

EFFECT OF DIETARY FOLIC ACID SUPPLEMENTATION ON LAYING HENS PRODUCTIVE PERFORMANCE AND IMMUNITY

By

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Abstract: *A study was designed to determine the response of one hundred and twelve Hy-sex Brown laying hens at 28 weeks of age (n: 28/ diet) fed a corn-based ration containing 0, 10, 20, or 30 mg/ kg of crystalline folic acid for 12 weeks experimental period. Response criteria included production parameters, measures of blood physiological parameters, serum folate status, and egg quality measurements content. Dietary folate (F) did not significantly affect most of the production parameters including body weight, egg production, feed consumption, feed conversion ratio, egg mass, and also egg quality parameters. Significant increase ($P \leq 0.05$) in yolk index, serum folate 47.7 vs. 16.7 ng/mL (30 mg/ kg F vs. control; 4th wk), 48.00 vs. 17.7 ng/mL (30 mg/ kg F vs. control; 12th wk), cholesterol 174.62 (control) vs. 151.92 mg dl (20 mg/ kg F), total protein 5.58 vs. 5.17 mg/ dl (20 mg/ kg F vs. control: recovery period) and globulin 4.16 vs. 3.72 g/dl (30 mg/ kg F vs. 10 mg kg F; recovery period) were observed as F dietary supplementation increased. White blood cells differentiation showed that heterophills were increased ($P \leq 0.05$) with supplementing diets with 30 mg/ kg F (20.67 mg/ dl) when compared to other treatments especially the control (18.67 mg/ dl), respectively. Overall, production parameters were maintained due to feeding dietary treatments, while serum folate content was maximized as dietary crystalline folic acid was supplemented to diets at 10 mg/ kg or higher with a possible deposition in egg yolk.*

INTRODUCTION

Folate (F) is an essential micronutrient that, in mammals, must be obtained from exogenous sources via intestinal absorption (Said, *et al.*, 2000). Folic acid is required in the methylation of homocysteine to form

methionine and in the biosynthesis of amino acids and deoxynucleotides needed for DNA replication and repair (Selhub *et al.*, 1996 and Tapiero *et al.*, 2001). It is a collective term for a group of different water compounds with a pteroylglutamic acid backbone but differing oxidation states (*i.e.*, F, 5-methyltetra- hydrofolate) whose primary function includes one- carbon transfer reactions (Selhub and Rosenberg, 1996). Examples of one carbon transfer reactions include the remethylation of homocysteine, glycine-serine interconversion and purine synthesis (Selhub and Rosenberg, 1996).

It is generally known that eggs are an excellent dietary source of many essential and non-essential nutrients (Surai and Sparks, 2001) and are one of the least expensive livestock products. Eggs potentially may contribute 10-20% of daily intake of folate and total saturated and polyunsaturated fat (Song and Kerver, 2000). More than 95% of F in egg is located in the yolk and enrichment of eggs with F is possible when dietary F levels are increased (Sherwood *et al.*, 1993; Hebert *et al.*, 2005). Hence, increasing F in egg through dietary supplementation may be necessary for pregnant women, older adults and people in rural areas. Also, enhancing levels of such a vitamin in eggs may promote better health of these individuals, as human malnutrition and undernourishment are considered major problems that occur in many developing countries including Egypt. Vitamin deficiencies, including F, often occur in residents of rural area (Stabler and Allen, 2004; Singla *et al.*, 2006). Low vitamin levels (folate, B₁₂ and B₆) in serum are also related to the etiology of atherosclerosis and coronary heart disease because these deficiencies lead to inadequate production of S-adenosylmethionine, creating a condition of hypomethylation (Newman, 1999) and hyperhomocysteinemia (Ventura *et al.*, 2004). However, too much folate is a general phenomenon that affects other systems in the body, and might be a factor in some other diseases (Morris *et al.*, 2007).

Usually, serum F is associated with vitamin B₁₂ deficiency, *ie.* low doses (300-800 g/d) of folic acid can mask hematologic signs and may aggravate neurologic symptoms (Chosy *et al.*, 1962; Savage and Lindenbaum, 1995) and that the severity of neurologic impairment increases with rising serum F concentrations (Savage *et al.*, 1994). Furthermore, Troen *et al.*, (2006) studied an index of immune function-natural killer (NK) cell cytotoxicity in postmenopausal women and also showed advantage sides of dietary folic acid supplementation. NK cells are an important part of the non-specific immune response and can kill tumor cells and virally infected cells, studies showed a highly significant inverse linear association

between the amount of folic acid in plasma and NK cytotoxicity. Raising the hypothesis that excess folic acid from supplements or from fortified food can suppress NK function, which is vital for normal immune function. Therefore, this experiment aimed to study the effect of F supplementation on the performance, egg quality and blood physiological status of Hy-sex Brown laying hens at early production.

MATERIALS AND METHODS

Experimental birds and management:

This study was conducted at the Animal and Poultry Production Department, Faculty of Agriculture, South Valley University. One hundred and twelve Hy-sex Brown laying hens at 28 weeks of age were randomized into individual layer cages and fed one of four dietary treatment diets differing in F vitamin content (0, 10, 20 and 30 mg/ kg feed), hens within each dietary treatment were randomized into 28 individual replicates. Feed and water were provided *ad-libitum* all over the experimental period (12 weeks) from 28 to 40 weeks of age. Light was provided for 16 h daily and temperature was maintained throughout the experiment.

Experimental diets:

The corn-soybean basal diet composition is presented in (Table 1). Treatments consisted of the basal diet which was a commercial ration containing approximately 0.05 mg folate/ kg feed, to which had been added the following amounts of synthetic F (0, 10, 20 and 30 mg/ kg feed). Folate was added to the basal diet in a synthetic commercial form¹, and dietary treatments were formulated to be iso-caloric (2830 kcal ME/ kg) and iso-nitrogenous (19.37% CP). Experimental diets were formulated to meet nutrients requirements according to the recommended levels of (NRC, 1994).

Measurements:

Daily feed consumption (FI) per hen, hen-day production (EP) percent were recorded on daily basis, while one day egg production were collected and weighed (EW) weekly during the experimental period (12 weeks). Records of EP and EW were used to calculate egg mass (EM; g/ hen), which was used to calculate feed conversion ratio (FCR) during specific period and body weights (BW) were recorded every 4 weeks and at the end of the experimental period (40 weeks of age). Every 4 weeks of the experiment, a freshly collected sample of five eggs was collected from each

¹ Egypt for Feed Additives Company (EFAC)

treatment for egg quality measurements where: Yolk index according to (Well, 1968). Also, yolk color using color fan score was determined. Breaking strength was measured following the method of Shafey, (1991).

Blood Serum Measurements:

Plasma F concentrations were determined through the use of a competitive binding assay, Quantaphase II B₁₂/ folate radioassay with an Elecsys Folate II reagent kit² according to the manufacturer's recommended protocol (Hebert, *et al.*, 2005). Approximately 5 ml of blood was collected from hen's wing vein on the 4th, 12th weeks of the experimental period and after one month recovery period. Blood samples were centrifuged and the serum was collected and serum samples were stored at -20° C for further analysis of plasma total proteins and lipids (Fisher and Leveille, 1957) by using commercial kits produced by Diamond Diagnostic³.

Serum total immunoglobulin titre (STIT) was also determined according to Van der Zipp *et al.*, (1983). Plasma total lipids were determined according to the method of Zollner and Kirsch (1962). Determination of plasma cholesterol was carried out according to the method of Ratliff and Hall (1973). Plasma triglyceride was determined according to the method of Fossati, (1983).

Statistical analysis:

All data was analyzed using the GLM procedures of SAS for a Complete Randomized Design (CRD). Treatments (4 F inclusion levels) were considered fixed effects. Significant treatment differences were established using the LSMEANS statement in SAS (SAS, 2003). The following model was used to determine differences $Y_{ij} = \mu + a_i + e_{ij}$; where

Y_{ij} variable measured; μ overall mean; a_i = effect of the i^{th} level and e_{ij} = error component. Significance of difference was based on the probability of a type I error set at ($P \leq 0.05$). The differences among means were tested utilizing Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Effects of supplementing F on EP and FI are presented in (Table 2). No differences were noted in BW of hens fed supplemented dietary F levels. As feeding the control diet resulted in numerically heavier BW followed by hens fed F (10 mg/ kg diet) as compared to all other dietary treatments.

² Bio-Rad, Shimadzu, Mantech, Guelph, Ontario, Canada

³ Diamond diagnostic: 333 Fiske Street, Holliston, Massachusetts 01746 USA

Supplementation with F had not affected any of the productive performance parameters including BW, EP, EW, FI, FCR, EN or EM. These findings are consistent with those reported by Hebert *et al.*, (2004) and El-Husseiny *et al.*, (2005) who concluded no significant differences due to folic acid levels on LBWG were observed. Keshavarz (2003), Leeson and Caston (2003) and Hebert *et al.*, (2005) reported that egg production was not reduced by folate deficiencies as long as the FI was not significantly affected. In the present study, the vitamins contained in the basal diet met the requirements of hens (NRC 1994), and thus this diet was sufficient for normal egg production. Therefore, our study suggests that dietary folate levels in a diet based on corn and soybean do not affect EP and FI of laying hens (28-40 weeks of age). Accordingly, data of Husseiny *et al.*, (2005) showed that feeding diet supplemented with folate (6.0 - 12.0 mg/ kg, respectively) did not give any significant effect on egg production. Although, our results of EW were not significantly different, they agree with those results of Bunchasak and Kachana (2009) and House *et al.*, (2002) who concluded that there were differences in EW among different F levels. Keshavarz (2003) found that reducing dietary F resulted in reducing EW, as our results reported here were following a similar trend but insignificantly.

Some indices of internal and external egg quality measurements are presented in (Table 3). No effects of experimental diets on egg components were observed. Increasing dietary F levels resulted in a numerical reduction in albumin percent. At the end of the experimental period, feeding the control diet or that of 10 mg/ kg F diet resulted in insignificantly ($P \geq 0.05$) lighter shell weight percent as compared to other dietary treatments of 20 or 30 mg/ kg F. Abas *et al.*, (2008) reported similar results as of shell weights being similar as hens were fed dietary F up to 10 mg/ kg diet. Other studied egg quality measurements were significantly similar among dietary F levels. It is known that weight changes of both albumen and yolk are directly related to changes in egg weights and hens age, especially the egg albumen (Silversides and Budgell 2004). In this experiment, EW were statistically similar and hens being at the beginning of their production, as EW was lighter and hens were still young, which may be the reason of these results. Shell breaking strength (g. force/ g) was not affected by any of the dietary treatments, as averaged 3.29 g. force /g (4th week) and 3.32 g. force /g (12th week).

The effect of dietary F on blood physiological parameters is shown in (Table 4). Serum glucose, GOT, GPT and Albumen percentages were not affected by dietary F levels. Serum F was significantly increased ($P \leq 0.05$)

at the 4th and 12th weeks of the experiment, by increasing dietary F levels from 0 up to 30 mg/ kg. Plasma serves as the precursor pool for egg yolk deposition. Miller and White (1986) have identified the existence of a binding protein-mediated mechanism for the transfer of riboflavin into the egg yolk, but a similar mechanism has yet to be elucidated for folate. So, plasma folate concentrations may serve as the limiting factor for egg folate concentrations (Sherwood *et al.*, 1993), as both compartments appear to saturate at dietary folic acid concentrations between 2 and 4 mg/ kg of diet. Plasma folate concentrations were higher in birds consuming 30 mg of folic acid/kg diet than those consuming 0, 10 or 20 mg/ kg. This increase should be sufficient to translate into higher egg folate concentrations (*yolk Folate data are not available*). Therefore, a greater understanding of the factors regulating the absorption of dietary folic acid and its appearance in the systemic circulation in the form of 5-methyltetrahydrofolate in the laying hen, is warranted.

During the 4th, 12th and after removal of F from diets, serum cholesterol values were significantly ($P \leq 0.05$) higher as hens were fed the control diet and then were reduced by supplementing F at 10, 20 or 30 mg/ kg diet. Furthermore, feeding diets supplemented with F 30 mg/ kg diet at 12th week of the experiment resulted in a significantly ($P \leq 0.05$) higher total lipids values as compared to the control (12.36 vs. 12.15 mg/ dl), while the lowest total lipids were 12.07 mg/dl (F; 20 mg/ dl). Hens fed F (30 mg./ kg) at 4th, 12th weeks and recovery period of the experimental period had significantly ($P \leq 0.05$) higher serum total protein of 6.29, 6.23 and 5.58 mg/ dl; respectively. Furthermore, diets supplemented with higher dietary F levels (30 mg/ kg) at all experimental periods resulted in higher globulin values of 4.70, 4.69 and 4.16 mg/ dl at 4th, 12th and recovery period; respectively.

In poultry species, fatty acids synthesis in liver is very active in adult females producing eggs (Klasing, 1998) and the liver of the laying hen synthesizes lipid 15-25 times (on a cellular basis) as rapidly as that of immature birds (Hawkins and Heald, 1966). Two thirds of the total lipid biosynthesis in avian occurs in the liver (Saadoun and Leclercq, 1983), in laying hens liver must work harder, in a relative sense, to produce the fat and protein necessary for egg yolks. During production period, excess or low folate intake may induce more lipid metabolic disorders. In the present study, however, total lipids in serum in laying hen (28-40 weeks of age) were not significantly affected by F supplementation, which is in agreement with the report by Kuksis (1992) who concluded that the lipid class composition of yolk appeared to be

minimally affected by dietary influences and that the transport of triglycerides from the blood to egg yolk was not limited. These results suggest that triglyceride concentrations in the liver, serum and egg are not affected when hens receive folate at 10 to 30 mg/ kg diet.

Generally, dietary folate supplementation did not have any significant effects on white blood cells differentiations (Table 5). However on the 12th week heterophills were increased ($P \leq 0.05$) as dietary F levels increased with the control group being the lowest of 18.67 mg./ dl and all other F levels linearly higher with the highest at (30 mg./ kg) F of 20.67 mg/ dl. Data suggest that the increase in lymphocytes might be due to feeding F supplemented diets, and this could be an indicator to an increase in antibody formation. This is also, confirmed by lower heterocytes: lemphocytes ratio which is less than 0.5, which was supported by the increase in IGG values. Increasing dietary F levels increased IGG ($P \leq 0.01$) as diets were supplemented with 30 mg/ kg F resulted in a 7.79% increase when compared to the control group at the 12th week of age, respectively. In the same context, providing that F is required to maintain immunity and sustain production under higher ambient temperature, Sahin *et al.*, (2003) reported that low concentrations of folic acid under stress conditions are needed and have been reported, while higher dietary folic acid levels may be required for quail exposed to high ambient temperatures. This represents the case in our study, as the ambient temperature is higher in south valley region and may affect poultry production, so dietary F levels may play a role in maintaining production of these birds.

In conclusion, results of the present study suggested that folic acid had statistically similar effects on laying hens resulting in maintaining production especially with lower dietary levels 0 and 10 mg/ kg feed, with no negative impacts on immunity. Supplementing folic acid may offer a potential protective management practice in preventing heat stress-related depression in the performance of laying hens in warmer Egyptian regions.

Table (1): Composition and calculated analysis of the experimental diet

Ingredients,	%
Yellow corn	61.40
Soybean meal (44% CP)	18.00
Corn gluten meal (60% CP)	8.90
Vit. & Min. Premix ¹	0.30
Di-Calcium Phosphate	1.69
Limestone	8.33
Salt (NaCl)	0.40
DL-Methionine	0.06
L-Lysine	0.03
Filler (sand)	0.89
Total	100.00
Calculated Analysis, %	
ME, (kcal/ kg)	2830.00
Crude Protein	19.37
Calcium	3.60
Available Phosphorus	0.45
Lysine	0.77
Methionine	0.42
Folate (mg/ kg)	0.05

¹Premix provides by kg: Vit A, 5500 IU; Vit E, 11 IU; Vit D3, 1100 IU; riboflavin, 4.4 mg; Ca pantothenate, 12 mg; nicotinic acid, 44 mg; choline chloride, 191 mg; vitamin B₁₂, 12.1 ug; vitamin B₆, 2.2mg; thiamine (as thiamine mononitrate), 2.2 mg; folic acid, 0.55 mg; d-biotin, 0.11 mg. Trace mineral (mg /kg diet): Mn, 60; Zn, 50; Fe, 30; Cu, 5; Se, 0.3

Table 2: Effects of supplementing folate on productive performance traits

Parameter	Control		T1		T2		T3		Probabilities	
	4 wks	12 wk	4 wks	12 wk	4 wks	12 wk	4 wks	12 wk	4 wks	12 wk
Body weight (g.)	1718.57±2.25	1995.18±1.39	1660.19±2.30	1923.81±1.71	1702.13±1.88	1961.90±1.39	1617.12±1.25	1921.43±1.09	NS ²	NS
Egg production (%)	68.78± 1.18	69.84± 1.27	70.37± 2.00	71.95± 1.99	70.90± 2.03	69.32± 1.87	69.84± 1.16	69.32± 1.36	NS	NS
Egg weight (g.)	69.12±4.74	65.65±0.64	64.09±0.73	62.54±1.51	64.31±1.03	63.89±0.63	63.88±1.07	61.76±2.43	NS	NS
Feed consumption (g./h)	120.41±5.14	114.29±6.15	117.69±2.97	127.21±3.92	121.09±2.45	123.81±2.72	122.45±2.04	125.51±5.30	NS	NS
FCR ¹	2.70±0.06	2.50±0.05	2.67±0.04	2.73±0.06	2.69±0.05	2.65±0.06	2.84±0.07	2.78±0.06	NS	NS
Egg number	43.33±1.45	44.00±3.06	44.33±1.76	45.33±0.33	44.67±3.38	43.67±1.20	44.00±1.53	43.67±2.67	NS	NS
Egg mass (g.)	44.62±1.1	45.78±0.9	44.08±0.9	46.65±1.1	44.95±0.9	46.73±1.2	43.11±0.8	45.15±0.9	NS	NS

¹FCR: Feed conversion ratio (g. feed/ g. egg mass)²NS= not significant

Table 3: Effects of supplementing fōlate on egg quality measurements

Parameter	Control		T1		T2		T3		Probabilities	
	4 wks	12 wk	4 wks	12 wk	4 wks	12 wk	4 wks	12 wk	4 wks	12 wk
Egg weight (g.)	69.12±4.74	65.65±0.64	64.09±0.73	62.54±1.51	64.31±1.03	63.89±0.63	63.88±1.07	61.76±2.43	NS	NS
Albumen (%)	63.39±2.00	60.33±0.11	62.11±0.25	59.93±0.72	62.60±0.31	58.51±0.41	63.25±0.14	57.93±1.30	NS	NS
Yolk (%)	23.96±1.59	26.73±0.37	25.36±0.15	27.03±0.75	24.68±0.37	28.14±0.14	24.37±0.19	28.46±0.81	NS	NS
Shell (%)	12.65±0.43	12.93±0.27	12.53±0.17	13.04±0.12	12.72±0.26	13.35±0.28	12.37±0.19	13.61±0.54	NS	NS
Yolk color	6.50± 0.14	5.78± 0.29	6.33± 0.22	5.58± 0.22	6.83± 0.17	6.00± 0.00	6.42± 0.22	6.00± 0.00	NS	NS
Shell thickness (mm.)	0.39± 0.01	0.39± 0.01	0.38± 0.00	0.38± 0.00	0.37± 0.00	0.38± 1.66	0.37± 0.00	0.38± 0.01	NS	NS
Shape index	74.78±1.00	78.65±1.78	76.26±1.28	78.16±1.50	76.94±0.53	79.34±0.41	83.69±5.52	78.90±0.16	NS	NS
Yolk index	46.90±0.60 ^a	44.24±0.84	47.03±0.32 ^a	43.44±0.81	44.87±0.85 ^b	40.02±3.31	43.18±0.49 ^b	42.26±0.26	**	NS
Breaking strength (g force/g)	3.29± 0.33	3.30± 0.16	3.30± 0.16	3.35± 0.21	3.28± 0.09	3.30± 0.15	3.27± 0.13	3.32± 0.16	NS	NS

^{a,b} Means within a column with no common superscripts differ significantly ($P \leq 0.05$).

**= $P \leq 0.01$ NS= not significant

Table 4: Effects of supplementing folate on blood physiological parameters

Parameter	Control			T1			T2			T3			Probabilities		
	4 wks	12 wk	Recov ¹	4 wks	12 wk	Recov	4 wks	12 wk	Recov	4 wks	12 wk	Recov	4 wks	12 wk	Recov
Folate (ng/mL)	16.7 ^d ± 2.38	17.7 ^d ± 2.23	--	24.0 ^c ± 3.18	28.32 ^c ± 3.03	--	37.1 ^b ± 1.09	39.12 ^b ± 1.19	--	47.9 ^a ± 1.81	48.00 ^a ± 1.98	--	*	**	--
Glucose (mg/dl)	149.68 ± 2.68	150.94 ± 0.71	152.79 ± 0.97	143.63 ± 5.53	146.53 ± 2.55	151.22 ± 4.45	152.03 ± 4.97	149.52 ± 2.91	156.24 ± 1.93	147.15 ± 4.38	151.35 ± 1.01	152.96 ± 1.17	NS	NS	NS
Cholesterol (mg/dl)	174.5 ^a ± 5.46	174.6 ^a ± 3.01	176.5 ^a ± 0.52	133.3 ^c ± 3.53	142.4 ^b ± 1.76	150.4 ^b ± 2.93	148.0 ^b ± 3.92	151.9 ^c ± 1.94	165.1 ^a ± 5.23	167.6 ^a ± 3.40	161.8 ^b ± 2.83	176.2 ^a ± 5.41	**	**	**
GOT (U/L)	8.00 ± 1.00	8.00 ± 1.00	8.00 ± 1.00	7.00 ± 1.00	8.00 ± 1.00	9.00 ± 2.00	7.00 ± 1.00	8.00 ± 1.00	8.00 ± 1.00	7.00 ± 0.00	8.00 ± 1.00	11.00 ± 2.00	NS	NS	NS
GPT (U/L)	65.33 ± 1.67	63.67 ± 1.67	67.00 ± 2.89	65.33 ± 1.67	65.33 ± 1.67	70.33 ± 1.67	68.67 ± 1.67	65.33 ± 1.67	70.33 ± 1.67	65.33 ± 1.67	67.00 ± 1.33	70.33 ± 1.67	NS	NS	NS
Total Lipids (g/dl)	12.11 ± 0.26	12.15 ^{ab} ± 0.09	12.13 ± 0.40	12.09 ± 0.12	12.15 ^{ab} ± 0.08	12.27 ± 0.35	11.64 ± 0.36	12.07 ^b ± 0.19	12.32 ± 0.05	12.30 ± 0.19	12.36 ^a ± 0.18	12.72 ± 0.14	NS	*	NS
Total protein (g/dl)	5.66 ^c ± 0.12	5.50 ^c ± 0.15	5.40 ^{ab} ± 0.08	5.84 ^{bc} ± 0.15	5.69 ^{bc} ± 0.16	5.17 ^b ± 0.08	6.16 ^{ab} ± 0.13	6.00 ^{ab} ± 0.05	5.51 ^a ± 0.16	6.29 ^a ± 0.06	6.23 ^a ± 0.01	5.58 ^a ± 0.32	*	*	**
Albumen (g/dl)	1.56 ± 0.05	1.36 ± 0.04	1.37 ± 0.04	1.63 ± 0.06	1.51 ± 0.03	1.45 ± 0.04	1.54 ± 0.03	1.46 ± 0.08	1.41 ± 0.10	1.60 ± 0.03	1.54 ± 0.01	1.43 ± 0.02	NS	NS	NS
Globulin (g/dl)	4.10 ^b ± 0.07	4.14 ^b ± 0.10	4.03 ^b ± 0.08	4.20 ^b ± 0.19	4.18 ^b ± 0.14	3.72 ^c ± 0.12	4.39 ^a ± 0.02	4.54 ^a ± 0.04	4.10 ^a ± 0.26	4.70 ^a ± 0.09	4.69 ^a ± 0.01	4.16 ^a ± 0.34	*	*	**
Alb./Glob ratio	0.38 ± 0.01	0.33 ± 0.01	0.34 ± 0.01	0.39 ± 0.03	0.36 ± 0.01	0.39 ± 0.02	0.33 ± 0.01	0.32 ± 0.02	0.35 ± 0.05	0.34 ± 0.01	0.33 ± 0.02	0.35 ± 0.03	NS	NS	NS

¹Recov: recovery at 16 weeks, 4 weeks after removal of folate from dietary diets. ^{a-c} Means within a column with no common superscripts differ significantly ($P \leq 0.05$).

** = $P \leq 0.01$ * = $P \leq 0.05$ NS = not significant

Table 5: Effects of supplementing folate on white blood cells differentiation

Parameter	Control			T1			T2			T3			Probabilities		
	4 wks	12 wk	Recov ¹	4 wks	12 wk	Recov	4 wks	12 wk	Recov	4 wks	12 wk	Recov	4 wks	12 wk	Recov
Monocyte (mg/ dl)	2.33± 0.33	2.33± 0.33	2.33± 0.33	2.33± 0.33	2.33± 0.33	2.33± 0.33	2.33± 0.33	2.33± 0.33	2.33± 0.67	2.67± 0.33	2.33± 0.33	2.33± 0.33	NS	NS	NS
Heterophil (mg/ dl)	20.00 ±0.58	18.67 ^b ±0.33	19.33± 0.88	21.00± 0.58	19.67 ^{ab} ±0.33	20.67± 0.88	21.33± 0.33	20.00 ^{ab} ±0.58	18.00 ±2.65	21.67± 0.33	20.67 ^a ±0.33	19.67± 0.33	NS	*	NS
Lymphocyte (mg/ dl)	58.33± 0.88	66.00± 0.58	73.67± 0.88	59.33± 0.33	66.67± 0.88	74.33± 0.88	59.67± 0.67	67.00± 1.15	74.67 ±1.45	60.33± 0.88	68.00± 0.58	76.67± 0.88	NS	NS	NS
Hetero/ Lymph	0.34± 0.01	0.28± 0.01	0.26± 0.02	0.35± 0.01	0.30± 0.00	0.28± 0.01	0.36± 0.01	0.30± 0.00	0.24± 0.04	0.24± 0.04	0.30± 0.00	0.26± 0.00	NS	NS	NS
IGG	3.90 ^c ± 0.10	5.52 ^c ± 0.05	--	4.5 ^b ± 0.11	5.75 ^b ± 0.08	--	4.93 ^a ± 0.05	6.05 ^a ± 0.06	--	5.17 ^a ± 0.08	5.95 ^{ab} ± 0.08	--	**	**	--

¹Recov: recovery at 16 weeks, 4 weeks after removal of folic from dietary diets. ^{a-c} Means within a column with no common superscripts differ significantly ($P \leq 0.05$).

**= $P \leq 0.01$ * = $P \leq 0.05$ NS= not significant

REFERENCES

- Abas, I. R. Kahraman, H. Eseceli and N. Toker (2008).** *The effect of higher levels of folic acid on performance and egg quality of laying hens fed diets with or without ascorbic acid from 28-36 weeks of age.* *Journal of Animal and Veterinary Advances* 7 (4): 389-395
- Bunchasak, C., and S. Kachana (2009).** *Dietary folate and vitamin B12 supplementation and consequent vitamin deposition in chicken eggs.* *Trop. Anim. Health Prod.* 41:1583-1589.
- Chosy, J, D. Clatanoff, R. Schilling (1962).** *Responses to small doses of folic acid in pernicious anemia.* *Am. J Clin. Nutr.* 10: 349-50.
- Duncan, D. B. (1955).** *The multiple range and F-tests.* *Biometrics*, 11: 1-24.
- El-Husseiny, O. M., A. Z. Soliman, M. O. Abd-Elsamee and I. I. Omara (2005):** *Effect of dietary energy, methionine, choline and folic acid levels on layers performance.* *Egy. Poult. Sci. J.*, 25: 931-956.
- Fisher, H., and G. A., Leveille (1957).** *Observation on the cholesterol, linoleic and linolenic acid content of eggs as influenced by dietary fats.* *Journal of Nutrition* 63:119-129.
- Fossati, P. Principe (1983).** *Determination of triglyceride.* *L. Clin. Chem.*, 28:2077.
- Grobas, S., J. Mendez, C. Lopez Bote, C. De Blas. and G. G. Mateos (2002).** *Effect of vitamin E and A supplementation on egg yolk α -tocopherol concentration.* *Poult. Sci.* 81: 376-381.
- Hebert, K., House, J. D., W. Guenter (2005).** *Effect of dietary folic acid supplementation on egg folate content and the performance and folate status of two strains of laying hens.* *Poult. Sci.* 84: 1533-1538.
- Hebert, K., J., D. House and W. Guenter (2004).** *Efficiency of folate deposition in eggs through-out the production cycle of Hy-line W98 and W36 laying hens.* *Poult. Sci.*, 83: (suppl.1).
- House, J. D., K. Braun, C. P. Balance, C. P. O'Connor and W. Guenter (2002).** *The enrichment of eggs with folic acid through supplementation of the laying hen diet.* *Poult. Sci.*, 81: 1332-1337.
- Hawkins, R. A. and P. J. Heald (1966).** *Lipid metabolism and laying hens. IV. The synthesis of triglycerides by slices of avian liver in vitro.* *Biochim. biophys. Acta*, 116, 41.

- Keshavarz, K. (2003).** *Effects of reducing dietary protein, methionine, choline, folic acid, and vitamin B₁₂ during the late stages of the egg production cycle on performance and eggshell quality.* *Poult. Sci.* 82:1407-1414.
- Kuksis, A. (1992).** *Lipids.* In *J. Chromatogr. Library. Vol. 51. Part B.*, pp. B171-B227 (edited by E. Heftmann. Elsevier. Amsterdam).
- Leeson S., and L. J. Caston (2003).** *Vitamin Enrichment of Eggs.* *J. Appl. Poult. Res.* 12:24-26
- Miller, M. S., and H. B. White, III. (1986).** *Isolation of avian riboflavin-binding protein.* *Methods Enzymol.* 122: 227-234.
- Morris, M. S., Jacques, P. F., Rosenberg, I. H., J. Selhub (2007).** *Folate and vitamin B₁₂ status in relation to anemia, macrocytosis, and cognitive impairment in older Americans in the age of folic acid fortification.* *Am. J. Clin. Nutr.* 85, 193-200.
- Newman, P. E. (1999).** *Can reduced folic acid and vitamin B₁₂ levels cause deficient DNA methylation producing mutations which initiate atherosclerosis?.* *Medical Hypotheses,* 53, 421-424.
- NRC (1994).** *Nutrient Requirements of Poultry. 9th Edition.* National Academy press. Washington DC. USA.
- Ratcliff, C. R. and F. Hall (1973).** *Method for Determination of Plasma Cholesterol.* *Laboratory Manual of Clinical Biochemistry*
- Saadoun A., B. Leclercq (1983).** *Comparison of in vivo fatty acid synthesis of the genetically lean and fat chickens,* *Comp. Biochem. Physiol.* 75B: 641-644.
- Sahin K., M. Onderci, N. Sahin, M. F. Gursu and O. Kucuk (2003).** *Dietary Vitamin C and Folic Acid Supplementation Ameliorates the Detrimental Effects of Heat Stress in Japanese Quail.* *J. Nutr.* 133: 1882-1886.
- SAS (2003).** *SAS user's Guide: statistics. 9th Edition* SAS Institute, Inc., Cary, N.C., USA.
- Said, H. M., N. Chatterjee, R. Ul Haq, V. S. Subramanian, A. Ortiz, L. H. Matherly, F. M. Sirotnak, C. H. and S. A. Rubin (2000).** *Adaptive regulation of intestinal folate uptake: effect of dietary folate deficiency.* *Am. J. Physiol. Cell Physiol.* 279: 1889-1895.

- Savage, D., J. Lindenbaum (1995).** *Folate-cyanocobalamin interactions.* In: Bailey L, ed. *Folate in health and disease.* New York, NY: Marcel Dekker pp: 237- 285.
- Savage, D., I. Gangaidzo, J. Lindenbaum (1994).** *Vitamin B12 deficiency is the primary cause of megaloblastic anaemia in Zimbabwe.* *Br. J. Haematol.* 86: 844-50.
- Selhub, J. and I. H. Rosenberg (1996).** *Folic acid.* Pages 206-219 In *present knowledge in nutrition.* 7th Ed. E. E. Ziegler and L. J. Filer (Eds.). ILSI Press, Washington, DC.
- Silversides, F. G., and K. Budgell (2004).** *The relationships among measures of egg albumen heights, pH and whipping volume.* *Poult. Sci.*, 83:1619-1623.
- Shafey, T. M. (1991).** *The effects of dietary calcium, phosphorus and protein on the performance and nutrient utilization of broiler chickens.* *Poult. Sci.* 70: 548-553.
- Selhub, J., Jacques, P. F., Botsom, A. G., D'agostino, R. B., Wilson, P. W. F., Belanger, A. J., O'Leary, D. H., Wolf, P. A., Rush, D., Schaefer, E. J. & Rosenberg, I. H. (1996).** *Relationship between plasma homocysteine, vitamin status and extracranial carotid-artery stenosis in the Framingham study population.* *J. Nutr.* 126: 1258S-1265S.
- Sherwood, T. A., R. L. Alphin, W. W. Saylor, H. B. White (1993).** *Folate metabolism and deposition in eggs by laying hens.* *Archives of Biochemistry and Biophysics.* 307: 66-72.
- Singla, A., S. Kaushik, J. Kaur (2006).** *Folate deficiency results in alteration in intestinal brush border membrane composition and enzyme activities in weanling rats.* *Journal of Nutritional Science and Vitaminology*, 52, 163-167.
- Song, W. O., J. M. Kerver (2000).** *Nutritional contribution of eggs to American diets.* *Journal of American College of Nutrition.* 19. 556S-562S.
- Stabler, S. P., and R. H. Allen (2004).** *Vitamin B12 deficiency as a worldwide problem.* *Annual Review of Nutrition*, 24, 299 - 326.
- Surai, P. F., and N. H. C. Sparks (2001).** *Designer eggs: from improvement of egg composition to functional food,* *Trends in Food Science and Technology*, 12, 7-16.

- Tapiero, H., K. D. Tew, L. Gate, and D. Machover (2001).** *Prevention of pathologies associated with oxidative stress and dietary intake deficiencies: folate deficiency and requirements. Biomed. Pharmacother. 55: 381-390.*
- Troen, A. M., B. Mitchell, B. Sorensen (2006).** *Unmetabolized folic acid in plasma is associated with reduced natural killer cell cytotoxicity among postmenopausal women. J. Nutr. 136:189-94.*
- Van der Zipp, A. J., K. Frankena, J. Boneschancher and M. G. B. Nieumland (1983).** *Genetic analysis of primary and secondary immune response in the chicken. Poult. Sci., 62: 565-572.*
- Ventura, P., R. Panini, S. Tremosini, G. Salvioli, (2004).** *A role for homocysteine increase in haemolysis of megaloblastic anaemias due to vitamin B₁₂ and folate deficiency: results from an in vitro experience. Biochimica et Biophysica Acta/ Proteins and Proteomics, 1739, 33-42*
- Well, R. G. (1968).** *The measurement of certain egg quality. A study of the hens egg. T.C. Carter (Ed.). Published by Oliver and Boy Edinbrugh, pp: 220, 226 and 235-236.*
- Zollner, N., and K. Kirsch (1962).** *A colorimetric method to determine total lipid. Z. Ges. Exp. Med. 135:545*

الملخص العربي

تأثير إضافة حمض الفوليك في أعلاف الدجاج البيض على الأداء الإنتاجي وصفات المناعة

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تم تصميم دراسة لتحديد مدى استجابة ١١٢ دجاجة من سلالة الهاي سكس البنية على عمر ٢٨ اسبوع (ن : ٢٨ / عليقة) بالتغذية على اعلاف تتكون من الذرة الصفراء تحتوي على ١٠ ، ٠ ، ٢٠ أو ٣٠ ملجم/كجم من حمض الفوليك لمدة ١٢ أسبوعا. شملت القياسات الصفات الإنتاجية، مقاييس فسيولوجيا بالدم، ومستوى الفولات في سيرم الدم، وكذلك قياسات جودة البيض. لم تؤثر التغذية على حمض الفوليك بشكل ملحوظ على صفات الإنتاجية بما في ذلك وزن الجسم، إنتاج البيض، استهلاك العلف، الكفاءة التحويلية للعلف، كتلة البيض، وكذلك جودة البيض. لوحظ زيادة معنوية ($P \leq 0.05$) في مؤشر الصفار، مستوى الفولات في السيرم ، مقابل ٤٧.٧ ١٦.٧ ناتوجرام/ مل (٣٠ ملجم/كجم مقابل الكنترول؛ الاسبوع ٤) ، مقابل ٤٨.٠٠ ١٧.٧ ناتوجرام/ مل (٣٠ ملجم/كجم مقابل الكنترول؛ الاسبوع ١٢)، والكولسترول ١٧٤.٦٢ (كنترول) مقابل ١٥١.٩٢ ملجم/ ديسيلتر (٢٠ جم/كجم، البروتين الكلي ٥.٥٨ مقابل ٥.١٧ ملجم/ ديسيلتر (٢٠ ملجم/كجم مقابل الكنترول ؛ فترة النقاهة) والجلوبيولين ٤.١٦ مقابل ٣.٧٢ (٣٠ ملجم/كجم (١٠ ملجم/كجم؛ فترة النقاهة) وذلك مع زيادة حمض الفوليك في الأعلاف. وأظهرتقسيم خلايا كرات الدم البيضاء زيادة في heterophills ($P \leq 0.05$) بالنسبة للمعاملة ٣٠ ملجم / كجم من العلف (٢٠.٦٧ ملجم/ ديسيلتر) مقارنة بالمعاملات الأخرى وخاصة مجموعة الكنترول (١٨.٦٧ ملجم/ ديسيلتر)، على التوالي. بشكل عام، فإن مؤشرات الإنتاجية للدجاج البيض لم تتأثر سلبيا بالمعاملات الغذائية المستخدمة ، في حين ارتفع محتوى سيرم الدم من حمض الفوليك بزيادة نسبة الحامض في العلف بدأ من ١٠ مل / كجم عليقة وأعلى مع امكانية ترسيب حمض الفوليك في صفار البيض.