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Effect of folic acid supplementation to Lohmann Brown layers diet on immune response and intestinal morphology

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***Abstract*---**Sometimes, producer use the folic acid to enhance shell brown color of the brown hens` eggs. Therefore, this study was carried out to investigate the effect of supplementation of folic acid enriched diet on immune response and intestinal morphology of laying hens. Five thousand Lohmann Classic Brown laying hen type were use in this experiment . At the first period, hens were fed on basal diet according to Lohmann Classic Brown nutrition requirement guide. The Second period of the experiment, all hens were fed same basal diet enriched with folic acid with 1mg/kg of feed. Blood samples were collected from the hens at the end of the first and second periods for measuring the effect of folic acid on immune response which tested by using ELIZA technique. Duodenum, jejunum and ileum were taken from intestine for measuring villi height and crypt depth. Immune tolerance of ND showed a significant increase after administrated folic acid as compare with before administration folic acid. Villi height and crept depth showed a significant difference in second periods after supplementation of folic acid compare with first period before folic acid supplementation. The villi height and crypt depth of ileum was showed no significant deference between two periods. In conclusion, immune response and intestinal morphology of layer hens may be enhance after supplementation of folic acid during enriched diet with folic to enhance egg shell brown color.

***Keywords*---**folic acid, immune response, villi height, crypt depth, Lohmann hens.

Introduction

Folate is a generic term for both naturally occurring folate found in foods and synthesis folic. Folates are vitamins that cannot be synthesized by animals, so it is required to process efficient intestinal absorption, after that all shapes of folates are delivered via hepatic portal system to liver (Jing et al., 2010). Humans cannot synthesize folate. Therefore, folate has to be supplied through the diet to meet their daily requirements. The remaining amounts of the vitamin leave the body through the urine. Folic acid may also called Polyglutamyl folacin, Pteroyl monoglutamate, folate and B9 (Dietrich et al., 2005; Ulrich & Potter., 2006).

Folic acid has antioxidant activity to be involved in these effects of folic acid on health against reactive oxygen species (ROS) (Sarna et al., 2012). It is possible to significantly increase folate content of eggs through fortification of the laying hen diet with synthetic crystalline FA, and eggs can be changed to one of the rich sources of natural folate (House et al., 2002; Hebert et al., 2005; Hoey et al., 2009; Tactacan et al., 2010; Dickson et al., 2010).

Folic acid also maintenance of nervous system's integrity and decrease of neurological and neuropsychiatric disorders and reduction of the risk of neural tube defects (Gilbody et al., 2007; De Wals et al., 2007). Folate deficiency during pregnancy may also increase the risk of preterm delivery, infant low birth weight, and fetal growth retardation so that increase folic acid will decrease neurological and neuropsychiatric disorders and cardiac diseases (Yajnik & Deshmukh, 2008).

Folate has more health benefits in serum of pregnant women including their prevention of neural tube defects (NTD) in child during the first trimester (Smithells et al., 1976). Besides, its possibility of reducing infertility also folic acid may also improve pregnancy reduce their risk of miscarriage (Grodnitskaya & Kurtser, 2012).

The current study was aimed to evaluate the effect of folic acid that was supplemented to Lohmann layer diet on immune response by studying the antibody titer against Newcastle disease ND and Avian Influenza after 10 days of vaccinated of two periods before and after supplementation of folic acid also, to studying intestine morphology by measuring of villi height (VH) and crypt depth (CD).

Material and Methods

Five thousand laying hens Lohmann Classic Brown type were reared for table egg production at AL –Diwaniya province, Albudayr city, private layer hen's house. This study was carried out for three months divided into two periods. The first period extended from 11/12/2021 to 25/1/2022 (before supplementation folic acid) and the age of the hens were 39 to 45 weeks old. All hens were fed basal diet according to Lohmann Classic Brown nutrition requirement guide.

Second period extended from 25/1/2022 to10/3/2022 (after supplementation of folic acid). All hens were fed same basal diet administrated with 1mg /kg folic acid (Province supplier in Erbil city, Iraq).This products was manufacture by

Bregan. Co. At the end of the first and second periods (10 days after vaccination), blood samples were collected from 20 hens from wing vein. Five ml (5 ml) of blood was drawn from the wing vein. And sterile Medical sterile syringes of 5 ml were used. The blood was placed in special gel tube not containing an anticoagulant.

The serum was separated by a centrifuge at a speed of 3000 r / min for 5 minutes. The separated serum put in an Eppendorf's tubes and kept in freeze at -20 ° C until the completion of the measurements for calculating serum folic acid and to measure antibody titer against ND and AI viruses by using ELISA. Tissue samples (intestine) were applied with 10% formalin solution for 2 days. The samples then dehydrated in ascending grades of alcohol and used xylene to clearing, and paraffin wax to bury. The blocks were carefully oriented to have the cross-sections to be cut (5) µm thickness serial sections. The sections were de-paraffinized and hydrated for hematoxinen and eosin stain (Suvarna et al.,2018).

Results and Discussion

Results of stained sections with hematoxylin and eosin stain taken from the intestine showed as significant increase in intestine villi high and crypt depth of duodenum and jejunum of the folic acid administration as compare with before folic acid administration as shown in table 1

Table (1) effect of after administration of folic acid on intestine morphology

Parameter(µm)	Pre administration of folic acid	Post administration of folic acid
Duodenum		
Villi height	991±0.34 B	1207.7±0.33 A
Crypt height	94.7±0.26 B	164.2±0.32 A
Jejunum		
Villi height	858±0.51 B	997.5±0.76 A
Crypt height	81.59±0.3 B	102±0.24 A
illume		
Villi height	676.1±0.6 A	751.7±0.49 A
Crypt depth	77.19±0.07 A	79.2±0.06 A

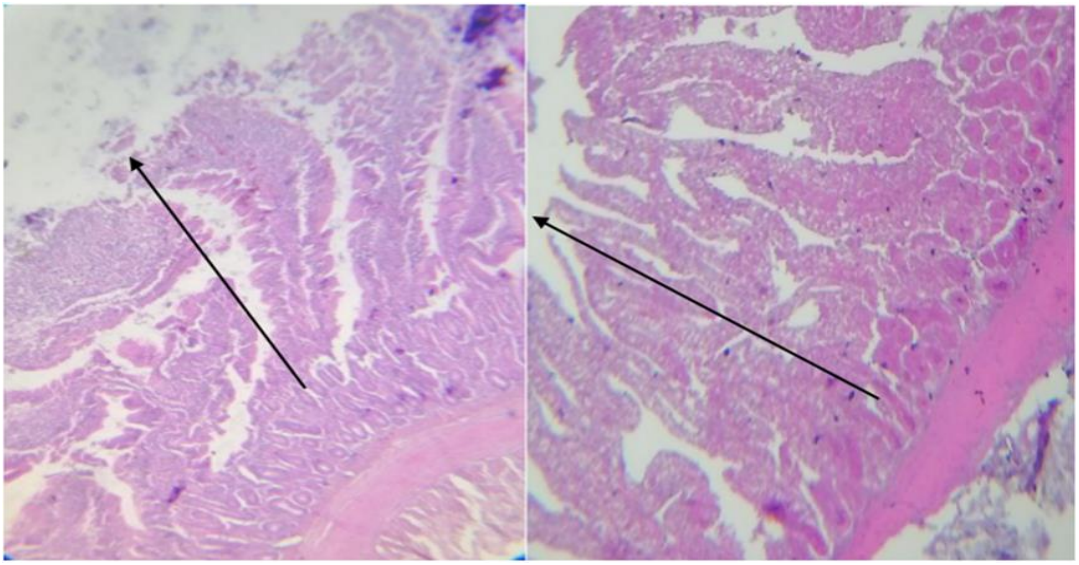

Figure (1): effect of folic acid on duodenum (A) before administration folic acid, (B) after administration folic acid. (H&E4X)

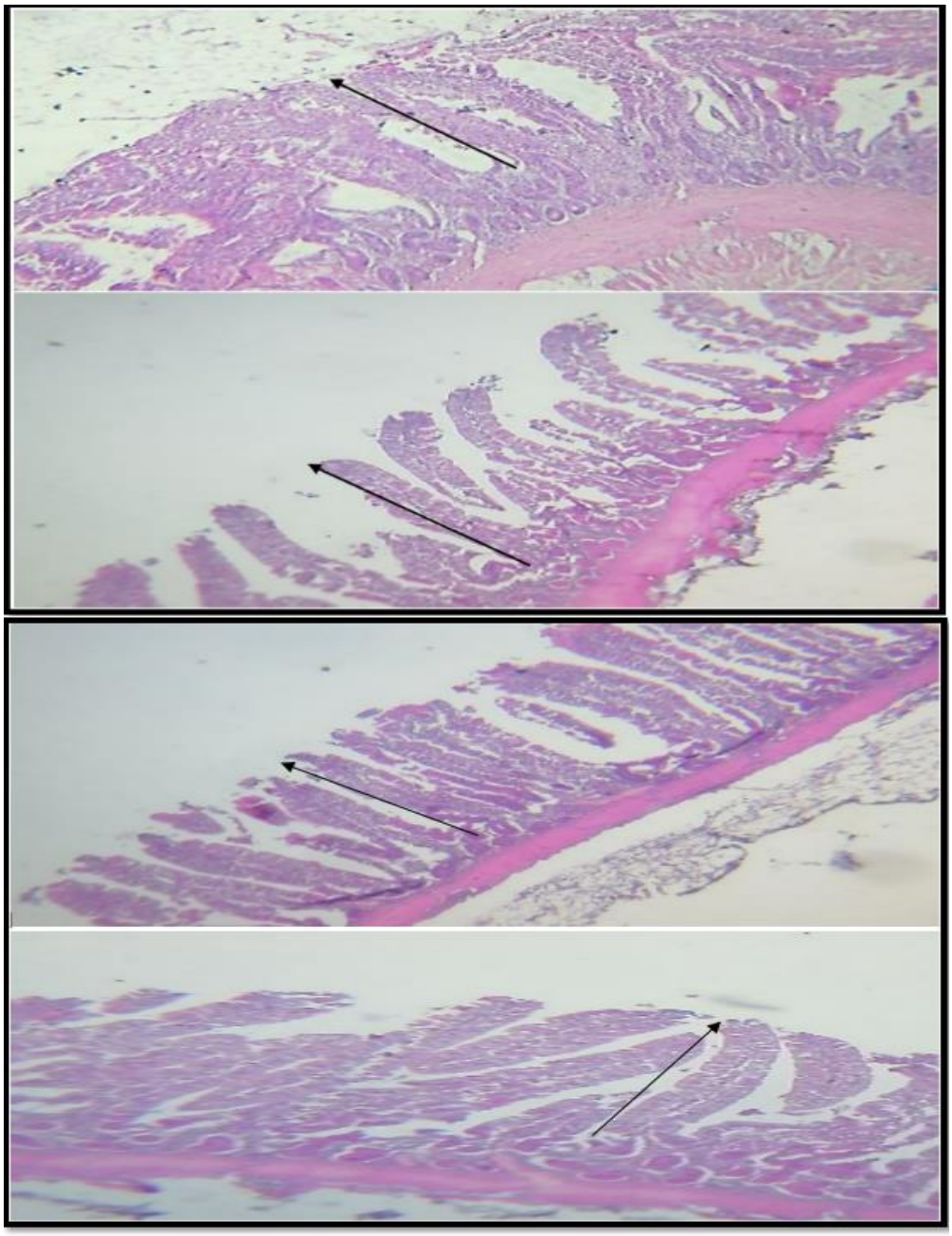

Figure (2) effect on administration of folic acid on intestine villi (A): jejunum before administration folic acid (B) jejunum after administration folic acid, (C) ileum before administration folic acid, (D)ileum after administration folic acid

The result showed a significant (p≤0.05) increase in the villi height and crypt depth after folic acid administration as compare with before folic acid administration. This rustle may be occur due to the ability of folic acid to reduce apoptosis in the cells and tissue. (Wang et al., 2021) Also the intestinal villi may be increase in high due to the ability of folic acid to increase proliferation of villi cells (Hwang et al., 2018).

Besides folic acid plays an important role in increasing the expression of mRNA in the cells. It can cause an increase in the growth and proliferation of cells and thus an increase in the length of the villi and this is was observe on after folic acid administration as compare with before folic acid administration (Li et al., 2020). Immune tolerance of IGg of ND showed a significant (p≤0.05) increase after administration of folic acid when compared with before administration of folic acid.

Table (2) effect of before administration of folic acid on immune titer of Lohmann classic Layer hens

Parameter	before administration of folic acid	after administration of folic acid
ND titer	16900±200 B	19523±220 A

Dietary FA had beneficial effects on the level of IgG in these hens possibly through enhancement of the level, or the activities of the B type lymphocytes (Venter et al., 2020). Folic acid has multiple roles and has received considerable attention in animal studies including neurotransmission regulation and gene expression and has a protective role in the immune system (Mikkelsen et al., 2019; Kunisawa et al., 2012).

Folic acid is also involved in T cell and mitogen regulation, which is essential for immunity and growth. Moreover, folic acid influences the methylation cycle, and DNA and RNA biosynthesis (James et al., 1994).

Dietary FA supplementation may modulate some immune responses in young laying hens, enhancing the level of biochemical constituents as well as the generation of immunoglobulins necessary for immune responses in response to bacterial and viral infections (Munyaka et al., 2012).

Conclusion

From the data collected, we conclude that there is an increase in the length of the villi of the small intestine, an increase in the proportion of folic acid in the diet, , and there is also a relationship between an increase in folic acid in the diet and an increase in the immune index of birds.

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