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IMPROVEMENT OF GROWTH PERFORMANCE AND INTESTINAL DIGESTIVE FUNCTION IN BROILER CHICKENS BY SUPPLEMENTATION OF SOY ISOFLAVONE IN CORN-SOY DIET

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ABSTRACT

This study was conducted to investigate the effect of dietary soy isoflavone (ISF) on growth performance, ileal nutrients digestibility and intestinal morphology of broiler chickens. A total of 240 mixed sex day-old Ross 308 chicks were allocated to 4 treatments with 3 replicates of 20 birds each. The birds were fed with corn-soybean based diet supplemented with either 0 (control), 25, 50 or 100 ppm ISF for a period of 21 days. Intestinal content and tissue samples were collected on day 21. During the experimental period, dietary supplementation with ISF had non-significant effect on feed intake, while improved weight gain and feed conversion ratio ($P<0.01$). Supplementation of 50 and 100 ppm ISF significantly ($P<0.01$) increased ileal digestibility of dry matter, protein and fat. Ileal digestibility of energy was increased at 50 ppm ISF ($P<0.01$). Jejunal villus height and surface area, and villus height to crypt depth were increased ($P<0.01$) whereas crypt depth was decreased ($P<0.05$) by 50 or 100 ppm ISF supplementation. Relative weight of pancreas decreased due to additional 25 and 50 ppm ISF ($P<0.05$). Findings of this study showed the beneficial effects of supplemental ISF on performance and digestive function of broilers. The level of 100 ppm soy ISF can be recommended for use in chicken diets.

Key words: broiler, digestibility, morphometry, performance, soy isoflavone.

INTRODUCTION

The main purpose of using feed additives is to improve growth performance of commercial poultry. Nowadays, new commercial additives of plant origin are introduced to poultry producers and accepted by consumers due to their safety and origin from natural sources. Herbs, spices, extracts, essential oils and other phytogenic compounds have received increased attention as an alternative to growth promoter antibiotics in poultry rations. Isoflavones (ISFs) are a group of phytogenics classified as phytoestrogens because of their similarity in structure and function to natural intrinsic estrogens (Whitten and Naftolin, 1998). They are present in many foods and feedstuffs, and it seems that every plant is able to synthesize them (Wuttke *et al*., 2007). Among the feedstuffs, soybean and its products contain the highest levels of ISF (Mazur and Adlercreutz, 1998; Nurmi *et al*., 2002; Umphress *et al*., 2005). Soybean ISFs are mainly including daidzein, genistein, and glycitein (Thomas, 1997).

Numerous biological activities are evidenced for ISFs such as estrogenic and anti-estrogenic functions (Kurzer and Xu, 1997), antioxidant property (Rimbach *et al*., 2003; Jiang *et al*., 2007a,b), immune function enhancer (Zhang *et al*., 1997) improving carcass quality (Jiang *et al*., 2007b) and increasing bone mineralization (Sahin *et al*., 2006).

Jiang *et al*. (2007b) used glycitein, as an ISF, in male broilers diet and observed increased weight gain (WG) and feed intake (FI), and decreased feed conversion ratio (FCR) during 43 to 63 days. However, Payne *et al*. (2001) reported increased FCR and no effect of ISF on daily gain and FI. The studies on intact broilers (Iqbal *et al*. 2014; Kamboh and Zhu, 2014) and those fed with oxidized fish oil (Jiang *et al*. 2007a) has shown no influence of supplemental ISF on growth parameters. Likewise, non-significant effect on growth performance in Japanese quail as a result of supplemental ISF were reported by Wilhelms *et al*. (2006) and Yilmaz *et al*. (2014).

Studies on the effect of ISF on nutrient utilization and intestinal morphology in broiler chickens are limited. In one recent study Kamboh and Zhu (2014) did not display any influence of genistein on villus height and crypt depth of intestinal segments. However, there are evidences of improved nutrients utilization due to ISFs in Japanese quail (Sahin *et al*., 2006) and humans (Mendez *et al*., 2002). In general, the effects on broilers growth response and gut function are contradictory and there is a need for further investigation in this area. The objective of this research was to determine the effects of a commercial soy ISF supplementation in corn-soybean diet on growth performance, ileal nutrients digestibility, jejunal morphology and carcass traits of commercial broilers.

MATERIALS AND METHODS

Birds and experimental diets: The *in vivo* study of this experiment was performed at broiler research unit of the University of Mohaghegh Ardabili, Iran. A total of 240 mixed sex broiler chicks (Ross 308) were obtained from a local hatchery and then randomly distributed into twelve deep floor litter pens (1.5 m × 3 m). A completely randomized design was employed with 4 dietary treatments, 3 replicates and 20 birds each. The birds reared under standard conditions with free access to water and feed for 23 hours per day. The temperature was gradually reduced from 29˚C to approximately 22˚C at the end of the growing period. The basal diet based on corn and soybean meal (Table 1) was formulated to meet or exceed the nutrient requirements recommended by the National Research Council (1994). Dietary treatments were 0 (control), 25, 50 or 100 ppm supplemental ISF and fed to birds from 1 to 21 day. Supplemented ISF (Genistein® powder 3.53 oz, a soy isoflavone supplement supplied by Source Naturals Co, USA) had 400 mg genistein, 1700 mg daidzein and 1000 mg glycitein per 100 g. During the experimental period, FI and WG of the birds per each pen were recorded weekly. The FCR was calculated using FI and WG data. To determine the apparent ileal nutrients digestibility, chromic oxide as an inert marker was added (by 0.3%) to each experimental diet and fed to birds from 18 to 21 days. All procedures used in the experiment approved by the research council of the University of Mohaghegh Ardabili.

Digesta sampling and carcass traits: On day 21, two birds (one male and one female) were chosen at random from each pen and euthanized by CO_2 asphyxiation. After eviscerating, ileum, from Meckel's diverticulum to the ileo-cecal junction, was dissected to obtain its digesta sample. The ileal contents of two birds per pen were collected into the plastic containers, pooled per cage and frozen at -20 °C for subsequent nutrient digestibility analyses. To determine the morphometric parameters of the mucosa, a tissue sample (3 cm) of the proximal jejunum was obtained and its digesta being washed with saline buffer was fixed in 10% buffered formalin (pH 7.0).

The weight of breast, gizzard (empty), proventriculus (empty), pancreas, liver, bursa of fabricius, spleen, and abdominal fat of each slaughtered bird were recorded and considered as g of 100 g of live weight.

Physico-chemical analyses: The collected ileal samples were oven dried (60 °C, 72 h). The dietary samples and ileal contents after being ground passed through a 1 mm sieve prior to analysis. Gross energy of ground feed and ileal samples was measured by an adiabatic calorimeter bomb (Parr 1341, USA). Dry matter was determined using an oven at 105 °C for 24 h. Organic matter, dry matter, protein and fat content of diet and ileal samples were determined according to standard procedures of AOAC (2000). Chromic oxide content of the samples was also measured by Fenton and Fenton (1979) method. The apparent ileal digestibility of nutrients was calculated according to equation suggested by Saha and Gilbreath (1993).

Jejunal morphometery: Formalin-fixed jejunal tissue samples were processed and embedded in paraffin. A thickness of 5 µm sections were cut and stained with haematoxylin and eosin. The morphometric parameters were measured using computer-aided light microscope image analyses (Iji *et al*., 2001). The measurements of villus height (from the tip of villus to the villus-crypt junction), villus width, crypt depth, villus height: crypt depth ratio and thickness of muscle layer were made. Apparent villus surface area was calculated from the villus height and width. The values of means from 9 adjacent and vertically orientated villi and crypts were used for further analysis.

Statistical analysis: The obtained data were subjected to statistical analyses using the General Linear Model procedure of SAS software (SAS Institute, 2002). Duncan's Multiple Range Test was used to compare the significant difference between means. Relative weights (n) of carcass traits were transformed to arcsin√n before analyzing.

RESULTS

Growth performance: Growth performance responses of the chicks on different dietary treatments are presented in Table 2. During the first week of study, dietary supplementation of 25, 50 or 100 ppm ISF had no significant effect on birds FI, WG and FCR. Addition of 100 ppm ISF with no effect on FI, however, significantly improved WG and FCR of the chicks during 1-14 days ($P<0.01$). Through the period, the other levels of ISF did not show any significant influence on bird's performance compared with the control diet. Throughout the experimental period (1-21 days), while all chicks had the same FI, those receiving 50 or 100 ppm ISF diets grew faster and showed better FCR ($P<0.01$). The chicks on 25 ppm ISF also had better WG and FCR compared with the control ($P<0.01$). There was a significant linear reduction of FCR values by increasingly levels of ISF in the diets.

Ileal nutrients digestibility: Dietary supplementation with 25, 50 or 100 ppm ISF had non-significant effect on ileal organic matter digestibility (Table 3). However, addition of 50 and 100 ppm ISF significantly increased ileal dry matter, protein and fat digestibility of the diets ($P<0.01$). Ileal energy digestibility was only improved by

50 ppm ISF (P<0.01). Inclusion of 25 ppm ISF had no positive effect on nutrients digestibility.

Jejunal morphology: Villus height and surface area were higher (P<0.05) in birds receiving ISF than those in control group (Table 4). Birds fed on 50 and 100 ppm ISF had longer villus height and wider surface area. Crypt depth was shorter (P<0.05) and villus height to crypt depth was higher (P<0.01) in chicks receiving 50 and 100 ppm supplemental ISF than those on o and 25 ppm.

Carcass traits: Among the studied carcass traits, only relative weight of pancreas decreased due to addition of 25 and 50 ppm ISF (P<0.05; Table 5). Addition of different levels of soy ISF had non-significant effect on spleen, bursa of fabricius, breast, gizzard, proventriculus and liver relative weights. Relative weight of abdominal fat gradually decreased by increasing level of the supplemented ISF, although was not significant (P>0.05).

Table 1. Ingredient and composition of the basal diet for broilers from 1 to 21 d of age.

Ingredients	Percentage
Yellow corn	54.41
Soybean meal (CP 44%)	38.06
Soybean oil	3.54
Oyster shell	1.3
Dicalcium phosphate	1.48
Common salt	0.44
Vitamin Permix[†]	0.25
Mineral Permix[††]	0.25
DL-Methionine	0.18
L-Lysine HCL	0.09
Total	100
Chemical composition (calculated)	
ME (kcal/kg)	3000
CP (%)	21.56
Met (%)	0.81
Met+Cys (%)	1.05
Lys (%)	1.2
Arg (%)	1.36
Ca (%)	0.94
Available P (%)	0.42
Na (%)	0.19

[†] Supplied per kg of diet: vitamin A, 18000 IU; vitamin D_3, 4000 IU; vitamin E, 72 mg; vitamin K_3, 4 mg; vitamin B_1, 3.55 mg; vitamin B_2, 13.2 mg; vitamin B_6, 5.88 mg; vitamin B_9, 2 mg; vitamin B_{12}, 0.03 mg; niacin, 59.4 mg; calcium D-pantothenate, 19.6 mg; choline chloride, 1 g.
[††] Supplied per kg of diet:, Mn, 198.4 mg; Zn, 169.4 mg; Fe, 100 mg; Cu, 20 mg; I, 1.985 mg; Se, 0.4 mg.

Table 2. Effects of different levels of soy isoflavone (ISF) on growth performance of broiler chickens (1-21d).

	ISF levels (ppm)					
Parameters	0	25	50	100	SEM	P-value
1-7d						
Feed intake (g/bird)	91.9	90.2	91.1	92.3	1.43	0.7474
Weight gain (g/bird)	72.0	74.6	71.8	79.2	2.124	0.1263
Feed conversion ratio	1.28	1.21	1.27	1.17	0.034	0.1445
1-14d						
Feed intake (g/bird)	389.5	382.4	378.6	382.9	6.95	0.7410
Weight gain (g/bird)*	240.3^{b}	253.8^{ab}	254.8^{ab}	271.8^{a}	5.26	0.0189
Feed conversion ratio	1.62^{a}	1.51^{ab}	1.49^{ab}	1.41^{b}	0.041	0.0377
1-21d						
Feed intake (g/bird)	1020.2	1026.6	1019.5	994.6	15.60	0.5202
Weight gain (g/bird)	553.8^{c}	666.5^{b}	701.0^{a}	715.3^{a}	7.00	<0.0001
Feed conversion ratio	1.84^{a}	1.54^{b}	1.46^{c}	1.39^{d}	0.014	<0.0001

[a,b,c] Means in the same row with different superscripts are significantly different (P<0.05). SEM: standard error of means.

Table 3. Effects of different levels of soy isoflavone (ISF) on ileal nutrients digestibility of broiler chickens (21d).

	ISF levels (ppm)					
Parameters	0	25	50	100	SEM	P-value
Dry matter	69.43^{b}	69.49^{b}	72.28^{a}	71.83^{a}	0.481	0.0046
Organic matter	68.76	68.36	71.57	70.78	0.803	0.0605
Protein	74.46^{b}	74.83^{b}	78.11^{a}	77.34^{a}	0.426	0.0006
Fat	73.00^{b}	73.23^{b}	76.53^{a}	75.72^{a}	0.525	0.0029
Energy	71.72^{b}	70.02^{c}	73.61^{a}	71.72^{b}	0.372	0.0011

^{a,b,c} Means in the same row with different superscripts are significantly different (P<0.05). SEM: standard error of means.

Table 4. Effects of different levels of soy isoflavone (ISF) on jejunal mucosal morphometry of broiler chickens (21d).

	ISF levels (ppm)					
Parameters	0	25	50	100	SEM	P-value
Villus height (µm)	654.9^{c}	674.9^{b}	779.4^{a}	781.1^{a}	2.93	<0.0001
Crypt depth (µm)	87.3^{a}	87.0^{a}	84.2^{b}	83.5^{b}	0.73	0.0127
Villus height:crypt depth	7.5^{b}	7.8^{b}	9.3^{a}	9.4^{a}	0.08	<0.0001
Villus surface area (mm^2)	0.066^{c}	0.069^{b}	0.080^{a}	0.081^{a}	0.0001	<0.0001
Muscle layer thickness (µm)	116.3	118.0	117.1	116.5	0.2445	0.2445

^{a,b,c} Means in the same row with different superscripts are significantly different (P<0.05). SEM: standard error of means.

Table 5. Effects of different soy isoflavone (ISF) levels on carcass traits of broiler chickens (21d).

	ISF levels (ppm)					
Parameters	0	25	50	100	SEM	P-valaue
Breast	17.45	17.50	18.62	18.18	0.472	0.3042
Gizzard (empty)	2.62	2.24	2.37	2.42	0.084	0.0679
Proventriculus (empty)	0.74	0.64	0.73	0.65	0.028	0.0559
Pancreas	0.48^{a}	0.41^{b}	0.38^{b}	0.48^{a}	0.014	0.0019
Liver	3.90	3.50	3.85	3.53	0.118	0.0844
Bursa of fabricius	0.28	0.26	0.22	0.22	0.022	0.1568
Spleen	0.11	0.11	0.08	0.10	0.010	0.2004
Abdominal fat	1.46	1.36	1.35	1.29	0.051	0.2776

^{a,b} Means in the same row with different superscripts are significantly different (P<0.05). SEM: standard error of means.

DISCUSSION

In the present study improved broiler performance with better FCR was more pronounced with high level of ISF (100 ppm). It indicates that ISF response depends on broiler age and usage dose. In agreement with this result, such improvement has also been reported by Jiang *et al*. (2007b). They noted better FCR in broilers by supplementation of 10 ppm glycitein in an ISF free diet. In contrast, Payne *et al*. (2001) reported that supplemental ISF in excess of those in a corn-soybean meal diet decreased gain to feed ratio. No influence of ISF on growth parameters has been shown by other studies in broilers (Iqbal *et al*. 2014; Kamboh and Zhu, 2014) and Japanese quail (Wilhelms *et al*., 2006; Yilmaz *et al*. 2014). The possible reason for these differences in results may be related to the environmental condition and production system. It seems that the beneficial effects of dietary soy ISFs are more pronounced in animals exposed to stress conditions as improved growth performance in virally challenged pigs (Greiner *et al*., 2001) and nutrients use in heat stressed Japanese quail (Sahin *et al*., 2006) was noted. Moreover, lower positive effect of ISFs were reported in birds reared into battery cages (Payne *et al.*, 2001; Wilhelms *et al*. 2006) compared with those kept in floor pens (Jiang *et al*., 2007a, b) and subjected to more stressors and lower hygiene condition. It is also shown that other phytogenic compounds display more satisfactory performance in broiler chickens at low hygiene condition (Giannenas *et al*., 2003). Thus, the improved growth performance of young broilers in this experiment due to excess soy ISF supplementation might partly be accounted for by supportive effect of this component from susceptible chicks raised in relatively lower hygiene floor pens. Moreover, applying combined soy ISF in this study and using one pure ISF in other similar studies (Iqbal *et al*., 2014; Kamboh and Zhu, 2014; Yilmaz *et al*., 2014) may also be other explanation for different observations.

ISF supplementation in the diet without any effect on FI, by improving WG decreased FCR (Table 2), suggesting that consumed feed has been utilized with higher efficiency in ISF receiving birds than those on the control. The data illustrated in Table 3 confirmed the fact since the ileal digestibility of dry matter, energy, protein and fat was increased by high levels of ISF supplementation to the diet. As there are no available reports on ISF effect on ileal nutrient digestibility in broilers, comparison of the obtained data is difficult. However, the observed improvement in nutrient digestibility as a result of supplemental soy ISF agrees with the results obtained by Sahin *et al*. (2006) in quails reared at high ambient temperature and Mendez *et al*. (2002) in human.

The mechanism by which the high levels of ISF supplementation increased digestibility of dietary nutrients compared with the control diet, might be explained by this phytoestrogen beneficial effect on intestinal mucosal structure at least in two ways. Firstly, by increasing the length of jejunal mucosal villi and their surface area (Table 4) that can result in greater digestive and absorptive capacity of intestine to prepare more nutrients for host growth. Such finding concerning villi structure and improved growth performance of broiler chicks has been illustrated by application of other additives like microbial enzyme (Shakouri *et al*., 2009), mannanoligosaccharide and growth promoting antibiotic (Chee *et al*., 2010). Secondly, by decreasing crypt depth and increasing villus height to crypt depth. It has already been displayed that crypts are the main center of intestinal cell proliferation (Geyra *et al*., 2001; Iji *et al*., 2001). Consequently, the large crypt suggests a high request for nutrients to maintain the intestine, hence less nutrients will be available for growth. However, the mode of action by which soy ISFs affect mucosal structure, directly or in directly via gut microbial activity or etc., still remained unknown. It is reported that ISF can alter human gut bacterial communities and increase *Lactobacillus* group (Clovel *et al*., 2005). The results of mucosal morphometry are not in agreement with the findings of Komboh and Zhu (2014), who found no change in villi height and crypt depth of duodenum, jejunum and ileum of broilers on genistein containing diets. Unlike the alteration in mucosal layer of intestine, there were non-significant changes in relative weight of the studied digestive organs, except pancreas, the decrease of which does not clearly rely on the digestibility of nutrients.

In conclusion, supplementation of commercial soy ISF (Genistein®) to corn-soybean meal diet of broiler chicks during 1 to 21 d by increasing intestinal digestive capacity and improving nutrients digestibility prepare more nutrients for growth performance. Due to the significant beneficial effects on studied traits compared with the control, 100 ppm of the product is recommended for application in the diet.

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