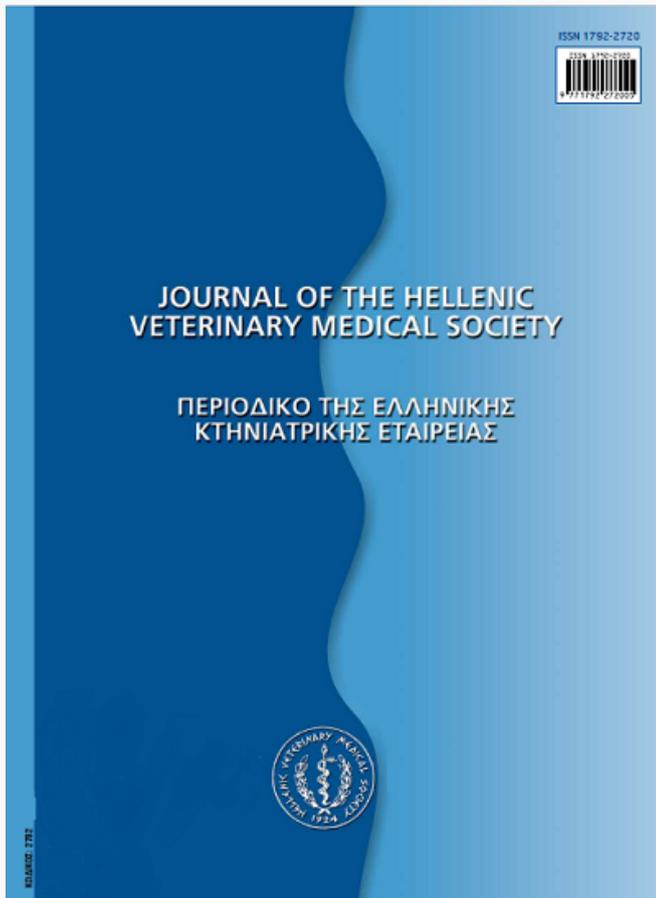


Περιοδικό της Ελληνικής Κτηνιατρικής Εταιρείας

Τόμ. 68, Αρ. 2 (2017)



Η επίδραση της βιταμίνης Ε σε μαστοκύτταρα του λεπτού εντέρου των κοτόπουλων κρεατοπαραγωγής κάτω από θερμικό στρες

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doi: [10.12681/jhvms.15600](https://doi.org/10.12681/jhvms.15600)

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Βιβλιογραφική αναφορά:

YILDIZ, M., KUM, S., & EREN, U. (2018). Η επίδραση της βιταμίνης Ε σε μαστοκύτταρα του λεπτού εντέρου των κοτόπουλων κρεατοπαραγωγής κάτω από θερμικό στρες. *Περιοδικό της Ελληνικής Κτηνιατρικής Εταιρείας*, 68(2), 159–166. <https://doi.org/10.12681/jhvms.15600>

The effect of vitamin E on mast cells in small intestine of broilers under heat stress

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ABSTRACT. The aim of this study is to identify the effect of vitamin E (DL- α -tocopherol acetate) (300 IU/kg) on mast cells in the small intestine (duodenum, jejunum and ileum) under heat stress. In the study, 42 one-day-old Ross 308 male broiler chicks were used. The chicks were randomly separated into 3 groups as follows; control (22 \pm 2°C), heat stress (35°C, 5 hours/per day) and vitamin E (300 IU/kg/per day) + heat stress (35°C, 5 hours/per day). The applications of heat stress and vitamin E began on the fifteenth day and ended on the thirty-fifth day. Tissue samples were taken from animals in each group of four and five-week-old chickens. Tissue samples were fixed in BLA (Basic Lead Acetate) solution. The sections were stained with toluidine blue (TB) (pH 0.5) and alcian blue-critical electrolyte concentration (AB-CEC) (pH 5.8, 0.3 M MgCl₂) / Safranin O (SO) (pH 1.0) combined method. It was determined that increasing of the exposure duration to heat stress increased the number of mast cells in the small intestine of the boilers. Also, it was revealed that vitamin E reduced mast cell population under heat stress. Consequently, heat stress may play a role in the pathogenesis of small intestine-associated with disorders and the supplementation of vitamin E can contribute to regulate small intestine functions of broilers by decreasing mast cell proliferation and activation under heat stress.

Keywords: Broiler, heat stress, mast cell, vitamin E

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Date of initial submission: 20.1.2016
Date of revised submission: 2.3.2016
Date of acceptance: 1.6.2016

INTRODUCTION

In poultry breeding, animals remain under pressure of many stress factors and one of the most important of these factors is heat stress. High ambient temperature reduces body weight and food consumption of laying hens. Besides, it suppresses the antibody production and the number of white blood cell but increases the rate of mortality (Mashaly et al., 2004). However, under the influence of heat stress, the rate of apoptosis in human intestinal epithelial Caco-2 cells increases and tight junction damage occurs (Xiao et al., 2013). Moreover, heat stress increases serum tumor necrosis factor- α and interleukin-1 (IL-1) levels, as well as the number of mast cells in laying hens. Unlike, it decreases the numbers of intra-epithelial lymphocytes and IgA-secreting cells in the small intestine (Deng et al., 2012). On the other hand, under the influence of heat stress, serum glutathione (GSH), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) activities decrease in black-boned chickens (Liu et al., 2014). On the contrary, dienecephalic and mitochondrial malondialdehyde (MDA) levels increase (Chowdhury et al., 2014; Mujahid et al., 2007).

Mast cells are classified as mucosal and connective tissue mast cells (Enerbäck, 1966a; Enerbäck, 1966b). Mast cells are associated with blood vessels and nerves, and they particularly located in close proximity to the surface of interaction with the environment (Metcalf et al., 1997). They play a role in regulating visceral sensitivity and vascular permeability (Ramsay et al., 2010). They also have a protective role in parasitic or microbial infections (Penissi et al., 2003). Degranulation of mast cells leads to the intestinal release of mast cell mediators such as histamine and so, the epithelial ion transport is induced (Collins et al., 2007). Besides, mast cells contribute to regulation of sensation (Chen et al., 2004). On the other hand, they can phagocytize various particles and secrete numerous cytokines to improve the functions of lymphocytes, as well as activate B cells to produce Ig-E (Henz et al., 2001). Moreover, the number of mast cells increases in gastrointestinal diseases such as acute appendicitis (Coskun et al., 2003), irritable bowel syndrome, mastocytic enterocolitis and systemic mastocytosis in the intestinal mucosa (Ramsay et al., 2010).

Vitamin E is an important antioxidant (Wallert et al., 2014) and protects cells from oxidative damage (Rengaraj and Hong, 2015). In studies investigating the antioxidant role of vitamin E, it has been found that the levels of GPx, SOD, CAT (Kabay et al., 2009) and GSH (Ajith et al., 2007) increased, whereas the value of MDA decreased (Kabay et al., 2009; Eid et al., 2006). On the other hand; vitamin E has an anti-inflammatory effect (Wallert et al., 2014). It reduces the activation (Shaik-Dasthagirisaheb et al., 2013) and proliferation of mast cells (Reiter et al., 2007; Kempná et al., 2004). It suppresses neutrophil infiltration (Ohta et al., 2006; Rocksén et al., 2003) and the degranulation of mast cells, thus it reduces histamine release (Tsuduki et al., 2013). Rather, it increases lymphocyte proliferation (Puthongsiriporn et al., 2001; Moriguchi, 1998) and the number of immunoglobulins producing cells (Kum et al., 2013). In its deficiency, it reduces resistance to helminth infections (Au Yeung et al., 2005).

In the present study, it is aimed to determine the numerical density of mast cells in all segments in the small intestine of broilers under heat stress and to reveal the effect of vitamin E application on mast cells.

MATERIALS AND METHODS

Animals and experimental procedure

In the study, 42 one day old Ross 308 male broiler chicks were used. The chicks were divided into three groups [control group ($22\pm 2^\circ\text{C}$) ($n=7$), heat stress group (35°C , 5 hours/per day) ($n=7$) and vitamin E (DL- α -tocopherol acetate) (Merck) (300 IU/kg/per day) plus heat stress group (35°C , 5 hours/per day) ($n=7$)]. Every 21 chicks were used for groups of fourth and fifth weeks. The chickens were fed *ad libitum* a starter diet (days 1-21) and growth/finishing diet (days 22-35) (Table 1). The experiment was carried out in May and June months. The ambient temperature on the first day was set at $32\pm 1^\circ\text{C}$ and then gradually decreased until it reached at $24\pm 1^\circ\text{C}$ on day 14. The applications of heat stress and vitamin E began on the fifteenth day and ended on the thirty-fifth day. Vitamin E was administrated by oral gavage. Heat stress and vitamin E were applied 14 days for the chickens of 4th week and 21 days for the chickens of 5th week. In the control group, air conditioning was used. The temperature in

Table 1: The chemical composition of basal diets distributed to broilers for 35 days

	Starter (1-21 days)	Growth/Finisher (22-35 days)
Metabolizable energy (MJ/kg)	12.97	13.60
Crude proteins (g/kg)	220	205
Lysine (g/kg)	11	13.5
Methionine+cysteine (g/kg)	9	9
Methionine (g/kg)	5	5
Calcium (g/kg)	10	9
Phosphorus (g/kg)	7	6.5

the heat stress groups was provided by utilizing the electric heater. The relative humidity was maintained at 50±5%. The lighting was continued. All procedures were approved by Adnan Menderes University Animal Ethics Committee.

Examination with light microscopy

The animals were euthanized by decapitation and samples of their small intestines (duodenum, jejunum and ileum) were removed from four and five-week-old chickens. Then, each tissue sample was fixed in basic lead acetate (BLA). After routine histologic processing, the tissues were embedded in paraffin and five serial sections, each 5 µm thick, were taken at 100 µm intervals. Then, the sections were stained with toluidine blue (TB, pH 0.5) (Uslu and Yoruk, 2008) and Alcian blue-critical electrolyte concentration (AB-CEC) (pH 5.8, 0.3 M MgCl₂) / Safranin O (SO, pH 1.0) combined method (Scott and Dorling, 1965; Harem and Liman, 2009). Finally, the sections were examined under a light microscope (Leica DMLB) equipped with an image-analysis system (Leica Q Win Standard) and appropriate locations were photographed.

Cell count and statistical analyses

The number of mast cells was determined in the TB-stained sections at fourth and fifth weeks. Therefore, mast cells were counted in ten different 36475, 3 µm² microscopic fields in lamina propria of the duodenum, jejunum and ileum at 40x magnification. All analyses were performed using the SPSS 17.0 program

package. Data were analysed with one-way analyses of variance (ANOVA) and the source of the group's differences was determined *post hoc* with a Duncan's test (Park et al., 2011). The mast cell counts between same groups of fifth and fourth weeks were compared with independent-samples T test (Bingley et al., 2003). Values were presented as mean ± standard error. Values for which P<0.05 (*), P<0.01 (**) and P<0.001 (***) were considered statistically significant.

RESULTS

Mast cell distributions were demonstrated in the duodenum, jejunum and ileum of broilers. For this reason, TB and AB-CEC/SO methods were applied to the tissues. Mast cells were stained metachromatically with TB and AB-CEC (+) and SO (-) with AB-CEC/SO staining methods in all segments of the small intestine (Figure 1 and 2). Furthermore, mast cells were observed in the lamina propria, submucosa, tunica muscularis and serosa. They were especially noticeable in the connective tissue of the villi, surrounding the blood vessels, the glands and the peripheral nerves.

The mast cell numbers were determined in the duodenum, jejunum and ileum of broilers at fourth and fifth weeks and were shown in Table 2-4, Figure 1 and 2. While, a significant difference wasn't found between the numbers of the mast cell in duodenum and jejunum, it was significantly lower in the ileum of heat stress group compare to control group at the fourth week. Besides, the number of mast cells was significantly lower in vitamin E+heat stress group compare to heat

Table 2. The number of the mast cells in duodenum

Groups	n	Fourth week	n	Fifth week	P
Control	7	5,00±0,16 ^a	7	4,65±0,17 ^a	NS
Heat stress	7	4,70±0,25 ^a	7	5,14±0,15 ^a	NS
Vitamin E+Heat stress	7	2,43±0,17 ^{B,b}	7	3,67±0,12 ^{A,c}	***
P		***		***	

^{a,b,c}: Different superscripts in the same column indicate the significant difference. ^{A,B}: Different superscripts in the same row indicate the significant difference. NS: Non- significant, ***: P<0,001

Table 3. The number of the mast cells in jejunum

Groups	n	Fourth week	n	Fifth week	P
Control	7	5.26±0,25 ^a	7	6,09±0,22 ^{a,b}	NS
Heat stress	7	4,95±0,17 ^{B,a}	7	6,61±0,19 ^{A,a}	***
Vitamin E+Heat stress	7	3,31±0,12 ^{B,b}	7	5,34±0,14 ^{A,b}	***
P		***		**	

^{a,b}: Different superscripts in the same column indicate the significant difference. ^{A,B}: Different superscripts in the same row indicate the significant difference. NS: Non-significant **:P<0,01, ***: P<0,001

Table 4. The number of the mast cells in ileum

Groups	n	Fourth week	n	Fifth week	P
Control	7	6,37±0,24 ^a	7	5,68±0,19	NS
Heat stress	7	5,20±0,30 ^{B,b}	7	6,06±0,16 ^A	*
Vitamin E+Heat stress	7	4,10±0,18 ^{B,c}	7	5,92±0,16 ^A	***
P		***		NS	

^{a,b,c}: Different superscripts in the same column indicate the significant difference. ^{A,B}: Different superscripts in the same row indicate the significant difference. NS: Non- significant, *:P<0,05, ***: P<0,001

stress group in all segments of the small intestine at the fourth week ($P<0,001$). At the fifth week, a significant difference wasn't determined in terms of the number of mast cells in all segments of the small intestine of heat stress group compare to the control group. On the contrary, it was significantly lower in the duodenum and jejunum of vitamin E + heat stress group compare to heat stress group ($P<0,001$, $P<0,01$). However, a significant difference wasn't found in the ileum. On the other hand, the number of mast cells was significantly higher in duodenum at the fifth week compare to the fourth week in vitamin E+heat stress group. Similarly, it was significantly higher in jejunum and ileum at the fifth week compare to the fourth week in heat stress and vitamin E+heat stress groups ($P<0,05$, $P<0,001$). But there wasn't a significant difference in terms of control groups between fifth and fourth weeks.

DISCUSSION

There are two types of mast cells as mucosal and connective tissue (Enerbäck, 1966a; Enerbäck, 1966b). Mucosal mast cells are stained with AB (+) and connective tissue mast cells are stained with SO (+) (Enerbäck, 1966b). In studies; while AB (+), SO (+) and AB/SO (+) mast cells were observed in proventriculus of *Gallus gallus domestica* (Aksoy and Cinar, 2008). It was detected that mucosal mast cells had AB (+) reaction in the digestive tract of chickens and quails. Also, SO (+) and less frequently AB/SO (+) mast cells were observed in some organs of the digestive system (Karaca and Yoruk, 2004). In the intestine of dogs, AB (+) and SO (-) mast cells were found (Eren et al., 2000). Besides, it was determined that mast cells had different glycosaminoglycan types with aldehyde fuchsin (AF) and AB-CEC (pH 5.8, 0.3 M MgCl₂) combined method stained in tissue sections of rats and quails (Harem and Liman, 2009). In our study, it was detected that mast cells showed AB-CEC (+), SO (-) reaction with the AB-CEC/SO staining method in all segments of the small intestine. Therefore, it was concluded that mast cells in the small intestine of the broiler were mucosal mast cells.

After being activated, mast cells release granule-associated mediators and they produce lipid-derived substrates in order to induce the allergic inflammation

(Metcalf, 1997). Similarly, degranulation of mast cells leads to release histamine in the intestinal tract and so, epithelial ion transport is stimulated (Collins et al., 2007). Heat stress increases the apoptosis rate in human intestinal epithelial Caco-2 cells and it induces tight junction damage and this leads to dysfunction of the intestinal epithelial barrier (Xiao et al., 2013). It has been reported that the numbers of intra epithelial lymphocytes and IgA-secreting cells decreased in the small intestine of the laying hens under the influence of heat stress whereas the number of mast cells increased. For that reason, it was stated that it may be contributed to the formation of epithelial damage in the small intestine of laying hens (Deng et al., 2012). Also, it was determined that the number of mast cells increased in the diseases such as appendicitis (Coskun et al., 2003; Singh et al., 2008), inflammatory bowel disease (Goral et al., 2010), mastocytes enterocolitis (Jakate et al., 2006) and ulcerative colitis (Stasikowsk A-Kanick et al., 2012; Zhao et al., 2009; Magro et al., 2006). In the present study, the number of mast cells was significantly higher in duodenum at the fifth week compare to the fourth week in vitamin E+heat stress group. Also, it was significantly higher in jejunum and ileum at the fifth week compare to the fourth week both heat stress and vitamin E+heat stress groups. Based on these findings; it was revealed that increasing of the exposure duration to heat stress increased the number of mast cells in the small intestine of the boiler. Therefore, it is thought that various pathophysiological diseases or disorders involving mast cells may occur in the small intestine. However, they might have a positive effect in the intestine, too. Because, water loss increases under the heat status. Mast cells play a role in controlling the permeability of the intestine (Jacob et al., 2005). Thus, the number of mast cells may increase in intestine under heat stress in order to decrease the water loss and increase the absorption.

Vitamin E has an anti-inflammatory effect (Wallert et al., 2014). It increases the number of immunoglobulin-producing cells in the intestine (Kum et al., 2013). In the deficiency of it, resistance to helminth infections reduces (Au Yeung et al., 2005). On the other hand, it suppresses the activation (Shaik-Dasthagirisaheb et al., 2013) and proliferation of mast cells (Reiter et al., 2007; Kempná et al., 2004). Also, it contributes to prevent inflammatory diseases by reducing the release and

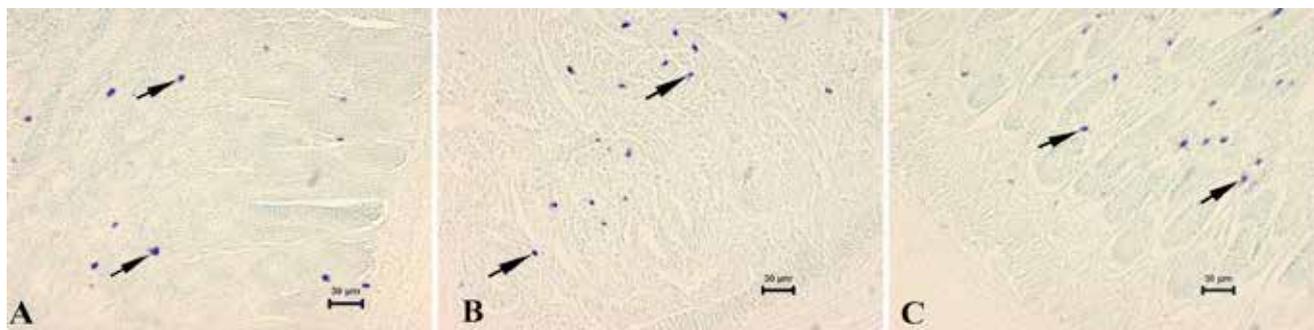


Figure 1. The metachromatic mast cells are seen in lamina propria folds of the duodenum (A), jejunum (B), ileum (C) in the control group (Arrows). TB staining method. Bar: 30 μ m (A-C)

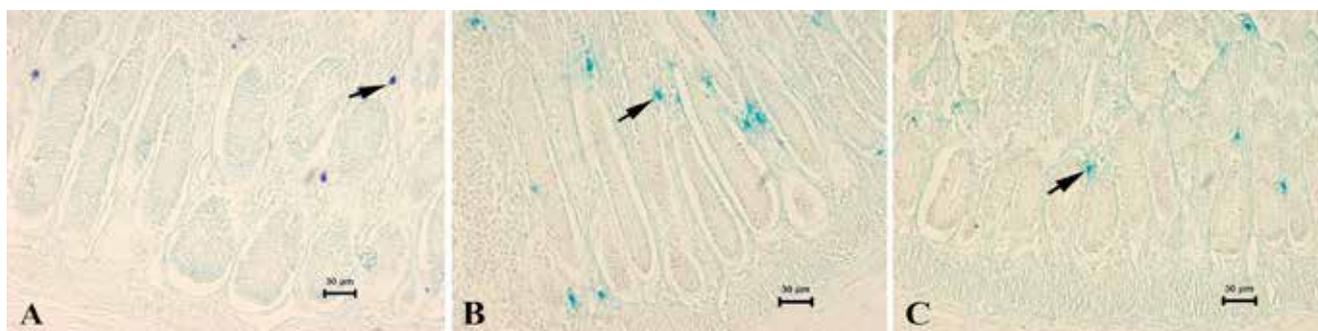


Figure 2. The metachromatic mast cells are observed in lamina propria fold of duodenum in vitamin E+heat stress group (A). Also, AB-CEC (+) / SO (-) mast cells are seen in lamina propria folds of jejunums in control (B) and vitamin E+heat stress groups (C) (Arrows). TB staining method (A), AB-CEC/SO staining method (B, C). Bar: 30 μ m (A-C)

production of inflammatory mediators in mastocytoma cells (Gueck et al., 2002). In our study, it was detected that the number of mast cells was significantly lower in all segments of the small intestine at the fourth week and in duodenum and jejunum at the fifth week in vitamin E + heat stress group compared to heat stress group. Therefore; it can be considered that vitamin E may suppress the proliferation of mast cells in the small intestine under heat stress and may have an inhibitory effect on the formation of inflammatory diseases.

CONCLUDING REMARKS

Under the influence of heat stress, the alteration in the number of mast cells in the small intestine of

broilers may play a role in the pathogenesis of intestine associated lesions. The mast cell proliferation and activation may be suppressed with the application of vitamin E. So, it can be contributed to the regulation of intestine functions of broilers under stress.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST STATEMENT

The authors report no conflicts of interests. ■

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