THE EFFECTS OF VITAMIN E ON IMMUNGLOBULIN-CONTAINING PLASMA CELLS IN GUT-ASSOCIATED LYMPHOID TISSUE (GALT) OF BROILERS UNDER HEAT STRESS

Sadiye Kum[1], Ulker Eren[1], Deniz Korkmaz[1], Mustafa Sandikci[1], Isil Aydemir[2]
[1]*Department of Histology and Embryology, Faculty of Veterinary Medicine, University of Adnan Menderes 09016, Isikli, Aydin, Turkey, tel. +9002562470700*
[2]*Department of Histology and Embryology, Faculty of Medicine, University of Celal Bayar 45016, Manisa, Turkey, tel. +900232331920*
Corresponding Author: skum@adu.edu.tr

Abstract. The effects of vitamin E (DL-α-tocopherol acetate) (300 IU/kg) on the immunglobuling-containing plasma cells (IgA, IgG and IgM) in oesophageal tonsils, pyloric tonsils, jejunum, ileum and caecal tonsils of broilers under heat stress were investigated. In the experiments, sixty-three one day-old Ross 308 breeds male broiler chicks were used. The chicks were divided into three groups [control group (22±2 °C), heat stress group (35 °C, 5h/day) and vitamin E (300 IU/kg) + heat stress (35 °C, 5h/day) group]. Each group consisted of twenty one chicks. Tissue samples were taken from seven animals in each group of four, five and six- week- old chickens and fixed in ice-cold PLP (Periodate-lysine-paraformaldehyde), and then embedded in paraffin. Sections were immunostained by the indirect immunoperoxidase method. The present study demonstrated that vitamin E could increase the number of the immunoglobulins producing cells. Consequently, supplementation of vitamin E in the diet can improve immunocompetence of broilers when in the summer months.
Keywords: heat stress, vitamin E, Ig-containing plasma cells, broiler.

VITAMINO E POVEIKIS BROILERIŲ VIRŠKINAMOJO TRAKTO LIMFOIDINIO AUDINIO IMUNOGLOBULINĄ GAMINANČIOMS PLAZMOS LĄSTELĖMS TERMINIO STRESO SĄLYGOMIS

Sadiye Kum[1], Ulker Eren[1], Deniz Korkmaz[1], Mustafa Sandikci[1], Isil Aydemir[2]
[1]*Histologijos ir embriologijos katedra, Veterinarinės medicinos katedra, Adnan Menderes universitetas 09016, Isikli, Aydin, Turkija; tel. +90 02 562 470 700*
[2]*Histologijos ir embriologijos katedra, Medicinos fakultetas, Celal Bayar universitetas 45016, Manisa, Turkija; tel. +90 02 32 33 19 20; el. paštas: skum@adu.edu.tr*

Santrauka. Buvo tirtas vitamino E (DL-α-tokoferolio acetatas) (300 IU/kg) poveikis imunoglobuliną gaminančioms plazmos ląstelėms (IgA, IgG and IgM) broilerių stemplės tonzilėse, prievarčio skrandžio liaukoje, tuščiojoje, klubinėje ir aklojoje žarnose terminio streso sąlygomis. Bandymas atliktas su 63 vienos dienos *Ross 308* veislės viščiukais broileriais. Viščiukai suskirstyti į tris grupes: kontrolinę (22±2°C), terminio streso (35°C, 5 h/d.) ir vitamino E (300 IU/kg) + terminio streso (35°C, 5 h/d.). Kiekvienoje grupėje buvo po 21 viščiuką. Paimti keturių, penkių ir šešių savaičių septynių iš kiekvienos grupės viščiukų audinių pavyzdžiai. Audiniai fiksuoti naudojant perjodato-lizino paraformaldehidą ir įtvirtinti parafine. Parafino blokų nuopjovos tirtos imunoperoksidazės metodu. Nustatyta, kad vitaminas E gali padidinti imunoglobuliną gaminančių ląstelių skaičių. Vadinasi, vitaminu E papildyta dieta galėtų sustiprinti broilerių imuninį atsaką vasaros mėnesiais.
Raktažodžiai: terminis stresas, vitaminas E, Ig gaminančios plazmos ląstelės, broileris.

Introduction. Commercial broiler farms play a significant role in avoiding malnutrition of people. However, some viral and bacterial diseases and vitamin deficiencies, lead to significant losses in poultry industry. These losses are even greater when birds are exposed to heat stress. The heat stress has detrimental effects on the performance of broiler birds reared in the open-sided poultry houses, principally through reducing feed intake and growth rate, and negatively affects feed efficiency and carcass quality (Carmen *et al.,* 1991; Yahav *et al.,* 1996; Temim *et al.,* 2000; Har *et al.,* 2000; Oskan *et al.,* 2003; Abu-Dieyeh, 2006). The heat stress affects the development of a specific immune response in chickens (Thaxton and Siegel, 1972; Niu *et al.,* 2009). While mammals have a large number of lymph nodes and rather complex lymphoid system birds, except some species such as ducks, geese and swans, do not have lymph nodes (Hodges, 1974; Casteleyn *et al.,* 2010). For this reason, GALT is important for the function of the immune system in chickens. In birds, the GALT is a lymphoid structure containing lymphoid aggregates distributed within the epithelium of the oesophageal tonsil (Olah *et al.,* 2003; Nagy *et al.,* 2005; Kum *et al.,* 2006; Sagsoz and Liman, 2009), pyloric tonsil (Nagy and Olah, 2007), lymphoid tissues in the jejunum (Olah *et al.,* 1984), and in the ileum (Befus *et al.,* 1980) and caecal tonsils (Olah and Glick, 1979). Vitamin E, major lipid-soluble antioxidant present in all cellular membranes, is an important nutrient for

optimal immune function. When animals are fed nutritionally complete diets lacking vitamin E, immune responses are adversely affected (Bendich, 1988).

Studies concerning the effect of vitamin E on the humoral immune response are rather limited (Muir *et al.,* 2002; Heffels-Redmann *et al.,* 2003; Khan *et al.,* 2008; Khan *et al.,* 2012). In this study, it was aimed to understand the effect of vitamin E on the localization of Ig containing plasma cells in oesophageal tonsil, pyloric tonsil, jejunum and ileum and caecal tonsils of broilers under heat stress.

Materials and Methods. In this study, sixty-three one day-old Ross 308 breeds male broiler chicks were used. All studies with animals described in this article were reviewed and approved by University of Adnan Menderes Institutional Animal Ethic Committee (B.30.2.ADU.0.06.00.00/124-HEK/2008/057). The chicks were divided into three groups [control group (22±2 ^{o}C), heat stress group (35 ^{o}C, 5h/day) and vitamin E (DL-α-tocopherol acetate) (Merck) (300 IU/kg) + heat stress (35 ^{o}C, 5h/day) group]. Each group consisted of twenty one chicks. The chickens were fed *ad libitum* a starter diet (days 1-21, ME, 12.97 MJ/kg; CP, 220 g/kg; lysine, 11 g/kg; methionine+cysteine, 9 g/kg; methionine, 5 g/kg; calcium, 10 g/kg; phosphorus, 7 g/kg), and growth/finishing diet (days 22-42, ME, 13.60 MJ/kg; CP, 205 g/kg; lysine, 13.5 g/kg; methionine+cysteine, 9 g/kg; methionine, 5 g/kg; calcium, 9 g/kg; phosphorus, 6.5 g/kg). The experiment was conducted in May and June. Ambient temperature on day one was set at 32±1 ^{o}C and then gradually decreased until it reached at 24±1 ^{o}C on day 15. Application of heat stress (35 ^{o}C) (5h/day) began on the sixteenth day. In the control group, air conditioning was used. Temperature in the heat stress groups was provided by utilizing the electric heater. The relative humidity was maintained at 50±5%. The lighting was continuous. Body weight gain and food consumption were recorded at weekly interval.

Tissue samples (oesophageal junction with proventriculus, pylorus junction with the duodenum, jejunum-ileum and left proximal caceum) were taken from seven animals in each group (control 7, heat stress 7 and vitamin E + heat stress 7) at four, five and six- week-old chickens. Tissue samples were fixed in ice-cold PLP (Periodate-lysine-paraformaldehyde), dehydrated in a series of graded alcohol, cleared in xylene, and embedded in paraffin. 6 µm thick paraffin sections for immunohistochemistry were cut at intervals of 50 µm. Tissue sections taken serially were placed on organosilane (3-aminopropyltriethoxy-silane, A3648; Sigma) coated slides. Paraffin sections were immunostained by the indirect immunoperoxidase method (Khan *et al.,* 2008). After endogenous peroxidase was inhibited with methanol and H_2O_2, sections were overlayed with 2% normal rabbit serum diluted with 0.01 M phosphate-buffered saline (PBS) for 2 h, followed by incubation with goat anti-chicken IgG (1:250, Bethyl Lab), goat anti-chicken IgA (1:250, Bethyl Lab), goat anti-chicken IgM (1:250, Bethyl Lab), for 18h at 4 ^{0}C. After washing with PBS for 5 min, sections were treated with HRP-conjugated rabbit anti-goat IgG (1:1000, Bethyl Lab), for 1h at room temperature. The sections were placed in TBS for 5 min and sections were finally colored with substrate-chromogen solution (3.3-diaminobenzidine-tetrahydrochloride dehydrate D-5905; Sigma) and slightly counterstained with hematoxyline. In control sections PBS was used instead of primary antisera. The prepared sections were examined under a light microscope (Leica DMLB microscope), localization and the frequency of the IgG, IgA and IgM containing plasma cells in the different tissues were determined (Bianchi *et al.,* 1992). Each tissue examined fifteen microscopic fields (40X) for each antibody. The symbols indicate that depending on the number of cells. Photographs of the tissues were taken with a Leica DC-200 camera. All data were checked for normal distribution with Shapiro-Wilk and homogeneity of variance with Levene's test. The data were compared among groups using Kruskall-Wallis analysis of variance (ANOVA), according to which the data were not normally distributed. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) software. Differences were considered statistically significant if $P<0.01$ and $P<0.001$ (Conover, 1980).

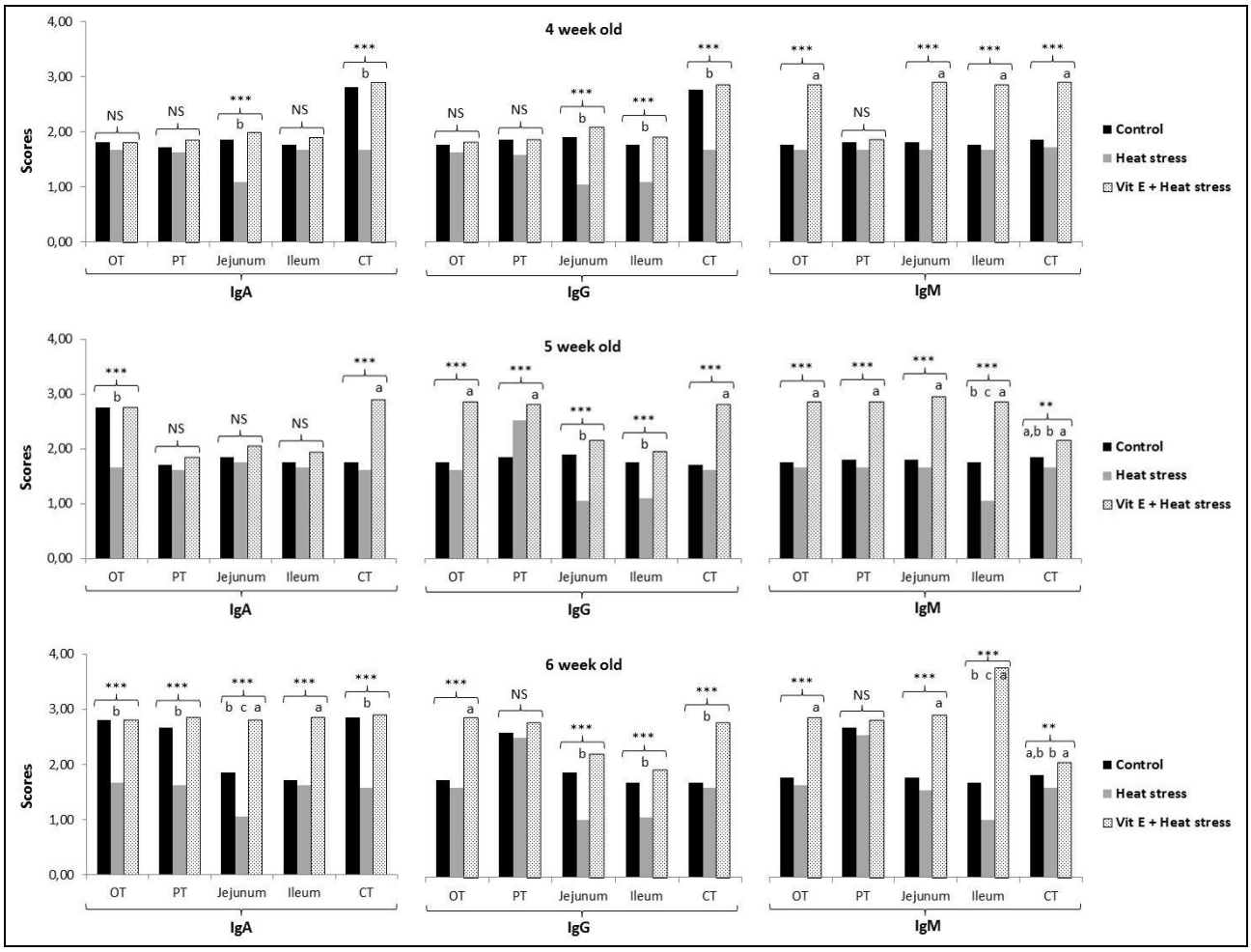
Results and Discussion. Oesophageal tonsils. The frequency of IgA, IgG, IgM positive cells in the oesophagus at all groups is shown in Table I and Graph I. Anti-immunoglobulin antibodies recognized IgA, IgG and IgM positive cells distinctly distributed in the lymphoid tissue. At four weeks in the control groups, IgA positive cells were seen as more intense in the subepithelial lamina propria (Fig. 1A).They were also found in the germinal centers (GC) (Fig. 1B). IgG positive cells were seen subepithelial, germinal center of the tonsils and between lamina propia of the mucous gland. IgM positive cells were observed subepithelial and between the corpus gland (Fig. 1C). At five weeks in the control groups, IgA positive cells were seen subepithelal lymphoid tissue and a few cells were seen intraepithelial. They also were observed in the germinal centers. IgG positive cells were found in the germinal center and between lymphoid tissues of the corpus glandula (Fig. 1D). A few IgM positive cells were seen subepithelial and between lymphoid tissue of the corpus glandula. At six weeks in the control groups, IgA, IgG and IgM positive cells were seen subepithelial lymphoid tissue and also in the germinal centers (Fig. 1E). Arai et al. (1988) drew attention that the avian oesophageal tonsil not only produces IgA for mucosal immunity, but also IgG systemic immunity. Olah et al. (2003) found that B lymphocytes in the oesophageal tonsils localized in the germinal centers and a few B cells occured in the interfollicular areas. At four weeks in the heat stress groups, a few IgA positive cells were seen intraepithelial and under the epithelium. IgG positive cells were observed between the mucous glands in little more than IgA positive cells (Fig. 1F). A few IgM positive cells were seen between corpus glandula and in the tonsils. At five weeks in the heat stress groups, IgA positive cells were observed in the lamina propria or under the epithelium and in the germinal centers. IgG positive cells

were seen in the lymphoid tissue between corpus glandula. IgM positive cells were observed intraepithelial, lamina propria under the epithelium and lymphoid tissue between corpus glandula (Fig. 1G). At six weeks in the heat stress groups, IgA positive cells were seen in the germinal centers of the tonsils and periphery of the tonsils (Fig. 1H). They were observed also intra epithelium and lamina propria between corpus glandula. IgG positive cells were distributed within the lamina propria and around glands. IgM positive cells were seen lamina propria under the epithelium and germinal centers. At four weeks in the vitamin E + heat stress group, a few IgA positive cells were observed under the epithelium and in the tonsils. IgG positive cells were seen as more intensive in the lamina propria under the epithelium and between the glands (Fig. 1I). A few IgM positive cells were observed in the germinal centers and periphery of the tonsils. At five weeks in the vitamin E + heat stress groups, IgA positive cells were observed in the lamina propria under the epithelium. IgG positive cells were encountered in the lamina propria under the epithelium (Fig. 1J) and lymphoid tissue between glands. IgM positive cells were seen in the germinal centers and the under the epithelium. At six weeks in the vitamin E + heat stress groups, IgA positive cells were observed in the lamina propria on top of the lymphoid tissue (Fig. 1K). IgG positive cells were seen in the germinal centers. A few IgM positive cells were observed in the lamina propria under the epithelium and between glands but more positive IgM cells were seen in the germinal centers (Fig. 1L).

Table 1. ***Frequencies of IgA, IgG, IgM containing plasma cells in different groups, olds and tissues***

			Oesophageal tonsils	Pyloric tonsils	Jejunum	Ileum	Caecal tonsils
4-week-old	Control	IgA	++	++	++	++	+++
		IgG	++	++	++	++	+++
		IgM	++	++	++	++	++
	Heat stress	IgA	++	++	+	++	++
		IgG	++	++	+	+	++
		IgM	++	++	++	++	++
	Vit E+Heat stress	IgA	++	++	++	++	+++
		IgG	++	++	++	++	+++
		IgM	+++	++	+++	+++	+++
5-week-old	Control	IgA	+++	++	++	++	++
		IgG	++	++	++	++	++
		IgM	++	+++	++	++	++
	Heat stress	IgA	++	++	++	++	++
		IgG	++	+++	+	+	++
		IgM	++	++	++	+	++
	Vit E+Heat stress	IgA	+++	++	++	++	+++
		IgG	+++	+++	++	++	+++
		IgM	+++	+++	+++	+++	++
6-week-old	Control	IgA	+++	+++	++	++	+++
		IgG	++	+++	++	++	++
		IgM	++	+++	++	++	++
	Heat stress	IgA	++	++	+	++	++
		IgG	++	+++	+	+	++
		IgM	++	+++	++	+	++
	Vit E+Heat stress	IgA	+++	+++	+++	+++	+++
		IgG	+++	+++	++	++	+++
		IgM	+++	+++	+++	++++	++

+ – few, ++ – little, +++ – moderate, ++++ – marked, Vit E – Vitamin as DL-α-tocopherol acetate



Graph 1. **Scores of IgA, IgG, IgM containing plasma cells in different groups, olds and tissues**

OT – Oesophageal tonsils, PT – Pyloric tonsils, CT – Caecal tonsils, Vit E – Vitamin as DL-α-tocopherol acetate, ** – $P<0.01$, *** – $P<0.001$, NS – Not significant

Pyloric tonsils. The frequency of IgA, IgG, IgM positive cells in the pyloric tonsils at all groups is shown in Table I and Graph I. At four weeks in the control groups, a few IgA positive cells were observed in the germinal centers. Usually positive cells were seen in the lamina propria around the crypts. Also IgG positive cells were distributed in the lamina propria around the crypts (Fig. 2A). IgM positive cells were seen in the lamina propria under the epithelium and a few positive cells were observed in the periphery of the tonsils. At five weeks in the control groups, IgA, IgG and IgM positive cells were seen in the lamina propria periphery of the tonsils (Fig. 2B). At six weeks in the control groups, IgA positive cells were observed in the interfollicular regions. Numerous IgG positive cells were seen in the lamina propria and germinal centers (Fig. 2C). A few IgM positive cells accumulated in the germinal centers. Nagy and Olah (2007) reported that B cells restricted to the germinal centers in the pyloric tonsils. At four weeks in the heat stress groups, a few IgA and IgM positive cells were seen interfollicular regions. More IgG positive cells were observed interfollicular regions and around the crypts (Fig. 2D). At five weeks in the heat stress groups, IgA, IgG and IgM positive cells were observed in the interfollicular regions (Fig. 2E). A few positive cells were seen in the germinal centers. At six weeks in the heat stress groups, a few IgA positive cells were observed in the lamina propria under the epithelium and germinal centers. Numerous IgG positive cells were seen in the interfollicular regions (Fig. 2F). A few IgM positive cells were observed in the interfollicular regions (Fig. 2G). At four weeks in the vitamin E + heat stress groups, IgA, IgG and IgM positive cells were observed in the interfollicular regions (Fig. 2H). At five weeks in the vitamin E + heat stress groups IgA, IgG and IgM positive cells were seen in the interfollicular regions (Fig. 2I). At six weeks in the vitamin E + heat stress groups, numerous IgA positive cells were observed in the lamina propria under the epithelium and a few positive cells were seen the germinal centers (Fig. 2J).

Jejunum and Ileum. The frequency of IgA, IgG, IgM positive cells in the jejunum and ileum at all groups is shown in Table I and Graph I. At four weeks in the control groups, a few IgA positive cells were observed on the top of the villi and in the lamina propria. IgG positive cells were seen in the lamina propria and crypt of the villi

(Fig. 3A). IgM positive cells were detected on the lymphoid tissue top of the villi. A few IgM positive cells were seen around the crypts. At five weeks in the control groups, IgA positive cells were distributed in the lamina propria of the villi. IgG positive cells were observed around the crypts. IgM positive cells were seen in the lamina propria top of the villi (Fig. 3B). At six weeks in the control groups, the IgA, IgG and IgM positive cells were distributed in the lamina propria, around the glands. IgM positive cells also were seen in the germinal centers (Fig. 3C). Khan et al. (2008) found that IgA, IgG and IgM-containing plasma cells were distributed in the lamina propria, around the intestinal glands and in the core of villi. These results were similar to our results. At four weeks in the heat stress groups, a few IgA positive cells were distributed in the lamina propria. IgG positive cells were seen in the lamina propria and crypt of the villi (Fig. 3D). A few IgM positive cells were observed in the lamina propria of the villi. At five weeks in the heat stress groups, IgA positive cells were distributed in the lamina propria of the villi. IgG positive cells were found in the top of the lymphoid tissue. IgM positive cells were observed in both of crypts and villi (Fig. 3E). At six weeks in the heat stress groups, the IgA, IgG and IgM positive cells were distributed in the lamina propria (Fig. 3F). At four weeks in the vitamin E + heat stress groups, the IgA, IgG and IgM positive cells were observed in the lamina propria (Fig. 3G). At five weeks in the vitamin E + heat stress groups, IgA positive cells were distributed in the lamina propria of the villi. IgG positive cells were seen both in the villi and lymphoid tissue (Fig. 3H). IgM positive cells were observed both in crypts and villi. At six weeks in the vitamin E + heat stress groups, IgA were seen both in the lamina propria of the villi and crypts (Fig. 3I). More IgG positive cells were observed in the lamina propria of the villi than in the control and heat stress groups (Fig. 3J). IgM positive cells were detected in the crypts, lamina propria of the villi and lymphoid tissue. Khan et al. (2008) reported that these cells increased in lamina propria and core of the villi of 150 mg and 300 mg vitamin E supplemented chickens in comparison with the control chickens. These results are consistent with our findings.

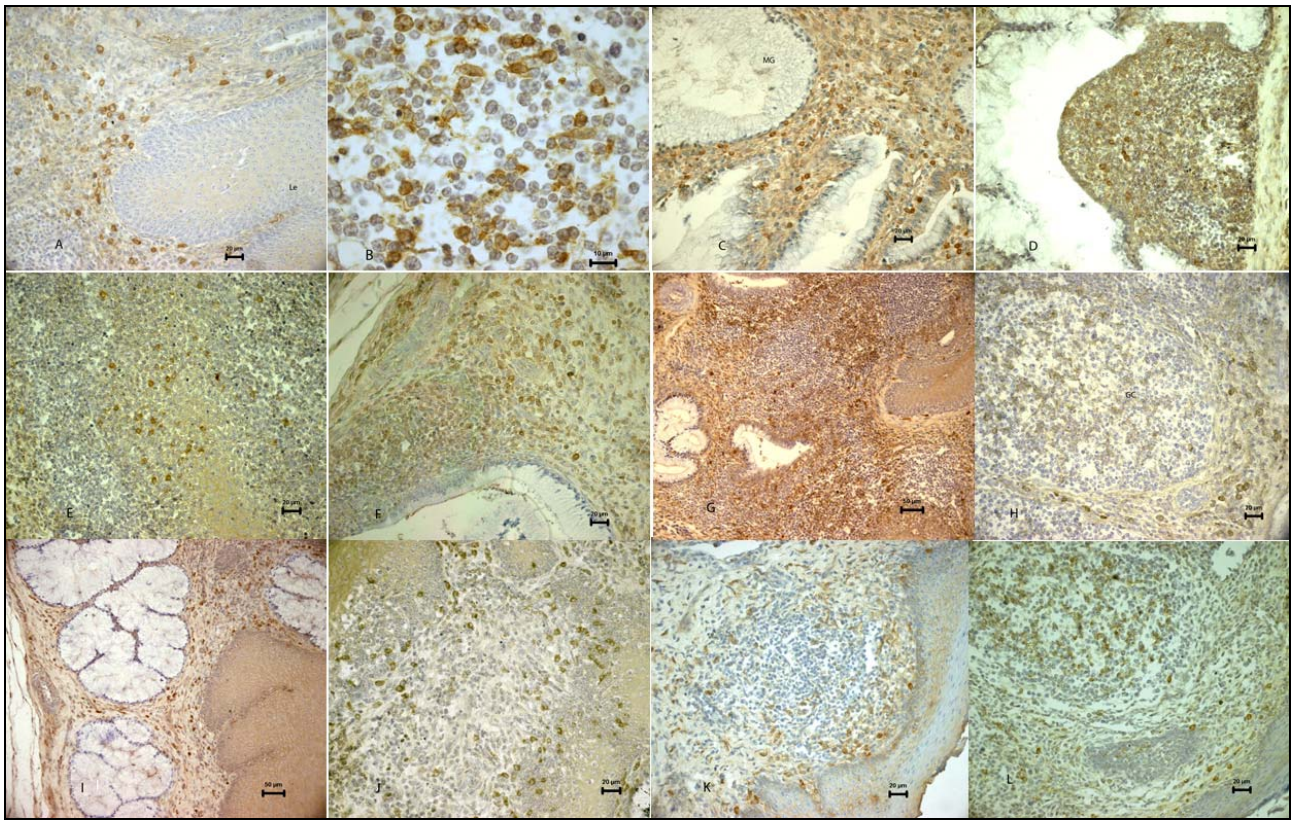


Fig. 1. A) In control group, IgA positive cells in the oesophageal tonsils. 4-week-old chicken, Bar: 20 µm. B) In control group, IgA positive cells in the germinal centers (GC). 4-week-old chicken, Bar: 10 µm. C) In control group, IgM positive cells in the oesophageal tonsils. 4-week-old chicken, Bar: 20 µm. D) In control group, IgG positive cells in the oesophageal tonsils. 5-week-old chicken, Bar: 20 µm. E) In control group, IgA positive cells. 6-week-old chicken, Bar: 20 µm. F) In heat stress group IG positive cells. 4-week-old chicken, Bar: 20 µm. G) In heat stress group, IgM positive cells. 5-week-old chicken, Bar: 50 µm. H) In heat stress group, IgA positive cells. 6-week-old chicken, Bar: 20 µm. I) In heat stress group with treated vitamin E, IgG positive cells. 4-week-old chicken, Bar: 50 µm. J) In heat stress group with treated vitamin E, IgG positive cells. 5-week-old chicken, Bar: 20 µm. K) In heat stress group with treated vitamin E, IgA positive cells. 6-week-old chicken, Bar: 20 µm. L) In heat stress group with treated vitamin E, IgM positive cells. 6-week-old chicken, Bar: 20 µm. Le: lamina epithelium, GC: Germinal center, MG: Mucous gland

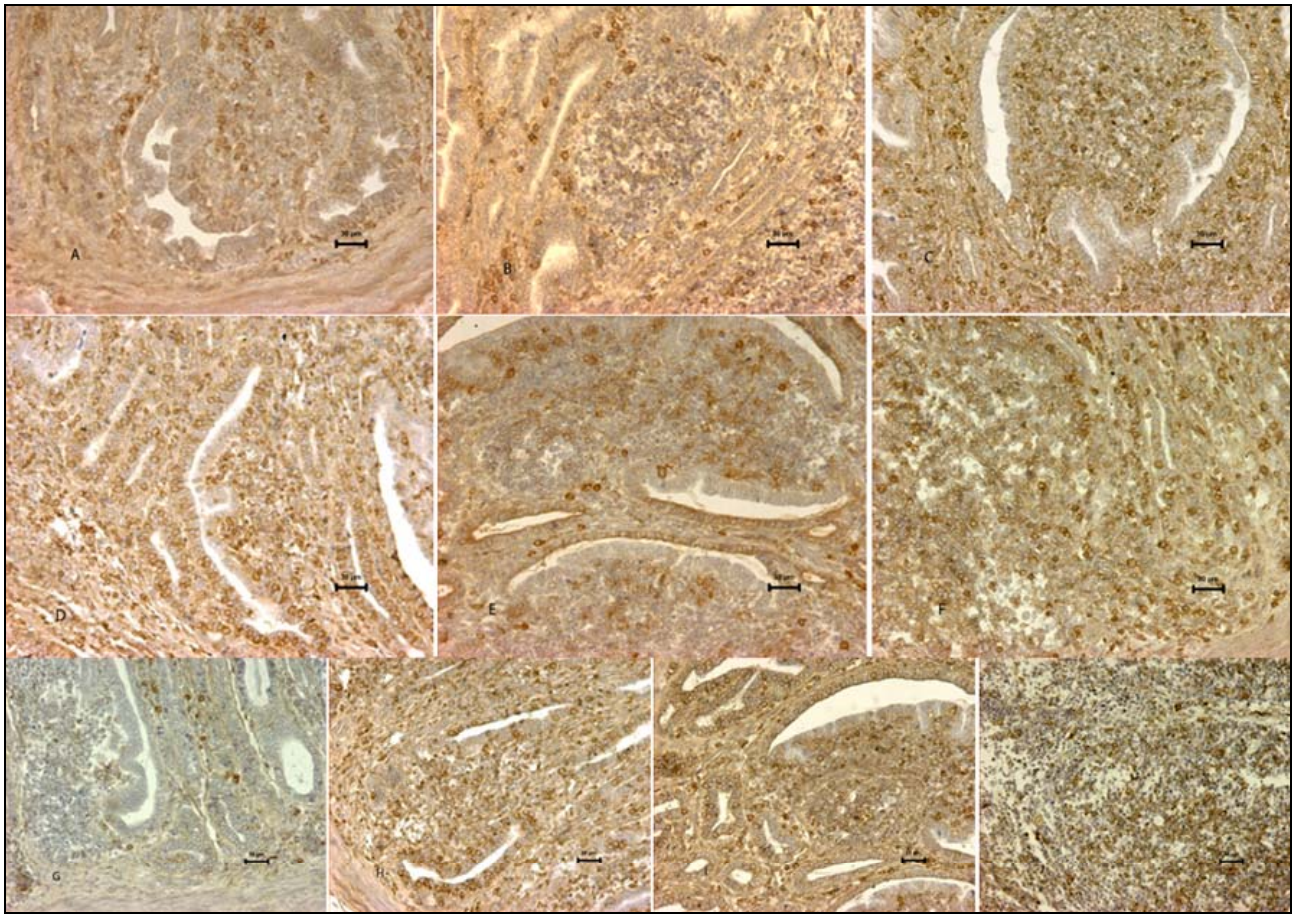


Fig. 2. A) In control group, IgG positive cells in the pyloric tonsil. 4-week-old chicken. Bar: 30 µm. B) In control group, IgA positive cells. 5-week-old chicken. Bar: 30 µm. C) In control group, IgG positive cells. 6-week-old chicken. Bar: 30 µm. D) In heat stress group, IgG positive cells. 5-week-old chicken. Bar: 30 µm. E). In heat stress group, IgM positive cells. 5-week-old chicken. Bar: 30 µm. F) In heat stress group, IgG positive cells. 6-week-old chicken, Bar: 30 µm. G). In heat stress group, IgM positive cells. 6-week-old chicken, Bar: 30 µm. H) In heat stress group with treated vitamin E, IgM positive cells. 4-week-old chicken, Bar: 30 µm. I) In heat stress group with treated vitamin E, IgG positive cells. 5-week-old chicken, Bar: 30 µm. J) In heat stress group with treated vitamin E, IgA positive cells. 6-week-old chicken, Bar: 30 µm

Caecal tonsils. The frequency of IgA, IgG, IgM positive cells in the caecal tonsils in all groups is shown in Table I and Graph I. At four weeks in the control groups, IgA positive cells were seen in the lamina propria under the epithelium and in the germinal centers (Fig. 4A). IgG positive cells were observed under the epithelium but no cells were seen in the germinal centers. IgM positive cells were seen in the lamina propria under the epithelium. At five weeks in the control groups, IgA positive cells were distributed in the lamina propria of the villi. IgG positive cells were seen in the germinal centers (Fig. 4B). IgM positive cells were more frequent in lymphoid tissue and germinal centers than IgG positive cells. At six weeks in the control groups, IgA and IgG positive cells were distributed in the lymphoid tissue (Fig. 4C). IgM positive cells were also observed in the germinal centers. In the caecal tonsils, IgA, IgG and IgM positive cells were distributed within lymphatic nodules, lamina propria and in the core of the villi (Hussan *et al*., 2009; Khan *et al.,* 2008; Islam *et al.,* 2008). These findings were similar with the results of the present study. Cacho et al. (1993) reported that both IgA and IgM positive cells were found in the subepithelial diffuse lymphoid tissue and underneath the crypt epithelium. IgM-expressing cells also were present in the germinal centers. Whereas IgA positive cells were not present in follicles. They also showed that numerous IgG positive cells were found subepithelial zone as well as in the deep and the mid portion of the lamina propria and under the crypt epithelium and also in the peripheral zone of the germinal centers. Hussan et al. (2009) found that IgA positive cells were more numerous than IgG and IgM positive cells. But Gomez Del Moral et al. (1998) said that in caecal tonsils, B lymphocytes, mainly expressed either IgM or IgA, predominate. At four weeks in the heat stress groups, a few IgA positive cells were seen lamina propria under the epithelium (Fig. 4D) A few IgG and IgM positive cells were observed in the lymphoid tissue.

At five weeks in the heat stress groups, a few IgA, IgG and IgM positive cells were seen in the lymphoid tissue (Fig. 4E). At six weeks in the heat stress groups, IgA positive cells were observed in the lymphoid tissue. IgG positive cells were seen under the epithelium and germinal centers (Fig. 4F). A few IgM positive cells were encountered in the lymphoid tissue. At four weeks in the vitamin E + heat stress groups, IgA, IgG and IgM positive cells were observed in the lamina propria under the epithelium and germinal centers (Fig. 4G). At five weeks in the vitamin E + heat stress groups, numerous IgA, IgG and IgM positive cells were distributed in the lymphoid tissue (Fig. 4H). At six weeks in the vitamin E + heat stress groups, IgA positive cells were observed lymphoid tissue under the epithelium and tonsils. IgG positive cells were distributed in the lymphoid tissue. Numerous IgM positive cells were seen in the lymphoid tissue around the crypts (Fig. 4I).

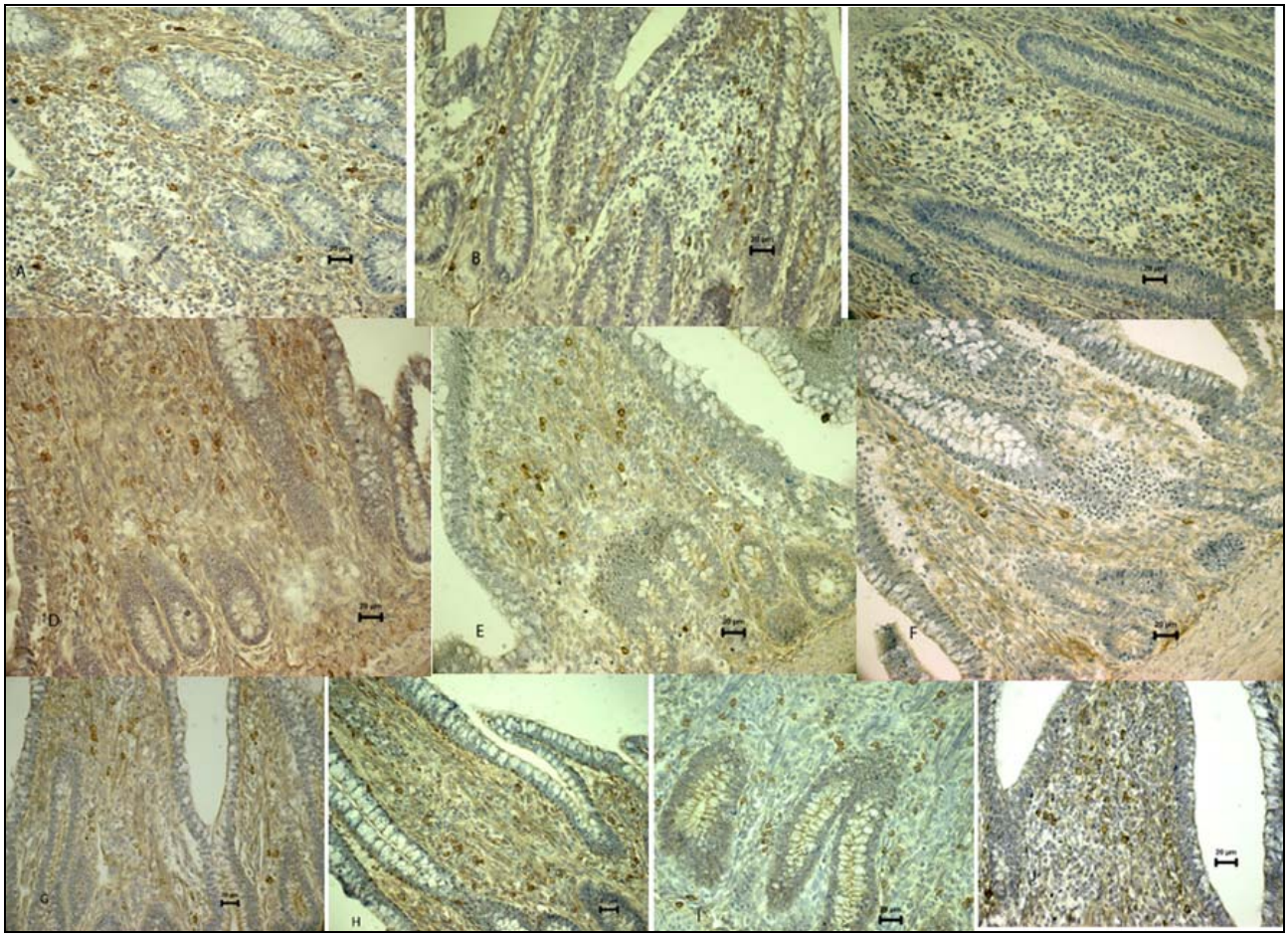


Fig. 3. A) In control group, IgG positive cells in the ileum. 4-week-old chicken, Bar: 20 µm. B) In control group, IgM positive cells. 5-week-old chicken, Bar: 20 µm. C) In control group, IgM positive cells. 6-week-old chicken, Bar: 20 µm. D) In heat stress group, IgG positive cells. 4-week-old chicken, Bar: 20 µm. E) In heat stress group, IgM positive cells. 5-week-old chicken. Bar: 20 µm. F) In heat stress group, IgM positive cells. 6-week-old chicken, Bar: 20 µm. G) In heat stress group with treated vitamin E, IgM positive cells. 4-week-old chicken, Bar: 20 µm. H) In heat stress group with treated vitamin E, IgG positive cells. 5-week-old chicken, Bar: 30 µm. I) In heat stress group with treated vitamin E, IgA. 6-week-old chicken, Bar: 20 µm. J) In heat stress group with treated vitamin E, IgG positive cells. 6-week-old chicken, Bar: 20 µm

High temperature significantly reduces the body weight, feed intake and feed conversion (Niu *et al.,* 2009). At the same time, heat stress negatively affects the development of embryo (Oznurlu *et al.,* 2010) and reduction of antibody synthesis (Zulkifli *et al.,* 2000; Mashaly *et al.,* 2004). This reduction could be indirectly due to an increase in inflammatory cytokines under stress (Ogle *et al.,* 1997), which stimulate the hypothalamic production of corticotrophin releasing factor (Sapolsky *et al.,* 1987). In this study it is shown that, the heat produces an adverse effect on the antibody producing cells. These results are in agreement with the results obtained by (Zulkifli *et al.,* 2000; Mashaly *et al.,* 2004; Niu *et al.,* 2009; Oznurlu *et al.,* 2010).

Vitamin E, the major lipid-soluble antioxidant present in all cellular membranes, is an important nutrient for optimal immune function. When animals are fed nutritionally complete diets lacking vitamin E immune responses are adversely affected (Bendich, 1988). The present study demonstrated that vitamin E could increase

the number of the immunoglobulins producing cells. Reports on the effect of vitamin E on the IgA, IgG and IgM-containing plasma cells in the gut-associated lymphoid tissue are scanty (Boa-Amponsem *et al.,* 2000; Muir *et al.,* 2002; Khan *et al.,* 2008).

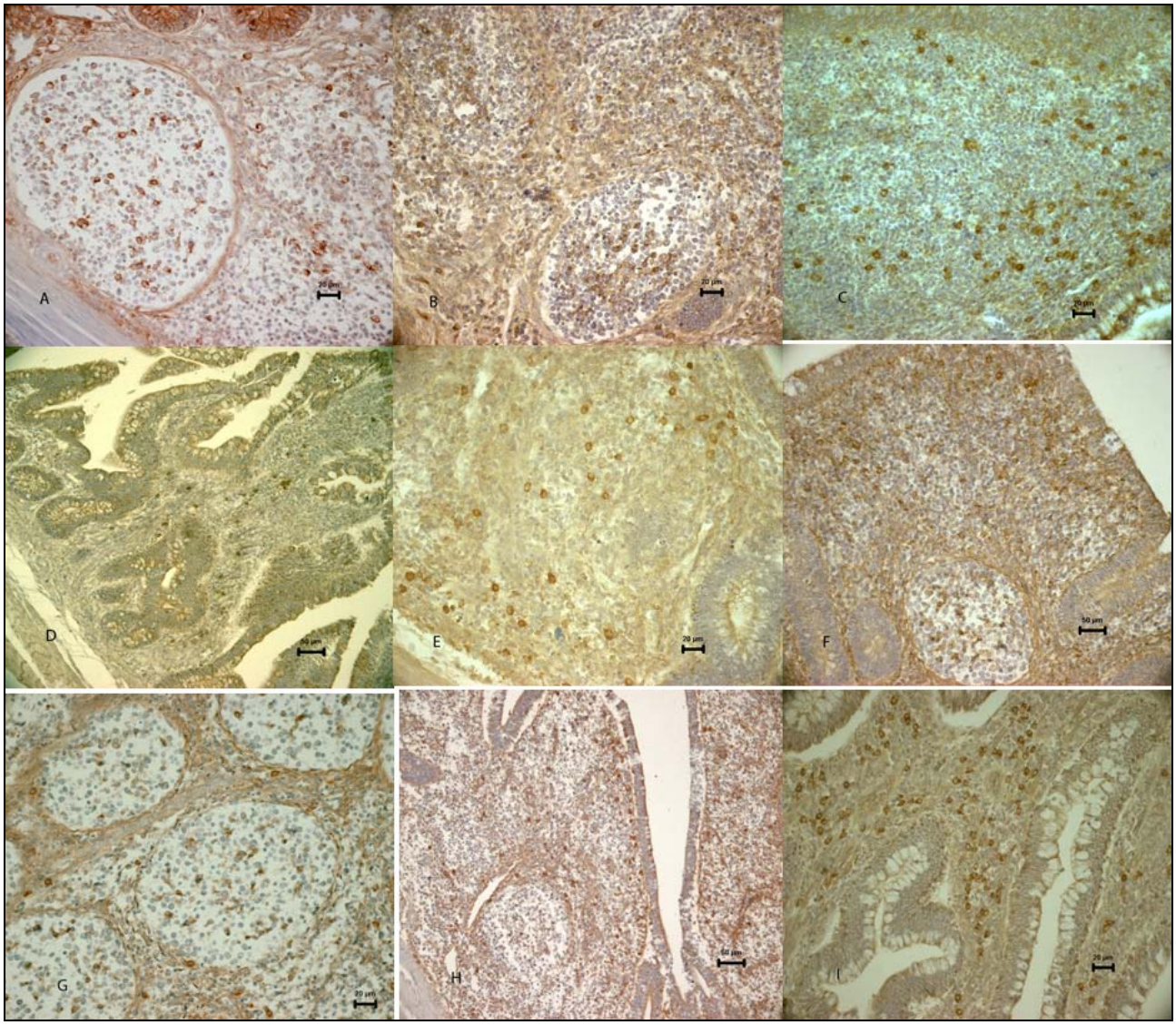


Fig. 4. A) In control group, IgA positive cells in the caecal tonsil. 4-week-old chicken, Bar: 20 µm. B) In control group, IgG positive cells. 5-week-old chicken, Bar: 20 µm. C) In control group, IgG positive cells. 6-week-old chicken, Bar: 20 µm. D) In heat stress group, IgA positive cells. 4-week-old chicken, Bar: 50 µm. E) In heat stress group, IgM positive cells. 5-week-old chicken, Bar: 20 µm. F) In heat stress group, IgG positive cells. 6-week-old chicken, Bar: 50 µm. G) In heat stress group with treated vitamin E, IgM positive cells. 4-week-old chicken, Bar: 20 µm. H) In heat stress group with treated vitamin E, IgM positive cells. 5-week-old chicken, Bar: 50 µm. I) In heat stress group with treated vitamin E, IgM positive cells. 6-week-old chicken, Bar: 20 µm.

In conclusion, Ig-containing plasma cells in oesophageal tonsils, pyloric tonsils, jejunum and ileum, ceacal tonsils of the broilers decreased or were not affected by heat stress and increased by vitamin E supplementation (300 IU/kg). This result suggests that supplementation of vitamin E in the diet can improve immunocompetence of broilers when in the summer months.

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