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EFFECT OF FOLIC ACID ADDITION INTO FEED MIXTURE OF LAYING HENS ON ITS CONTENT IN EGGS

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ABSTRACT

Experiment was conducted on two hen lines according to egg type marked as SH-OL and SH-ML. The feed mixtures were enriched for folic acid. The content of folic acid was estimated by chemical analysis at 0.5 mg/kg in control feed mixture (CFM), at 1.5 mg/kg in first experimental group (FM E1) and at 3.5 mg/kg of feed mixture in the second experimental group (FM E2). Analysis of eggs showed higher content of folic acid in the experimental group E1 (0.06 – 0.11 mg/kg of egg melange), the highest content was in the experimental group E2 (0.10-0.16 mg/kg of egg melange) in comparison with control (0.05 mg/kg of egg melange). The highest content of folic acid was found in mother line (SH-ML) eggs in the E2 group where the repeated analysis showed values of 0.11 and 0.16 mg of folic acid/kg melange. In father line (SH-OL) in the E2 group we found 0.10 and 0.11mg/ kg content of folic acid. The results show that in the E2 group the content of folic acid in both lines was more than twice higher compared to control. Therefore, it is possible to use the folic acid- enriched hen eggs as health beneficial food for human nutrition.

Key words: folic acid, enriched eggs, egg quality

INTRODUCTION

Folic acid (B9 vitamin) is essential for cell division in body function and therefore very important for embryo development. Consequences of folic acid deficiency were described by Labuda (1971), who reported that avitaminosis inhibits synthesis of purine bases what may have adverse effect on tissue metabolism. The requirement of all animal species in folic acid, with exception of poultry, is fulfilled through intestinal synthesis. Avitaminosis inhibits growth, deteriorates feathering of chickens, decreases hatchability and liveability. The folic acid, although has no beneficial effect on embryo development, however lowers homocysteine level in the blood and, therefore reduces a risk of cardiovascular diseases in human population. In hospitalized patients of higher age groups a correlation between deficient plasma level of folic acid and increased level of homocysteine was found (Boushey et. al., 1995; Rasmussen et al., 2000; Rader, 2002).

The high level of homocysteine may impair also blood vessels and cause formation of sediments. Therefore, folic acid is important tool against cardiovascular diseases. Also people with depression often have a deficiency in folic acid. Preventive role of folic acid against Alzheimer disease is also assumed. Folic acid lowers also a risk of polyps and colon carcinoma, ulcer colitis and Crohn disease (Rohan et al., 2000). Preventive dose for embryos in healthy population is 400–800 µg /day. Recommended dose for risk pregnancy is a 10-fold higher i.e. 5 mg /day. The common foods rich for folic acid could not assure daily intake of 400 µg per day. In natural products folic acid is contained in folate form, but their biological efficiency is lower than 30-50 %. This is caused by storage, cooking, and also by non-absorption of polyglutamic form and this form must be degraded into mono- glutamic form. Therefore, fortified foods with folic acid in monoglutamic form are reasonable because are absorbed without previous enzymatic change. In national health programs of USA, Canada and the Great

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Britain a support for folic acid uptake during pregnancy is appreciable. In USA and Canada since 1998 folic acid is compulsory added to meal in dose of 140 or 150 µg per kg of meal. Similarly, in Hungary folic acid is willingly added to the yeast at the production of bread (Czeizel, 2002). But the folic acid content in the food should be appropriately marked.

Effect of folic acid addition to the feed mixture on the folate content in egg was studied by House, (2002); Hebert et al. (2005). They found significant effect of crystalline folic acid addition at 0 – 128 mg/kg level into feed mixture for two egg type Hyline hen lines (W 98 and W 36) on increase of folate in eggs and also in plasma; hereby the homocysteine concentration was decreased. The content of folate increased after folic acid addition from 2mg/kg and more. Significant increase in the folate content was observed also in eggs of W 98 line; also the egg quality parameters were better in W 98 line in comparison with W 36 line. Caffola et al. (2002) analyzed effect of folic acid and vitamin C addition on blood levels of folate and homocysteine in smokers. Arockiam et al. (2005) studied interaction between vitamin C and folic acid on growth parameters and behavior of broiler chickens. At various levels of vitamin C (300 mg/kg) and folic acid (25 mg/kg) addition, they recorded higher body mass, growth intensity, food conversion and lower mobility in chickens. The lowest body mass was measured in chickens with feed addition 5mg/kg of folic acid and 300 mg/kg of vitamin C. Temporal changes in biochemical indices of sulfur amino acid (SAA) metabolism in the folate deficient piglet were described Zhang and House, (2007).

The purpose of this experiment was to study the effect of folic acid addition into feed mixture of laying hens on its content in eggs.

MATERIAL AND METHODS

The experiment was performed in a detached branch of Animal Production Research Centre (APRC) at Ivanka pri Dunaji on egg type laying hens marked as SH-OL line (father line) and SH-ML line (mother line). The hens were housed in windowless brick building in two flat individual cages. The hens were divided according to lines and to feed mixture into 6 groups. The first 3 groups were hens of SH-OL line and the others 3 groups were hens of SH-ML line. Each line was divided into one control group (C) and two experimental groups: E1 with addition of 4,5 mg folic acid/kg feed mixture, E2 with addition of 8,46 mg folic acid/kg feed mixture. In each group 20 experimental hens were housed. In the building a light regime was regulated, i.e. 14 hour light and 10 hours dark. The feed mixtures were produced and analyzed by the feed mixture manufacturer Agrorama Ltd., (Šaľa, Slovak Republic).

The hens were fed with these feed mixtures *ad libitum* during 36 days egg of laying period. During this time we recorded an individual egg production and egg weight of each hen. After ending of the experiment the samples of individual feed mixtures and eggs were analyzed for folic acid concentration.

After this time period the folic acid content was determined in feed mixtures and in laid eggs from control and experimental groups. The analysis was carried out by accredited company EL Ltd. (Spišská Nová Ves, Slovak Republic). Each sample for analysis was a mixture of 20 eggs. From melange an average sample was prepared and two analyses were done from each sample. The determination of folic acid in feed mixture as well as in eggs was done in EL Ltd (Spišská Nová Ves) by high performance liquid chromatography with diode field (HPLC/DAD), VARIAN STAR. Except chemical analysis of folic acid, we evaluated also egg production and physical parameters of both controls and experimental groups. Physical parameters were estimated also on 20 eggs from each analyzed group. We evaluated also individual egg production (n), laying intensity (%), egg weight (g), yolk weight (g), albumen weight (g), egg shell weight (g) and index of egg shape in %. Yolk color intensity was evaluated using Hoffman-La Roche color spectrum in °HLR. Differences in egg production and physical parameters of eggs between two experimental lines were estimated by one-way analysis of variance and t-test.

RESULTS AND DISCUSSION

In our experiment egg-type hens, marked as SH-OL and SH-ML, were fed with feed mixtures. Basic

Table 1: Compostition of the feed mixture for layers (HYD-10)

Raw materials	% of raw materials in feed mixture		
	CFM (control)	FM E1 (exp. 1)	FM E2 (exp.2)
Wheat 11	35,00	34,30	33,20
Maize 7.5	28,10	28,00	28,00
Soda	0,10	0,10	0,10
Soya exctract	23,30	23,30	23,30
Colza oil	2,00	2,00	2,00
L-lysine HCL	0,05	0,05	0,05
DL-methionine 99	0,17	0,17	0,17
L-threonine 98	0,09	0,09	0,09
MCP 22.7 revised	1,20	1,20	1,20
Feed calcite	9,14	9,14	9,14
Sodium chloride	0,03	0,03	0,03
Kemzyme WP New	0,05	0,05	0,05
PX of folic acid	0,20	1,00	2,10
Amivit SK 0.3 % pigment	0,30	0,30	0,30

components of the mixtures were wheat, maize and soybean extracted scrap. The proportion of raw materials is shown in Table 1.

The standard feed mixture (CFM) contained 0.20 % folic acid, whilst the experimental feed mixture E1 (FM E1) contained 1 % and feed mixture E2 (FM E2) contained 2.1 % of folic acid provided from premix Aminovitan.

Tables 2-4 show the content of tracked nutrients. Metabolic energy varied in individual FM in range 11.324-11.572 MJ, the content of nitrogenous substances was in range 165.909 – 168.043/ kg of feed mixture. Also content of most important amino acids: lysine, methionine, cysteine, threonine, trypthopan, linoleic acid, and also the most important mineral elements like calcium, phosphorus, sodium, iron, zinc, manganese and vitamins A, D and E was determined.

The addition of folic acid into CFM was 1.2 mg and through raw materials - 0.4 mg, in FM E1 - 4.2 mg through premix and 0.3 mg through raw materials. In FM E2, folic acid addition through a premix was 8.1 mg and through raw materials - 0.3 mg/kg of feed mixture. Analysis of folic acid content in feed mixture and in eggs was performed in EL Ltd Spišská Nová Ves. The values are shown in tables 5 and 6.

According to this analysis the content of folic acid in CFM was 0.5 mg/kg and in FM E1 1.05 mg/kg and FM E2 - 3.5 mg/kg.

We found higher content of folic acid in the experimental group in comparison to control ones; the highest content was in E2 group. Overall, a highest content of folic acid was revealed in mother line (SH-ML) from the second experimental group (E2), where repeated analysis documented values of 0.11 and 0.16 mg/kg of folic acid. At father line (SH-OL) in the second group (E2) the content of folic acid was 0.10 and 0.11 mg/kg.

Our results correspond with those of Haus (2002) and Herbert (2005), who reported that the content of folic acid in eggs can be influenced by line and that the increase of folic acid in feed mixture has a positive effect on the folic acid content in eggs.

In E2 experiment the content of folic acid in eggs of both lines was a twice as much higher than in control. Concerning the egg production (Tab.7), the hen's 36-day egg production was 31.8 – 33.11 eggs; the laying intensity in SH-OL line was highest in E2 group (91.18 %). In SH-ML line laying intensity was highest in control group (92.05 %). Differences between analyzed groups were not statistically significant. The egg shape

Table 2: Content of spotted nutritives in 1 kg of control mixture (CFM)

	Unit	Calculation	Minimum	Maximum
ME_N	MJ	11,572	11,60	12,25
NL	g	168,043	168,00	
LYSINE	g	8,510	8,50	
METHIONINE	g	4,213	4,20	
MET+CYS	g	7,211	6,90	
THREONINE	g	6,837	6,80	
TRYPTOPHANE	g	2,079	1,70	
FOLIC ACID	g	14,414	12,50	
CALCIUM	g	37,736	38,00	39,00
P total	g	6,915	1,60	
P unphyt.	g	4,016	50,00	
Sodium	g	1,673	50,00	
Iron	mg	134,443	70,00	
Zinc	mg	99,941	10,00	
Manganese	mg	119,385	2,00	
Vit. A	kilo m.j.	12,617	15,00	
Vit. D	kilo m.j.	2,700		
Vit. E	mg	29,762		
Content of other nutrients in 1 kg				
Folic acid total	mg	1,63	0,50	
Per px	mg	1,2	6,40	
Per raw materials	mg	0,4	4,00	

Table 3: Content of spotted nutritives in 1 kg of experimental mixture E1 (FM E1)

	Unit	Calculation	Minimum	Maximum
ME_N	MJ	11,468	11,60	12,25
NL	g	167,167	168,00	
LYSINE	g	8,486	8,50	
METHIONINE	g	4,198	4,20	
MET+CYS	g	7,178	6,90	
THREONINE	g	6,811	6,80	
TRYPTOPHANE	g	2,068	1,70	
FOLIC ACID	g	14,319	12,50	
CALCIUM	g	37,732	38,00	39,00
P total	g	6,885	6,40	
P unphyt.	g	4,007	4,00	
Sodium	g	1,672	1,60	
Iron	mg	134,093	50,00	
Zinc	mg	99,722	50,00	
Manganese	mg	119,168	70,00	
Vit. A	kilo m.j.	12,590	10,00	
Vit. D	kilo m.j.	2,700	2,00	
Vit. E	mg	29,654	15,00	
Content of other nutrients in 1 kg				
Folic acid total	mg	4,508	0,50	
Per px	mg	4,2		
Per raw materials	mg	0,3		

Table 4: Content of spotted nutritives in 1 kg of experimental mixture E2 (FM E2)

	Unit	Calculation	Minimum	Maximum
ME_N	MJ	11,324	11,60	12,25
NL	g	165,909	168,00	
LYSINE	g	8,451	8,50	
METHIONINE	g	4,178	4,20	
MET+CYS	g	7,130	6,90	
THREONINE	g	6,774	6,80	
TRYPTOPHANE	g	2,053	1,70	
FOLIC ACID	g	14,206	12,50	
CALCIUM	g	37,725	38,00	39,00
P total	g	6,843	6,40	
P unphyt.	g	3,995	4,00	
Sodium	g	1,670	1,60	
Iron	mg	133,598	50,00	
Zinc	mg	99,407	50,00	
Manganese	mg	118,842	70,00	
Vit. A	kilo m.j.	12,548	10,00	
Vit. D	kilo m.j.	2,700	2,00	
Vit. E	mg	29,503	15,00	
Content of other nutrients in 1 kg				
Folic acid total	mg	8,465	0,50	
Per px	mg	8,1		
Per raw materials	mg	0,3		

index varied at father line between 74.26 – 76.39 %, at mother line - between 73.25 – 74.39 %. At SH -OL line the egg weight, yolk weight and albumen weight in E2 group were insignificantly highest (66.65g, 19.16 g, resp. 40.05 g); the egg shell weight was significantly higher also in the E2 group.

At SH-ML line the differences of egg quality parameters between E1 and E2 group were not of the same tendency as in line SH-OL. The yolk color estimated by Hoffman-La Roche spectrum was most intensive at SH-OL and SH-ML lines in the E2 group (11.70 – 11.50 resp.), but the differences between groups were statistically insignificant. Similar results were reported also by Herbert et al. (2005), who found different values of these parameters in two lines of Hylines hens.

In conclusion, we can state, that hen's eggs can be used, as fortified food enriched for folic acid, for human nutrition.

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Table 5: Results of feed mixture analysis on folic acid content in time of eggs gathering

Sample	Unit	Result of test	Uncertainty of measure	Examination method	Estimation interface	Methodical formula	Type of test
CFM	mg/kg	0,5	25%	HPLC/DAD	0,1	IP 4,14	A
FM E1	mg/kg	1,5	15%	HPLC/DAD	0,1	IP 4,14	A
FM E2	mg/kg	3,5	15%	HPLC/DAD	0,1	IP 4,14	A

Table 6: Content of folic acid in hens eggs from two hen lines

Line	Unit	Sample	Result of test	Uncertainty of measure	Examination method	Estimation interface	Methodical formula	Type of test
Control	mg/kg	1.	0,05	25%	HPLC/DAD	0,05	IP 4.14	A
SH-OL	mg/kg	2.	0,05	25%	HPLC/DAD	0,05	IP 4.14	A
E1	mg/kg	1.	0,06	25%	HPLC/DAD	0,05	IP 4.14	A
SH-OL	mg/kg	2.	0,07	25%	HPLC/DAD	0,05	IP 4.14	A
E2	mg/kg	1.	0,11	25%	HPLC/DAD	0,05	IP 4.14	A
SH-OL	mg/kg	2.	0,10	25%	HPLC/DAD	0,05	IP 4.14	A
Control	mg/kg	1.	0,07	25%	HPLC/DAD	0,05	IP 4.14	A
SH-ML	mg/kg	2.	0,07	25%	HPLC/DAD	0,05	IP 4.14	A
E1	mg/kg	1.	0,09	25%	HPLC/DAD	0,05	IP 4.14	A
SH-ML	mg/kg	2.	0,11	25%	HPLC/DAD	0,05	IP 4.14	A
E2	mg/kg	1.	0,11	25%	HPLC/DAD	0,05	IP 4.14	A
SH-ML	mg/kg	2.	0,16	25%	HPLC/DAD	0,05	IP 4.14	A

Table 7: Egg production and physical parameters of eggs of two experimental lines

Parameters	Line SH-OL			Line SH-ML				
	C	E1	E2	C	E1	E2	F-test	S
36-days egg prod. (number)	32,33	32,13	32,80	33,11	31,80	32,90	0,399	-
Laying intensity (%)	89,81	89,23	91,18	92,05	88,47	91,94	0,459	-
Egg shape index (%)	74,26	75,99	76,39	74,39	73,62	73,25	1,571	-
Egg weight (g)	64,56	62,85	66,65	64,72	63,16	64,01	0,619	-
Yolk weight (g)	18,14	17,93	19,16	18,93	18,22	18,27	0,968	-
Albumen weight (g)	39,34	37,92	40,05	39,09	38,55	38,98	0,256	-
Egg shell weight (g)	7,02	6,99	7,46	6,70	6,39	6,84	3,193	+
Yolk color (°HLR)	11,30	11,50	11,70	11,30	11,20	11,50	1,409	-

S - significance; +P< 0.05; -P< 0.05 = n.s

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