Thesis of Doctoral (PhD) Dissertation

ALLEVIATING THE ADVERSE EFFECT OF CHRONIC HEAT STRESS ON SELECTED ANTIOXIDANT PARAMETERS AND PERFORMANCE OF MEAT TYPE DUCKS BY DIETARY VITAMIN E, VITAMIN C, SELENIUM AND ZINC SUPPLEMENTATION

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1. INTRODUCTION AND AIM OF THE THESIS

The increasing ambient temperature and inordinate types of weather caused by climate change have impacts on agriculture: on plant, crop and livestock production and even on product quality.

The thermoneutral zone is the temperature zone in which the animals are able to keep their body temperature constant with the help of physical heat regulation. Unfavourable temperatures (too cold or too hot environments) caused by different reasons lead to increased heat production by the animal, there is more loss of energy, and less energy remains for production at the same level of energy intake, and the efficiency of energy utilization deteriorates. The lowest temperature in the thermoneutral zone is called the lowest critical temperature (LCT). If temperatures falls under this temperature, the bird will start to use feed energy to warm itself and will consume more feed. The highest temperature is called the highest critical temperature (HCT). If the temperature rises above this temperature the birds can no longer dissipate their heat, this will cause "heat shock" (BABINSZKY et al., 2019; NOBLET et al., 2001).

Based on the literature data, it is known that higher environmental temperatures may have consequences that are more serious and lead to heat stress in poultry if the birds can no longer dissipate their heat. Heat stress can decrease the resistance capacity and 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 a,b; 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 rapid, they have 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).

However, there is not much relevant information about their nutrition and physiological changes, especially under high environmental conditions, and a very limited number of scientific studies carried out with ducks. A solution for prevention of heat stress in animals includes biological (e.g. genetics, thermal conditioning, nutrition) (DAGHIR, 2008; LIN et al., 2006) or keeping technology devices (e.g. air conditioning, intensive ventilation, humidification) (ARMSTRONG, 1994; WOLFENSON et al., 2001).

However, housing methods are expensive and the service costs are high and mostly not always adequate. Therefore, reducing the biochemical and physiological negative effects of heat stress with different nutritional tools is one of the primary interests for the economical production of food produced from animals. In this dissertation, we present the nutritional solutions. According to several studies (DAGHIR, 2009; GOUS AND MORRIS, 2005; LEESON, 1986; LIN et al., 2006; MUJAHID, 2011; SAHIN et al., 2009), we selected the primary vitamins and micro minerals, which are most commonly used in poultry nutrition as supplementation during times of heat stress.

Aim of the thesis

The aim of the present thesis is to evaluate different levels of antioxidant supplementation such as Vitamin E, Vitamin C, Se and Zn in the growing period (14th day of age - 42nd day of age) of Cherry Valley meat type ducks exposed to constant (chronic) high environmental temperature (30±1°C for 24h during 28 days) with consideration on the

- some physiological trials including antioxidant defence mechanisms,
- nutrient utilization regarding digestibility and efficiency of energy and protein utilization as well as
- growth performance and chemical composition of valuable meat parts

Moreover, this study presents the elimination of the negative effects of heat stress using vitamin and micro mineral supplementation (Vitamin C, E, Se and Zn) in the diet of this species of duck.

2. MATERIALS AND METHODS

2.1. Ethics Statement

The experiment procedures were done according to the Hungarian Animal Protection and Welfare Act (Act XXVIII of 1998, 3.§).

2.2. Animals and experimental design

Birds. A total of 900-mixed sex 14-day-old (14d) Cherry Valley type hybrid ducks were raised under standard management conditions based on the standard practice of Transitker Trading Company. They were fed the same compound feed (meet the nutrient requirements according to NRC, 1994 for ducks) for 14 days before being randomly placed into three experiment groups, each containing 20 birds in five replicates (n=100 birds/treatment group; n=300 birds /experiment). The experiment was performed in three replicates (n=300; 3x300 ducks= 900 ducks in total). Birds were marked individually by wing tag.

Housing. The birds were housed in uniform circumstances from 1 day of age until 14 days of age. At 14 days of age (14d), the ducks were housed in an environmentally controlled room in the duck housing and subjected to the following treatment over 28 days (until 42 days of age- 42d): high environmental temperature (HT) constant 30±1°C for 24 hours with the relative humidity at (62±5%). The temperature and humidity were monitored twelve times daily at different locations of the animal house. 15 pens were placed in the duck housing. Each pen (3x3x1, 5m) consisted of 20 birds. The lightning schedule of 23L: 1D (2Lux) was provided (CHERRY AND MORRIS, 2008). Ducks were kept in floor pens covered with straw.

Duration of the study. The experiment started at 14d and lasted until 42 days of age.

Feeding and drinking. All ducks were provided with free access to feed and water.

Dietary treatments, composition of diets and analysed nutrient content. As mentioned previously, the experimental diets were supplemented by two vitamins (Vitamin C and E) and two micro minerals (Zn and Se).

The three experimental diets were corn-soybean meal based and formulated in the study to meet NRC requirements for ducks (NRC, 1994). The composition and analysed nutrient contents of the experimental diets are summarized in *Table 1*. The diets were

prepared by using a control diet, which can be used in normal environmental temperatures in practice (near to the thermoneutral zone of ducks) and two experimental diets (supplemented with antioxidant feed additives). In treatment 1 (**T1**), the diet contained: Vitamin E (40 mg/kg diet), Vitamin C (0 mg/kg diet), Se (0.45 mg/kg diet) and Zn (50 mg/kg diet) by the premix. 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 and Treatment 3 (**T3**) had more increased Vitamin E (1540 mg/kg diet), Vitamin C (1996 mg/kg diet), Se (0.90 mg/kg diet) and Zn (148 mg/kg diet) supplementation (*Table 2*).

Table 1

Composition and analysed nutrient contents of experimental diets

Ingredients (%)	**Diets**		
	1 (T1)[T]	**2 (T2)[T]**	**3 (T3)[T]**
Corn	25.0	38.7	35.0
Wheat	20.0	10.0	10.0
Triticale	25.5	20.0	20.0
Ext. Soybean meal (CP: 46%)	3.6	14.2	10.6
Full fat soy	5.0	5.0	5.0
Ext. Sunflower granulate	13.5	6.3	10.2
Wheat meal	3.0	3.0	6.0
Premix[1]	0.5	0.5	0.5
Others[2]	3.9	2.3	2.7
Antioxidant supplementation[T]	-	+	++
Calculated energy and analysed nutrient content (100g dry matter)			
ME poultry (MJ/kg)	12.3	12.11	12.0
Crude protein (%)	16.2	16.2	16.6
Crude fiber (%)	4.2	4.2	4.3
Crude fat (%)	3.5	3.3	3.8
Lysine (%)	1.0	1.0	1.0
Methionine (%)	0.42	0.47	0.52
Ca (%)	0.58	0.58	0.68
Si_2O (%)	0.5	0.5	0.5

[T] Treatments and codes are defined in Table 2.

[1] 1 kg premix contains: Vitamin A (retinyl acetate): 2 000 000 IU; Vitamin D3 (cholecalciferol): 600 000 IU; Vitamin E (all-rac-alfa-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; Zink (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.

[2] Amino acid supplementation : L-lysine HCl, DL- Methionine, L-Threonine; Sunflower oil; Limestone; MCP; Salt; $NaHCO_3$; premix (0.5%)

Table 2

Dietary treatments

	Treatments		
	T1	**T2**	**T3**
Supplementation*	-	+	++
Vitamin E (mg/kg)[1]	40	540	1540
Vitamin C (mg/kg)[2]	-	998	1996
Se (mg/kg)[3]	0.45	0.60	0.90
Zn (mg/kg)[4]	50	97	148

*Analysed
[1] all-rac-alfa-tocopheril acetate form
[2] ascorbic acid form
[3]organic selenomethionine form
[4] inorganic zinc oxide form

2.3 Sampling and sample preparation

15 ducks/ treatment were randomly euthanized by cervical dislocation at d42 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 1000xg at 4°C for 10 min. The supernatant plasma was further divided into 300 µl aliquots (to determine the parameters describing the antioxidant status: MDA, SOD, ACW, ACL, GPx, GR, GSH) and 200 µl (to determine plasma Vitamin C and E) and stored at -20°C until analysis (n=15 ducks/treatment).

Digesta. The digestibility of nutrients in the small intestine was determined by post mortem digestibility trial using silicon dioxide (Si_2O) sand (Sigma-Aldrich, St. Louis, Missouri, USA) as the indicator to increase the amount of HCl insoluble ash concentration. 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=15 ducks/treatment).

Meat. The skinny leg and breast meat of the ducks were collected individually in plastic bags at 42d. The samples were stored at -20°C until analysis. (n=15 ducks/treatment).

2.4. Measurements

Body temperature (BT). A thermometer was inserted 3 cm into the rectum for 10-15s for BT measurement. BT was measured by sex (two sexes/pen; n=30) two times (at 9 am and 4 pm) three times a week (n=240 data/treatment).

Performance. The body weight (BW) of the ducks was measured individually (n=300) at d14 and d42 (g/bird). The daily weight gain (dWG) was calculated also individually (g/day/bird). The daily feed intake (FI) was recorded by each pen (n=15, 3 repeat x 15=45 pen in total) and was divided with the number of birds to get FI g/day/bird. The feed conversion ratio (FCR) and the energy (Energy CR) and protein (Protein CR) conversion were also calculated by each pen (n=15). The number of dead birds was recorded and after autopsy veterinarian determined the reason.

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 (α-tocopherol) and malondialdehyde (MDA).

2.5. Chemical analysis

Diet. The nutrient, vitamin and mineral content of the diet were determined using standard procedures of AOAC Official Methods (AOAC, 2012).

Blood. SOD, ACL, ACW was determined using commercially available kit (Analytik Jena AG, Jena, Germany) with PhotoChem© (POPOV AND LEWIN, 1999). The GR, GPx, GSH activity (Abcam, Cambridge, UK) was determined using commercially available assay kits. The GR was measured with SpectroStar^{Nano} microplate reader (BMG Labtech, Offenburg, Germany). The calculation was done according to the assay protocol. The concentration of MDA was determined using a commercially available assay kit (Sigma-Aldrich, St. Louis, Missouri). The concentration of Vitamin E was determined using ELISA kit (Blue Gene Biotech LTD, Shanghai, China). To determine the plasma Vitamin C content the plasma samples were pre-treated due to the high

protein content of the samples. The supernatant was used for determination using a commercially available assay kit (Abcam, Cambridge, UK).

Digesta. The digesta samples (DM, crude protein) were analysed using standard procedures of Proximate Analysis (AOAC, 2012). and SiO_2 was measured according to AOAC Authors, 2006; AOAC Official Method 920.08.

Meat. The meat samples (DM, protein, fat) were analysed using standard procedures of Proximate Analysis (AOAC, 2012). DALRYMPLE AND HAMM (1973) method measured glycogen content in terms of glucose.

2.6. Calculations

Performance

FCR, Energy CR, Protein CR and Specific feed cost were calculated as follows:

n=15 (1 data/cage):

- FCR=FI (kg) / WG (kg)
- Energy Conversion Ratio= energy content of the diet (MJ AME_n) / WG (kg)
- Protein Conversion Ratio= protein content of the diet (g) / WG (kg)
- Specific feed cost= price (US $) x WG (kg).

Digestibility.

We used the following formula to calculate the apparent digestibility of nutrients according to ELBERTS et al. (1989) and REFSTIE et al (1999):

Nutrient digestibility= 1- (conc. $_{\text{indicator in the diet}}$ / conc. $_{\text{of indicator in the digesta}}$) x (nutrient conc. $_{\text{in digesta}}$ / nutrient conc$_{\text{. in diet}}$)

2.7. Statistical analysis

The first step of data analysis was to test normality by Kolmogorov-Smirnov (K-S) (SAS 2010, version 9.3). Then the experimental data was analysed using the PROC GLM (mixed models) procedure of SAS. When repeat and treatment x repeat interaction was not significant, it was omitted and the model was recalculated without these variables. Sex and time of the day was also not significant therefore, these variables were also omitted from the model. Live performance parameters (FI, FCR,

energy and protein conversion, feed cost) were analysed using the pen as the investigational unit, while each duck was considered as an investigator unit for WG, live weight (d14d, 42d), blood, BT, 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.). Correlation analysis was done by SAS Correlation Analysis (SAS 2010, version 9.3) between the following variants:

- "A" (supplementation: daily vitamin and mineral supply, g/day) and "B" (antioxidant parameters) and between
- "C" (supplementation) and "D" (performance).

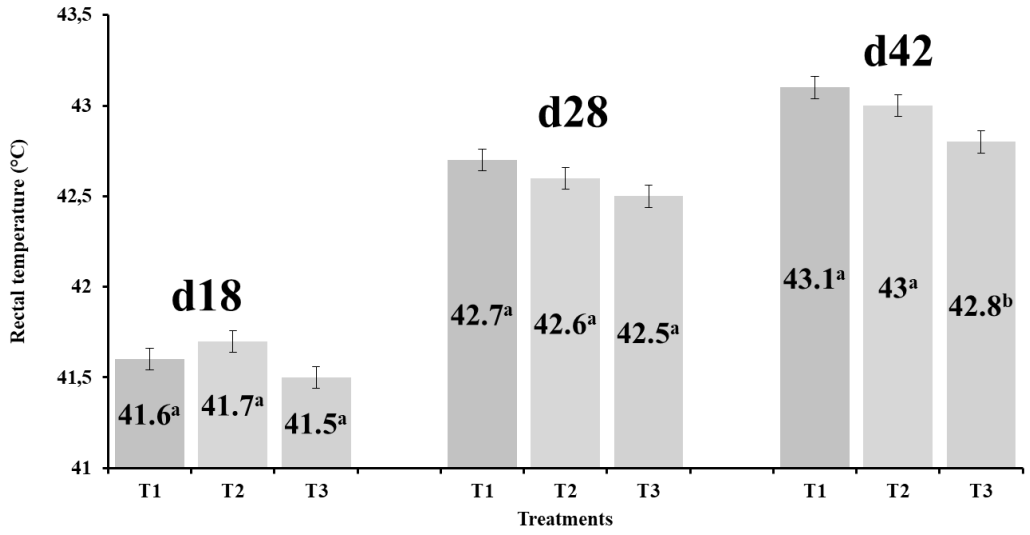
3. RESULTS AND DISCUSSION

3.1. Body temperature of ducks

The results show that supplementation decreased the body temperature of ducks under heat stress significantly at d42; however, only by several degrees ($P<0.05$) (Figure 1). At d28, there was no significant difference between treatments. We suggest this could be because of the adaptation ability of ducks against high environmental temperature. These results show that during constant high environmental temperature, the adaptation ability of ducks decreased; therefore, supplementation is recommended to reduce body temperature. According to different studies, the body temperature of poultry during short-term heat stress increased (ALTAN et al., 1999; XIE et al., 2014); however, during long term (constant) heat stress, the rectal temperature is not elevated in poultry (XIE at al., 2014).

Figure 1 **Effect of heat stress on the rectal temperature of ducks in different treatments at 18d, 28d and 42d (LS means ± SEM^{+})**

(n=240datas/treatment)



*LS means=least squares means; SEM=standard error of the mean.
[a, b] Different superscripts indicate significant differences between groups at $P<0.05$ level.

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3.2. Antioxidant defence mechanisms

The results of the antioxidant parameters of ducks under heat stress can be seen in *Table 3.*

Table 3

Effects of heat stress on antioxidant parameters of ducks (LS means ± SEM[+])

Antioxidant parameters (n=15/treatment)	Treatments[T]			P-value	Probability Treatment
	T1	T2	T3		
MDA (nMol/µl)	13.2 ± 1.45[a]	4.4 ± 1.87[b]	4.7 ± 1.45[b]	0.001	***
Vitamin E (µg/ml)	1.15 ± 0.08[a]	0.98 ± 0.08[ab]	0.84 ± 0.08[b]	0.02	*
Vitamin C (µg/µl)	2.2 ± 0.30[a]	3.3 ± 0.30[b]	2.8 ± 0.30[a]	0.05	*
SOD (U/ml)	104.2 ± 8.22[a]	113.2 ± 8.22 [a]	70 ± 9.19[b]	0.005	**
ACW (µg/ml ascorbic acid)	19.6 ± 3.95[a]	31.01 ± 3.95[b]	68.2 ± 4.16[c]	0.0001	***
ACL (µg/ml trolox)	21.05 ± 1.36[a]	22.1 ± 1.52[a]	33.9 ± 1.36[b]	0.0001	***
GPx (mU/ml)	88.95 ± 2.91[a]	70.4 ± 3.06[b]	79.3 ± 3.06[c]	0.001	***
GR (mU/ml)	46.01 ± 4.26[a]	40.6 ± 4.26[a]	36.3 ± 4.49[a]	0.3	NS
GSH (µM/ml)	14.4 ± 2.28[a]	22.1 ± 2.28[b]	13.2 ± 2.28[a]	0.02	*

[T] Treatments and codes are defined in Table 2.
[+]LS means=least squares means; SEM=standard error of the mean.
*[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).

Increased environmental temperature will cause increased lipid peroxidation and induced formation of MDA; therefore, the antioxidant defence system is altered. According to other studies with Vitamin C, E, Se and chromium (Cr) supplementation (LESCOVEC et al., 2018; MAINI et al., 2007; SAhin et al, 2001a, 2001b; 2002a, 2002b) decreased MDA concentration. As expected, our results show that MDA level significantly decreased in T2 and T3 (P<0.05) due to the supplementation. Lipid peroxidation decreased and enzymatic and non-enzymatic antioxidant systems improved with supplementation under heat stress (HARSINI et al., 2012; MAINI et al., 2007; SAHIN et al., 2002b).

The concentration of Vitamin E in blood decreased in line with MDA formation in T2 and T3 (P<0.05) however no significance difference between T2 and T3 (P>0.05).

Vitamin E and Se have moderate negative correlation (-0.77), Vitamin C and Zn have a very strong negative correlation with MDA (>-0.8) (*Table 4*). The supplementations

decreased the MDA concentration, therefore it can be concluded that they all influence the lipid peroxidation, however Vitamin C and Zn have a major part in decreasing oxidative stress (HARSINI et al., 2012; LESCOVEC et al., 2018; MAINI et al., 2007; SAHIN et al, 2001a; 2002 a, b).

Table 4

Correlation between the supplementation and some antioxidant parameters

Supplementation	Antioxidant parameters	y-value	Coefficient of determination (R^2)	Pearson's Coefficient of Correlation
Vitamin E	ACL	y=0.0088x+20.13	0.99	0.99
Vitamin C	ACW	y=0.0243x+15.317	0.91	0.96
Vitamin E	GR	y=-0.0035x+44.698	0.66	-0.81
Vitamin C		y=-0.003x+45.213	0.82	-0.91
Se		y=-11.644x+49.798	0.66	-0.81
Zn		y=0.0602x+48.148	0.80	-0.90
Vitamin E	MDA	y=0.0049x+11.043	0.59	-0.77
Vitamin C		y=-0.0043x+11.837	0.77	-0.88
Se		y=-16.353x+18.206	0.59	-0.77
Zn		y=-0,0858x+16,012	0.75	-0.87
Se	Vitamin E	y=-0.6731x+1.4266	0.94	-0.97

y= dependent variable
x=independent variable

We found that concentration of Vitamin C increased significantly in T2 and T3 ($P<0.05$). Vitamin C supplementation in the diet increased Vitamin C concentration in serum of broilers, similar results were found in different studies (KUMAR et al., 2017; MAHMOUND et al., 2004; SAHIN et al., 2002a).

The results showed that the amount of SOD in T2 did not change ($P>0.05$) however decreased significantly in T3 ($P<0.05$). We supposed that this was because the supplemented ascorbic acid, which quickly reacted with the superoxide anions; therefore, the activity of SOD enzyme was not much required. In other studies, SOD activity also decreased by antioxidant supplementation under heat stress in poultry (LIN et al., 2006; MAINI et al., 2007).

Due to the increased vitamin and micro mineral supplementation in the diets, the results of ACW show significant enlargement in T2 and T3 ($P<0.05$). The concentration of ACL did not change in T2 ($P>0.05$) however increased in T3 ($P<0.05$). According to the correlation analysis we found that Vitamin E - ACL; and Vitamin C - ACW both have a

strong, positive correlation (>0.9) (*Table 4*). The increased amount of Vitamin E increased the amount of ACL in blood (TOMAZIN et al., 2013); it is the same with Vitamin C and ACW (KUMAR et al., 2017; MAHMOUND et al., 2004; SAHIN et al., 2002a). This is because these vitamins are components of the water and lipid soluble antioxidant compounds therefore increasing the supplementation improves the amount.

Elimination of H_2O_2 is done by GPx with GSH consumption. We found that GPx significantly decreased in T2 and T3 ($P<0.05$). The activity of GPx in continuous and depends on presence of GSH. GR enzyme regenerates GSH from glutathione disulphide (GSSG) and requires NADPH as source of energy (SISEIN, 2014). Vitamin E, C, Se and Zn has a strong inverse correlation with GR (>-0.8) (*Table 4*). According to the supplementation in T2 and T3 the activity of GR reduced, therefore less GSH was produced (MAINI et al, 2007) which led to reduced GPx activity.

This means that Vitamin C and Vitamin E, the small molecule antioxidants „took over" the primary antioxidant activity from the enzymatic pathway. In case of Vitamin C, -GR is also responsible for Vitamin C conversion therefore when more Vitamin C is supplemented to the diet there were more „active„ forms of Vitamin C in the blood, so decreased amount of GR was needed. Increased concentration of Zn led to increased SOD activity, therefore SOD „take part" the primary antioxidant function from GPx. The increased amount of Se could be cofactor for more GPx, this could result increased GPx activity, therefor less GR activity was needed (LIN et al., 2006; MAINI et al., 2007, SAHIN et al, 2006). With this finding can be explained why in our results 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 supposed 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 with Vitamin E supplementation (MAINI et al., 2007).

In our study Se, supplementation has a very strong negative correlation with Vitamin E (-0.97) (*Table 4*). This means that increased amount of Se decreased the amount of Vitamin E concentration in blood. This could be because the Se is cofactor for GPx (HARSINI et al, 2012), therefore the enzyme activity increased (the enzymatic pathway is dominate) therefore less Vitamin E (small molecule antioxidant) was „needed" in the blood to scavenge the increased amount of free radicals.

3.3. Digestibility

Our results show that digestibility of DM and CP in T1, T2 and T3 did not change significantly (P<0.05) due to the supplementation under hot environmental conditions (*Table 5*).

Table 5

Effects of heat stress on digestibility of DM and CP (LS means ± SEM[+])

Digestibility (%) (n=15/treatment)	**Treatments[T]**			**Probability Treatment**
	T1	T2	T3	
Dry matter	77.9 ± 0.60	79.0 ± 0.54	79.6 ± 0.54	NS
Crude protein	73.1 ± 1.00	74.3 ± 0.91	73.5 ± 0.91	NS

[T] Treatments and codes are defined in Table 2.
[+]LS means=least squares means; SEM=standard error of the mean.
NS=non-significant (P>0.05).

Results of crude protein (CP) in our experiment show the same data as in other studies, in which digestibility of CP ranges from 67% (BONNET et al. 1997; SEVEN AND SEVEN 2011) to 80-84% (HOSSEINI et al., 2016). Digestibility of DM in broilers can vary from 66% (BONNET et al., 1997) to 73% (SEVEN AND SEVEN, 2011). Vitamin C addition under heat stress in general improved the digestibility of nutrients, although in our study this was not expressive.

It can be concluded that digestibility of nutrients (DM, 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). Based on different experiments, it was found that heat stress has negative effects on nutrient digestibility (DAGHIR, 2008; HAI et al., 2000). However, it should be noted that there are inconsequence and limited number of scientific results on the digestibility of nutrients in ducks under heat stress.

3.4. Performance and efficiency of nutrient conversion

The results of the production parameters are presented in *Table 6*.

The number of dead birds was very low and there was no difference (P>0.05) between the treatments. The feed intake significantly increased in T2 and T3 (P<0.05). The live weight of the birds at 14d did not differ between the treatments (P>0.05). Our results show that live weight at d42 (at the end of the experiment) significantly increased in T2 and T3 (P<0.05). The daily weight gain also improved in T2 and T3 (P<0.05). Feed conversion ration decreased in T2 and T3 (P<0.05). Energy and protein conversion ratio both decreased significantly (P<0.05) in T2 and T3. The most important economical parameter is specific feed cost which decreased in T2 and T3 (P>0.05) due to the supplementation.

Table 6

Effects of heat stress on performance (LS means ± SEM[+])

Production parameters	Treatments[T]			P-value	Probability Treatment	n
	T1	T2	T3			
Dead birds	2 ± 0.43	2 ± 0.43	1 ± 0.43	0.81	NS	300
FI (g/day/bird)	125 ± 0.44[a]	127 ± 0.44[b]	126 ± 0.44[b]	0.002	**	15[x]
Live weight at 14d (g/bird)	692 ± 16.49	687 ± 16.49	672 ± 16.49	0.69	NS	300
Live weight at 42d (g/bird)	1899 ± 47.84[a]	2127 ± 46.21[b]	2176 ± 46.21[b]	0.0003	**	300
dWG (g/day/bird)	40.6 ± 2.41[a]	49.1 ± 2.32 [b]	51.8 ± 2.32[b]	0.005	**	300
FCR (kg/kg WG)	2.8 ± 0.08[a]	2.6 ± 0.08[b]	2.4 ± 0.08[b]	0.009	**	15[x]
Energy CR (MJ AME_n/ kg WG)	345.3 ± 10.11[a]	309. 2 ± 9.76[b]	285.1 ± 9.76[b]	0.0005	***	15[x]
Protein CR (g protein / kg WG)	459.9 ± 14.43	449.9 ± 13.94	428 ± 13.94	0.28	NS	15[x]
Specific feed cost ($/WG)	0.83 ± 0.58	0.71 ± 0.58	0.68 ± 0.58	0.2	NS	15[x]

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.
[x] =5 data/treatment (1 data/cage).
[+]LS means=least squares means; SEM=standard error of the mean.
[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).

Based on other studies performance of poultry improved under various high temperature conditions with Vitamin C (ATTIA et al., 2011; FAROOQI et al., 2005; SAHIN et al., 2001a; 2002a) and Vitamin E supplementation (HABIBIAN et al., 2014; HARSINI et al., 2012; HASHIZAWA et al., 2013; SAHIN et al., 2001a), which can be explained by their function as scavenging free radicals. Zinc supplementation has positive effects on broiler performance (e.g. FI, WG, FCR) because it works as a cofactor for antioxidant enzyme (KUCUK, 2008; NOVA AND ZEIN, 2020; SAHIN et al., 2003, 2006, 2009). Selenium is also improving production parameters of broilers (BW, FI, FCR) based on its cofactor function (HARSINI et al, 2012; NIU et al., 2009; SUCHY et al, 2014).

Our results of the energy conversion decreased significantly due to supplementation. This could be because the ducks needed larger energy demand for maintenance, principally for body temperature regulation. The termoregulation in birds include diverse mechanisms which cause increased energetic expense and cause lower energy efficiency (DE SOUZA et al., 2016). The protein conversion decreased in line with the energy. The feed cost decreased with the vitamin and mineral supply under heat stress because the FCR improved greater than the difference between the price of the different diets.

Vitamin E, C, Se and Zn both have a strong positive correlation with live weight at 42d (>0.85) and with dWG (>0.88) *(Table 7*). This means that supplementation improves the weight of the ducks. The correlation between FCR and Vitamin E, Se and Zn is very strong negative (-9), therefore increased supplementation in the diet improved FCR (*Table 7*). These results show similar effects in other studies done with poultry (ATTIA et al., 2011; FAROOQI et al., 2005; HABIBIAN et al., 2014; HARSINI et al., 2012; NIU et al., 2009).

Table 7

Correlation analysis between the supplementation and some production parameters

Supplementation	Production parameters	y-value	Coefficient of determination (R^2)	Pearson's Coefficient of Correlation
Vitamin E	Live weight at 42d (g/bird)	y=0.1653x+1950,5	0.73	0.85
Vitamin C		y=0.1388x+1928.8	0.88	0.93
Se		y=550.95x+1709.2	0.73	0.85
Zn		y=2.8001x+1792	0.86	0.93
Vitamin E	dWG	y=0.0068x+42.371	0.78	0.88
Vitamin C		y=0.0056x+41.567	0.91	0.95
Se		y=22.619x+32.464	0.78	0.88
Zn		y=0.1134x+36.014	0.90	0.95
Vitamin E	FCR	y=0.0003x+2.7817	0.94	-0,98
Se		y=-0.8571x+3.1571	0.96	-0,98
Zn		y=-0.0041x+3.0011	0.99	-0,99

y= dependent variable
x=independent variable

3.5. Chemical composition of valuable meat parts

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 (*Table 8*). Therefore, according to our results, the supplementation does not have improved effects on the chemical composition of duck leg meat under high environmental temperatures. The same tendency as the results for breast meat (*Table 9*). Mostly, the parameters were not affected by the supplementation; however, the DM content decreased in breast meat in T2 ($P<0.05$). 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; ZABOLi et al., 2018). The chemical composition of duck breast in different experiments determined as 20-21% protein and 1.5-3.6% fat content, as sampled under thermoneutral conditions and without increased vitamin supplementation (GALAL et al., 2011; HEO et al., 2015). TANKSON et al (2001) reported that high environmental temperature caused reduction in protein content of poultry carcass.

According to our 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 level. Based on the literature, heat stress has negative effects on muscle glycogen level because glycogen breakdown and depletion increased (FLETCHER, 2002; KHAN, 1971).

Table 8

Effects of heat stress on chemical composition of duck leg meat (LS means ± SEM[+])

Thigh meat composition (%) (n=15/treatment)	Treatments[T]			Probability Treatment
	T1	T2	T3	
Dry matter content before drying (%)	23.8 ± 0.21	23.5 ± 0.21	23.6 ± 0.24	NS
Protein [1]	83.6 ± 0.92	84 ± 0.99	86.3 ± 1.03	NS
Fat [1]	9.9 ± 0.94	9.9 ± 0.91	7.9 ± 0.98	NS
Glycogen [1]	0.56 ± 0.02[a]	0.49 ± 0.02[b]	0.48 ± 0.02[b]	*

[T] Treatments and codes are defined in Table 11.
[+] LS means=least squares means; SEM=standard error of the mean.
[1] =based on 100g dried sample.
[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 9

Effects of heat stress on chemical composition of duck breast meat (LS means ± SEM[+])

Breast meat composition (%) (n=15/treatment)	Treatments[T]			Probability Treatment
	T1	T2	T3	
Dry matter content before drying (%)	23.6 ± 0.18[a]	22.9 ± 0.19[b]	23.5 ± 0.19[a]	*
Protein [1]	90.3 ± 0.57	89.8 ± 0.57	90.8 ± 0.59	NS
Fat [1]	2.5 ± 0.42	3.3 ± 0.44	2.2 ± 0.46	NS
Glycogen [1]	0.85 ± 0.03[a]	0.69 ± 0.03[b]	0.7 ± 0.03[b]	**

[T] Treatments and codes are defined in Table 11.
[+] LS means=least squares means; SEM=standard error of the mean
[1] =based on 100g dried sample.
[a, b] Different superscripts in the same row indicate significant differences between groups at P<0.05 level. NS=non-significant (P>0.05).
**P<0.01;*P<0.05; NS=non-significant (P>0.05).

4. NEW SCIENTIFIC RESULTS

1. The serum antioxidant capacity (reflected by ACL= lipid soluble antioxidant compounds and ACW=water soluble antioxidant compounds) improved, while the lipid peroxidation (indicated by MDA=malondialdehyde) significantly decreased in meat type ducks ($P<0.05$) under heat stress (30 ± 1°C between d14-42) if the feed is supplemented with 1540 mg Vitamin E, 998 mg Vitamin C, 0.60 mg Se and 97 mg Zn on kg feed basis.

2. Different levels of Vitamin E, Vitamin C, selenium and zinc supplementation improved the performance parameters (live weight, weight gain, feed conversion) of meat type ducks exposed to chronic heat stress.

3. There is a positive correlation (>0.9) between Vitamin E supply of the diet-serum lipid soluble antioxidant compounds (ACL) and the Vitamin C supply of the diet – serum water soluble antioxidant compounds (ACW). The Vitamin E and Se supply of the diet have moderate negative correlations (-0.77) with serum malondialdehyde (MDA) level, while Vitamin C and Zn supply of the diet have very strong negative correlation with malondialdehyde (MDA) level (>-0.8) under heat stress in meat type ducks.

4. Dietary levels of Vitamin E, C, Se and Zn have a strong positive correlation with live weight at 42d (>0.85) and with dWG (>0.88) of meat type ducks, while Vitamin E, Se, Zn supply of the diet and feed conversion ratio (FCR) have very strong negative correlation (-0.9) under heat stress (30 ± 1°C).

5. The digestibility of crude protein and dry matter, the chemical composition of duck meat (thigh and breast) are not influenced ($P>0.05$) under constant environmental temperature due to antioxidant vitamin and mineral supplementation (Vitamin E: 1540 mg/kg diet, Vitamin C: 998 mg/kg diet, Se: 0.60 mg/kg diet and Zn: 97 mg/kg diet) of meat type ducks under heat stress (30 ± 1°C).

6. High dose of dietary supplementation of antioxidants (1540 mg Vitamin E, 998 mg Vitamin C, 0.60 mg Se and 97 mg Zn on kg feed basis) can alleviate heat stress induced elevation of body temperature in meat type ducks and substantially increase the level of both the lipid soluble (ACL) and water soluble antioxidant compounds (ACW) in the blood.

5. IMPORTANT RESULTS OF THE THESIS FOR PRACTICE

1. The deterioration in production parameters (FI, dWG, FCR) of the meat-type ducks under heat stress could be reduced significantly if the Vitamin E-, C, selenium and zinc content of the diets are higher than the used concentrations in the practice nowadays.
2. According to our findings, at constant high ambient temperature (30 ± 1°C) we recommend to use in practical duck nutrition the following vitamin and micro-mineral concentration in the diet: Vitamin E: 540 mg/kg diet, Vitamin C: 998 mg/kg diet, Se: 0.60 mg/kg diet and Zn: 97 mg/kg diet.
3. A "special summer" premix (increased Vitamin E, Vitamin C, Se and Zn content in diet like the amounts in our study) should be developed for practice to reduce the harmful effects of high ambient temperature (heat shock) in intensive duck meat production.

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7. LIST OF PUBLICATIONS

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List of publications related to the dissertation

Foreign language international book chapters (1)

1. Babinszky, L., **Horváth, M.**, Gálné Remenyik, J., Verstegen, M. W. A.: The adverse effects of heat stress on the antioxidant status and performance of pigs and poultry and reducing these effects with nutritional tools.
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Hungarian scientific articles in Hungarian journals (2)

2. **Horváth, M.**, Pesti-Asbóth, G., Gálné Remenyik, J., Babinszky, L.: A hőstressz káros hatása a brojler antioxidáns státuszára és ezen hatás csökkentése takarmányozással: I. rész A hőstressz és az antioxidáns védelmi rendszer.
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7. **Horváth, M.**, Pesti-Asbóth, G., Gálné Remenyik, J., Babinszky, L.: A tartós hő-stressz káros hatásának csökkentése takarmányozási módszerekkel a pecsenyekacsa tartásban.
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List of other publications

Hungarian book chapters (1)

11. **Horváth, M.**, Babinszky, L.: A takarmányok kémiai összetétele: Vitaminok és kölcsönhatásaik. In: Innovatív takarmányozás. Szerk.: Babinszky Lástló, Halas Veronika, Akadémiai Kiadó, Budapest, 55-104, 2019. ISBN: 9789634540571

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12. **Horváth, M.**, Babinszky, L.: A hőstressz káros hatásának csökkentése nagy genetikai kapacitású hízósertésekben speciális diétákkal.
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13. **Horváth, M.**, Pesti-Asbóth, G., Gálné Remenyik, J., Babinszky, L.: Mikrobiális fermentációs eljárással előállított új probiotikum felhasználásának lehetősége a brojler takarmányozásban. In: Tavaszi szél 2018 : Tanulmánykötet. Szerk.: Szabó Csaba, Doktoranduszok Országos Szövetsége, Budapest, 44-53, 2018. ISBN: 9786155586316

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Total IF of journals (all publications): 1,493
Total IF of journals (publications related to the dissertation): 1,386

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

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