Effect of dietary orange peel essential oil on physiological, biochemical and metabolic responses of Japanese quails as affected by early age thermal conditioning and fasting

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SUMMARY

High ambient temperature is one of the most important problems for poultry production in many regions of the world. Heat acclimatization has been experienced in many poultry species for economic losses due to heat stress. Therefore, the effect of orange peel essential oil (OEO) dietary supplementation on compensatory growth parameters of short-term fasted and thermal conditioned quail chicks at an early age was studied. 168 seven-day-old quail chicks were randomly assigned to six groups as the control, thermal conditioning (36°±1°C and 70-80 % RH for 24 h) and fasting group (feed withdrawal for 24 h). Each group was split into two and received a basal feed or the same basal feed with 300 ppm OEO supplemented feed. Thermal conditioning and fasting at early age significantly resulted in growth retardation between 7-14 d of the experiment. Essential oil supplementing positively influenced weight gain and feed conversion ratio of the quails at 7-42 d with similar feed intake. Early age thermal challenge with essential oil supplementation increased the breast percentage whereas thigh and waist-neck percentage was decreased. Thermal conditioning at early age demonstrated better capacity to cope with heat stress through greater reduction in serum T3 levels compared to quails that were not thermal conditioned.
These results suggest that early exposure to thermal conditioning may promote quails' ability to cope with the subsequent heat load by altering thermoregulatory physiological responses. OEO supplementation demonstrated the best improvements in thermotolerance acquisition. In conclusion, OEO supplementation can be used as a natural growth promoter either early age treated or normal breeding chicks.

Keywords: Japanese quails, Orange peel essential oil, thermotolerance, growth performance, biochemical parameters

RÉSUMÉ

Effet de l'ajout d'huile essentielle de zeste d'orange dans l'alimentation sur les réponses physiologiques, biochimiques et métaboliques au cours d'un stress thermique et d'un jeûne chez les cailles japonaises.

Une température ambiante élevée est l'un des problèmes les plus importants pour la production de volailles dans de nombreuses régions du monde. L'acclimatation à la chaleur a été expérimentée dans plusieurs espèces de volailles pour prévenir les pertes économiques dues au stress thermique. L'effet de l'huile essentielle d'orange (OEO) en supplémentation dans l'aliment sur les paramètres de croissance compensatoire lors d'un stress thermique et d'un jeûne court a été étudié sur poussins de cailles. Des poussins de cailles âgés de 7 jours (n = 168) ont été répartis au hasard dans trois groupes : un groupe contrôle, un groupe avec stress thermique (36 ° ± 1 ° C et 70-80% d'humidité relative pendant 24 h) et groupe subissant un jeûne court (privation de nourriture pendant 24 h). Chaque groupe a été divisé en deux et a reçu un aliment de base ou le même aliment de base avec 300 mg/kg d'OEO. Le stress thermique et le jeûne ont entraîné un retard de croissance significatif entre 7 et 14 jours. L'ajout d'OEO a eu une influence positive sur le gain de poids et le taux de conversion d'alimentation entre 7-42 jours avec prise alimentaire similaire. Les animaux exposés à un stress thermique précoce recevant de l'OEO ont montré une variation de répartition des masses corporelles associées à une plus forte réduction des taux sériques de T3 par rapport aux témoins.
Ces résultats suggèrent que l'exposition précoce à stress thermique peut augmenter la capacité des cailles à faire face à un stress thermique ultérieur en modifiant leurs capacités physiologiques de thermorégulation. L'ajout d'OEO a amélioré l'acquisition de la thermotolérance.

Mots-clés : Caille, Alimentation, Huile essentielle de zeste d'orange, Stress thermique, Performances, Biochimie

Introduction

Thermotolerance or "heat acclimatization" has been experienced in many poultry species for economic losses due to heat stress by thermal conditioning or short-term fasting or feed restriction at an early age [3, 23, 37, 38, 40]. High ambient temperature has a negative effect on broiler production efficiency also behavioral, physiological, hormonal and molecular changes occur during the heat stress [17]. On the other hand, broilers can adjust to heat stress by physiological mechanisms that help to cope with high environmental temperatures [8, 37-39]. The mechanisms associated with the induction of thermotolerance by early-age temperature conditioning involve: 1) modulation of heat production through reduction in plasma triiodothyronine (T3) concentration; 2) hemodynamic changes (decrease in heart weight and hematocrit); 3) increase in sensible heat loss; and 4) pronounced ability to control the body water economy during thermal challenge [37-39].

Thermal conditioning (TC) at an early age has been reported to result in reduced weight gain during the

1st week of life, followed by an accelerated growth which leads to a higher body weight than that of non-conditioned chickens at marketing age [39]. Accelerated growth and thermoregulation in broiler chickens and quails have also been observed [14, 23, 27-29]. In natural environments, the supply of feed is often dependent on ambient temperature, precipitation, wind speed and other meteorological factors [24, 35].

Recently, the natural feed additives like (including) herbs and medicinal plants have been emerged as safe growth enhancers' alternative to synthetic drugs. These natural feed additives are given to animals/birds to improve their productive performance under normal or stress conditions [11, 20, 21]. Orange oil is an essential oil produced by cells within the rind of an orange fruit (Citrus sinensis fruit). In contrast to most essential oils, it is extracted as a by-product of orange juice production by centrifugation, producing cold-pressed oil. It is composed of mostly (greater than 90%) d-limonene and (2.0-2.1%) β-myrcene as a minor constituent [33]. D-limonene is listed in the Code of Federal Regulation as generally recognized as safe (GRAS) for a flavoring agent [5]. Recently, the orange peel essential oil has been used as antiparasitic [2], antifungal [30], antioxidant, antimicrobial and growth promoter [18] agents in a few studies.

Japanese quail (*Coturnix japonica*) is becoming more popular as a source of meat and eggs in various parts of world. It has also assumed worldwide importance as a laboratory animal with distinct characteristics such as rapid growth enabling quail to be marketed for human consumption at 5-6 weeks of age [22].

To our knowledge, there are no studies on the use of essential oil of orange peel (OEO) as growth promoter approach in thermotolerance acquisition studies of Japanese quails. Therefore, the current study was conducted to evaluate the growth promoter activity of OEO supplemented to the feed of Japanese quails which were fasted and heat stressed at an early age.

Material and Methods

This study was aimed to determine the growth promoter effect of orange peel essential oil in compensatory growth of quail chicks that were stressed at early age. All procedures were approved by Firat University Institutional Animal Care and Use Committee (FUHADEK, verdict no: 04.04.2013/55). One hundred and sixty eight 1-d-old Japanese quail chicks were kept together for seven days. When chicks were 7-d-old, they were weighed individually and randomly assigned to six groups of 4 replicate pens (30×80 cm), containing 7 chicks using a completely randomized design (CRD). Then early age (7 d-old) stress factors were applied to four of six groups for 24 hours so the two groups of four were subjected to fasting and the other two groups of four were subjected to thermal conditioning at 36±1˚C with %70–80 relative humidity in another room. After treatments all of the chicks were raised in a temperature-controlled room at 26±2°C with 50-60% relative humidity throughout the experiment. The diet was formulated to meet the nutrient requirements of the Japanese quails (24% CP and 2900 kcal/kg ME for starting and growing periods) according to the National Research Council [26] recommendations. Zeolite as a carrier was added at one kg per hundred kg to the basal diets. For essential oil groups 30 g orange peel essential oil + 970 g zeolite were mixed and then added to basal diet to obtain 300 ppm of essential oil concentration. The ingredients and compositions of the diets are presented in Table I. Orange peel essential oil was obtained from Agromix Livestock Feed Additives Food

Feed ingredients	Starting and Growing	Calculated analysis	
Maize	564.3	Crude protein	236.0
Soybean meal	315.0	ME, MJ/kg	12.7
Vegetable oil	30.0	Ether extract	46.5
Fish meal	58.0	Crude cellulose	25.5
Dicalcium phosphate	8.0	Crude ash	63.5
Calcium carbonate	8.0	Calcium	8.1
Salt	2.5	Available Phosphorus	3.6
DL-Methionine	0.5	Methionine+Cystine	8.4
L-Lysine	0.2	Lysine	13.9
Vitamin-Mineral Premix*	2.5		
Zeolite**	10.0		
Total	1000.0		

* Provided per kg of diet: retinol, 2.64 mg; cholecalciferol,0.04 mg; dl-a-tocopherol-acetate, 11 mg; riboflavin, 9.0 mg; pantothenic acid, 11.0 mg; vitamin B12, 0.013 mg; niacin, 26 mg; choline, 900 mg; vitamin K, 1.5 mg; folic acid, 1.5 mg; biotin, 0.25 mg; iron, 30 mg; zinc, 40 mg; manganese, 60 mg; copper, 8 mg; selenium, 0.2 mg..

**No added orange peel essential oil groups (10 g zeolit); 300 ppm orange peel essential oil added groups (3 g orange oil+7 g zeolite).

Table I: Ingredients and chemical composition of standard diet (g/kg)

Industry and Trade Ltd. (Izmir/Turkey) and was sent to Food Control Laboratory of Ege University (Izmir/Turkey) for analyzing the chemical composition by GC-MS (Table ii). The experimental groups were assigned as supplementing orange peel oil or not and stress factors that fasted and thermal conditioned at 7-d-old chicks for 24 hours. So the groups were: 1) Unstressed and no OEO (Negative Control, **NC**) Group, 2) Fasted and no OEO Group (**F**), 3) Thermal Conditioned and no OEO Group (**TC**), 4) Unstressed but OEO added (Positive Control, **PC**) Group, 5) Fasted+OEO Group (**F+OEO**) and 6) Thermal Conditioned+OEO Group (**TC+OEO**). During the experimental period (35 days), conventional breeding and management procedures were employed after the stress factors were applied. The birds were exposed to continuous lighting and birds were fed and watered *ad libitum*.

Birds were weighed individually and feed intake per pen was recorded every week. Mortality was recorded as it occurred and percentage mortality was determined at the end of study.

At the end of the study, when chicks were 42-d-old, all groups were subjected to heat stress at 33±1 ˚C for 6 hours to measure the thermotolerance ability. After heat stress birds were weighed individually and waste feed was recorded. Body temperatures from cloaca of birds were measured by digital thermometer after heat or fasting stress applications at the beginning and end of the experiment. Then 10 birds from each experimental group were weighed and slaughtered by cutting the jugular vein, blood was drained to tubes for hematological and biochemical analyses. Following slaughtering, hot and cold carcass characteristics were evaluated. From these chickens were removed feathers, head, legs and inner organs (except kidneys and lungs). Carcasses were kept at +4 ºC for 24 h, before remove legs (from articulatio coxe), breast (from articulatio sternocostalis), wings (from articulatio humeri), neck and back, according to Institute of Turkish Standards rules [4]. These pieces were weighted together with skin. Carcass yield, liver, heart and spleen percentages were calculated from whole body (slaughter weight), whereas breast, thigh, waist and neck, wings and abdominal fat percentages were estimated from carcass weight.

The taken blood samples were immediately transported to Firat University Central Laboratory for serum and plasma analyses. Whole blood (red cells, white cells, and platelets), glucose, cholesterol, alkaline phosphatase (ALP), insulin, insulin like growth hormone (IGF_1), sodium, potassium, calcium, magnesium, free T_3 and T_4 were analyzed in the laboratory. Chemical composition of feed ingredients (dry matter, crude protein, ash and ether extract) were analyzed according to the AOAC [6] procedures and crude fiber was determined by the methods of CRAMPTON and MAYNARD [13].

Data were subjected to two-way anova by using GLM (General Linear Model) procedure. Significant differences were further subjected to Duncan's multiple range tests by using SPSS 11.5 for Windows [32]. Mortality rate was determined for each treatment and evaluated by X^2 test. The results were considered as significant when $p<0.05$, $p<0.01$ and $p<0.001$. The results were given as Mean±S.E.M (Standard Error Mean).

Results

When performance parameters were examined in Table III, mean body weights of the 6 groups were similar before the early-age treatments at 7 days. The chemical composition of OEO used in the study was presented in Table II. The major component was Limonene as 92.3% of the compound.

Mortality was very low (1 death in F and F+EOE, 2 deaths in TC groups) throughout the experimental period (7 to 42 d). No significant difference was found about viability of the birds (Table III). The worse results were obtained in the fasting groups whatever the diets (Table III). The lowest body weight means were found in the fasting groups whatever the diet. On the contrary, the best body weight means were observed in the group TC+OEO from 21 days old to 42 days old (Table III).

Daily weight gain and feed conversion ratio means of 7-42 days of the experiment were affected by OEO supplementation. Daily feed intake was only affected at 14-21 days of the experiment by OEO supplementation (Table III).

Analysis	Result*
Limonene	92.3%
Beta Myrcene	3.3%
Alpha Pinen	1.4 %
Linalool	0.9 %
Sabinen	0.6 %
Delta 3 Caren	0.2 %
Octanal	0.2 %
Undefined	1.1 %

*: obtained by GC-MS analysis

Table II: The concentration of the volatile components in OEO

	Days	NC	F	TC	PC	F+OEO	TC+OEO	P OEO	P Treat.	P OxT
Body Weight	7.	22.3±0.5	22.2±0.6	22.3±0.4	22.3±0.7	22.2±0.3	22.2±0.6	NS	NS	NS
	14.	47.8±1.0^{A}	40.8±1.3^{B}	46.8±1.2^{A}	45.1±1.7^{a}	39.6±1.4^{b}	46.4±1.8^{a}	NS	***	NS
	21.	86. 5±2.3^{A}	77.8±2.3^{B}	84.7±2.0^{AB}	86.1±2.1^{ab}	78.9±2.3^{b}	86.9±2.4^{a}	NS	***	NS
	28.	123.5±3.0	117.8±2.8	124.6±2.4	125.4±2.7^{ab}	118.0±2.6^{b}	128.0±2.7^{a}	NS	**	NS
	35.	157.6±3.3	153.7±3.4	159.2±2.7	160.6±3.5^{ab}	158.0±3.0^{b}	168.0±3.2^{a}	*	*	NS
	42.	177.7±4.7	167.6±5.1	172.9±4.6	183.7±5.5	178.8±4.3	188.0±5.4	**	NS	NS
Weight Gain	7-14	3.7±0.1^{A}	2.7±0.1^{B}	3.5±0.1^{A}	3.3±0.3^{ab}	2.5±0.3^{b}	3.5±0.2^{a}	NS	***	NS
	14-21	5.5±0.2	5.3±0.2	5.4±0.1	5.9±0.4	5.6±0.4	5.8±0.1	NS	NS	NS
	21-28	5.3±0.2	5.7±0.2	5.7±0.2	5.6±0.2	5.6±0.2	5.9±0.3	NS	NS	NS
	28-35	4.9±0.2	5.1±0.2	4.9±0.3	5.0±0.4	5.7±0.2	5.7±0.4	*	NS	NS
	35-42	2.9±0.5	2.0±0.5	2.0±0.7	3.3±0.3	3.0±0.4	2.9±0.7	NS	NS	NS
	7-42	4.4±0.1	4.2±0.1	4.3±0.2	4.6±0.1	4.5±0.2	4.7±0.7	*	NS	NS
Feed Intake	7-14	9.0±0.1	8.5±0.3	8.3±0.4	9.5±0.2^{a}	7.9±0.3^{b}	8.6±0.1^{ab}	NS	**	NS
	14-21	12.9±0.4	14.7±0.7	13.3±0.4	14.9±0.4	14.3±0.6	14.4±0.5	*	NS	NS
	21-28	17.1±0.1	17.0±0.3	17.0±0.2	17.0±0.5	16.7±0.5	17.8±0.5	NS	NS	NS
	28-35	25.8±0.9	23.5±0.9	25.7±0.2	24.3±1.2	24.1±1.9	26.0±0.7	NS	NS	NS
	35-42	22.5±1.9	21.4±0.7	21.1±1.0	20.9±0.8	21.8±2.7	21.9±1.9	NS	NS	NS
	7-42	17.5±0.6	17.0±0.0	17.1±0.2	17.3±0.3	17.0±1.0	17.7±0.4	NS	NS	NS
FCR	7-14	2.5±0.1^{B}	3.2±0.1^{A}	2.4±0.1^{B}	2.9±0.4	3.2±0.4	2.5±0.1	NS	*	NS
	14-21	2.3±0.2	2.8±0.1	2.5±0.1	2.6±0.1	2.6±0.2	2.5±0.1	NS	NS	NS
	21-28	3.2±0.1	3.0±0.0	3.0±0.1	3.0±0.1	3.0±0.1	3.0±0.2	NS	NS	NS
	28-35	5.3±0.4	4.6±0.3	5.2±0.3	4.8±0.3	4.2±0.3	4.5±0.3	NS	NS	NS
	35-42	7.9±1.4	10.8±2.2	10.8±2.5	6.3±1.0	7.3±2.1	7.7±1.9	NS	NS	NS
	7-42	3.9±0.2	4.1±0.5	4.0±0.5	3.8±0.2	3.8±0.3	3.7±0.3	*	NS	NS
Mortality rate		0.0	3.6	7.1	0.00	3.6	0.0	X^2:0.97 P:0.32	X^2:4.35 P:0.36	-

-NC: Negative control, F: Fasted, TC: Thermal conditioned, PC: Positive control, OEO: Orange peel essential oil

-NS: Non significant, *: p<0.05, **: p<0.01, ***: p<0.001, -: Not statistically evaluated

-The differences between the mean values with different superscripts ($^{A, B}$: p<0.05) in the same row within the no added groups are statistically significant according to the ANOVA and post-hoc Duncan's multiple range tests. -The differences between the mean values with different superscripts ($^{a, b}$: p<0.05) in the same row within the orange peel essential oil groups are statistically significant according to the ANOVA and post-hoc Duncan's multiple range tests.

-Mortality rate was determined for each treatment and evaluated by X^2 test.

Table III: Effects of 300 ppm OEO supplementation on body weight (g), body weight gain (g/d), feed intake (g/d), feed conversion ratio (FCR) and mortality rate (%) of quails exposed to thermal conditioning and fasting early in life, Mean±S.E.M

	NC	F	TC	PC	F+OEO	TC+OEO	P OEO	P Treat.	P OxT
8. day	41.0±0.1	40.8±0.1	41.2±0.2	41.1±0.2	41.0±0.2	41.2±0.1	NS	NS	NS
42. day	41.2±0.1	41.2±0.1	41.3±0.1	41.1±0.1	41.2±0.1	41.3±0.1	NS	NS	NS

-NC: Negative control, F: Fasted, TC: Thermal conditioned, PC: Positive control, OEO: Orange peel essential oil, NS: Non significant

Table IV: Effects of 300 ppm OEO supplementation on rectal temperature measurements after heat challenge when birds were 8 and 42 days old that exposed to thermal conditioning and fasting early in life, Mean±S.E.M

In this study, both 8 and 42 days rectal temperatures of birds were similar among groups (Table IV).

Breast and thigh percentage changed significantly as a result of OEO supplementation (Table V). Thigh percentage was decreased and breast percentage was increased with essential oil supplementation. Early age fasting or thermal conditioning affected significantly the percentages of breast, waist and neck. Neither essential oil supplementation nor early age treatments had a significant effect on the other carcass characteristics such as carcass yield, wings, liver, heart, spleen and abdominal fat percentages (Table V).

Heat challenge as 33±1 ˚C for 6 hours at 42 d of age markedly reduced plasma T3 level only in thermal conditioning groups (Table VI). Plasma glucose levels were influenced by early age treatments. Either essential oil supplementation or early age treatments had a significant effect on plasma IGF-I. Plasma Cholesterol, T4, Insulin, ALP, Calcium, Sodium, Potassium and Magnesium levels were similar in all groups (Table VI).

Whole blood analysis results were presented in Table VII. Essential oil supplementation significantly increased plasma hemoglobin and erythrocyte hemoglobin concentration

							P		
Percentage, %	**NC**	**F**	**TC**	**PC**	**F+OEO**	**TC+OEO**	**OEO**	**Treat.**	**OxT**
Carcass yield[#]	66.2±2.6	66.8±1.0	67.5±1.5	69.2±1.8	66.9±1.1	66.0±1.3	NS	NS	NS
Breast[##]	33.2±0.8	33.3±0.8	34.2±0.6	35.0±0.3^{ab}	33.9±1.0^{b}	36.5±0.5^{a}	**	*	NS
Thigh[##]	36.7±0.4	37.5±0.6	36.8±0.6	35.3±0.3	36.3±0.7	36.0±0.4	**	NS	NS
Waist and neck[##]	21.7±0.8^{A}	19.9±0.7^{B}	19.5±0.4^{B}	21.2±0.4^{a}	21.0±0.7^{a}	19.1±0.8^{b}	NS	**	NS
Wings[##]	8.4±0.4	9.4±0.3	9.5±0.3	8.5±0.4	8.8±0.3	8.5±0.4	NS	NS	NS
Liver[#]	2.6±0.2	2.4±0.2	2.4±0.1	2.5±0.1	2.3±0.1	2.4±0.2	NS	NS	NS
Heart[#]	1.0±0.1	1.0±0.1	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.1	NS	NS	NS
Spleen[#]	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.2±0.1	0.1±0.0	NS	NS	NS
Abdominal fat[##]	1.1±0.2	0.9±0.2	1.0±0.2	1.2±0.2	0.9±0.1	1.2±02	NS	NS	NS

[#]: Percentage of slaughter weight, %; [##]: percentage of carcass weight, %

-NC: Negative control, F: Fasted, TC: Thermal conditioned, PC: Positive control, OEO: Orange peel essential oil

-NS: Non significant, *: p<0.05, **: p<0.01

-The differences between the mean values with different superscripts ($^{A, B}$: p<0.05) in the same row within the no added groups are statistically significant according to the ANOVA and post-hoc Duncan's multiple range tests.

-The differences between the mean values with different superscripts ($^{a, b}$: p<0.05) in the same row within the orange peel essential oil groups are statistically significant according to the ANOVA and post-hoc Duncan's multiple range tests.

Table V: Effects of 300 ppm OEO supplementation on carcass characteristics of quails exposed to thermal conditioning (TC) and fasting (F) early in life, Mean+S.E.M.

Parameters							P		
	NC	**F**	**TC**	**PC**	**F+OEO**	**TC+OEO**	**OEO**	**Treat.**	**OxT**
IGF-I	37.2±3.7^{A}	25.0±0.0^{B}	25.9±0.9^{B}	25.0±0.0	25.0±0.0	26.8±1.7	**	**	***
Cholesterol	175.2±18.8	172.2±17.5	167.2±13.3	163.2±19.2	165.4±17.1	159.3±19.3	NS	NS	NS
T4	1.3±0.2	1.3±0.3	1.7±0.3	1.8±0.5	1.2±0.3	1.2±0.0	NS	NS	NS
T3	3.0±0.5^{A}	2.1±0.3^{AB}	1.6±0.2^{B}	2.1±0.4	2.8±0.4	1.6±0.2	NS	**	NS
Insulin	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	NS	NS	NS
Glucose	242.8±13.3	219.1±14.2	239.2±9.9	234.7±10.9^{b}	229.9±12.0^{b}	293.2±12.9^{a}	NS	**	*
ALP	902.8±103.7	660.2±51.5	725.2±45.1	756.1±53.8	762.3±67.8	748.7±65.0	NS	NS	NS
Na (Sodium)	139.2±2.1	141.0±0.9	140.3±0.9	141.8±2.6	141.9±1.4	141.0±1.0	NS	NS	NS
K (Potassium)	8.3±0.5	7.9±0.5	8.2±0.6	8.3±0.6	8.2±0.4	8.1±0.8	NS	NS	NS
Ca (Calcium)	17.8±3.2	14.2±2.4	13.4±2.0	13.0±1.7	12.2±1.7	11.9±1.8	NS	NS	NS
Mg (Magnesium)	6.9±0.4	6.8±0.5	6.7±0.3	6.8±0.4	6.7±0.3	6.8±0.4	NS	NS	NS

-NC: Negative control, F: Fasted, TC: Thermal conditioned, PC: Positive control, OEO: Orange peel essential oil

-NS: Non significant, *: p<0.05, **: p<0.01, ***: p<0.001

-The differences between the mean values with different superscripts ($^{A, B}$: p<0.05) in the same row within the no added groups are statistically significant according to the ANOVA and post-hoc Duncan's multiple range tests. -The differences between the mean values with different superscripts ($^{a, b}$: p<0.05) in the same row within the orange peel essential oil groups are statistically significant according to the ANOVA and post-hoc Duncan's multiple range tests.

Table VI: Effects of 300 ppm OEO supplementation on serum biochemical parameters of quails taken when birds were 42 d old that exposed to thermal conditioning and fasting early in life, Mean±S.E.M

							P		
	NC	F	TC	PC	F+OEO	TC+OEO	OEO	Treat.	OxT
BA%	2.0±0.9	2.2±0.8	1.6±0.8	1.6±0.4	1.9±0.3	1.2±0.7	NS	NS	NS
EO%	2.1±0.5	3.1±1.1	1.7±0.5	1.4±0.5	2.7±0.7	1.3±0.6	NS	NS	NS
HCT	42.9±1.7	41.7±1.7	41.5±1.6	44.0±1.5	43.4±1.3	41.3±1.6	NS	NS	NS
HGB	12.6±0.8	11.9±0.5	13.2±0.6	13.4±0.5	13.8±0.7	14.2±0.6	*	NS	NS
MCH	41.2±2.0	40.4±1.2	42.1±1.3	43.7±0.9	43.7±1.7	47.1±1.1	**	NS	NS
MCHC	29.3±1.2	28.4±0.5	31.8±0.9	$30.6±0.6^{b}$	$31.8±1.2^{ab}$	$34.6±0.8^{a}$	**	**	NS
MCV	$140.4±2.6^{A}$	$141.4±2.4^{A}$	$132.7±1.7^{B}$	$143.0±1.2^{a}$	$137.6±1.6^{b}$	$136.3±1.6^{b}$	NS	**	NS
MO%	2.2±0.5	2.2±0.8	2.5±0.6	2.3±0.8	2.1±0.4	2.4±0.5	NS	NS	NS
MPV	25.4±0.9	24.4±0.3	27.3±1.5	26.1±1.2	25.0±0.5	26.9±1.9	NS	NS	NS
PCT	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	*	NS	NS
PDW	38.4±0.7	38.4±0.6	39.3±1.8	40.1±2.0	38.8±0.7	36.4±1.3	NS	NS	NS
PLT	100.2±19.0	98.7±11.3	70.0±10.1	76.3±12.7	81.8±11.2	62.1±11.8	NS	NS	NS
RBC	3.1±0.1	3.0±0.1	3.1±0.1	3.1±0.1	3.2±0.1	3.0±0.1	NS	NS	NS
RDW	$16.9±0.4^{B}$	$17.5±0.5^{A}$	$17.8±0.2^{A}$	$16.8±0.3^{b}$	$17.6±0.2^{ab}$	$18.1±0.3^{a}$	NS	**	NS
WBC	0.6±0.1	0.6±0.1	0.4±0.1	0.5±0.1	0.6±0.1	0.5±0.1	NS	NS	NS

-NC: Negative control, F: Fasted, TC: Thermal conditioned, PC: Positive control, OEO: Orange peel essential oil; NS: Non significant, *: p<0.05, **: p<0.01

-The differences between the mean values with different superscripts ($^{A, B}$: p<0.05) in the same row within the no added groups are statistically significant according to the ANOVA and post-hoc Duncan's multiple range tests. -The differences between the mean values with different superscripts ($^{a, b}$: p<0.05) in the same row within the orange peel essential oil groups are statistically significant according to the ANOVA and post-hoc Duncan's multiple range tests.

BA%: Basophile, %; EO%: Eosinophil, %; HCT: Hematocrit; HGB: Hemoglobin, g/dl; MCH: Mean Corpuscular Hemoglobin, pg; MCHC: Mean Corpuscular Hemoglobin Concentration, g/dl; MCV: Mean Corpuscular Volume, μm^3; MO%: Monocyte; MPV: Mean Platelet Volume, μm^3; PCT: Platelet crit; PDW: Platelet Distribution Width, %; PLT: Platelet, $10^3/mm^3$; RBC: Red Blood Cell, $10^6/mm^3$; RDW: Red Blood Distribution Width, %; WBC: White Blood Cell $10^3/mm^3$.

Table VII: Effects of 300 ppm OEO supplementation on whole blood analysis of quails taken when birds were 42 d old that exposed to thermal conditioning and fasting early in life, Mean±S.E.M

presented as HGB, MCH, and MCHC but decreased plasma thrombocyte concentration presented as PCT in Table VII. Hemoglobin concentration of erythrocyte and distribution width of erythrocyte was markedly increased whereas plasma erythrocyte concentration was decreased by early age treatments. These values are presented as MCHC, RDW and MCV, respectively (Table VII). The other values of whole blood analysis were not affected from any supplementation or treatment.

Discussion

This study demonstrates that early age treatment of short-term thermal conditioning can induce much better improvement either in thermotolerance or in compensatory growth of quails than short-term fasting. However, it is proved for the first time that 300 ppm orange peel essential oil supplementation to the feeds of early age treated quails can indicate the best improvements against short-term thermal conditioning or short-term fasting.

Continuous exposure of chicks to over or below the recommended temperature or any stresses from day 1 has been found to result in growth retardation, impaired feed efficiency and increased mortality [35]. However, short-term thermal conditioning at an early age of pre-post hatch period has been found to induce thermoregulatory adjustments which altered the response to thermal or other kind of stresses later in life, without impairing performance, either in chickens [8, 19, 24, 37, 39] or in quails [1, 23]. It is also well documented that during the perinatal period, thermal stress can induce epigenetic adaptation—expressed by alteration in the thermoregulatory threshold response in the pre-optic anterior hypothalamus (PO/AH) center [7].

In the present study, short-term fasting and exposure to heat at seven days-old for 24 h, resulted in growth retardation during the 2nd week of life (Table III). However, accelerated growth was gradually observed in these birds following termination of the treatments except only fasting group. Similarly to our results, growth retardation during first week and then compensatory growth have been proved formerly after early age thermal challenge or feed restriction treatments in many poultry researches [8, 27-29, 37]. The best body weights and weight gains were seen in thermal conditioned with feeding essential oil supplementation group. The differences in ability to compensate for growth retardation induced by the respective methods might be related to the duration and severity of the treatments or any supplementation [11, 38, 39].

The lowest body weights and weight gains were seen in the fasted group throughout the experiment, but it is striking that essential oil supplementation has significantly affected compensatory growth of birds fasted as well as thermal conditioned at the early age or unstressed birds. Feed intake was statistically affected only 2nd week of the experiment by essential oil supplementation but the best feed conversion ratio means were observed in essential oil groups at 7-42 days of the experiment. However, essential oil supplementation was significantly improved 42. d body weights as 3.4%, 6.7%, 8.7% and 7-42 days feed conversion ratio as 4.8%, 7. 6%, 5.8% much better when compared parallelly with no added control, fasting and thermal conditioning groups respectively. Such improvements in body weight, body weight gain, feed intake and feed conversion ratio means of essential oil groups may be related to the palatability and digestion stimulating effects of essential oils. Our results are in agreement with JAMROZ and KAMEL [20] who observed improvements of 8.1% in daily gain and 7.7% in feed conversion ratios in 17-d-old poults fed a diet supplemented with a plant extract containing capsaicin, cinnamaldehyde, and carvacrol at 300 ppm. In contrast, BOTSOGLOU et al. [10] showed that oregano oil exerted no growth-promoting effect when administered at 50 or 100 mg/kg of feed. It is well documented that essential oils have been used safely in feeds and revealed positive improvements on performance of animals [11, 12, 16, 18, 25]. It is reported that herbs, spices, and various plant extracts have appetite- and digestion-stimulating properties and antimicrobial effects [21].

ARAD [7] has been reported that the ability of chicks to regulate body temperature during the post-natal period to increase in an age-dependent manner, and to reach a completely homoeothermic level by the age of 10 days. This may describe that 8 and 42 days rectal temperatures were similar in all groups after thermal challenge (Table IV). In contrast to our findings, rectal temperatures were found higher in control groups than thermal conditioned groups after thermal challenge [37-39]. However, ALTAN et al. [3] reported that rectal temperatures were high in both control and thermal conditioned groups and no statistical difference was appeared by early age thermal challenge.

Early age thermal challenge with essential oil supplementation increased the breast percentage which is the valuable part of poultry carcass, while thigh and waist-neck percentage was decreased (Table V). In this study, carcass yield was not influenced by treatments. Similarly, some researchers have found no statistical difference in carcass characteristics of broilers by essential supplementation [10, 12] or early age treatments [8]. However, URDANETA-RINCON and LEESON [34] reported broilers restricted to 95, 90 or 85 percent of *ad libitum* intake of control group during different intervals showed *ad libitum* fed birds had the heaviest carcass weight and breast meat yields and a progressive reduction in both parameters was noted with increasing feed restriction. Similarly, SIMSEK et al., [31] observed that hot and cold carcass percentage increased by the dietary essential oils.

Under heat stress challenge of 33˚C at the end of the experiment; quails that received thermal conditioning at early age demonstrated better capacity to cope with heat stress through greater reduction in serum T3 (triiodothyronine) and Insulin-like growth hormone (IGF-I or called as somatomedin-C) levels compared to quails that were not thermal conditioned (Table VI). As previously demonstrated, T3 plays a major role in thermoregulation. The advantage of this mechanism may involve manipulating the system at an early stage of development or immaturity. Previous studies [8, 37-40], have applied short-term thermal conditioning during early post-hatch age to induce thermal tolerance at older age in chickens. Results from these studies showed that the mechanism also involved an improved capability to reduce T3 levels. The current data suggests that thermal conditioning involving the manipulation of the thyroid function can be achieved at an earlier stage of development. However, IQBAL et al. [19] and YAHAV [36] illustrated that the ability to reduce plasma T3 concentration, especially during a thermal challenge, suggests an improvement in thermotolerance.

Heat stress affects mineral balance and plasma concentrations in birds. It has been reported that high temperatures can cause a decline in blood hematocrit, K, Na and monocyte levels [9, 15, 39]. Results for plasma Na and K concentrations in the current study are inconsistent with these reports. Plasma Na, K, Ca and Mg did not significantly differ among groups in either the unexposed or the previously exposed groups. Similarly, ALTAN et al. [3] reported that exposing of broiler chickens to 38±1 °C for 2 h at 35 d of age did not affect acid-base balance and hematocrit values of blood between strains in either the unexposed control or the previously exposed group.

It can be concluded that early-age thermal conditioning improves the performance and thermotolerance of the quails. Fasting as an additional treatment did not further improve thermotolerance. It is essential that the best improvements are obtained by orange peel essential oil supplementation either in thermotolerance acquisition or compensatory growth. Therefore, supplementation of 300 ppm orange peel essential oil to the poultry feeds may be of practical value in any stress.

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