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Effects of Isoflavones in Feed on Plasma Estradiol Concentration and Productive Performances in Laying Hens

Research Article

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ABSTRACT

Isoflavones are phytoestrogens which have estrogen-like activity in animals and humans with structure similar to 17-β-estradiol and capable of binding to estrogen receptors (ERs).This experiment was conducted to demonstrate the effect of isoflavones on estradiol concentration in blood and its influence on the productive performances in ISA Brown laying hens fed with supplemented feed with large amount of isoflavones. Isoflavones as non steroid phytoestrogens were added in large amounts to the feed of laying hens in 0, 1000, 2000 and 3000 mg kg^{-1} of feed. Estradiol concentration in blood of the isoflavone-treated chickens was significantly higher in comparison with the control group (P<0.01). The supplemented isoflavones in the feed have significant improvement on the egg weight during the 1st month (P<0.05) and 2nd month (P<0.01) and also for the whole experimental period (P<0.05). Further studies are required to investigate the effect of isoflavones on other reproductive hormones (follicle stimulating hormone (FSH) and progesterone) which are related with the egg production.

KEY WORDS estradiol, isoflavones, laying hens.

INTRODUCTION

Soy isoflavones are functional phytoestrogenic products that have some properties similar with estrogens. They have estrogen-like biological activity and can influence animal and human reproduction by changing the sexual behaviour and morphology as well as the function of reproductive organs. Pilsakova *et al.* (2010) demonstrated that isoflavones are natural phytoestrogens with structure similar to 17-β-estradiol and capable of binding to estrogen receptors (ERs). The research (Kurzer and Xu, 1997; Murkies *et al.* 1998) focus was that they have oestrogen-like biological activity and can influence animal's and human's reproduction by changing the sexual behavior and morphology as well as the function of reproductive organs. They may act as either estrogen agonists or antagonists depending on the dose, duration of use, individual metabolism, intrinsic estrogenic state, age of the animal, and number of repeated exposures as demonstrated (Barrett, 1996; Cassidy, 2003; Zhao *et al.* 2005). Dibb (1995) reported that the ability for the effects to be reversed also depends on the same factors. In addition to these effects of isoflavones, some other reports describe fertility-enhancing effects as well as reproductive problems (James *et al.* 1994) and thymic atrophy (Yellayi *et al.* 2002). This experiment was conducted to demonstrate the effect of isoflavones on estradiol concentration in blood and its influence on the productive performances of hens fed with supplemented feed with large amount of isoflavones.

MATERIALS AND METHODS

Experimental animals and rearing systems

The experiment was performed on ISA Brown laying hens in a commercial poultry farm. Eighty laying hens, 60 wks old, were divided into 4 groups, 20 in each (4 cages for a group), set to a 16L:8D cycle and fed with different amount of supplemented isoflavones in the feed. The control group 1 was blank and fed with no supplemented isoflavones in the feed and the other 3 experimental groups was fed with supplemented isoflavones in the feed in amount of 1000, 2000 and 3000 mg kg^{-1}. Feed consumption was restricted on 120 g/hen/d. Water was offered for *ad libitum* consumption throughout the experiment, which was conducted for three months.

The experiment is conducted under permitted ethical regulations and rules. The experimental feed was supplemented with a concentrated product, 40% isoflavones, produced by the north China Pharmaceutical Corporation. The isoflavone composition of the product is presented in Table 1. The composition and nutritive value of the basal diet was presented in Table 2.

Table 1 Composition of the 40% isoflavone product

Isoflavone	%
1. Genistin	7.30
2. Genistein	1.26
3. Daidzin	22.12
4. Daidzein	1.74
5. Glycitin	8.01
6. Glycitein	0.45
Total	40.88

Measurements of body weight and productive parameters

Body weights of the laying hens were determined on the beginning and end of the experiment. The results were reported as means ± SEM. Daily egg production was monitored during the whole experiment. Egg production is expressed as average hen-day production, calculated from the total eggs divided with the total number of hen-days. Feed conversion is expressed as g feed consumed/g egg produced, and conversion efficiency as g feed/g egg.

Egg collection and egg quality analysis

The 6 eggs were taken on the 3rd, 7th, 14th, 21st, 30th, 60th and 90th days. The eggs were weighed, and then yolks were separated with an egg separator and weighed. The shell was wiped clean and weighed. The albumen weight was also weight.

Blood collection and estradiol radioimmunoassay

Blood samples were taken from 5 laying hens in each group directly from the heart (cardiac puncture) on the 3rd, 7th, 14th, 21st, 30th, 60th and 90th days at 11 a.m. Blood samples were centrifuged at 419 g for 15 minutes, not more than one hour after the cardiac puncture and the collected plasma was kept deep-frozen prior to analysis. The experiment was conducted under permitted ethical regulations. A quantitative analysis of total serum estradiol was performed by 125I-based radioimmunoassay kit (IBL International GmbH, Hamburg, Germany) according to the instructions of the manufacturer.

Statistical analysis

Statistical analysis was performed by Stat graph 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. The results were considered significant at ($P<0.05$) and ($P<0.01$).

RESULTS AND DISCUSSION

The body weight and productive parameters

The effects of supplemented isoflavones on the productive performance of experimental layers are presented in Table 3.

Body weight changes at the end of the experiment were not significantly different ($P>0.05$) in the treated groups compared with the control. Egg production and daily egg mass production also were not significantly different ($P>0.05$) in hens treated with supplemented isoflavones compared with the control group.

The laying intensity was significantly different among the control group and group fed with diet supplemented with 1000 mg kg^{-1} isoflavones at the end of the experimental period ($P<0.05$).

In this study, the laying hens are kept in the same poultry house and the light regime, feed and water availability were the same. The number of the experimental laying hens was 20 birds in the beginning of the experiment. Opposite with to the effects of the soy isoflavones in some other experiments in both mammals and birds, there was no effect of the soy isoflavones on body weight changes in the end of the experiment.

This study is in agreement with experiment done by Wilhelms *et al.* (2006) in which quails are feed with supplemented isoflavones on 1% and 5% level. The authors reported that there is no effect of the isoflavones on the average daily gain in female Japaenese quails. The present study showed that isoflavones had significant improvement on egg production.

Table 2 Composition and nutritive value of the experimental diets

Ingredient, g/kg	Control	1000 SI	2000 SI	3000 SI
Yellow corn	431.3	428.8	426.3	423.8
Soybean meal, 44% protein	144.3	144.3	144.3	144.3
Sunflower meal, 33% protein	153.0	153.0	153.0	153.0
Wheat middilings	107.0	107.0	107.0	107.0
Soybean oil	43.2	43.2	43.2	43.2
Methionine, 99%	0.40	0.40	0.40	0.40
Calcium carbonate	99.4	99.4	99.4	99.4
Mono calcium phosphate	7.6	7.6	7.6	7.6
$NaHCO_3$	3.0	3.0	3.0	3.0
Potassium carbonate	0.90	0.90	0.90	0.90
Zeolites	3.0	3.0	3.0	3.0
Salt	1.9	1.9	1.9	1.9
Vitamin and mineral premix	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				
Dry matter, g kg^{-1}	903.1	903.1	903.1	903.1
Metabolic energy, kcal kg^{-1}	2750	2742	2734	2726
Crude proteins, g kg^{-1}	165.0	164.0	163.0	162.0
Crude fat, g kg^{-1}	65.2	65.2	65.2	65.2
Crude fiber, g kg^{-1}	70.0	70.0	70.0	70.0
Total ash, g kg^{-1}	130.0	130.0	130.0	130.0
Calcium, g kg^{-1}	40.0	40.0	40.0	40.0
Phosphorus (available), g kg^{-1}	3.0	3.0	3.0	3.0
Lysine, g kg^{-1}	7.4	7.4	7.4	7.4
DL-methionine, g kg^{-1}	3.6	3.6	3.6	3.6
Methionine + cysteine, g kg^{-1}	6.1	6.1	6.1	6.1
Supplemented isoflavones, mg kg^{-1}	0	1000	2000	3000
Soybean meal isoflavones, mg kg^{-1*}	32.2	32.2	32.2	32.2

* Native isoflavones in soybean meal.
SI: supplemented isoflavones.

Table 3 Productive performance of the experimental hens

Number of experimental hens	Control	1000 SI	2000 SI	3000 SI
Initial	20	20	20	20
Final	15	17	18	19
Average body weight, g				
Initial	2049±204	1842±256	2063±204	2016±204
Final	1940±176	1851±220	1919±168	1860±178
Average egg production, number	66.88	59.04	52.83	59.65
Average produced egg mass, g/d	49.74	46.91	40.50	44.34
Laying intensity, %				
Initial	64.28	59.28	57.14	66.43
First month	74.04	55.71	63.63	60.15
Final	72.22^{a}	80.39^{b}	68.42^{a}	75.00^{a}
Feed expenditure				
Feed consumption, g/d	120	120	120	120
Feed consumption, g/d	161.49	182.93	204.43	181.08
Feed conversion ratio, g feed/g egg	2.42	2.55	2.94	2.71

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SI: supplemented isoflavones.

This is in agreement with the experiment conducted by Akintola *et al.* (2011) who reported that oestrogen in little doses can stimulate the egg production. The study conducted by Cai *et al.* (2013) with Hyline Brown laying hens fed with diets supplemented with 10 mg kg^{-1}, 50 mg kg^{-1} and 100 mg kg^{-1} daidzein provide an evidence that dietary daidzein significantly effected on egg production during the post peak period of egg laying. In our study the large doses of supplemented isoflavones in the feed reduced the egg production observed in the treatments with 2000 and 3000 mg SI kg^{-1} feed. Chickens are reported to be more sensitive to multiple toxicants, so in the present study the large doses of supplemented isoflavones demonstrate adverse effect on laying egg production. Also, the supplementing isoflavones affect the feed conversion efficiency in comparison with the control.

Egg components analysis

The effect of the isoflavones on the egg components of experimental eggs was presented in Table 4. Differences of the egg weight among the control group and groups fed with supplemented isoflavones were significant during the 1st month (P<0.05), 2nd month (P<0.01) and average for the whole experimental period (P<0.05). During the 2nd month the differences of the egg white weight among the control and groups fed with supplemented isoflavones are noticed (P<0.05). In this experiment average weight of experimental eggs and white egg weight were significantly increased in the experimental groups fed with supplemented diets with 1000, 2000 and 3000 mg kg^{-1} isoflavones (Table 4). The improvement of these two egg quality characteristics was therefore probably due to increases the albumen formation which is the biggest egg component. Previous studies from Saitoh *et al.* (2001) and Zhao *et al.* (2005) have also shown that soy isoflavone supplementation improved egg production and egg quality in poultry.

Estradiol concentration

The content of the estradiol in the plasma of the experimental laying hens is presented in Table 5.

Table 4 Comparison of egg components during the experimental period

Egg components[1]		Control[1]	1000 SI[1]	2000 SI[1]	3000 SI[1]
1st month	Egg weight, g	64.15±4.33[a]	67.70±5.69[b]	69.27±5.21[b]	67.56±4.51[b]
	Egg white, g	40.03±3.85	42.34±4.52	43.64±4.20	42.66±3.31
	Egg yolk, g	17.37±1.31	17.83±1.34	18.57±1.92	17.80±1.60
	Egg shell, g	6.74±0.86	7.53±0.67	7.06±0.91	7.10±0.51
2nd month	Egg weight, g	67.62±3.66[a]	73.96±1.77[b]	71.50±5.28[b]	70.09±5.81[b]
	Egg white, g	43.84±4.45[a]	48.47±1.76[b]	45.76±3.08[b]	45.35±4.49[b]
	Egg yolk, g	16.92±0.96	17.84±0.91	18.00±1.91	17.23±1.58
	Egg shell, g	6.85±1.29	7.65±0.24	7.74±0.79	7.51±0.79
3rd month	Egg weight, g	66.28±1.59	68.96±3.74	67.99±4.93	66.11±5.17
	Egg white, g	42.14±1.40	43.21±3.20	42.83±4.04	42.25±3.66
	Egg yolk, g	16.94±0.73	17.94±1.22	17.55±1.07	15.65±1.67
	Egg shell, g	7.21±0.46	7.80±0.30	7.60±0.53	7.21±0.44
Average	Egg weight, g	65.07±4.11[a]	69.03±5.37[b]	69.44±5.13[b]	67.77±4.89[b]
	Egg white, g	41.01±3.91	43.57±4.52	43.88±3.99	43.10±3.67
	Egg yolk, g	17.23±1.18	17.85±1.22	18.28±1.79	17.47±1.62
	Egg shell, g	6.83±0.83	7.60±0.56	7.28±0.87	7.20±0.56

[1] Values are means ± standard deviation (SD).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SI: supplemented isoflavones.

Table 5 Concentration of estradiol in the plasma of experimental laying hens

Period	Control[1]	1000 SI[1]	2000 SI[1]	3000 SI[1]
1st month	0.29±0.19	0.43±0.10	0.41±0.09	0.47±0.13
2nd month	0.39±0.04	0.37±0.06	0.39±0.12	0.34±0.06
3rd month	0.34±0.09	0.42±0.10	0.42±0.11	0.41±0.09
Average	0.32±0.16[a]	0.42±0.10[b]	0.40±0.09[b]	0.41±0.12[b]

[1] Values are means ± standard deviation (SD).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SI: supplemented isoflavones.

The average amount of estradiol was significantly higher (P<0.01) in the plasma of laying hens fed with 1000 mg, 2000 mg and 3000 mg of supplemented isoflavones per kg feed, compared with those plasma samples of layers fed the control feed, but differences among the groups fed with supplemented isoflavones groups were not found. Stimulation of estrogen production by genistein and daidzein is probably due to estrogen receptor blocking effects of both phytoestrogens. In our findings the supplemented feed with 1000, 2000 and 3000 mg isoflavones per kg significantly increased the average amount of estradiol in the plasma of the experimental laying hens.

CONCLUSION

In conclusion, data obtained from the present study showed that genistein and daidzein supplemention in feed of laying hens could affect the concentration of estradiol in the blood plasma, egg production and egg weight. The average amount of estradiol in the plasma of the control group was low (0.31 ng mL^{-1}) and in the other groups was 0.42, 0.40 and 0.42 ng mL^{-1} with supplemented feed with 1000, 2000 and 3000 mg isoflavones, respectively. The differences in the average amount of estradiol among the control and isoflavone-treated groups were significant (P<0.01). The experiment revealed the significant improvement on egg production with 1000 mg SI kg^{-1} feed over the control group and the large dosages of SI (2000 and 3000 mg SI kg^{-1}). It can be concluded that isoflavones had significant improvement on egg production at low dosage of isoflavones 1000 mg kg^{-1} and significant improvement on the egg weight during the 1^{st} and 2^{nd} months and also for the whole experimental period. Further studies are required to investigate the effect of isoflavones on other reproductive hormones (FSH and progesterone) which are related with the egg production.

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