Journal of Animal and Feed Sciences, 22, 2013, 144–148
The Kielanowski Institute of Animal Physiology and Nutrition, PAS, Jabłonna

Transfer of daidzein and genistein from feed into the egg yolk of hens

N. Gjorgovska[1,3] and F. Kiril[2]

Ss Cyril and Methodius University in Skopje,
[1] Institute of Animal Science,
bul. Ilinden br. 92a, 1000 Skopje, Macedonia
[2] Faculty of Agricultural Sciences and Food
bul. Aleksandar Makedonski bb, 1000 Skopje, Macedonia

KEY WORDS: isoflavones, daidzein, genistein, transfer, eggs, laying hens

Received: 17 July 2012
Revised: 13 February 2013
Accepted: 17 June 2013

[3] Corresponding author:
e-mail: natashagjorgovska@gmail.com

ABSTRACT. Soya isoflavones are compounds with phytoestrogenic characteristics, i.e. because of their structure, their effects are similar to those of animal estrogens. This is why they are used for medicinal purposes in aging women. The presented experiment with laying hens was conducted to demonstrate the possibility of transferring soya isoflavones from feed into egg yolk to produce isoflavone-enriched eggs. Laying hens were randomly assigned to a control and 3 experimental groups and fed a basal diet or the basal diet supplemented with different concentrations of isoflavones: 1000, 2000 or 3000 mg · kg^{-1} feed. The transfer of isoflavones (daidzein and genistein) from the feed into the egg yolk of treated hens was found to be significant. At the end of the experiment, the content of the analysed isoflavones was from 396.0 to 412.4 µg.

Introduction

Soya isoflavones are functional phytoestrogenic products with a similar structure and function as human oestrogens. The benefits of soya bean for human health include prevention of certain types of cancer (Adlercreutz, 1995), reduction of the risk of osteoporosis (Adlercreutz, 1995), mitigation of climacteric symptoms (Burton and Wells, 2002), regulation of mineral levels (Greendale et al., 2002), reduction of plasma cholesterol (Ho et al., 2000), stress relief because of the antioxidant activity of some soya bean substances (Arora et al., 1998). 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).

Some authors have suggested that isoflavones cause growth problems and mortality in birds (Payne et al., 2001). They also reported that isoflavones influence carcass characteristics and broiler growth.

Lin et al. (2004) reported that isoflavones could be transferred into chicken and quail eggs. The transfer of isoflavones into egg yolk was also reported by Sanshiroh et al. (2001), who noticed that isoflavone transfer peaked on the 10th day of administration to poultry. Woo-Keun et al. (2004) analysed the possibility of transferring genistein and daidzein from soya germ into egg yolk and reported that 5% to 10% supplementation of soya germ to hen feed significantly increased the amount of these isoflavones in the yolk.

This experiment was conducted to demonstrate isoflavone transfer into egg yolk with the aim to examine the possibility of enriching eggs with these compounds (daidzein and genistein).

Material and methods

The experiment was performed on ISA Brown laying hens, 27 weeks old at the beginning of the experiment, randomly assigned to 4 groups, 20 birds per group. The 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 isoflavone-supplemented feed per hen in the experimental groups. Water was offered for *ad libitum* consumption throughout the experiment, which was conducted for three months.

Laying hens were randomly assigned to receive basal feed (without additional isoflavones), and 1000, 2000 or 3000 mg supplemented isoflavones per kilogram of feed. 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.

Table 1. Composition of the 40% isoflavone product

Isoflavone	%
Genistin	7.30
Genistein	1.26
Daidzin	22.12
Daidzein	1.74
Glycitin	8.01
Glycitein	0.45
Total	40.88

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

The basal feed was the control, and the supplemented basal feeds were experimental.

The body weight of the experimental hens was determined every week during the experiment. Mortality was recorded daily. Egg production was monitored daily.

Egg samples, 6 eggs per group, were taken on the 3rd, 7th, 14th, 21st, and 30th day, then at the end of the 2nd and 3rd month. Egg samples from the control group were collected only on the 3rd, 7th, 14th and 21st, days because analysis showed that the results were similar. The eggs were measured, cracked, after which the shells were measured and discarded. The egg white was measured and the yolks were separated and measured, then mixed, homogenized, stored frozen and analysed no later than by 7 days.

Extraction of isoflavones (genistein and daidzein) from the egg yolk was performed according to Lin et al. (2004). A Perkin Elmer 2000 HPLC system was used for analysis of genistein and daidzein. The equipment consisted of a pump, UV-visible detector, an autosampler, computer system, and a Nova-Pak C18 column (3.9 × 150 mm 4 μm, Waters Corp., Ireland).

Table 2. Composition and nutritive value of the experimental diet, $g \cdot g^{-1}$

Indices	Basal feed (BF)	BF + 1000 mg AI · kg^{-1}	BF + 2000 mg AI · kg^{-1}	BF + 3000 mg AI · kg^{-1}
Ingredient				
maize	400.1	397.6	395.1	392.6
soya bean 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
monocalcium 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
NaCl	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
Chemical composition, calculated				
dry matter	904.3	904.3	904.3	904.3
metabolizable energy, kcal · kg^{-1}	2800	2792	2784	2776
crude protein	179.0	178.0	178.0	174.0
crude fat	71.5	71.5	71.5	71.5
calcium	37.5	37.5	37.5	37.5
phosphorus (available)	3.8	3.8	3.8	3.8
lysine	8.5	8.5	8.5	8.5
DL methionine	4.6	4.6	4.6	4.6
methionine + cystine	7.3	7.3	7.3	7.3
added isoflavones, mg·kg^{-1}	0	1000	2000	3000
soya bean meal isoflavones, mg · kg^{-1}*	43.6	43.6	43.6	43.6

* native isoflavones in soya bean meal; AI – added isoflavones

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

Results

The effects of additional 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, egg weight and daily egg mass production also were not significantly different ($p > 0.05$) in hens treated with additional isoflavones compared with the control group. Supplementing isoflavones did not affect the structure of the eggs or feed conversion efficiency in comparison with the control.

Table 3. Productive performance of the experimental hens

Parameters	Group 1 Basal feed (FB)	Group 2 BF+1000 mg AI · kg^{-1}	Group 3 BF+2000 mg AI · kg^{-1}	Group 4 BF+3000 mg AI ·kg^{-1}
Duration of the experiment, days	90	90	90	90
Number of experimental hens at the				
beginning	20	20	20	20
end	16	17	19	17
Livebility, %	80.00	85.00	95.00	85.00
Body weight, g				
initial	1674	1688	1660	1680
final	1769	1766	1749	1748
Egg production				
egg production, number	83.76	81.05	83.62	86.79
laying intensity, %	92.86	89.32	91.88	95.62
produced egg mass, g · day^{-1}	56.21	56.12	56.89	60.15
Structure of egg, g				
egg weight	60.40	62.32	61.23	62.38
egg white	38.72	40.38	39.59	40.18
egg yolkg	14.90	14.96	14.84	15.18
egg shell	6.79	6.97	6.80	7.01
Feed expenditure				
feed consumption, g · egg^{-1}	128.94	133.25	129.15	124.44
feed conversion efficiency, g feed · g^{-1} egg	2.29	2.37	2.27	2.07

AI – added isoflavones

The transfer of genistein into egg yolk is presented in Table 4.

The concentration of genistein in eggs from the treated hens increased from the 7th day of the experiment and this trend continued to the end of the study (3rd month).

Table 4. Transfer of genistein into egg yolk, μg · g^{-1}

Day of treatment	Group 1 Basal feed (BF)	Group 2 BF+1000 mg AI · kg^{-1}	Group 3 BF+2000 mg AI · kg^{-1}	Group 4 BF+3000 mg AI · kg^{-1}
3rd	2.7	3.0	2.2	2.6
7th	3.0	7.0	6.5	6.5
14th	2.4	7.0	7.0	7.0
21st	2.6	7.0	6.0	8.0
30th	NA	6.7	8.0	8.0
60th	NA	9.5	8.8	9.2
90th	NA	11.8	9.1	9.4
Average	2.67^{A}	7.43^{B}	6.80^{B}	7.24^{B}

$^{A, B}$ – values in the same row with no common superscript differ significantly ($P < 0.01$); NA – not analyzed; AI – added isoflavones

The average amount of genistein in the egg yolk was significantly higher ($P < 0.01$) in the eggs produced by laying hens fed with 1 g (214.0 mg genistein and genistin), 2 g (428.0 mg genistein and genistin), and 3 g of additional isoflavones (642.0 mg genistein and genistin) per kg feed, compared with those eggs produced by layers fed the basal feed, but differences among the experimental groups were not found. There was a positive correlation between the transfer of genistein from the supplemented feed into the egg yolk and the duration of the experimental period ($r = 0.795$) lasting 3 months.

The transfer of daidzein (Table 5) was found to occur when feed was supplemented with isoflavones at 1, 2, and 3 g · kg^{-1} (596.5, 1193.0 and 1789.5 mg daidzein and daidzin per kg feed).

The content of daidzein in the egg yolk increased significantly in the experimental groups compared with the control ($P < 0.01$). Also, the increase of daidzein in egg yolk correlated with the duration of treatment ($r = 0.858$).

Table 5. Transfer of daidzein into egg yolk, μg · g^{-1} yolk

Day of treatment	Group 1 Basal feed (BF)	Group 2 BF+1000 mg AI · kg^{-1}	Group 3 BF+2000 mg AI · kg^{-1}	Group 4 BF+3000 mg AI · kg^{-1}
3rd	2.4	4.0	3.3	3.8
7th	4.0	7.0	7.0	6.5
14th	3.4	8.0	8.5	8.0
21st	3.8	9.5	7.5	10.5
30th	NA	8.6	9.0	10.7
60th	NA	8.1	10.1	11.0
90th	NA	12.3	13.7	13.6
Average	3.40^{A}	8.21^{B}	8.44^{B}	9.16^{B}

$^{A, B}$ – values in the same row with no common superscript differ significantly ($P < 0.01$); NA – not analysed; AI – added isoflavones

Thee total amount of genistein and daidzein transferred into hen eggs is presented in Table 6.

Table 6. Total amounts of genistein and daidzein transferred into egg yolk, μg · g^{-1} yolk

Day of treatment	Group 1 Basal feed (BF)	Group 2 BF + 1000 mg AI · kg^{-1}	Group 3 BF + 2000 mg AI · kg^{-1}	Group 4 BF + 3000 mg AI · kg^{-1}
3rd	5.1	7.0	5.5	6.4
7th	7.0	14.0	13.5	13.0
14th	5.8	15.0	15.5	15.0
21st	6.4	16.5	13.5	18.5
30th	NA	15.3	17.0	18.7
60th	NA	17.6	18.9	20.2
90th	NA	24.1	22.8	23.0
Average	6.07^{A}	15.64^{B}	15.24^{B}	16.40^{B}

$^{A, B}$ – values in the same row with no common superscript differ significantly ($P < 0.01$); NA – not analysed; AI – added isoflavones

The highest level of phytoestrogens (genistein and daidzein) was recorded on the 90th day of treatment in the yolk from the experimental hens. The content of genistein and daidzein in egg yolk was significantly increased ($P < 0.01$) in the experimen-

tal groups, but the higher supplementation (2 and 3 g · kg^{-1}) did not affect the concentration of genistein and daidzein in egg yolk. The increase in the total amount of genistein and daidzein in the egg yolk of the hens treated with different levels of isoflavones correlated (r = 0.843) with the duration of treatment. The differences in the average amount of isoflavones among the control and experimental groups are significant ($P < 0.01$).

Discussion

Isoflavones act as antioxidants *in vitro* and *in vivo* in animals and humans. Genistein directly scavenges free radicals by inhibiting formation of hydrogen peroxide. These biological effects may also apply to poultry. Isoflavones act as oestrogens and play a key role in the biosynthesis of egg components. Feeding high concentrations of these compounds enhanced the egg production rate (Sanchiroh et al., 2001).

The present study demonstrates that inclusion of isoflavones in the diets of laying hens had no adverse effects on egg production, egg quality, feed consumption or conversion efficiency (Table 3). There was a positive effect on laying intensity in the experimental group fed 3000 mg AI per kg feed in comparison with the other experimental groups (1000 and 2000 mg AI) and the control group. Akdemir and Sahin (2009) reported that the use of 800 mg genistein per 1 kg feed increased the egg production of the quails and improved egg weight.

In this experiment, addition of the largest (3 g · kg^{-1}) amount of isoflavones (642 mg genistein and genistin) to the diet was not more effective than the lower amounts, 2 g · kg^{-1} (428 mg genistein and genistin) and 1 g · kg^{-1} (214 mg genistein and genistin) in transferring genistein into egg yolk (Table 4). The supplemented diets increased the amount of genistein significantly ($P < 0.01$) in comparison with the control group. The obtained results demonstrate a clear tendency towards increasing the amount of genistein in egg yolk in correlation with the duration of the experiment (r = 0.795). These results are supported by the earlier research by Sanshiroh et al. (2001).

The transfer of daidzein from supplemented isoflavones (1, 2 and 3 g · kg^{-1}) containing 596.5, 1193 and 1789.5 mg (daidzein and daidzin) per kg feed did not correlate with its concentration in the feed (Table 5), but was significantly increased ($P < 0.01$). The concentration of daidzein increased with the duration of treatment (r = 0.795). In this study, the lowest level of the two phytoestrogens was on the third day and the highest level, on the 90th day of treatment. Sanshiroh et al. (2001) also reported that an isoflavone-enriched diet (1240 mg · kg^{-1}) slightly increased the isoflavone content in egg yolk by the third day of feeding and reached a 65.29 μg · 100 g^{-1} on the 12th day. Woo-Keun et al. (2004) supplemented soya germ to feed (5% and 10%) and found that the genistein level in egg yolk peaked at the end of the second week of treatment. Their experiment lasted 3 weeks. In it, the daidzein concentration was also found to increase to 8 ppm during 3 weeks when 10% soya germ was added to the feed, which is a result similar to ours. In our experiment, however, the concentration continued to rise until the end of the 3rd month.

The sum of genistein and daidzein concentrations in egg yolk had the same increasing trend as that of the individual results of genistein and daidzein; the results were summed to show the total quantity of isoflavones enriching eggs.

The daily requirements of isoflavones for humans with hormone deficits vary, but these enriched eggs can be considered a good source of phytoestrogen hormones with better availability than crude isoflavones from the soya bean (Sanshiroh et al., 2001,2004; Lin et al., 2004; Woo-Keun et al., 2004).

Additional studies on the availability of animal-based isoflavones and evaluation of the effect of isoflavone-enriched eggs on human health are nonetheless required. Also, more models should be developed to investigate the question on how to increase the isoflavone concentrations in eggs.

Conclusions

Feeding laying hens with high concentrations of isoflavones supplemented to feed affected the concentration of genistein and daidzein in the yolk. The level of genistein and daidzein in the egg yolk at the beginning of the treatment was low (5.1 to 7.0 μg · g^{-1}) and reached a maximum on the 90th day. Enrichment ranged from 3.44 (daidzein) to 4.47-fold (genistein) in comparison with the control group.

In one average yolk, at the end of the experiment the isoflavone concentration was from 396.0 to 412.4 μg, making it a good source of oestrogen-like compounds.

Oestrogens play an important role in hen egg production, and in the functional properties of eggs as food products for promoting human and animal health.

References

Adlercreutz H., 1995. Phytoestrogens: Epidemiology and a possible role in cancer prevention. Environ. Health Perspect. 103, Suppl. 7, 103–112

Akdemir F., Sahin K., 2009. Genistein supplementation to the quail: Effects on egg production and egg yolk genistein, daidzein, and peroxidation levels. Poultry Sci. 88, 2125–2131

Arora A., Nair M.G., Strasburg G.M., 1998. Antioxidant activities of isoflavones and their biological metabolites in a liposomal system. Arch. Biochem. Biophys. 356, 133–141

Burton J.L., Wells M., 2002. The effect of phytoestrogens on the female genital tract. J. Clin. Pathol. 55, 401–407

Greendale G.A., FitzGerald G., Huang M.H., Sternfeld B., Gold E., Seeman T., Sherman S., Sowers M., 2002. Dietary soy isoflavones and bone mineral density: Results from the study of women's health across the nation. Amer. J. Epidemiol. 155, 746–754

Ho S.C., Woo J.L.F., Leung S.S.F., Sham A.L.K., Lam T.H., Janus E.D., 2000. Intake of soy products is associated with better plasma lipid profiles in the Hong Kong Chinese population. J. Nutr. 130, 2590–2593

James R.F., James V.A., Woodhams D.J., Fitzpatrick M.C., 1994. The toxicity of Soybeans & Related Products. Auckland, New Zealand

Lin F., Wu J., Abdelnabi M.A., Ottinger M.A., Giusti M.M., 2004. Effects of dose and glycosylation on the transfer of genistein into the eggs of the Japanese quail (*Coturnix japonica*). J. Agr. Food Chem. 52, 2397–2403

Payne R.L., Bidner T.D., Soyhern L.L., McMillin K.W., 2001. Dietary effects of soy isoflavones on growth and carcass traits of commercial broilers. Poultry Sci. 80, 1201–1207

Sanshiroh S., Sato T., Harada H., Matsuda T., 2004. Biotransformation of soy isoflavone-glycosides in laying hens: intestinal absorption and preferential accumulation into egg yolk of equol, a more estrogenic metabolite of daidzein. Biochim. Biophys. Acta 1674, 122–130

Sanshiroh S., Sato T., Harada H., Takita T., 2001. Transfer of soy isoflavone into the egg yolk of chickens. Biosci. Biotechnol. Biochem. 65, 2220–2225

Snedecor W.G., Cochran G.W., 1989. Statistical Methods. 8th Edition. Iowa State University Press (USA)

Woo-Keun K., Lim H.A., Kim J.H., Kim J.H., Kim J.G., 2004. Anticancerogenic isoflavones in soy germ is transferred to egg yolk. J. Korean Ass. Cancer Prev. 9 (4), 287–290

Yellayi S., Naaz A., Szewczykowski M.A., Sato T., Woods J.A., Chang J., Segre M., Allred C.D., Helferich W.G., Cooke P.S., 2002. The phytoestrogen genistein induces thymic and immune changes: a human health concern? Proc. Nat. Acad. Sci. USA 99, 7616–7621