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Original Research Article

Laying hen performance, egg quality improved and yolk 5-methyltetrahydrofolate content increased by dietary supplementation of folic acid

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ABSTRACT

This study was performed to evaluate the effect of folic acid (FA) on performance, egg quality and yolk 5-methyltetrahydrofolate (5-MTHF) content. A total of 384 Hy-line W36 strain hens from 52 to 58 weeks of age were randomly assigned to 4 groups, and each group received one of following dietary treatments: 0, 5, 10 and 15 mg FA/kg diet. A completely randomized design was used. Egg production percentage, egg mass and egg weight were increased significantly ($P < 0.05$) and feed conversion ratio (FCR) was reduced significantly ($P < 0.05$) by increasing FA content in diets. No significant differences were detected among treatments on egg quality except for shell thickness. The dietary supplementations of laying hens diets with FA significantly increased yolk 5-MTHF content ($P = 0.02$). Overall, these data demonstrate that dietary supplementation with FA raised 5-MTHF content of yolk and productivity of egg production in laying hens.

1. Introduction

Folates have 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). Folate is an important vitamin among the B vitamins participated in one-carbon transfer reactions which is required within the cell for purine and pyrimidine biosynthesis (DNA and RNA) and inter conversion of amino acids, such as serine and glycine, and for the synthesis of methionine from homocysteine (Bagley and Shane, 2005). 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., 2009). From 1991 to 1999, some studies began on micronutrient deficiency in Iran. From 2001, flour fortification with iron and folic acid (FA) started locally in different provinces of Iran. Results showed that fortification of flour with FA prevented nearly 2,388 cases of NTD in Iran per year (Bell and Oakley, 2006; Mahboob et al., 2006). It is possible to significantly increase folate content of eggs through fortification of the laying hen diet with synthetic crystalline FA, and table eggs can be changed to one of the rich sources of natural folate (Sherwood et al., 1993; House et al., 2002; Hebert et al., 2005; Hoey et al., 2009; Tactacan et al., 2010; Dickson et al., 2010). The folate requirement of laying hens was reported by the National Research Council (1994), which was 0.25 mg FA/kg of diet. 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 increasing dietary methionine and FA in diet improved feed conversion ratio (FCR) and hen day egg production. However, House et al. (2002) and Hebert et al. (2005) reported that FA had no effect on the production of laying poultry.

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More than 80% of the folate in eggs is 5-methyltetrahydrofolate (5-MTHF). It exists mainly in the monoglutamate form (McKillop, 2003). Nearly 95% of egg folate is found in yolk (Sherwood et al., 1993). Hebert et al. (2011) reported that the type of dietary cereal may influence the deposition of folate in eggs. One of the important strategies to promote health in society is enrichment of egg with FA to prevent relevant folate deficiency diseases in human.

Because of improved production traits in new strains of poultry, it seems the 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, it is necessary to conduct some relevant examinations. The aim of current study was to detect the effect of fortified diets with FA on layer poultry production performance, egg quality and yolk 5-MTHF content.

2. Materials and methods

2.1. General

The bird procedures were according to the 2006 Guidelines on Animal Care of Agriculture Organization of East Azerbaijan, Tabriz, Iran. A total of 384 commercial laying hens during 6 weeks (white leghorn Hy-line W36; 52 weeks of age) were fed 4 levels of FA (0, 5, 10, 15 mg/kg of diet) added to the basal diet (Table 1). Experiment was conducted in an industrial farm. Each experimental group consisted of 8 replications. Three adjacent cages with 4 hens per cage were used as an experimental unit. Hens received 16 h/d of artificial lighting and ventilation at a natural ambient temperature. Feed and water were provided *ad libitum* throughout the experiment. Feed consumption was recorded weekly throughout the entire experimental period. Feed efficiency was calculated as grams of feed consumed per gram of egg mass produced. Egg production was recorded daily and calculated as of hen day egg production percentage (HDEPP). Eggs laid in each week were weighed to give an average egg weight. After 6 weeks, 8 eggs were randomly selected from each dietary treatment to evaluate egg internal and external quality and 2 eggs were selected from each treatment. Their yolks were separated, frozen and stored at −20 °C until analysis of 5-MTHF content.

2.2. Experimental diets

A corn-soybean meal basal diet was formulated according to nutrient requirements of National Research Council (1994) (Table 1). Experimental diets were obtained by supplementing the basal diet with synthetic FA at 4 levels (0, 5, 10, 15 mg/kg of diet). Folic acid was purchased from the representative of Lohman animal health company in Iran with a trade name of CUXAVIT FOLIC ACID. The purity was at least 95% (as dry matter).

2.3. Chemicals for analysis

The analytical standard of 5-MTHF (Product Number: M0132, CAS Number: 68792-52-9, Formula: $C_{20}H_{23}N_7Na_2O_6$, Weight: 503.42 g/mol) as disodium salt of 5-MTHF with the highest available purity ≥88% (UV-vis) was purchased from Sigma (St. louis, MO, USA) and used without further purification. All of the chemicals and HPLC grade solvents were prepared by E. Merck (Darmstadt, Germany).

2.4. Yolk folate analysis

The analytical procedure for extraction and determination of the 5-MTHF content in the egg yolk was described by House et al. (2002). The yolks of egg samples were separated, weighed and stored in −20 °C until analysis.

Table 1
Ingredients and chemical composition of basal diet (%, as fed basis).

Ingredients	Content	Chemical composition[1]	Content
Yellow corn	43.03	ME, kcal/kg	2,700
Soybean meal (38%)	26.78	Protein	15.28
Wheat	15	Ca	4.27
Loma shell	5	Available P	0.45
Oyster shell	4.74	Sodium	0.16
Vegetable oil	2	Arg	0.95
Dicalcium phosphate	1	Lys	0.80
Bone meal	0.71	Thr	0.57
Wheat bran	0.7	Try	0.22
Salt	0.33	Met	0.35
Mineral premix[2]	0.25	Met + Cys	0.66
Vitamin premix[3]	0.25	Folate, mg/kg	1.06
DL-methionine	0.16	Zn, mg/kg	88
L-Lys·HCl	0.05		
Total	100		

[1] Refer to National Research Council (1994).
[2] Mineral premix supplied per kilogram of diet: 2.4 mg Cu, 0.34 mg I, 30 mg Fe, 29.76 mg Mn, 0.08 mg Se, 25.87 mg Zn.
[3] Vitamin premix supplied per kilogram of diet: 3,520 IU vitamin A, 0.59 mg vitamin B_1, 1.6 mg vitamin B_2, 13.86 mg niacin, 3.13 mg pantothonic acid, 1 mg vitamin B_6, 0.06 mg biotin, 80 mg choline, 0.004 mg vitamin B_{12}, 0.19 mg vitamin B_9, 1,000 IU vitamin D_3, 8.8 IU vitamin E, 0.88 mg vitamin K_3.

Separation, detection and quantitation of 5-MTHF content of egg yolk samples after extraction into an ascorbate buffer (pH 7.8) under nitrogen atmosphere were performed with an HPLC (Model: Aglient 1100 series, Agilent Technologies, Wilmington – DE, USA) fitted a C18 column (25 cm × 4.6 mm i.d.), controlled by Chemstation software, equipped with a diode array detector (DAD) and a 20-μL injection loop. An external standard curve was prepared by pure 5-MTHF, used for quantification of 5-MTHF content in egg samples. The laboratory measurements about 5-MTHF were done in the research department of chromatography, Iranian Academic Center for Education, Culture and Research (ACECR), Urmia, Iran.

2.5. Egg quality assessments

The height of yolk and albumen was measured using a Japanese tripod altimeter OSK-3470 with an accuracy of 0.01 mm. The Haugh unit was calculated according to the formula described by Haugh (1937). Egg shell strength (kg/cm^2) was measured using an egg shell strength measuring device (Japanese model OSK-13473). Egg shell thickness was evaluated using a micrometer with an accuracy of 0.01 mm (model F.K.H) in the middle of the lumbar region of shells.

2.6. Statistical analysis

A completely randomized design with 4 levels of FA (0, 5, 10, 15 mg/kg of diet) was used. Production performance, egg quality and yolk of folate contents data were subjected to using the PROC GLM procedure of SAS software. Data were log-transformed before analyzing in case of unequal variances. Means were compared using Duncan's multiple range test at $\alpha = 0.05$.

3. Results

3.1. Laying hen performance

Effect of dietary FA on mean values of laying hen performance is shown in Table 2. Hen day egg production percentage was significantly improved by dietary FA supplementation at all levels ($P < 0.05$), but there was no significant difference among FA treatments ($P > 0.05$). Feed consumption was not significantly affected by FA supplementation at all levels ($P > 0.05$). Feed conversion ratio was

Table 2
Effect of dietary folic acid (FA) levels on laying hen performance ($n = 8$).

Item	Feed conversion ratio	Hen day egg production percentage, %	Feed consumption, g/(hen·d)	Egg mass, g	Egg weight, g
0 mg/kg FA	2.02[a]	80.47[a]	89	41.46[a]	49.08[a]
5 mg/kg FA	1.78[b]	84.23[b]	88	42.10[a]	49.98[a]
10 mg/kg FA	1.77[b]	84.29[b]	89.91	42.84[b]	50.43[b]
15 mg/kg FA	1.76[b]	84.64[b]	88.75	42.68[b]	50.81[b]
SEM	0.043	0.27	1.83	1.03	1.25

[a, b] Means within each column with different superscripts are significantly different ($P < 0.05$).

considerably lower for FA treatments than for non-FA treatments ($P < 0.05$). Egg mass was significantly affected by dietary FA supplementation ($P < 0.05$). It was around 1 g more for FA treatments than for the non-FA treatment. Egg weight was significantly improved by FA supplementation at 10 and 15 mg/kg ($P < 0.05$).

3.2. Egg quality: internal and external egg quality traits

No significant differences were detected on egg Haugh unit, albumen and yolk pH, and yolk index because of dietary FA supplementation (Table 3). Shell thickness was reduced significantly in the FA group of 10 mg/kg compared with the non-FA group ($P < 0.05$). Shell strength, shape index and egg shell weight were not significantly affected because of dietary FA supplementation. Shape index was significantly increased ($P < 0.05$) by FA supplementation at 5 and 15 mg/kg (Table 3).

3.3. 5-MTHF content of yolk

The results from egg folate analysis showed that eggs has a good capacity to be enriched with 5-MTHF by supplementation of laying poultry diet with FA. Folate content of eggs is shown in 3 forms (μg/egg, μg/100 g egg and μg/100 g yolk) (Table 4). The 5-MTHF content in yolks was strongly enhanced by supplementation of the laying hen diet with FA ($P = 0.02$). In this experiment, the 5-MTHF content of enriched eggs was raised nearly up to 24 folds by dietary FA supplementation at 15 mg/kg compared with the non-FAgroup.

4. Discussion

Effect of dietary FA in different levels enhanced laying hens performance traits (Table 2). These results showed FA supplementation that was higher than the recommended from National Research Council (1994) enhanced the productivity of laying hens. Islam et al. (2009) observed that increasing dietary methionine and FA in diets improved FCR and HDEPP. Dickson et al. (2010) reported that the supplementation at 4 mg FA/kg diet improved feed efficiency during the entire production cycle of Hy-line W36, Hy-line W98 and CV20 laying hens. But House et al. (2002) and Hebert et al. (2005) reported that FA had no effect on the overall performance of laying hens.

Folate is required for reactions of one-carbon transfer involving to help maintain normal plasma homocysteine and remethylate it to methionine (Pillai et al., 2006). In a different study, Tactacan et al. (2010) reported plasma homocysteine was significantly reduced and serum folate was increased in laying hen bloods when the diet of laying hens was supplemented with FA (10 mg/kg diet) and 5-MTHF (11.3 mg/kg diet) for 3 weeks. They also observed larger eggs were produced by dietary supplementation with 5-MTHF compared with dietary supplementation with FA and control diet. In another research, plasma homocysteine was significantly reduced when laying poultry diet was fortified with FA (Hebert et al., 2005).

The folate requirement recommended from National Research Council (1994) for poultry goes back to the last decades. Modern laying hen strains need more folate because of their improved FCR and production traits in a short period of time. The results reported that at this experiment it seems related to high dietary FA. Furthermore, folates reduce homocysteine and increase methionine, which is a primarily limiting amino acid in diets based on corn-soybean meal.

Haugh unit is often referred as an albumen quality (Silversides and Villeneuve, 1994). It has not been majorly affected by nutrition in a specific case. Important factors to determine albumen height is egg storage time. Effect of storage on egg quality can also be measured by measuring the increase of albumen pH (Monsey et al., 1997). Zang et al. (2011) mentioned that different dietary vitamin levels do not have effect in egg quality parameters such as egg shape index, egg shell gravity, Haugh unit and egg shell thickness, but vitamin supplementation significantly reduces the crashed egg percentage, dirty egg percentage and egg shell strength. These findings were opposite result series of examinations indicating that reducing dietary methionine, FA, vitamin B_{12} and choline reduced egg size and improved shell quality (Keshavarz, 2003). Generally dietary FA significantly reduced shell thickness in all supplemented levels, but shell strength was not reduced. The related mechanism is not clear. Shape index was significantly increased in groups supplemented with FA at 5 and 15 mg/kg in diets. How FA has effects on shape index is not clear.

The 5-MTHF content of yolks was strongly enhanced by supplementation of laying hens diet with FA. Our findings confirmed other authors who reported fortification of laying hen diets significantly increased the folate content of eggs (Sherwood et al., 1993; House et al., 2002; Hebert et al., 2005; Tactacan et al., 2010; Dickson et al., 2010).

Eggs have a saturation characteristic for deposition of folate into yolk. Higher levels of FA supplementation did not show the maximum level of egg folate. (Sherwood et al., 1993; House et al., 2002; Hebert et al., 2005). Hebert et al. (2005) observed the same pattern in blood folate content which showed saturation nature when laying hens were fed diets high in FA. So due to the saturation of blood which serves as the precursor for egg folate deposition, identification of the points which has an important role in folate absorption from alimentary system such as enzymes (polyglutamylfolate deconjugation) and intestinal pH is essential. Tactacan et al. (2011) demonstrated a FA transport system in the entire intestine of hens. Maximum uptake rate of FA was observed at acidic pH 6.0 and was increased in the duodenum and jejunum and decreased in the ileum and ceca.

In our study, 5-MTHF content in non-enriched eggs was very low (2.3 μg/egg), opposite to 17.5, 16.7, 28.2 and 15.3 μg/egg reported by House et al. (2002), Hebert et al. (2005), Tactacan et al. (2010), and Dickson et al. (2010), respectively. They claimed that they enriched eggs 2 to 3 folds with FA compared with regular eggs, but in our research we enriched eggs nearly 24 folds compared with the usual eggs. An enriched egg nearly weighed 60 g and contained 54 μg natural folate. It can provide 13.5%, 36% and 9% of the folate recommended daily allowance for adults (400 μg DFE/d), infant (150 μg DFE/d) and pregnant women (600 μg DFE/d), respectively. One microgram food folate or 0.6 μg synthetic FA taken with food or 0.5 μg synthetic FA taken on an empty stomach equals to one dietary folate equivalent (DFE).

Table 3
Effect of dietary folic acid (FA) levels on egg quality ($n = 8$).

Item	Egg shell weight, g	Egg shell strength, g/cm^2	Haugh unit	Yolk index	Shape index	Yolk pH	Albumen pH	Egg shell thickness, mm
0 mg/kg FA	5.93	0.78	74.02	43.577	74.09^a	5.94	9.10	0.303^a
5 mg/kg FA	5.85	0.94	75.23	50.275	74.66^b	5.92	9.09	0.304^a
10 mg/kg FA	5.61	0.72	76.55	50.292	74.09^a	5.92	9.14	0.27^b
15 mg/kg FA	5.74	0.95	74.51	50.782	74.66^b	5.93	9.14	0.29^{ab}
SEM	0.09	0.1	1.76	0.82	0.159	0.005	0.03	0.007

$^{a, b}$Means within each column with different superscripts are significantly different ($P < 0.05$).

Table 4
Effect of dietary folic acid (FA) levels on folate content of eggs ($n = 8$).

Item	5-MTHF, μg/egg	5-MTHF, μg/100 g egg	5-MTHF, μg/100 g yolk
0 mg/kg FA	2.3^a	3.7^a	11.9^a
5 mg/kg FA	23.8^{ab}	37.9^{ab}	127.9^{ab}
10 mg/kg FA	48.5^b	79.6^{bc}	252.4^b
15 mg/kg FA	54.5^b	88.8^c	286.6^b
SEM	8.65	13.63	44.27

$^{a, b}$Means within each column with different superscripts are significantly different ($P < 0.05$).

Specific advantages of natural 5-MTHF over synthetic FA that have been declared recently include the following: 1) well absorption even when gastrointestinal pH is altered; 2) the bioavailability is not diminished by defects of metabolic; 3) it does not mask the hematological symptoms of vitamin B_{12} deficiency; 4) it reduces interaction with drugs that inhibit dihydrofolate reductase and overcomes the defects produced by methylenetetrahydrofolate reductase polymorphism. According to Scaglione and Giscardo (2014), 5-MTHF also reduces the negative effects of nonmetabolizable FA in the peripheral circulation.

5. Conclusion

In conclusion, results of the present study demonstrated that hens receiving supplemented diets with FA not only presented a high percentage of egg production and a less FCR but also could produce eggs with the good source of natural folate (5-MTHF), which has more advantages than crystalline FA on human health.

Conflicts of interest

The authors report no conflicts of interest. The authors by themselves are responsible for the writing and content of the text.

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