EFFECT OF SUPPLEMENTED ISOFLAVONES IN FEED ON PLASMA AND EGG CHOLESTEROL CONCENTRATION IN ISA BROWN LAYERS

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Abstract. This experiment was performed to investigate the influence of the dietary daidzein and genistein on the plasma cholesterol and egg cholesterol concentration. A total of 80 ISA Brown Laying hens 27-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. Plasma cholesterol, yolk cholesterol and yolk total fat was monitored during the three month period. The average content of cholesterol in the plasma of the control hens was lower, then in the plasma of the experimental groups. The supplemented isoflavones in the feed reduced the content of yolk cholesterol during the 3-month feeding period ($P<0.05$). The supplemented isoflavones have no influence on the concentration of fat in the egg yolk. Therefore, supplementation of the commercial feed with isoflavones could be used as a tool for the reduction of the yolk cholesterol.

Keywords: isoflavones, cholesterol, laying hens

Introduction. Soy isoflavones are functional phytoestrogenic products that have some properties similar with estrogens. Isoflavones are diphenolic compounds in aglycone (unconjugated) and conjugated forms (Kudou et al., 1991; Shigemitsu et al., 1991). The aglycone forms of isoflavones are daidzein, genistein and glycitein. The benefits of soybean on the human health are: prevention of certain types of cancer (Adlercreutz, 1995), reduction of the risk of osteoporosis (Adlercreutz, 1995), mineral regulation (Greendale et al., 2002), decrease of plasma cholesterol (Ho et al., 2000), decrease of stress because of the antioxidant activity of some soybean substances (Arora et al., 1998) and a lot of other physiological effects. A large proportion of soybean is composed in the animal feed. The isoflavones from the soybean can be transferred into the animal products such as meat products (Kuhnle et al., 2008), milk (Flachowsky et al., 2011; Kuhnle et al., 2008), and eggs (Sanshiroh et al., 2001; Lin et al., 2004; Kuhnle et al., 2008). Vorlova et al., (2001) reported that the average 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).

The objective of this experiment was to investigate the influence of the dietary daidzein and genistein on the plasma cholesterol, egg cholesterol concentration, and fat concentration in the yolk.

Materials and Methods

The experiment was performed with ISA Brown laying hens, 27 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 lasted for three months.

The 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 Table1.

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.

The basal diet was the control, and the supplemented basal diets were experimental.

The concentration of cholesterol was measured in the blood of the experimental hens. For this purpose blood

samples were taken from the marked hens directly from the heart (cardiac puncture) at the end of 1st, 2nd and 3rd month. Blood samples were centrifuged 15 minutes on 3500 rpm, not later than one hour after the cardiac puncture and the collected plasma was frozen at –20°C in a freezer until analysis. The content of the plasma cholesterol was analyzed using suitable ready kit BioSystems S.A., Barcelona, Spain.

Table 2. **Composition and nutritive value of the experimental diet**

Ingredients, g/kg	Basal feed (BF)	BF + 1000 mg SI/kg	BF + 2000 mg SI/kg	BF + 3000 mg SI/kg
Maize	400.1	397.6	395.1	392.6
Soybean meal, 44% protein	189.6	189.6	189.6	189.6
Sunflower meal, 33% protein	150.0	150.0	150.0	150.0
Wheat bran	94.0	94.0	94.0	94.0
Vegetable oil	51.0	51.0	51.0	51.0
Methionine, 99%	1.20	1.20	1.20	1.20
Calcium carbonate	91.2	91.2	91.2	91.2
Mono calcium phosphate	11.0	11.0	11.0	11.0
$NaHCO_3$	1.5	1.5	1.5	1.5
Zeolites	3.0	3.0	3.0	3.0
Salt	2.4	2.4	2.4	2.4
Vitamin and mineral mixture	5.0	5.0	5.0	5.0
Isoflavones, 40%	0.0	2.5	5.0	7.5
Total	1000	1000	1000	1000
Chemical composition, calculated				
Dry matter, g/kg	904.3	904.3	904.3	904.3
Metabolic energy, Kcal/kg	2800	2792	2784	2776
Crude protein, g/kg	179.0	178.0	178.0	174.0
Crude fat, g/kg	71.5	71.5	71.5	71.5
Calcium, g/kg	37.5	37.5	37.5	37.5
Phosphorus (available), g/kg	3.8	3.8	3.8	3.8
Lysine, g/kg	8.5	8.5	8.5	8.5
DL Methionine, g/kg	4.6	4.6	4.6	4.6
Methionine + cystine, g/kg	7.3	7.3	7.3	7.3
Supplemented isoflavones, mg/kg	0	1000	2000	3000
Soybean meal isoflavones, mg/kg [1]	43.6	43.6	43.6	43.6
[1] Isoflavones from soybean meal were calculated; SI - supplemented isoflavones				

The concentrations of total fat and cholesterol were measured in the yolks produced from the experimental hens. Egg samples of 6 eggs per group, were collected at the end of 1st, 2nd, and 3rd month. 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). The lipid extract was treated according the method of Pearson et al. (1953) 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.

Data were tested for significance using the analysis of variance, the F-test according to Snedecor and Cochran (1989).

Results

The data for effect of the supplemented isoflavones on concentration of the plasma total cholesterol over the 3 months period are presented in Table 3. None of the parameters were affected significantly by the level of isoflavones in the diet at any of the time points tested ($P>0.05$).

Table 3. **Effect of supplemented isoflavones on plasma cholesterol**

	1st month	2nd month	3rd month	Average
Basal feed (BF)				
mmol/l	3.38±1.36	2.47±0.30	2.29±1.37	2.71±1.30
BF + 1000 mg SI/kg				
mmol/l	4.06±1.07	4.01±0.60	3.99±0.30	4.04±0.91
BF + 2000 mg SI/kg				
mmol/l	3.98±1.55	5.40±2.05	2.57±0.57	4.07±1.70
BF + 3000 mg SI/kg				
mmol/l	3.59±0.91	3.68±0.49	2.31±0.30	3.42±0.91
SI, supplemented isoflavones Values are means ± S.D Data statistically insignificant (P>0.05)				

The concentration of the plasma cholesterol in the experimental groups during the 3 months of the experiment had a trend of decreasing. The concentration of the plasma cholesterol in the control group was from 3.38 mmol/l in the 1st month to 2.29 mmol/l in the 3rd month in the experimental hens fed with supplemented diets, 4.06 to 3.19 mmol/l in the group with additional 1000 mg supplemented isoflavones in the diet, 3.98 to 2.57 mmol/l in the group with additional 2000 mg isoflavones per kg of feed, and 3.59 to 2.31mmol/l in the group with additional 3000 mg isoflavones in the diet. But, the values of the average content of cholesterol in the plasma for the whole experimental period of the control hens were lower than in the plasma of the experimental groups.

The obtained data reported in Table 4 show an obvious 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. But there was an increase of total cholesterol per egg in the control group (from 196.29 to 201.65 mg). The inclusion of 1000, 2000, and 3000 mg isoflavones per kg of feed, significantly affected the yolk cholesterol contents expressed as mg/g of yolk or as mg/yolk) (P<0.05). There was a reduction of the total cholesterol in egg yolk in the experimental groups fed with diet supplemented with isoflavones (1000 mg, 2000 mg and 3000 mg).

Table 4. **Content of cholesterol in egg yolk produced from the experimental hens**

	1st month	2nd month	3rd month	Average
Basal feed (BF)				
Yolk, g	13.33±0.20	17.72±0.43	17.17±0.24	14.82±2.10
mg/g yolk	14.73±1.12	13.17±0.69	11.71±0.05	13.90±1.52a
mg/yolk	196.29±14.98	233.60±17.91	201.65±1.92	204.04±19.63a
BF + 1000 mg SI/kg				
Yolk, g	14.10±0.62	17.23±0.27	16.43±0.28	15.19±1.51
mg/g yolk	13.10±0.32	7.86±3.14	9.65±1.23	11.53±2.49b
mg/yolk	186.64±11.65	134.99±52.04	172.59±17.18	172.36±28.67b
BF + 2000 mg SI/kg				
Yolk, g	13.80±0.83	16.66±0.25	17.44±0.64	14.98±1.79
mg/g yolk	12.72±1.09	8.78±2.91	9.97±1.84	11.74±2.09b
mg/yolk	175.45±13.23	145.91±46.29	197.15±39.33	173.77±27.20b
BF + 3000 mg SI/kg				
Yolk, g	14.23±0.92	17.24±0.38	17.93±0.07	15.57±1.88
mg/g yolk	12.73±0.98	7.80±1.23	8.46±1.38	11.08±2.39b
mg/yolk	181.07±6.74	134.33±18.16	169.15±25.41	168.94±22.08b
SI, supplemented isoflavones Values are means ± S.D a, b – values in the same column with no common superscript differ significantly (P<0.05).				

It is also important to note that the eggs from the control (without dietary supplement) group of hens have larger concentration of cholesterol in the yolks (0.84 mg/g yolk) in comparison to all experimental (supplemented) groups (0.64 mg/g yolk).

The average content of the total fat in 100 g egg yolk in the control group was 28.43 g, and in the experimental groups fed with different amount of supplemented diet were 28.02 g, 28.48 g and 28.32 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 obtained results are presented in Table 5.

Table 5. **Content of total fat in egg yolk produced from experimental hens**

	1st month	2nd month	3rd month	Average
Basal feed (BF)				
Yolk, g	13.33±0.20	17.72±0.43	17.17±0.24	14.82±2.10
g/yolk	3.74±0.32	5.22±0.08	4.91±0.07	4.27±0.73
g/100g yolk	28.03±2.26	29.47±0.45	28.58±0.40	28.43±1.79
BF + 1000 mg SI/kg				
Yolk, g	14.10±0.62	17.23±0.27	16.43±0.28	15.19±1.51
g/yolk	3.87±0.35	5.09±0.08	4.65±0.05	4.27±0.60
g/100g yolk	27.40±1.66	29.56±0.45	28.33±0.28	28.02±1.54
BF + 2000 mg SI/kg				
Yolk, g	13.80±0.83	16.66±0.25	17.44±0.64	14.98±1.79
g/yolk	3.81±0.13	4.79±0.20	5.03±0.06	4.25±0.58
g/100g yolk	28.28±0.70	28.73±1.17	28.82±0.37	28.48±0.71
BF + 3000 mg SI/kg				
Yolk, g	14.23±0.92	17.24±0.38	17.93±0.07	15.57±1.88
g/yolk	3.96±019	4.74±0.03	4.91±0.03	4.30±0.47
g/100g yolk	28.93±0.95	27.48±0.17	27.36±0.14	28.32±1.06
SI, supplemented isoflavones; Data statistically insignificant (P>0.05)				

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

Discussion

There are few investigations about the effect of additional isoflavones on plasma and egg-yolk cholesterol. During the present research which lasted for 3 months, no significant alterations were observed in plasma cholesterol concentration. Some authors, using ingredients rich in phytoestrogens as supplements in the diet of laying hens, found that this application has no beneficial effect on plasma cholesterol concentration (Fujiwara et al., 2008; Shin et al., 2008). Shin et al. (2008) investigated the effect of the germinated and fermented unmarketable soybean on egg laying performance and egg quality. No significant differences were observed in the concentration of total cholesterol and HDL – cholesterol. On the contrary, Kanpai et al. (2004) reported that isoflavones had an effect on the plasma cholesterol. They conducted an experiment with administration of White Kwao Krua (*Pueraria mirifica)* which contains potent phytoestrogens in chicken feed. The results showed that plasma cholesterol was significantly different in the control group compared with the treated groups (P<0.05). The reduction of plasma cholesterol, in our experiment during the second and third month of the experimental period could be the result of increasing laying intensity, weight of the yolk, and total cholesterol deposition in the yolk.

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

However, there was no significant difference in cholesterol content among the treatments with supplemented 1000, 2000 and 3000 mg kg $^{-1}$ isoflavones in basal feed. Egg cholesterol content (mg/g yolk) decreased by 33.54% in the group fed with the supplementation of 3000 mg/kg isoflavones in the diet. Yin et al. (2004) reported that cholesterol levels in egg yolks decreased by 19 % when the hens were fed with addition of 40 mg/kg daidzein in the diet. Suppression of yolk cholesterol by adding a fermented soybean "Natto" supplement in the layer feed was reported by Fujiwara et al. (2008). Our finding of the reduction in yolk cholesterol agrees with these studies. Other study was conducted with ingredients which are rich with phytoestrogens. Kanpai et al. (2004) reported that administration of White Kwao Krua (*Pueraria mirifica*) in feed which contained potent phytoestrogen did not influence significantly the egg yolk cholesterol levels in the treatment groups. Hong et al. (2010) investigated the effect of the dietary soybean fermented with *Aspergillus oryzae* or *Bacillus natto* on egg lipid composition. The groups fed with fermented soybean showed reduction in cholesterol content of egg yolk. Egg yolk cholesterol was significantly lower (P<0.05) (1.411 and 1.387 mg/100g) than that of the control group (1.614 mg/100g).

The amount of total fat in egg yolk was not affected by the diet in the mentioned study. Nasra et al. (2010) conducted an experiment with fenugreek and licorice, which are source of phytoestrogens, and the major findings in this study are that the percent of yolk in the egg decreased significantly by 0.5 % with fenugreek compared to the control group. The significant differences between day 0 and day 3 (P<0.05) in the content of yolk cholesterol were noticed in the experiment conducted by Saitoh et al. (2001) with diet containing high concentration of soy isoflavones, but no significant differences were noticed among days 0, 1, 6, 12 and 18 (P>0.05).

The disagreement between the obtained results may be due to different amount and source of isoflavones and different rearing periods of the animals.

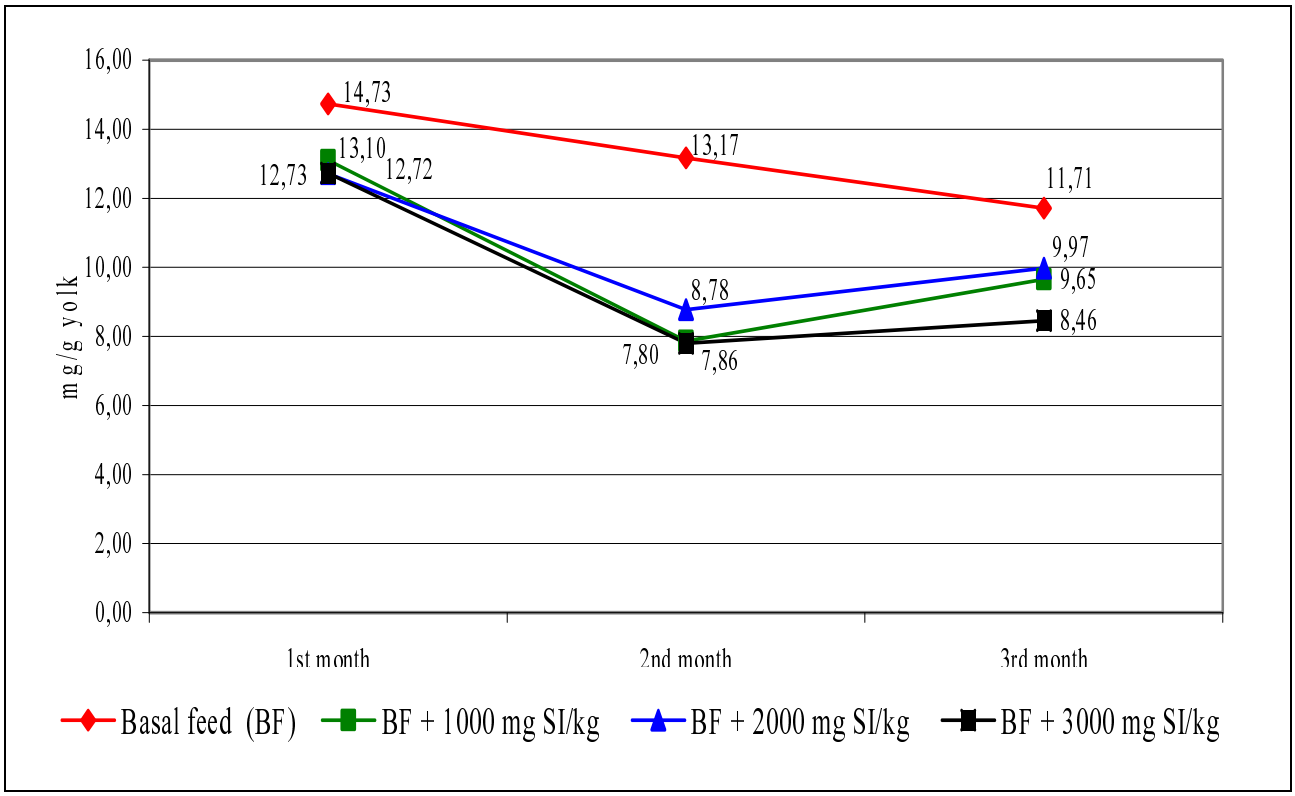

Fig 1. **Changes in yolk cholesterol content of layers fed with supplemented isoflavones**

Conclusions

The content of cholesterol in plasma of laying hens is not significantly influenced by the amount of additional isoflavones in the feed. The average content in the plasma of the control hens was lower then in the plasma of the experimental groups but the difference was insignificant ($P>0.05$).

The results demonstrate that supplementation with isoflavones in the diet of the experimental laying hens has a beneficial effect. The supplemented isoflavones in the feed 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 have no influence on the concentration of fat in the egg yolk.

Therefore, our findings suggest that enriched feed with isoflavones for laying hens improve the egg quality to provide low-cholesterol eggs.

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