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Research Article

Side Effects of *In ovo* Given Sunset Yellow FCF on the Embryonic Development of the Spleen by Means of Histological and Enzyme Histochemical Methods

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Abstract

Background: E110 is one of the food colorants which is widely used in dairy products, fast foods, jam and dry beverage powders, aqueous drug solutions, tablets, capsules, toothpastes mouthwashes, hair care products and cosmetics. Doubts have accumulated in recent years that food additives might cause allergic reactions in humans or increase these ailments. In this study, side effects of Sunset yellow FCF (E110) on the embryonic development of chicken spleen were evaluated by means of histological, histomorphometrical and enzyme histochemical methods.

Methods: In the study, 250 fertilized broiler eggs obtained from a commercial broodstock were used. Eggs were divided into 5 groups each having 50 eggs. Control eggs were either nontreated or distilled water injected *via* air sac. The eggs in the experimental groups were injected with 100ng/egg, 500ng/egg and 1.000ng/egg E110 prior to incubation. Blood and spleen samples were taken from randomly selected 10 eggs of each group at 11^{th}, 15^{th}, 18^{th} and 21^{st} days of incubation. Leukocyte formula, alpha-naphtyl acetate esterase-positive and acid phosphatase-positive lymphocyte percentages were determined in the blood samples and embriyonic development of the spleen was assessed in the tissue sections.

Results: In the 500ng/egg and 1.000ng/egg E110 injected experimental groups, embryonic development of the spleen retarded, alpha-naphtyl acetate esterase-positive and acid phosphatase-positive lymphocyte rates were significantly depressed.

Conclusions: Significant disturbances in the immune system functions of the affected animals might occure at post-natal period of their life.

Keywords: E110; Spleen; Immune system; Acid phosphatase; Alpha-naphthyl acetate esterase

Abbreviations

ACPase: Acid Phosphatase; ADI: Acceptable Daily Intake; ADHD: Attention Deficit Hyper-Activity Disorder; ANAE: Alpha-Naphthyl Acetate Esterase; BW: Body Weight; BW/D: Body Weight/Day; CAS: Chemical Abstracts Service; CHEST: Embryotoxicity Screening Test; ChEs: pseudo Cholinesterases; E110: Sunset Yellow FCF; E123, Amaranth; EFSA: European Food Safety Authority; FAO: Food and Agriculture Organization; GCs: Germinal Centers; IRDC: International Research and Development Corporation; LD50: Lethal Dose-50; LOAEL: Lowest Observed Adverse Effect Level; MN: Micronucleus; NOAEL: No-Observed-Adverse-Effect Level; NTP: National Toxicology Program; PALS: Periarteriolar Lymphoid Sheath; SCE: Sister Chromatid Exchange; UNESDA: Union of European Soft Drinks Associations.

Introduction

Sunset Yellow FCF is a very important food colorant azo dye, which is used to increase appealing of the foods. E110 is widely used in beverages, gel confectionery, cereal products, macaroni, desserts, snacks, ice cream and canned fish. It also participates as a colorant in the drugs [1]. E110 is often used in combination with E123 to achieve brown color in the chocolate and caramel and it is the most commonly used food colorant in soft beverages in many European countries and Turkey [2].

E110 was first introduced as a food colorant in 1929 and its reliability as food additive was evaluated in 1982. ADI level of E110 was determined as 0-2.5 mg/kg body weight/day [3]. The LD50 doses of E110 are >6.000mg/kg BW in the mouse and >10.000mg/kg BW in the rats [4].

Although E110 did not have significant side effects when the permitted safe limits were not exceeded, these limits are often exceeded in daily practice. Moreover, because of E110 is a sulfonated form of sudan I, which is considered a possible carcinogen, there is a certain amount of sudan I in the produced final E110 product, although it is not desired. E110 is suspected to be responsible for the health problems such as, allergic reactions, diarrhea, vomiting and urticaria [5], angioedema [6,7], rarely anaphylactic shock and headache in the children with aspirin intolerance [8]. These reactions

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are common symptoms of ailments caused by azo group food colorings, including E110. In recent years, it has been suggested that E110 is also associated with childhood hyperactivity and also might trigger aggression in children [9]. The affected children return to normal, when artificial food colorants including E110 are removed from the drinks of hyperactive children, but behavioral disorders reappear by reintroducing the beverages containing these substances [10].

Water solubility of E110 is 190.000mg/L at 25°C and 200.000mg/L at 60°C [11]. Since E110 does not contain functional groups such as ester, amide, acetal, epoxide, lactone, which are hydrolyzed in water, its reaction potential in water depends on the desulfonation of aromatic sulfonic acid or its equivalent sulfonic acid salt. Since aromatic sulfonic acids are not desulfonated in the natural environment, E110 is not biodegradable in the natural conditions and tends to maintain its stable structure in water [12].

Metabolism studies showed that 3.6% of orally ingested E110 is absorbed through the digestive tract, and only 0.8% of 100mg single dose was observed in the feces. The main metabolic pathway in the breakdown of the dye is probably occurs *via* bacterial activity in the gut. This activity provides the breakdown of aromatic amines and aminosulfonic acids, and the resulting products are partially absorbed from the intestine [13]. In the rat, relatively important (20-30%) part of intravenously administered E110 is excreted *via* bile without destruction after 6 hours, and urine is also a significant excretion route of E110 and its metabolites [14].

Results of the cell metabolism experiments showed that E110 reversibly inhibits true and ChEs in a mixed manner *in vitro*; both types of inhibition occur, *via* competitive and non-competitive mechanisms [15]. In previous mutagenicity and clastogenicity studies [16-19] on E110, consistent and inconsistent results have been revealed. In the SCE assay with E110, concentrations up to 5.000µg/ml gave incompatible results [20]. MN induction test results showed that E110 administered orally at a dose of 500 or 1.000 mg/kg BW, increased the MN frequency in the bone marrow of male rats [21]. E110 caused clastogenic effects [22]. And increased MN frequency in Chinese hamster fibroblasts [23], whereas similar effects were not observed in the *in vivo* studies on different laboratory animals [24]. Results of the previous genotoxicity studies on E110 are also contradictory. In a previous experiment, E110 administered orally at 500mg/kg BW did not change the timing of DNA synthesis [25]. E110 did not cause chromosomal disorders [26]. Similarly, Ishidate et al. [23] suggested that metabolically inactive E110 caused chromosomal damage at 6,000µg/mL concentration. However, in another study, 5.000µg/mL of both metabolically activated and inactive E110 was found to be ineffective [20]. NOAEL of E110 was determined as 6.000ppm for female and 12.500ppm for male rat [27]. In the mouse, 2.000mg/kg BW E110 given twice at 24 hours intervals increased mitosis frequency in the intestinal epithelial cells, whereas MN frequency did not change [28]. In the embryotoxicity trials, side effects were not observed in Charles River CD rats of 100, 300 or 1.000 mg/kg BW/D E110 administered *via* nasogastric gauge. Similarly, there were no negative effects on the reproductive system [29,30]. However, Mathur et al. [31] have observed significant effects on the testes of the rats received 250 and 1.500 mg/kg BW/D E110 for 90 days accepted LOAEL as 250mg/kg BW/D.

In the skin tests, the people with eczematous hypersensitivity to p-phenylenediamine gave cross-sensitivity to E110 [32]. This cross-reaction is explained by the ability of the dye molecule to easily transform into compounds similar to quinone structure binding structural molecules [33].

Because the lack of a placental barrier in the avian species, chicken eggs have become a widely preferred test material in experimental studies to determine the negative effects of external factors on the embryonic development of the immune system. Spleen, which is the largest peripheral lymphoid organ in both the adult mammals and poultry species, mainly allows the removal of foreign organisms and aged red blood cells from the circulating blood [34].

The white pulp is mainly constituted of lymphoid follicles and lymphatic cords, and red pulp contains red pulp regions and venous sinuses all those form parenchym of the organ. The most abundant cells are lymphocytes in the white pulp of the spleen. Specific regions populated by T- and B-lymphocytes are distinguished in the spleen, as in the other secondary lymphoid organs. T-lymphocytes mainly populate adventitial layer of the central artery and form PALSs, while B-lymphocytes mostly occupy the GCs of the lymphoid follicles. Because that the lymph nodes are not well developed in the avails, the role of the spleen in the chicken immune system is vital and its embryonic development should not to be adversely affected by either external or internal factors. Therefore, disorders occurring during embryonic development of the spleen may result in significant deficiencies in both cellular and humoral immune functions in the post-hatch period of the chicken [35].

Precursors of T and B lymphocytes migrate to primordium of thymus and bursa of Fabricius *via* blood circulation and mature in these central lymphoid organs [36]. In the chicken embryo, the migration begins between 10^{th}-14^{th} days of day of incubation [37]. These cells form organ-specific lymphatic cords and lymphoid follicles in later periods of the embryonic development. In these follicles, B-lymphocytes populate GCs and T-lymphocytes mainly locate in cortical areas of the folicles and PALS [35,36].

In many animal species including chickens, ruminants, dogs and humans, mature T-lymphocytes give ANAE positivity with a very specific localized granular color reaction [38,39]. While the null cells give a fine granular staining [37], monocytes/macrophages display a strong and diffuse ANAE positivity [38-40].

ACPase is also a lysosomal enzyme of myelocytes, PMNLs, lymphocytes, plasma cells, megakaryocytes, blood platelets and mononuclear phagocytes. Lymphocytes give large granular positivity, whereas the reaction product is diffuse granular in monocytes [40]. In the avian species, the ACPase positivity has been suggested to be specific for B-lymphocytes [40-42].

In the present study, effects of E110 on the development of spleen, migration and localization of T- and B-lymphocytes were determined by means of histological, and enzyme histochemical methods during embryonic development of the chicken embryo.

Materials and Methods

From Ross 508 line, 250 fertilized eggs were used as egg material. The eggs were weighed and then disinfected by fumigating with

130ml of formaldehyde 37% and 80g of potassium permanganate vapor. Relative embryo weight was calculated with the following formula; (embryo weight/egg weight) X 100. The eggs were grouped as given in the Table 1, each group having 50 eggs. Sterile 20µl of test solution was injected *via* blunt end of each egg, immediately sealed with liquid paraffin and incubated in 1.000 egg-capacity incubator (Veyisoğlu, Istanbul, Turkey) under optimum conditions (37.8°C and 65% relative humidity).

Randomly selected 10 eggs from each group were weighed and then opened at 11th, 15th, 18th and 21st days of incubation. Embryos were weighed, heparinized cardiac blood samples were taken and spleen were dissected out. Some of the blood smears were stained with May grünwald-Giemsa. In the remaining blood smears ANAE and ACPase were histochemically demonstrated. Splenic tissue samples were divided into 2 pieces and fixed in appropriate fixatives. Paraffin sections were used in Crossmon's trichrome stain and Papenheim's panoptic stain. In the frozen sections, ANAE and ACPase were demonstrated as demonstrated in the blood smears.

The specimens were examined under light microscope (Nikon Eclipse, E-400 equipped with Nikon DS Camera Control Unit DS-L1 with DS Camera Head DS-5M) and digital images of the required regions were recorded.

The data of peripheral blood smears was transformed by arc-sine method and analyzed by Duncan test. Other parameters were analyzed by ANOVA and student's t test. The significance of the differences between mean values of the groups was determined and $P < 0.05$ was concerned as statistically significant.

Results

Embryonic developmental stages were compatible with Hamburger-Hamilton [43]. Scale in the control groups, 100ng/egg and 500ng/egg E110 administered groups. Nevertheless, some embryos were developmentally retarded and ectopia viscera was common in the 1,000ng/egg E110 given group.

Mean relative embryo weight of the 1,000 ng/egg group were significantly ($P<0.05$) lower than those of the other groups at 11th and 15th days of incubation, whereas the remaining groups had similar ($P>0.05$) relative embryo weights in the same embryonic periods. However there was no significant differences between mean relative embryo weights of the groups at 18th and 21st days of incubation.

The cells with lymphoid morphology were rarely seen in the blood samples of the control embryos taken at 11th day of incubation. Their frequency increased at 15th and 21st days of incubation (Figure 1) in both controls and experimental. Nevertheless, mitotic figures in erythrocytes were frequently seen in the 500 and 1.000 ng/egg E110 injected groups (Figure 2) at 15th day of incubation. In the later periods, results of the experimental groups were quite similar to those of the controls.

In the control groups, ANAE and ACPase+ lymphoid cells were first seen at 13th day of incubation (Figure 3 and 4).

Blood cell percentages were determined starting from the 15th day of the incubation. Except monocyte percentages of the experimental group -I and -II were significantly ($P<0.05$) higher. There were no significant ($P>0.05$) differences in the percentages of other blood cell types between controls and experimental groups ($P<0.05$) at 15th and 18th days of incubation. In the same embriyonic periods, 1.000ng/egg E110 administered animals had significantly ($P<0.05$) lower ANAE and ACPase+ lymphocyte percentages (Figure 5 and 6).

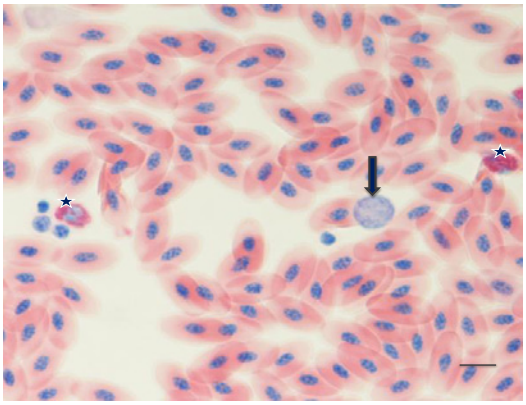

Figure 1: A peripheral blood sample of an animal from control-I group at 15th day of incubation. Dominant cell type are heterophils. A lymphocyte (arrow) and granulocytic cells (asterisks) are also seen. May Grünwald-Giemsa. Magnification bar: 10µm.

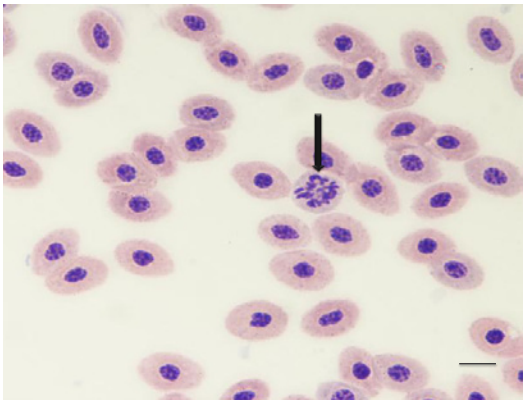

Figure 2: A mitotic figure (arrow) is seen in the peripheral blood sample of an animal from 500ng/egg E110 injected experiment-II group at 15th day of incubation. May Grünwald-Giemsa. Magnification bar: 10µm.

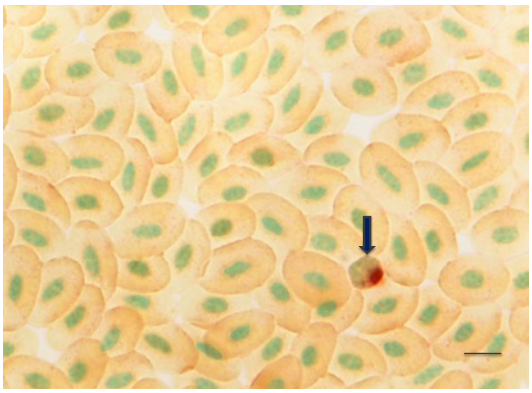

Figure 3: An ANAE-positive lymphocyte (arrow) is seen in the peripheral blood sample of an animal from control-I group at 13th day of incubation. ANAE demostration. Magnification bar: 10µm.

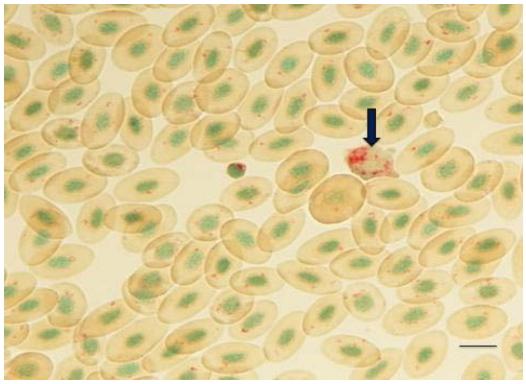

Figure 4: An ACPase-positive lymphocyte (arrow) is seen in the peripheral blood sample of an animal from control-II group at 13th day of incubation. ACPase demostration. Magnification bar: 10µm.

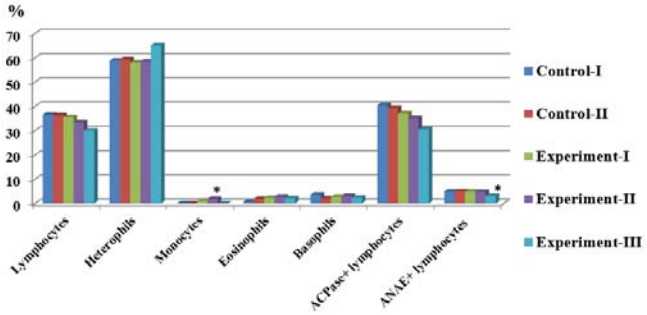

Figure 5: Percentages of leucocyte types, ANAE+ and ACPase+ lymphocyte percentages of the groups at 15th day of the experiment. *Mean monocyte level of the experiment - III group is significantly (p <0.05) higher, mean ANAE+ lymphocyte percentage of the same group is significantly (p <0.05) lower.

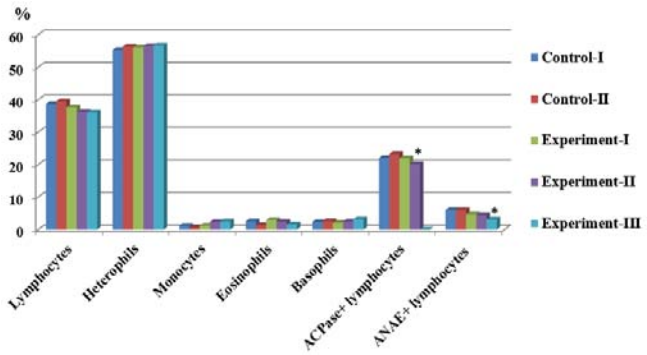

Figure 6: Percentages of leucocyte types, ANAE+ and ACPase+ lymphocyte percentages of the groups at 18th day of the experiment. *Mean ACPase+ and mean ANAE+ lymphocyte percentages of the experiment-III group are significantly (p<0.05) lower.

Leucocyte percentages of the 500 and 1.000 ng/egg E110 administered groups were significantly (P<0.05) lower than those of the other groups of the experiment at 21st day. Experimental group-II had the highest (P<0.05) monocyte percentage. However the remaining groups had similar (P >0.05) monocyte levels. Experimental groups-II and -III displayed significantly (P<0.05) lower ANAE+ and ACPase+ lymphocyte percentages in the same period (Figure 7).

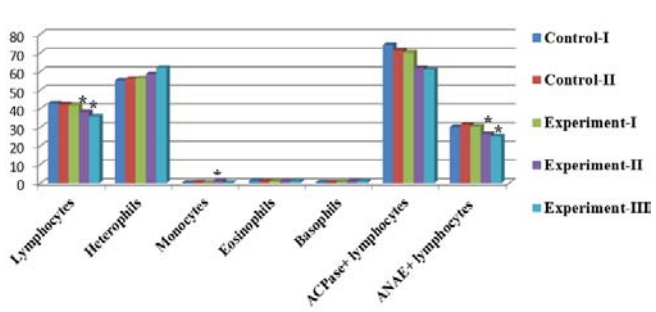

Figure 7: Percentages of leukocyte types, ANAE+ and ACPase+ lymphocyte the groups at 21st day of the experiment. *Mean lymphocyte levels of the experiment -I and -II are significantly (p<0.05) lower, mean monocyte level of the experiment-II is significantly (p <0.05) higher, both ACPase and ANAE+ lymphocyte levels are significantly (p<0.05) lower.

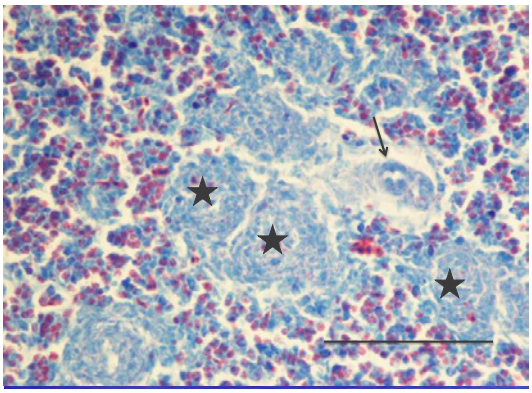

Figure 8: A section of the spleen of an animal from control - I group at 18th day of incubation. Histolological organization of the lymphoid follicles (asterisks) is advanced. Population of the PALS (arrow) by lymphocytes is relatively weak. Pappenheim's panoptik stain. Magnification bar: 100µm.

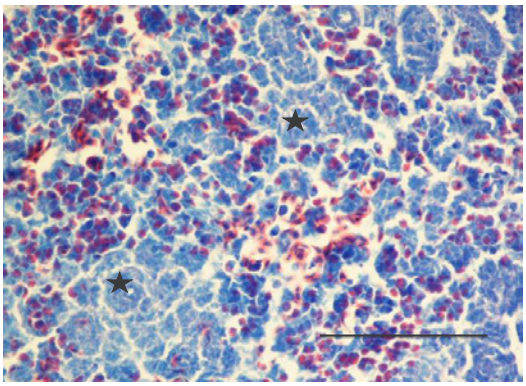

Figure 9: A section of the spleen of an animal from experiment - III group at 18th day of incubation. Lymphoid follicle (asterisks) development is retarded. Pappenheim's panoptik stain. Magnification bar: 100µm.

Hemopoietin foci and the cells with lymphoid cell morphology were first seen in splenic primordium of the control groups and the experiment-I group at 11th day of incubation. ANAE+ and ACPase+ cells did appear in this period of the development. In the experiment-II and -III groups, hemopoietin foci and lymphoid cells were less in number.

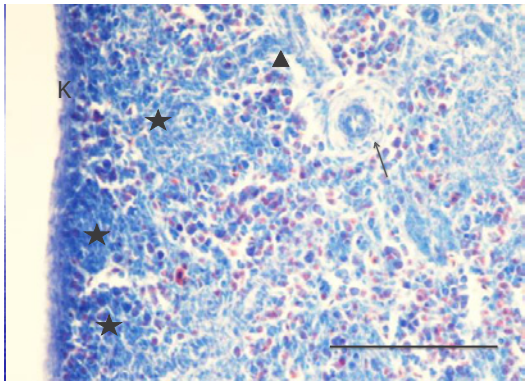

Figure 10: A section from spleen of an animal from control - I group at 21st day of incubation. Organ is covered with a continuous fibro muscular capsule (C), blood vessels are surrounded with lymphatic cords (arrow head), lymphoid follicles (asterisks) are organized and GCs are definite. Pappenheim's panoptic stain. Magnification bar: 100µm.

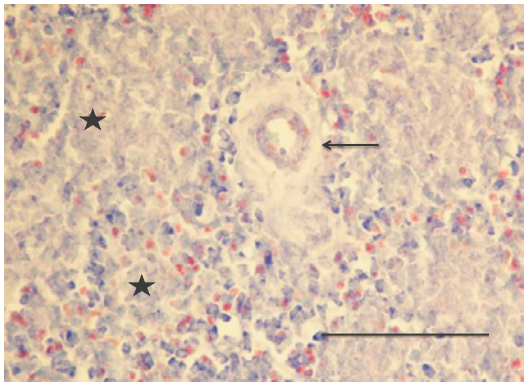

Figure 11: A section from spleen of an animal from experiment - II group at 21st day of incubation. Pale staining due to low cellularity in lymphoid follicles (asterisks) and in PALS (arrow) is striking. Pappenheim's panoptic stain. Magnification bar: 100µm.

Lymphoid tissue development progressed and early signs of PALS formation was definite in the control groups at 15th day of incubation. Small number of ANAE+ lymphocytes infiltrating into the adventitial layer of central arteries were seen, ACPase+ lymphocytes were also very scarce. Although experimental groups also displayed similar developmental pattern, development of the lymphoid tissue was weaker especially in the experiment -II and -III groups.

At 18th day of incubation, development of spleen progressed; ANAE+ cells heavily populated the PALS, lymphatic cords enlarged and organization of primary lymphoid follicles were completed (Figure 8). Developmental process was definitely retarded in the experiment -II and -III groups (Figure 9).

At 21st day of incubation, embryonic development of the spleen completed and organ gained typical histological structure. Centers of the lymphoid follicles stained as pale areas (Figure 10). ANAE+ lymphocytes preferentially located in the PALS, whereas ACPase+ lymphocytes mostly populated central regions of the lymphoid follicles.

In the experimental groups -II and -III, lymphoid tissue weakly developed, ANAE+ and ACP+ lymphocytes were less in number (Figure 11).

Table 1: Experimental groups and procedures.

Groups N=5	Procedures
Control - I	Non treated.
Control - II	20µl of sterilized distilled water was injected.
Experiment - I	20µl of 100ng/egg E110 containing test solution was injected.
Experiment - II	20µl of 500ng/egg E110 containing test solution was injected.
Experiment - III	20µl of 1000ng/egg E110 containg test solution was injected.

Discussion

Since food colorings increase appealing of foodstuffs, they have an important use in the food industry. E110 is one of the food colorants which is widely used in dairy products, fast foods, jam and dry beverage powders, aqueous drug solutions, tablets, capsules, toothpastes mouthwashes, hair care products and cosmetics [44].

Doubts have accumulated in recent years that food additives might cause allergic reactions in humans or increase these ailments. Nevertheless, there are insufficient experimental results on the negative effects of food colorings on the embryological development of the immune system. In the present study, effects of E110 on the embriyonic development and histological maturation of avian spleen have been determined. Also, migration and populating of ANAE+ T-lymphocytes and ACPase+ B-lymphocytes the splenic primordium were evaluated in the experiment. E110 was given at 100ng/egg, 500ng/egg and 1,000ng/egg doses *via* air sac into the fertilized chicken eggs, prior to incubation.

In the European countries, daily E110 intake levels *via* foods have been determined for children (1-10 years of age) between 0.3-6.7 mg/kg BW/D. The highest E110 levels in the beverages were detected as 50mg/L in a survey study by UNESDA and these levels were between the determined limits (1-48 mg/L) by UNESDA [45]. However the exposure levels of the children (0.2-2.1 and 0.6-5.8 mg/kg BW/D) are higher than the determined ADI levels or near to the upper limits. Moreover, it has been pointed out that the levels of sulfonated colorings such as orange II and sudan I, sodium chloride and sodium sulfate levels should be taken into consideration while determining the upper level limits for E110 [46].

Ching et al. [47] administered to rats 1.000 and 2.000 mg/kg BW/D E110, which was mixed with egg yolk, for 3 days. Macroscopically, they found ulcerous lesions and hemorrhages in gastric antrum, moderate splenomegaly, hepatomegaly and enlarged kidneys in both 1.000 and 2.000 mg/kg BW/d E110 administered animals. Nevertheless, the observed findings might not originated from E110 itself because that E110 was not used alone [46]. In the long term feeding experiments, any adverse effects on body weight gain, liver, kidney, hematology, organ weights and their histology were not observed in pigs received 0, 250, 500 and 1.000 mg/kg BW/D E110 for 98 days [47]. Mathur et al. (2005a) observed degenerative changes in the testes of animals received 250 and 1.500 mg/kg E110 BWD for 90 days. About 50% of seminiferous tubuli were affected. Thus, The Scientific Committee (SCF) determined 250mg/kg BWD dose as LOAEL.

High doses (0.15%, 0.30% and 0.60% of total feed) of E110

significantly affected swimming direction of successive F1 generation of the mice [48]. E110 had no genotoxicity neither on Escherichia coli [49] nor Salmonella typhimurium [16]. Ames test results gave similar results [50]. E110 did not increase mitotic gene conversion in Saccharomyces cerevisiae [51]. Also, Tema Nord evaluation system did show genotoxic effects of E110 [52]. *In ovo* given single dose 2.000mg/kg BW E110 does did not increase MN frequency in the mouse myeloid tissue [21]. Nevertheless, Durnev et al. [53] showed that 0.17 or 1.7 mg/kg ingested BW E110 increased the frequency of the cells with chromosomal damage in the mouse. Based on these results, EFSA concerned as non genotoxic the doses lower than 2.000mg/kg BW [46].

Mitotic figures in erythrocytes were more frequently seen in 500 and 1.000 ng/egg E110 injected groups at 15th day of incubation in the present study. Nevertheless, Kuş and Eroglu [54], reported high doses of Brilliant Blue and E110 decreased mitotic index and replication ratios. The authors [54] suggested that food colorings might potentially exert genotoxic and cytotoxic effects when consumed at high amounts. According to Bhattacharjee [55], E110 reduces mitosis index in the apical root cells of garlic (*Allium sativum*) in a dose and exposure period dependent manner. This effects might have arisen either from blockage of the G1phase [56] or prevention of entrance to the G2 phase [57] and finally, blocking DNA synthesis in the S phase [58]. Possibly, serious decline in ATP level results in arrest of DNA synthesis [59].

Fertilized chicken egg is a perfect test material for testing drugs and chemicals including E110 [60] since the avian species have no placental barrier. A test method known as CHEST has been developed by Jelinek [61]. Results of the test can be adopted the mammals, by multiplying 10^{-2} the determined toxic dose, the toxic level can be expressed as kg/BW of a pregnant [62,63]. The test is easily applicable and gives reproducible results, reduces number of experimental subjects, free from ethical concerns, complies with ethical rules and regulations of most countries, relatively cheaper and time saving [63,64].

In the present study, 100ng, 500ng, 1.000ng/egg doses of E110 were used as suggested by Brown et al [65]. These levels corresponds to 1, 5 and 10 ng/kg BW dose of E110 that the pregnant will ingest [62].

During the embryonic development of the spleen, reticular cells and smooth muscles comprising the stroma originate from local mesenchyme while thymus and bursa of Fabricius originated T- and B-lymphocytes immigrate into organ primordium *via* blood stream [34]. The lymphocyte migration begins between 10-14th days of incubation. ACPase+ lymphocytes were frequently seen in the primordium at 13th of incubation in the present study. This finding is in accordance with previous results showing the peripheral migration of ACPase+ lymphocytes from bursa of Fabricius takes place between 10th-14th days of incubation [66,67].

Hashem et al. [68] showed that 3 times the ADI levels of E123, E110 and curcumin-3 suppress cellular immunity. Yadav et al. [69] suggested that E100 diminishes functions of the splenic cells. In this study, spleen development retarded in the 500 and 1.000 ng/egg E100 injected experimental groups. Percentages of peripheral blood ACPase+ lymphocytes, which are presumably immunocompetent but not primed B-lymphocytes, those will transform into plasma cells significantly declined in 500ng/egg and 1.000ng/egg E110 groups at 11th, 18th and 21th days of the incubation. Also, germinal centers of the splenic lymphoid follicles were poorly developed. In a previous study, Berktay [60] observed retarded development of thymus and bursa of Fabricius in 1.000ng/egg E110 received embryos. These results might show that E110 might adversely affect embryonic development of the peripheral lymphoid organs, those of the spleen, which is the largest peripheral lymphoid organ. The failure of the splenic development might result in insufficient or adverse immune reactions, such as hypersensitivity or allergic reactions in the postnatal life. These results may be morphological evidence for diminishing effect of E110 administered in early embryonic period on the antibody response. Slowik et al. [41] found poorly developed germinal centers and fewer ACPase+ cells in the chicks bursectomized at neonatal period. Similarly, Graczyk [42,70] observed serious decreases in ACPase+ cell population in the peripheral blood and antibody production against sheep red blood cells in both of the chicks bursectomized immediately after hatching and administered anti-bursa serum.

Güler and Başımoğlu [71] showed that 2.5mg/kg E110 injected into the vitelline sac at 15th day of incubation caused partial degranulation of dermal mast cells after 12 hours, and degranulation of mucosal mast cells after 6 hours. Their results might support that E110 is responsible for allergic reactions. Among the adverse effects food colorings, ADHD which is a behavioral disorder of children, allergic reactions and other side effects are the most suspicious side effects and thus attract most attention. Because E110 is an azo dye, cross reactions might develop in the individuals sensitive to salicylates. Moreover, as it has been shown by Güler and Başımoğlu [71], E110 might cause histamine releasing through mast cell degranulation. This effect might strengthen asthma symptoms and hyper activity when ingested together with benzoates.

In this study, E110 administrated into the air sac of fertilized chicken egg at 500ng/egg and 1.000ng/egg doses caused retarded spleen development and declined ACPase+ and ANAE+ lymphocyte percentages in the peripheral blood. These results imply that the affected animals might exert suppressed or adverse immune functions in their post-natal life. Although the results of the present experiment did not introduce convincing evidences on the action mechanism, depression in the development of thymus and bursa of Fabricius, which both are central immune system organs, might result in underdeveloped spleen. The results of the present study imply that E110 might adversely affect embryonic development of the immune system. The humoral system appears would be more affected. However future experiments are necessary to elucidate the degree and mode of influence, especially on the mammalian animal models.

Conclusion

Based on the results it was concluded that that early pregnant women and young children should consume synthetic food additives under strict control.

Ethical Review

All test procedures used in the experiments were approved by the

Ethical Committee of Veterinary Faculty of Selçuk University (Date: December 27th, 2013 and no: 2013/061), Konya, Turkey.

Credit Authorship Contribution Statement

Selime Çelik: Formal analysis, methodology, investigation, validation.

İlhami Çelik: Conceptualization, methodology, formal analysis, writing-original draft, resources, funding acquisition, project administration, supervision.

Elif Berktay: Formal analysis, methodology, investigation, validation. Writing-review and editing.

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