Effect of Orotic Acid on Egg Production and Egg Fat of Laying Hens

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ABSTRACT

The present study was undertaken to investigate the potential effects of different concentrations of dietary orotic acid (OA) on egg production rate and egg lipid accumulation in two breeds of mature laying hens—Hisex white (HSW) and Hisex brown (HSB). The chicks of the two breeds were raised on a cholesterol free basal diet for 16 weeks after that each breed was divided into two groups. One group was continued on the free cholesterol diet while the second group was fed the same diet containing 2, 1, 0.5, 0.1 or 0.05% OA, respectively, by changing the OA concentration monthly. The response to dietary OA as indicated by the increase in rate of egg production was significant in both breeds at the 0.05% level. The experimental HSW and the experimental HSB exhibited an elevation of 15 and 14% daily increase in the rate of egg production, respectively.

Furthermore, the effect of dietary OA on total egg cholesterol was more dramatically in HSW as compared to HSB. The reduction of total egg cholesterol at all OA levels was between 28–35% in the experimental HSW hens. However, the HSB hens showed a reduction of 21–26% and at only 2, 1 and 0.5% OA feeding levels. This reduction of total egg cholesterol was accompanied by elevation of egg total neutral lipids (NL) and free fatty acids (FFA). There was no concomitant change in egg total phospholipids (PL).

INTRODUCTION

It has recently been shown that OA is one of at least two inhibitors of cholesterol synthesis present in bovine milk (1). When OA is fed as 1% of the diet to rats, it results in a fatty liver which is unique in that the ultra structural changes accompanying hepatic lipid accumulation occurred in the absence of cellular necrosis (13). Furthermore, levels of very low density lipoprotein (VLDL) levels were reduced by 90% in plasma, however, removal of OA from the diet resulted in a rapid return of plasma and liver lipid levels to normal (13).

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Various factors and dietary manipulations have been used to study their effect on cholesterol composition of egg and egg production rate of laying hens. These include triparanol (2), diazacholesterol (14) and β-sistosterol (10). The latter appears to interfere with intestinal absorption of dietary and enterohepatically circulating cholesterol (10). This in turn resulted in an increase of fecal excretion rate of sterols and their degradation products (6, 7). Clarenburg *et al.* (3) reported that over 60% of added plant sterol was absorbed by the chicken and egg cholesterol levels declined by 35% while the incorporation of sistosterol to egg increased. Triparanol, when fed to laying hens, has been shown to result in almost complete replacement of egg cholesterol by desmosterol (4).

MATERIALS AND METHODS

Hens and diets

Two available laying breeds at the Faculty Farm (HSW and HSB) were used for this study. The chicks of the two breeds were raised on a basal free diet* until the age of 16 weeks, after that each breed was divided into two groups (each group consisted of 54 brids). The control group continued on the basal diet, while the other group was fed a basal diet plus OA. The two groups of each breed were housed separately in a controlled room with a temperature of 23°C, humidity of 50% and 12-hour light cycles. The two groups of each breed were maintained under these conditions for a period of 5 months. The % of OA in the diet of the experimental groups were as follows:

2, 1, 0.5, 0.1 and 0.05% (weight/weight), respectively.

Assay of egg lipids

Egg lipid assays were carried out 4 times on each group (control and experimental) during the last 2 weeks of each OA feeding level period. Sampling was performed by discarding the eggs between periods of analysis, and collecting what was layed within a period. Then 24 randomly selected eggs out of the total collected were homogenized in a stainless steel waring blender for subsequent analysis.

The lipids were extracted from the homogenate according to Folch *et al.* (5). The total PL were isolated using the modified Hirsch and Ahrens silicic acid chromatographic column (8). Total NL and total FFA were fractioned using the McCarthy and Duthie alkaline silicic acid chromatographic column (11). Total cholesterol was determined applying a modified procedure of Gilck *et al.* (8). The aliquots, after being evaporated under nitrogen, 0.4 ml absolute ethanol was added followed by 0.4 ml color reagent (2.5% $Fecl_3 6H_2O$ in 86% aquous solution of H_3PO_4). Tubes were mixed well immediately following color reagent addition and the absorbance determined after 1 hour at 558 nm in Model 25 Spectrophotometer (Beckman Instruments, Fullerton, CA). For each set of samples, appropriate blanks were employed using standard cholesterol (Supelco, Inc., Bellfonte, PA).

*The basal diet contained 12% moisture, 21% protein, 3% fat, 4% fiber, 6.5% ash, 1.6% calcium, 1% phosphorous, 8,000 IU vitamin A, 2,000 IU vitamin D, 4 mg Biotin, 0.016 mg B_{12} and 16 mg panthothenic acid.

Statistical analysis

The data for either egg production or lipid analysis were subjected to analysis of T test (12).

RESULTS AND DISCUSSION

Only at the lowest dietary level of OA, there was a significant difference ($P<0.05$) in egg production as shown in Table 1. The significant elevations of egg production at the 0.05% level were 15.9 and 14.5% daily for HSW and HSB, respectively. The standard error values indicate the high degree of consistency when the control hens were compared to the experimental laying hens of both breeds.

The addition of OA to the diet resulted in a significant ($P<0.05$) reduction of total cholesterol at all OA levels (Table 2). These reductions were 35.2, 27.6, 32.9, 32.5 and

Table 1. Effect of orotic acid supplementation on egg production in Hisex White and Hisex Brown laying hens.

Orotic acid suppl.	Hisex White		Hisex Brown	
	Control[a]	Experimental[b]	Control[a]	Experimental[b]
(%)	Egg production %[c] 1 day ± SE		Egg production %[c] 1 day ± SE	
2	58.67 ± 14.79	50.77 ± 8.68	52.88 ± 9.00	54.71 ± 6.82
1	61.63 ± 9.82	55.46 ± 8.20	54.13 ± 10.73	54.68 ± 7.92
0.5	60.95 ± 9.59	58.60 ± 10.21	44.89 ± 7.15	49.33 ± 13.42
0.1	64.77 ± 4.19	63.39 ± 5.83	46.12 ± 8.59	42.79 ± 4.08
0.05	61.71 ± 4.48A	77.59 ± 2.93A	45.03 ± 3.74B	59.49 ± 2.81B

[a] Hens on basal diet.
[b] Hens on basal diet plus orotic acid.
[c] Each value is the mean ± SE of egg layed by 54 laying hens in the last 2 weeks of one month trial. Means within the same level and breed followed by the same letter superscript are significantly different ($P<0.05$).

Table 2. Effect of orotic acid supplementation on total egg cholesterol of Hisex White and Hisex Brown hens.

Orotic acid suppl.	Hisex White		Hisex Brown	
	Control[a]	Experimental[b]	Control[a]	Experimental[b]
(%)	Egg total cholesterol/100 gm edible portion[c]		Egg total cholesterol/100 gm edible portion[c]	
2	0.71 ± 0.01A	0.46 ± 0.02A	0.77 ± 0.03F	0.057 ± 0.02F
1	0.76 ± 0.00B	0.55 ± 0.02B	0.70 ± 0.03G	0.53 ± 0.03G
0.5	0.73 ± 0.04C	0.49 ± 0.02C	0.71 ± 0.018H	0.56 ± 0.02H
0.1	0.77 ± 0.01D	0.52 ± 0.04D	0.59 ± 0.02I	0.62 ± 0.03I
0.05	0.72 ± 0.01E	0.52 ± 0.00E	0.49 ± 0.03	0.47 ± 0.01

[a] Hens on basal diet.
[b] Hens on basal diet plus orotic acid.
[c] Each value is the Means ± SE of 4 replicates of 24 eggs layed by 54 hens in the last 2 weeks of one month trial. Means with the same level and breed followed by the same letter superscripts are significantly different ($P<0.05$).

27.8% at 2, 1, 0.5, 0.1 and 0.05% OA levels, respectively. The corresponding data for HSB are also shown in Table 2. Dramatic differences in the total cholesterol across the two groups were observed. After 2 weeks on the different levels of dietary OA (2, 1 and 0.05%), the experimental HSB showed a significantly ($P<0.05$) lower total cholesterol than control hens. The reductions were 25.0, 24.3 and 21.1% at OA levels of 2, 1 and 0.5%, respectively. No significant differences ($P<0.05$) in total cholesterol due to OA levels of 0.1 or 0.05% were observed in the experimental and control laying hens of HSB. The total cholesterol levels remained nearly constant over these feeding trials. Table 3 shows the total egg NL of control and experimental HSW. All levels of OA

Table 3. Effect of orotic acid supplementation on egg total neutral lipids of Hisex White and Hisex Brown.

Orotic acid suppl.	Hisex White		Hisex Brown	
	Control[a]	Experimental[b]	Control[a]	Experimental[b]
(%)	Egg total neutral lipids/100 gm edible portion[c]		Egg total neutral lipids/100 gm edible portion[c]	
2	4.15 ± 0.09A	7.39 ± 0.34A	4.62 ± 0.13F	6.52 ± 0.25F
1	4.17 ± 0.334B	6.26 ± 0.30B	4.10 ± 0.20G	5.95 ± 0.21G
0.5	4.05 ± 0.21C	6.53 ± 0.13C	4.31 ± 0.17H	6.72 ± 0.17H
0.1	4.91 ± 0.12D	6.48 ± 0.01D	4.36 ± 0.71I	6.10 ± 0.15I
0.05	3.67 ± 0.13E	6.34 ± 0.17E	3.96 ± 0.10J	4.90 ± 0.30J

[a] Hens on basal diet.
[b] Hens on basal diet plus orotic acid.
[c] Each value is the mean ± SE of 4 replicates of 24 eggs layed by 54 hens in the last 2 weeks of one month trial. Means within the same level and breed followed by the same letter superscripts are significantly different ($P<0.05$).

resulted in a significant ($P<0.05$) increase in the total NL content of egg obtained from experimental laying hens. The elevations were 78.1, 50.1, 61.2, 32.0 and 42.1 at OA levels of 2, 1, 0.5, 0.1 and 0.5%, respectively. The same trend has been observed for the HSB (Table 3). The elevations of total NL in the experimental hens were 41.1, 45.1, 55.9, and 23.7% higher than control hens at OA levels of 2, 1, 0.5, 0.1 and 0.05%, respectively.

Table 4 presents the total FFA composition of the eggs produced by HSW hens. Diets containing OA at levels of 2 and 1% caused a higher significant elevation of total FFA ($P<0.05$) than the corresponding unsupplemented diets. These elevations were 190.0 and 218.8% at 2 and 1% OA levels, respectively. The supplementation of the diets with 0.5% OA or less showed no significant differences in the amount of total FFA among the control and experimental HSW hens ($P<0.05$). The results of the total FFA at 2 and 1% levels with comparison to the HSW breed. The supplementation of the diet with 2 and 1% OA increased the relative concentration of total FFA with 39.1 and 92.0%, respectively. The low concentration of dietary OA (0.5% or less) caused no significant changes in the total egg FFA in HSB hens. Table 5 presents the total PL composition of eggs produced from HSW and HSB supplemented with or without OA. The variations in dietary OA had no apparent effect on egg total PL concentrations in either breeds ($P<0.05$).

Table 4. Effect of orotic acid supplementation on egg total free fatty acids of Hisex White and Hisex Brown.

Orotic acid suppl.	Hisex White		Hisex Brown	
	Control[a]	Experimental[b]	Control[a]	Experimental[b]
(%)	Egg total free fatty acids/100 gm edible portion[c]		Egg total free fatty acids/100 gm edible portion[c]	
2	0.11 ± 0.02A	0.32 ± 0.01A	0.23 ± 0.03C	0.32 ± 0.01C
1	0.16 ± 0.01B	0.51 ± 0.02B	0.25 ± 0.02D	0.48 ± 0.03D
0.5	0.19 ± 0.02	0.18 ± 0.10	0.24 ± 0.02	0.25 ± 0.02
0.1	0.21 ± 0.03	0.20 ± 0.02	0.23 ± 0.04	0.21 ± 0.02
0.05	0.14 ± 0.03	0.20 ± 0.01	0.16 ± 0.03	0.19 ± 0.01

[a] Hens on basal diet.
[b] Hens on basal diet plus orotic acid.
[c] Each value is the mean ± SE of replicates of 24 eggs layed by 54 hens in the last 2 weeks of one month trial. Means within the same level and breed followed by the same letter superscripts are significantly different ($P < 0.05$).

Table 5. Effect of orotic acid supplementation on egg total phospholipids in Hisex White and Hisex Brown laying hens.

Orotic acid suppl.	Hisex White		Hisex Brown	
	Control[a]	Experimental[b]	Control[a]	Experimental[b]
(%)	Egg total phospholipids/100 gm edible portion[c]		Egg total phospholipids/100 gm edible portion[c]	
2	4.90 ± 0.29	4.14 ± 0.21	4.62 ± 0.14	4.32 ± 0.14
1	4.80 ± 0.35	4.02 ± 0.13	4.68 ± 0.14	4.95 ± 0.14
0.5	4.98 ± 0.13	4.31 ± 0.09	4.73 ± 0.16	4.79 ± 0.08
0.1	5.14 ± 0.10	4.99 ± 0.06	5.74 ± 0.17	5.42 ± 0.19
0.05	4.78 ± 0.24	5.21 ± 0.18	4.50 ± 0.14	4.81 ± 0.19

[a] Hens on basal diet.
[b] Hens on basal diet plus orotic acid.
[c] Each value is the mean ± SE of 4 replicates of 24 egg samples layed by 54 hens in the last 2 weeks of one month trial. Means within the same level and breed followed by the same letter superscripts are significantly different ($P < 0.05$).

The results of this study establish the suitability of low OA diets for controlling egg production. On the basis of the present observations, the optimum dietary OA content for inducing elevation in egg laying would appear to be in the range of 0.05%. Diets containing between 0.1–2% OA did not significantly increase or decrease egg production.

Burgess *et al.* (2) when tested high levels of triparanol (0.5%) diet on Leghorn hens, noted that egg production ceased within 3 weeks. Dam *et al.* (4) used one tenth of this level (500 mg/kg diet), this resulted in a sharp decline in feed consumption and rate of laying of Coturnix quail. The same study (4) showed that when the level of triparanol was reduced to 5 mg/kg diet, the egg production was maintained at levels equal to that of the control. The results with respect to the response of mature females clearly show

enhanced egg lipid accumulations due to dietary OA. The low egg cholesterol may be explained by inhibition of endogenous cholesterol synthesis which is reflected in the transfer of hepatic cholesterol to the egg. The cholesterol-lowering effects of OA in eggs again resemble those observed with triparanol. The latter revealed a complete diminishing of egg cholesterol at 0.5% level diet in leghorns (2). When the concentration of triparanol was reduced to a level of 0.05% or 0.00005% in the diet of Coutanix quail, the egg cholesterol was reduced to 27 and 41% of the control, respectively (4).

These findings suggest that introducing a proper level of OA in the diet of laying hens will improve the rate of egg production and also could reduce egg cholesterol.

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تأثير حامض الأورتيك على انتاج البيض وعلى الدهنيات في الدجاج البياض

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مستخلـــص

أجري البحث التالي لدراسة تأثير قدرة حامض الأورتيك بتركيزات مختلفة على زيادة إنتاج البيض وتراكم اللبيدات (الدهنيات) في نوعين من الدجاج البياض (هاي سيكس وايت والهاي سيكس براون) غذيت البدارى على عليقة خالية من الكولسترول لمدة ستة عشر أسبوعاً ثم بعد ذلك قسم كل نوع إلى مجموعتين. إحدهما إستمرت على العليقة الخالية من الكولسترول والثانية أعطيت نفس العليقة مع إضافة نسب مختلفة من حامض الأورتيك بنسبة ٢ ، ١ ، ٠,٥ ، ٠,١ ، ٠,٠٥٪ بالتوالي لمدة شهر على كل تركيز من هذه التركيزات.

وقد كانت الإستجابة لإضافة حامض الأورتيك للعليقة تأثير جوهري على إنتاج البيض في كلا النوعين على مستوى ٠,٠٥٪. وقد كانت نسبة الزيادة في إنتاج البيض ١٥ و١٤٪ يومياً في الهاي سيكس وايت والهاي سيكس براون على التوالي.

ولقد كان تأثير إضافة حامض الأورتيك على كولسترول البيض أكثر استجابة في الهاي سيكس وايت عن الهاي سيكس براون وقد كان انخفاض كولسترول البيض بصفة عامة لكل التركيزات بالنسبة التي استخدم فيها حامض الأورتيك بين ٢٨ و٣٥٪ — بالنسبة للهاي سيكس وايت وبين ٢١ و٢٦٪ عند مستوى ١,٢ و٠,٥٪ بالنسبة للهاي سيكس براون. هذا الإنخفاض في كولسترول البيض كان مصحوباً بارتفاع في نسبة اللبيدات المتعادلة الكلية والأحماض الدهنية الحرة. ولم يلاحظ اي تغير في نسبة الفوسفولبيدات الكلية في البيض.