN et al., 2018; SOBCZAK et al., 2018). It was found, for instance, that increased TBARS values resulting from HS were mitigated with the addition of probiotics (CRAMER et al., 2018). On the 21st day of the present experiment, it was determined that the incorporation of 400 mg/kg LRE to the diet caused an increase in the TBARS value in the groups under HS. Conversely, on the 42nd day, it was found that the incorporation of the probiotic had no effect on TBARS in the CT groups. In groups under HS, however, a statistically significant ($p < 0.05$) increase of TBARS values was found. Finally the results of the present study are similar to other published results (LODDI et al., 2000; ZHANG et al., 2012; PARK and KIM, 2014; BAI et al., 2016; JAHROMI et al., 2016; LAN et al., 2017; SOBCZAK et al., 2018; CRAMER et al., 2018; KHAN et al., 2018). It is possible to conclude that the increase in TBARS in groups under HS is due to reactive oxygen species deriving from oxidative stress caused by overheating.

Depending on the latest technological developments, various factors (live production, cutting, processing factors, packaging, etc.) affect consumer satisfaction (AKSU et al., 2005). When buying meat products, the main criterion for the consumer is the colour (CHEN et al., 2013; D'ALESSANDRO and ZOLLA, 2013). There are many factors that can change the meat colour. Although the exact causes of colour changes in meat are not known, many factors such as variety, feeding, growth conditions and slicing before or after slaughter may be decisive (D'ALESSANDRO and ZOLLA, 2013). In some studies no effect on meat colour related to probiotic supplementation was reported (LODDI et al., 2000; ZHANG et al., 2012; PARK and KIM, 2014; BAI et al., 2016; JAHROMI et al., 2016; LAN et al., 2017; SOBCZAK et al., 2018; CRAMER et al., 2018; KHAN et al., 2018). In contrast, a number of other studies have shown that addition of probiotics can lead to a decrease in the L* value compared to control groups (CHEN et al., 2013; CENGIZ et al., 2015; KIM et al., 2017). There are also studies indicating that the use of probiotics in diet improves meat colour (PELICANO et al., 2003; ZHANG et al., 2012). It is assumed that the decrease in L* value in groups under HS is a consequence of oxidation due to stress (ADEBIYI et al., 2011). Finally, the results of the present study are compatible with some literature references (CENGIZ et al., 2015; KIM et al., 2017), but also contradict some other studies (BAI et al., 2016; JAHROMI et al., 2016; CRAMER et al., 2018; KHAN et al., 2018). It is assumed that this difference is due to the use of a different type of probiotics and to different doses added to the feed.

Conclusion

In conclusion, the addition of *Lactobacillus reuteri* E81 to the diet of quail (*Coturnix coturnix japonica*) in varying doses had no effect on the performance or quality of meat in groups under HS. Similarly, there was no differences on the weight of internal organs and intestinal pH between the HS and CT groups. In addition, this probiotic strain showed positive effects on performance and meat quality in the CT groups. Since these differences depend on many factors such as the probiotic dose, the time of application and the genetic structure, further studies are necessary. The present research aimed at improving animal welfare and performance as well as meat quality in poultry under conditions of high temperatures and the knowledge gained can be important for poultry producers.

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