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Influence of Lysolecithin on the Performance of Laying Hens, Interior and Exterior Egg Quality as well as Fat Soluble Vitamin and Cholesterol Content in the Yolk

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Abstract: This study was conducted to evaluate the effects of feeding lysolecithin on laying hen performance and to determine its effects on interior and exterior egg quality as well as the fat soluble vitamin and cholesterol content of egg yolks. About 64 Lohmann Brown-Lite laying hens were fed diets based on corn, wheat and soybean meal with lesser quantities of lupin meal, corn gluten meal and corn distiller's dried grains with solubles providing additional supplementary protein for a 6 week period. The diets contained 0.0, 0.05, 0.10 or 0.15% lysolecithin. There were eight cages per treatment and two hens per cage. There was no difference in the rate of egg production as a result of feeding lysolecithin with all treatments exceeding 97%. Adding lysolecithin to the diet linearly ($p<0.01$) increased egg weight. The general increase in egg weight was associated with a dramatic increase ($p = 0.02$) in the number of large (63-72.9 g) size eggs with a concomitant reduction in the number of medium (53-62.9 g) size eggs. Feed intake declined linearly ($p<0.01$) while there was a linear ($p<0.01$) improvement in feed efficiency (g feed/g egg) with increasing lysolecithin level. The increase in egg weight appeared to result from an increase in albumen weight ($p<0.01$). No significant differences were detected in egg shell weight, yolk color or Haugh units. However, the weight of the egg yolk declined linearly ($p<0.01$) with increasing level of lysolecithin supplementation. Treatment with lysolecithin appeared to increase the vitamin A ($p = 0.05$) and vitamin E ($p = 0.06$) content of egg yolks. In addition, there was a linear ($p<0.01$) increase in the cholesterol content of egg yolks with increasing level of lysolecithin supplementation. In summary, supplementation of lysolecithin in the diet of laying hens significantly increased egg weight and feed efficiency. In addition, treatment with lysolecithin resulted in linear increases in the vitamin A and E content of egg yolk. However, these advantages may be more than offset by a significant increase in the cholesterol content of the egg yolk.

Key words: Laying hens, egg production, egg quality, lysolecithin, concomitant reduction, cholestrol content

INTRODUCTION

Lysolecithins are an important metabolite produced by many cells and are widely distributed in a variety of tissues and are capable of increasing ion permeation in membranes (Lee and Chan, 1977). Lysolecithin can function as a membrane transducer by diffusing rapidly through the lipid portions of cellular membranes to modify the activity of various membrane associated enzymes which may alter the general properties of the membrane such as increasing fluidity and permeability (Shier *et al*., 1976).

Lysolecithin may also alter mucosal barrier function and increase the gut permeability to macromolecules such as proteins (Tagesson *et al*., 1985).

Although, lysolecithins are a potent membrane transport modifier, their role in animal performance has not been widely studied. Xing *et al*. (2004) and Van Heugten and Odle (2000) reported increases in weight gain and nutrient digestibility as a result of lysolecithin supplementation of weanling pig diets. In contrast, Gatlin *et al*. (2005) reported no improvement in lipid digestibility from lysolecithin supplementation of finishing swine diets.

Few studies have monitored the effects of lysolecithin in laying hens. Calton *et al*. (1998) showed that the addition of lysolecithin produced heavier egg weights due largely to an increase in the weight of the egg yolk. These results were ascribed to a better assimilation of nutrients at the gut level. The aim of the present study

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was to further evaluate the effects of lysolecithin supplementation on laying hen performance and to determine its effects on interior and exterior egg quality as well as fat soluble vitamin and cholesterol content in egg yolk.

MATERIALS AND METHODS

Production of lysolecithin: The lysolecithin used in the present experiment was obtained from Devenish Nutrition (Belfast, North Ireland) and is marketed under the trade name Lipidol®. The lysolecithin was prepared from phospholipids present in soybean lecithin. Phospholipase enzymes were used to remove one of the fatty acid chains from the soybean lecithin resulting in a small charged lipid which encourages interaction with cell membranes. The charge connects water soluble nutrients with oil based nutrients thus facilitating nutrient absorption. The final product contained 50% lysolecithin along with an inert calcium silicate carrier.

Experimental design, animals and housing: All procedures used in this experiment were approved by the Animal Ethics Committee of Sungkyunkwan University (Suwon, Korea) following the Guidelines for the Care and Use of Animals in Research published by the Korean Ministry for Food, Agriculture, Forestry and Fisheries (2008).

Sixty four, 18 week old Lohmann Brown-Lite laying hens were obtained from a commercial supplier (Join farm, Songtan, Korea). The birds were allowed 6 weeks to adapt to the experimental facilities and then were randomly allocated to one of four dietary treatments. The dietary treatments were based on corn, wheat and soybean meal with lesser quantities of lupin meal, corn gluten meal and corn distiller's dried grains with solubles providing additional supplementary protein.

The basal diet was fed either unsupplemented or supplemented with 0.05, 0.10 or 0.15% lysolecithin (Table 1). The diets were formulated to provide 11.6 MJ ME kg^{-1} and supplied 17% crude protein, 0.85% lysine and 0.72% methionine and cystine (Table 2). These levels met or exceeded the nutrient requirements suggested in the Lohmann Brown Management Guide (Lohmann, 2008). The diets were fed in mash form. At the initiation of the experiment, the average laying rate of the hens was 96.3±3.19%.

During the 6 week study, all hens were housed in a windowless and environmentally controlled room. The room temperature was maintained between 21 and 23°C and incandescent lighting (10 lux) was provided with a photoperiod of 16 h of light and 8 h dark. The hens were housed in pairs in galvanized metal wire cages (approximately 25×35×50 cm) in double-decked rows providing 430 cm^2/hen. There were eight cages per treatment and two hens per cage. One upper deck and one lower deck of cages were used for this experiment. Each cage had a nipple waterer and a continuous, plastic feed trough was divided by replicate to insure that the hens were not able to consume feed assigned to the adjoining replicate. Feed and water were available *ad libitum*. Feed consumption was measured on a weekly basis.

Table 1: Ingredient composition of experimental diets (percentage as fed)

	Level of lysolecithin (%)			
Ingredients	0.00	0.05	0.10	0.15
Corn	50.45	50.40	50.35	50.30
Wheat	8.00	8.00	8.00	8.00
Lupin meal	2.00	2.00	2.00	2.00
Soybean meal, extracted	18.40	18.40	18.40	18.40
Corn gluten meal	2.90	2.90	2.90	2.90
Corn distillers dried grains with solubles	4.00	4.00	4.00	4.00
Meat and bone meal	2.50	2.50	2.50	2.50
Animal fat	0.80	0.80	0.80	0.80
Sodium bicarbonate	0.06	0.06	0.06	0.06
L-Lysine HCl	0.05	0.05	0.05	0.05
Methionine	0.17	0.17	0.17	0.17
Limestone	8.56	8.56	8.56	8.56
Oyster shell	1.50	1.50	1.50	1.50
Phytase (Optiphos-1000®)	0.05	0.05	0.05	0.05
Carbohydrase (Endopower®)	0.10	0.10	0.10	0.10
Vitamin-mineral premix[a]	0.24	0.24	0.24	0.24
Choline	0.02	0.02	0.02	0.02
NaCl	0.20	0.20	0.20	0.20
Lysolecithin (50%)	0.00	0.05	0.10	0.15

[a]Provided the following per kg diet: vitamin A, 12,000 IU; vitamin D3, 3,500 IU; vitamin E, 30 IU; vitamin K3, 3.0 mg; thiamin, 3.0 mg; riboflavin, 7.0 mg; pyridoxine, 5.0 mg; vitamin B12, 0.025 mg; niacin, 40.0 mg; pantothenic acid, 10 mg; folic acid, 1.0 mg; biotin, 0.15 mg; Fe, 75.0 mg; Zn, 97.5 mg; Mn, 97.5 mg; Cu, 7.5 mg; I, 1.5 mg; Se, 0.2 mg

Table 2: Chemical composition of experimental diets (percentage as fed)[a]

	Level of lysolecithin (%)			
Items	0.0	0.05	0.10	0.15
Moisture	12.20	12.23	12.24	12.25
Crude protein	17.05	17.05	17.04	17.04
Ether extract	3.97	3.99	3.98	3.99
Crude fibre	2.72	2.74	2.74	2.73
Ash	13.28	13.32	13.36	13.36
Calcium	4.08	4.09	4.09	4.09
Phosphorus	0.44	0.45	0.45	0.46
Lysine	0.85	0.87	0.86	0.87
Methionine	0.43	0.43	0.43	0.46
Methionine+Cystine	0.72	0.72	0.75	0.76

[a]Data are the mean of a chemical analysis conducted in triplicate

Sampling and analyses: A wire egg collector was installed in the front of each cage to prevent eggs from different replicates from being mixed. The eggs were collected and weighed daily. Egg production was calculated per cage. Egg weight (g of egg hen day^{-1}) and feed

conversion (g of feed per g of egg produced) were also calculated. Eggs were sorted by four weight groups (very large, large, medium and small) according to the European Union Marketing Standards (European Commission, 2008).

On the last 2 days of each week, the weight of the albumen, yolk and egg shell were measured using two eggs of average weight obtained from each cage yolk color and Haugh units were measured using an EMT-5200 Egg Multi-Tester (Robotmation Company, Tokyo, Japan). Haugh Units (HU) were calculated from the records of egg weight and albumen height using the formula: HU = 100 $\log^{10}$ (H-1.7 $W^{0.37}$+7.56) where HU = Haugh Unit, H = height of the albumen (mm) and W = egg weight (g). The yolks were separated from the tester tray (yolk, albumen and tray) using a Teflon spoon. Before the yolk weight was determined, the chalaza was removed with a spatula. Albumen weight was calculated by subtracting the weight of the tester tray from the remaining egg contents. The total weight of the egg contents produced on a daily basis was calculated (% egg production x (daily yolk weight + daily albumen weight) from egg production, yolk weight and albumen weight. The shells were weighed without drying.

The total cholesterol content of the eggs was determined without saponification according to the procedures described by Zhang *et al.* (1999). The extraction and HPLC analysis of fat soluble vitamins of egg yolk were basically the same as the method used for the analysis of milk by Gong and Ho (1997). Briefly, 2 g samples of egg yolk were accurately weighed and diluted to 20 mL with distilled water and then mixed completely. Diluted egg yolk (1 mL) was pipetted into a 15 mL centrifuge tube fitted with a stopper and 1.0 mL of 95% ethanol was added into the same tube and thoroughly mixed to facilitate the subsequent extraction.

The mixture was first mixed with 2.5 mL of ether and then mixed with 2.5 mL of petroleum ether (b.p. 35-60°C, Sigma-Aldrich, St Louis, MO). After standing for 30 min at 20°C, 1.0 mL of the organic phase was pipetted into an Eppendorf tube (1.5 mL) and evaporated to dryness under a stream of nitrogen in a fume hood. The residue was dissolved in 1.0 mL of ethanol and filtered through a 0.45 μm micro-filter (Whatman, NJ) and the sample was stored in a 1.8 mL auto-sampler vial with screw cap prior to analysis. Samples of feed were analyzed in triplicate according to the methods of the AOAC (1990). Analyses were conducted for moisture (method 930.15), ether extract (method 920.39), crude protein (method 984.13), crude fiber (method 978.10) and ash (method 942.05).

Calcium was determined by a Shimazu AA625 Atomic Absorption Spectrophotometer (Shimazu, Kyoto, Japan) and phosphorus was analyzed using a UV-Vis. Spectrophotometer (Hitachi, Tokyo, Japan). An amino acid analysis of the feed was performed using a L8500-Hitachi Amino Acid Analyzer (Hitachi, Tokyo, Japan) after hydrolysis for 24 h in 6 N HCl. Performic acid (85%) hydrolysis was performed for analysis of sulfur-containing amino acids.

Statistical analysis: Data were analyzed as a randomized block design (Snedecor and Cochran, 1989) using the appropriate General Analysis of Variance procedures of Statistix (1996). Hens were blocked on the basis of hen day egg production during the adaptation period and the cage was considered the experimental unit for all analyses. The model included the effects of replication (i.e., block), treatment and replication x treatment (error). Polynomial contrasts were constructed to determine the nature of response variables to increasing levels of supplemental lysolecithin.

RESULTS AND DISCUSSION

There was no difference in the rate of egg production as a result of feeding the different levels of lysolecithin (Table 3). However, since the rate of production exceeded 97% for all treatments there was a very small window of opportunity for lysolecithin to increase the rate of egg production.

In commercial poultry production, peak production usually occurs when birds reach 24-26 weeks of age and production steadily declines until the flock is taken out of production at approximately 76 weeks of age (Bell, 2002). The present study was conducted relatively close to the period of peak production and it would be interesting to repeat the study to determine whether or

Table 3: Performance of laying hens fed diets supplemented with lysolecithin

	Level of lysolecithin (%)					p-values		
Parameters	0.0	0.05	0.10	0.15	SEM	Linear	Quadratic	Cubic
Egg production (%)	97.50	97.60	99.10	98.30	1.04	0.40	0.48	0.56
Egg weight (g/hen/day)	54.40	54.70	56.60	57.70	0.76	<0.01	0.83	0.34
Feed intake (g/hen/day)	106.50	104.90	105.20	102.10	0.79	<0.01	0.36	0.11
Feed conversion (g feed/g egg)	1.96	1.94	1.88	1.79	0.02	<0.01	0.48	0.97

Table 4: Exterior egg quality for laying hens fed diets supplemented with lysolecithin

	Level of lysolecithin (%)					p-values		
Egg quality	0.00	0.05	0.10	0.15	SEM	Linear	Quadratic	Cubic
Cracked eggs (%)	1.76	0.96	0.79	0.32	0.50	0.32	0.78	0.08
Dirty eggs (%)	3.75	2.75	2.00	1.63	1.99	0.27	0.34	0.99
Egg grade (%)								
Very large (<73 g)	0.98	0.63	0.16	0.47	0.47	0.39	0.38	0.87
Large (63-72.9 g)	4.56	5.85	7.18	21.40	4.81	0.02	0.25	0.65
Medium (53-62.9 g)	68.50	64.82	77.55	58.11	6.57	0.50	0.14	0.19
Small (>53 g)	25.96	28.70	15.12	20.02	6.52	0.27	0.56	0.32

Table 5: Egg components and egg quality for laying hens fed diets supplemented with lysolecithin

	Level of lysolecithin (%)					p-values		
Components and quality of egg	0.0	0.05	0.10	0.15	SEM	Linear	Quadratic	Cubic
Egg weight (g)	54.4	54.7	56.6	57.7	0.76	<0.01	0.83	0.34
Weight of egg contents (g)	47.0	47.2	49.1	50.0	0.70	<0.01	0.91	0.53
Albumen weight (g)	33.7	33.9	35.2	36.4	0.53	<0.01	0.79	0.69
Albumen (% of egg weight)	61.4	61.4	61.5	62.7	0.27	<0.01	0.06	0.50
Yolk weight (g)	13.4	13.3	13.8	13.6	0.25	0.33	0.40	0.35
Yolk (% of egg weight)	25.8	25.6	25.3	24.4	0.25	<0.01	0.23	0.60
Yolk:albumen (%)	39.8	39.5	39.3	37.4	0.63	<0.01	0.27	0.58
Egg shell weight (g)	7.6	7.2	7.5	7.7	0.17	0.34	0.18	0.11
Egg shell (% of egg weight)	12.8	12.9	13.1	12.9	0.13	0.54	0.09	0.69
Yolk color	7.2	7.2	7.2	7.3	0.11	0.47	0.78	0.83
Haugh units	88.7	89.4	87.2	88.6	0.82	0.43	0.35	0.13

not lysolecithin has any impact on the productivity of laying hens during the later stages of the production cycle. Adding lysolecithin to the diet linearly ($p<0.01$) improved egg weight (Table 3). These results support the earlier research of Calton *et al*. (1998) who also observed increases in egg weight as a result of lysolecithin supplementation. The general increase in egg weight was associated with a dramatic increase ($p = 0.02$) in the number of large (63-72.9 g) size eggs with a concomitant reduction in the number of medium (53-62.9 g) size eggs (Table 4). As many countries pay a premium for larger sized eggs (Food and Agriculture Organization of the United Nations, 2003) there may be an economic incentive for producers to utilize lysolecithin as a means of increasing the percentage of large size eggs produced.

Feed intake declined linearly ($p<0.01$) with increasing level of lysolecithin supplementation (Table 3). This finding is in conflict with previous research conducted with lysolecithin using swine where no negative effects on feed intake were observed (Xing *et al*., 2004; Gatlin *et al*., 2005). However, as a result of the increase in egg weight and the reduced feed intake, there was a linear ($p<0.01$) improvement in feed efficiency (g feed/g egg) with increasing lysolecithin level.

The total weight of the egg contents (albumen+egg yolk) increased linearly ($p<0.01$) with increasing levels of lysolecithin supplementation. The increase in the weight of the egg contents appeared to result from an increase in albumen weight ($p<0.01$; Table 5). The weight of the egg yolk declined linearly ($p<0.01$) with increasing level of lysolecithin supplementation (Table 5). The decline in the weight of the egg yolk conflicts with the earlier research of Calton *et al*. (1998) who observed an increase in egg yolk weight as a result of supplementation with lysolecithin. No significant differences were detected egg shell weight, yolk color and Haugh units as a result of lysolecithin supplementation (Table 5).

The effects of lysolecithin supplementation on the fat-soluble vitamin content of egg yolk are shown in Table 6. Treatment with lysolecithin resulted in linear increases in the vitamin A ($p = 0.05$) and vitamin E ($p = 0.06$) content of egg yolk. Eggs are considered a good source of vitamin A and E in the human diet as two large eggs can contribute 12 and 6%, respectively of the recommended daily allowance of these nutrients (Applegate, 2000). The increases as a result of lysolecithin supplementation can only enhance the reputation of eggs as a good source of vitamins.

The effects of lysolecithin supplementation on the cholesterol content of egg yolk are shown in Table 6. There was a linear ($p<0.01$) increase in the cholesterol content of egg yolks with increasing level of lysolecithin supplementation. This finding has vast implications for the potential adoption of lysolecithin as a feed additive for use in the poultry industry as consumers are very concerned about the cholesterol content of foods (Applegate, 2000). This concern is based on >40 years of speculation regarding the association between

Table 6: Fat soluble vitamin and cholesterol content in the egg yolk of laying hens fed diets supplemented with lysolecithin

	Level of lysolecithin (%)					p-values		
Vitamins	0.0	0.05	0.10	0.15	SEM	Linear	Quadratic	Cubic
Vitamin A (μg/100 g)	930.0	940.0	940.0	950.0	6.00	0.05	0.92	0.77
Vitamin D (μg/100 g)	43.9	44.1	44.2	44.1	0.23	0.62	0.42	0.95
Vitamin E (μg/100 g)	1950.0	1970.0	1970.0	2000.0	15.00	0.06	0.93	0.46
Cholesterol (mg/100 g)	1106.0	1163.0	1299.0	1395.0	52.10	<0.01	0.43	<0.01

the consumption of high cholesterol foods, blood cholesterol and coronary heart disease (Dawber *et al*., 1982; Paik and Blair, 1996; Kritchevsky and Kritchevsky, 2000; Kritchevsky, 2004; Steinberg, 2004). Current dietary guidelines from the American Heart Association Nutrition Committee recommend that in order to reduce the risk of cardiovascular disease, consumers should limit their intake of cholesterol to less than 300 mg day^{-1} (Lichtenstein *et al*., 2006). Therefore, it is likely that consumers may reduce the number of eggs they consume should the cholesterol content of eggs increase due to widespread use of lysolecithin as a feed additive.

CONCLUSION

In this study, supplementation of lysolecithin in the diet of laying hens significantly increased egg weight and feed efficiency. In addition, treatment with lysolecithin resulted in linear increases in the vitamin A and E content of egg yolk. However these advantages may be more than offset by a significant increase in the cholesterol content of the egg yolk.

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