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## Response of Japanese quails (*Coturnix coturnix japonica*) to dietary inclusion of *Moringa oleifera* essential oil under heat stress condition

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### ABSTRACT

The present study was aimed at investigating the effects of the dietary use of different doses of *Moringa oleifera* essential oil (MOEO) on performance parameters, internal organ weights and meat quality in Japanese quails (*Coturnix coturnix japonica*) exposed to Heat Stress (HS). For this purpose, 320 male day-old quail chicks were used. Seven days of preliminary (physical exercise) and a 35-day fattening period were applied. The animals were divided into 8 groups, each including 40 animals according to temperature (C: 25 °C and SC: 37 °C) and dietary doses (C, 200, 400 and 600). Each group was divided into 4 subgroups, each comprising 10 animals. The consequences of this study showed that, in the stress-free (HSF) groups, while all of the different doses of dietary MOEO positively affected growth performance, feed intake and feed efficiency, in the HS groups, similar positive effects were observed with the use of 200 ppm of dietary MOEO. While no effect was detected on internal organ weights on Days 21 and 42 in the HSF and HS groups, a dose-dependent increase was determined in the intestinal pH value on day 42 in the HS group ( $p < .05$ ). Additionally, in the analysis of meat quality on the 21st and 42 days compared to the controls, no effect was observed on the meat colour parameters value and TBARS level in the HS and HSF groups. Based on the consequences of this study, it is considered that, MOEO could be used as an alternative product in poultry, at a dose of 200 ppm, to reduce the detrimental effects of HS.

### HIGHLIGHTS

- Temperature stress, performance parameters and serious problems in the health of poultry.
- *Moringa oleifera* helps regulate performance parameters in poultry exposed to heat stress.
- Since the addition of *Moringa oleifera* to the diets does not affect the organ weight and meat quality in 21 and 42 days, it does not cause any negative effects for the consumer.

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Thermal stress; performance parameters; meat colour; *Moringa oleifera*

## Introduction

Heat stress (HS) is great environmental trouble that adversely affects the poultry sector in terms of (mortality, growth performance and product quality) throughout the world (Sahin et al. 2013; Aljubori et al. 2017; Attia, Al-Harhi, et al. 2018). Poultry is continuously exposed to several endogenous (nutritional disorders, rapid growth, infection, etc.) and exogenous stress factors (HS, high stocking density, poor ventilation, etc.) (Tekce and Gül 2017; Attia and Hassan, 2017; Chegini et al. 2019). Poultry species are homeothermic animals capable of maintaining their organism

temperature within a certain sequence, independently of the ambient temperature. Therefore, poultry needs to be raised under conditions of a minimum energy requirement for thermoregulation purposes and maximum net energy available for production purposes. Conditions other than these place poultry under stress. When exposed to stress, the growth performance of poultry species decreases (Attia et al. 2011, 2016; Hassan et al. 2016; Attia, El-Hamid, et al. 2018; Attia, Al-Harhi, et al. 2018). With an aim to eliminate the adverse effects of stress, in 1940, the use of sub-therapeutic doses of antibiotics was introduced to the

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**Table 1.** Basal diet ration nutrient content and analysis (The dry matter content is given as %).

Raw materials	Quail chick feed (0–21 d)	Quail growing feed (22–42 d)
Corn	52.70	58.12
Soybean meal (% 44 CP)	26.35	10.65
Corn gluten meal	15.21	26.14
Di-kalsiyum fosfat	1.95	1.60
Lisin	1.20	1.10
Limestone	1.18	1.04
Metiyonin	0.50	0.44
Sodyum klorur	0.31	0.31
Sodyum bikarbonat	0.20	0.20
Salt	0.20	0.20
Vitamin-mineral premix <sup>a</sup>	0.20	0.20
ME (Mj/kg)	12.97	13.50
Crude protein %	24	20
Moisture %	13.20	13.20
ASH %	5.19	3.85
Crude oil %	2.61	2.50
Lysine (%)	1.28	1.04
Calcium (%)	1.00	0.90
Total phosphorus (%)	0.72	0.60
Total sulphur amino acids %	0.71	0.71
Methionine (%)	0.50	0.38

<sup>a</sup>The vitamin-mineral premix provided the following (per kg of diet): vitamin A, 6000 U; vitamin D<sub>3</sub>, 1000 U; vitamin E, 15 mg/kg; vitamin K, 2 mg/kg; vitamin B<sub>1</sub>, 3 mg; vitamin B<sub>2</sub>, 4 mg; vitamin B<sub>6</sub>, 4 mg; vitamin B<sub>10</sub>, 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.

ME: Metabolisable Energy; Mj: megajoules

poultry sector. However, the development of anti-microbial resistance in consequence of the mis-dosage of antibiotics, the public wellness risks associated with antibiotic residues in meat, and consumer demands have all caused the forbidden of the use of antibiotics as feed admixture in food-producing animals (Tekce and Gul 2016; Attia, Bakhawain, et al. 2018; Attia, El-Naggar, et al. 2019; Guo et al. 2020).

In response to the increased interest of consumers in natural products and with a view to meet evolving market demands, over time, the poultry sector focussed on how to increase the growth performance and regulate the intestinal microflora of animals, and targeted at developing new products that would not harm human health (Alishah et al. 2013). The essential oils of natural aromatic herbs, which are purified from all hazardous synthetic products, do not pose any risk to human health and do not leave any residues in animal products, are among the primary of these newly developed products (Attia and Al-Harhi 2015; Attia, Bakhawain, et al. 2017; Guo et al. 2020). *Moringa oleifera*, which grows in the tropical and subtropical climate zones, is one of these aromatic herbs from which essential oil is derived. The leaves of *M. oleifera* contain minerals (potassium, calcium, phosphorus and iron), vitamins (A, D, and C), carotenoids (neoxanthin, violaxanthin,  $\beta$ -cryptoxanthin,  $\beta$ -carotene epoxide,  $\beta$ -carotene, and lutein), phenolic acids (gallic, chlorogenic, ellagic and ferulic acid) and flavonoids (zeaxanthin, quercetin, kaempferol, and apigenin) (Teixeira

et al. 2014; Helal et al. 2018). Previous studies suggest that MOEO positively affects growth performance (Ayssiwede et al. 2011; Onu and Aniebo 2011; Cui et al. 2018), shows no adverse effect on blood parameters (Onu and Aniebo 2011), reduces oxidative stress (Helal et al. 2018) and antimicrobial effects (El-Badawi et al. 2018).

In line with the current literature, studies have been conducted on the effect of temperature stress on performance parameters, internal organ weights and meat quality, but studies on the reduction of temperature stress are limited. Temperature stress is a problem that causes serious economic losses, especially in the poultry industry. In addition, consumer preferences change as a result of the negative effects of meat quality due to stress. Therefore, in this study was aimed at the investigation of the impact of different doses of dietary MOEO (200, 400 and 600 ppm) on performance parameters (body weight (BW), daily body weight gain (DBWG), feed efficiency/conversion (FCR) and feed intake (Fi), internal organ weights, meat colour parameters (L\*, a\* and b\*) and meat pH value in Japanese quails (*Coturnix coturnix japonica*) exposed to HSF and HS (C: 25 °C and SC: 37 °C).

## Materials and methods

### Animals and experimental design

A total of, 320 one-day-old male Japanese quail (*Coturnix japonica*) chicks constituted the material of

the study. Male quails were obtained from the Çukurova University poultry unit. The study covered a 7-day preliminary period followed by 35 days of fattening. The trial was guided at the Poultry Unit of the Food, Agriculture and Livestock Research and Application Centre of Bayburt University on groups of 40 animals, which were galvanised wire cages in batteries (Each floor of the cages has a capacity of 15 animals) with standard dimensions (100 × 50 × 100 cm) in an environmentally controlled lightproof house (close system; controlled for temperature, humidity, and light). The animals were divided into 8 groups according to temperature (C: 25 °C and SC: 37 °C) and dietary doses (200, 400 and 600) and based on their average body weight in the 7<sup>th</sup> days of the trial. Each group was divided into 4 subgroups and 10 animals in each. The trial lasted 35 days in total. Starting from Day 7 until Day 42 of the trial, the HSF groups were raised at a comfort (thermoneutral) temperature of 25 °C and the HS groups were raised at a temperature of 37 °C, which was above the thermoneutral zone. This study was guided pursuant to the approval (dated 12.11.2019 and numbered 2019/15) of the Local Ethics Board for Animal Experiments of Directorate of Veterinary Control Centre Research Institute.

### Feed

In order 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 on a basal diet, the chemical composition of which is presented in 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 manufactured and supplied in the laboratories of Mega analytical company operating in Trabzon province. The feed provided to the animals was analysed according to the official methods of analysis described by the Association of Official Analytical Chemists (AOAC 2005), and the nutrient composition of the feed is shown in Table 1.

### Birds heat, lighting and humidity

The quails were taken from Çukurova University poultry husbandry Research Centre at the age of 1 day with veterinarians health control and vaccinations. The general heat of the quails section was kept constant at 32–33 °C for the first 2 days and at 27–28 °C for the next 5 days. The animals were divided into 8 groups according to temperature (C: 25 °C (55–60% (relative)

humidity) and SC: 37 °C 75–85% (relative) humidity) and dietary doses (200, 400 and 600) and based on their average body weight in the 7<sup>th</sup> days of the trial. At the same time each day, the remaining feeds in front of the quail were weighed and new feeds added to the MOEO mixture were weighed and given to groups other than the control groups. Furthermore, 24 h of lighting (60 W) was applied to all the groups. Japanese quail were given fresh drinking water *ad libitum*. The 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 metre (TFA Dostmann, GERMANY) thermometers placed at 4 different points of the coop to control the temperature in the coop.

### Chemical composition of *Moringa oleifera* essential oil

Based on analysis results (ASU 64 LFGB L 07.00.40, HPLC-UV/FLD), the MOEO used 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, saturate level of 22.2 g/100 g, monounsaturated level of 74.2 g/100 g, and polyunsaturated level of 3.2 g/100 g. MOEO contained vitamin A (<5 µg/100 g), vitamin D<sub>3</sub> (<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).

### Performance parameters

The quails were weighed individually at the beginning of the probationary period after which the body weight (BW) of the quails and feed consumption were weekly (14, 21, 28, 35 and 42) recorded by replicate. FCR was estimated as total Fi (g)/BWG (g). Mortality was recorded when it occurred.

### Internal organ weights

At the end of the experiment, 96 male Japanese quail (*C. coturnix japonica*) (3 animals in each subgroup total

**Table 2.** Effects of diet, temperature and their interaction on quail fattening performance under stress.

	N	BW, g		DBWG, g		Fi, g		FCR, g/g	
		25 °C	37 °C	25 °C	37 °C	25 °C	37 °C	25 °C	37 °C
Control	80	185.88 <sup>a</sup>	178.56 <sup>a</sup>	5.31 <sup>ac</sup>	5.10 <sup>a</sup>	18.07 <sup>ad</sup>	17.09 <sup>ad</sup>	3.40 <sup>a</sup>	3.35 <sup>ad</sup>
MOEO 200 mg/kg	80	200.41 <sup>ac</sup>	204.36 <sup>cd</sup>	5.73 <sup>ac</sup>	5.84 <sup>bc</sup>	14.84 <sup>b</sup>	18.21 <sup>de</sup>	2.60 <sup>b</sup>	3.12 <sup>de</sup>
MOEO 400 mg/kg	80	209.99 <sup>bc</sup>	178.53 <sup>a</sup>	6.00 <sup>b</sup>	5.10 <sup>a</sup>	16.97 <sup>a</sup>	17.58 <sup>ae</sup>	2.86 <sup>ce</sup>	3.44 <sup>a</sup>
MOEO 600 mg/kg	80	185.63 <sup>ad</sup>	199.38 <sup>ac</sup>	5.30 <sup>ac</sup>	5.69 <sup>ab</sup>	14.50 <sup>b</sup>	19.75 <sup>c</sup>	2.74 <sup>bc</sup>	3.47 <sup>a</sup>
	SEM	7.53		0.22		0.40		0.09	
Diet									
Control		182.22		5.21		17.59		3.38 <sup>a</sup>	
MOEO 200 mg/kg		202.38		5.78		16.53		2.86 <sup>c</sup>	
MOEO 400 mg/kg		194.27		5.60		17.28		3.16 <sup>b</sup>	
MOEO 600 mg/kg		192.51		5.50		17.13		3.11 <sup>b</sup>	
	SEM	5.33		0.15		0.29		0.06	
Temperature									
25 °C		195.48		5.58		16.10		2.90	
37 °C		190.21		5.44		18.16		3.35	
	SEM	3.77		0.11		0.20		0.04	
Source of variation ( <i>p</i> -values)									
Diet		.09		.09		.09		.00	
Temperature		.33		.33		.00		.00	
Temperature × Diet		.04		.04		.04		.00	

a,b,c: Means within a column showing different superscripts are significantly different ( $p < 0.05$ ). SEM: standard error of the mean; MOEO: Moringa oleifera essential oil; BW: body weight; DBWG: daily body weight gain; FCR: feed efficiency/conversion; Fi: feed intake.

12 animals in each group) were randomly selected and subjected to cervical dislocation method and their internal organs were taken on 21st and 42nd days. The internal organs were then weighed on a digital precision scale (Shimadzu BI-3200h, Germany) with a sensitivity of 0.001 g in the Application and Research Centre laboratory of Bayburt University Food, Agriculture and Livestock Department.

### Quality and antioxidant properties of meat

At the end of the experiment, the above-mentioned parameters were applied for the cervical dislocation method of breast meat samples taken on 21st and 42nd days from randomly selected 3 animals of each group, making 12 pieces and in total 96 pieces male Japanese quail (*C. coturnix japonica*). The analysis was conducted in the Bayburt University Food Engineering Department's Meat Technology Laboratory.

### pH determination

The pH determination of the breast meats was performed by using samples weighing 10 g that was mixed with 100 ml of distilled water and subjected to homogenisation using ultra-turrax (IKA Werk7 T 25, Germany) for 1 min. The pH of the prepared homogenate was measured with a pH metre (Mettler-Toledo AG, 8603 Schwerzenbach, Switzerland). The pH metre was calibrated with appropriate buffer solutions (pH 4.00 and pH 7.00) before use (Gökalp et al. 2012; Kirkpinar et al. 2014).

### TBARS value determination

The determination of TBARS value of the samples was first, the analysis sample with a weight of approximately 2 g was weighed and following the addition of 12 ml trichloroacetic acid (TCA) solution (7.5% 28 TCA, 0.1% EDTA (ethylenediaminetetraacetic acid), and 0.1% propyl gallate (dissolved in 3 ml ethanol)) the homogenisation was applied with ultra turrax (IKA \* Werk T25, Germany). Later the homogenates were filtered through Whatman N° 1 filter paper and 3 ml of the filtrate was transferred to a glass tube. 3 ml 0.02 M thiobarbituric 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 centrifuged 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 240 V, Japan). The calculation of TBARS values was performed by using the absorbance values obtained with the help of the formula below and the results were given in mg MDA/kg.

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

### Colour values determination

The determination of colour values of samples (L\* (lightness), a\* (redness), b\* (yellowness)) were performed by using Chroma Metre (CR-400 Konika Minolta, Japan) colorimeter. Colour measurements were evaluated according to the criteria set by the



**Table 3.** Effects of diet, temperature and their interaction on internal organ weights (g/100g BW) on 21st-day quail under stress.

	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	4.11	2.51	6.80	6.97
MOEO 200 mg/kg	24	1.23	0.83	5.40	3.27	4.33	2.81	7.20	6.89
MOEO 400 mg/kg	24	1.25	0.95	4.57	3.01	3.90	3.45	6.80	6.95
MOEO 600 mg/kg	24	1.69	0.92	6.13	3.75	5.09	3.53	6.85	6.90
SEM		0.15		0.48		0.38		0.11	
Main effect means diet									
Control		0.92		3.79		3.31		6.88	
MOEO 200 mg/kg		1.03		4.34		3.57		7.05	
MOEO 400 mg/kg		1.09		3.79		3.68		6.89	
MOEO 600 mg/kg		1.31		4.94		4.31		6.87	
SEM		0.11		0.34		0.27		0.07	
Temperature									
25 °C		1.30		5.29		4.36		6.92	
37 °C		0.88		3.14		3.08		6.93	
SEM		0.08		0.24		0.19		0.05	
Source of variation (p-values)									
Diet		.09		.08		.09		.25	
Temperature		.00		.00		.00		.87	
Temperature × Diet		.24		.75		.39		.09	

a,b,c: Means within a column showing different superscripts are significantly different ( $p < .05$ ). SEM: standard error of the mean; MOEO: Moringa oleifera essential oil; BW: body weight.

International Commission on Lighting (Commission Internationale De L'Eclairage).

### 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 normal distribution and except liver weight, all of them distributed normally. The square root transformation was used for liver weights. 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,  $\mu$  = 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) × 3 (three dose levels) factorial design. The Duncan multiple comparison test was used for comparing the group means. The statistical analyses were performed by using SAS software, version 9.0 (SAS Institute, Cary, NC, USA).

## Results

### Performance parameters

The effects of different doses of dietary MOEO on the performance of HS Japanese quails are shown in Table 2. In the HSF groups, which were raised at 25 °C, in comparison to the controls, growth performance, feed intake and feed efficiency were observed to have increased ( $p < .05$ ). On the other hand, in the HS groups, which were raised at 37 °C, compared to the controls, the groups that received dietary MOEO presented with improved BW, BWG, Fi and FCR values and the best results were obtained with dietary MOEO supplementation at a dose of 200 ppm.

### Internal organ weights

The statistical analysis results obtained for internal organ weights (g/100g BW) on Days 21 and 42 of the study are presented in Tables 3 and 4. These results showed that, on 21 and 42 days, when compared to the controls, of the HSF and HS groups, no effect was observed on the Internal organ weights (g/100g BW) parameters ( $p > .05$ ). However, on Day 42, when compared to the controls, the HS groups the intestinal pH value showed a dose-dependent increase ( $p < .05$ ).

### Quality and antioxidant properties of meat

The results obtained for meat colour parameters on Days 21 and 42, as an indication of meat quality, are presented in Tables 5 and 6. On Day 21, when compared to the controls, of the HSF groups, no effect was observed on the meat colour parameters ( $p > .05$ ). The meat pH value was ascertained to have increased in all of the HSF groups ( $p < .05$ ). In the HS groups, dietary MOEO was observed not to have affected the meat colour parameters, pH value and TBARS level ( $p < .05$ ). The analysis results demonstrated that, on Day 42, in the HSF and HS groups, when compared to the controls, no effect was observed on the meat colour parameters and TBARS levels in the groups. Compared to the controls, the meat pH value was ascertained to have some group decreased and some group increased in the groups that received dietary MOEO ( $p < .05$ ).

## Discussion

### Performance parameters

In the present study, the effects of dietary MOEO on growth performance, internal organ weights, and

**Table 4.** Effects of diet, temperature and their interaction on internal organ weights (g/100g BW) on 42st-day quail under stress.

	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 <sup>ac</sup>	7.76 <sup>a</sup>
MOEO 200 mg/kg	24	1.19	0.78	0.09	0.09	0.66	0.57	4.15	2.98	2.63	2.33	7.89 <sup>ac</sup>	8.05 <sup>cd</sup>
MOEO 400 mg/kg	24	1.07	0.95	0.07	0.10	0.60	0.53	3.34	3.28	2.44	2.71	7.88 <sup>ac</sup>	7.97 <sup>a</sup>
MOEO 600 mg/kg	24	1.42	1.04	0.09	0.08	0.80	0.60	6.53	3.17	3.13	2.57	7.67 <sup>c</sup>	8.34 <sup>bd</sup>
SEM		0.11		0.01		0.08		0.64		0.23		0.12	
Main effect means diet													
Control		1.04		0.08		0.62		3.17		2.33		7.83	
MOEO 200 mg/kg		0.98		0.09		0.62		3.57		2.48		7.97	
MOEO 400 mg/kg		1.01		0.09		0.56		3.31		2.57		7.93	
MOEO 600 mg/kg		1.23		0.09		0.70		4.85		2.85		8.00	
SEM		0.08		0.01		0.05		0.45		0.16		0.08	
Temperature													
25 °C		1.18		0.09		0.69		4.38		2.62		7.84	
37 °C		0.95		0.09		0.56		3.07		2.50		8.03	
SEM		0.06		0.01		0.04		0.32		0.11		0.06	
Source of variation (p-values)													
Diet		.15		.97		.35		.06		.17		.49	
Temperature		.01		.68		.03		.01		.46		.04	
Temperature × Diet		.29		.37		.85		.08		.28		.01	

a,b,c: Means within a column showing different superscripts are significantly different ( $p < .05$ ). SEM: standard error of the mean; MOEO: Moringa oleifera essential oil; BW: body weight.

**Table 5.** Effects of diet, temperature and their interaction on meat quality and colour parameters on 21st-day quail under stress.

	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	10.32	0.62	0.58	6.17 <sup>a</sup>	6.35
MOEO 200 mg/kg	24	40.69	43.14	14.05	16.78	11.45	14.71	0.52	0.81	6.32 <sup>ab</sup>	6.28
MOEO 400 mg/kg	24	52.87	42.45	17.42	15.43	13.18	13.44	0.30	1.10	6.24 <sup>ab</sup>	6.36
MOEO 600 mg/kg	24	39.31	39.18	15.53	16.33	13.85	10.69	0.79	0.45	6.43 <sup>b</sup>	6.38
SEM		3.99		1.78		1.73		0.19		0.03	
Main effect means											
Control		42.65		15.92		12.89		0.59		6.26 <sup>a</sup>	
MOEO 200 mg/kg		41.92		15.42		13.08		0.66		6.30 <sup>ab</sup>	
MOEO 400 mg/kg		47.66		16.43		13.31		0.70		6.30 <sup>ab</sup>	
MOEO 600 mg/kg		39.25		15.93		12.27		0.62		6.40 <sup>b</sup>	
SEM		2.82		1.26		1.22		0.14		0.02	
Temperature											
25°C		44.93		15.34		13.49		0.56		6.29	
37°C		40.81		16.51		12.29		0.74		6.34	
SEM		1.99		0.89		0.87		0.09		0.01	
Source of variation (p-values)											
Diet		.24		.95		.94		.95		.00	
Temperature		.16		.36		.34		.22		.03	
Temperature × Diet		.33		.48		.12		.06		.00	

a,b,c: Means within a column showing different superscripts are significantly different ( $p < .05$ ). SEM: standard error of the mean; MOEO: Moringa oleifera essential oil; BW: body weight; TBARS: thiobarbituric reactive substances; MDA: malondialdehyde.

meat quality were investigated in Japanese quails exposed to HS. Environmental temperatures above 30 °C can be causing HS in poultry species (Attia, Bakhawain, et al. 2017; Seifi et al. 2018; Attia, Bakhawain, et al. 2018). High ambient temperature is known to adversely affect growth performance (Attia, Bakhawain, et al. 2018; El-Deep et al. 2019). For minimising HS-induced adverse effects, pathogenic feed additives are used, and when incorporated into feed, these additives improve performance and gut health and reduce stress (Mehdi et al. 2018; Attia, Al-Harhi, et al. 2019). While some searcher has declared that dietary MOEO improves growth performance in

stressed poultry (Ayssiwede et al. 2011; Hassan et al. 2016; Cui et al. 2018; Vedendar 2018), some other searchers have suggested that dietary MOEO has no such effect (El Shoukary and Mousa 2018; El-Deep et al. 2019). Previous studies have reported that the application of *Moringa olifera* leaf, powder or essential oil to animals on feed-in HSF revealed a positive effect on performance (Abbas and Ahmed 2012; Paguia et al. 2014). However, in contrast, some other studies have reported no effect from the application of moringa olifera leaf, powder or essential oil (Banjo 2012; Donkor et al. 2013; Alabi et al. 2017; Khan et al. 2017). In the present study, it was observed that, in the HSF groups,

**Table 6.** Effects of diet, temperature and their interaction on meat quality and colour parameters on 42st-day quail under stress.

	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	55.35	24.75	16.17	19.54	17.38	0.62	1.16	6.04	5.81
MOEO 200 mg/kg	24	50.88	47.53	17.41	22.31	14.89	14.80	0.89	1.52	6.07	6.04
MOEO 400 mg/kg	24	51.28	49.01	18.89	17.02	16.17	15.91	0.32	0.59	6.05	5.98
MOEO 600 mg/kg	24	49.92	45.32	20.60	19.72	19.64	11.47	1.57	0.78	5.84	5.79
	SEM	2.18		1.94		1.66		0.29		0.04	
Main effect means											
Control		50.64		20.46		18.46		0.89		5.92 <sup>ab</sup>	
MOEO 200 mg/kg		49.20		19.86		14.85		1.21		6.05 <sup>b</sup>	
MOEO 400 mg/kg		50.15		17.96		16.04		0.45		6.02 <sup>ab</sup>	
MOEO 600 mg/kg		47.62		20.16		15.55		1.17		5.82 <sup>a</sup>	
	SEM	1.54		1.37		1.18		0.21		0.03	
Temperature											
25°C		49.92		20.16		16.65		1.05		5.99	
37°C		48.88		19.06		15.78		0.81		5.92	
	SEM	1.09		0.97		0.83		0.15		0.02	
Source of variation ( <i>p</i> -values)											
Diet		.24		.95		.94		.95		.00	
Temperature		.16		.36		.34		.22		.03	
Temperature × Diet		.33		.48		.12		.06		.00	

a,b,c: Means within a column showing different superscripts are significantly different ( $p < 0.05$ ). SEM: standard error of the mean; MOEO: Moringa oleifera essential oil; BW: body weight; TBARS: thiobarbituric reactive substances; MDA: malondialdehyde.

when compared to the controls, dietary MOEO improved growth performance, feed intake and feed efficiency ( $p < .05$ ). In the HS groups, comparison with the controls demonstrated that dietary MOEO positively affected BW, BWG, Fi, and FCR, and that the best results were obtained with the use of 200 ppm of MOEO. While these results are consistent with some literature reports (Ayssiwede et al. 2011; Banjo 2012; Donkor et al. 2013; Hassan et al. 2016; Alabi et al. 2017; Khan et al. 2017; Cui et al. 2018; Vedendar 2018), they disagree with some other reports (Abbas and Ahmed 2012; Paguia et al. 2014; El Shoukary and Mousa 2018; El-Deep et al. 2019). Differences between the consequences of the present study and previous research are attributed to differences in the form and doses of MOEO incorporated into a feed and the preparation method of the MOEO supplements used.

### Internal organ weights

In poultry species, HS is described as a non-specific response to high levels of humidity and temperature. Some literature reports indicate that, in animals exposed to HS, internal organ weights are affected in consequence of immunosuppression (Lara and Rostagno 2013). In such cases, medicinal aromatic herbs improve gastrointestinal functions owing to the secondary metabolites they contain (Kiczorowska et al. 2016). Previous studies on the effects of secondary metabolites, found in the powder, leaves and essential oil of MOEO, on internal organ weights in HS poultry have suggested either the absence of an effect

(Hassan et al. 2016; Vedendar 2018) or visceral organ weights vary with weight gain and age the presence of effects (Aderinola et al. 2013; Abou Sekken 2015). In the present study it was determined that, On 21 and 42 days, when compared to the controls, of the HSF and HS groups, no effect was observed on the internal organ weights (g/100g BW) parameters ( $p > .05$ ). Furthermore, on Day 42 of the trial, the HS groups given dietary MOEO displayed the intestinal pH value showed a dose-dependent increase ( $p < .05$ ). In studies on internal organ weight of addition of *Moringa olifera* leaf, powder or essential oil to feeds in HSF environment, there is no effect of organ weight (Abbas and Ahmed 2012; El-Tazi 2014; Alabi et al. 2017) and some studies have indicated that it increases organ weight (Onunkwo and George 2015). Analysis results demonstrated that dietary supplementation with MOEO did not affect internal organ weights in HS Japanese quails. While these results are in agreement with some literature reports (Abbas and Ahmed 2012; El-Tazi 2014; Hassan et al. 2016; Alabi et al. 2017; Vedendar 2018), they disagree with some other reports (Aderinola et al. 2013; Abou Sekken 2015; Onunkwo and George 2015). Differences among the consequences of the available study and those acquired in some previously conducted studies are dedicated to variation in the content, form, and doses of MOEO used.

### Quality and antioxidant properties of meat

Poultry meat is a perishable food product. Following the slaughter of poultry, raw poultry meat, even when



stored below the chilling temperature, tends to spoil within 4–10 days (Lin et al. 2004). The post-mortem metabolism of muscle tissue influences meat properties. The rate and degree of acidification have a particularly strong impact on the organoleptic properties and technological parameters of meat, which in the end define meat quality (Duclos et al. 2007). Some literature reports suggest that the incorporation of the powder, leaves and essential oil of MOEO into poultry feed does not adversely affect the pH value of meat (Wapi et al. 2014; Nkukwana et al. 2014; Cui et al. 2018; Ng'ambi et al. 2017). On the other hand, another study suggests that dietary MOEO increases the meat pH value (Rehman et al. 2018). On Day 21 of the trial, an increase in meat pH values was observed in all of the HSF groups ( $p < .05$ ), whereas among the HS groups, dietary MOEO was observed not to have affected the meat pH value ( $p < .05$ ). The analysis results demonstrated that, on Day 42 of the trial, among the HSF and HS groups was observed not to have affected the meat pH value ( $p < .05$ ). While these results of the present study are compatible with some literature reports (Rehman et al. 2018), they disagree with some other reports (Wapi et al. 2014; Nkukwana et al. 2014; Ng'ambi et al. 2017); Cui et al. 2018. Differences between the results of the present study and those obtained in some previously conducted studies are dedicated to variety in the conditions to which the animals were exposed (HS) and the composition, form, and doses of MOEO incorporated into feed.

HS increases lipid peroxidation by increasing the generation of free radicals. Some plants are known to include natural antioxidants (Hassan et al. 2016; Attia, Bakhawain, et al. 2017; Attia, Bakhawain, et al. 2018). The essential oil derived from MOEO leaves also contains natural antioxidants and displays a high level of oxidative stability (Nkukwana et al. 2014). It has been demonstrated that, the level of lipid peroxidation is inversely proportional to the dose of the powder or essential oil of MOEO leaves incorporated into the ration (Nkukwana et al. 2014; Cui et al. 2018; Vedendar 2018). In another study, it was ascertained that while the total antioxidant capacity (TAC) decreased in broiler chickens exposed to HS, dietary MOEO supplementation reduced the stress-induced detrimental effects (El-Deep et al. 2019). On Day 21 and 42 of the trial, among the HSF and HS groups, when compared to the controls, no effect was observed on the TBARS levels ( $p > .05$ ). Differences among the consequences of the present study and some previously conducted studies are considered to

have arisen from differences in the composition, form, and doses of MOEO incorporated into the feed.

Meat quality is a complex parameter defined not only by the composition and visual and sensory properties of meat but also by the carcass ratio. In the broiler industry, the colour of the skin, bones, and meat is of particular significance. MOEO foliage comprises xanthophylls and carotenoids, which are natural colourants and have an important place in poultry nutrition (Nkukwana et al. 2014). In a previous study, it was reported that while the  $L^*$  and  $a^*$  levels displayed a dose-dependent increase proportionate to the level of powdered *M. oleifera* leaves incorporated into the feed, no effect was observed on the  $b^*$  value with the use of MOEO powder (Wapi et al. 2014). In another study, it was observed that dietary supplementation with *M. oleifera* leaves decreased the  $b^*$  value, increased the  $a^*$  value, and showed no effect on the  $L^*$  value (Cui et al. 2018). It has also been reported that dietary supplementation with the flour of *M. oleifera* seeds showed no effect on meat colour (Ng'ambi et al. 2017). Another published report indicates that dietary supplementation with powdered MOEO leaves decreased the  $L^*$  value, and increased the  $a^*$  and  $b^*$  values (Nkukwana et al. 2014). Previous studies have reported that the application of moringa *oleifera* leaf, powder or essential oil to animals on feed in the HSF environment affects meat quality (Wapi et al., 2014; Nkukwana et al. 2014; Rehman et al. 2018). However, in contrast, some other studies have reported no effect from the application of moringa *oleifera* leaf, powder or essential oil (Mukumbo et al., 2015; Alabi et al., 2017). In the present study, it was ascertained that, on Day 21 and 42 of the trial, in the HSF and HS groups, compared to the controls, no effect was observed on the meat colour parameters with the incorporation of MOEO into feed yet these differences were statistically insignificant ( $p > .05$ ). Based on the results of the present study and published literature reports, the differences observed between study results are attributed to differences in the composition, form, and doses of MOEO incorporated into feed.

## Conclusions

Different doses of dietary MOEO were determined to have positively affected growth performance, internal organ weights, and meat quality on Days 21 and 42 in Japanese quails exposed to HS and HSF. In the HSF groups, of the different doses (200 and 400 ppm) of dietary MOEO positively affected growth performance.

In the HS groups, the best growth performance results were achieved with the use of 200 ppm of MOEO. But 21 and 42 days in the HSF and HS groups had no effect on internal organ weights, meat quality and TBARS value. As a result, our research aims to increase the fattening performance, meat quality and animal welfare of poultry, which is one of the animals most affected by high temperatures, especially in summer. Furthermore, detailed studies are required for a better understanding of the potential MOEO offers to the poultry sector in reducing economic losses and improving product quality.

### Ethical approval

This study was guided under the approval (dated 12.11.2019 and numbered 2019/15) of the Local Ethics Board for Animal Experiments of Directorate of Veterinary Control Centre Research Institute.

### Disclosure statement

We certify that there is no conflict of interest with any financial organisation regarding the material discussed in the manuscript.

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