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EFFECT OF DIETARY FOLIC ACID SUPPLEMENTATION ON PRODUCTION AND HATCHING PERFORMANCE IN BAHEIJ CHICKEN STRAIN

By

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Abstract: *The objectives of this study were to investigate the effect of dietary folic acid levels on productive performance of Baheij as a local Egyptian chicken strain and the transfer of folic acid in the eggs as related to hatchability and egg weight loss during incubation and hatched chick weight. One hundred and five laying hens and 21 cocks aged 32 weeks of age from Baheij chicken strain were randomly divided into 7 treatment groups with 3 replicates each. First treatment was considered as a negative control diet and fed a diet containing 0 folic acid (premix free from folic acid). The second one was considered as positive control containing 1 mg folic acid/kg diet. The other experimented diet groups contained 2, 4, 8, 16 and 32 mg folic acid/kg diet, respectively. Feeding the treatment diets began 14 days before the start of egg collection and last for 8 weeks. Results 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 eggshell thickness, egg shape index and yolk percentage. The increase of folic acid levels in the diet from 0 to 2 mg/kg diet had increased the total egg folate concentration from 12.26 to 34.64 µg/egg. This study provides strong evidence of sensitivity of egg folate concentration to dietary folic acid levels. 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 32 mg folic/kg diet while the other used levels had numerical increase of chick body weight.*

INTRODUCTION

There has been growing awareness of the need for increased consumption of folate to human. Egg is an important source of dietary folate and contain at approximately 22 µg folate per large egg (USDA, 2001), which is equivalent to 6% of the adult daily requirements for folate (Institute of Medicine, 1998). Folic acid is the synthetic form of this vitamin which found in vitamin supplements, fortified foods and vitamin premixes. Sherwood *et al*., (1993) reported that plasma and yolk folate are proportionate across the range of dietary folic acid levels investigated (0-7 mg folic acid/kg) and concluded that regulatory processes controlling plasma folate levels might be the point of metabolic control. Also, Roble (1993) reported that dietary supplemental folic acid levels produced a positive linear response pattern on the transfer of folic acid in eggs ,but did not result in a hatchability increase. Ferguson *et al* ., (1961) and Sirbu *et al*., (1981) reported that hatchability and poult weight are related to folic acid in the egg and it is due to the role of folic acid in cellular development and higher supplementation of folic acid levels which could be required for rapid embryonic development.

Snetsinger *et al*., (1963) reported that folic acid is important in poult livability and growth. Keshavarz (2003) reported that certain manipulations of the combination of folic acid and other vitamins have the potential effect to reduce egg weight and improve shell quality without affecting egg production. House *et al* ., (2002) showed that the addition of folic acid to laying hen diets did not impact the performance of the birds, as reflected by no significant differences in egg production or egg weights and there was a marginal, but significant, effect of folic acid, especially at higher levels on feed intake.

The current study was conducted to investigate the effect of dietary folic acid levels on productive performance of Baheij chicken as a local Egyptian strain and the transfer of folic acid in the eggs as related to hatchability, egg weight loss during incubation and chick weight at hatch.

MATERIALS AND METHODS

The present study was carried out at El-Sabahia Poultry Research Station, Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture. Total number of 105 Baheij laying hens and 21 cocks aged 32 weeks of age were randomly assigned to 7 dietary treatments groups with 3 replicates each and each one consists of 6 birds. The birds were kept in floor pens. The birds were kept in floor pens.

A basal experimental diet was formulated as shown in Table 1. The first treatment group was considered as a negative control (-C) and fed on diet containing 0 folic acid (premix free from folic acid). The second one was considered as positive control (+C) containing 1 mg folic acid/kg diet as recommended level from NRC (1994). The other experimental groups were fed on diets containing 2, 4, 8, 16 and 32 mg folic acid/kg diet, respectively. Feeding of the treatment diets began 14 days before the start of egg collection and last for 8 weeks. Feed and water were provided *ad libtuim*.

During the experimental period, egg number and egg weight were recorded daily per pen. The average daily egg production and the daily feed consumption per replicate were calculated for fortnight intervals. The values of feed conversion ratio (feed consumption/egg mass) were calculated. Also, external and internal egg qualities were measured.

Hatching eggs were numbered consequently and weighed to nearest 0.01 g before setting in the incubator. All eggs were weighed individually again during incubation on the 5^{th}, 10^{th}, 15^{th} and 18^{th} day in order to obtain egg weight loss percentage for each incubation interval. Chicks that had fully emerged from eggs were removed and wing banded, weighed to the nearest 0.1 g and recorded as chick body weight at hatch then placed again to the incubator. All chicks were weighed again at the time of removal from the hatch. Chick body weight loss percentage during incubation was calculated:

$$\text{Chick weight loss percentage} = \frac{\text{chick weigh at hatch} - \text{chick weight at pull out}}{\text{chick weight at hatch}}$$

All percentage data of fertility and hatchability were subjected to arcsine square root percentage transformation prior to analysis.

Extraction of egg yolk folate:

Five eggs from each treatment were weighed and processed for egg folate determinations. All chemical used in the extraction and analysis of folate were purchased from Sigma Chemical Company. Eggs were weighed and boiled for 10 minutes and then were immersed immediately in chilled water. Once the eggs were cooled, yolks were removed, weighed, and lyophilized, after that the dry weights were noted. Dried yolks were stored at -20C until being analyzed. With respect to egg folate contents, previous studies have clearly shown that virtually of the folate found in eggs is first; limited to the yolk fraction (Sherwood *et al*., 1993), second; present as 5-methyltetrahydrofolate (Seyoum and Selhub, 1998); and third; present as the

monoglutamate (Seyoum and Selhub, 1998). The initial investigations support these findings to deconjugate glutamate residues or to determine multiple species forms for folate. Approximately 0.5 g of dried yolk was weighted into glass tubes with lids. Ten milliliters of an extraction buffer (20g/L sodium ascorbate; 12.1 g/L Trizma base; pH 7.8) were added to each tube, and the tubes were topped with nitrogen gas, vortexed, and placed in a boiling water bath for 60 minutes after boiling, the tubes were centrifuged at 4000Xg for 30 minutes then the supernatant from each tubes was decanted and retained. An additional 10 ml of extraction buffers was added to each tube, and the tubes were vortexed and centrifuged as before. The supernatants were pooled, and the final volume brought to 25 ml. A sample from each flask was placed into microcenterfuge tubes and frozen at -20C until being analyzed.

Analysis of egg yolk folate content.

The concentration of 5-methyltetrahydrofolate in egg yolk extracts was determined by reserve-phase HPLC with fluorescence detection, as previously described (Vahteristio *et al*., 1997). An external standard curve with purified 5-methyltetrahydrofolate was used to quantify egg folate concentrations. The inter- and intra-assay CV for determinations was < 3%, and recovery of 5-methyltetrahydrofolate added to dried egg yolk was 98.9%. The folate content was expressed as micrograms of folic acid per egg.

Statistical analysis:

The data were subjected to analysis of variance using the GLM Procedure of SAS (2001). Significant differences among individual means were analyzed by Duncan's multiple range test (Duncan's, 1955).

The following model was used:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where Y_{ij} = observed trait, μ = the overall mean, T_i = the effect of dietary folic acid levels and e_{ij}= random error.

RESULTS AND DISCUSSION

There were no significant differences among the dietary treatments groups fed the different levels of folic acid with respect to egg number per hen per day and during 56 days, egg weight, feed intake, egg mass and feed conversion (Table 2). Also, there were no apparent differences between negative and positive control with respect to the previous mentioned parameters. The best numerical results of feed conversion and egg mass

had been realized with the group supplemented with 32 mg folic acid/kg diet. It is clear from the results herein that increasing of folic acid supplementation in the diet up to 32 mg/kg diet had no significant effect on the previous mentioned parameters of laying hens production, whereas egg mass had increased numerically for groups of birds fed levels of 16 and 32 mg folic/kg diet. These results are in harmony with those reported by House *et al*., (2002) who showed that there was no significant difference due to folic acid supplementation on egg weigh or productivity. Also, they stated that feed consumption and egg weights were slightly depressed at level of 4-16 mg folic/kg intake, while, Robel (1993) in his research on turkey mentioned that egg weight was significantly increased with the increase of folic acid supplementation.

Table 3 reveals that increasing folic acid levels in the diet had significantly ($P<0.01$) affected egg shape index, egg shell thickness and yolk percentage. Whereas albumen height, yolk index and shell and albumen percentages had not been affected significantly by dietary folic acid supplementation. However, it is clear from the results of eggshell thickness in Table 3 that increasing the dietary folic acid supplementation up to 8 mg folic acid/kg diet and more had affected and increased egg shell thickness significantly. The previous report of Keshavarz (2003) support this finding and indicated that folic acid have the potential effect to improve shell quality. Moreover, there is a little information regarding the folic acid supplementation on egg quality characters.

The increase of folic acid levels in the diet from 0 to 2 mg/kg diet had increased the total egg folate concentration from 12.265 to 34.641 µg /egg (Fig 1). The increase of folic acid in the diet from 4 to 32 mg/kg diet had nearly a little effect on the increase of total egg folate concentration as increased from 40.979 to 47.123 µg /egg. It means that more addition of folic acid to the diet above 2 mg/kg diet had a limited effect on secreted concentration of folate in the egg. In addition to, it is observed the same trend of linear increase of folate in egg yolk with the increase of folic acid added levels to diet from 0 to 2 mg/kg diet and following that the increase of egg yolk folate concentration had not increased with the increase of folic acid supplementation in the diet (4-32 mg/kg diet).

The current study provides strong evidence of sensitivity of egg folate concentrations to dietary folic acid levels. The result from the present study are in accordance with those obtained by Sherwood *et al*., (1993) and House *et al*., (2002) who examined the relationship between dietary folate and egg yolk folate concentration.

Table 4 demonstrates that no apparent significant effect of added levels of dietary folic acid on fertility, hatchability of fertile eggs and chick weigh loss percentage. These results are contradicted with the results of Fergusan *et al*., (1961) who reported that hatchability of turkey is related to folic acid in the egg. Whereas, data of chick body weight at hatch in Table 4 had increased significantly ($P<0.01$) with the increase of dietary folic acid supplementation. The best significant weight of baby hatched chicks had been produced with the dose of 32 mg folic/kg diet while the other used levels had numerical increase of chick body weight. Supporting to the results herein, Sirbu *et al*., (1981) reported that poult weights are related to folic acid levels and their effect on cellular development and higher supplementation of folic acid levels which could be required for rapid embryonic development.

The results in Table 5 show that egg weight loss during the setting period (0-18 day) had significantly decreased ($P<0.01$) with the increase of folic acid supplementation. This result is normal and could be explained with the increase of egg shell thickness as it appears in Table 3, as shell thickness is the main barrier for egg weight loss. Egg weight loss is an accurate measure of the egg shell functional ability to resist water vapor passage (Paganell *et al*., 1978). The results herein were coincided with the results of Tullett and Board (1977) who reported that rate of egg weight loss is inversely proportional to shell thickness. Also, Peebles and Brake (1987) found that eggshell weight and thickness were negatively correlated to the relative rate of water loss from eggs. The decline in eggshell thickness and weight coincided with increase percentage of incubational egg weight losses during incubational intervals (Peebles *et al*., 2001).

It can be concluded from the present study that addition of folic acid to laying diet did not impact the performance of the birds as reflected by no significant differences in egg production and egg weight. Eggshell thickness and chick body weight at hatch are the important variable traits which had been affected significantly with folic acid supplementation. Besides we can recommend to increase of folic acid levels in the diet as a good source of folic acid for the human.

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Table 1: Composition and calculated nutrient composition of experimental diet.

Ingredients	**%**	**Calculated nutrient composition**	
Yellow corn	63.14	ME (kcal/kg)	2735
Soybean meal (44% CP)	27.10	Crude protein (% analysed)	17.29
Limestone	7.60	Calcium (%)	3.05
Dicalcium Phosphat	1.50	Phosphorus (%)	0.41
Sodium chloride	0.30	Folate, mg/kg	0.605
D L.Methionine	0.06		
Premix*	0.30		

*Premix contain per 3Kg:Vit.A 10,000,000 IU, D_3 2,000,000 IU, E 10,000 mg, K_3 1,000 mg, B_1 1,0000 mg, B_2 5000mg, B_6 1500 mg, $_{B12}$ 10mg, Niacin 30000 mg, Pantothenic acid 1000mg, MnO 60,000 mg, ZnO 50,000 mg, Fe_2SO_4 30,000 mg, $CuSO_4$ 4000 mg, Calcium iodide 300 mg, Co 100mg, Choline Chloride 250mg, $CaCO_3$ carrier till3000g.

Table 2: Effect of dietary folic acid supplementation on egg production traits, feed intake and feed conversion for Baheij chicken strain.

Folic acid levels (mg/kg diet)	Egg number (egg/hen/56 d)	Egg number (egg/hen/d)	Egg weight (g)	Feed intake (g/hen/d)	Egg mass (kg egg/hen/56d)	Feed conversion (g feed/ g egg)
0 (-C)	38.00±5.20	0.68±0.09	52.89±1.64	137.56±13.41	2.01±0.32	3.83±0.70
1 (+C)	38.80±3.11	0.69±0.06	52.47±0.54	134.59±20.30	2.04±0.18	3.69±0.38
2	41.73±3.97	0.75±0.07	52.08±0.47	137.51±06.50	2.18±0.22	3.53±0.56
4	39.93±2.25	0.71±0.04	50.92±0.71	135.32±08.70	2.04±0.14	3.71±0.46
8	38.33±4.33	0.68±0.07	51.50±0.88	132.54±11.69	1.96±0.19	3.87±0.37
16	42.20±3.74	0.75±0.07	51.62±1.15	135.79±08.49	2.19±0.23	3.47±0.59
32	42.87±2.31	0.76±0.04	52.07±0.17	134.13±12.11	2.23±0.11	3.37±0.37
Significant	NS	NS	NS	NS	NS	NS

NS : Not Significant

Table 3 : Effect of dietary folic acid supplementation on some egg quality for Baheij chicken strain

Folic acid levels (mg/kg diet)	Egg shape index	Egg shell thickness (mm)	Albumen height (mm)	Yolk index	Shell (%)	Yolk (%)	Albumen (%)
0 (-C)	77.03±1.14ab	36.00±1.2ab	6.59±0.57	0.44±0.01	12.24±1.03	34.57±1.59a	53.19±1.33
1 (+C)	79.82±0.20a	35.60±0.39b	7.26±0.65	0.42±0.01	12.35±0.79	31.03±0.43bc	56.62±1.08
2	79.09±1.20a	37.20±1.77b	7.46±0.52	0.44±0.02	13.11±0.88	30.04±0.47bc	56.85±0.43
4	73.16±1.20c	36.80±0.96b	6.57±0.46	0.46±0.02	13.41±0.36	30.89±0.56bc	51.03±4.52
8	75.49±0.73bc	39.80±0.73a	7.29±0.69	0.44±0.02	13.33±0.45	29.98±1.39bc	56.69±1.43
16	75.62±0.15bc	39.00±0.77a	6.33±0.57	0.43±0.01	13.66±1.24	29.20±0.64c	57.14±0.86
32	74.92±0.12bc	39.60±1.63a	6.71±0.67	0.44±0.02	12.85±0.80	32.49±1.10ab	54.66±1.53
Significant	**	**	NS	NS	NS	**	NS

Means within the same column with different superscripts are significantly different.

** Significantly at 0.01 NS : Not Significant

Table 4 :Effect of dietary folic acid supplementation on fertility, hatchability, body weight at hatch and chick weight loss for Baheij chicken strain

Folic acid levels (mg/kg diet)	Fertility (%)	Hatchability of fertile eggs (%)	Body weight at hatch (g)	Chick weight loss (%)
0 (-C)	96.08±1.44	91.24±1.44	35.91±0.59b	5.03±1.18
1 (+C)	91.36±8.64	91.27±5.44	36.61±0.83b	4.18±0.83
2	94.54±2.19	88.77±4.31	36.47±0.82b	4.06±0.15
4	97.06±1.70	86.26±3.36	37.69±0.67b	4.49±1.04
8	95.94±1.38	87.31±4.74	37.58±0.87b	5.73±1.60
16	94.39±2.31	87.96±2.80	37.10±0.71b	4.79±1.64
32	97.72±0.89	88.16±3.09	40.00±1.28a	5.20±0.71
Significant	NS	NS	**	NS

NS : Not Significant ** Significantly at 0.01

Table 5: Effect of dietary folic acid supplementation on egg weight loss percentage during incubation for Baheij chicken strain

Folic acid levels (mg/kg diet)	0-5 days	5-10 days	10-18 days	0-18 days
0 (-C)	3.65±0.16ab	3.41±0.12a	7.30±0.26a	13.72±0.33a
1 (+C)	3.37±0.17bc	3.46±0.14a	6.29±0.29b	12.54±0.41b
2	3.63±0.11ab	3.45±0.12a	5.49±0.18c	12.05±0.27bc
4	3.25±0.14bc	3.27±0.16a	4.52±0.17d	10.63±0.29de
8	3.92±0.15a	3.22±0.13a	4.80±0.14d	11.49±0.21c
16	2.98±0.15c	3.22±0.14a	4.62±0.18d	10.43±0.29e
32	3.82±0.12a	2.46±0.11b	5.47±0.15c	11.32±0.18cd
Significant	**	**	**	**

Means within the same column with different superscripts are significantly different.
** Significantly at 0.01

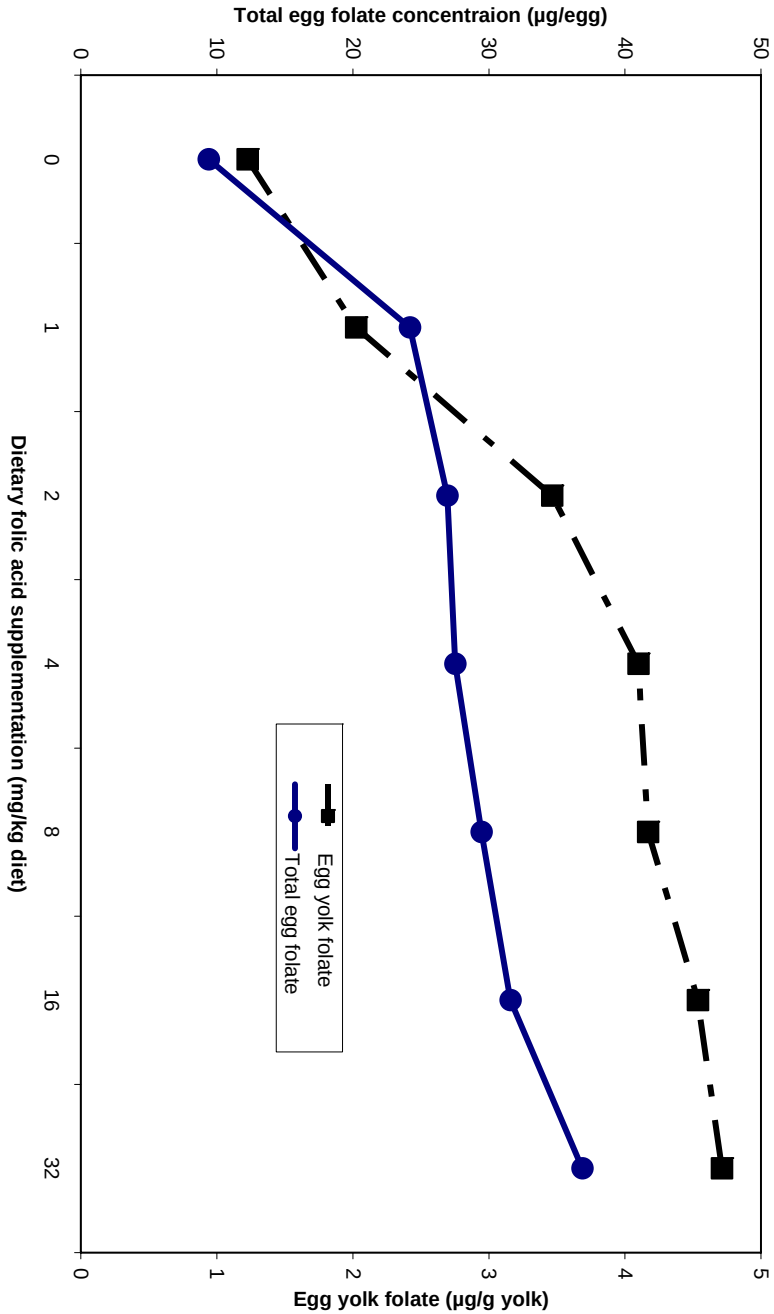

Fig (1) : Dose-response relationship between dietrary folic acid levels and folate concentration in total egg and egg yolk

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الملخص العربى

تأثير أضافه حامض الفوليك للعلف على الإنتاج وصفات التفريخ فى سلالة البهيج

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أستهدفت هذه الدراسة معرفه تأثيرَ حامض الفوليكِ على الاداء الانتاجى لسلاله البهيج ومدى انتقال هذا الحامض الى البيض وعلاقه ذلك بصفات التفريخ ومعدل الفقد فى وزن البيضه داخل ماكينه التفريخ. تم توزيع عدد ١٠٥ دجاجه بياضه و ٢١ ديك عمر ٣٢ اسبوع عشوائيا على ٧ معاملات تجريبيه بكل منها ٣ مكررات. المجموعه الاولى وقد اعتبرت ككنترول سالب تغذى على عليقه تحتوى على صفر حامض الفوليك (البريمكس خالى من حامض الفوليك). المجموعه الثانيه اعتبرت ككنترول موجب تغذت على عليقه تحتوى على ١ مجم حامض فوليك/كجم علف. باقى المجاميع التجريبيه تغذت على علائق تحتوى على ٢، ٤، ٨، ١٦ أو ٣٢ مجم حامض الفوليك/كجم علف على التوالى.

اوضحت النتائج ان مستويات حامض الفوليك ليس لها تاثير معنوى على كل من انتاج البيض، استهلاك العلف ومعدل التحويل الغذائى بجانب ذلك لم يكن لحامض الفوليك اى تاثير معنوى على خواص جودة البيض غير ان سمك القشرة، معامل شكل البيضه و نسبه الصفار قد تزداد معنويا بزيادة مستوى الفوليك فى العلف. وقد ادى زياده مستوى حامض الفوليك فى العلف من ٠ الى ٢ مجم حامض الفوليك/كجم علف الى زيادة تركيز الفولات الكلى فى البيضه من ١٢,٢٦ الى ٣٤,٤٦µجم/للبيضه. وقد امدتنا هذه الدراسه بمؤشر قوى على مدى حسّاسيةِ تركيز الفولات فى البيضه لمستوى الفوليك فى العلف. ولم يكن لمستويات حامض الفوليكِ فى العلف تاثير معنوى على كل من الخصوبةِ، التفريخ والفقد فى وزن البيضة اثناء التفريخ. احسن زيادة معنويه فى وزن الكتاكيت عند الفقس كانت عند المستوى ٣٢ مجم فوليك/كجم علف بينما لم تكن هناك اى زياده معنويه فى باقى المستويات الاخرى المستخدمه .