

Effect of dietary antioxidant supplementations on antioxidant parameters, rectal temperature, digestibility of nutrients, performance and chemical composition of meat in Cherry Valley type ducks under chronic heat stress

Wirkung von antioxidativen Nahrungsergänzungen auf antioxidative Parameter, Rektaltemperatur, Nährstoffverdaulichkeit, Leistung und chemische Fleischbeschaffenheit bei Enten vom Typ Cherry Valley unter chronischem Hitzestress

M. Horvath^{1*}, G. Pesti-Asbóth², J. Galne Remenyik² and L. Babinszky¹

¹ Department of Feed and Food Biotechnology, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen, Debrecen, Hungary

² Institute of Food Technology, Biochemistry Laboratory, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen, Debrecen, Hungary

*Correspondence: mhorvath@agr.unideb.hu; martahorvath87@yahoo.co.uk

Manuscript received 7 November 2019, accepted 3 February 2020

Abstract

The aim of the study was to determine the effect of dietary antioxidant supplementation to ducks kept under constant high environmental temperature ($30 \pm 1^\circ\text{C}$ for 24 h per day during days 14 to 42 d) on their antioxidant status, rectal temperature, production parameters, nutrient digestibility and the chemical composition of their meat. Fourteen days old mixed-sex Cherry Valley type hybrid ducks were randomly placed into three experimental groups, each containing 20 birds in 5 replicates. This was repeated two times and in total 900 ducks was used. Ducks were housed in a high environmental temperature room (HT: constant $30 \pm 1^\circ\text{C}$ for 24 h, relative humidity $62 \pm 5\%$) during 28 days. The three experimental diets (**T1**, **T2**, **T3**) were maize-soybean meal based. **T1** was a control diet only with premix. **T2** and **T3** had differently increased concentration of Vitamin E, C, selenium and zinc (**T2**: 540 mg/kg, 998 mg/kg, 0.60 mg/kg, 97 mg/kg and **T3**: 1540 mg/kg, 1996 mg/kg, 0.90 mg/kg, 148 mg/kg) respectively. It was concluded that the digestibility of crude protein and dry matter, the rectal temperature and the chemical composition of duck breast and leg meat were not affected ($P > 0.05$) by dietary treatments under heat stress. However, several production parameters (feed intake, live weight at 14 d and 42 d, daily weight gain; feed conversion ratio) and serum antioxidant capacity (malondialdehyde, superoxide-dismutase, antioxidant capacity of water- and lipid-soluble compounds, glutathione-peroxidase) of 14–42 day old ducks were significantly improved ($P < 0.05$) with antioxidant supplementations (**T2**, **T3**). The improved antioxidant capacity of the serum in the **T2** and **T3** groups thus led to an increase in duck performance under heat stress. Based on the present results, at high environmental temperature in the summer we recommend using **T2** (Vitamin E: 540 mg/kg diet, Vitamin C: 998 mg/kg diet, Se: 0.60 mg/kg diet and Zn: 97 mg/kg diet) supplementation in practical duck nutrition.

Key words

Heat stress; duck; Vitamin C; Vitamin E; selenium; zinc

Zusammenfassung

Ziel der Studie war es, die Wirkung von antioxidativen Nahrungsergänzungen bei konstant hoher Umgebungstemperatur ($30 \pm 1^\circ\text{C}$ für 24 h über 28 Tage) auf den antioxidativen Status der Enten sowie auf Rektaltemperatur, Produktionsparameter, Nährstoffverdaulichkeit und chemische Zusammensetzung des Entenfleisches während der Wachstumsperiode (Tag 14 – 42) zu bestimmen. Die Untersuchungen wurden an insgesamt 900 Hybridenten vom Typ Cherry Valley im Alter von 14 Tagen und beiderlei Geschlechts durchgeführt. Zunächst wurden die Enten nach dem Zufallsprinzip auf drei Versuchsgruppen mit jeweils fünf Wiederholungen zu je 20 Tieren verteilt. Diese Versuchsanordnung wurde dann noch zweimal wiederholt. Die Enten wurden 28 Tage in einem Raum mit hoher Umgebungstemperatur (HT: konstant $30 \pm 1^\circ\text{C}$ für 24 h, relative Luftfeuchtigkeit $62 \pm 5\%$) gehalten. Die drei Versuchsfuttermittel (**T1**, **T2**, **T3**) basierten auf Mais-Sojabohnenmehl. Das Kontrollfutter (**T1**) entsprach einer üblichen Futtermischung. **T2** und **T3** wiesen eine unterschiedlich erhöhte Konzentration von Vitamin E, C, Selen und Zink auf (**T2**: 540 mg/kg, 998 mg/kg, 0,60 mg/kg, 97 mg/kg und **T3**: 1540 mg/kg, 1996 mg/kg, 0,90 mg/kg, 148 mg/kg). Es wurde festgestellt, dass die Verdaulichkeit von Rohprotein und Trockensubstanz, die Rektaltemperatur und die chemische Zusammensetzung von Entenbrust- und -keulenfleisch durch diätetische Behandlungen unter Hitzestress nicht beeinflusst wurden ($P > 0,05$). Jedoch wurden mehrere Produktionsparameter (Futterraufnahme, Lebendmasse am Tag 14 und 42, tägliche Lebendmassezunahme, Futterverwertung) und die antioxidative Kapazität des Serums (Malondialdehyd, Superoxid-Dismutase, antioxidative Kapazität von wasser- und fettlöslichen Verbindungen, Glutathion-Peroxidase) von 14 – 42 Tage alten Enten durch Supplementierung von Antioxidantien (**T2**, **T3**) signifikant verbessert ($P < 0,05$). Die verbesserte antioxidative Kapazität des Serums in den **T2**- und **T3**-Gruppen führte somit zu einer Leistungssteigerung der Enten unter Hitzestress. Auf der Basis dieser Ergebnisse wird empfohlen, bei hoher Umgebungstemperatur im Sommer eine antioxidative Nahrungsergänzung wie beispielsweise in der **T2**-Gruppe (Vitamin E: 540 mg/kg Futter, Vitamin C: 998 mg/kg Futter, Se: 0,60 mg/kg Futter und Zn: 97 mg/kg Futter) für die Ernährung von Enten in der Praxis einzusetzen.

Stichworte

Hitzestress; Ente; Vitamin C; Vitamin E; Selen; Zink

Introduction

Climate change is a research area of particular importance today, as it has an effect on everyday life, including on agriculture and food production. High environmental temperatures may lead to heat stress, which in turn can decrease antioxidant status, change energy and nutrient metabolism, reduce performance, increase mortality and also affect the product quality of livestock (AKBARIAN et al., 2015; ATTIA et al., 2011; BABINSZKY et al., 2011; HABIBIAN et al., 2014; HARSINI et al., 2012; HORVÁTH and BABINSZKY, 2019; LI, 2011; LIAO et al., 2012; KUMAR et al., 2017; YANG et al., 2010).

Poultry, especially ducks, are more sensitive to heat stress than other livestock, because their metabolism is high, causing high body temperatures, their body is covered with feathers and they do not have sweat glands (AVILÉS-ESQUIVEL et al., 2018; LARA and ROSTAGNO, 2013). The importance of duck meat is increasing worldwide. Global duck meat production was about 4.2 million tonnes in 2011 and increased 3.5% each year according to FAO data. Successful and economic production is possible with intensive nutrition and concentrated nutrient content, under intensive conditions such as those used in the broiler industry. However, there is a lack of information about their nutrition, especially under high environmental conditions. The negative effects of high environmental temperatures can be reduced by biological approaches (e.g. genetics, thermal conditioning, nutrition) (DAGHIR, 2008; LIN et al., 2006) or housing equipment (e.g. air conditioning, intensive ventilation, humidification) (ARMSTRONG, 1994; WOLFENSON et al., 2001). However, in most cases, housing methods are expensive and not adequate. Therefore, intensive research is being conducted on different nutritional tools, especially feed additives. In a situation of heat stress, feed additives with direct and indirect antioxidant effects can be supplemented alone (e.g. Vitamin C, E and A), or together with other micro minerals (e.g. chromium, selenium and zinc).

It is well known that the elimination of the free radicals activates the three level antioxidant system, which helps to protect livestock against heat stress (ARAI et al., 1987; HORVÁTH and BABINSZKY, 2019; IRSHAD and CHAUDHURI, 2002). According to most research, results show that influencing the second level of the antioxidant system (the

small molecule antioxidant group) provides an opportunity to reduce the negative effects of heat stress. These compounds take part in detoxification and recovery reactions. The most important are: Vitamin C, E and glutathione (CHAN and DECKER, 1994; GROB et al., 2013; NIKI et al., 1983; PACKER, 1992).

Very few studies on ducks under long-term heat stress have been published. Therefore, the aim of this study was to determine the effect of dietary supplementation of Vitamin C, E, Se and Zn to ducks kept under constant high environmental temperature ($30 \pm 1^\circ\text{C}$ for 24 h during 28 days) on their antioxidant status, rectal temperature, production parameters, nutrient digestibility and the chemical composition of meat in the growing period from 14 days to 42 days of age.

Materials and Methods

Ethics Statement

The experiment procedures were approved by the University of Debrecen Animal Care Committee (Debrecen, Hungary).

Animals and experimental design

Birds. A total of 900 mixed sex 14 d old Cherry Valley type hybrid ducks were raised under standard management conditions. They were fed by same compound feed (meeting NRC requirements) for 14 days before being randomly placed into three experimental groups each containing 20 birds in five replicates ($n = 100$ birds/treatment group; $n = 300$ birds/experiment) and was repeated twice (3×300 ducks = 900 ducks in total).

Housing. The birds were housed in uniform circumstances from 1 day of age until 14 days of age (as recommended by CHERRY and MORRIS, 2008). At 14 days of age (14 d), the ducks were housed in an environmentally controlled room for 28 days (until 42 days of age- 42 d) to a high environmental temperature of $30 \pm 1^\circ\text{C}$ and RH of $62 \pm 5\%$ for 24 h/day. The temperature was monitored twelve times daily at different locations of the animal house. Fifteen pens were used. Each pen ($3 \times 3 \times 1.5$ m) held 20 birds. A lightning schedule of 23L:1D (2 lux) was provided (CHERRY and MORRIS, 2008). Ducks were kept in floor pens covered with straw. All ducks were provided with free access to feed and water.

Dietary treatments, composition of diets and calculated nutrient content. The three experimental diets were maize-soybean meal based and formulated in the study to meet NRC requirements for ducks (NRC, 1994). The experimental diet (Table 1) was prepared by using a control diet, which can be used in normal environmental temperatures in practice (near to the thermo-neutral zone of ducks). In treatment 1 (T1), we used a control diet only with premix supplementation (Table 2). Treatment 2 (T2) had increased concentration of Vitamin E (540 mg/kg diet), Vitamin C (998 mg/kg diet), Se (0.60 mg/kg diet) and Zn (97 mg/kg diet) respectively (Table 2). Treatment 3 (T3) had even higher concentrations of Vitamin E (1540 mg/kg diet), Vitamin C (1996 mg/kg diet), Se (0.90 mg/kg diet) and Zn (148 mg/kg diet) (Table 2). The composition and calculated nutrient contents of the diet is summarised in Table 1. The supplementations in T2 and T3 were added directly to the feed beyond the premix.

Table 1. Composition and analysed or calculated nutrient content of the experimental diet
Zusammensetzung und analysierter bzw. berechneter Nährstoffgehalt des Futters

Ingredients (%)	Diet
Maize	38.7
Wheat	10.0
Triticale	20.0
Ext. soybean meal (CP: 46%)	14.2
Full fat soy	5.0
Ext. sunflower granulate	6.3
Wheat meal	3.0
Premix ¹	0.5
Others*	2.3*
Analysed and calculated nutrient content (100 g dry matter)	
ME poultry (MJ/kg) ^y	12.11
Crude protein (%) ^x	17.7
Crude fibre (%) ^x	4.5
Crude fat (%) ^x	3.3
Lysine (%) ^x	1.0
Methionine (%) ^x	0.47
Ca (%) ^x	0.58
Si ₂ O (%) ^x	0.5

¹ 1 kg premix contains: Vitamin A (retinyl acetate): 2 000 000 IU; Vitamin D3 (cholecalciferol): 600 000 IU; Vitamin E (all-rac- α -tocopheril acetate): 8 000 mg; Vitamin K3: 600 000 mg; Thiamine: 400 mg; Riboflavin: 1400 mg; Pantothenic acid (calcium D-pantothenate): 2000 mg; Pyridoxine: 1000 mg; Vitamin B12: 4 mg; Niacin: 7 999 mg; Folic acid: 200 mg; Biotin: 20 mg; Iron (Iron-II-sulphate-monohydrate): 10 000 mg; Manganese (Manganese-II-oxide): 20 000 mg; Zinc (Zinc-oxide): 10 000 mg; Iodine (granulated anhydrous calcium-iodate): 400 mg; Selenium: 90 mg; Copper (methionine-hydroxyl-analogue copper-chelate): 4000 mg; Zinc (methionine-hydroxyl-analogue-zinc-chelate): 10 000 mg; Butylated hydroxytoluene (BHT): 1 440 mg; Propyl gallate (E310): 738 mg; Butylated hydroxyanisole (BHA): 1 152 mg.

* Amino acid supplementation: L-lysine HCl, DL- Methionine, L-Threonine; Sunflower oil; Limestone; MCP; Sodium chloride; NaHCO₃

^x Analysed

^y Calculated

Table 2. Dietary treatments
Fütterungsgruppen

	Treatments		
	T1	T2	T3
<i>Supplementation</i>	–	+	++
Vitamin E (mg/kg)*	40	540	1540
Vitamin C (mg/kg)*	–	998	1996
Se (mg/kg)*	0.45	0.60	0.90
Zn (mg/kg)*	50	97	148

* Analysed

Sampling and sample preparation. Three ducks per pen (5 pens/treatment = 15 ducks/treatment) were chosen randomly and killed by cervical dislocation at d 42 for blood, digesta and meat sampling.

Blood: Plasma blood was collected into EDTA-coated vacutainer tubes (BD, Franklin Lakes, NJ, USA). 1 ml of the samples was centrifuged at 1000 g at 4°C for 10 min. The supernatant plasma was further divided into 300 µl aliquots and stored at –20°C until analysis (n = 3 ducks/pen, 15 ducks/treatment).

Digesta: The digestibility of nutrients in the small intestine was determined by *post mortem* digestibility trial using Si₂O as the indicator. Digesta was collected from the distal duodenum to the ileocecal junction (the entire ileum) according to [JIN et al. \(2000\)](#). The samples were placed in plastic holders and stored at -20°C until laboratory analysis (n = 3 ducks/pen, 15 ducks/treatment).

Meat: The skinny (without bones) leg and breast meat of the ducks were collected individually in plastic bags at d 42. The samples were stored at -20°C until analysis (n = 3 ducks/pen, 15 ducks/treatment).

Measurements. Rectal temperature (RT): A thermometer was inserted 3 cm into the rectum for 10–15 s for RT measurement. RT was measured (n = two birds/pen) two times a day (at 9 am and 4 pm).

Performance trial: The body weight (**BW**) of the ducks was measured individually (n = 900) at d 14 and d 42 (g/bird). The daily weight gain (**dWG**) was calculated individually (g/day/bird). The daily feed intake (**FI**) was recorded by each pen (n = 15 pen) and was calculated individually (g/day/bird). Each duck had a wing-mark for identification. The feed conversion ratio (**FCR**) and the energy (**Energy CR**) and protein (**Protein CR**) conversion ratio were calculated for each pen (n = 15 pen). The number of dead birds was recorded.

Antioxidant status: The following antioxidant parameters were measured: Superoxide dismutase (**SOD**), antioxidant capacity of water-soluble (**ACW**) and lipid soluble (**ACL**) compounds, glutathione-reductase (**GR**), glutathione-peroxidase (**GPx**), reduced glutathione (**GSH**), ascorbic acid (**AsA**), **Vitamin E** and malondialdehyde (**MDA**).

Chemical analysis. Diet: The nutrient, vitamin and mineral contents of the diet were determined using standard procedures of AOAC Official Methods ([AOAC, 2012](#)). The following contents were determined: crude protein ([AOAC, 2006e](#)), crude fibre ([AOAC, 2006f](#)) crude fat ([AOAC, 2006g](#)), lysine and methionine ([AOAC, 2006a](#)), Ca ([AOAC, 2006c](#)), Vitamin E ([AOAC, 2013](#)), Vitamin C ([AOAC, 2006h](#)), Se and Zn ([AOAC, 2006b](#)) and Si₂O ([AOAC, 2006d](#)).

Blood: To determine SOD activity, erythrocyte containing pellets were washed four times with 3 moles of 0.9% NaCl solution and centrifuged at 2555 g at 4°C for 10 min. The erythrocytes were re-suspended in 2 ml cold distilled water and incubated at 4°C for 15 min. SOD activity was measured after incubation. SOD, ACW and ACL was determined using commercially available kits (Analytik Jena AG, Jena, Germany) with PhotoChem© ([POPOV and LEWIN, 1999](#)).

The GR, GPx, GSH, AsA, Vitamin E and MDA were measured with SpectroStar^{Nano micro plate} reader (BMG Labtech, Offenburg, Germany). The GR and GPx activity (Abcam, Cambridge, UK) and the concentration of GSH (BioVision, INC. Headquarters Milpitas, California, USA) and MDA (Sigma-Aldrich, St. Louis, Missouri, USA) were determined using commercially available assay kits. The concentration of Vitamin E was determined using ELISA kits (Blue Gene Biotech LTD, Shanghai, China). To determine the plasma AsA content the plasma samples were pre-treated due to the high protein content of the samples. First, chemical destruction was done using 37% HCl acid: 96% ethanol solution (1:7) ([EUROPEAN PHARMACOPEIA SUPPLEMENT, 2001](#)). A sample of 100 µl plasma was added to 500 µl of HCl: ethanol solution. This was incubated for 30 min at 25°C. The samples were centrifuged at 13000 g at 4°C for 5 min. The supernatant was used for determination using a commercially available assay kit (Abcam, Cambridge, UK). The results were multiplied by the molecular weight of ascorbic acid (176.12 g).

Digesta: The digesta samples were analysed using standard procedures of Proximate Analysis ([AOAC, 2012](#)). We used Si₂O sand (Sigma-Aldrich, St. Louis, Missouri, USA) to increase the amount of HCl insoluble ash concentration.

Meat: The meat samples were analysed using standard procedures of Proximate Analysis ([AOAC, 2012](#)). Glycogen content in terms of glucose was measured by the method of [DALRYMPLE and HAMM \(1973\)](#).

Calculations. Production parameters: FCR, Energy CR, Protein CR and Specific feed cost were calculated as follows:

FCR = feed intake (kg)/weight gain (kg)

Energy CR = used energy (MJ AME_n)/weight gain (kg)

Protein CR = used protein (g)/weight gain (kg)

Specific feed cost = price (US) × WG (kg). The specific feed cost is a ratio between weight gain and the price of 1 kg of the diet.

Digestibility: We used a formula to calculate the apparent digestibility of nutrients according to [ELBERS et al. \(1989\)](#).

Statistical analysis

The experimental data was analysed using the PROC GLM (mixed models) procedure of SAS (SAS, 2010, version 9.3; SAS Inst. Inc., Cary, NC, USA). The general model was as follows:

$$Y_{ijk} = \mu + T_i + R_j + (TxR)_{ij} + e_{ijk}$$

Where: Y = dependent variable; μ = overall mean; T = treatment (I = 3); R = repeat (j = 2); TR = interaction between treatment and repeat; e = residual error

Live performance was analysed using the pen as the investigation unit, while each duck was considered as statistical unit for blood, RT, digestibility and carcass parameters. When significant effects were obtained, differences between means were compared by Tukey's multiple range test at a significance level of $P < 0.05$ (SAS, 2010). Repeat and treatment-repeat interaction was omitted because these effects were not significant.

Results and discussion

Rectal temperature of ducks

The results of rectal temperature of ducks in different treatments at 18 d, 28 d and 42 d are presented in Figure 1. Effects of time of the day were not significant; therefore, this variable was omitted. Based on the present results, we suppose that the supplementation affected the rectal temperature of ducks, irrespective of time of day.

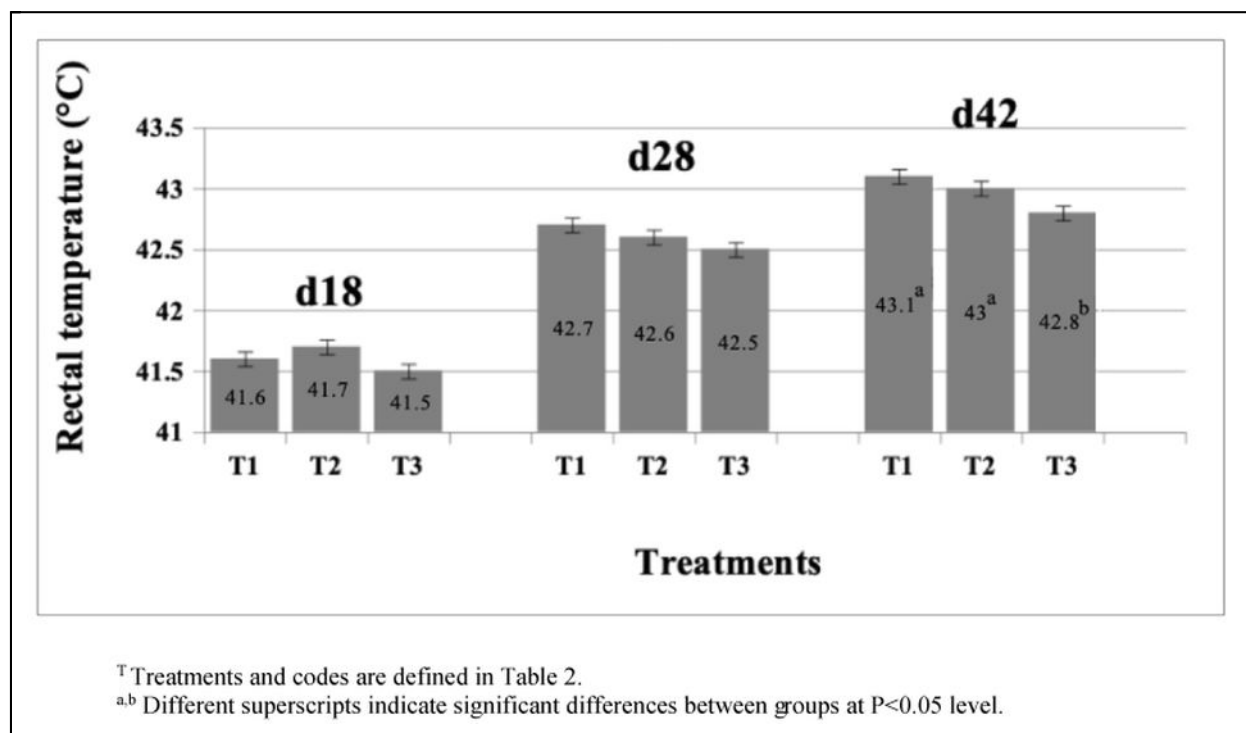


Figure 1. Effect of heat stress on the rectal temperature of ducks in different treatments at d18, d28 and d42 (least square means $\pm$ SEM)

Auswirkung von Hitzestress auf die Rektaltemperatur von Enten in verschiedenen Fütterungsgruppen am Tag 18, 28 und 42 (von links nach rechts; kleinste mittlere Quadrate $\pm$ SEM)

It can be concluded that T3 supplementation decreased the rectal temperature of ducks under heat stress significantly at 42 d; however, only by some degrees ($P < 0.05$). At 18 d and 28 d, there was no significant difference between treatments. We suggest this could be because of the adaptation ability of ducks against high environmental temperature. The rectal temperature decrease in T3 at 42 d shows that during constant high environmental temperature, the adaptation ability of ducks is decreased. Therefore, supplementation is recommended to reduce rectal temperature.

The body temperature of poultry under normal conditions is in the range of 41 to 42°C and increases when environmental temperature increases (COOPER and WASHBURN, 1998) and during short term heat stress (ALTAN et al.,

2003; XIE et al., 2014). However, during long term (constant) heat stress, the rectal temperature is not elevated in poultry (XIE et al., 2014).

Antioxidant parameters

The results of the antioxidant parameters of ducks under heat stress can be seen in Table 3. The results showed that in T3, the amount of SOD decreased due to the supplementation. This could be because the supplemented ascorbic acid reacted with the superoxide anions; therefore there was less requirement for SOD enzyme activity. In other studies, SOD activity also decreased by antioxidant supplementation under heat stress in poultry (LIN et al., 2006; MAINI et al., 2007). The results of ACW and ACL contain all water and lipid soluble antioxidants in the blood. Due to the increased vitamin and micro mineral supplementation in the diets, a significant increase in T2 and T3 is seen ($P < 0.05$). This is in accordance with TOMAŽIN et al. (2013), but LESKOVEC et al. (2018) found that Vitamin E supplementation did not increase the ACL levels in serum. It is suggested that the metabolic role of selenium is to protect cells against oxidation and tissue damage. Elimination of H_2O_2 is done by GP with GSH consumption. In the present study, we found that GPx significantly decreased in T2 and T3 ($P < 0.05$). GPx activity increases in broiler (AKBARIAN et al., 2015) and turkey (MIKULSKI et al., 2009) under heat stress under selenium supplementation. The activity of GPx is continuous and depends on the presence of GSH. GR enzyme regenerates GSH from glutathione disulphide (GSSG) and requires NADPH as source of energy (SISEIN, 2014). Therefore, in the present study the GR activity decreased ($P > 0.05$) in T2 and T3 in parallel with the GPx activity ($P < 0.05$). The increased amount of GSH did not increase the activity of GPx in T2. We suggest that presence of GSH affects another pathway of H_2O_2 dissociation, which is catalysed by ascorbate peroxidase. However, GSH concentration decreased under heat stress in broilers under Vitamin E supplementation (MAINI et al., 2007). Se supplementation can increase production of GSH (SUCHÝ et al., 2014). Zn may play an important role in suppressing free radicals because it works as a cofactor (Cu/Zn-SOD) and inhibits NADPH-dependent lipid peroxidation (PRASAD and KUCUK, 2002, SAHIN et al., 2006).

Table 3. Effects of heat stress on antioxidant parameters of ducks (least square means $\pm$ SEM)

Einfluss von Hitzestress auf antioxidative Parameter von Enten (kleinste mittlere Quadrate $\pm$ SEM)

Antioxidant parameters	Treatments			P-value	Probability Treatment
	T1	T2	T3		
Malondialdehyde (MDA) (nMol/ μ l)	13.2 $\pm$ 1.45 ^a	4.4 $\pm$ 1.87 ^b	4.7 $\pm$ 1.45 ^b	0.001	***
Vitamin E (μ g/ml)	1.15 $\pm$ 0.08 ^a	0.98 $\pm$ 0.08 ^{ab}	0.84 $\pm$ 0.08 ^b	0.02	*
Vitamin C (μ g/ μ l)	2.2 $\pm$ 0.30 ^a	3.3 $\pm$ 0.30 ^b	2.8 $\pm$ 0.30 ^{ab}	0.05	*
Superoxide-dismutase (SOD) (U/ml)	104.2 $\pm$ 8.22 ^a	113.2 $\pm$ 8.22 ^a	70 $\pm$ 9.19 ^b	0.005	**
Antioxidant capacity of water-soluble compounds (ACW) (μ g/ml ascorbic acid)	19.6 $\pm$ 3.95 ^a	31.01 $\pm$ 3.95 ^b	68.2 $\pm$ 4.16 ^c	0.0001	***
Antioxidant capacity of lipid soluble compounds (ACL) (μ g/ml trolox)	21.05 $\pm$ 1.36 ^a	22.1 $\pm$ 1.52 ^a	33.9 $\pm$ 1.36 ^b	0.0001	***
Glutathione-peroxidase (GPx) (mU/ml)	88.95 $\pm$ 2.91 ^a	70.4 $\pm$ 3.06 ^b	79.3 $\pm$ 3.06 ^c	0.001	***
Glutathione-reductase (GR) (mU/ml)	46.01 $\pm$ 4.26 ^a	40.6 $\pm$ 4.26 ^a	36.3 $\pm$ 4.49 ^a	0.3	NS
Reduced glutathione (GSH) (μ M/ml)	14.4 $\pm$ 2.28 ^a	22.1 $\pm$ 2.28 ^b	13.2 $\pm$ 2.28 ^a	0.02	*

^T Treatments and codes are defined in Table 2.

^{a, b} Different superscripts in the same row indicate significant differences between groups at $P < 0.05$ level.

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; NS = non-significant ($P > 0.05$).

According to other studies with Vitamin C, E, Se and chromium (Cr) supplementation (LESKOVEC et al., 2018; MAINI et al., 2007; SAHIN and KUCUK, 2001; SAHIN et al., 2002a, b) decreased MDA concentration. As expected, the present results show that MDA level significantly decreased in T2 and T3 ($P < 0.05$) (Table 3) due to the supplementation. Increased environmental temperature will cause increased lipid peroxidation and induced formation of MDA; therefore, the antioxidant defence system is altered (ALTAN et al., 2003; COSTANTINI and VERHULST, 2009; LARA and ROSTAGNO, 2013). Lipid peroxidation decreased and enzymatic and non-enzymatic antioxidant systems activity increased with supplementation under heat stress (HARSINI et al., 2012; MAINI et al., 2007; SAHIN et al., 2002b). The concentration of Vitamin E decreased in line with MDA formation; however, the supplemented Vitamin E in T2 and T3 did not affect ($P > 0.05$) the concentration of Vitamin E in the blood. This could be because the ascorbic acid, which is necessary for the Vitamin E regeneration, was used for dissociation of superoxide anions and H_2O_2 and was used by GR for maintaining the glutathione level. In the present study, we found that concentration of Vitamin C increased significantly in T2 but not in T3 ($P < 0.05$) (Table 3). Vitamin C supplementation in the diet increased Vitamin C concentration in serum of broilers, similar results were found in other studies (KUMAR et al., 2017; MAHMOUD et al., 2004; SAHIN et al., 2002a). High environmental temperature may decrease the concentrations of vitamins and micro minerals in serum and increase their excretion (KHAN et al., 2012; KUTLU and FORBES, 1993). Therefore, supplementation of direct or indirect antioxidant compounds (e.g. vitamins and micronutrients) at higher levels is recommended (YUN et al., 2012). The increased amount of pro-oxidants, which cannot be eliminated by the small molecule antioxidants (Vitamin C and E), activates at the same time the direct enzymatic pathway of the three level antioxidant system.

As mentioned before, ducks are more sensitive to heat stress than other livestock. Therefore, we suggest that the supplementation used in the present study improved the antioxidant status of ducks under heat stress. These additives support mechanisms against lipid peroxidation, improve antioxidant and immune status and performance in other studies, too (HARSINI et al., 2012; MAHMOUD and EDENS, 2003; SAHIN and KUCUK, 2001). Lipid peroxidation decreased and the enzymatic and non-enzymatic antioxidant systems improved with supplementation under heat stress (HARSINI et al., 2012; MAINI et al., 2007; SAHIN et al., 2002a, b).

Digestibility of nutrients

The digestibility coefficients of dry matter and crude protein are summarised in Table 4. Digestibility of DM in T1, T2 and T3 were all higher than otherwise seen in the literature. Digestibility of crude protein (CP) in the present experiment show the same level as in other studies, in which digestibility of CP ranges from 67% (BONNET et al., 1997; SEVEN and SEVEN, 2008) to 80–84% (HOSSEINI and AFSHAR, 2016). Vitamin C addition under heat stress in general improved the digestibility of nutrients, although in the present study this was not significant.

Table 4. Effects of heat stress on digestibility of dry matter and crude protein (least square means $\pm$ SEM)

Einfluss von Hitzestress auf die Verdaulichkeit von Trockenmasse und Rohprotein (kleinste mittlere Quadrate $\pm$ SEM)

Digestibility (%)	Treatments			Probability Treatment
	T1	T2	T3	
Dry matter	77.9 $\pm$ 0.60	79.0 $\pm$ 0.54	79.6 $\pm$ 0.54	NS
Crude protein	73.1 $\pm$ 1.00	74.3 $\pm$ 0.91	73.5 $\pm$ 0.91	NS

^T Treatments and codes are defined in Table 2.
NS = non-significant ($P > 0.05$).

It can be concluded that digestibility of nutrients (dry matter, CP) in ducks was not affected by the increased vitamin and mineral supplementation under long-term heat stress. The digestibility of DM and CP did not change significantly ($P > 0.05$).

Heat stress has negative effects on nutrient digestibility (DAGHIR, 2008; HAI et al., 2000). However, there are only a limited number of scientific studies on the digestibility of nutrients in ducks under heat stress. Digestibility of DM in broilers can vary from 66% (BONNET et al., 1997) to 73% (SEVEN and SEVEN, 2008).

Production parameters

The results of the production parameters are presented in Table 5. In the present study, the number of dead birds was very low and caused by sudden death syndrome SDS (n = 2). The d14 weight, which did not differ significantly between treatments, confirmed an equal distribution to the treatments with regard to body weight. The weight of the ducks at the end of the experiment (d42) was significantly higher in T2 and T3. In T2 and T3, the dWG and FCR was significantly higher (P < 0.05). The energy and protein CR is an index used to represent the applied energy and protein used for weight gain. The energy conversion ratio showed same tendency as other parameters: improved values in T2 and T3. However, protein conversion ratio did not differ significantly in treatments. The production costs did not differ significantly between treatments (P > 0.05).

Table 5. Effects of heat stress on production parameters (least square means ± SEM)

Einfluss von Hitzestress auf Produktionsparameter (kleinste mittlere Quadrate ± SEM)

Production parameters	Treatments			P-value	Probability Treatment
	T1	T2	T3		
Dead birds	2 ± 0.43	2 ± 0.43	1 ± 0.43	0.81	NS
FI (g/day/bird)	25 ± 0.44 ^a	27 ± 0.44 ^b	26 ± 0.44 ^b	0.002	**
Live weight at 14d (g/bird)	692 ± 16.49	687 ± 16.49	672 ± 16.49	0.69	NS
Live weight at 42d (g/bird)	1899 ± 47.84 ^a	2127 ± 46.21 ^b	2176 ± 46.21 ^b	0.0003	**
dWG (g/day/bird)	40.6 ± 2.41 ^a	49.1 ± 2.32 ^b	51.8 ± 2.32 ^b	0.005	**
FCR (kg feed/kg WG)	2.8 ± 0.08 ^a	2.6 ± 0.08 ^b	2.4 ± 0.08 ^b	0.009	**
Energy CR (MJ AME _n /kg WG)	345.3 ± 10.11 ^a	309.2 ± 9.76 ^b	285.1 ± 9.76 ^b	0.0005	***
Protein CR (g protein/kg WG)	459.9 ± 14.43	449.9 ± 13.94	428 ± 13.94	0.28	NS
Specific feed cost (/WG)	0.83 ± 0.58	0.71 ± 0.58	0.68 ± 0.58	0.2	NS

FI = feed intake; dWG = daily weight gain; FCR = feed conversion ratio; Energy CR = energy conversion ratio; Protein CR = protein conversion ratio.

^T Treatments and codes are defined in Table 2.

^{a, b} Different superscripts in the same row indicate significant differences between groups at P < 0.05 level.

*** P < 0.001; ** P < 0.01; NS = non-significant (P > 0.05).

Production parameters significantly improved with higher vitamin and mineral supplementation under heat stress in ducks. The T2 level did not differ significantly from T3 (P < 0.05) so it seems that supplementation of vitamins and minerals above T2 is not necessary.

Production parameters of poultry kept under high temperature conditions improved with Vitamin C (ATTIA et al., 2011; FAROOQI et al., 2005; KUTLU and FORBES, 1993; MCKEE et al., 1997; SAHIN and KUCUK, 2001; SAHIN et al., 2002a) and Vitamin E supplementation (HABIBIAN et al., 2014; HARSINI et al., 2012; HASHIZAWA et al., 2013; NIU et al., 2009; SAHIN and KUCUK, 2001), which can be explained by their function free radical scavengers.

Chemical composition of meat

The effects of heat stress on chemical composition of duck leg and breast meat are summarised in Table 6 and Table 7. As can be seen in Table 6, the DM, protein and fat content of duck leg meat was not affected by higher vitamin C, E, zinc and selenium supplementation under heat stress. Therefore, according to the present results, antioxidant supplementation does not improve the chemical composition of duck leg meat under high environmental temperatures. Breast meat (Table 7) shows the same tendency as the results for leg meat. Mostly there were no effects of supplementation. However, the DM content decreased in breast meat in T2 (P < 0.05). According to the present results, the glycogen content of duck meat significantly decreased in both leg and breast samples in T2 and T3. This suggests that the increased vitamin and mineral supplementation could not inhibit the reduction of glycogen levels.

Table 6. Effects of heat stress on chemical composition of duck leg meat (least square means $\pm$ SEM)Einfluss von Hitzestress auf die chemische Zusammensetzung von Entenkeulenfleisch (kleinste mittlere Quadrate $\pm$ SEM)

Leg meat composition (%)	Treatments			Probability Treatment
	T1	T2	T3	
Dry matter (%)	23.8 $\pm$ 0.21	23.5 $\pm$ 0.21	23.6 $\pm$ 0.24	NS
Protein (DMb ¹)	83.6 $\pm$ 0.92	84 $\pm$ 0.99	86.3 $\pm$ 1.03	NS
Fat (DMb ¹)	9.9 $\pm$ 0.94	9.9 $\pm$ 0.91	7.9 $\pm$ 0.98	NS
Glycogen (DMb ¹)	0.56 $\pm$ 0.02 ^a	0.49 $\pm$ 0.02 ^b	0.48 $\pm$ 0.02 ^b	*

^T Treatments and codes are defined in Table 2.¹ DMb = Dry matter based.^{a, b} Different superscripts in the same row indicate significant differences between groups at $P < 0.05$ level.* $P < 0.05$; NS = non-significant ($P > 0.05$).**Table 7. Effects of heat stress on chemical composition of duck breast meat (least square means $\pm$ SEM)**Einfluss von Hitzestress auf die chemische Zusammensetzung von Entenbrustfleisch (kleinste mittlere Quadrate $\pm$ SEM)

Breast meat composition (%)	Treatments			Probability Treatment
	T1	T2	T3	
Dry matter (%)	23.6 $\pm$ 0.18 ^a	22.9 $\pm$ 0.19 ^b	23.5 $\pm$ 0.19 ^a	*
Protein (DMb ¹)	90.3 $\pm$ 0.57	89.8 $\pm$ 0.57	90.8 $\pm$ 0.59	NS
Fat (DMb ¹)	2.5 $\pm$ 0.42	3.3 $\pm$ 0.44	2.2 $\pm$ 0.46	NS
Glycogen (DMb ¹)	0.85 $\pm$ 0.03 ^a	0.69 $\pm$ 0.03 ^b	0.7 $\pm$ 0.03 ^b	**

^T Treatments and codes are defined in Table 2.¹ DMb = Dry matter based.^{a, b} Different superscripts in the same row indicate significant differences between groups at $P < 0.05$ level.** $P < 0.01$; * $P < 0.05$; NS = non-significant ($P > 0.05$).

Different conditions (e.g. heat stress, feed withdrawal, transport) have been known to affect meat characteristics and quality in poultry (BERRI et al., 2005; FLETCHER, 2002; SANDERCOCK et al., 2001) by changing the aerobic metabolism and glycolysis (ZABOLI et al., 2018). The chemical composition of duck breast meat in different experiments were determined to be around 20–21% protein and 1.5–3.6% fat, when sampled under thermos-neutral conditions and without supplementation (GALAL et al., 2011; HEO et al., 2015). AKŞIT et al. (2006) and TANKSON et al. (2001) reported that high environmental temperature caused a reduction in protein content of poultry carcasses.

One of the physical changes during the *post mortem* ageing of animal meat is the breakdown of glycogen. Based on the literature, heat stress has negative effects on muscle glycogen levels because glycogen breakdown and depletion is increased (FLETCHER, 2002; KHAN, 1971). Due to the decreased glycogen level, the concentration of lactic acid also decreases, meaning that the pH of the animal meat will increase. This process occurs in PSE meat (WANG et al., 2017; ZABOLI et al., 2018). Different studies show that muscle glycogen levels is decreased under stress (SONG and KING, 2015; WANG et al., 2017).

Conclusion

It can be concluded that the digestibility of crude protein and dry matter, the rectal temperature and the chemical composition of duck breast and leg meat were not affected ($P > 0.05$) by dietary treatments under heat stress. On the other hand several production parameters (FI, live weight at 14 d and 42 d, dWG, FCR) and the serum antioxidant capacity (MDA, SOD, ACW, ACL, GPx) of 14–42 day old ducks were significantly improved ($P < 0.05$) with supplementation (**T2**, **T3**). Based on the present results, we recommend using **T2** (Vitamin E: 540 mg/kg diet, Vitamin C: 998 mg/kg diet, Se: 0.60 mg/kg diet and Zn: 97 mg/kg diet) supplementation in practical duck nutrition at high environmental temperatures (in the summer).

Acknowledgement

The work is supported by the EFOP-3.6.2–16–2017–00001 project, entitled “Complex rural economic development and sustainability research, development of the service network in the Carpathian Basin”.

This study was supported in part by Hungarian Research Found (TÁMOP 4.2.2D-15/1). The authors gratefully acknowledge the generous sponsorship of Tranzitker Trading Company, Debrecen, Hungary (www.transitker.hu).

Disclosure statement

No potential conflict of interest was reported by the authors.

References

- AKBARIAN, A., A. GOLIAN, H. KERMANSHAH, S. DE SMET, J. MICHIELS, 2015: Antioxidant Enzyme Activities, Plasma Hormone Levels and Serum Metabolites of Finishing Broiler Chickens Reared under High Ambient Temperature and Fed Lemon and Orange Peel Extracts and *Curcuma Xanthorrhiza* Essential Oil. *J. Anim. Physiol. Anim. Nutr.* **99** (1), 150-162.
- AKŞİT, M., S. YALÇIN, S. OZKAN, K. METİN, D. OZDEMİR, 2006: Effects of temperature during rearing and crating on stress parameters and meat quality of broilers. *Poult. Sci.* **85**, 1867-1874.
- ALTAN, Ö., A. PABUÇCUOĞLU, A. ALTAN, S. KONYALIOĞLU, H. BAYRAKTAR, 2003: Effect of Heat Stress on Oxidative Stress, Lipid Peroxidation and Some Stress Parameters in Broilers. *Br. Poult. Sci.* **44** (4), 545-550.
- AOAC, 2006a: Official Methods of Analysis Amino Acids Analysis Methionine, Lysine or Hydroxyproline (M, L Or Hp) - item 70. Association of Analytical Communities, Gaithersburg, MD, 17th Edition. Reference Data: Method 982.30 E(A); Nfnap; Nitr; Aa.
- AOAC, 2006b: Official Methods of Analysis Minerals Analysis Arsenic, Cadmium, Lead, Selenium and Zinc Human and Pet Foods - Item 64. Association of Analytical Communities, Gaithersburg, Md, 17th Edition. Reference Data: Method 986.15 (9.1.01); Nfnap; Min.
- AOAC, 2006c: Official Methods of Analysis Minerals Analysis Ca, Cu, Fe, Mg, Mn, P, K, Na and Zn Infant Formula - Item 62. Association of Analytical Communities, Gaithersburg, Md, 17th Edition. Reference Data: Method 984.27; Nfnap; Min.
- AOAC, 2006d: Official Methods of Analysis Minerals Analysis Silicate, Gravimetric - Item 48. Association Of Analytical Communities, Gaithersburg, Md, 17th Edition. Reference Data: Method 920.08; Nfnap; Min; Si.
- AOAC, 2006e: Official Methods of Analysis Proximate Analysis and Calculations Crude Protein Meat and Meat Products Including Pet Foods - Item 80. Association of Analytical Communities, Gaithersburg, Md, 17th Edition. Reference Data: Method 992.15 (39.1.16); Nfnap; Nitr; Nt.
- AOAC, 2006f: Official Methods of Analysis Proximate Analysis and Calculations Crude Fiber (Cfiber) - Item 4. Association of Analytical Communities, Gaithersburg, Md, 17th Edition. Reference Data: Method 978.10; Cho; Fib.

- AOAC, 2006g: Official Methods of Analysis Proximate Analysis and Calculations Crude Fat (Cf) - Item 17. Association of Analytical Communities, Gaithersburg, Md, 17th Edition. Reference Data: Method 920.39 (A); Lipid; Fa.
- AOAC, 2006h: Official Methods of Analysis Vitamin Analysis L-Ascorbic Acid Vitamin Preparations - Item 100. Association of Analytical Communities, Gaithersburg, Md, 17th Edition. Reference Data: Method 967.22 (45.1.15); Nfnap; Vit; Ascl.
- AOAC, 2012: Official Methods of Analysis of AOAC International. 19th Edition. 2012: AOAC International, Gaithersburg, Maryland, Usa. [Www.Eoma.Aoac.Org](http://www.eoma.aoc.org). Access date: 6.May 2019.
- AOAC, 2013: Official Method 2012.10.Simultaneous Determination of 13-cis and all-trans Vitamin A Palmitate (Retinyl Palmitate), Vitamin A Acetate (Retinyl Acetate), and Total Vitamin E (dl-a-Tocopherol and dl-a-Tocopherol Acetate) in Infant Formula and Adult Nutritionals.
- ARAI, K., S. MAGUCHI, S. FUJII, H. ISHIBASHI, K. OIKAWA, N. TANIGUCHI, 1987: Glycation and inactivation of Human Cu-Zn-Superoxide dismutase. Identification of the in vitro glycat-ed sites. *J. Biol. Chem.* **262**, 16969-16972.
- ARMSTRONG, D., 1994: Heat Stress Interaction with Shade and Cooling. *J. Dairy Sci.* **77** (7), 2044-2050.
- ATTIA, Y.A., R.A. HASSAN, A.E. TAG EL-DIN, B.M. ABOU-SHEHEMA, 2011: Effect of ascorbic acid or increasing metabolizable energy level with or without supplementation of some essential amino acids on productive and physiological traits of slow-growing chicks exposed to chronic heat stress. *J. Anim. Physiol. Anim. Nutr.* **95** (6), 744-755.
- AVILÉS-ESQUIVEL, D.F., M.A. MONTERO, H. ZURITA-VÁSQUEZ, M. BARROS-RODRÍGUEZ, 2018: Animal Welfare and Poultry Productivity, A Short Review. *Trop. Subtrop. Agroecosyst.* **21** (1), 114-123.
- BABINSZKY, L., V. HALAS, M.W.A. VERSTEGEN, 2011: Impacts of Climate Change on Animal Production and Quality Of Animal Food Products. In: Blanco, J.A. – Kheradmand, H. (Eds.): *Climate Change – Socioeconomic Effects*. Intech Open Access Publisher. Rijeka, Croatia, 165-190.
- BERRI, C., M. DEBUT, V. SANTÉ-LHOUTELLIER, C. ARNOULD, B. BOUTTEN, N. SELLIER, E. BAÉZA, N. JEHL, Y. JÉGO, M.J. DUCLOS, E. LE BIHAN-DUVAL, 2005: Variations in Chicken Breast Meat Quality: Implications of Struggle and Muscle Glycogen Content At Death. *Br. Poult. Sci.* **46** (5), 572-579.
- BONNET, S., P.A. GERAERT, M. LESSIRE, B. CARRE, S. GUILLAUMIN, 1997: Effect Of High Ambient Temperature On Feed Digestibility In Broilers. *Poult. Sci.* **76** (6), 857-863.
- CHAN, K.M., E.A. DECKER, 1994: Endogenous Skeletal Muscle Anti-Oxidants. *Crit. Rev. Food Sci. Nutr.* **34**, 403-426.
- CHERRY, P., T.R. MORRIS, 2008: *Domestic Duck Production: Science and Practice*. Wallingford, Oxfordshire, Uk Cabi.
- COOPER, M.A., K.W. WASHBURN, 1998: The Relationships of Body Temperature to Weight Gain, Feed Consumption, and Feed Utilization in Broilers Under Heat Stress. *Poult. Sci.* **77** (2), 237-242.
- COSTANTINI, D., S. VERHULST, 2009: Does High Antioxidant Capacity Indicate Low Oxidative Stress? *Funct. Ecol.* **23** (3), 506-509.
- DAGHIR, N.J., 2008: Broiler Feeding and Management in Hot Climates. In: Daghir, N.J. (Ed.) *Poultry Production in Hot Climate*, 227-260. Cab International, Cromwell Press, Trowbridge.
- DAGHIR, N.J., 2009: Nutritional Strategies to Reduce Heat Stress in Broilers and Broiler Breeders." *Lohmann Information.* **44** (1), 6-15.
- DALRYMPLE, R.H., R. HAMM, 1973: A Method for the Extraction of Glycogen and Metabolites from a Single Muscle Sample. *Int. J. Food Sci. Tech.* **8** (4), 439-444.
- ELBERS, A.R.W., L.A. DEN HARTOG, M.W.A. VERSTEGEN, T. ZANDSTRA, 1989: Between-and within-Herd Variation in the Digestibility of Feed for Growing-Finishing Pigs. *Livest. Prod. Sci.* **23** (1), 183-193.
- EUROPEAN PHARMACOPEIA SUPPLEMENT (3rd Ed.), Council of Europe, Strasbourg, 2001: *FAO Food and Agriculture Organization*. [Http://Www.Fao.Org/Faostat/En/#Home](http://www.fao.org/faostat/en/#Home). Access date: 6.May 2019.

- FAROOQI, H.A.G., M.S. KHAN, M.A. KHAN, M. RABBANI, K. PERVEZ, J.A. KHAN, 2005: Evaluation of Betaine and Vitamin C in Alleviation of Heat Stress in Broilers. *Int. J. Agric. Biol.* **5**, 744-746.
- FLETCHER, D.L., 2002: Poultry Meat Quality. *Worlds Poultry Sci. J.* **58** (2), 131-145.
- GALAL, A., W.A.H. ALI, A.M.H. AHMED, K.A. ALI, 2011: Performance and Carcass Characteristics of Dumyati, Muscovy, Peking and Sudani Duck Breeds. *Egypt. J. Animal Prod.* **48** (2), 191-202.
- GROB, F., J. DURNER, F. GAUPELS, 2013: Nitric Oxide, Antioxidants and Prooxidants in Plant Defence Responses. *Front. Plant Sci.* **4**, 419. Doi: 10.3389/Fpls.2013.00419.
- HABIBIAN, M., S. GHAZI, M.M. MOEINI, A. ABDOLMOHAMMADI, 2014: Effects of Dietary Selenium and Vitamin E on Immune Response and Biological Blood Parameters of Broilers Reared under Thermoneutral or Heat Stress Conditions. *Int. J. Biometeorol.* **58** (5), 741-752.
- HAI, L., D. RONG, Z.Y. ZHANG, 2000: The Effect of Thermal Environment on the Digestion of Broilers. *Anim. Physiol. Anim. Nutr.* **83**, 57-64.
- HARSINI, S.G., M. HABIBIAN, M.M. MOEINI, A.R. ABDOLMOHAMMADI, 2012: Effects of Dietary Selenium, Vitamin E, and their Combination on Growth, Serum Metabolites, and Antioxidant Defense System in Skeletal Muscle of Broilers under Heat Stress. *Biol. Trace Elem. Res.* **148** (3), 322-330.
- HASHIZAWA, Y., M. KUBOTA, M. KADOWAKI, S. FUJIMURA, 2013: Effect of Dietary Vitamin E on Broiler Meat Qualities, Colour, Water-Holding Capacity and Shear Force Value, under Heat Stress Conditions. *Anim. Sci.* **84** (11), 732-736.
- HEO, K.N., E.C. HONG, C.D. KIM, H.K. KIM, M.J. LEE, H.J. CHOO, J.H. KIM, 2015: Growth Performance, Carcass Yield, and Quality and Chemical Traits of Meat from Commercial Korean Native Ducks with 2-Way Crossbreeding. *Asian-Austral. Anim. Sci.* **28** (3), 382-390.
- HORVÁTH, M., L. BABINSZKY, 2019: Impact of Selected Antioxidant Vitamins (Vitamin A, E and C) and Micro Minerals (Zn, Se) on the Antioxidant Status and Performance under High Environmental Temperature in Poultry. A Review. *Acta Agric. Scand. A Anim. Sci.* Doi: 10.1080/09064702.2019.1611913.
- HOSSEINI, S.M., M. AFSHAR, 2016: Effects of Feed Form and Xylanase Supplementation on Performance and Ileal Nutrients Digestibility of Heat-Stressed Broilers Fed Wheat-Soybean Diet. *J. Appl. Anim. Res.* **45** (1), 550-556.
- IRSHAD, M., P.S. CHAUDHURI, 2002: Oxidant-Antioxidant System: Role and Significance in Human Body. *NISCAIR-CSIR, India.* **40** (11), 1233-1239.
- JIN, L.Z., Y.W. HO, N. ABDULLAH, S. JALALUDIN, 2000: Digestive and Bacterial Enzyme Activities in Broilers Fed Diets Supplemented with Lactobacillus Cultures. *Poult. Sci.* **79** (6), 886-891.
- KHAN, A.W., 1971: Effect of Temperature during Post-Mortem Glycolysis and Dephosphorylation of High Energy Phosphates on Poultry Meat Tenderness. *J. Food Sci.* **36** (1), 120-121.
- KHAN, R., S. NAZ, Z. NIKOUSEFAT, M. SELVAGGI, V. LAUDADIO, V. TUFARELLI, 2012: Effect of Ascorbic Acid in Heat-Stressed Poultry. *Worlds Poultry Sci. J.* **68** (3), 477-490.
- KUMAR, K., A. MISHRA, A.A. SHEIKH, P. PATEL, M.K. AHIRWAR, S.M. BASHIR, A. ALI, 2017: Effect of Ascorbic Acid on Some Biochemical Parameters during Heat Stress in Commercial Broilers. *Int. Curr. Microbiol. Appl. Sci.* **6** (11), 5425-5434.
- KUTLU, H.R., J.M. FORBES, 1993: Changes in Growth and Blood Parameters in Heat-Stressed Broiler Chicks in Response to Dietary Ascorbic Acid. *Livest. Prod. Sci.* **36** (4), 335-350.
- LARA, L., M. ROSTAGNO, 2013: Impact of Heat Stress on Poultry Production. *Animals.* **3** (2), 356-369.
- LESKOVEC, J., A. LEVART, A. NEMEC SVETE, L. PERIC, M. ĐUKIC STOJIC, D. ŽIKIC, J. SALOBIR, V. REZAR, 2018: Effects of Supplementation with A-Tocopherol, Ascorbic Acid, Selenium, or their Combination in Linseed Oil-Enriched Diets on the Oxidative Status in Broilers. *Poult. Sci.* **97** (5), 1641-1650.

- LI, Y. R., 2011: Free Radicals and Related Reactive Species. In Free Radical Biomedicine: Principles, Clinical Correlations, and Methodologies. Chapter 2:10-39. Bentham Science Publishers, Sharjah, United Arab Emirates.
- LIAO, X., L. LU, S. LI, S. LIU, L. ZHANG, G. WANG, X. LUO, 2012: Effects of Selenium Source and Level on Growth Performance, Tissue Selenium Concentrations, Antioxidation, and Immune Functions of Heat-Stressed Broilers. Biol. Trace Elem. Res. **150** (1-3), 158-165.
- LIN, H., H.C. JIAO, J. BUYSE, E. DECUYPERE, 2006: Strategies for Preventing Heat Stress in Poultry. Worlds Poult. Sci. J. **62** (1), 71-86.
- MAHMOUD, K.Z., F.W. EDENS, 2003: Influence of Selenium Sources on Age-Related and Mild Heat Stress-Related Changes of Blood and Liver Glutathione Redox Cycle in Broiler Chickens (*Gallus Domesticus*). Comp. Biochem. Physiol. B, Biochem. Mol. Biol. **136** (4), 921-934.
- MAHMOUD, K.Z., F.W. EDENS, E.J. EISEN, G.B. HAVENSTEIN, 2004: Ascorbic Acid Decreases Heat Shock Protein 70 and Plasma Corticosterone Response in Broilers (*Gallus Gallus Domesticus*) Subjected to Cyclic Heat Stress. Comp. Biochem. Physiol. B, Biochem. Mol. Biol. **137** (1), 35-42.
- MAINI, S., S.K. RASTOGI, J.P. KORDE, A.K. MADAN, S.K. SHUKLA, 2007: Evaluation of Oxidative Stress and its Amelioration through Certain Antioxidants in Broilers during Summer. J. Poult. Sci. **44** (3), 339-347.
- McKEE, J.S., P.C. HARRISON, G.L. RISKOWSKI, 1997: Effects of Supplemental Ascorbic Acid on the Energy Conversion of Broiler Chicks during Heat Stress and Feed Withdrawal. Poult. Sci. **76** (9), 1278-1286.
- MIKULSKI, D., J. JANKOWSKI, Z. ZDUNCZYK, M. WRÓBLEWSKA, K. SARTOWSKA, T. MAJEWSKA, 2009: The Effect of Selenium Source on Performance, Carcass Traits, Oxidative Status of the Organism, and Meat Quality of Turkeys. J. Anim. Feed Sci. **18** (3), 518-530.
- NIKI, E., T. SAITO, Y. KAMIYA, 1983: The Role of Vitamin C as an Anti-Oxidant. Chem. Lett. **12** (5), 631-632.
- NIU, Z., F. LIU, Q. YAN, L. LI, 2009: Effects of Different Levels of Selenium on Growth Performance and Immunocompetence of Broilers under Heat Stress. Arch. Anim. Nutr. **63** (1), 56-65.
- NRC, 1994: (National Research Council) Nutrient Requirements of Poultry. The National Academies Press. Washington Dc, Usa.
- PACKER, L., 1992: New Horizons in Vitamin E Research- the Vitamin E Cycle, Biochemistry, and Clinical Applications. In: Lipidsoluble Antioxidants: Biochemistry and Clinical Applications. Birkhauser Verlag. Boston. 1-16.
- POPOV, I., G. LEWIN, 1999: Antioxidative Homeostasis: Characterization by Means of Chemiluminescent Technique. In: Methods in Enzymology. Academic Press. **300**, 437-456.
- PRASAD, A.S., O. KUCUK, 2002: Zinc in Cancer Prevention. Cancer Metastasis Rev. **21** (3-4), 291-295.
- SAHIN, K., O. KUCUK, 2001: Effects of Vitamin E and Selenium on Performance, Digestibility of Nutrients, and Carcass Characteristics of Japanese Quails Reared under Heat Stress (34 C). Anim. Physiol. Anim. Nutr. **85** (11-12), 342-348.
- SAHIN, K., O. KUCUK, N. SAHIN, M. SARI, 2002a: Effects of Vitamin C and Vitamin E on Lipid Peroxidation Status, Serum Hormone, Metabolite, and Mineral Concentrations of Japanese Quails Reared under Heat Stress (34°C). Int. J. Vitam. Nutr. Res. **72** (2), 91-100.
- SAHIN, K., N. SAHIN, M. SARI, M.F. GURSU, 2002b: Effects of Vitamins E and A Supplementation on Lipid Peroxidation and Concentration of Some Mineral in Broilers Reared under Heat Stress (32°C). Nutr. Res. **22**, 723-731.
- SAHIN, K., M. ONDERCI, N. SAHIN, F. GULCU, N. YILDIZ, M. AVCI, O. KUCUK, 2006: Responses of Quail to Dietary Vitamin E and Zinc Picolinate at Different Environmental Temperatures. Anim. Feed Sci. Tech. **129** (1-2), 39-48.

- SANDERCOCK, D.A., R.R. HUNTER, G.R. NUTE, M.A. MITCHELL, P.M. HOCKING, 2001: Acute Heat Stress-Induced Alterations in Blood Acid-Base Status and Skeletal Muscle Membrane Integrity in Broiler Chickens at two Ages: Implications for Meat Quality. *Poult. Sci.* **80** (4), 418-425.
- SAS, 2010: Sas Institute Inc., Cary, Nc, Usa.
- SEVEN, P.T., I. SEVEN, 2008: Effect of Dietary Turkish Propolis as Alternative to Antibiotic on Performance and Digestibility in Broilers Exposed to Heat Stress. *J. Appl. Anim. Res.* **34** (2), 193-196.
- SISEIN, E. A., 2014: Biochemistry of Free Radicals and Antioxidants. *Scholars Academic J. Biosci.* **2** (2), 110-118.
- SONG, D.J., A.J. KING, 2015: Effects of Heat Stress on Broiler Meat Quality. *Worlds Poult. Sci. J.* **71** (4), 701-709.
- SUCHÝ, P., E. STRAKOVÁ, I. HERZIG, 2014: Selenium in Poultry Nutrition: A Review. *Czech. J. Anim. Sci.* **59** (11), 495-503.
- TANKSON, J.D., Y. VIZZIER-THAXTON, J.P. THAXTON, J.D. MAY, J.A. CAMERON, 2001: Stress and Nutritional Quality of Broilers. *Poult. Sci.* **80** (9), 1384-1389.
- TOMAŽIN, U., T. FRANKIC, M. VOLJC, V. REZAR, A. LEVART, J. SALOBIR, 2013: The Potency of Γ -And G-Tocopherol, and their Combination. In: Reducing Dietary Induced Oxidative Stress *in-vivo* and Improving Meat Lipid Stability in Broilers. *Arch. Geflügelk.* **77**, 266-274.
- WANG, R.H., R R. LIANG, H. LIN, L.X. ZHU, Y.M. ZHANG, Y.W. MAO, X. LUO, 2017: Effect of Acute Heat Stress and Slaughter Processing on Poultry Meat Quality and *post-mortem* Carbohydrate Metabolism. *Poult. Sci.* **96** (3), 738-746.
- WOLFENSON, D., D. BACHRACH, M. MAMAN, Y. GRABER, I. ROZENBOIM, 2001: Evaporative Cooling of Ventral Regions of the Skin in Heat-Stressed Laying Hens. *Poult. Sci.* **80** (7), 958-964.
- XIE, J., L. TANG, L. LU, L. ZHANG, L. XI, H. C. LIU, J. ODLE, X. LUO, 2014: Differential Expression of Heat Shock Transcription Factors and Heat Shock Proteins after acute and chronic Heat Stress in Laying Chickens (*Gallus Gallus*). *PlosOne* **9** (7), E102204.
- YANG, L., G.Y. TAN, Y.Q. FU, J.H. FENG, M.H. ZHANG, 2010: Effects of Acute Heat Stress and Subsequent Stress Removal on Function of Hepatic Mitochondrial Respiration, Ros Production and Lipid Peroxidation in Broiler Chickens. *Comp. Biochem. Physiol. C, Toxicol. Pharmacol.* **151** (2), 204-208.
- YUN, S.H., Y.S. MOON, S.H. SOHN, I.S. JANG, 2012: Effects of Cyclic Heat Stress or Vitamin C Supplementation During Cyclic Heat Stress on Hsp70, Inflammatory Cytokines, and the Antioxidant Defense System. In: Sprague Dawley Rats. *Exper. Animals.* **61** (5), 543-553.
- ZABOLI, G., X. HUANG, X. FENG, D.U. AHN, 2018: How Can Heat Stress Affect Chicken Meat Quality? – A Review. *Poult. Sci.* **98** (3), 1551-1556.