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Effect of folic acid supplementation to Lohmann Brown layers diet on performance, egg quality and folic acid content of eggs and serum

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***Abstract*---**Sometimes, customers added folic acid to layer diet to enhance brown egg shell color. This supplementation may lead to increase the content of folic acid in serum and eggs. The increment of folic acid in egg lead to produce folate egg that it will be beneficial to customers health especially pregnant women. This study was carried out to investigate the effect of supplementation of folic acid enriched diet on layer performance, egg quality, and folic acid content of egg and serum. Five thousand Lohmann classic brown laying hen's 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. Eggs were collected and blood samples were collected from the layers at the end of the first and second periods for measuring folic acid content of egg and serum by using HPLC & ELIZA respectively. Egg quality were measuring by calculating of egg weight, albumin height, albumin diameter, yolk height, yolk diameter, yolk color, shell thickness and Haugh unit. The results showed a significant increase of folic acid content of serum and egg after supplementation of 1 mg /kg feed of folic acid. Elisa serum folic acid content were recorded 145.4 ng/mL after supplementation of folic acid as Compare before supplementation which was recorded 79 ng/mL. On the other hand, HPLC folic acid content after supplementation of folic acid was recorded 64.9 ppm as Compare before supplementation that was recorded 32 ppm. Egg quality was enhance after supplementation of folic acid. Egg weight, albumin height diameter, yolk height, yolk

diameter, yolk color and Haugh unit were enhanced significantly by ($p \leq 0.05$) after supplementation of folic as compare with before supplementation of folic acid. Egg shall thickness showed there is no significant change between the two period. In conclusions, adding 1mg /kg folic acid to layer hen's diet may lead to increase of serum folic acid content then increase egg folic acid content. The highest folic egg content will produce folic acid egg called folate egg this will help pregnant woman to reduce spinal cord defect in fetus. A rich laying hens diet may be enhance of egg quality.

***Keywords*---**folic acid, serum, egg, egg quality, Lohmann layer.

Introduction

Folic acid is the term represents the group of B vitamins (Vitamin B9) which have similar biological activity to folic acid which is converted into folate by the body, is used as a dietary supplement and in food fortification as it is more stable during processing and storage (Choi *et al.,* 2014). Folate deficiency during pregnancy women 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 requirement recommended by NRC for laying poultry is very low (0.25 mg/kg diet). Thus, to meet the real requirement of folate in industrial poultries (Bagheri et al., 2019). Dickson et al (2010) reported that FA supplementation improved feed efficiency over the entire production cycle of laying hens under a long-term production condition. These results were confirmed in another study which was conducted by Islam et al. (2009) who observed that sk of miscarriage increasing dietary methionine and FA in diet improved feed conversion ratio (FCR) and improved shell color (Grodnitskaya, & Kurtser., 2012).

More than 80% of the folate in eggs is 5-methyltetrahydrofolate (5-MTHF) and it exists mainly in the yolk which that contain 95% of egg (Bagheri et al., 2019). One of the important strategies to promote health in society is enrichment of egg with FA to prevent relevant folate deficiency diseases in human. Also found that increase in serum folic acid led to increase precipitation in egg folic acid and consequently, the color of the yolk increased, the amount of albumin increased thus increasing the human desire to consume eggs, which is useful for pregnant women and prevents fetal abnormalities (Tactacan, 2011; Munyaka et al., 2012). The current study was aimed to evaluate the effect of folic acid that was supplemented to Lohmann layer diet on folic acid content of eggs and serum by using Elisa and HPLC. Study of egg quality by calculating of egg weight, albumin height, albumin diameter, yolk diameter, yolk height, yolk color, egg shell thickness and Haugh unit.

Materials and Methods

Five thousand laying hens Lohmann Classic Brown type were reared for table egg production at AL –Diwaniya province, Albudayr city, private layer hens 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. Basal diet contain a calculated amount from folic acid

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 Brgan. Co. The Netherland (500mg/kg) of product folic acid was provided to basal diet. At the end of the first and second periods 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. Serum folic acid was measured by use Folic Acid ELISA Kit Elabscience ® Egg folic acid was measured by use High Performance Liquid Chromatography (HPLC) Sykam® Egg weight were measured by use electronic balance.

Shell thickness was measured using gauge by taking from the narrow side (sharp region), the middle side (equatorial region) and the broad-end side (blunt region) of eggs. Finally, average Shell thickness was calculated as average of these three measurements. The yolk diameter and Albumin diameter was measured with Vernier caliper. Yolk height and Albumin height using tripod micrometer after the eggs were broken out on a flat mirror. Yolk color (YC) was measured by Roch Color Scale which has 15 color gradation from very pale to deep yellow (North and Bell, 1990).

Results and Discussions

The current study showed that there is a significant increase in the egg weight in folic acid when compared to the non-folic acid diet The mean values of egg weight on the end of the experiment was (61.74, 64.23) for the non-folic acid and folic acid respectively , Albumin height on the end of the experiment was (7.55, 9.94) for the non-folic acid and folic acid respectively, Albumin diameter on the end of the experiment was (6.84, 7.51) for the non-folic acid and folic acid respectively, Yolk height on the end of the experiment was (15.12±, 17.31) for the non-folic acid and folic acid respectively, Yolk diameter on the end of the experiment was (40, 41.99) for the non-folic acid and folic acid respectively, Yolk color on the end of the experiment was (5.01, 5.95) for the non-folic acid and folic acid respectively, Serum folic acid on the end of the experiment was (79.3, 145.4) for the non-folic acid and folic acid respectively and Egg folic acid on the end of the experiment was (32.8, 64.9) for the non-folic acid and folic acid respectively. While there was non-significant difference in egg Shell thickness between the non-folic acid and folic acid which was (0.317, 0.314) for the non-folic acid and folic acid respectively.

Table (1): effect of before administration of folic acid and after administration of folic acid on external egg quality

Parameter	Before supplementation of folic acid	After supplementation of folic acid
Egg weight(gm)	61.74±0.17 B	64.23±0.13 A
Shell thickness (mm)	0.317±0.002A	0.314±0.001 A
Albumin height (mm)	7.55±0.15 B	9.94±0.14 A
Albumin diameter(cm)	6.84±3.45 B	7.51±1.42 A
Yolk height (mm)	15.12±0.75 B	17.31±0.12 A
Yolk diameter(mm)	40±0.05 B	41.99±0.06 A
Yolk color	5.01±0.01 B	5.95±0.11 A

Table (2): effect of before administration of folic acid and after administration of folic acid on serum folate and egg folate

Parameter	Befor administration of folic acid	after administration of folic acid
Serum folic acid((ng/mL)	79.3±0.57 B	145.4±1.39 A
Egg folic acid(ppm)	32.8±0.2 B	64.9±0.24

Our results of the current study showed a significant increase (P≤0.05) in the serum and egg folic acid after administration of 1 mg /kg folic acid as compere with before FA supplementation. Where the results after administration folic acid were 145.4±1.39 for serum and 64.9±0.24 for egg as showed in table (4-1).This improve result agreed with (House et al., 2002; Hoey et al., 2008 and Bailey et al., 2015). The improvement of serum folic acid may be due to prescreens of γ-glutamylcarboxy conjugase which is convert polyglutamated to monoglutamyl forms that can easily travel throw the brush border membrane of the duodenum and jejunum.

The easy monoglutamate forms are transported across the enterocytes through the action of specific transporters: folate receptor (FOLR), reduced folate carrier (RFC), and proton-coupled folate transporter (PCFT). Once inside the enterocytes, synthetic FA is reduced to tetrahydrofolate (THF) by dihydrofolate reductase (DHFR) to gain a metabolic activity similar to other folate species. The results of the current study showed that there was a significant increase (P≤0.05) in egg folic acid after administrated folic acid as compare with before administrated folic acid. Perhaps the reason for the current increase of egg folic acid is due to increase in folic acid in the blood and in the body as a result of the increase in folic acid administration in the food provided to laying hens, which contributed to improvement proportion of folic acid in the egg by passive diffusion and this is consistent with (House et al., 2002 and Altic et al., 2016).

Our results of the current study showed a significant increase (P≤0.05) in the Egg weight, Albumin height, Albumin diameter, yolk height, yolk diameter, yolk color

and Haugh unit after administration of 1 mg /kg folic acid as compere with before FA supplementation. And these results agrees with (Arzeni et al., 2015; Bagheri et al., 2019; Czarnowska-Kujawska et al., 2021 and Gu et al., 2022). On the other hand Egg shell thickness showed no significant difference (P>0.05) after administration of 1 mg /kg folic acid as compere with before FA supplementation. And this agree with (Gu et al., 2022).

The improvement of the egg weight, it is known that weight may be occur due to increase the amount of albumen and egg yolk in the egg which are directly related to egg weight. Furthermore it was found that folic acid helps to increase the level of estrogen which plays a key role in increasing the proportion of egg albums, which leads to an increase in the height and diameter of the egg albums (Wallock-Montelius et al., 2007). Which lead to increase egg weight so that adequate folic acid with the hen impairs the oviducts response to estrogen and ability to form albumen (Saleh et al., 2021).

Our study showed a significant increase (P≤0.05) in the Albumin height and Albumin diameter height and diameter after administration of 1 mg /kg folic acid as compere with before FA supplementation. this result may be occur due to the increase in the albumin amount in the egg. Since that folate play important role in the protein synthesis in the body. Folate has an essential role in one-carbon metabolism and is a strong anti-proliferative agent. Folate increases DNA stability, being crucial for DNA synthesis and repair the methylation cycle and preventing oxidation of DNA by free radicals which led to increase protein formation. And this agree with (Abbasi et al., 2018).

Our study showed a significant increase (P≤0.05) in the egg yolk height and diameter after administration of 1 mg /kg folic acid as compere with before FA supplementation. This result may be occur due to the ability of folic acid to increase follicle stimulating hormone secretion from the pituitary gland. In the process of follicular growth and maturation, leading role by stimulating the follicle to secrete estrogen through the FSH receptor of the ovary, promoting the deposition of yolk material which lead to increase the height and diameter of egg yolk .and this agree with (Cui et al.,2020).

The study showed that there was a significant increase (P≤0.05) in the egg yolk color after folic acid supplement when compared with before folic acid administration. And this result is agreement with (Czarnowska-Kujawska et al., 2021 and Gu et al., 2022). The yellow or orange color in egg yolk is due to group called carotenoids. The most important and most available for egg yolk coloration are zeaxanthin and lutein. Which is a powerful antioxidant that defend against unstable molecules called free radicals (Zaheer, K. 2017).

Conclusion

The data from the current study indicate that eggs from laying hens have a significant increase in the some of internal and external quality when administrated folic acid. Also, the layer hens when fed diet enriched with folic acid to enhance egg shell color will be led to increase the folic acid content of serum and eggs. This will lead to produce folate egg enriched with folic acid.

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