Scientific Papers. Series D. Animal Science. Vol. LX, 2017
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

EVALUATING ISOFLAVONES ON CHOLESTEROL AND FAT DEPOSITION IN EGG YOLK DURING LAST FAZE OF EGG

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Abstract

The average content of cholesterol per egg varied from 153.45 to263.90 mg and it varies depending on genotype and, mainly,on the diet. During past decades, there are a lot of experiments with different supplementation of the diet (mineral, probiotic, vegetable oil) of laying hens to decreased the level of the cholesterol in the egg yolk. This experiment was performed to evaluate dietary daidzein and genistein on cholesterol and fat deposition in egg yolk during late phase of egg production. A total of 80 ISA Brown Laying hens 63-wks old were randomly assigned to 4 treatment groups containing 20 hens each. Birds were fed commercial feed diet containing: 0 (BF), 1000 (BF +1000 mg SI), 2000 (BF +2000 mg SI) and 3000 (BF +3000 mg SI) supplemented isoflavones. Water was offered for ad libitum consumption throughout the experiment. Yolk cholesterol and yolk total fat was monitored during the three month period. The supplemented isoflavones in the feed decreased the content of yolk cholesterol during the 3-month feeding trial ($P<0.05$). The supplemented isoflavones has not any influence on the concentration of fat in the egg yolk and egg yolk mass. Therefore, supplementation of the commercial feed with isoflavones could be used as a tool for the reduction of the yolk cholesterol.

Key words*: isoflavones, cholesterol, fat, egg yolk.*

INTRODUCTION

During past decades, the lipids composition (30–40%) of chicken egg has been an area of consumer concern, due to the relationship between specific dietary lipids and the development of coronary heart diseases, atherosclerosis, increasing stroke rate, high proportion of gallstones, enhancing depression rate and as a consequence is deleterious to human health and life expectancy (Imran M. et al., 2015). Vorlova et al., (2001) reported that theaverage content of cholesterol per egg was from 153.45 to 263.90 mg and it varies depending on genotype and, mainly,on the diet (Campo, 1995; Pesti and Bakalli, 1998). Recently, different dietary supplementations as probiotic strains (Mikulski etal., 2012; Abdelqader et al., 2013; Lei et al., 2013), vegetable oils (Faitarone et al., 2013) and fermented feed ingredient (Loh et al., 2009; Zhao et al., 2013) have been used to decreased the egg yolk cholesterol content. Soy isoflavones as functional phytoestrogenic products content in soybean preventing certain types of cancer (Adlercreutz, 1995), reducing the risk of osteoporosis (Adlercreutz, 1995), mineral regulation (Greendale et al., 2002) and also decreasing plasma cholesterol (Ho et al., 2000) in human population.

The objective of this experiment was to evaluate the influence of the isoflavones (dietary daidzein and genistein) on the egg cholesterol content and fat concentration in the yolk during late faze of egg production.

MATERIALS AND METHODS

The experiment was performed with ISA Brown laying hens, 63 weeks old at the beginning of the experiment. The experimental laying hens were randomly assigned to 4 groups, 20 birds per group. The laying hens were housed in laying cages (2 birds per cage)

in a standard poultry house set to a 16L:8D cycle. The laying hens were fed 120 g basal feed per day (control group) and the same amount of the isoflavones supplemented feed per hen of the experimental groups. Water was offered for ad-libitum consumption throughout the experiment. The experiment was conducted under permitted ethical regulations and rules. The experiment was lasting three months.

Laying hens were randomly assigned to receive basal feed (without supplemented isoflavones), and 1000, 2000 and 3000 mg/kg supplemented isoflavones in feed. The experimental feed was supplemented with concentrated product, 408.8g isoflavones per kg product, produced by the North China Pharmaceutical Corporation. The isoflavone composition of the product is presented in Table 1.

Table 1.Composition of the isoflavonic product

Isoflavone	g/kg
1. Genistin	73.0
2. Genistein	12.6
3. Daidzin	221.2
4. Daidzein	17.4
5. Glycitin	80.1
6. Glycitein	4.5
7. Total	408.8

The composition and nutritive value of the experimental diet is presented in Table 2.

Table 2.Composition and nutritive value of the experimental diet

Ingredients, g/kg	Basal feed (BF)
Maize	430.1
Soybean meal, 44% protein	144.3
Sunflower meal, 33% protein	153.0
Wheat bran	107.0
Vegetable oil	43.2
Methionine, 99%	0.40
Calcium carbonate	99.4
Mono calcium phosphate	7.6
$NaHCO_3$	3.0
Potassium carbonate	0.9
Zeolites	3.0
Salt	1.9
Vitamin and mineral mixture	5.0
Isoflavones, 40%	0.0
Total	1000
Chemical composition, calculated	
Dry matter, g/kg	903.1
Metabolic energy, Kcal/kg	2750
Crude protein, g/kg	165.0
Crude fat, g/kg	65.2
Calcium, g/kg	40.0
Phosphorus (available), g/kg	3.0
Lysine, g/kg	7.4
DL Methionine, g/kg	3.6
Methionine + cystine, g/kg	6.1

The control group was blank and fed without SI (basal diet - BF) in the feed and the other 3 experimental groups was fed with SI in the feed in amount of 1000, 2000 and 3000 mg in kg feed.

Concentration of total fat and cholesterol was measured in the yolks produced from the experimental hens. Egg samples of 6 eggs per group, were collected at the beginning and at the end of 1st, 2nd, and 3rdmonth. The eggs were measured, cracked, the yolks were separated and measured, then mixed, homogenized, stored frozen and analyzed up to 7 days.

The total cholesterol in the egg yolks was measured using the modified method according Washburn and Nix (1974) and Pearson et al. (1953). Briefly, total lipid was extracted by solution of chloroform and methanol 2:1 (v/v). Cholesterol determination wasdone using a commercial test kit for cholesterol analysis (BioSystems S.A., Barcelona, Spain) and analyzed spectrophotometrically at the wavelength of 625 nm.

The results were reported as means ±SEM.

The total fat was analyzed with extraction with diethyl ether according Soxhlet protocol.

Statistical analysis was performed by Statgraph 3 software package.

One-way analysis of variance (ANOVA) was used for the differences between groups. When the F values were significant, the Duncan's Multiple Range Test was performed.

RESULTS AND DISCUSSIONS

From the obtain data reported in table 3, there can be clear noticed the reduction of cholesterol concentration per gram of yolk, from the 1st till the 3rd month of experiment in the groups fed with supplemented isoflavones in the feed.

The inclusion of 1000, 2000, and 3000 mg isoflavones per kg of feed, at the end of the experiment (3rd month) significantly affect the yolk cholesterol contents expressed as mg/g of yolk or as mg/yolk) ($P<0.05$).

There is reduction of the cholesterol in egg yolk in the experimental groups fed with diet supplemented with isoflavones (1000 mg, 2000 mg and 3000 mg).

Table 3. Content of cholesterol in egg yolk produced from the experimental hens

	At the begining	1st month	2nd month	3rd month
Basal feed (BF)				
Yolk, g	16.92±0.61	17.85±1.52	17.23±0.96	16.72±1.00
mg/g yolk	15.04±0.21	14.72±1.31a	13.73±0.59a	14.39±1.37a
mg/yolk	254.44±2.63	262.75±2.38a	236.57±3.88	240.60±3.68a
BF +1000 mg SI/kg				
Yolk, g	16.90±0.66	17.86±1.09	17.95±1.22	17.73±0.35
mg/g yolk	14.43±0.36	16.53±1.58a	12.30±2.38a	12.74±0.54b
mg/yolk	243.87±2.05	295.29±3.11a	220.85±4.25	225.83±3.51b
BF +2000 mg SI/kg				
Yolk, g	17.46±0.03	17.10±1.93	17.55±1.07	17.13±1.67
mg/g yolk	15.54±0.50	12.85±0.24b	11.00±2.38b	12.68±0.46b
mg/yolk	271.40±2.75	219.68±4.02b	193.10±4.17	217.26±3.88b
BF +3000 mg SI/kg				
Yolk, g	16.17±1.18	17.67±1.50	16.65±1.67	17.08±0.69
mg/g yolk	14.38±1.15	12.31±0.66b	13.28±0.38ab	11.00±0.23b
mg/yolk	232.52±3.52	217.46±2.67b	221.10±3.38	187.93±3.93b

SI, supplemented isoflavonesValues are means ± S.D
a, b – values in the same column with no common superscript differ significantly (P<0.05).

The content of the total fat in 100 g egg yolk in the control group at the end of the experiment was 25.50 g, and in the experimental groups fed with different amount of supplemented diet were 28.50 g, 28.78 g and 27.95 g in group with 1000 mg, 2000 mg and 3000 mg supplemented isoflavones, respectively. The diet had no significant influence on total fat in the egg yolk (P>0.05). The obtain results are presented in Table 4.

Table 4.Content of total fat in egg yolk produced from experimental hens

	At the begining	1st month	2nd month	3rd month
Basal feed (BF)				
Yolk, g	16.92±0.61	17.85±1.52	17.23±0.96	16.72±1.00
g/yolk	4.95±0.08	5.25±0.13	4.26±0.07	4.26±0.12
g/100g	29.23±0.48	29.42±0.74	24.73±0.43	25.50±0.70
BF + 1000 mg SI/kg				
Yolk, g	16.90±0.66	17.86±1.09	17.95±1.22	17.73±0.35
g/yolk	5.07±0.21	5.10±0.11	4.87±0.07	5.05±0.01
g/100g	30.00±1.23	28.55±0.59	27.16±0.39	28.50±0.04
BF + 2000 mg SI/kg				
Yolk, g	17.46±0.03	17.10±1.93	17.55±1.07	17.13±1.67
g/yolk	5.22±0.01	5.15±0.06	5.09±0.03	4.93±0.07
g/100g	29.90±0.02	30.14±0.36	29.01±0.18	28.78±0.43
BF + 3000 mg SI/kg				
Yolk, g	16.17±1.18	17.67±1.50	16.65±1.67	17.08±0.69
g/yolk	4.67±0.04	5.14±0.11	4.59±0.04	4.77±0.17
g/100g	28.88±0.28	29.11±0.60	27.54±0.25	27.95±0.97

SI, supplemented isoflavones; Data statistically insignificant (P>0.05)

The content of total fat in the egg yolk in the experimental groups has a similar trend as a content of total fat in the control group.

There are a few investigation conducted to investigate the effect of additional isoflavones on egg-yolk cholesterol.

The present results demonstrated that inclusion of isoflavones in different addition levels in layer diet decreased significantly the yolk cholesterol after the 3 months feeding period (P<0.05) (Figure 1).

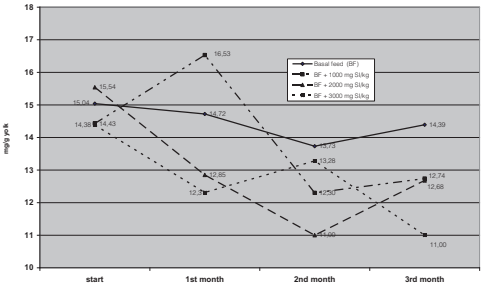

Figure 1. Changes in yolk cholesterol content of layers fed with supplemented isoflavones

However, there was no significant difference in cholesterol contents among the treatments with supplemented 1000, 2000 and 3000 mg kg^{-1} isoflavones in basal feed. Egg cholesterol content (mg/g yolk) decreased by 23.56% in the group fed with the supplementation of 3000 mg/kg isoflavones in the diet. The decreasing of 19% was reported by Yin et al. (2004) in his study when the hens were fed with addition of 40 mg/kg daidzein in the diet. Also Fujiwara et al. (2008) reported the suppression of yolk cholesterol by adding a fermented soybean "Natto" supplement in the layer diet. Our finding of decreasing of yolk cholesterol are in agreement with these mentioned studies. Other study was conducted by Hong H. et al. (2010) with fermented soybean with *A. Oryzae* and with *B. subtilis var. Natto.* The results showed that egg cholesterol content in egg yolk was lower (p<0.05) than those in the control group.There were no significant differences in total fat in egg yolkin mentioned study. Kanpai et al. (2004) reported that administration of White KwaoKrua (*Pueraria mirifica*) in feed which containing potent phytoestrogen not influenced significantly in egg yolk cholesterol content among the experimental groups. Nasra et al. (2010) conducted experiment with fenugreek and licorice which are source of phytoestrogens, and the major findings in this study are that the yolk percent of the egg was decreased significantly by 0.5% fenugreek compared to control group. The significant differences between day 0 and day 3 (P<0.05) in the content of yolk cholesterol are also reported by Saitoh et al. (2001)in the

experiment conducted with diet contained high concentration of soy isoflavones, but no significant differences are noticed among days 0, 1, 6, 12 and 18 ($P>0.05$). Disagreement about the results may be due of different amount and source of isoflavones and different rearing periods of the animals.

CONCLUSIONS

The results demonstrate that supplementation diet with isoflavones has a beneficial effect. The supplemented isoflavones in the diet reduced the content of yolk cholesterol during the 3-months feeding period ($P<0.05$).

There are no differences between the concentration of the total fat in the yolk of the control group and the fat concentration in the yolk of the experimental groups. The supplemented isoflavones in the diet has not affect the concentration of fat in the egg yolk.

Therefore, our findings suggest that supplemented diet with isoflavones for laying hens in late faze of egg production improve the egg quality to provide low-cholesterol eggs.

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