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Effect of folic acid on fertility of layer breeder of Novogen brown strain

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Abstract

Folic acid (FA or folate) is a type of vitamin (B9) naturally found in some foods that help the body to produce red blood cells. It is an essential vitamin B needed for chickens of all ages. This study was initiated to analyze the effect of FA on fertility of layer breeder. A total of 3,200 cocks of the sire line and 33,800 hens of the dam-line were randomly assigned to 4 tiers 4 rows colony cage shed and mated at a ratio of 1 cock: 10.5 hens. Four dietary treatments were used supplemented practical corn-soybean meal basal diet (control group) and the basal diet supplemented with 10 mg of FA/kg of diet (group-II), the basal diet supplemented with 15 mg of FA/kg of diet (group-III) and the basal diet supplemented with 20 mg of FA/kg of diet (group-IV). Egg production, egg weight, feed intake, mortality, fertility, hatchability and related parameters were measured during 39 to 42 weeks of age. The egg production was increased gradually in all folic acid treated feed groups and was highest in group-III (89.98±0.25), in comparison to control group (89.83±0.14). The fertility and hatchability were increased non-significantly (*p<0.05*). But FA supplementation was reduced the hatch loss and cull chicks. There were no significant (P<0.05) effects in mortality and body weight gain. The administration of supplementation folic acid to layer breeders via dietary supplementation was effective in improving the fertility and related parameters.

Keywords: Folic acid, fertility, hatchability, Novogen, chicks

Introduction

Folic acid is an N-acyl-amino acid that is a form of the water-soluble vitamin B9. Folic acid known as folate or vitamin B9 is an essential for all tissues with a high rate of cellular division and growth and therefore very important in reproduction (Pertiwi *et al*. 2022). Folacin is the generic descriptor not only for the original vitamin, folic acid, but also for related compounds that qualitatively show folic acid activity; the pure substance being designated *pteroylmonoglutamic* acid (Hussin *et al.* 2020). Its chemical structure contains three distinct parts, consisting of glutamic acid, a para-amino benzoic acid (PABA) residue and a pteridine nucleus. The PABA portion of the vitamin structure was once thought to be a vitamin. Research has shown that if the folic acid requirement of the organism is met, there is no need to add PABA to the diet (Kenny and Kemp 2005).

The beneficial effects of folic acid (FA) on reproduction in humans, polytocous species and in some livestock, animals have been well documented (Blair 2018). A study conducted in humans in the 1950s and 1960s led to the recognition of prenatal FA supplementation as a means to prevent pregnancy-induced megaloblastic anemia (Tamura and Picciano 2006). It has been experimentally demonstrated that FA is critical for embryo survival and fetal development in rats (Reddy 2017), hamsters and guinea pigs (Alonge *et al.* 2018). In sows, supplemental FA has been associated with increases of about 10% in litter size at parturition (Alonge *et al.* 2018). On the contrary, FA supplementation did not improve the reproductive performance of prolific and non-prolific ewes either in the estrous season or in the anestrous period (Alonge *et al.* 2018). Various contradictory results on the influence of supplementing diets with excess of FA on the egg folate concentrations have been reported in laying hens (Hebert *et al.* 2005, Abas *et al.* 2008) reported that the addition of 4 mg of FA/kg of laying hen diet led to an increase of approximately 3-fold in egg folate concentration relative to a regular commercial egg (Hebert *et al.* 2005). B9 vitamin is essential for cell division in body function and therefore very important for embryo development (Reddy 2017, El-Husseiny *et al*. 2018).

Poultry industry is one of the important industries in Bangladesh in terms of employment avenue and source of protein supply at cheaper price for the nation system. About 90% of rural Bangladeshi households keep poultry (DLS 2024). Commercial poultry farms are growing at a rate of 15% a year, with investment in the sector expected to double in the next decade. The poultry sector is gearing up for exporting by 2024. On the other hand, 1 million entrepreneurs and 8 million people commercially produce 10.22 billion eggs and 1.46 million tons of poultry meat annually. According to the most recent data, there are up to 70,000 commercial farms, 206 parent farms (PS), and 08 Grand Parent (GP) farms in Bangladesh (DLS 2024).

Chickens are more susceptible to deficiency of folic acid than other farm livestock, as a deficiency can readily be produced by feeding a folic acid-deficient diet (Korver 2023). Folic acid deficiency, as indicated by retarded growth and feed efficiency, could be produced in 15-day-old chicks fed corn-soybean meal diets (Tercic and Pestotnik 2014). In folic acid deficiency, megaloblastic arrest of erythrocyte formation in bone marrow causes severe macrocytic anemia as one of the first signs. Folic acid deficiency in chicks is also characterized by poor growth, very poor feathering, an anemic appearance, and perosis (Korver 2023). The chicks become lethargic, and feed intake declines. As anemia develops, the comb becomes waxy white and the mucous membrane of the mouth becomes pale (Tercic and Pestotnik 2014). Limited studies were done on the role of feeding with folic acid on performance of layer breeder (Nouri *et al.* 2018). Considering the above facts, the present study was planned to study the effect of folic acid on egg production and to evaluate the effect of folic acid on fertility and hatchability of layer breeder of Novogen brown strain.

Material and methods

Study area: The Experiment was conducted at Diamond Chicks Limited (Layer Breeder Farm), Kapasia, Gazipur and the protocol was approved by the Department of Pathobiology, Faculty of Veterinary Medicine and Animal Science, Gazipur Agricultural University (GAU), Gazipur.

Selection of birds: The present study was planned to see the effect of FA supplementation on productive and reproductive performance of layer breeders. A total of 37,000 parent stock chickens were used with an environmentally controlled shed. Total 3,200 cocks of the sire line and 33,800 hens of the dam-line of Novogen brown strain were randomly assigned to 4 tiers 4 rows colony cage shed and mated at a ratio of 1 cock: 10.5 hens. Four dietary treatments were used supplemented practical corn-soybean meal basal diet (For control group) and the basal diet supplemented with 10 mg of FA/kg of diet (For group-II), the basal diet supplemented with 15 mg of FA/kg of diet (For group-III) and the basal diet supplemented with 20 mg of FA/kg of diet (For group-IV) in each experimental group. The basal diet consisted of antibiotic-free layer mash formulated to meet minimum nutrient requirement for layers established by NRC (1994) and included no crystalline FA or commercially produced 5-methyltetrahydrofolate. Egg production, egg weight, feed intake, mortality, fertility, hatchability and related parameters were measured during 39 to 42 weeks of age.

The photoperiod was fixed at 16 hours with illumination provided by fluorescent lights at an intensity range of 3 to 5 lux at chicken head height. Egg production and mortality were recorded daily. Feed consumption from all birds in each replicate was recorded on a weekly basis and the intake was calculated per bird per day. For the analyses of fertility, hatchability and body weight of newborn chicks, eggs were collected daily saved for hatching in a cool room and incubated at the end of the weeks. Before setting in the incubators eggs from the different treatments/replicates were labeled with a pen number, weighed and fumigated. A total of 48,550 hatching eggs were used each day. The incubation was carried out in a single-stage electronically controlled setter, Petersime S168 (Petersime, Zulte, Belgium), at 37.8 °C and 60% RH (Relative Humidity). On day 18, the eggs were transferred to a hatcher, Petersime H168 (Petersime, Zulte, Belgium), with 37.2°C and 70% RH (Relative Humidity) to complete incubation. After 21 days of incubation, chicks that had fully emerged from their shells were removed, weighed and their number was recorded. Unhatched eggs were broken, and classified by macroscopic examination as dead-in-shells or as infertile. Dead-in-shells also included pipped eggs. Pipped eggs contained chicks that were not able to complete hatching successfully or were already dead. Percentage fertility and hatchability for each treatment group were calculated. Hatchability was calculated and expressed as a percentage of fertile and total eggs set. Daily egg production was calculated as percentage hen-day egg production.

Data analysis: Data were subjected to ANOVA, using the PROC GLM procedure of SAS software (SAS Institute Inc., 2004). All data in percentage form were transformed using arc sine transformations prior to analysis. Pen constituted the experimental unit. The model tested the main effects of treatment, hatch time (16 hatches - consecutive ages of layer breeders), replication (row) as well as the interaction terms using residual error. Because two-way interactions were not significant (P>0.05), data were analyzed for the main effects. Values are expressed as least squares mean (LSM) ±standard error of the mean (SE). Treatment means were separated using Tukey›s test. Statistical significance was accepted at P < 0.05.

Results and discussion

The study revealed that dietary folic acid supplementation levels had no significant effect on egg production, feed consumption and feed conversion. Besides folic acid levels had no significant effect on most of the egg quality traits except for egg shell thickness, egg shape index and yolk percentage. Also, dietary folic acid levels had no significant effect on fertility, hatchability and egg weight loss during incubation. Best significant weight of baby hatched chicks had been produced with the dose of 15 mg folic/kg diet while the other used levels had numerical increase of chick body weight. Poultry is more susceptible to lack of folic acid than other farm livestock, as a deficiency can readily be produced by feeding a folic acid-deficient diet (Hussin *et al*. 2020). Folic acid deficiency, as indicated by retarded growth and feed efficiency, could be produced in 15-day-old chicks fed corn-soybean meal diets (Ekmay *et al*. 2013, Tercic and Pestotnik 2014).

Effect of folic acid on mortality: The mortality was 13±2.06, 11±1.58 and 11±1.66, respectively in three treatment group. In control group the mortality was 12±1.5 (Table 1) and hatched chicks is also showed poor growth, very poor feathering, an anemic appearance, and perosis. No clinical signs were not found in the treatment group. The finding was non-significant (*p<0.05*) among the treatment and control group.

The finding in this study was corresponded with others (Nouri *et al*. 2018, Alonge *et al*. 2019).

Effect of folic acid on feed intake: The feed intake was 113.25±1.26, 113.75±1.71 and 113.75±2.22 grams, respectively in three treatments but 113.5±1.29 grams was in the control group (Table 1). Feed intake of Novogen Breed at the age of 39, 40, 41 and 42 weeks was 113.5 gm per day but the standard was 120 grams/ day. The data was non-significant (*p<0.05*) among all groups.

Feed intake may be increase or decrease due to environmental condition. Standard feed requirement for Novogen Breed at the age of 39, 40, 41 and 42 weeks but actual feed intake 113 or 114 gm per day (6/7 gm less than the standard) because of there is no wastage of feed due the cage rearing. The similar results were reported by Whitehead *et al.* (1995) and Nouri *et. al.* (2018).

Effect of folic acid on egg production: The effects of folic acid on egg production were found (Table 1). Egg production of control group was showed 89.83±0.14 and the standard egg production was 91%. In case of control group egg production is less than 1.13 % due to the nutritional and environmental factors. The egg production was increased gradually in all folic acid treated feed groups and was highest in group-III (G3T2) (89.98±0.25), in comparison to control group (89.83±0.14). It indicated that the dose of folic acid at 15 mg/kg diet may increase the egg production or not Analysis of variance of the results revealed that the differences in the changes of doses among the different groups (both control and treated) were non-significant (*p<0.05*).

The egg production was increased gradually in all folic acid treated feed groups and was highest in group-III (G3T2) (89.98±0.25), in comparison to control group (89.83±0.14). It indicated that the dose of folic acid at 15 mg/kg diet may increase the egg production which was supported by Tercic and Pestotnik (2014) and Hussin *et al*. (2020).

Table 1. Effect of folic acid on mortality, feed intake, egg production, egg weight, fertility, hatchability, cull chicks and hatch loss (Mean± S.E)

Parameter	Chicks	Group-I (Control)	Group-II (10 mg/ kg Diet)	Group-III (15 mg/ kg Diet)	Group-IV (20 mg/ kg Diet)	P- Value
Mortality	-	12±1.5	13±2.06	11±1.58	11±1.66	NS
Feed Intake (grams/bird)	-	113.5±1.29	113.25±1.26	113.75±1.71	113.75±2.22	NS
Egg Production	-	89.83±0.14	89.86±0.13	89.98±0.25	89.89±0.13	NS
Hatching Eggs	-	91.19±0.23	91.7±0.22	91.67±0.22	91.31±0.19	NS
Egg Weight	-	59.5±0.30	59.2±0.41	59.2±0.41	59.2±0.41	NS
Fertility	-	86.4±0.16	86.8±0.22	87.3±0.41	87.3±0.19	NS
Hatchability	Female	39.3±0.21	39.3±0.37	39.6±0.35	39.2±0.26	NS
	Male	39.8±0.19	39.5±0.38	39.7±0.43	39.8±0.3	NS
Cull Chicks	-	1.6±0.13	1.6±0.12	1.6±0.08	1.6±0.05	NS
Hatch Loss	-	5.1±0.43	5±0.46	5.8±0.11	5.4±0.25	NS

S.E. = Standard Error =Significance at the level of ($P \geq 0.05$). NS=Non-Significant.

Effect of folic acid on hatching eggs production: The effects of folic acid on egg production were seen. Hatching eggs production of control group was showed 91.19±0.23 and the standard production percentage (%) was 95 (Table 1). In case of control group hatching eggs percentage (%) is less than 3.81 % due to the structure of cage and excessive mating tendency of male. The Hatching egg production were increased in all folic acid treated feed groups and was highest in group-II (G2T1) (91.7±0.22), in comparison to control group (91.19±0.23). It indicated that the dose of folic acid at 10 mg/kg diet may increase the hatching egg production. Analysis of variance of the results revealed that the differences in the changes of doses among the different groups (both control and treated) were non-significant (*p<0.05*).

The Hatching egg production were increased in all folic acid treated feed groups and was highest in group-II (G2T1) (91.7±0.22), in comparison to control group (91.19±0.23). It indicated that the dose of folic acid at 10 mg/kg diet may increase the hatching egg production. Analysis of variance of the results revealed that the differences in the changes of doses among the different groups (both control and treated) were non-significant (*p<0.05*). The similar results were reported by other authors Benkova *et al.* (2009), Tercic and Pestotnik (2014) and Alonge *et al*. (2019).

Effect of folic acid on egg weight: The effects of folic acid on egg weight were recorded (Table 1). Egg weight of Control group was showed 59.5 gm but in group G2T1, G3T2 and G4T3 the egg weight 59.2 gm and actually the standard egg weight was 60.1 gm. The weights of the eggs (Fig.05) were increased in Control group and the treated group G2T1, G3T2, G4T3 (59.2±0.41), in comparison to G1C (59.5±0.30). Analysis of variance of the results revealed that the differences in the changes of folic acid doses among the different groups (both control and treated) were non-significant (*p<0.05*).

The hatching weights of the eggs were increased in Control group and the treated group G2T1, G3T2, G4T3 (59.2±0.41), in comparison to G1C (59.5±0.30). These findings were also supported by Whitehead *et al*. (1995), Benkova *et al.* (2009), Kachana (2009) and Korver (2023).

Effect of folic acid on fertility of eggs: The beneficial effects of folic acid (FA) on fertility of eggs in poultry were documented (Figure 1 and Table 1). The fertility hatching eggs of Control group was showed 86.4±0.16 but in group G2T1, G3T2 and G4T3 the fertility was 86.8±0.22, 87.3±0.41, and 87.3±0.19, respectively. Compare to the Control group with the treated group fertility % of G3T2(15 mg/kg diet) is higher than the all-other groups but at the G4T3 (20 mg/kg diet) fertility remain the

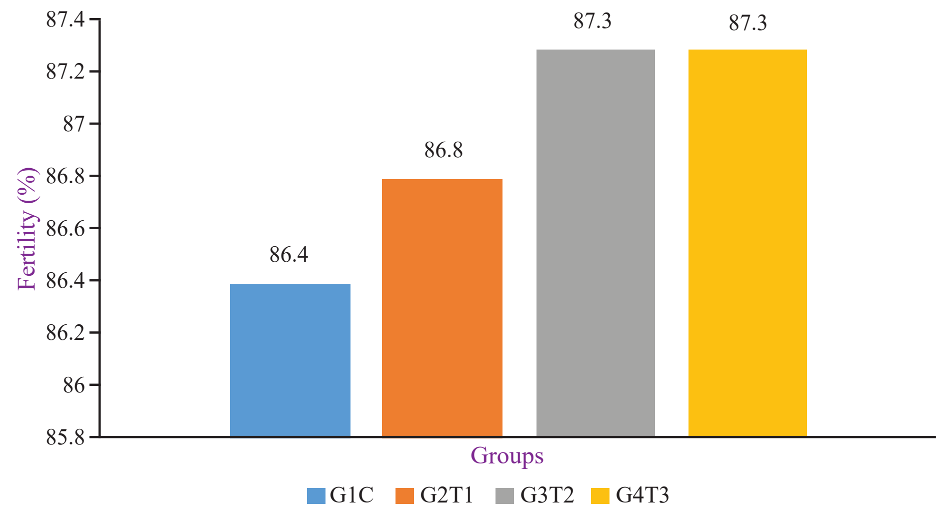

Figure 1. Effect of folic acid on Fertility (Figure is self-explanatory.

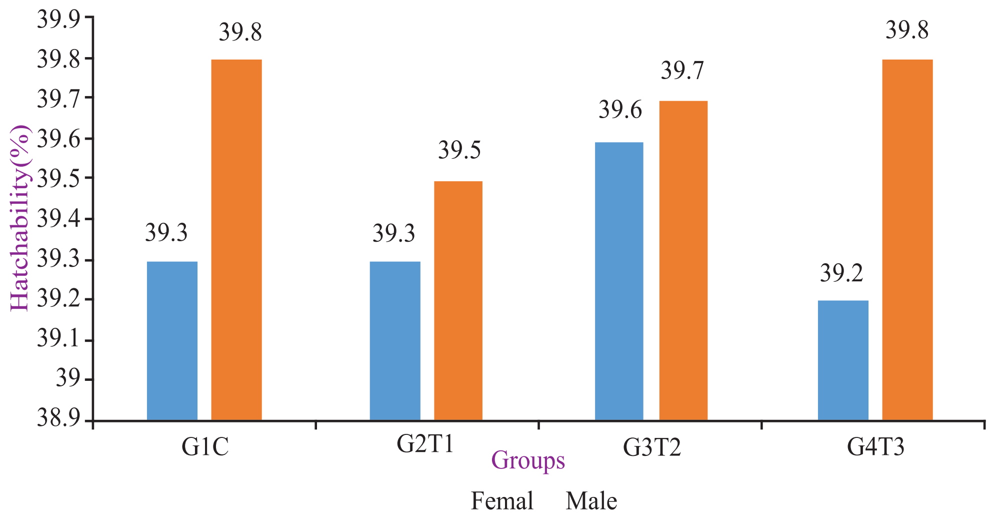

Figure 2. Effect of folic acid on Hatchability.

same. Analysis of variance of the results revealed that the differences in the changes of folic acid doses among the different groups (both control and treated) were non-significant (*p<0.05*).

The fertility hatching eggs of Control group was showed 86.4±0.16 but in group G2T1, G3T2 and G4T3 the fertility was 86.8±0.22, 87.3±0.41, and 87.3±0.19 respectively. Compare to the Control group with the treated group fertility % of G3T2(15 mg/kg diet) is higher than the all-other groups but at the G4T3 (20 mg/kg diet) fertility remain the same. Ekmay *et al*. (2013), Urso *et al*. (2015) and Sadegheymojarad *et al*. (2018) were also reported the similar findings.

Effect of folic acid on hatchability: Percentage fertility and hatchability for each treatment group were calculated. Hatchability was calculated and expressed as a percentage of fertile and total eggs set. The effect of folic acid on hatchability was varied in different treated and control group (Figure 2). From the table 1 hatchability percentage (%) is higher in G3T2 (15 mg/kg diet) and also lower in G4T3 (20 mg/kg diet). Hatchability were increases by the effect of folic acid because folic acid prevents the megaloblastic anemias, early embryonic death and perosis of chicks. Compare to the Control group with the treated group hatchability percentage (%) of female chicks is lower than the male chicks. Analysis of variance of the results revealed that the differences in the changes of folic acid doses among the different groups (both control and treated) were non-significant (*$p<0.05$*).

The effect of folic acid on hatchability was varied in different treated and control group. From the table hatchability % is higher in G3T2 (15 mg/kg diet) and also lower in G4T3 (20 mg/kg diet). Compare to the Control group with the treated group hatchability percent of female chicks is lower than the male chicks. The result was also corresponded by others Robel *et al*. (1993), Chung *et al.* (2005), Tercic and Pestotnik (2014), Urso *et al*. (2015) and El-Husseiny *et al*. (2018).

Effect of folic acid on cull chicks: Significant weight of baby gains hatched chicks had been produced with the dose of 15 mg folic/kg diet while the other used levels had numerical increase of chick body weight. Cull chicks were decreased (Table 1). Analysis of variance of the results revealed that the differences in the changes of folic acid doses among the different groups (both control and treated) were non-significant (*$p<0.05$*).

Significant weight of baby hatched chicks had been produced with the dose of 15 mg folic/kg diet while the other used levels had numerical increase of chick body weight. Cull chicks were decreased which were also reported by Abas *et al*. (2008), Benkova *et al*. (2009) and Blair (2018).

Effect of folic acid on hatch loss: Unhatched eggs were broken, and classified by macroscopic examination as dead-in-shells or as infertile. Dead-in-shells also included pipped eggs. Pipped eggs contained chicks that were not able to complete hatching successfully or were already dead. The BW of the newly hatched chicks was increased ($P<0.05$) with the supplementation of FA to the diet when compared with the control treatment. Hatch loss was reduced (Table 1) but statistically non-significant (*$p<0.05$*).

Unhatched eggs were broken, and classified by macroscopic examination as dead-in-shells or as infertile. Dead-in-shells also included pipped eggs. Pipped eggs contained chicks that were not able to complete hatching successfully or were already dead. The BW of the newly hatched chicks was increased with the supplementation of FA to the diet when compared with the control treatment. Hatch loss was reduced. The finding was also corresponded with Khalifah *et al*. (2006) and Korver (2023).

Conclusions

Supplementation of folic acid in diet of layer breeders was effective in improving body weight of newborn chicks. Egg production and feed intake were also positively affected by adding supplementation of folic acid. Dietary folic acid supplementation levels had no significant effect on egg production, feed consumption and feed conversion. However, the FA in diet reduced hatch loss and culled chicks. As overall performance was not significantly (*$p<0.05$*) affected by foliate concentration in the feed. It is suggested that further researches are needed to determine exact cellular and molecular mechanism of the folic acid metabolism in the layer breeder.

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