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Short Communication

Effect of Orange Peel Essential Oil and Thermotolerance Acquisition on Oxidative Stress Parameters of Liver, Heart and Spleen in Heat Stressed Japanese Quails [1]

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Abstract

The aim of this study was to determine the effects of orange peel essential oil intake by feed and thermotolerance acquisition by early age treatments on antioxidant status of liver, heart and spleen in Japanese quails which subjected to acute heat stress. 168 seven-day-old quail chicks were randomly assigned to six groups as the control; thermal conditioning and fasting for 24 h fed with basal feed or 300 ppm orange peel essential oil added feed. Acute heat stress at marketing age was affected antioxidant systems of selected organs. Orange peel essential oil supplementation decreased Malondialdehyde (MDA) levels while no effect was observed on enzyme activities. Fasting and thermal conditioning at early age negatively affected the antioxidant status of selected organs. Orange peel essential oil may prevent tissues from lipid peroxidation and can be used as a natural antioxidant supplement in heat stress.

Keywords: *Antioxidant, Heat stress, Orange peel essential oil, Thermotolerance*

Portakal Kabuğu Esansiyel Yağı ve Termotolerans Kazandırmanın Sıcaklık Stresine Tabi Tutulan Japon Bıldırcınlarında Karaciğer, Kalp ve Dalakta Oksidatif Stres Parametrelerine Etkisi

Özet

Bu çalışmada, Japon bıldırcınlarında erken yaşta stres uygulamaları sonucu termotolerans kazandırılması ve portakal kabuğu esansiyel yağının yeme ilavesinin akut sıcaklık stresinde karaciğer, kalp ve dalak dokularının antioksidan durumlarına etkileri incelenmiştir. 168 adet 7 günlük yaşta Japon bıldırcınları, 24 saatlik açlık veya sıcaklık stresi uygulaması ve bunların yeme portakal kabuğu esansiyel yağı (300 ppm) katılan alt grupları olacak şekilde altı gruba ayrılmıştır. Kesim yaşına gelen bıldırcınlara uygulanan akut sıcaklık stresi organlarda antioksidan sistemleri etkilemiştir. Portakal kabuğu esansiyel yağı ilavesi Malondialdehit (MDA) düzeylerinde düşmeye neden olurken enzim aktiviteleri üzerine etki etmemiştir. Erken yaşta sıcaklık ve/veya açlık uygulamaları ile seçilen organlarda antioksidan sistemler olumsuz yönde etkilenmiştir. Portakal kabuğu esansiyel yağı dokuları lipid peroksidasyonundan koruyabilir ve sıcaklık stresinde doğal antioksidan yem katkısı olarak kullanılabileceği kanısına varılmıştır.

Anahtar sözcükler: *Antioksidan, Portakal kabuğu esansiyel yağı, Sıcak stresi, Termotolerans*

INTRODUCTION

Animals under heat stress simultaneously exhibit oxidative stress, which causes further cellular damage. Dietary modifications are among the most preferred and practical ways to alleviate the effect of high environmental temperature on poultry performance. Studies have shown that under stressful conditions, the requirements for antioxidants are thought to be increased to protect tissues from lipid peroxidation and antioxidant nutrient supplementation could be used to attenuate the negative effects of environmental stress [1]. Orange oil is an essential oil produced by cells within the rind of an orange fruit. Recently, the orange peel essential oil has been used as

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antioxidant, antimicrobial and growth promoter agent [2].

Thermal conditioning is one of the management tools that partially enable organisms to cope with extreme environmental conditions and 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]. The objective of the present study was to test the effects of orange peel essential oil and acquisition of thermotolerance by early age thermal conditioning or fasting on the antioxidant function of liver, heart and spleen in quail chicks when they are subjected to a high ambient temperature in the finishing phase.

MATERIAL and METHODS

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 7-d-old Japanese quail chicks 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 stress factors of fasting and thermal conditioning were applied to four of six groups for 24 h. 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 for 24 h. Thermal conditioning was applied by electric heater with water vaporization to obtain heat stress. Then the experiment was continued as: 1) Unstressed and no added (Negative Control) Group (NC), 2) Fasted Group (F), 3) Thermal Conditioned Group (TC), 4) Unstressed but Orange Essential Oil added (Positive Control) Group (PC + OEO), 5) Fasted + Orange Essential Oil Group (F + OEO) and 6) Thermal Conditioned + Orange Essential Oil Group (TC + OEO).

The diet was formulated to meet the nutrient requirements of the Japanese quails according to the NRC [4] recommendations. The ingredients and compositions of the basal diet are presented in *Table 1*. Orange peel essential oil was mixed in a carrier (Zeolite), and added to the basal diet at a level of 10 kg/ton to obtain 300 ppm of essential oil concentration. The concentration of the volatile component of orange peel essential oil was shown at *Table 2*.

Chemical composition of feed ingredients (dry matter, crude protein, ash and ether extract) were analyzed according to the AOAC [5] procedures and crude fiber was determined by the methods of Crampton and Maynard [6].

At the end of the study, all groups were subjected to heat stress at 33±1°C for 6 h. Then 10 quails from each experimental group whose body weight near the group average were slaughtered by cutting the jugular vein. Then liver, heart and spleen weights were measured and kept at -20°C until laboratory analyses.

Table 1. *Ingredients and chemical composition of standard diet (g/kg)*
Tablo 1. *Bazal diyetin kompozisyonu ve bileşimi (g/kg)*

Feed Ingredients	Starting and Growing 0-42 Days	Calculated Analysis	
Maize	564.3	Crude protein	236.0
Soybean meal	315.0	ME, MJ/kg	12.74
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*	3.5		
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 B_{12}, 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 groups (10 g zeolite/kg feed); 300 ppm orange peel essential oil added groups (3 g orange oil + 7 g zeolite/kg feed)*

Table 2. *The concentration of the volatile components in orange peel essential oil (%)*
Tablo 2. *Portakal kabuğu esansiyel yağındaki uçucu bileşenlerin konsantrasyonu (%)*

Analysis	Result*
Limonene	92.31
Beta Myrcene	3.25
Alpha Pinen	1.41
Linalool	0.89
Sabinen	0.61
Delta 3 Caren	0.22
Octanal	0.21
Undefined	1.10

** Obtained by GC-MS analysis*

Malondialdehyde (MDA) concentration of the tissue homogenates expressed as the thiobarbituric acid reactive substances (TBARS), were assayed spectrophotometrically according to the method of Placer *et al*.[7]. Catalase (CAT) activity was estimated by measuring the breakdown of H_2O_2 at 240 nm according to the method of Aebi [8]. Glutathione Peroxidase (GSH-Px) activity was determined using the method of Lawrance and Burk [9], which records at 340 nm, the disappearance of NADPH. Glutathione (GSH) concentrations of the tissue homogenates were measured by an assay using the dithionitrobenzoic acid recycling method described by Sedlak and Lindsay [10]. Superoxide

Dismutase (SOD) activity were evaluated in accordance with the method described by Sun *et al*.[11]. Tissue protein contents were determined by the method of Lowry *et al*.[12].

Data were subjected to two-way Anova by using GLM (General Linear Model) procedure. Significant differences were further subjected to Duncan's multiple range test [13].

RESULTS

Either early age treatments or essential oil supplementation were not influenced the liver, heart and spleen weights of quails *(Table 3)*.

The effect of orange peel essential oil supplementation and thermotolerance acquisition on antioxidant status of liver, heart and spleen are presented in *Table 4*. Essential oil supplementation was significantly decreased Malondialdehyde (MDA) levels of liver (P<0.001), heart (P<0.05) and spleen (P<0.05). It is strange that the unexpected values were occurred by thermotolerance acquisition. MDA (P<0.001), CAT (P<0.01), GSH (P<0.01) levels of liver and MDA (P<0.05), CAT (P<0.001), GSH-Px (P<0.05), GSH (P<0.01) levels of heart and MDA (P<0.001), CAT (P<0.05) levels of spleen were negatively affected by fasting and thermal conditioning at early age.

DISCUSSION

Organ weights were similar among groups in the current study. Similarly, Simsek *et al*.[14] and Ciftci *et al*.[15] have found no effect on relative weights of liver, heart

Table 3. *Effect of orange peel essential oil supplementation and thermotolerance acquisition on liver, heart and spleen weights,(g)*
Tablo 3. *Termotolerans kazandırma ve portakal kabuğu esansiyel yağı ilavesinin karaciğer, kalp ve dalak ağırlıklarına etkisi, (g)*

Organ	Basal Diet, No Added			Orange Peel Essential Oil, 300 ppm			P	
	NC	F	TC	PC	F	TC	O	T
Liver	4.88±0.68	4.01±0.44	3.99±0.35	4.34±0.33	4.05±0.28	4.32±0.51	NS	NS
Heart	1.72±0.12	1.62±0.08	1.67±0.06	1.76±0.08	1.63±0.06	1.79±0.07	NS	NS
Spleen	0.11±0.01	0.20±0.03	0.14±0.01	0.21±0.03	0.27±0.00	0.17±0.02	NS	NS

NS: *Not significant,* ***NC:*** *Negative control,* ***PC:*** *Positive Control,* ***F:*** *Fasted,* ***TC:*** *Thermal Conditioned,* ***O:*** *Oil,* ***T:*** *Treatment*

Table 4. *Effect of orange peel essential oil supplementation and thermotolerance acquisition on oxidative stress parameters of liver, heart and spleen*
Tablo 4. *Termotolerans kazandırma ve portakal kabuğu esansiyel yağı ilavesinin karaciğer, kalp ve dalak oksidatif stres parametrelerine etkisi*

Parameter	Basal Diet, No Added			Orange Peel Essential Oil, 300 ppm			P	
	NC	F	TC	PC	F	TC	O	T
Liver								
MDA	2.48 ± 0.12^{c}	3.56 ± 0.39^{b}	5.37 ± 0.41^{a}	1.98 ± 0.25^{B}	2.60 ± 0.18^{AB}	3.14 ± 0.20^{A}	***	***
CAT	1.52 ± 0.16^{a}	0.75 ± 0.15^{b}	0.71 ± 0.12^{b}	1.51±0.09	1.15±0.06	0.98±0.21	NS	**
GSH-Px	0.05±0.00	0.08±0.04	0.03±0.00	0.05±0.00	0.04±0.00	0.03±0.00	NS	NS
GSH	0.07±0.01	0.06±0.00	0.04±0.00	0.10 ± 0.01^{A}	0.07 ± 0.00^{AB}	0.05 ± 0.00^{B}	NS	**
SOD	33.30±3.63	30.03±2.05	30.85±5.65	28.70±1.72	29.87±0.83	29.52±2.29	NS	NS
Heart								
MDA	4.01 ± 0.25^{b}	7.02 ± 2.06^{a}	6.12 ± 1.54^{ab}	2.26 ± 0.85^{B}	6.67 ± 0.86^{A}	5.09 ± 0.82^{AB}	*	*
CAT	0.12 ± 0.01^{a}	0.03 ± 0.00^{b}	0.04 ± 0.01^{b}	0.07 ± 0.00^{A}	0.04 ± 0.00^{B}	0.04 ± 0.00^{B}	NS	***
GSH-Px	0.23 ± 0.01^{a}	0.19 ± 0.00^{b}	0.12 ± 0.02^{b}	0.20±0.01	0.22±0.01	0.20±0.01	NS	*
GSH	0.20 ± 0.01^{a}	0.19 ± 0.01^{ab}	0.12 ± 0.01^{b}	0.15 ± 0.01^{B}	0.23 ± 0.01^{A}	0.15 ± 0.01^{B}	NS	**
SOD	111.39±9.22	95.54±4.14	73.70±22.21	104.56±6.73	100.03±10.31	102.60±8.06	NS	NS
Spleen								
MDA	14.33 ± 1.00^{b}	18.10 ± 1.02^{ab}	28.85 ± 4.30^{a}	7.30 ± 1.16^{B}	16.50 ± 2.46^{A}	21.30 ± 1.78^{A}	*	***
CAT	0.07 ± 0.00^{a}	0.05 ± 0.00^{ab}	0.03 ± 0.00^{b}	0.05±0.00	0.04±0.00	0.04±0.00	NS	*
GSH-Px	0.05±0.00	0.05±0.00	0.04±0.00	0.04±0.00	0.04±0.00	0.06±0.00	NS	NS
GSH	0.05±0.00	0.04±0.01	0.04±0.00	0.06±0.00	0.04±0.00	0.06±0.01	NS	NS
SOD	32.42±8.04	33.15±2.06	21.54±5.38	21.96±3.96	20.51±2.59	33.88±2.51	NS	NS

NS: *Not significant, * P<0.05, ** P<0.01, *** P<0.001;* ***NC:*** *Negative control,* ***PC:*** *Positive Control,* ***F:*** *Fasted,* ***TC:*** *Thermal Conditioned,* ***O:*** *Oil,* ***T:*** *Treatment;* ***MDA:*** *Malondialdehyde, nmol/mg protein;* ***CAT:*** *Catalase, k/g protein;* ***GSH-Px:*** *Glutathione Peroxidase, U/g protein;* ***GSH:*** *Glutathione, nmol/mg protein;* ***SOD:*** *Superoxide Dismutase, U/g protein*

and spleen of broilers with dietary thyme and cinnamon essential oil supplementation, but it is reported that liver weights were increased by anise essential oil supplementation [16].

Increasing MDA levels of liver, heart and spleen in the current study might be due to the heat stress condition. Similarly, Yang *et al*.[17] mentioned about heat stress induced a significant production of reactive oxygen species (ROS), function of the mitochondrial respiratory chain, antioxidative enzymes such as SOD, CAT, GSH-Px activity and formation of MDA. Supplementation of orange peel essential oil to diet reduced MDA production in liver, heart and kidney, under heat stress condition. Likewise, Simsek *et al*.[18] reported that cinnamon oil supplementation decreased the MDA levels in liver, kidney and heart tissues of Japanese quails under heat stress condition. Faix *et al*.[19] observed that 0.1% level *C. zeylanicum* essential oil supplementation has significantly decreased the concentration of MDA in plasma and duodenal mucosa; however it had no significant effect on the concentration of MDA in the liver and kidney tissues.

In this study orange peel essential oil supplementation had no effect on antioxidant enzyme activities of liver, heart and spleen. But, these results are in contrast with the findings of Akbarian *et al*.[20] whom recorded that the dietary supplementation of the bird's diet with orange peel essential oil had significant differences in glutathione peroxidase and superoxide dismutase activity, when compared with the control group. It is possible that the antioxidant properties of essential oils are being utilized by the cells, thus sparing the intracellular antioxidant system. Barja de Quiroga *et al*.[21] suggested that cellular antioxidants are under homeostatic control and that dietary antioxidant supplementation depresses endogenous antioxidant synthesis so as to nullify the expected beneficial effect of the supplement.

In conclusion, orange peel essential oil supplementation has protective effects to tissues in heat stress, but early age thermal conditioning or fasting had no positive effects. However, future research experiments based on different doses of essential oils or in combination (to explore their possible synergic effects) and thermotolerance acquisition could be designed to elucidate more precisely their effects on the oxidative status of avian species.

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