Effects of Dietary Folic Acid Supplementation Level on Laying Performance, Egg Quality, Reproductive Organs, and Plasma Reproductive Hormone Indices in Shanma Ducks (Postprint)

Authors: Xia Weiguang, Wang Shenglin, Chen Wei, Ruan Dong, Wang Shuang, A. M. Fouad, Lin Yingcai, Zheng Chuntian

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Abstract

This experiment aimed to investigate the effects of dietary folic acid supplementation levels on laying performance, egg quality, reproductive organs, and plasma reproductive hormone indices in Shanma ducks, in order to determine the folic acid (FA) requirement during the laying period (18-32 weeks of age). A total of 360 Shanma ducks at 16 weeks of age were randomly divided into 6 groups with 5 replicates per group and 12 ducks per replicate, housed individually in cages. After feeding the experimental ducks a basal diet without FA supplementation for 2 weeks, they were fed experimental diets supplemented with 0, 0.5, 1.0, 2.0, 4.0, and 8.0 mg/kg FA in the basal diet for a 15-week experimental period. The results showed: 1) Dietary FA supplementation level had no significant effect on laying performance indices of ducks during the early laying period, peak laying period, and the entire experimental period ($P>0.05$). 2) With increasing dietary FA supplementation levels, eggshell relative weight and eggshell thickness first increased and then decreased, with the 1.0 mg/kg FA supplementation group having significantly higher eggshell relative weight than other groups ($P<0.05$). 3) With increasing dietary FA supplementation levels, dominant follicle weight and dominant follicle weight/ovary weight first increased and then decreased; dietary FA supplementation level had a significant effect on dominant follicle weight and dominant follicle weight/ovary weight ($P<0.05$), with the 4.0 mg/kg folic acid supplementation group being the highest. 4) With increasing dietary FA supplementation levels, plasma progesterone concentration first increased and then decreased, with the 2.0 mg/kg FA supplementation group having the highest plasma progesterone concentration; the 8.0 mg/kg FA supplementation group had significantly higher plasma luteinizing hormone concentration than other groups ($P<0.05$). It can be concluded that FA supplementation in corn-soybean meal diets is beneficial for promoting dominant follicle development to a certain extent, but did not significantly affect laying performance in Shanma ducks. Considering only laying performance, folic acid supplementation is not required in corn-soybean meal diets; using eggshell relative weight and eggshell thickness as evaluation indices, the recommended appropriate folic acid supplementation level in corn-soybean meal diets is 1.0 mg/kg.

Full Text

Effects of Dietary Folic Acid Supplemental Level on Laying Performance, Egg Quality, Reproductive Organ and Plasma Reproductive Hormone Indices of Shanma Laying Ducks

XIA Weiguang[1], WANG Shenglin[1], CHEN Wei[1], RUAN Dong[1], WANG Shuang[1], A. M. Fouad[1,2], LIN Yingcai[1]*, ZHENG Chuntian[1]*

[1]Institute of Animal Science, Guangdong Academy of Agricultural Sciences, State Key Laboratory of Livestock and Poultry Breeding, Key Laboratory of Animal Nutrition and Feed Science in South China, Ministry of Agriculture, Guangdong Public Laboratory of Animal Breeding and Nutrition, Guangdong Key Laboratory of Animal Breeding and Nutrition, Guangzhou 510640, China
[2]Department of Animal Production, Faculty of Agriculture, Cairo University, Giza 12613, Egypt

Abstract

This experiment was conducted to investigate the effects of dietary folic acid (FA) supplemental level on laying performance, egg quality, reproductive organ development, and plasma reproductive hormone indices of Shanma laying ducks, and to determine the dietary FA requirement during the laying period (18–32 weeks of age). A total of 360 healthy 16-week-old Shanma laying ducks were randomly allocated to 6 groups with 5 replicates per group and 12 ducks per replicate. All ducks were individually housed in cages and fed a basal diet without FA supplementation for 2 weeks, followed by experimental diets supplemented with 0, 0.5, 1.0, 2.0, 4.0, or 8.0 mg/kg FA for 15 weeks. The results showed that: 1) Dietary FA supplemental level had no significant effect on laying performance indices during the early laying period, peak laying period, or the entire experimental period ($P>0.05$). 2) Eggshell relative weight and thickness increased initially and then decreased with increasing dietary FA levels, with the 1.0 mg/kg FA group exhibiting significantly higher eggshell relative weight than other groups ($P<0.05$). 3) Large yellow follicle weight and the ratio of large yellow follicle weight to ovarian weight showed a similar trend, with dietary FA level significantly affecting these parameters ($P<0.05$), peaking at the 4.0 mg/kg FA supplementation level. 4) Plasma progesterone concentration increased initially and then decreased with increasing dietary FA levels, reaching its maximum in the 2.0 mg/kg FA group, while plasma luteinizing hormone concentration in the 8.0 mg/kg FA group was significantly higher than in other groups ($P<0.05$). In conclusion, FA supplementation in corn-soybean meal diets may promote large yellow follicle development to some extent but does not significantly affect laying performance of Shanma ducks. Based solely on laying performance, FA supplementation is unnecessary in corn-soybean meal diets; however, considering eggshell relative weight and thickness, the optimal dietary FA supplemental level is recommended to be 1.0 mg/kg.

Keywords: folic acid; Shanma ducks; laying performance; egg quality; reproductive organ

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Introduction

Folic acid (FA), also known as pteroylglutamic acid, is a crucial water-soluble vitamin in animal growth and development. FA exists widely in nature in its reduced forms, dihydrofolate and tetrahydrofolate, and exhibits various structural forms in animal liver and kidney tissues, including 5-methyltetrahydrofolate, 10-formyltetrahydrofolate, 5-formyltetrahydrofolate, 5,10-methenyltetrahydrofolate, 5,10-methylenetetrahydrofolate, and formiminotetrahydrofolate. The N5 or N10 positions, or both, of tetrahydrofolate can bind one-carbon groups, enabling it to carry and donate these groups during metabolic processes such as purine synthesis, deoxythymidylate and methionine synthesis, and DNA methylation. The recommended FA levels in feeding standards represent the minimum requirements to prevent clinical deficiency symptoms; however, optimal FA supplemental levels for maximal animal production performance remain a focal point of research.

Previous studies have reported varying effects of FA supplementation in poultry. Ge Wenxia [1] found that supplementing corn-soybean meal diets with 0, 0.75, 1.50, or 3.00 mg/kg FA progressively increased serum immunoglobulin G (IgG) content in 21-day-old broilers, though differences among supplemented groups were not significant. Abas et al. [2] demonstrated that supplementing corn-soybean meal diets with 0, 5, or 10 mg/kg FA for 8 weeks significantly increased egg weight in early-laying hens, with no significant difference between the 5 and 10 mg/kg groups. Bunchasak et al. [3] reported that dietary supplementation with 0, 0.5, 4.0, or 10.0 mg/kg FA for 8 weeks (64–72 weeks of age) had no significant effects on daily egg mass, laying rate, egg weight, or feed intake in laying hens, though serum and egg FA content increased, with significant elevations in serum FA at supplementation levels $ $4.0 mg/kg. Meng Lingfeng et al. [4] estimated optimal dietary FA levels of 2.45 mg/kg for goslings and 2.08 mg/kg for growing geese based on growth performance regression analysis. While FA effects on poultry performance have been investigated in broilers, laying hens, and geese, no studies have been reported in laying ducks. Therefore, this experiment aimed to evaluate the effects of different dietary FA supplemental levels on laying performance, egg quality, reproductive organ development, and plasma reproductive hormone indices in Shanma laying ducks, and to determine the optimal dietary FA requirement during the laying period, providing a scientific basis for rational FA utilization in duck production.

Materials and Methods

Experimental Animals and Management

A total of 360 healthy 16-week-old Shanma laying ducks with normal feed intake and no significant differences in initial body weight (P>0.05) were randomly divided into 6 groups, each consisting of 5 replicates of 12 ducks. All experimental ducks were individually housed in two-tier stainless steel galvanized cages (27.8 cm × 40 cm × 55 cm) throughout the trial. Following a 2-week adaptation period during which all ducks received a basal diet without FA supplementation, experimental diets containing 0, 0.5, 1.0, 2.0, 4.0, or 8.0 mg/kg FA were fed for 15 weeks. Ducks were vaccinated against duck viral hepatitis, infectious serositis, and avian influenza according to routine immunization programs during the rearing period. Feed and water were provided ad libitum, with a 16-hour daily photoperiod (intensity $15lx/m^{2}$). Ambient temperature, humidity, and weather conditions were recorded daily at 06:00, 12:00, and 18:00.

Experimental Design and Diet Composition

A single-factor randomized design was employed. The experimental diets were based on a corn-soybean meal formulation with nutrient levels determined according to previous studies by our research group [5–10]. The composition and nutrient levels of the basal diet are presented in Table 1 , with a calculated FA content of 0.04 mg/kg.

Laying Performance

During the pre-trial period, feed allowance was adjusted based on the previous day' s intake, with consistent feed allocation per replicate. Ducks were fed to maximize intake while ensuring complete consumption of provided feed, with accurate records of feed offered and residual amounts. Once laying rate reached 50%, feed allowance was fixed at 145 g per duck per day. Laying performance was monitored by replicate, with accurate daily records of egg number and egg weight used to calculate average laying rate, average egg weight, daily egg mass, and feed-to-egg ratio throughout the laying period.

Egg Composition and Quality

Every 4 weeks, three eggs were collected from each replicate. All egg samples were analyzed within 48 hours of collection for eggshell weight, yolk weight, albumen weight, egg shape index, eggshell thickness, eggshell strength, yolk color, and Haugh unit. Final values were calculated as the mean across all batches for statistical analysis. Egg shape index was determined using vernier calipers (Shanghai 01120028) to measure longitudinal and transverse diameters, with the ratio (longitudinal/transverse) calculated as the index. Eggshell thickness was measured at the blunt end, middle, and sharp end using a digital micrometer (MODEL-1061), with the mean value reported. Eggshell strength, yolk color, and Haugh unit were measured using an ORKA eggshell force gauge (EFR-01, Israel) and a fully automatic egg quality analyzer (EMT-5200, Israel), respectively.

Reproductive Organs

At week 16 of the experiment, two ducks from each replicate were randomly selected for slaughter. Ovaries were excised and weighed to calculate ovarian index (ovarian weight/body weight). Follicular development and ovarian morphology were examined, with the number and weight of large yellow follicles (mature follicles filled with yolk, diameter >8 mm) and small yellow follicles (3-8 mm diameter) recorded. The ratios of large yellow follicle weight and small yellow follicle weight to ovarian weight were calculated. Oviduct length and weight were measured to determine oviduct length index (oviduct length/body weight) and weight index (oviduct weight/body weight).

Plasma Reproductive Hormones

At 18:00 on the day before slaughter, 5 mL of blood was collected from the wing vein of each selected duck using anticoagulant vacuum tubes. Plasma was prepared by centrifugation at 3,000 r/min for 15 min, aliquoted into Eppendorf tubes, and stored at -80°C. Plasma concentrations of luteinizing hormone (LH) and progesterone (PG) were determined by radioimmunoassay.

Data Processing and Statistical Analysis

Experimental data were analyzed using the GLM procedure of SAS 9.0 software for one-way ANOVA. When significant effects were detected, means were compared using Student-Newman-Keuls multiple range test. Differences were considered significant at $P<0.05$.

Results

Effects of Dietary FA Supplemental Level on Laying Performance of Laying Ducks

As shown in Table 2 , dietary FA supplemental level had no significant effect on laying rate, average egg weight, daily egg mass, or feed-to-egg ratio during the early laying period, peak laying period, or the entire experimental period ($P>0.05$).

Effects of Dietary FA Supplemental Level on Egg Composition and Quality of Laying Ducks

Table 3 shows that eggshell relative weight and thickness increased initially and then decreased with increasing dietary FA levels. The 1.0 mg/kg FA group exhibited significantly higher eggshell relative weight than other groups ($P<0.05$) and greater eggshell thickness than the 0.5 mg/kg FA group ($P<0.05$). Dietary FA supplemental level had no significant effect on yolk relative weight, albumen relative weight, egg shape index, eggshell strength, yolk color, or Haugh unit ($P>0.05$).

Effects of Dietary FA Supplemental Level on Reproductive Organ Indices of Laying Ducks

As presented in Table 4 , large yellow follicle weight and the ratio of large yellow follicle weight to ovarian weight increased initially and then decreased with increasing dietary FA levels. Dietary FA supplemental level significantly affected these parameters ($P<0.05$), with maximal values observed in the 4.0 mg/kg FA group.

Effects of Dietary FA Supplemental Level on Plasma LH and PG Concentrations of Laying Ducks

Table 5 indicates that plasma PG concentration increased initially and then decreased with increasing dietary FA levels, peaking in the 2.0 mg/kg FA group. Plasma LH concentration in the 8.0 mg/kg FA group was significantly higher than in all other groups ($P<0.05$).

Discussion

Effects of Dietary FA Supplemental Level on Laying Performance of Laying Ducks

In nature, FA exists widely in green forages, vegetables, grass seeds, and alfalfa meal in its reduced forms, dihydrofolate and tetrahydrofolate. As an essential water-soluble vitamin, accumulating evidence demonstrates that FA can regulate laying performance in poultry, though effects vary depending on species, diet composition, and treatment duration. Jing et al. [11] reported that supplementing wheat-soybean meal diets with 4 mg/kg FA for 8 weeks significantly increased egg weight and daily egg mass in laying hens. Conversely, Hebert et al. [12] found that dietary supplementation with 0, 2, 4, 8, 16, 32, 64, or 128 mg/kg FA for 21 days had no significant effects on laying rate, feed intake, egg weight, or feed-to-egg ratio. Tactacan et al. [13] observed that supplementation with 10 or 100 mg/kg FA in wheat-soybean meal diets for 21 days did not affect average daily feed intake, laying rate, egg weight, or egg mass, though it significantly increased serum and egg FA content. These findings align with our results, which showed that dietary supplementation with 0, 0.5, 1.0, 2.0, 4.0, or 8.0 mg/kg FA in a corn-soybean meal diet had no significant effects on laying rate, average egg weight, daily egg mass, or feed-to-egg ratio in Shanma ducks.

Effects of Dietary FA Supplemental Level on Egg Composition and Quality of Laying Ducks

Egg quality is typically assessed through composition indices (yolk, albumen, and eggshell weights and their relative proportions) and quality parameters (egg shape index, eggshell thickness, eggshell strength, Haugh unit, and yolk color). The Haugh unit, influenced primarily by thick albumen content and viscosity, serves as an important indicator of egg freshness. As eggs age, air cell size increases and moisture loss accelerates, resulting in decreased specific gravity. Freshness can thus be evaluated using the saline flotation method with salt solutions ranging from 1.058 to 1.112 in 0.004 increments [14], where specific gravity >1.080 indicates fresh eggs, >1.060 indicates slightly stale eggs, >1.050 indicates stale eggs, and <1.050 indicates spoiled eggs [15]. Based on preliminary experiments, eggs collected within 48 hours of laying had specific gravity values of 1.083–1.092; therefore, all egg composition and quality analyses in this study were completed within 48 hours post-collection. Our results demonstrated that dietary FA supplementation had no significant effect on Haugh unit. Stern et al. [16] reported that FA deficiency elevates plasma homocysteine concentration, which promotes protein degradation by affecting hormone levels (cortisol, growth hormone, insulin-like growth factor) or the phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin signaling pathway. Conversely, FA supplementation reduces plasma homocysteine concentration [12], potentially promoting protein synthesis. However, we found no significant effect of FA supplementation on albumen relative weight, though the 1.0 mg/kg FA level significantly increased eggshell relative weight and yielded greater eggshell thickness. Eggshell formation involves the interaction of calcium carbonate crystals and eggshell matrix proteins to create a shell with adequate thickness and strength. This suggests that 1.0 mg/kg FA supplementation may enhance eggshell matrix protein synthesis, though the underlying regulatory mechanisms require further investigation. Additionally, dietary FA supplementation showed no significant effect on yolk color in our study. Shi Tianhong et al. [17] similarly reported that supplementing corn-soybean meal diets with 5 mg/kg FA did not significantly improve yolk color in duck eggs. Since poultry cannot synthesize pigments, yolk color primarily depends on the type and amount of lipophilic pigments consumed in the diet [18], indicating that FA supplementation does not affect lipophilic pigment deposition in yolk.

Effects of Dietary FA Supplemental Level on Reproductive Organs and Endocrine Function

Reproductive axis hormones influence ovarian and oviduct development [19-20], and both dietary nutrient levels and feeding duration can regulate reproductive organ development through effects on hormone secretion [21-22]. Early studies indicated that FA deficiency reduces sex hormone secretion and inhibits amino acid composition and development of the oviduct [23]. Our results showed that 4.0 mg/kg FA supplementation yielded the highest large yellow follicle weight and large yellow follicle weight/ovarian weight ratio, though these values did not differ significantly from the 2.0 and 8.0 mg/kg FA groups. Yang et al. [24] demonstrated that plasma reproductive hormone levels undergo continuous dynamic changes, with LH and PG being two critical hormones promoting ovulation in ducks. Plasma PG is secreted by the largest preovulatory follicle, peaking 4-6 hours before ovulation, while plasma LH reaches its maximum following the PG peak [25]. Our analysis of plasma LH and PG concentrations revealed that the 2.0 mg/kg FA group had significantly higher PG concentration than the unsupplemented group, though not significantly different from other FA groups. The 8.0 mg/kg FA group exhibited significantly higher plasma LH concentration than all other groups. Follicular development and maturation constitute a complex multi-factorial process regulated not only by endocrine factors but also by growth factors and cytokines. Therefore, the significant increase in large yellow follicle weight and its ratio to ovarian weight observed with dietary FA supplementation may be mediated through pathways involving growth factors or cytokines other than PG and LH, warranting further investigation into the mechanisms of FA action on follicular maturation.

In conclusion, dietary FA supplementation in corn-soybean meal diets may promote large yellow follicle development to some extent but does not significantly affect laying performance in Shanma ducks. Based on eggshell relative weight and thickness, the optimal dietary FA supplemental level is recommended to be 1.0 mg/kg.

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Note: Figure translations are in progress. See original paper for figures.