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Kaposvár University, Faculty of Agricultural and Environmental Sciences, Kaposvár

Effect of excess folic acid on egg production, fertility and hatchability in layer breeders

D. Terčič, M. Pestotnik
University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Groblje 3, Domžale, Slovenia

ABSTRACT

*Folic acid (**FA**) (also known as folate or vitamin B9) is essential for all tissues with a high rate of cellular division and growth and therefore very important in reproduction. The present study was planned to see the effect of FA supplementation on productive and reproductive performance of layer breeders. A total of 105 cocks of the sire line and 906 hens of the dam-line were randomly assigned to 4 floor pens in a deep litter trial and mated at a ratio of 1 cock : 8.6 hens. Two dietary treatments were used: an unsupplemented practical corn-soybean meal basal diet and the basal diet supplemented with 50 mg of FA/kg of diet. Egg production, feed intake, mortality, fertility, hatchability and related parameters were measured during 36 to 39 wk of age. Compared with birds fed control diet, excess FA supplementation reduced (P<0.0001) feed intake and percentage hen day egg production (P<0.05). Egg weight, fertility and hatchability were similar in the laying hens fed the two dietary treatments. 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. Percent mortality of layer breeders was unaffected by FA dietary treatment. Clearly, supplemental folic acid is not required to maximize layer breeders fertility and hatchability.*
(Key words: folic acid, layer breeders, fertility, hatchability)

INTRODUCTION

The beneficial effects of folic acid (FA) on reproduction in humans, polytocous species and in some livestock animals have been well documented. Studies 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. The second major achievement with the use of FA occurred in the 1990s when periconceptional FA supplementation was found to reduce both the recurrence and occurrence of neural tube defects such as spina bifida, in children *(Tamura and Picciano*, 2006*)*. It has been experimentaly demonstrated that FA is critical for embryo survival and fetal development in rats *(Tagbo and Hill*, 1977*)*, hamsters *(Moiij et al.*, 1993*)* and guinea pigs *(Habibzadeh et al.*, 1986*)*. In sows supplemental FA has been associated with increases of about 10% in litter size at parturition *(Matte et al.*, 2006*)*. 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 *(Méthot et al.*, 2008).

Several *observations suggest* that poultry reproduction might be influenced by dietary supplements of FA *(Taylor*, 1947; *Sunde et al.*, 1950; *Robel*, 1993*)*. The current estimated requirement for FA for laying hens, based on experiments conducted in the

1950s, is between 0.21 to 0.31 mg/kg (*NRC,* 1994). Various contradictory results on the influence of supplementing diets with excess of FA on the egg folate concentrations have been reported in laying hens. *Dickson et al.* (2010) reported that the addition of 4 mg of FA/kg of laying hen diet lead to an increase of approximately 3-fold in egg folate concentration relative to a regular commercial egg. *Hebert et al*. (2005) supplemented as much as 128 mg of FA/kg of the laying hen diet but did not find any significant increase in egg folate concentration beyond the saturation level achieved at 4 mg of FA/kg in the diet. However, contrary to these observations, *House et al.* (2002) observed that egg folate concentrations increased above a plateau value when the level of FA in the diet was 32 mg/kg of diet. *Naber and Squires* (1993) found that the transfer efficiency of FA from diet to egg is very low. Considering the results obtained in the latter two studies we hypothesized that excess dietary FA might increase the amount of FA deposited in the egg and consequently resulting in improved reproductive performance success. Research on this topic is scarce. The goal of our study was, therefore, to assess the effect of two practical chicken diets, with various FA concentrations on the reproductive parameters of layer breeders.

MATERIAL AND METHODS

Experiment was conducted at a poultry research station (Biotechnical Faculty, University of Ljubljana, Slovenia) and the animal care and use protocol was approved by the Animal Welfare Council of the same Faculty. 1011 parent stock chickens (906 hens of dam line and 105 roosters of sire line) of Slovenian provenance Barred Prelux were selected at 35 wk of age, randomly allocated to control (n = 473 hens + 55 roosters) and FA-treated groups (n = 433 hens + 50 roosters), and raised in floor pens covered with wood shavings in an environmentally controlled facility. Each experimental group was replicated 2 times. The control group was fed with the basal diet, and the FA group was fed with the basal diet supplemented with 50 mg of crystalline FA/kg of diet (Farmalabor s.r.l., Milano, Italy). 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. Composition and calculated nutrient content of the basal diet is given in *Table 1*. The basal diet contained 0.85 mg of total FA (from natural FA in feed ingredients) per kilogram of diet. Birds were fed with the experimental diets for a period of 4 wk (the first wk served as the adaptation period). Feed and water were available to permit ad libitum consumption. The photoperiod was fixed at 14h with illumination provided by fluorescent lights at an intensity range of 5 to 10 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 2 times at 10 d intervals. Before setting in the incubators eggs from the different treatments/replicates were labeled with a pen number, weighed and fumigated. A total of 11005 hatching eggs were used. The incubation was carried out in a single-stage electronically controlled setter, Petersime S168 (Petersime, Zulte, Belgium), at 37.8 °C and 60% RH. On day 18, the eggs were transferred to a hatcher, Petersime H168, with 37.2°C and 70% RH to complete incubation. After 21 d 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 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 (two hatches - two ages of layer breeders), replication (pen) 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 means (LSM) ± standard error of the mean (SE). Treatment means were separated using Tukey's test. Statistical significance was accepted at $P < 0.05$.

Table 1

Ingredient and calculated nutrient composition of standard layer basal diet

Ingredient	Percentage of ingredient
Maize (8.5% CP)	60.0
Soybean meal (49.5% CP)	17.0
Soybean oil	1.0
Sunflower meal (41.5% CP)	6.0
Maize gluten meal (60% CP)	1.0
Sugar beet molasses (10.1% CP)	3.0
Calcium carbonate	8.8
Calcium sodium phosphate	2.0
Mono-dicalcium phosphate	0.7
Sodium bicarbonate	0.2
Sodium chloride	0.2
Dietary supplements[1]	0.03
Calculated nutrient composition	
Metabolizable energy	2698.96 kcal / kg
Crude protein	16.2 %
Crude fibre	3.2 %
Crude fat	3.6 %
Crude ash	11.6 %
Lysine	0.75 %
Methionine	0.40 %
Calcium	3.50 %
Available phosphorus	0.50 %
Sodium	0.15 %
Folic acid	0.85 mg/kg

[1]Provided (per kg of diet): vitamin A, 10000 IU; vitamin D3, 2500 IU; 25 mg iron (from $FeSO_4 \times H_2O$); 6 mg copper (from $CuSO_4 \times 5H_2O$); 100 mg manganese (from MnO); 50 mg zinc (from $ZnSO_4 \times H_2O$); 0.3 mg cobalt (from $2CoCO_3 \times 3Co(OH)_2 \times H_2O$); 0.68 mg iodine (from KI); 0.15 mg selenium (from Na_2SeO_3); 5.4 mg butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT); 9.33 mg ethoxyquin; 1 mg **ethyl ester of ß-apo-8'-carotenic acid; 4 mg c**anthaxanthin; 10 mg lutein; 100 mg 6-phytase

RESULTS AND DISCUSSION

The supplementation of corn based diet with 50 mg crystalline FA did not affect egg weight before incubation (*Table 2*). These results agree with observations of *Hebert et al*. (2005) who researched two Leghorn strains of laying hens supplemented with 2, 4, 8, 16, 32, 64 and 128 mg/kg of FA and observed no difference in egg weight during a 3-wk experimental period. Similar results were also reported by *Tactacan et al*. (2012), who noted no effect on egg weight when Shaver White laying hens were fed diet supplemented with 10 or 100 mg of FA/kg of diet. Similar results were obtained by *Khalifah and Shahein* (2006), who determined that the inclusion of 0–32 mg FA/kg diet in the Baheij chicken strain did not affect the egg weight.

Table 2

The effects of folic acid (FA) supplementation of the diets of layer breeders on egg weight, one day old chick weight, hatchability, percentage of infertile eggs and dead-in-shells

Trait	Group	LSM ± SE	P-value
Egg weight (g)[1]	FA	61.32 ± 0.11	0.6486
	Control	61.25 ± 0.09	
Chick body weight at hatching (g)[2]	FA	40.62 ± 0.13	0.0223
	Control	40.19 ± 0.12	
Hatchability of total eggs (%)	FA	85.96 ± 0.56	0.6534
	Control	85.62 ± 0.51	
Hatchability of fertile eggs (%)	FA	89.16 ± 0.48	0.8084
	Control	89.00 ± 0.44	
Infertile eggs (%)	FA	3.58 ± 0.29	0.5707
	Control	3.80 ± 0.26	
Dead-in-shells (%)	FA	10.44 ± 0.46	0.8225
	Control	10.58 ± 0.41	
Hen-day egg production (%)	FA	88.98 ± 0.60	0.0352
	Control	90.84 ± 0.60	
Feed consumption (g/bird per day)	FA	136.16 ± 1.38	0.0001
	Control	150.11 ± 1.38	

[1] Number of eggs weighed and set in incubators: 4917 (FA group), 6088 (control group)
[2] Number of chicks hatched and weighed: 4218 (FA group), 5200 (control group)

Krishnan (2010) reported that the supplementation of 0, 2 and 4 ppm of dietary FA affected egg weight in 29-week-old Single Comb White Leghorn Bovan hens. The highest egg weight was observed at 0 ppm of FA supplementation compared to 2 and 4 ppm.

In the present study, supplementation of the maternal diet with FA increased BW at hatch by about 1% ($P<0.05$) compared with that of chicks from hens fed basal diet (*Table 2*). The reasons for this improvement are unclear. The fact that eggs from FA hens were not havier than eggs from control hens may indicate that the amount of water that was lost by diffusion through pores in the eggshell during the incubation process was higher in eggs from control groups in comparison with eggs from FA groups. However, in the present study, eggs were not weighed individually for the 0 to 18 d

incubational period to determine variation in egg weight loss among eggs. Data referring to chick weights are in agreement with the results reported in turkey hens by *Robel* (1993), who has shown that poult weights were increased when turkey hens received higher dietary FA (5.51 mg FA/kg of diet) and when eggs were injected with FA.

In the current experiment, the fertility and hatchability were not affected by the inclusion of FA in the diet (*Table 2*). The same outcome was observed in the studies with turkey breeders. *Schweigert et al.* (1947) did not observe any difference in the hatchability of turkey breeders when the FA content of the diet was increased from 0.42 mg of FA/kg to 2 mg of FA/kg. *Robel* (1993) reported that neither supplementing practical turkey breeder diets nor injecting 25-d embryonated eggs with FA improved hatchability.

In the analysis of nonhatched eggs, no differences in percentage of infertile and percentage of dead-in-shells were detected between treatments (*Table 2*). In this evaluation, nonhatched eggs were opened and classified through a macroscopic visual examination as infertile and consequently some eggs that were classified as infertile eggs may have been fertile eggs that contained a dead embryo.

The inclusion of FA in the diet reduced the rate of egg production and feed intake compared of hens fed the control diet (*Table 2*). Several studies have been conducted to examine the effects of supplemental dietary FA on the productive performance of the chickens and reported results are somewhat contradictory. *Tactacan et al.* (2012) showed that percentage hen-day egg production and feed consumption were similar in the laying hens fed 0, 10 or 100 mg of FA/kg of diet. Similarly, *Keshavarz* (2003), *Bunchasak and Kachana* (2009), *Hebert et al.* (2011) and *Benkova et al.* (2009) were unable to improve egg production by supplementing practical diets for laying hens with FA. On the other hand *Krishnan* (2010) reported improved egg production with FA supplementation at higher levels (2 and 4 ppm) compared to no (0 ppm) supplementation. *House et al.* (2002) reported that the addition of supplemental FA in graded levels (0, 1, 2, 4, 8, 16, or 32 mg FA/kg) to laying hen diets did not have any effect on egg production. However, average daily feed consumption was higher for birds consuming the diets fortified with 32 mg/kg FA when compared to those consuming diets containing FA at lower levels (4 mg/kg; 8 mg/kg; 16 mg/kg), but not when compared to birds eating FA at 0 mg/kg. This result is to a certain degree in agreement with later study by *Krishnan* (2010), wherein addition of supplemental FA to laying hen diets had a marginal, but significant effect on feed intake.

Contrary to above mentioned reports, in the present experiment, the inclusion of FA to the diet reduced egg production and feed intake. It is well known that FA requirement is affected by many factors such as layer hen strain and age, stage of production, basal diet, FA level, form in which FA is fed, feed and management protocol, environmental factors (heat, light, moisture), etc (*House et al.*, 2002; *Krishnan*, 2010; *Hebert et al.*, 2011). Because of the different experimental materials and methods applied, it is very difficult to directly compare the results from other experiments with those of the current study. The reduced feed intake and consequently decreased egg production might have been the result of appetite depression or low palatibility of high levels of FA. However, further works are needed to test these hypotheses. The livability of males from sire line and females from dam line was not affected by the dietary treatment.

CONCLUSIONS

1. The administration of supplemental FA to layer breeders via dietary supplementation was effective in improving body weight of newborn chicks.
2. Egg production and feed intake were adversely affected by adding supplemental FA.
3. Folic acid at a concentration of 0.85 mg/kg was not limiting for fertility and hatchability in the basal diet as indicated by similar fertility and hatchability of birds that received the basal diet and the basal diet plus additional FA.

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Corresponding author:

Dušan Terčič
University of Ljubljana, Biotechnical Faculty, Department of Animal Science
Groblje 3, 1230 Domžale, Slovenia
Phone +386 1 320 3 915
E-mail: dusan.tercic@bf.uni-lj.si