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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

By
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TABLE OF CONTENTS

List of Abbreviations

14 d= 14 days

18 d= 18 days

28 d= 28 days

42 d=42 days

Aa= ascorbic acid

ACL= lipid soluble antioxidant compounds

ACW=water soluble antioxidant compounds

AOAC= Association of Official Agricultural Chemists

BT = body temperature

BW= body weight

C3= 3-carbon

C4 = 4-carbon

CAM= crassulacean acid metabolism

CAT= catalase

Co= copper

CO_2= carbon-dioxide

CP= crude protein

Cr= chromium

DM= dry matter

dWG= daily weight gain

EDTA= ethylenediaminetetraacetic acid

EFSA= European Food Safety Authority

ELISA= enzyme-linked immunosorbent assay

ER= endoplasmatic reticulum

FAO= Food and Agriculture Organization

FCR= feed conversion ratio

Fe= iron

FI= feed intake

GLM= general linear model

GPx= glutathione peroxidase

GR= glutathione reductase

GSH= glutathione

GSSG= glutathione disulphide

H_2O= water

H_2O_2= hydrogen peroxide

HCl= hydrochloric acid

HCT= high critical temperature

HSF= heat shock factor

HSP70= heat shock protein 70

LCT= low critical temperature

LDL= low-density lipoprotein

LS means= least squares means

MDA= malondialdehyde

Mn= manganese

NaCl= sodium chloride

NADH= nicotinamide-adenine-dinucleotide

$NADP^+$= oxidised nicotinamide-adenine-dinucleotide-phosphate

NADPH= nicotinamide-adenine-dinucleotide-phosphate

NRC= National Research Council

O_2^- = superoxide anion radical

OH^-= hydroxyl radical

PSE= pale soft exudative meat

PUFA= polyunsaturated fatty acid

RNS= reactive nitrogen species

ROO^-= peroxyl radical

ROOH= hydro peroxide

ROS= reactive oxygen species

Se= selenium

SEM= standard error of the mean

SiO_2= silicon dioxide

SOD= superoxide dismutase

T-AOC= total antioxidant capacity

WG= weight gain

Zn= zinc

1. INTRODUCTION

The effects of climate change are multidisciplinary in nature: human–plant–animal health and environmental, and they can cause severe damage in production animal origin foodstuffs for human consumption. Climate change may enhance or modify the occurrence and intensity of some food origin diseases, which are harmful to plants and animal health. It can increase exposure to known hazards or contribute to the emergence of new hazards, as well as change the micronutrients and macronutrients in food and feed items (EFSA STRATEGY 2020).

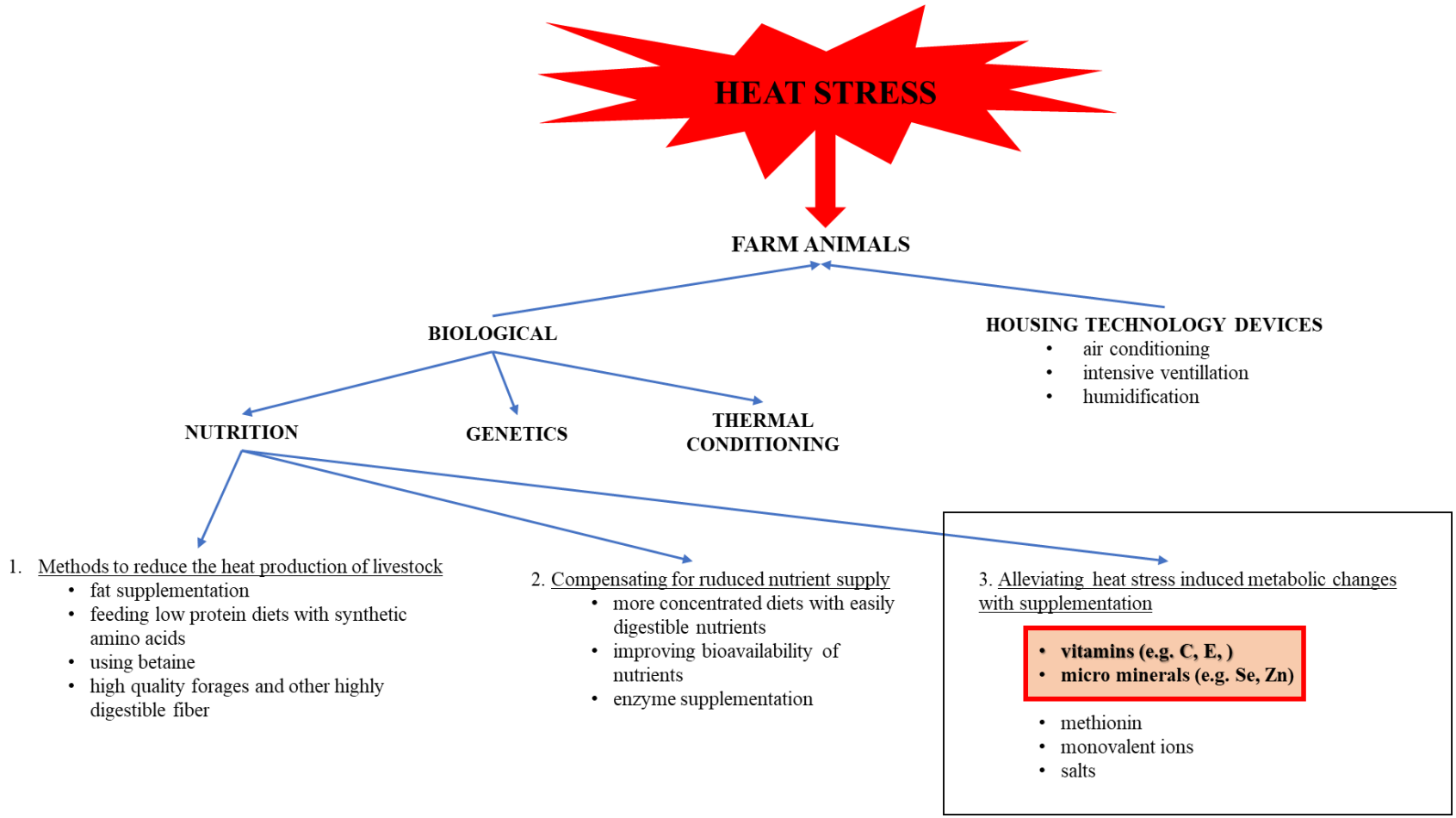
The increasing ambient temperature and inordinate types of weather caused by climate change have impacts on agriculture: on plant, crop and livestock production and even on product quality. Most of the grain cereals (e.g. wheat, soybean, barley, rice) are C3 plants; these plants fix and reduce inorganic CO2 into organic compounds using the C3 pathway in photosynthesis. C4 (e.g. corn, millet, sorghum) and CAM plants (e.g. cacti, agave) can use both C3 and C4 cycles. Because of the unfavourable climate, feed crop production should be changed: e.g. irrigation-based precipitation, feed crop varieties should be used which have improved drought resistance, more C_4 and CAM plants because both result in better water use efficiency and adapt better to arid conditions. (BABINSZKY et al., 2011a, 2011B; BABINSZKY et al., 2019).

Heat stress is one of the prominent environmental elements which can influence meat quality (LARA AND ROSTAGNO, 2013; WANG et al., 2017). High environmental temperature may cause reduced performance and increased mortality in farm animals. Based on the literature data, it is well known that higher environmental temperatures may have consequences that are more serious and lead to heat stress in poultry. 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 bodies are covered with feathers and they do not have sweat glands (AVILÉS-ESQUIVEL et al., 2018; LARA AND ROSTAGNO, 2013). Regardless of what is happening with climate change, the importance of duck meat is increasing worldwide. Duck meat is a highly appreciated meat source, and offers many

of the benefits of red meat. It contains a high composition of essential amino acids and a high percentage of polyunsaturated fatty acid (PUFA) (QIAO et al, 2017). The demand for duck meat is increasing, with production rising by 3% each year according to the latest FAO (2019) statistics. Worldwide duck production is expected to intensify to reduce the risk of losses due to the changing climate and infection, as well as because of successful and economic production. Therefore, it is necessary to use indoor, intensive production methods with intensive nutrition and concentrated nutrient content, in addition to the environmental temperature being equal to the animal's thermo neutral zone (CHERRY AND MORRIS, 2008). However, there is not much relevant information published about their nutrition, especially under severe environmental conditions and only a very limited number of scientific studies carried out specifically on 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. Generally, feed additives with direct or indirect antioxidant effects can be used, containing only added vitamins or even micro minerals (**Figure 1**).

Figure 1 **Reduction of negative effects of heat stress in farm 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 during times of heat stress, and we describe their significance.

It is well known that the elimination of the free radicals activates the three level antioxidant system (see later in "Literature overview") which helps to protect livestock against heat stress (ARAI et al, 1987; HORVÁTH AND BABINSZKY, 2019; IRSHAD AND CHAUDHURI, 2002). According to the latest 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 (GSH) (CHAN AND DECKER, 1994; GROSS et al, 2013; NIKI et al, 1983; PACKER, 1992).

Based on the scientific findings, it could be concluded that there is very limited relevant information and are hardly any systematic studies about ducks under long-term heat stress. Many researchers have been studying the effect of high environmental temperature or heat stress on performance and some antioxidant enzymes in broilers, but there are very limited comprehensive studies on the repair mechanisms of the entire antioxidant system (ATTIA et al., 2011; HABIBIAN et al., 2014; HARSINI et al., 2012; KUMAR et al., 2017; LIAO et al., 2012).Unfortunately, these studies mostly focus on a narrow part of the antioxidant system, usually to either level and only in broilers; an excellent review by BELHADJ SLIMEN et al. (2016) described the effects of heat stress on livestock according to their molecular, cellular and metabolic aspects. Therefore, it is demonstrated in this dissertation how the antioxidant system can be repaired with vitamin and mineral supplementation.

According to our view, knowing the impact of vitamin and mineral supply on the antioxidant system helps one to understand why it is necessary to supplement poultry diets while animals are suffering from 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. LITERATURE OVERWIEW

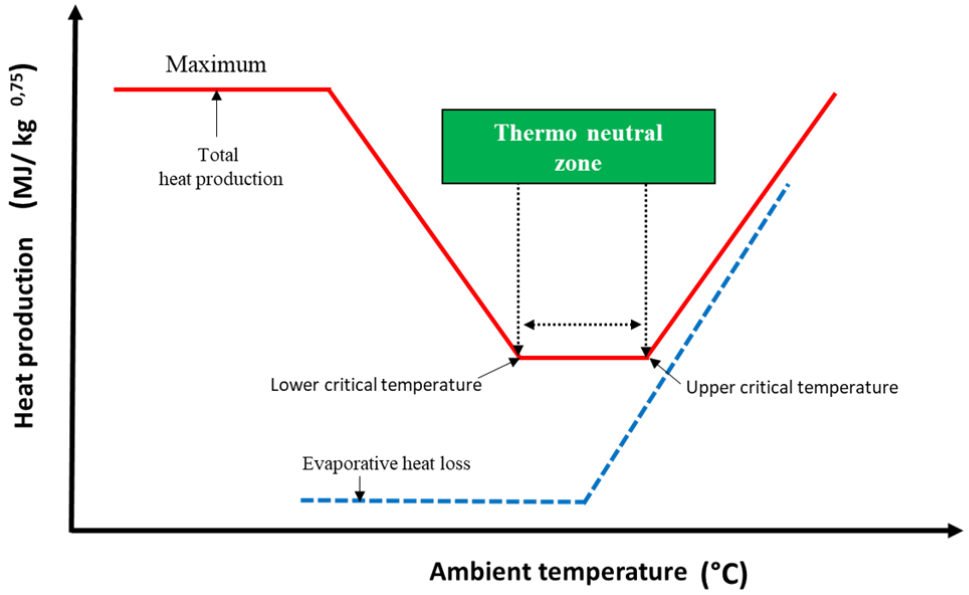
2.1. Effects of heat stress on energy metabolism in farm animals

Physiological processes are associated with heat production, which is the sum total of non-productive energy utilized by the animal and of the energy "lost" in the course of converting dietary nutrients. Non-productive energy is used for maintenance (e.g. the energy requirement of the maintenance of body temperature, the nervous system, for minimal activity). The extra heat (which is produced by digestion, excretion and metabolism of nutrients) is called the heat increment.

The comfort zone is defined as the temperature zone in which the animals are able to keep their body temperature constant with minimum effort. The thermo neutral zone is the temperature zone in which the birds are able to keep their body temperature constant with the help of physical heat regulation (**Figure 2**). 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 thermo neutral zone is called the lowest critical temperature (LCT). If temperature 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 entirely their heat, this will cause "heat shock". The energy and nutrition metabolism will be affected; therefore, the animals will start to consume less feed and the production will decrease (BABINSZKY et al., 2019; NOBLET et al., 2001).

Figure 2 **Relationship between ambient temperature and heat production of livestock**

(BABINSZKY et al., 2019)



2.2. Supporting the balance between redox homeostasis: the antioxidant system

Based on the literature data, it is known that heat stress can cause several kinds of damage to organisms. There can be biochemical and physiological changes, e.g. changes in the antioxidant status, increased free radical formation and stress hormone appearance, the homeostasis (acid/base balance) is upset and the resistance capacity decreases (AKBARIAN et al., 2015; LI, 2011; YANG et al., 2010). In order to better understand the main attributes of free radicals, antioxidants and the general mechanism of the three level antioxidant system will be demonstrated in the next sections.

2.2.1. Free radicals

A free radical can be an atom or molecule, which contains unpaired electrons, and are highly chemically reactive. They can also be reactive oxygen (ROS) or nitrogen species (RNS). ROS are produced by chemical, physical and biological mechanisms associated with metabolic processes in mitochondria, peroxisomes and endoplasmic reticulum (ER). They are important signal molecules, thought to be involved in cellular signals controlling gene expression. The healthful or damaging properties of ROS depends on the balance between ROS production and their scavenging (RAY et al., 2012). Lipid peroxidation is

a process which is generated naturally in small amounts in the organism through the effect of ROS (MYLONAS AND KOURETAS, 1999) and it can be measured by the concentration of malondialdehyde (MDA) which is produced from PUFAs, and the most measured biomarker of lipid peroxidation (TSIKAS, 2017). ROS levels are maintained with antioxidant scavenging systems and defence components; however, different conditions (e.g. high environmental temperature) can alter the balance between ROS levels and lead to more free radical formation. High levels of ROS can alter important molecules (DNA, proteins, lipids) and lead to pathological conditions (e.g. apoptosis, necrosis, inflammation) (LI, 2011). However, ROS play an important role as secondary messengers in cellular processes and also support defence mechanisms and repair (RAY et al, 2012).

2.2.2. Antioxidants in general

Antioxidants are elements that counteract the damage caused by radicals. They are capable of deactivating or stabilizing free radicals and they help to protect healthy cells (IRSHAD AND CHAUDHURI, 2002). Antioxidants can act in reducing the production of ROS (chelating agents), radical scavengers, hydrogen (H+) and electron (e-) donors, singlet oxygen quenchers, enzyme inhibitors or repairing enzyme systems. Antioxidants are produced in animal organisms under normal conditions, but not in amounts which would be enough to protect cells against increased free radicals. To protect the organism from damage, cells have different antioxidant systems (KURUTAS, 2016).

Exogenous antioxidants, such as vitamins, must be provided from exogenous sources, mostly in the diet or from biosynthesis by microorganisms in the gastrointestinal tract (ROMERO et al., 2013). There are enzymatic antioxidants: primary (catalase-Cat, superoxide dismutase-SOD, glutathione peroxidase-GPx) or secondary (glutathione reductase-GR). The antioxidant enzymes require co-factors, e.g. Se, Zn and others (Fe, Co, Mn) for optimum activity. Non-enzymatic antioxidants include, vitamins (e.g., C, A, E) and minerals (e.g., Se, Zn). (RAHMAN, 2007).

2.2.3. The Antioxidant System

The antioxidant defence system plays a very important role in the reduction of the heat stress generated lipid peroxidation process. In the acute phase (heat stress), the

antioxidant-prooxidant balance is upset, with the balance shifted to the prooxidant phase. Therefore, the lipid peroxidation processes can cause necrosis in the organism. To re-establish this balance, the antioxidant defence system will be activated. Stress leads to increased production of reducing equivalents: NADH, NADPH, GSH and increase the activity of enzymatic defences (BERRY AND KOHEN, 1999). Protection against ROS can be prevention, interception and repair (SIES, 1992). According to the latest research, the elimination of the free radicals activates the three level antioxidant system **(Figure 3.).**

As can be seen in **Figure 3,** NADPH oxidase is a superoxide and $NADP^+$ oxidase is a hydrogen peroxide (H_2O_2) forming enzymes of cytosol (**Figure 3, number 1**). Elimination occurs on by the first level of the antioxidant system, activated at the same time as the detoxification and regeneration pathways of the second level. The third level starts activating after the damage, to repair and eliminate damaged cells.

This first level (direct enzymatic pathway) includes the neutralization of the oxygen and nitrogen centred free radicals by enzymes. A large amount of free radicals forms under heat stress, which can be partially eliminated by the direct enzymatic pathway. A small part of oxygen will be transformed into superoxide anion radical (O_2^-) (BOVERIS, 1984). O_2^- has to be converted or scavenged. The principal enzymes, which regulate this, are SOD, GPx and CAT. SOD enzyme catalyses the dismutation of O_2^- to H_2O_2 (**Figure 3, number 2**). GPx enzyme is present in cytosol and mitochondria. It removes H_2O_2 and organic hydro peroxides using four Se cofactors. It also catalyses the oxidation of GSH.

Activity of GPx is dependent on the availability of GR and NADPH, which are supplied by the pentose phosphate pathway (**Figure 3, number 3**). CAT can remove H_2O_2 and converts it into water and molecular oxygen, using Fe or Mn cofactor. It is located in the peroxisomes and erythrocytes (CONNER AND GRISHAM, 1996; JEEVA et al., 2015) (**Figure 3, number 4**).

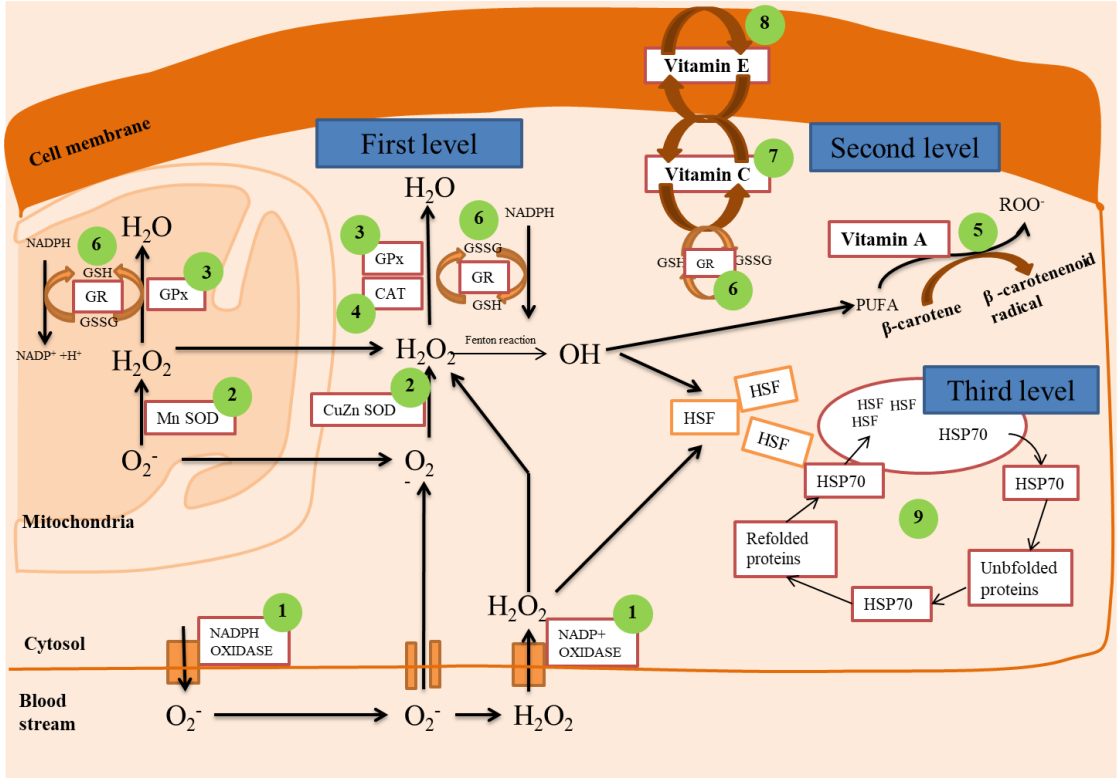
The second level includes the detoxification and regeneration reactions of the small molecule antioxidants. Vitamin A is a carotenoid and it can be produced as a result of the breakdown of β-carotene in the liver. However, most of the carotene is converted to Vitamin A by the carotenase enzyme in the epithelial cells of the small intestine during absorption. The efficiency of the conversion of β-carotene to Vitamin A depends on many

factors in addition to the species of the animal. The transformation in best is poultry (50%), medium (about 20%) in pigs and sheep, and worst (5-15%) in cattle. It is able to combine with peroxyl radical (ROO^-) before they propagate peroxidation to lipids (**Figure 3, number 5**). GSH can donate a hydrogen atom or an electron and regenerate ascorbate. GR is an enzyme which catalyses the reduction of GSSG to GSH-form using NADPH (**Figure 3, number 6**). Vitamin C is capable of scavenging hydroxyl radical (OH^-), singlet oxygen, H_2O_2 and O_2^-. It also regenerates Vitamin E: Vitamin C reinstates the antioxidant potential of Vitamin E by regenerating the tocopheroxyl radical into an intermediate form (CONNER AND GRISHAM, 1996; IRSHAD AND CHAUDHURI, 2002). (**Figure 3, number 7**). Vitamin E is present in cell membranes and known as a chain-breaking antioxidant. It can inhibit lipid peroxidation (**Figure 3, number 8**).

The third level is activated when damaged systems (proteins, DNA) have to be repaired and/or removed from the cells by chaperones and DNA-repair enzymes. The most known heat stress protein is heat shock protein 70 (HsP70) which is located in the cytoplasm. The expression of HSPs is regulated by heat shock factors (HSF) through their association to bind to heat shock elements (HSE) (IRSHAD AND CHAUDHURI, 2002.) (**Figure 3, number 8**).

Figure 3 **Three level antioxidant system**

(Based on BABINSZKY et al, 2019)



CAT= catalase; Cu SOD= copper superoxide dismutase; GPx= glutathione peroxidase; GR=glutathione reductase; GSH= glutathione; GSSG= glutathione disulphide; H_2O= water; H_2O_2= hydrogen peroxide; HSF= heat shock factors; HSP70= heat shock protein 70; Mn SOD= manganese superoxide dismutase; NADH=nicotinamide-adenine-dinucleotide; $NADP^+$= oxidised nicotinamide-adenine-dinucleotide-phosphate, NADPH=nicotinamide-adenine-dinucleotide-phosphate, O_2^-= superoxide anion radical; OH= hydroxyl radical; PUFA= polyunsaturated fatty acids; ROO^-= peroxyl radical

2.3. Selected antioxidant vitamins and their effects on the antioxidant system

According to the results of 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 during times of heat stress, and we describe their significance.

The vitamin requirements of livestock under various conditions have been the subjects of numerous excellent studies. Different external factors (e.g. temperature, physical stress, increased production) can affect the utilisation of the vitamins supplied. Heat stress decreases the concentration of vitamins and micronutrients in the serum and increase their excretion from the body (KHAN et al., 2012) due to their higher demand in redox repair mechanisms. Therefore, more supplementation is recommended to decrease the formation of free radicals, to support the mechanisms against lipid peroxidation, to improve immune status and performance (MCDOWELL, 1989).

The general properties of Vitamin E and C and micro minerals (Zn, Se) are summarized in many excellent reviews (BRIGELIUS-FLOHE AND TRABER, 1999; IQBAL et al., 2004; KURUTAS, 2016; O'BYRNE AND BLANER, 2013; PARDUE AND THAXTON, 1986; ROSS, 2010; SIES et al., 1992; SEMBA, 1998). Therefore, the present thesis focuses only on a discussion on their importance in the antioxidant system.

2.3.2. Vitamin E

Vitamin E functions as a fat-soluble antioxidant (BRIGELIUS-FLOHE AND TRABER, 1999; KURUTAS, 2016; SIES et al., 1992) which protects cellular and membrane lipids from peroxidation-catalysed free radicals due to heat stress (DAS et al, 2016). Tocopherols as antioxidants act as lipid peroxide-initiated chain reaction breakers, intercept $ROO^{\cdot}$, and protect lipids from oxidation in low-density lipoproteins (LDLs). α-tocopherols can protect the physiological properties of lipid membrane layers and can influence the activity of membrane proteins and enzymes (MAY et al., 1998b). In cell membranes and lipoproteins, the essential antioxidant function of Vitamin E is to trap $ROO^{\cdot}$ and to break the chain reaction of lipid peroxidation. While it cannot prevent their formation, it can reduce the formation of secondary radicals. Tocopherol inhibits the production of free radicals in tissues, due to the quickly reaction with $ROO^{\cdot}$ to form a

relatively stable tocopheroxyl radical (BLOKHINA et al., 2003). Tocopherol is able to transfer a hydrogen atom to a free radical thereby eliminating the free radical before it can interact with proteins and membranes or cause lipid peroxidation. When tocopherol (tocopherol-OH) combines with a free radical it is oxidised and becomes a tocopheroxyl radical (tocopherol-O). Its antioxidant capacity is lost resulting an unpaired electron on the oxygen and therefore becoming a radical itself. If ascorbic acid is available, it bonds with tocopherol-O and forms dehydroascorbate and tocopherol-OH. In this progression, which is shown in **Figure 4,** a small amount of dehydroascorbate is formed and also a tocopheroxyl radical, which will be reduced and return to its reduced state (BURTON AND INGOLD, 1986; TRABER AND STEVENS, 2011). In conclusion, ascorbate recycles the tocopherol radical and regenerates the tocopherol at the aqueous-lipid interface (MAY et al., 1998b).

A tocopheroxyl radical might react in several other ways. α- tocopherol can be regenerated with GR or ascorbate (MAY et al, 1998a)

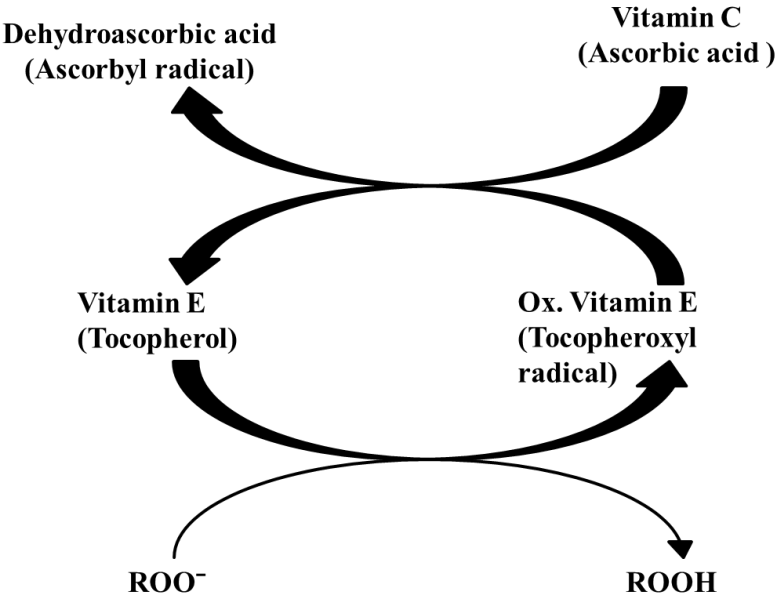
2.3.4. Vitamin C

Vitamin C (also known as ascorbate, ascorbic acid) protects against oxidative stress-induced cellular damage in action of scavenging of ROS, it is capable itself of inhibiting lipid peroxidation in plasma (RETSKY et al., 1993). Ascorbic acid can directly scavenge radicals in the aqueous compartment. In humans, ascorbate can act as a cofactor for enzymes (e.g. α-ketoglutarate-dependent dioxygenases) which catalyst hydroxylation reactions. It can also provide electrons for enzymes. Ascorbate can scavenge O_2^-, H_2O_2, the OH^-, hypochlorous acid, aqueous ROO^-, and singlet oxygen. Under its antioxidant activity, ascorbate has a two-electron reduction (semihydroascorbyl radical and dehydroascorbate). The semidehydroascorbyl radical is relatively stable; however, with the presence of a single electron over the three oxygen atoms, it can be readily detected in the presence of increased free radical production (PIETRI et al., 1994). Dehydroascorbate in plasma is rapidly taken up by red blood cells, so very small amount is present in plasma (KOSHIISHI et al., 1998). It is relatively unstable and can be reduced back to ascorbate by selenoenzyme thioredoxin reductase (MAY et al., 1998a) or by a non-enzyme reaction, which uses GR (MAY et al., 1996).

It has also been shown that ascorbate can inhibit the oxidation of LDL and is more potent than tocopherol (SÁNCHEZ-QUESADA et al., 1998); however, it can also act as a prooxidant, which can lead to oxidative damage in the presence of Fe *(*SUH et al., 1999). The connection between the mechanisms of Vitamin C and E can be seen in **Figure 5**.

As can be seen in **Figure 5**, Vitamin C can regenerate oxidized Vitamin E bound in the membrane. It reacts with the tocopheroxyl radical, resulting in tocopherol, while in this progression; Vitamin C is oxidized into dehydroascorbic acid. GSH can regenerate ascorbate from dehydroascorbate.

Figure 5 **Connection between the antioxidant mechanism of Vitamin E and C**



ROO⁻= peroxyl radical; ROOH= hydro peroxide.

2.4. Selected micro minerals and their effects on the antioxidant system

As mentioned previously, we selected the most common micro minerals used in poultry nutrition under heat stress (DAGHIR, 2009; GOUS AND MORRIS, 2005; LIN et al., 2006; MUJAHID, 2011; SAHIN et al., 2009). They are dietary antioxidants and play a significant role in the metabolism of other antioxidants.

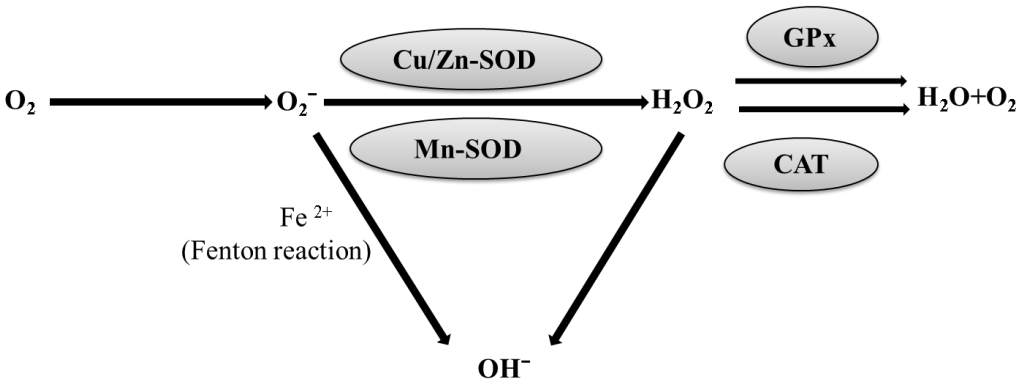
2.4.1. Zinc

Zn is a "member" of the antioxidant network because it is a cofactor of a very important antioxidant enzyme: CuZn-SOD. Zn plays a role in depressing the free radicals and inhibiting lipid peroxidation (PRASAD, 1997) and GSH depletion (GIBBS et al., 1985). Zn can have direct antioxidant function (OTEIZA et al., 1995; Powell, 2000), it is necessary for the prevention of free radical formation. However, it does not act directly against them. Zn is also an inhibitor of NADPH oxidase, which catalyses the production of singlet oxygen radicals from oxygen by using NADPH as an electron donor (SAHIN et al., 2009).

SOD is considered a first line defence against free radicals. Mammalian tissues have three forms of SOD, all of them with a specific subcellular location and different tissue distribution. They are present in the cytoplasm of almost all aerobic cells. SODs can have metal cofactors depending on the isozyme e.g. Zn, Fe, Mn, Co. Mitochondrial and bacterial SOD contain manganese, while cytosolic SOD contain copper and Zn (MEHTA AND GOWDER, 2015). Zn supports the production of an OH^- scavenger called metallothionein, thereby decreasing the production of free radicals (PRASAD et al., 2004).

Figure 6 shows the elimination of free radicals by the direct enzymatic pathway (first level of the antioxidant defence system).

Figure 6. **Enzymatic pathway of eliminating reactive oxygen spices: mechanism of SOD, GPx, and CAT**



CAT= catalase; Cu SOD= copper superoxide dismutase; Fe^{2+}= iron; GPx= glutathione peroxidase; H_2O= water; H_2O_2= hydrogen peroxide; Mn SOD= manganese superoxide dismutase; $O_{2=}$ oxygen; O_2^-= superoxide anion radical; OH-= hydroxyl radical.

Heat stress increase the activity of NADPH oxidise which catalyses the production of large amounts of O_2^-. It is relatively unreactive and spontaneously (or enzymatically) and rapidly dismutased to H_2O_2 and O_2 by SOD. H_2O_2 can still react with other free radicals; therefore, it needs to be removed by CAT or GPx and form into oxygen and H_2O. It is also known that H_2O_2 and O_2^- can interact with Fe to form highly reactive OH^- in Fenton reaction (CONNER AND GRISHAM, 1996, KURUTAS, 2016).

2.4.2. Selenium

Organoselenium compounds are essential micronutrients and are required for cellular defence against oxidative stress and optimal immune function. Se can be found in both inorganic (selenite and selenate) and organic (selenocysteine and selenomethionine) forms (SUCHÝ et al, 2014). Se is necessary for cellular function and is a component of antioxidant enzymes: an important part (cofactor) of GPx, which works as an important antioxidant enzyme, protecting cells against free radical damage and oxidative stress (GILL AND WALKER, 2008).

There are more than 30 distinctive selenoproteins, all containing selenocysteine, each with its own function and distribution (ARTHUR, 1997) which can be seen in **Table 1.**

Table 1

GPx enzyme types

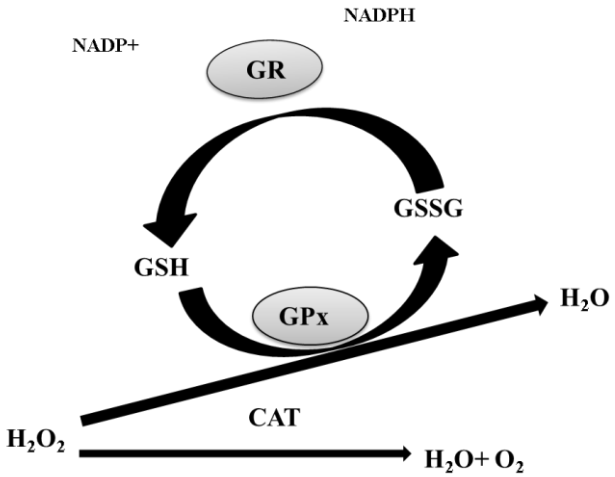
(ARTHUR, 1997)

Nomenclature	Selenoprotein type	Location	Function
GPx1	Cytosolic GSH peroxidase	Tissue cytosol	Antioxidant, storage
GPx2	Plasma GPx	Plasma, kidney, lung	Extracellular antioxidant
GPx3	Phospholipid hyper oxide GPx	Intracellular membranes	Intracellular antioxidant
GPx4	Gastrointestinal GPx	Intestinal mucosa	Mucosal antioxidant
Sel P	Selenoprotein P	Plasma	Antioxidant, transport, heavy metal detoxifier
Sel W	Selenoprotein W	Muscle	Antioxidant

Se is a part of antioxidant enzymes (metalloenzymes, GPx, and thioredoxin reductase) therefore does not act directly on free radicals (TABASSUM et al., 2010). GPx require selenium at the active site. Se deficiency may occur enzyme deficiency (NAKANE et al., 1998). Cytosolic peroxidase (GPx1) is the first identified and most studied from others. However, animals can survive without the gene for GPx1. This suggests that the enzyme itself is non-essential (CHENG et al., 1997). The highest concentration is found in the liver; however, GPx occurs in almost all tissues and is the main scavenger of H_2O_2 (HOLBEN AND SMITH, 1999).

GPx1 catalyses the oxidation of GSH at the expense of a hydro peroxide (TAKAHASHI AND COHEN, 1986). GR restores GSH (this molecule is critical in resisting oxidative stress) by reducing GSSG in the presence of NADPH. **Figure 7** presents the mechanism of GPx and GR. Other peroxides (e.g. lipid hydro peroxides) can also act as a substrate for these enzymes. This can be the reason for their important role in repairing the damage caused by lipid peroxidation. The reduced form of glutathione is GSH; the oxidised form is glutathione disulphide (GSSG). GSSG is accumulated inside the cells (RAHMAN, 2007). GPx removes H_2O_2 by using it to oxidize GR to GSSG. GR enzyme regenerate GSH from GSSG and requires NADPH as a source of power (SISEIN, 2014). The activity is dependent on the constant availability of GR (HOLBEN AND SMITH, 1999).

Figure 7 **Mechanism of GPx and GR**



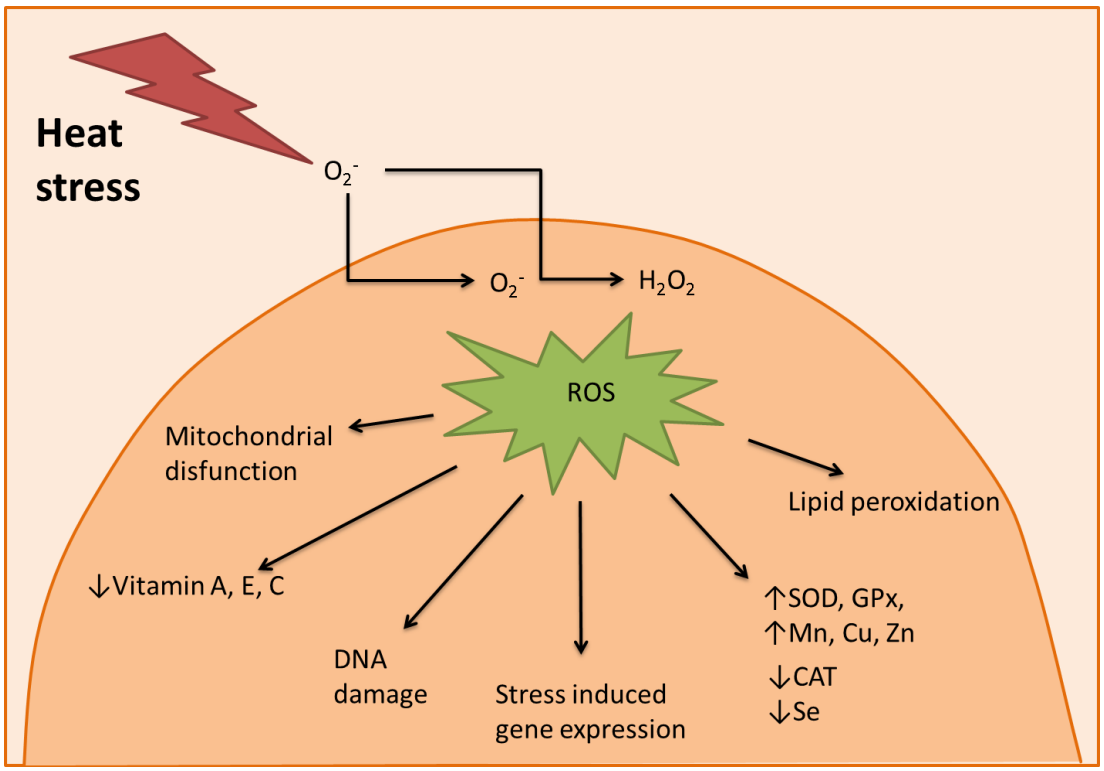
CAT= catalase; GPx= glutathione peroxidase; GSH= glutathione; GSSG= glutathione disulphide; GR= glutathione reductase; H_2O= water; H_2O_2= hydrogen peroxide; $NADP^+$= oxidised nicotinamide-adenine-dinucleotide-phosphate, NADPH=nicotinamide-adenine-dinucleotide-phosphate; $O_{2=}$ oxygen.

2.5. Impact of heat stress on the antioxidant system in poultry

Poultry is more sensitive to heat stress than other domestic animals, because they do not have sweat glands, their metabolism is rapid and they have high body temperatures. Increased environmental temperature caused increased lipid peroxidation (in addition induced formation of MDA, which is an indicator for lipid peroxidation). Therefore, the antioxidant defence system is altered (ALTAN et al., 2003; COSTANTINI et al., 2009; LARA AND ROSTAGNO, 2013).

Figure 8 shows the changes in the organism according to heat stress. Heat stress promotes oxidative stress through increased production of ROS and decreased antioxidant defence (CHAUHAN et al., 2014).

Figure 8 **Negative effects of heat stress on the antioxidant system in poultry**



CAT= catalase; Cu= copper; GPx= glutathione peroxidase; H_2O_2= hydrogen peroxide; Mn=manganese; O_2^-= superoxide anion radical; ROS= reactive oxygen species; Se= selenium; SOD= superoxide dismutase; Zn= zinc.

In general, it can be concluded that a large amount of ROS causes disruption of mitochondrial function, increased lipid peroxidation, decreased Vitamin concentrations, induce stress gene expression, leads to dysfunction in antioxidant enzymes and cause

DNA damage. According to YANG et al (2010), who studied short-term heat stress (35°C for 3h/day) in broilers, the activity of mitochondrial respiratory chain is reduced by heat stress, which leads to over-production of ROS. This resulted in lipid peroxidation and oxidative stress in the birds. Lipid peroxidation and SOD activity was measured in broilers under heat stress (32°C for 6 h/day) in another study (LIN et al, 2006). The results showed that high temperature disturbed the equilibrium between the synthesis and catabolism of ROS production. GPx and SOD activity increased under heat stress (34°C 5h/day from d28 to d38) (AKBARIAN et al., 2015). If SOD level increased, manganese (Mn) and Cu level increases within the cell but at the same time Zn level is remained stable. Consequently, in addition of increased SOD resulted in increased Cu/Zn ratio.

ROS production reduces Vitamin A and E levels. Vitamin C concentration decreased under heat stress in poultry (KUTLU AND FORBES, 1993). It has been reported that heat stress increases Zn mobilization from tissues and thus may cause marginal Zn deficiency and increase requirements (ANDERSON, 1994). ROS has effects on gene expression: heat stress proteins will be formed.

Birds burn fat as fuel and increase their metabolic rate to overwhelm oxidative damage. Birds can consume dietary antioxidants or upregulate their endogenous antioxidant system to mitigate the effects of the increased amount of ROS (COOPER-MULLIN AND MCWILLIAMS, 2016).

2.6. Antioxidant vitamins and microelement supplementation in poultry diets under heat stress

As mentioned before, several methods (biological or technological) are available for use to alleviate the negative effects of heat stress in poultry. As it is usually more expensive to cool buildings, most methods focus on nutritional tools. Different nutritional strategies are known to reduce the negative effects of heat stress, e.g. decreased protein level and different amino acid composition in the diet, increased fat intake, electrolytes in water, probiotics and betaine supplementation (BALNAVE AND BRAKE, 2005; DAGHIR, 2009; GOUS AND MORRIS, 2005; LIN et al., 2006; SZABÓ et al., 2013). High environmental temperatures decrease the concentrations of vitamins and micro minerals in serum and increase the excretion (KHAN et al., 2012); therefore, supplementation of direct or indirect antioxidant compounds (e.g. vitamins and micronutrients) at higher

levels is commonly recommended (YUN et al., 2012). These additives support mechanisms against lipid peroxidation, improve immune status and performance.

The vitamin and mineral requirements of broilers and ducks under thermo neutral conditions are in **Table 2.** As it can be seen in this table the ME does not differ however FHP is higher in ducks therefore more energy is required for maintenance. The higher demand for Zn and Se in ducks emphasizes the importance of dietary antioxidants.

Table 2

Some vitamin and mineral requirement of broilers and ducks

(NRC, 1994)

	Broilers	**Ducks**
Vitamin E	5-60 IU/kg	5-60 IU/kg
Vitamin C	-	-
Zinc	35 mg/kg	60 mg/kg
Selenium	0,01-0,05 mg/kg	0,2 mg/kg
Metabolizable Energy (ME) in grower phase	3050 kcal/kg =12,8 MJ/kg	3010 kcal/kg = 12,6 MJ/kg
Fastening heat production (FHP)	425-490 kJ/kg $BW^{0.75}$/ day *	570 kJ/kg $W^{0,75}$/day **

*NOBLET et al, 2017
** WANG et al., 2016

2.6.1. Antioxidant vitamins and microelement supplementation in duck diets under heat stress

It is very important to highlight that there are limited studies and results with ducks under heat stress. Almost all trials were conducted with broilers, laying hens or quails and only a few of them was carried out with ducks.

According to ZENG et al. (2013; 2014), SOD, MDA and CAT activity in duck liver increased under short-term heat stress (39°C for 1 hour then a 3-hour recovery at 20°C). The total antioxidant capacity (T-AOC) also increased significantly ($P<0.05$). However, these results show opposite trend in Peking ducks. It can be concluded that these enzymes

(SOD, MDA, CAT) can alter the balance between the production of ROS and the antioxidant system. The different thermal response of the two species (antioxidant enzymes unregulated in Muscovy and down regulated in Peking ducks) suggest that Muscovy ducks have better thermal tolerance than Peking ducks. However, MA et al (2014) found that SOD, GPx and T-AOC decreased while MDA increased in layer duck's plasma exposed to greater temperatures. Plasma concentration of uric acid and triiodothyronine (T3) increased but glucose decreased under high environmental temperature (30°C) in ducks (MA et al, 2014; ZHIGANG et al., 2013). The expression of HSP 70 increased under heat stress in ducks (MA et al, 2014; ZENG et al, 2013, 2014).

Because there are limited number and controversial results of studies done with different antioxidant supplementations in ducks under heat stress, we therefore present experiment results done with broilers. We assumed there would be similar results using vitamin and mineral supplementation with ducks as the following experiment results done in broilers and laying hens.

Vitamin A

Based on the studies used Vitamin A supplementation (15 000 IU/kg diet) in broilers under heat stress (32 and 34°C for 24 h), it can be concluded that performance improved. Feed intake (FI) and weight gain (WG) increased, feed conversion ratio (FCR) decreased. KUCUK et al. (2003), SAHIN et al. 2001c) and LIN et al.(2002) found that Vitamin A (9 000 IU/kg diet) increased FI, laying rate and egg weight in layers under heat stress (31,5°C for 24 h). Vitamin A can act as an effective radical-tapping antioxidant and quencher of singlet oxygen (SAHIN et al., 2001c; SAHIN et al., 2002b) (**Table 3**).

Table 3

Effects of Vitamin A supplementation under heat stress based on different studies
(HORVÁTH AND BABINSZKY, 2019)

Author(s)	Spices	Duration of the study	Environmental temperature	Amount of Vitamin A supplementation (IU/kg diet)	Effects on performance	Other effects
Kucuk et al., 2003	broiler	1-42 day	34°C (24h/day)	15 000	↑ BW, FI ↓FCR	↓MDA
Sahin et al, 2001b	broiler	21-42 day	32°C (24h/day)	15 000	↑ FI, WG ⌀ FCR	
Lin et al., 2002	layers	56 - 61week	31.5 °C (24h/day)	9 000	↑FI, laying rate, egg weight	

BW=Body weight (kg); FCR=Feed conversion ratio (kg gain /kg feed); FI=Feed intake (kg); MDA=malondialdehyde concentration in blood, WG=weight gain.
↑ Increase; ↓ decrease; ⌀ no effect.

Vitamin C

Although chickens are known to synthesize ***ascorbic acid*** in the kidney, increased supplementation has proved beneficial effects in broilers reared under heat stress (MAHMOUD et al., 2004). Ascorbic acid is actively transported into tissues. The requirements increase under heat stress; therefore, it is suggested that the bird's synthesizing capacity may become inefficient by reducing plasma ascorbic acid concentrations under high environmental conditions. According to different studies, ascorbic acid supplementation (200mg/kg feed) caused significant increase in plasma ascorbic acid levels (KUMAR et al., 2017; MAHMOUD et al., 2004) in broilers under heat stress.

Literature data on the effect on performance of poultry with different Vitamin C supplementation is presented in **Table 4**.

Production parameters of broilers improved under various high temperature conditions with Vitamin C supplementation (150-500mg/kg diet) (ATTIA et al., 2011; FAROOQI et al., 2005; KUTLU AND FORBES, 1993; MCKEE et al., 1997; SAHIN et al, 2001a; SAHIN et al., 2002a) which can be explained by its function of scavenging free radicals. Other results show that heat stress decreases the production of layers. However, ascorbic acid supplementation effected egg production, egg weight and egg mass in layers (AJAKAIYE et al., 2011; ASLI et al., 2007).

Table 4

Effects of Vitamin C supplementation under heat stress based on different studies

(HORVÁTH AND BABINSZKY, 2019)

Author(s)	Spices	Duration of the study	Environmental temperature	Amount of Vitamin C supplementation (mg/kg diet)	Effects on performance	Other effects
Ajakaiye et al., 2011	layers	39 week	35.9°C (24h/day)	150	↑egg weight	
Asli et al., 2007	layers	62 week	33±2°C (4h/day)	200	↑egg production, egg mass ↓ FCR	
Attia et al., 2011	broiler	21-84 day	38°C (4h/day)	250	↑FI, protein digestibility ↓FCR	
Farooqi et al. 2005	broiler	10-32 day	35°C (24h for first week) 32.5°C (24h for second week)	400 (20g/5kg)	↑BW, FI	
Kutlu and Forbes, 1993	broiler	1-28 day	36°C (6-10h/day)	250	↑BW, FI ↓FCR	
Mahmound and Edens, 2003.	broiler	1-42 day	30°C (3.5h/ for 3 days)	500		↑plasma Aa concentration ↓Hsp70 expression
Mckee et al., 1997	broiler	8-17 day	34°C (24h/day)	150	↑WG ∅ FI, FCR	
Kumar et al., 2017	broiler	1-45 day	37±5°C	200		↑plasma Aa concentration
Sahin et al., 2001a.	broiler	1-42 day	32°C (24h)	250	↑BW, FI ↓FCR	
Sahin et al., 2002a	Japanese quails	10-40 day	34°C (24h)	200	↑BW, FI ↓FCR	

Aa. = ascorbic acid; BW=Body weight (kg); FCR=Feed conversion ratio (kg gain /kg feed); FI=Feed intake (kg); MDA= malondialdehyde concentration in blood; WG=weight gain.
↑ Increase; ↓ decrease; ∅ no effect.

Vitamin E

Results of different studies of various Vitamin E supplementations on the performance of poultry are presented in **Table 5**.

Vitamin E is known as the first line of defence against lipid peroxidation caused by heat stress. It has free radical quenching activity and attacks free radicals in an early stage. In the following studies, which can be seen in **Table 5,** lipid peroxidation decreased and the enzymatic and non-enzymatic antioxidant systems improved (HARSINI et al., 2012; MAINI et al. 2007; SAHIN et al., 2002b). Heat stress also decreased the production parameters, which can be improved with Vitamin E supplementation (150-500mg/ kg diet). The results show that FI, BW and FCR improved in poultry (HABIBIAN et al., 2014; HARSINI et al., 2012; HASHIZAWA et al., 2013; SAHIN et al., 2001b) and, in addition, the nutrient digestibility also improved in Japanese quail (SAHIN et al., 2001a; 2001b SAHIN et al., 2002a). Egg production, egg weight and egg mass in layers were also positively affected with Vitamin E supplementation (AJAKAIYE et al., 2011; ASLI et al., 2007).

Table 5

Effects of Vitamin E supplementation under heat stress based on different studies

(HORVÁTH AND BABINSZKY, 2019)

Author(s)	Spices	Duration of the study	Environmental temperature	Amount of Vitamin E supplementation (mg/kg diet)	Effects on performance	Other effects
Ajakaiye et al., 2011	layer	39 day	35.9°C (24h/day)	150	↑egg weight	
Asli et al., 2007	layer	62 day	33±2°C (5h/day)	200	↑egg production, egg mass ↓ FCR	
Habibian et al. 2014	broiler	1-27 day	37 °C (8h/day)	125	⌀ BW, FI ↓FCR	
Harsini et al., 2012	broiler	1-49 day	37°C (8h/day)	250	↑BW,FI	↑Zn concentration in serum
Hashizawa et al., 2013	broiler	28-38 day	30°C (24h/day)	200	↑BW, FI ⌀ FCR	
Maini et al. 2007	broiler	1-49 day	38.6±1.3°C (24h/day)	200		↓MDA,GSH, CAT, SOD,GR
Sahin et al, 2001b	broiler	21-42 day	32°C (24h/day)	250	↑FI, WG ⌀ FCR	
Sahin et al, 2006	Japanese quail	10-4 2day	34°C (8h/day)	250	↑FI, WG ↓FCR	
Sahin et al., 2002b.	Japanese quails	10-40 day	34°C (24h/day)	500	↑BW, FI ↓FCR	
Sahin et al., 2002b	Japanese quail	10-40 day	34°C (24h/day)	250	↑FI, WG ↓FCR	↑Vitamin E, A concentration in serum ↓ MDA

BW=Body weight (kg); CAT=catalase concentration in blood; FCR=Feed conversion ratio (kg gain /kg feed); FI=Feed intake (kg); GPx=glutathione peroxidase concentration in blood; GR=glutathione reductase concentration in blood; GSH=reduced glutathione concentration in blood; MDA= malondialdehyde concentration in blood; SOD= superoxide dismutase concentration in blood; WG=weight gain;Zn=zinc.
↑ Increase; ↓ decrease; ⌀ no effect.

Zinc

Table 6 presents the literature data about Zn supplementation in poultry under heat stress.

Zn supplementation has positive effects on performance and antioxidant status of birds. (BRANDAE-NETO et al., 1995; NOVA AND ZEIN, 2020; ROBERSON AND EDWARDS, 1994). Zn has a protective role on pancreatic tissue against oxidative damage, which may improve nutrient digestibility and may therefore improve production parameters. Studies noted increased FI, WG and decreased FCR in broilers and quails (KUCUK et al., 2003; KUCUK, 2008; SAHIN et al., 2003; SAHIN et al., 2006; SAHIN et al., 2009) and ducks (NOVA AND ZEIN, 2020). 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), thus improving antioxidant status: increased serum Vitamin C and E concentrations (SAHIN et al., 2006) and decreased MDA levels (KUCUK et al., 2003; KUCUK, 2008).

Table 6

Effects of Zn supplementation under heat stress based on different studies
(HORVÁTH AND BABINSZKY, 2019)

Author(s)	Spices	Duration of the study	Environmental temperature	Amount of Zn supplementation (mg/kg diet)	Effects on performance	Other effects
Sahin et al., 2006	Japanese quail	10-42 day	34°C (8h/day)	30 or 60	↑FI, WG ↓FCR	↑Vitamin C concentration in serum ↓ MDA
Kucuk et al., 2003	broiler	1-42 day	34°C (24h/day)	30	↑BW, FI ↓FCR	↓MDA
Kucuk, 2008	Japanese quail	10-40 day	35°C (8h/day)	30	↑FI, WG ↓FCR	↓ MDA
Nova and Zein, 2020	Ducks	5-8 week	34°C	40 ppm	↑WG ↓FCR	
Sahin et al, 2006	Japanese quail	10-42 day	34°C (8h/day)	30	↑FI, WG ↓FCR	
Sahin and Kucuk, 2003b	Japanese quail	52-73 day	34°C (24h/day)	30 or 60	↑FI, egg production ↓FCR	

BW=Body weight (kg); FCR=Feed conversion ratio (kg gain /kg feed); FI=Feed intake (kg); MDA=malondialdehyde concentration in blood; WG=weight gain.
↑ Increase ↓ decrease ⦰ no effect.

Selenium

In can be seen in **Table 7** that heat stress induced oxidative stress can be partially ameliorated by supplementing Se in poultry due to its cofactor properties.

Performance (HARSINI et al., 2012; NIU et al., 2009; SAHIN et al., 2001b) and antioxidant status (HARSINI et al., 2012; MAHMOUND AND EDENS, 2003. SAHIN et al., 2001b) were both improved. It is suggested that the metabolic role of Se is to protect cells against oxidation and tissue damage. Rapid oxidation of GSH to GSSH is necessary to compensate the heat stress caused ROS production. However, the Se supplementation increases the level of available NADPH to promote the activation of GR, leading to increased GSSH reduction to GSH (SUCHÝ et al., 2014). Therefore, Se supplementation effected GPx activity and GPx/GSH ratio.

Table 7

Effects of Se supplementation under heat stress based on different studies

(HORVÁTH AND BABINSZKY, 2019)

Author(s)	Spices	Duration of the study	Environmental temperature	Amount of selenium supplementation (mg/kg diet)	Effects on performance	Other effects
Harsini et al., 2012	broiler	1-49 day	37°C (8h/day)	1	↑ BW, FI ↓FCR	⌀Zn concentration ↑GPx
Mahmound and Edens, 2003	broiler	1-28 day	33°C (24h/day for 4 weeks)	0.46ppm		↑GSH, GSSG, GPx, GPx/GR ratio
Liao et al., 2012	broiler	22-42 day	33±1°C (8h/day) 27±1 °C (8h/day)	0.30	↑FI	↑GPx, Se concentration
Niu et al., 2009	broilers	1-42 day	38°C (5h/day)	0.2	⌀ FI, WG ↓FCR	
Sahin et al., 2002b	Japanese quail	10-40 day	34°C (24h/day)	0.1 / 0.2	↑FI, WG ↓FCR	↑serum Vitamin E, A, Zn ↓ MDA

BW=Body weight (kg); FCR=Feed conversion ratio (kg gain /kg feed); FI=Feed intake (kg); GSH=reduced glutathione concentration in blood; GSSG=glutathione disulphide concentration in blood; GPx=glutathione peroxidase concentration in blood; GR=glutathione reductase concentration in blood; MDA=malondialdehyde concentration in blood; Se=selenium, WG=weight gain; Zn=zinc.
↑ Increase; ↓ decrease;
⌀ no effect.

2.6.2. Interactions between vitamins and micro minerals

Based on the studies done with separated supplementation of Vitamin A (9000-15000 IU/kg diet), Vitamin E (150-500 mg/kg diet), Vitamin C (150-500mg/kg diet), Zn (30 or 60 mg/kg diet) and Se (0,1-1mg/kg diet) it can be concluded that performance (FI, WG, FCR) and antioxidant status improved in poultry under heat stress. Antioxidant potential has been reported to be more efficient and important in combination than single antioxidant nutrients (GALLO-TORRES, 1980). The latest research studies show that interactions between vitamin-vitamin and vitamin-minerals used in combination have more improved effects than they do separately on the antioxidant status and performance of poultry under heat stress. Literature data of combination of vitamin and mineral supplementation can be seen in **Table 8.**

Table 8

Effects of vitamin and mineral interaction under heat stress based on different studies

(HORVÁTH AND BABINSZKY, 2019)

Supplementation	Author(s)	Spices	Duration of the study	Environmental temperature	Amount of supplementation	Effects on performance	Other effects
Vitamin C+ E	Sahin et al., 2002a.	Japanese quails	10-40 d	34°C (24h/day)	200 mg Vit. C +250 mg Vit. E	↑ BW ↓FCR	
Vitamin C+ E	Ajakaiye et al., 2011	Layer	39 w	35.9°C (24h/day)	150mg Vit C + 150mg Vit.E	↑ egg weight	
Vitamin C + Zn	Naila et al., 2014	broiler	22-42d	40°C (12h/day)	300mg Vit. C + 60 mg Zn	↑ WG, FCR ↓FI	↓mortality
Vitamin C + Zn	Al-Masad, 2012	broiler	22-28d	40°C	600mg Vit. C +35 mg Zn	↑ WG, FI ↓FCR	↑immunity ↓mortality
Vitamin C + Se	Lescovec et al., 2018	broiler	21-40d	32 -26°C	250 mg Vit. C + 0.2 mg Se	↓FI ⌀ BW, WG, FCR	↓MDA in meat and serum, ↓body temperature
Vitamin A + E	Sahin et al, 2001b	broiler	21-42d	32°C (24h/day)	15 000 IU/kg Vit. A +250mg Vit E	↑FI, WG ⌀ FCR	
Vitamin A+ Zn	Kucuk et al., 2003	broiler	1-42d	34°C (24h/day)	15 000IU Vit A + 30mg	↓FCR ↑BW, FI	↓MDA
Vitamin E+ Zn	Sahin et al, 2006	Japanese quail	10-42	34°C (8h/day)	30 mg Vit E + 250mg Zn	↑FI, WG ↓ FCR	
Vitamin E+ Zn	Hosseini-Mansoub et al. 2010	broilers	10-42	35 °C (24h/day)	100 mg Vit E + 50mg Zn	⌀ FCR ↑BW, FI	↓MDA
Vitamin E+ Se	Harsini et al., 2012	broiler	1-49d	37°C (8h/day)	0.5mg Se + 152mg Vit.E	↑ BW ↓ FCR	↓MDA ↑SOD
Vitamin E+ Se	Habibian et al., 2014	broiler	21-49d	37°C (8h/day)	0.5 mg Se +250mg Vit. E	⌀ BW, FI ↓FCR	↑antibody response

BW=Body weight (kg); FI=Feed intake (kg); FCR=Feed conversion ratio (kg gain /kg feed), WG=weight gain
MDA=malondialdehyde concentration in blood; SOD= superoxide dismutase concentration in blood, Se=selenium, Zn=zinc
↑ Increase; ↓ decrease

Vitamin C (200mg/kg diet) and ***Vitamin E*** (250mg/kg diet) supplementation in combination improved performance (BW and FCR) and meat quality in quails under heat stress (SAHIN AND KUCUK, 2001). Both vitamins had beneficial effects on egg quality under heat stressed layers (AJAKAIYE et al., 2011). Vitamin C (200mg/kg diet) and E (100mg/kg diet) supplementation increased the total antioxidant capacity, SOD, GPx enzyme activities in broilers under oxidative stress (EL-SENOUSEY et al., 2017). Vitamin C interacts with tocopheroxyl radical and regenerate the reduced tocopherol. Higher dietary level of Vitamin C increased the tissue and plasma concentrations of Vitamin E (MACHLIN AND BENDICH, 1987).

Vitamin A (15 000IU retinol/kg diet) and ***Vitamin E*** (250mg α-tocopherol-acetate/kg diet) supplementation in combination improved FI and WG in broilers under heat stress (32°C) and also decreased lipid peroxidation and increased serum Zn concentrations (SAHIN et al., 2001c; 2002b). Vitamin E has a supportive effect on the absorption and utilization of carotenoids. Vitamin A and E have synergistic effects due to the similarity between both, because both act as anti-stress agents (SAHIN et al, 2002b). Vitamin E can protect the double bonds of carotene from oxidation.

Vitamin A (15 000 IU retinol/ kg diet) and ***Zn*** (30 mg/kg diet) has a proved connection: Zn plays a role in Vitamin A transport mediated protein synthesis and in the oxidative alteration of retinol to retinal (Zn dependent retinol dehydrogenase enzyme) (CHRISTIAN AND WEST JR, 1998). Vitamin A supports the utilization and perhaps the transport of Zn and also the absorption in ileum mucosa in broilers (CHRISTIAN AND WEST JR, 1998); however, the absorption and transport of Vitamin A are affected by Zn status (BERZIN AND BAUMAN, 1987). Dietary supplementation of Vitamin A and Zn in combination had additive effects therefore increased the FI and BW and decreased FCR and lipid peroxidation of broilers under heat stress (KUCUK et al., 2003).

Vitamin E (100 mg/kg diet) and ***Zn*** (50 mg/kg diet) combination in diet increase the production parameters (FI, BW) and decreased FCR and lipid peroxidation in broilers under heat stress (HOSSEINI-MANSOUB et al. 2010; SAHIN et al, 2006). It is suggested that Vitamin E causes Zn release into the serum, thus increasing the serum Zn concentration (SAHIN et al., 2002a).

Vitamin E (152, 250 mg/kg diet) and ***Se*** (0.5 mg/kg diet) in combination decreased FCR and lipid peroxidation, increased enzyme activity (GPx, SOD) therefore indicated

oxidative stability in broilers under heat stress (HABIBIAN et al., 2014; HARSINI et al., 2012). Se increased the intestinal Vitamin E absorption and has a protective effect on pancreatic tissue against oxidative damage.

Vitamin C (300-600 mg/kg diet) and ***Zn*** (35-60 mg/kg diet) supplementation in the broiler diet improved performance (BW, daily gain, FCR, less mortality) and also indicated immune response under heat stress (40°) (AL-MASAD, 2012; CHAND et al., 2014; NAILA et al., 2014). The results of these experiments indicated that Vitamin C and Zn in combination improved the performance and immune status of broilers under heat stress (34°C, 40°C).

Supplementation of ***Vitamin C*** (250 mg/kg) and ***Se*** (0.2 mg/kg) did not influence WG, FCR however decreased FI and MDA content in plasma and breast meat (LESCOVEC et al, 2018). The supplementation increased digestibility of DM and CP and decreased MDA concentration and body temperature (ATTIA et al., 2015). The results show that Vitamin C and Se in combination improves antioxidant status and performance.

From the scientific findings, the following main conclusions can be drawn:

- Heat stress can cause harmful effects in birds (e.g. disruption of mitochondrial function, increased ROS production and lipid peroxidation, decreased vitamin concentrations, changed enzyme activity) and consequently in production parameters (e.g. FI, WG, FCR). However, the three level antioxidant defence system plays a crucial role in the reduction of the heat stress generated lipid peroxidation process.
- Vitamin A, E and C are capable of reacting with free radicals, thereby reducing their amounts and preventing lipid peroxidation in the poultry. Micro minerals (Zn, Se) are not directly capable of preventing or reducing ROS formation. However, they are essential cofactors for those enzymes which have the attribute of reacting with free radicals. Supplementation is necessary for preventing the negative effects of heat stress.
- Antioxidant potential of vitamins and micro minerals is more efficient in combination under heat stress in poultry nutrition. The latest research studies show that interactions have more improved effects on the antioxidant status and performance of poultry under heat stress.

- Because of the limited number and controversial results of studies done with different antioxidant supplementations in ducks under heat stress, we could not achieve clear and direct conclusions. Therefore, this study has great importance.
- It is necessary for duck farmers to understand the importance of heat stress and how to reduce these effects by using nutritional tools.

3. STUDY

3.1. MATERIALS AND METHODS

3.1.1. Ethics Statement

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

3.1.2. 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 (**Table 9**) 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.

Table 9

Composition and analysed nutrient contents of the starter diet (0-14d)

Ingredients (%)	**Starter diet**
Corn	25
Wheat	20
Triticale	18
Ext. Soybean meal (CP: 46%)	25
Full fat soy	4
Wheat meal	5
Premix[1]	0.5
Others[2]	2.5
Calculated energy and analysed nutrient content (100g dry matter)	
ME poultry (MJ/kg)	11,9
Crude protein (%)	20
Crude fiber (%)	3,5
Crude fat (%)	3,2
Lysine (%)	1,14
Methionine (%)	0,47
Ca (%)	0,82

[1] 1 kg Premix contains: Vitamin A (retinyl acetate):2400000 IU; Vitamin D3 (cholecalciferol): 600000 IU;Vitamin E (all-rac-alfa-tocopheril acetate):10000mg; Vitamin K: 999mg; Vitamin B1: 600mg; Vitamin B2: 1800mg; Pantothenic acid: 3000mg; Vitamin B6: 1400mg; Vitamin B12: 8mg; Niacin: 9997 mg; Folic acid: 400mg; Biotin: 30mg; 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

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 (*Appendix: Photo 1*), P 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 (*Appendix: Photo 7*).

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 (**Photo 1**).

Photo 1 **Housing**

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 (*Appendix: Photo 2 and 3*).

Dietary treatments, composition of diets and analysed nutrient content. As mentioned previously, the latest research studies show that interactions between vitamin-vitamin and/or vitamin-minerals used in combination have more improved effects than they do separately on the antioxidant status and performance of poultry under heat stress**.**

Therefore, we selected two vitamins (E and C) and two micro minerals (Se and Zn) for this study, because these are the most common used supplementations in poultry nutrition.

The three experimental diets were corn-soybean meal based crumbled feed 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 10**. The diets were prepared by using a control diet, which can be used in normal environmental temperatures in practice (near to the thermo neutral 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), Se (0.45 mg/kg diet) and Zn (50 mg/kg diet) by the premix **(Table 11).** 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 11**). 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 11**).

The general aim of the diet formulation was to provide same energy and nutrient content among the experimental diets. They also aimed to ensure that vitamin and micronutrient supplements did not significantly increase the price of compound feeds. This is because on the farm level, it is an important economic requirement that the feed costs have to be lower than the value of the bird loss caused by heat shock.

Table 10

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	-	+	++
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
SiO_2 (%)	0.5	0.5	0.5

[T] Treatments and codes are defined in Table 11,

[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 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%)

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

Table 11

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

[2] ascorbic acid

[3] organic selenomethionine

[4] inorganic zinc oxide

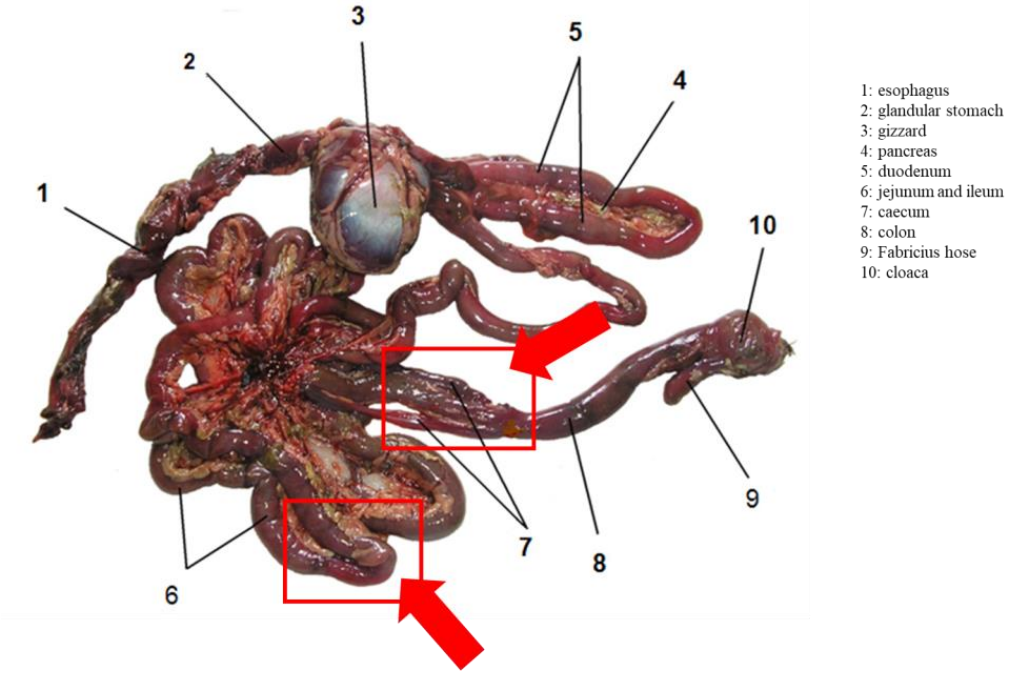
3.1.3. Sampling and sample preparation

15 ducks/ treatment were randomly euthanized by cervical dislocation at 42d 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) (*Appendix: Photo 4, 5 and 6*). The samples were placed in plastic holders and stored at -20°C until laboratory analysis (n=15 ducks/treatment). Red arrows on **Figure 9** show sampling locations.

Figure 9 **Sampling the digesta**



Meat. The skinny thigh 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).

3.1.4. Measurements

Body temperature (BT)

A thermometer was inserted 3 cm into the rectum for 10-15s for RT measurement (**Photo 2**). BT was measured by sex (two sexes/pen; n=30) two times a day (at 9 am and 4 pm) three times a week (n=240 data/treatment) however we only used the results at the days: d18, d28, d42. We used d18 because we wanted the ducks to adapt to the new environment, d28 because this date is 2 weeks from the start of the experiment and d42 because it is the last day of the experiment.

Photo 2 **Measuring the BT with thermometer**

Performance

The body weight (**BW**) of the ducks was measured individually (n=300) at 14d and 42d (g/bird). The daily **WG** was calculated also individually (g/day/bird). The daily **FI** was recorded by each pen (n=15, 3 repeat x 15=45 pen in total) and this result was-divided

with the number of birds to get FI g/day/bird. The **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 cause of death.

Antioxidant status

The following antioxidant parameters were measured: **SOD**, water soluble antioxidant compounds (**ACW**), lipid soluble antioxidant compounds (**ACL**), **GR**, **GPx, GSH, Vitamin C**, **Vitamin E (α-tocopherol)** and **MDA**.

3.1.5. Chemical analysis

Diet

The nutrient, vitamin and mineral content of the diet were determined using standard procedures of AOAC Official Methods (AOAC, 2012). The following contents were determined: crude protein (AOAC Authors, 2006; AOAC Official Method 992.15), crude fiber (AOAC Authors, 2006; AOAC Official Method 978.10) crude fat (AOAC Authors, 2006; AOAC Official Method 920.39), Lys and Met (AOAC Authors, 2006; AOAC Official Method 982.30), Ca (AOAC Authors, 2006; AOAC Official Method 984.27), Vitamin E (α-tocopherol) (AOAC Authors, 2006; AOAC Official Method 2012.10), Vitamin C (AOAC Authors, 2006; AOAC Official Method 967.22), Se and Zn (AOAC Authors, 2006; AOAC Official Method 989.15) and SiO_2 (AOAC Authors, 2006; AOAC Official Method 920.08).

Blood

SOD was determined using commercially available kit (Analytik Jena AG, Jena, Germany) with PhotoChem© (POPOV AND LEWIN, 1999). To determine SOD activity, erythrocyte containing pellet was washed four times with 3M of 0.9% NaCl solution and centrifuged at 2555xg at 4°C for 10 min. The erythrocytes were resuspended in 2ml cold distilled water and incubated at 4°C for 15 min. SOD activity was measured after incubation.

ACW and **ACL** was determined using a commercially available kit (Analytik Jena AG, Jena, Germany) with PhotoChem© (POPOV AND LEWIN, 1999). The antioxidants are quantified based on their inhibitory effects on luminescence generation by comparison with a standard. Ascorbic acid is used for generation of a calibration curve and the

antioxidative capacity is calculated as equivalent units of ascorbic acid (POPOV AND LEWIN, 1999).

The **GR** 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 samples were measured colorimetrically 405 nm the ΔA_{405nm} was determined for all samples. The calculation was done according to the assay protocol. The activity of GR was expressed as plasma GR activity (in nmol/min/ml=mU/ml) (SMITH et al., 1988).

The **GPx** activity (Abcam, Cambridge, UK) was determined using commercially available assay kits. The GPx was measured with SpectroStar$^{Nano\ microplate}$ reader (BMG Labtech, Offenburg, Germany). The GPx activity was measured by changing of the absorbance colorimetrically at 340 nm. The calculation was done according to the assay protocol. The NADPH standard curve was plotted and the equations were used which was suggested by the kit. The activity of GPx was expressed as a plasma GPx activity (in nmol/min/ml=mU/ml) (ANN et al, 2015).

The concentration of **GSH** (Bio Vision, Inc. Headquarters Milpitas, California, and USA) was determined using commercially available assay kits. The GSH was measured with SpectroStar^{Nano} microplate reader (BMG Labtech, Offenburg, Germany). The absorbance was measured at 412 nm colorimetrically. Sample GSH concentration was compared with GSH standard curve and the results were divided by the molecular weight of GSH. The concentration of GSH was expressed as plasma GSH concentration (in µM/mL) (BEUTLER et al. 1963).

The concentration of **MDA** was determined using a commercially available assay kit (Sigma-Aldrich, St. Louis, Missouri). The colour change was measured spectrophotometrically at a wavelength of 532 nm. The calculation was done according to the assay protocol. The MDA concentrations of the samples were expressed as plasma MDA concentration (in nmol/µL) (HAMDY et al., 2013).

The concentration of **Vitamin E** was determined using ELISA kit (Blue Gene Biotech LTD, Shanghai, China).

ELISA kit applies the competitive enzyme immunoassay technique utilizing a monoclonal anti-Vitamin E antibody and a Vitamin E- HRP (horse-radish peroxidase)

conjugate. The product of the enzyme substrate reaction forms a blue colored complex. The intensity of the colour was measured spectrophotometrically at 450 nm. The intensity of the colour was inversely proportional to the Vitamin E concentration since the calculation was done according to the assay protocol. The Vitamin E concentrations of the samples were expressed as plasma Vitamin E concentration (in µg/mL).

To determine the plasma **Vitamin C** content, the plasma samples were pre-treated due to the high protein content of the samples. First chemical destruction used 37% HCl acid: 96% ethanol solution (1:7) (EUROPEAN PHARMACOPOEIA, 2001). 100µl of plasma was added to a 500µl of HCl: ethanol solution. This was incubated for 30min at 25°C. The samples were centrifuged at 13000xg at 4°C for 5 min. The supernatant was used for determination using a commercially available assay kit (Abcam, Cambridge, UK). The absorbance was measured at 570 nm. The results were multiplied by the molecular weight of ascorbic acid (176.12g). The Vitamin C concentrations of the samples were expressed as a plasma Vitamin C concentration (in ng/µl) (BLASCHKE et al., 2013).

Digesta. The digesta samples (DM, crude protein) were analysed using standard procedures of Proximate Analysis (AOAC, 2012) and Si_2O 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.

3.1.6. 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). The feed prices were given from Transitker Trading Company and were calculated based on the cost of 1kg feed

(T1: 71 HUF, T2: 75 HUF, T3: 77 HUF) and we exchanged this price on the daily currency in 2016 summer (1 US $= 270 HUF).

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$_{\text{. in diet}}$)

3.1.7. 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 (SAS 2010). The general model was as follows:

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

Where: Y= dependent variable; μ= overall mean; T=treatment (i=3); R=repeat (j=2); TR= interaction between treatment and repeat; S=sex; Tday=time of the day; e= residual error.

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.).

Correlations analysis

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). Correlation is positive when both the variants move in the same direction and negative when the variables move in the opposite direction. The value of the coefficient of correlation always lies between ±1. Such as:

r=+1, perfect positive correlation; r=-1, perfect negative correlation, r=0, no correlation.

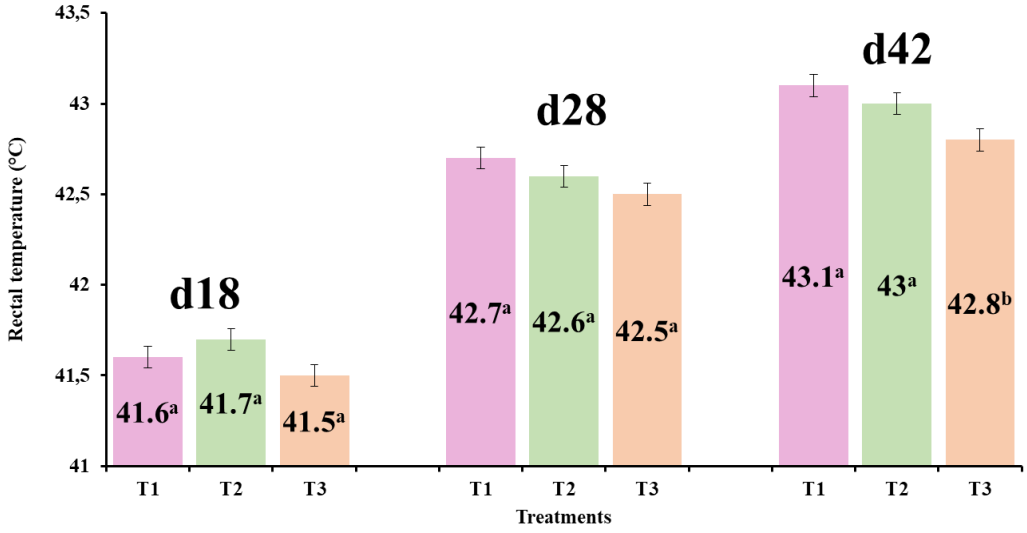
3.2. RESULTS

3.2.1. Body temperature of ducks

Our results of body temperature of ducks in different treatments at 18d, 28d and 42d are presented in **Figure 10**.

Figure 10 **Effect of heat stress on the body temperature of ducks in different treatments at 18d, 28d and 42d (LS means ± SEM[+])**

(n=240datas/treatment)



[T] Treatments and codes are defined in Table 11.
[+] 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.

Effects of sex and time of the day were not significant; therefore, these variables were omitted. Based on our results, we supposed that the supplementation affected the body temperature of ducks, irrespective of sex and time of day.

The results show that supplementation decreased the body temperature of ducks under heat stress significantly at 42d; however, only by several degrees (P<0.05). At 28d, there was no significant difference between treatments. The body temperature of ducks in T3 at 42d decreased significantly (P<0.05) as compared to T1 and T2.

3.2.2. Antioxidant parameters

The results of the antioxidant parameters of ducks under heat stress can be seen in **Table 12**.

MDA level significantly decreased in T2 and T3 ($P<0.05$) compared to T1. The concentration of Vitamin E in blood decreased in line with MDA formation in T1 and T3 ($P<0.05$) however no significance difference between T2 and T3 ($P>0.05$). We found that concentration of Vitamin C increased significantly in T2 but not in T3 ($P<0.05$) compared to T1. The results showed that the amount of SOD in T2 did not change ($P>0.05$) however decreased significantly in T3 ($P<0.05$) compared to the T1. Due to the increased vitamin and micro mineral supplementation in the diets, the results of ACW show significant enlargement in T2 and T3 ($P<0.05$) in dose dependent manner compared to the T1. The concentration of ACL did not change in T2 ($P>0.05$) however increased in T3 ($P<0.05$) compared to the T1. GPx significantly decreased in T2 and T3 ($P<0.05$) compared to the T1. GR activity decreased ($P>0.05$) however not significantly in T2 and T3 in parallel with the GPx activity ($P<0.05$). GSH concentration increased in T2 ($P<0.05$) but did not change in T3 ($P>0.05$) compared to the T1.

Table 12

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 11.
[+] 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).

3.2.3. Digestibility of nutrients

The digestibility coefficients of dry matter (DM) and crude protein (CP) are summarized in **Table 13.**

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 13

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 11.
[+] LS means=least squares means; SEM=standard error of the mean.
NS=non-significant (P>0.05).

3.2.4. Performance

The results of the production parameters are presented in **Table 14**.

The number of dead birds was very low and there was no difference ($P>0.05$) between the treatments. The FI significantly increased in T2 and T3 ($P<0.05$) compared to the T1. The live weight of the birds at 14d did not differ between the treatments ($P>0.05$). Our results show that live weight at 42d (at the end of the experiment) significantly increased in T2 and T3 ($P<0.05$) compared to the T1. The daily WG also improved in T2 and T3 ($P<0.05$) compared to the T1. FCR decreased in T2 and T3 ($P<0.05$) compared to the T1. The energy and protein CR is an indicator used to represent the applied energy and protein used for weighting gain. Energy and protein conversion ratio both decreased significantly ($P<0.05$) in T2 and T3 compared to the T1. The most important economical parameter is specific feed cost which decreased in T2 and T3 ($P>0.05$) compared to the T1 due to the supplementation.

Table 14

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 11.
[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<0.05; NS=non-significant (P>0.05).

3.2.5. Chemical composition of meat

The effects of heat stress on chemical composition of duck thigh and breast meat are summarized in **Table 15** and **Table 16**. The results on meat protein, fat and glycogen content is based on 100g of the dried sample.

As can be seen in Table 13, the DM, protein and fat content of duck thigh meat were not affected by higher vitamin C, E, Zn and Se supplementation under heat stress.

Table 14 shows the same tendency as the results for breast meat. Mostly, the parameters were not affected by the supplementation; however, the DM content decreased in breast meat in T2 ($P<0.05$). According to our results, the glycogen content of duck meat significantly decreased in both thigh and breast samples in T2 and T3 compared to the T1.

Table 15

Effects of heat stress on chemical composition of duck thigh **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 16

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. DISCUSSION

4.1. Physiological changes

4.1.1. Body temperature

The body temperature of poultry under normal conditions is in the range of 41 to 42°C and it can be concluded that BT of homoeothermic animals will increase due to heat stress (COOPER AND WASHBURN, 1998). Our results show that supplementation decreased the body temperature of ducks under heat stress significantly at 42d; however, only by several degrees ($P<0.05$). At 18d and 28d, there was no significant difference between treatments. According to the literature this could be because of the adaptation ability of ducks against high environmental temperature and the control by the neuroendocrine and endocrine system which is maintaining the normal body temperature. However, our results show that during constant high environmental temperature, the adaptation ability of ducks decreased or there was a positive effect of the supplementation on the neuro- and endocrine system; 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., 2003; XIE et al., 2014); however, during long-term (constant) heat stress, the body temperature is not elevated in poultry (XIE at al., 2014).

4.1.2. Antioxidant defence mechanisms

According to other studies with Vitamin C, E, Se and chromium (Cr) supplementation (LESKOVEC et al., 2018; MAINI et al., 2007; SAHIN et al, 2001a,b,c; 2002 a, b) decreased MDA concentration. As expected, our results show that MDA level significantly decreased in T2 and T3 ($P<0.05$) (Table 3) compared to the T1 due to the supplementation. Increased environmental temperature will cause increased lipid peroxidation and induced formation of MDA; therefore, the antioxidant defence system is altered and the concentration of MDA will increase (ALTAN et al., 2003; COSTANTINI et al., 2009; LARA AND ROSTAGNO, 2013). 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 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 O_2^- and H_2O_2 and was used by GR for maintaining the GSH level. In our study, we found that concentration of Vitamin C increased significantly in T2 and T3 (P<0.05) compared to the T1. 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). 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 commonly recommended (YUN et al., 2012).The increased amount of prooxidants, 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 systems.

The results showed that in T3, the amount of SOD decreased due to the supplementation. We supposed that this was because the supplemented ascorbic acid, which quickly reacted with the O_2^-; 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) compared to the T1. TOMAZIN et al. (2013) found same results as ours; however, LESKOVEC et al. (2018) found that Vitamin E supplementation did not increase the ACL level in serum. It is suggested that the metabolic role of Se is to protect cells against oxidation and tissue damage as being the active site of the potent GPx which contains selenocystein residues. 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) compared to the T1 however in poultry (AKBARIAN at al., 2015) and turkey studies (MIKULSKI et al., 2009) GPx has increased under heat stress due to Se supplementation. The activity of GPx in continuous and depends on presence of GSH. GR enzyme regenerates GSH from GSSG and requires NADPH as source of energy (SISEIN, 2014). With this finding, it 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). Se supplementation can increase production of GSH (SUCHY at 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).

As mentioned before, ducks are more sensitive to heat stress than other livestock. Therefore, we supposed that the supplementation used in our 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; MAHMOUND AND EDENS, 2003; SAHIN et al, 2001a). 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., 2002 a, b).

Correlation analysis- among antioxidant parameters

The results of the correlation analysis between the supplementation and some antioxidant parameters can be seen in Table 17. Other antioxidant parameters did not show strong correlation between the supplementation; therefore, these are not explained. The results are discussed that the supplements have individual effects, however the partial effect cannot be determined in our study it is suggested that the vitamins and minerals could interact with each other.

According to the correlation analysis, we found that Vitamin E content of the diet – serum ACL; and Vitamin C content of the diet – serum ACW both have a strong, positive correlation (>0.9). 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 supplementation improves their amounts.

Vitamin E, C, Se and Zn content of the diet has a strong inverse correlation with GR (>-0.8). GR is an enzyme for GSH production, which is necessary for GPx activity (see 2.2.3. Antioxidant system) 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 (see 2.2.3. Antioxidant system). 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 “takes part” in the primary antioxidant function of GPx. The increased amount of Se could be cofactor for more GPx, and could result in increased GPx activity; therefore, less GR activity is needed (see 2.4.2 Selenium) (LIN et al., 2006; MAINI et al., 2007, SAHIN et al, 2006).

Vitamin E and Se content of the diet have moderate negative correlations (-0.77), Vitamin C and Zn content of the diet have a very strong negative correlation with MDA (>-0.8). The supplementations decreased the MDA concentration; therefore, it can be concluded that they all influence lipid peroxidation. However, Vitamin C and Zn play a major role in decreasing oxidative stress (HARSINI et al., 2012; LESKOVEC et al., 2018; MAINI et al., 2007; SAHIN et al., 2001; 2002 a, b).

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

Table 17

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

4.2. Digestibility

Digestibility of DM in broilers can vary from 66% (BONNET et al., 1997) to 73% (SEVEN AND SEVEN, 2008). Our results show that digestibility of DM in T1, T2 and T3 are all higher than in the literature. Results of 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 2008) to 80-84% (HOSSEINI et al., 2016). According to the literature (BONETT et al., 1997; HAI et al., 2000) the changing in the nutrient digestibility might be explained by physiological modifications under heat exposure. Water consumption increase under heat stress, which could reduce absorption through the feed passage rate. Chronic heat stress also has shown reduced size of gastrointestinal tract and decreased intestinal villosity surface which can reduce absorption. The decrease in feed digestibility explains a part of decrease in growth performance in broilers under heat stress. Therefore, DM, CP digestibility were reduced under heat stress. Vitamin C addition under heat stress in general improved the digestibility of nutrients (SAHIN AND KUCUK, 2001a), although in our study this was not expressive. We suggest that the

higher DM values in our experiment could be because the vitamin and micro nutrient supply in the diet did not induce or maybe reduce the physiological changes described in the literature.

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 an inconsequential and limited number of scientific results on the digestibility of nutrients in ducks under heat stress.

4.3. Performance and efficiency of nutrient conversion

In our study, the number of dead birds was very low and caused by sudden death syndrome (SDS) (n=2). The equal distribution of the ducks was confirmed by the 14d weight, which did not differ significantly between treatments. Performance (live weight at 42d, dWG, FCR,) significantly improved by higher vitamin and mineral supplementation under heat stress in ducks. According to the performance results, T2 and T3 did not differ significantly ($P<0.05$) from each other; therefore, the increased supplementation of vitamins and minerals is not positively necessary.

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., 2001b), which can be explained by their function as scavenging free radicals. Zn 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). Se also improves the 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 thermoregulation 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.

Correlation analysis among performance

The results of the correlation analysis between the supplementation and some production parameters can be seen in **Table 18**. Other production parameters did not show strong correlation between the supplementation; therefore, as mentioned before, these are not explained.

Vitamin E, C, Se and Zn content of the diet both have a strong positive correlation with live weight at 42d (>0.85) and with dWG (>0.88). This means that supplementation improves the weight of the ducks. The correlation between FCR and Vitamin E, Se and Zn content of the diet is very strong negative (-9), therefore increased supplementation in the diet improved FCR. 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 18

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

4.4. Chemical composition of valuable meat parts

The DM, protein and fat content of duck thigh meat were not affected by higher vitamin C, E, Zn and Se supplementation under heat stress. Therefore, according to our results, the supplementation does not have improved effects on the chemical composition of duck thigh meat under high environmental temperatures. The same tendency as the results for breast meat. 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 in different experiments determined as 20-21% protein and 1.5-3.6% fat content, as sampled under thermo neutral conditions and without increased vitamin supplementation (GALAL et al., 2011; HEO et al., 2015). AKSIT et al (2006) and 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 thigh and breast samples in T2 and T3 compared to the T1. This suggests that the increased vitamin and mineral supplementation could not inhibit the reduction of glycogen level. One of the physical changes during the post mortem aging of animal meat is the breakdown of glycogen. Based on the literature, heat stress has negative effects on muscle glycogen level because glycogen breakdown and depletion 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 decreased under stress (SONG AND KING, 2015; WANG et al, 2017).

5. CONCLUSION

Based on the present study the following main conclusions can be drawn:

- The antioxidant capacity of blood (ACW, ACL), improved, the SOD and MDA concentration decreased by 1540 mg Vitamin E, 1996 mg Vitamin C, 0.90 mg Se and 148 mg Zn on kg feed basis supplementation under heat stress in meat type ducks between growing phase (14-42d).

- The Vitamin E supply - serum ACL, and Vitamin C supply - serum ACW both have a strong, positive correlation (>0.9). The Vitamin E and Se supply have moderate negative correlations (-0.77) with serum MDA, while Vitamin C and Zn supply have very strong negative correlation with MDA (>-0.8) under heat stress in meat type ducks.

- The Vitamin E, C, Se and Zn supply of the diet both 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 content of the diet and FCR have very strong negative correlation (-0.9) under heat stress (30 ± 1°C).

- The digestibility of crude protein and dry matter and the chemical composition of duck meat (thigh and breast) are not affected (P>0.05) under chronic heat stress due to antioxidant vitamin supplementation (Vitamin E: 1540 mg/kg diet, Vitamin C: 1996 mg/kg diet, Se: 0.90 mg/kg diet and Zn: 148 mg/kg

- There is no need to use the higher doses of Vitamin E, ascorbic acid, Se and Zn. Production parameters do not improve better and unfavourable changes are already induced in the antioxidant system, which indicate the prooxidant effect of the compounds and elements used. Therefore, at high environmental temperatures (30°C) (in the summer), we recommend to use the following supplementation: 540 mg Vitamin E, 998 mg Vitamin C, 0.60 mg Se and 97 mg Zn on kg feed basis.

- A “special summer” premix must be developed in practice to reduce the harmful effects of high ambient temperature (heat shock).

6. 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, 1996 mg Vitamin C, 0.90 mg Se and 148 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 and 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: 1996 mg/kg diet, Se: 0.90 mg/kg diet and Zn: 148 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, 1996 mg Vitamin C, 0.90 mg Se and 148 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.

7. 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°C) we recommend to use in practical duck nutrition the following vitamin and micro-mineral concentration in the diet: Vitamin E: 1540 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.

8. SUMMARY

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. 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. 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 and nutrition) or keeping technology devices (e.g. air conditioning, intensive ventilation and humidification). 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. According to the limited number of studies we selected the primary vitamins and micro minerals, which are most commonly used in poultry nutrition as supplementation during times of heat stress.

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 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

A total of 900-mixed sex 14-day-old Cherry Valley type hybrid ducks randomly placed into three experiment groups (3 treatment group). Each group containing 20 birds (20 birds/cage) and 5 cages/treatment. The experiment was repeated twice. The ducks were housed in an environmentally controlled room in the animal house and subjected to the following treatment during 28 days (until 42d): high environmental temperature (HT) constant 30±1°C for 24 hours with the relative humidity at (62±5%). Three experimental

diets were used. In **T1**, we used a control diet (which is usually used regardless of the season in meat type ducks). In treatment 1 (**T1**), the diet contained: Vitamin E (40 mg/kg diet), Se (0.45 mg/kg diet) and Zn (50 mg/kg diet) by 0.5% of the premix. **T2** had increased concentration of Vitamin E (1540 mg/kg diet). Vitamin C (998 mg/kg diet), Se (0.60 mg/kg diet) and Zn (97 mg/kg diet), respectively, and **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. 15 ducks/ treatment were randomly euthanized by cervical dislocation at 42d for blood, digesta and meat sampling. The body temperature (BT) was measured by sex two times every day. The nutrient, vitamin and mineral content of the diet were determined using standard procedures of AOAC Official Methods (AOAC, 2012). The experimental data was analysed using the PROC GLM (mixed models) procedure of SAS. 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.).

The results show that digestibility of nutrients (CP and DM) and the chemical composition of duck meat (thigh and breast) were not affected ($P>0.05$) by the dietary treatments under constant environmental temperature of 30 ± 1°C. Increased environmental temperature will cause increased lipid peroxidation and induced formation of MDA; therefore, the antioxidant defence system is altered and the concentration of MDA will increase. As expected, our results show that MDA level significantly decreased in T2 and T3 ($P<0.05$) as compared to T1 due to the supplementation. The concentration of Vitamin E in blood decreased in line with MDA formation in T1 and T3 ($P<0.05$), however, the supplemented Vitamin E in T2 and T3 did not affect ($P>0.05$) the concentration of Vitamin E in the blood. In our study, we found that concentration of Vitamin C increased significantly in T2 and T3 ($P<0.05$). Vitamin E and Se content of the diet have moderate negative correlation (-0.77), Vitamin C and Zn content of the diet have a very strong negative correlation with serum MDA (>-0.8). The supplementations decreased the MDA concentration; therefore, it can be concluded that they all influence lipid peroxidation. 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 O_2^-; therefore, the activity of SOD enzyme was not much required. 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 content of the diet - serum ACL; and Vitamin C content of the diet - serum ACW both have a strong, positive correlation (>0.9). This is because these vitamins are components of the water and lipid soluble antioxidant compounds therefore increasing the supplementation improves the amount. We found that GPx significantly decreased in T2 and T3 (P<0.05). Vitamin E, C, Se and Zn content of the diet has a strong inverse correlation with GR (>-0.8). According to the supplementation in T2 and T3 the activity of GR reduced; therefore, less GSH was produced, which led to reduced GPx activity. With this finding, it can be explained why the GR activity decreased (P>0.05) in our results in T2 and T3 in parallel with the GPx activity (P<0.05). In our study, Se supplementation has a very strong negative correlation with serum Vitamin E concentration (-0.97). This means that increased amount of Se decreased the amount of Vitamin E concentration in blood. This could be because the Se is a cofactor for GPx; therefore, the enzyme activity increased (the enzymatic pathway is dominating). Thus, less Vitamin E (small molecule antioxidant) was "needed" in the blood to scavenge the increased amount of free radicals. The FI significantly increased in T2 and T3 (P<0.05) as compared to T1. The live weight of the birds at 14d did not differ between the treatments (P>0.05). Our results show that live weight at 42d (at the end of the experiment) significantly increased in T2 and T3 (P<0.05) as compared to T1. The daily WG also improved in T2 and T3 (P<0.05). FCR decreased in T2 and T3 (P<0.05). Energy and protein CR and the specific feed cost all decreased significantly (P<0.05) in T2 and T3. Vitamin E, C, Se and Zn content of the diet both have a strong positive correlation with live weight at 42d (>0.85) and with dWG (>0.88). This means that this supplementation improves the weight of the ducks under heat stress. The correlation between FCR and Vitamin E, Se and Zn content of the diet is very strong negative (-0.9); therefore, increased supplementation in the diet improved FCR under heat stress.

The following main conclusions can be drawn:

- The antioxidant capacity of blood (ACW, ACL), improved, the SOD and MDA concentration decreased by 1540 mg Vitamin E, 1996 mg Vitamin C, 0.90 mg Se and 148 mg Zn on kg feed basis supplementation under heat stress in meat type ducks between growing phase (14-42d).

- The Vitamin E supply - serum ACL, and Vitamin C supply - serum ACW both have a strong, positive correlation (>0.9). The Vitamin E and Se supply have moderate negative correlations (-0.77) with serum MDA, while Vitamin C and Zn supply have very strong negative correlation with MDA (>-0.8) under heat stress in meat type ducks.
- The Vitamin E, C, Se and Zn supply of the diet both 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 content of the diet and FCR have very strong negative correlation (-0.9) under heat stress (30 ± 1°C).
- The digestibility of crude protein and dry matter and the chemical composition of duck meat (thigh and breast) are not affected (P>0.05) under chronic heat stress due to antioxidant vitamin supplementation (Vitamin E: 1540 mg/kg diet, Vitamin C: 1996 mg/kg diet, Se: 0.90 mg/kg diet and Zn: 148 mg/kg
- There is no need to use the higher doses of Vitamin E, ascorbic acid, Se and Zn. Production parameters do not improve better and unfavourable changes are already induced in the antioxidant system, which indicate the prooxidant effect of the compounds and elements used. Therefore, at high environmental temperatures (30°C) (in the summer), we recommend to use the following supplementation: 540 mg Vitamin E, 998 mg Vitamin C, 0.60 mg Se and 97 mg Zn on kg feed basis.
- A “special summer” premix must be developed in practice to reduce the harmful effects of high ambient temperature (heat shock).

The new scientific results are the following:

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, 1996 mg Vitamin C, 0.90 mg Se and 148 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 and 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: 1996 mg/kg diet, Se: 0.90mg/kg diet and Zn: 148 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, 1996 mg Vitamin C, 0.90 mg Se and 148 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.

9. ÖSSZEFOGLALÁS

A szakirodalomi adatok bizonyítják, hogy a magas környezeti hőmérséklet esetén hő-stressz alakulhat ki a baromfiban, amennyiben a madár a termelt hőt nem tudja leadni a környezetében. Ismeretes az is, hogy a baromfi, különösképpen a kacsák sokkal érzékenyebbek a hő stresszel szemben, mint más gazdasági állatfajok, mivel gyors anyagcserével rendelkeznek, magas a testhőmérsékletük, testük tollakkal borított és nincs izzadságmirigyük. Jelenleg azonban a nemzetközi szakirodalomban alig található szisztematikusan felépítette kísérlet és ennek okán kevés megbízható szakirodalmiadat áll rendelkezésre azzal kapcsolatban, hogy a magas környezeti hőmérséklet esetén, milyen fiziológiai változások történnek a kacsák szervezetében és ilyen körülmények között hogyan kell a kacsákat takarmányozni.

Általánosságban ismert, hogy az állatok esetében a hő-stressz káros hatása csökkenthető biológiai (pl. genetika, magas környezeti hőmérséklethez „szoktatás", takarmányozás) és tartástechnológiai eszközökkel (pl. légkondicionálás, intenzív ventilláció, párásítás). A tartástechnológiai módszerek megvalósítása és fenntartása is költséges és általában nem minden esetben elegendő. Ezért a gazdaságos termelés érdekében rendkívül fontos annak ismerete, hogy a hő-stressz negatív biokémiai és fiziológiai hatásai miképpen csökkenthetők különböző takarmányozási módszerekkel. Az ide vonatkozó kevés számú kutatások eredményei alapján azokat a legfontosabb vitamin és ásványi anyagokat, melyeket a hő-stressz kivédésére általánosságban a baromfitakarmányozásban, mint takarmány kiegészítőket alkalmaznak.

A disszertáció célja, annak vizsgálata volt, hogy a takarmány C- és E-vitamin, valamint a cink és szelén kiegészítése állandó magas környezeti hőmérséklet esetén (30±1°C 24 órán keresztül 28 napig) miképpen befolyásolja a pecsenye kacsák néhány élettani paraméterét beleértve az antioxidáns védelmi rendszert; a táplálóanyagok átalakulását, az emészthetőség és energia valamint fehérje hasznosítást, továbbá a termelési paramétereket és az értékes kacsa húsrészek kémiai összetételét a nevelés 14 és 42 napja között. Ezen kívül a disszertáció bemutatja a hőstressz negatív hatásának csökkentését a takarmány vitamin és ásványi anyag kiegészítésével (C-vitamin, E-vitamin, cink, szelén) a kacsa takarmányozásban.

A kísérletben 900 vegyes ivarú Cherry Valley típusú hús hibrid kacsát véletlenszerűen osztottunk szét 3 különböző takarmányozási csoportba (3 kezelési csoport). Mindegyik

kezelési csoportban, fülkénként 20 vegyes ivarú kacsát helyeztünk el (20 madár/fülke). Így 5 fülke állt rendelkezésünkre kezelésenként (5 fülke/kezelés). A kísérletet pedig még kétszer megismételtük. Az állatházban 28 napon keresztül (14-42 napos korig) állandó hőmérsékletet biztosítottunk: 30±1°C (24 órán keresztül), 62±5% relatív páratartalom mellett. A vizsgálatban három kísérleti takarmányt etettünk. A K1-es kezelésben kontroll takarmány került felhasználásra (évszaktól függetlenül általában ezt alkalmazzák a hús típusú kacsatartó telepeken), mely a 0,5%-os premixben az alábbiakat tartalmazta: E-vitamin: 40 mg/kg; C-vitamin: 0 mg/kg; szelén: 0,45 mg/kg; cink: 50 mg/kg. A K2-es kezelésben megnöveltük a vitaminok és mikroelemek mennyiségét. Ebben a kezelésben az E-vitamin tartalom 1540 mg/kg tak., C-vitamin tartalom 998 mg/kg tak., szelén tartalom 0,60 mg/kg tak. és cink tartalom pedig 97 mg/kg tak. volt. A K3-as kezelésben a K2-es kezelésben adott vitamin és mikroelem mennyiségét tovább növeltük, így a takarmány előbb említett vitamin és mikroelem tartalma a következő volt: E-vitamin (1540 mg/kg tak.), C-vitamin (1996 mg/kg tak.), szelén (0.90 mg/kg tak.) és cink (148 mg/kg tak.). 15 kacsát kezelésként véletlenszerűen kiválasztottunk és cervikális diszlokációval vér- bélsár és hús mintavétel céljából leöltük. A testhőmérséklet (BT) ivaronként került megmérésre. A takarmány táplálóanyag, vitamin és ásványi anyag tartalmát az AOAC szabványban (2012) meghatározott takarmány analitikai módszer szerint került elvégzésre. A kísérleti adatok statisztikai analízisét PROC GLM (mix modell) használatával, a SAS programmal végezzük el. A szignifikancia hatás meghatározása után az átlagok közötti különbségeket Tukey-teszttel határoztuk meg. A szignifikáns kezeléshatást $P<0,05$ szinten vizsgáltuk (SAS, 2010).

Magas környezeti hőmérséklet esetén a takarmányozási kezelések hatására nem változott a táplálóanyagok emészthetősége (nyers fehérje és szárazanyag) továbbá a kacsahús (mell és comb) kémiai összetétele sem.

Ismeretes, hogy a hő-stressz emelkedett lipid peroxidációt okoz, mely növeli az MDA koncentrációt továbbá zavart szenved az antioxidáns rendszer. Az általunk elvégzett kísérletben a várakozásunknak megfelelően az MDA koncentráció szignifikánsan csökkent a K2-es és K3-as kezelésben ($P<0,05$) a kontrolhoz képest. A vér E-vitamin koncentrációja az MDA-val párhuzamosan csökkent a K1 és K3-as kezelésben ($P<0,05$), azonban a K2 és K3-as kezelések esetén a különbség nem volt szignifikáns ($P>0,05$). A kísérletben a vér C-vitamin koncentrációja - hasonlóan más publikált kísérletek erdeményeihez-, szignifikánsan növekedett a K2 és K3-as kezelésben ($P<0,05$). Míg a

vér E-vitamin és a szeléntartalma között mérsékelt negatív korrelációt (-0,77) állapítottunk meg, addig a C-vitamin és cink között nagyon erős negatív korrelációt mutatott a vér MDA koncentrációjával (<0,8). Ez azt jelenti, hogy az alkalmazott takarmány kiegészítők alkalmazása csökkentette az MDA mennyiségét. Ez arra utal, hogy a kiegészítés a lipid peroxidáció mértékét pozitívan befolyásolta. A SOD mennyisége a K2-es kezelésben nem változott (P>0,05), de szignifikánsan csökkent a K3-as kezelésben (P<0,05). Ennek oka azzal magyarázható, hogy a takarmányhoz adott aszkorbinsav gyorsan reakcióba lépett a O_2^--dal, és emiatt a SOD enzim kevésbé volt aktív. Az ACW mennyisége szignifikáns növekedést mutatott a K2-es és K3-as kezelésben (P<0,05). Az ACL mennyisége nem változott (P<0,05) a K2-es kezelésben, azonban növekedett a K3-asban (P<0,05). A korreláció analízis eredményei azt mutatták, hogy a takarmány E-vitamin tartalma– a szérum ACL és a takarmány C-vitamin tartalma – szérum ACW között erős, pozitív korreláció áll fenn (>0,9). Ez azzal magyarázható, hogy az alkalmazott vitaminok a víz- és lipidoldékony antioxidáns vegyületek közzé tartoznak, vagyis a takarmányban történő koncentráció növekedés a vérben is ~~a~~ növekvő koncentrációt eredményez. A GPx szignifikánsan csökkent a K2 és K3-as kezelésben (P<0,05). A takarmány E-vitamin, C-vitamin, szelén és cink tartalma és a GR között erős negatív korrelációt tapasztaltunk. (>-0,8). Az alkalmazott vitamin és ásványi anyag kiegészítés hatására a GR koncentrációja csökkent, vagyis kevesebb GSH termelődött, ami csökkent GPx aktivitáshoz vezetett. Ezzel magyarázható, hogy a GR aktivitással párhuzamosan a K2-es, K3-as kezelésben csökkent a GPx aktivitása (P<0,05). A takarmány szeléntartalma és a vér E vitamin koncentrációja között erős negatív korrelációt állapítottunk meg, azaz a nagyobb szelén kiegészítés csökkentette az E-vitamin koncentrációt a vérben. Ez azzal magyarázható, hogy a szelén a GPx enzim kofaktora, vagyis a szelén megnövelt mennyisége az enzim aktivitását is növelte, és ezáltal a háromszintű antioxidáns védelmei rendszerben az ún. enzimatikus útvonal dominált. Ennek következtében kevesebb E-vitaminra (kis molekulájú, nagy antioxidáns kapacitású vegyületre) volt "szükség" a vérben ahhoz, hogy a madár semlegesítse a hő-stressz hatására megnőtt szabadgyök koncentrációt. A takarmányfelvétel szignifikánsan növekedett a K2 és K3-as kezelésekben (P<0,05) a K1-es kontroll csoporthoz képest. A 14 napos élősúly nem különbözött a kezelések között (P>0,05). A 42 napos élősúly (végsúly) szignifikánsan nagyobb volt a K2 és K3-as kezelésekben (P<0,05) mint a K1-ben. A napi súlygyarapodás a K2 és K3-as kezelésben ugyancsak nőtt (P<0,05) a K1-hez képest. A fajlagos takarmány értékesítés javult a K2 és K3-as kezelésekben a kontrollnak

tekintett K1 csoport értékeihez képest (P<0,05). A fajlagos energia- és fehérjeértékesítés valamint a fajlagos takarmányköltség szignifikánsan csökkent a K2 és K3 kezelésben (P<0,05). A takarmány E-vitamin, C-vitamin, szelén és cink tartalma és a 42 napos élősúly (>0,85) valamint a napi súlygyarapodás (>0,88) között erős pozitív korreláció áll fent. Ez azt jelenti, hogy a kísérletben alkalmazott kiegészítések növelték a súlygyarapodást a magas környezeti hőmérséklet esetén. A takarmány E-vitamin, szelén és cink tartalma és a fajlagos takarmányértékesítés között nagyon erős negatív korrelációt találtunk (-0,9). Ezek az eredmények azt is jelentik, hogy tartós meleg környezeti hőmérséklet (30±1°C) esetén is számíthatunk a takarmány E-vitaminnal, valamint szelénnel és cinkkel való kiegészítése esetén a fajlagos takarmány értékesítés javulására.

A kísérleti eredmények alapján az alábbi következtetések vonhatók le:

- A vérben oldható antioxidáns anyagok mennyisége (ACW, ACL) javult, a SOD és MDA koncentráció csökkent az alábbi takarmány kiegészítés hatására: E-vitamin: 1540 mg/kg tak., C-vitamin:1996 mg/kg tak., szelén: 0.90 mg/kg tak. és cink: 148 mg/kg tak. tartós hő-stressz esetén.
- A takarmány E-vitamin tartalma és a pecsekacsák szérum ACL valamint a takarmány C-vitamin tartalma és a szérum ACW között erős, pozitív korreláció áll fenn (>0,9) magas környezeti hőmérséklet esetén. A takarmány E-vitamin és szelén tartalma és az MDA között közepes negatív (-0,77), a takarmány C-vitamin és a cink tartalma és az MDA között pedig erős, negatív korreláció áll fent (>-0,8) a hús típusú kacsáknál hő-stressz során.
- A takarmány E-vitamin, C-vitamin, szelén és cink tartalma és a hús típusú kacsák 42 napos élősúlya (>0,85), valamint a napi súlygyarapodás (>0,88) között erős pozitív korreláció áll fenn krónikus hőstressz során. A takarmány E-vitamin, szelén és cink tartama és a hús típusú kacsák fajlagos takarmányértékesítő képesség között nagyon erős negatív korreláció áll fent (-0,9) hő-stressz során.
- A nyersfehérje és szárazanyag emészthetősége, valamint pecsenyekacsák húsának (comb és mell) kémiai összetétele nem változott az alkalmazott takarmányozási kezelések (E-vitamin: 1540 mg/kg tak., C-vitamin:1996 mg/kg tak., szelén: 0.90 mg/kg tak. és cink: 148 mg/kg tak. tartós hő-stressz esetén) hatására (P>0,05) állandó 30 ± 1°C teremhőmérséklet esetén.
- Az eredményeink alapján nem szükséges az emelt vitamin és ásványi anyag kiegészítést használni. A termelési paraméterek nem javultak jobban és az

antioxidáns rendszerre történő kedvezőtlen hatások kialakulását az alkalmazott kiegészítők csökkentették. Ezért magas környezeti hőmérséklet esetén (30℃) (nyáron) az alábbi takarmány kiegészítést javasoljuk a kacsák takarmányozáskor (E-vitamin: 540 mg/kg tak., C-vitamin:998 mg/kg tak., szelén: 0.60 mg/kg tak. és cink: 97 mg/kg tak.).

- Ugyancsak javasoljuk a gyakorlat számára egy "speciális" (megnövelt C-, és E-vitamin, valamint szelén és cink tartalommal) úgynevezett „nyári" premix összeállítását és alkalmazását a kacsa takarmányozásban a magas környezeti hőmérséklet káros hatásainak csökkentés érdekében.

Az új tudományos eredmények a következők:

1. A szérum antioxidáns kapacitása (melyet az ACL=zsíroldékony antioxidáns vegyületek és ACW=vízoldékony antioxidáns vegyületek határoznak meg) szignifikánsan javulnak, továbbá a lipid peroxidáció (melyet az MDA=malondialdehid koncentráció jelez) szignifikánsan csökkent hús típusú kacsák esetén, 30 ± 1°C-os állandó hőmérsékleten az alábbi takarmány kiegészítés használatával: E-vitamin: 1540 mg/kg tak., C-vitamin: 1996 mg/kg tak., szelén: 0.90 mg/kg tak. és cink: 148 mg/kg tak.
2. A takarmány különböző mennyiségű E-vitamin, C-vitamin, szelén és cink kiegészítés használatával javulnak a termelési paraméterek (élősúly, súlygyarapodás, takarmány értékesítés) hús típusú kacsák esetében tartós hő stressz során.
3. Állandó magas környezeti hőmérsékleten (30 ± 1°C) a takarmány E-vitaminkiegészítése és pecsenyekacsák szérum ACL, valamint a takarmány C-vitamin kiegészítése és a szérum ACW között erős, pozitív korreláció van (>0,9). A takarmány E-vitamin, szelén kiegészítése és a szérum MDA koncentrációja között közepes negatív korreláció állapítható meg (-0,77), míg a takarmány C-vitamin, cink kiegészítése és a szérum MDA koncentrációja között erős, negatív korreláció áll fent (>-0,8) hő-stressz esetén(30 ± 1°C).
4. A takarmány E-, C-vitamin, szelén és cink kiegészítése és a hús típusú kacsák 42 napos élősúlya (>0,85) és a napi súlygyarapodása (>0,88) között erős pozitív míg a takarmány E-vitamin, szelén és cink kiegészítése és a kacsák fajlagos takarmányértékesítésével között nagyon erős negatív korreláció (-0,9) áll fent tartós hő-stressz esetén (30 ± 1°C).

5. Tartósan magas környezeti hőmérséklet esetén (30 ± 1°C) a takarmány nyersfehérje és szárazanyag tartalmának emészthetősége továbbá a kacsahús (comb, mell) kémiai összetétele nem változik az alkalmazott takarmány kiegészítés hatására (E-vitamin: 1540 mg/kg tak., C-vitamin: 1996 mg/kg tak., szelén: 0.90 mg/kg tak., cink: 148 mg/kg tak.).
6. Emelt mennyiségű antioxidáns anyagok hatására (E-vitamin: 1540 mg/kg tak., C-vitamin: 1996 mg/kg tak., szelén: 0.60 mg/kg tak., cink: 97 mg/kg tak.) hús típusú kacsákban csökkenthető a hőstressz hatására emelkedett testhőmérséklet és nő a vér víz- (ACW) és zsíroldékony (ACL) antioxidáns vegyületeinek kapacitása.

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12. LIST OF PUBLICATIONS

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Registry number: DEENK/203/2021.PL
Subject: PhD Publication List

Candidate: Márta Horváth
Doctoral School: Doctoral School of Animal Husbandry
MTMT ID: 10056754

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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3. **Horváth, M.**, Pesti-Asbóth, G., Gálné Remenyik, J., Babinszky, L.: A hőstressz káros hatása a brojler antioxidáns státuszára és ezen hatás csökkentése takarmányozással: II. rész A hőstressz csökkentése takarmányozási módszerekkel.
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4. **Horváth, M.**, Babinszky, L.: Impact of chronic heat stress on digestibility of nutrients and performance of meat type ducks.
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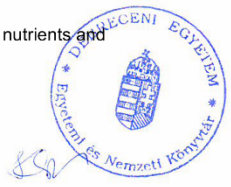

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5. **Horváth, M.**, Pesti-Asbóth, G., Gálné Remenyik, J., Babinszky, L.: Effect of dietary antioxidant supplementations on antioxidant parameters, rectal temperature, digestibility of nutrients, performance and chemical composition of meat in Cherry Valley type ducks under chronic heat stress.
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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

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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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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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14. **Horváth, M.**, Pesti-Asbóth, G., Gálné Remenyik, J., Babinszky, L.: Egy új mikrobiális fermentációs eljárással előállított probiotikum felhasználásának lehetősége a brojler takarmányozásban.
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19 April, 2021

13. APPENDIX

SOME PHOTOES TAKEN DURING THE ANIMAL STUDY

Photo 1 The animal house

Photo 2 Free access to water

Photo 3 Ad libitum feeding

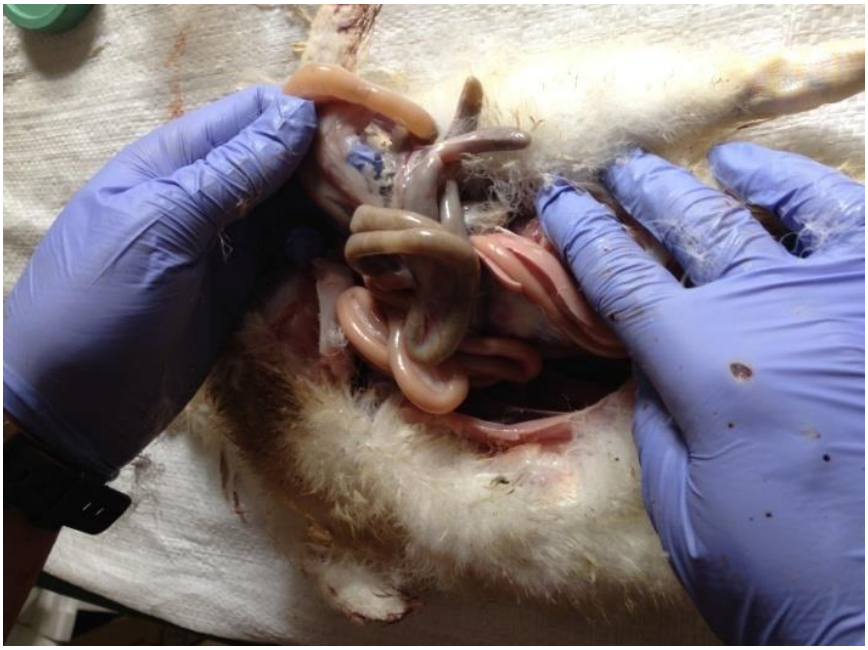

Photo 4 Opening the abdominal cavity

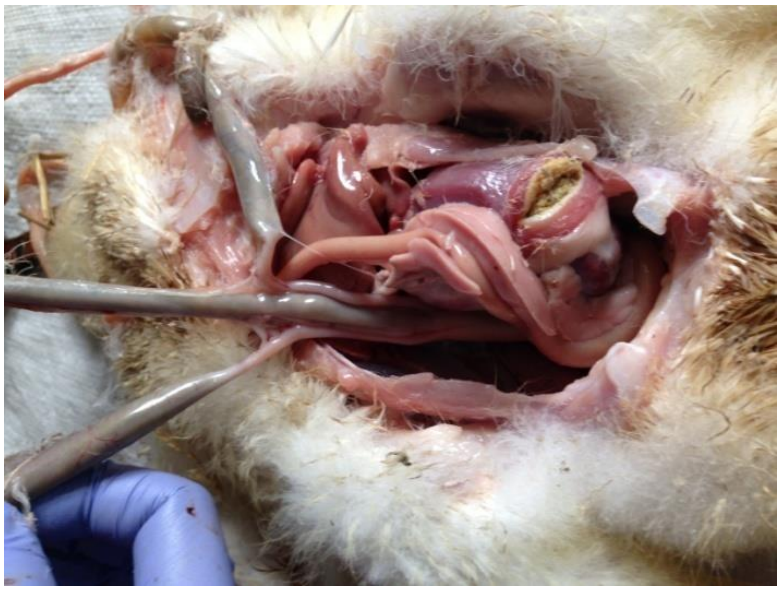

Photo 5 Determining the digesta sampling locations

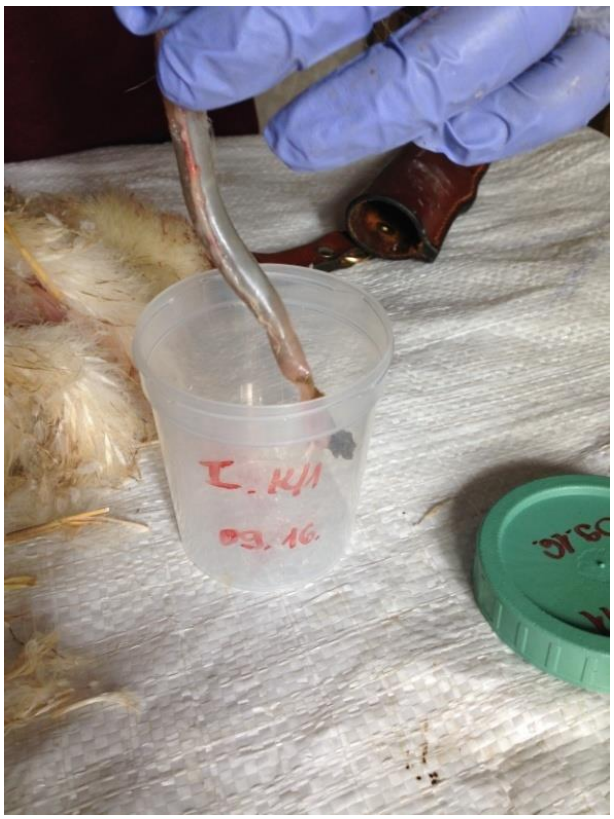

Photo 6 Digesta sampling

Photo 7 The temperature and humidity monitoring

14. NYILATKOZAT

Ezen értekezést a Debreceni Egyetem Állattenyésztési Tudományok Doktori Iskola keretében készítettem, a Debreceni Egyetem doktori (Ph.D.) fokozatának elnyerése céljából.

Debrecen, 2021. április 21.

……..

a jelölt aláírása

NYILATKOZAT

Tanúsítom, hogy **HORVÁTH MÁRTA** doktorjelölt **2015-2018** között a fent megnevezett Doktori Iskola keretében irányításommal/irányításunkkal végezte munkáját. Az értekezésben foglalt eredményekhez a jelölt önálló alkotó tevékenységével meghatározóan hozzájárult, az értekezés a jelölt önálló munkája. Az értekezés elfogadását javaslom/javasoljuk.

Debrecen, 2021. április 21.

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a témavezető(k) aláírása