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Effects of soybean meal substitution with isolated soya protein in pre-starter diet on immune system and performance of broiler chickens

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ABSTRACT. The aim of the study was to evaluate the effectiveness of soybean meal (SBM) substitution with isolated soya protein (ISP) in pre-starter diet on immune system and performance of broiler chickens. After sex identification, 200 male Ross 308 broiler chicks were randomly allotted to 4 treatment groups with 5 replicates, 10 birds in each, and fed for 35 days. Experimental treatments including 4 substitution levels of SBM with ISP (0, 10, 20 and 30%) were fed from 1 to 7 day of age. Then all birds were fed common grower (days 7–24) and finisher (days 24–35) commercial diets. Dietary ISP inclusion improved ($P < 0.05$) body weight gain and feed conversion ratio during days 1–7 and 1–24 of age. Dietary ISP supplementation increased ($P < 0.05$) European Production Efficiency Factor and protein efficiency ratio throughout the experiment. Dietary ISP inclusion did not have an effect on relative weight of immune organs at days 3 and 7 of age. Dietary ISP inclusion linearly increased total anti-sheep red blood cells (anti-SRBC) ($P = 0.017$), immunoglobulin (Ig) G ($P = 0.021$) and IgM ($P = 0.003$) at 35 day of age. So, the inclusion of ISP (20 or 30% substitution of the SBM) in the pre-starter diet may improve immune response and performance of broiler chickens.

Introduction

Digestive system, mainly its physiology and morphology, is not fully developed in newly hatched broiler chicks (Jin et al., 1998). Intestinal villus height rapidly increases during the first week of life (Wijtten et al., 2011), and pancreatic and biliary secretion may be insufficient in the first week after hatch, especially to digest proteins (Noy and Sklan, 1997). Due to this, feed is not completely digested in the pre-starter period. On the other hand, appropriate nutrient absorption from digestive tract is essential for maturity and development of some body systems (e.g., immune system) and thermoregulation. In addition, due to positive correlation between seven-day live weight and final weight of broiler chickens (Willemsen et al., 2008), feeding animals highly digestible ingredients can improve an economic efficiency (Sklan and Noy, 2003). Soybean meal (SBM) is commonly used as the main dietary source of protein for broiler chicks; however non-starch polysaccharides (NSPs) constitute a significant part of SBM. Total NSPs and soluble NSPs contents in SBM equal 192 and 27 mg/g, respectively (Irish and Balnave, 1993). As digesta viscosity increases, soluble NSPs prevent the enzymatic digestion and reduce protein and amino acid digestibilities (Siddhuraju and Becker, 2005). The soluble NSPs due to anti-nutritive activities may affect gut physiology, microflora and health of birds (Choct, 2015). Isolated soya protein (ISP) has higher nutritional value than SBM which is characterized by a high protein content (90%), low anti trypsin (less than 1 mg per kg), zero or negligible oligosaccharides and antigenic factors contents (Peisker, 2001). The non-protein components of ISP such as NSPs are removed during the protein isolation process (Grieshop and Fahey, 2000). Anti-nutritional contents of SBM may affect small intestine characteristics and decrease digestive enzymes activities, especially at starter phase (Saki et al., 2012). ISP that is characterized by zero or negligible oligosaccharides and antigenic factors content may be an appropriate source of protein in order to improve the immune system and performance of broiler chickens. Therefore, the purpose of this study was to evaluate the effects of SBM substitution with ISP in pre-starter diet on immune system and performance of broiler chickens.

Material and methods

The experimental protocol was approved by the Animal Welfare Committee, at the Islamic Azad University of Bojnourd (Iran).

Birds, housing and diets

In total, 200 1-day-old Ross 308 broiler chicks (average body weight = 44 g) were randomly assigned to 4 treatments of 5 replicates with 10 birds in each replicate. Replicate groups of chicks were housed in independent pens (100 × 100 × 70 cm), each equipped with two nipple drinkers and a hanging plastic feeder for manual feed distribution. The pens had a concrete floor and were bedded by clean wood shavings litter. All chicks had unrestricted access to feed and water throughout the experiment. All chicks were exposed to 23:1 light:dark cycle. Temperature was initially set at 32 °C on day 1 and then it was decreased by 0.5 °C per day to reach 21 °C on day 23 and kept constant thereafter. To protect birds against Newcastle Disease (ND) and Infectious Bursal Disease (IBD) all birds were vaccinated as scheduled followed by the veterinary official.

Treatments were at four substitution levels of SBM with ISP (0, 10, 20 and 30%). The ISP used in this study was a commercial product (Shangdong Yuxin Bio-Tech Co., Qing-dao, China) which contained: %: crude protein 90, moisture 5.2, fat 0.5, NSP 1.2 and ash 3.1. The 4 treatment diets were fed in a completely randomized design from day 1 to 7 of age. Then all birds were fed a common grower (days 7–24) and finisher (days 24–35) commercial diets based on Ross Broiler Nutrition Specification (Aviagen, 2014) in a mash form. The ingredients and chemical composition of the diets are shown in Tables 1 and 2, respectively.

Table 1. Ingredients of the pre-starter diets fed from 1 to 7 day of age[1]

Ingredient, %	Soybean meal substitution, %			
	0	10	20	30
Maize grain (8.5%)	56.64	56.64	56.64	56.64
Soybean meal (46%)	27.81	25.04	22.25	19.47
ISP[2] (90%)	0	1.46	2.91	4.37
Maize gluten meal (60%)	8.91	8.41	7.94	7.44
Casein (80%)	1	1	1	1
Wheat bran (15%)	1	2.58	4.17	5.79
Soybean oil	0.18	0.36	0.55	0.47
Calcium carbonate	1.39	1.40	1.41	1.42
Dicalcium phosphate	1.70	1.70	1.70	1.69
Sodium chloride	0.39	0.39	0.39	0.38
Lysine-HCl	0.3	0.3	0.3	0.3
DL-methionine	0.18	0.21	0.22	0.23
Threonine	–	0.01	0.02	0.03
Vitamin + mineral premix[3]	0.5	0.5	0.5	0.5

[1] similar commercial maize-soya grower and finisher diets were fed to all birds from days 7–24 and 24–35 of age, respectively; [2] ISP = isolated soy protein; [3] each kg of diet contained: IU: vit. A 9000, vit. D3 2000, vit. E 18; mg: vit. K_3 2, vit. B_1 1.8, vit. B_2 6.6, vit. B_3 10, vit. B_5 30, vit. B_6 3, vit. B_9 1, vit. B_{12} 0.015, vit. H_2 0.1, choline choloride 500; Mn 100, Fe 50, Zn 100, Cu 10, Mg 3.5, Se 0.2

Table 2. Chemical composition of the pre-starter diets fed from days 1 to 7 of age[1]

Nutrient, %	Soybean meal substitution, %			
	0	10	20	30
ME, kcal/kg	3000	3000	3000	3000
Crude protein[2]	22.87	22.96	22.85	22.97
Lysine	1.29	1.29	1.29	1.29
Met + Cys	0.95	0.95	0.95	0.95
Threonine	0.90	0.90	0.90	0.90
Ca	0.96	0.96	0.96	0.96
Available P	0.48	0.48	0.48	0.48
Na	0.17	0.17	0.17	0.17

ME – metabolizable energy; [1] similar commercial maize-soya grower and finisher diets were fed to all birds from days 7–24 and 24–35 of age, respectively; [2] analysed values

Performance measurements

Records for live body weight and feed intake were obtained on days 3, 7, 24 and 35. Feed intake (FI) was determined from the difference between supplied and residual feed in each pen. Mortality was recorded by pen number, date and weight of carcass and was added to the total pen live body weight for calculation of feed conversion ratio (FCR). The FCR was calculated by dividing total FI by body weight gain (BWG) of birds for each pen during every period. The following formulas were used to calculate European Production Efficiency Factor (EPEF) and protein efficiency ratio (PER):

$$\mathrm{EPEF} = \frac{\text{survival rate (\%)} \times \text{live weight (kg)}}{\mathrm{FCR} \times \text{age (day)}} \times 100$$

$$\mathrm{PER} = \frac{\text{weight gain (g)}}{\text{protein intake (g)}}$$

Immune organ weights

At 3 and 7 day of life, two chickens from each replicate were randomly selected, weighed and killed by cervical dislocation. Spleen, thymus and bursa of Fabricius were removed, weighed and expressed as:

$$\frac{\text{organ weight}}{\text{body weight}} \times 100$$

Humoral immune response to SRBC

Non-pathogenic antigen, sheep red blood cell (SRBC), was used to monitor the humoral immune response in broiler chickens. Total anti-SRBC antibody titer was measured by a haemagglutination test. Two chicks in each replicate were intramuscularly injected with 1 ml of a 15% suspension of SRBC at 21 and 28 day of age. Antiserums were collected 7 days after the immune challenge at 28 and 35 day of age. The serum from each sample was collected, inactivated by heating at 56 °C for 30 min and then analysed for total anti-SRBC, mercaptoethanol-resistant (MER) and mercaptoethanol-sensitive (MES) antibody titers. MER and MES titers are presumably immunoglobulin (Ig) G and IgM, respectively. The difference between the total and the IgG response was considered to be equal to the IgM antibody level (Cheema et al., 2003).

Statistical analysis

Data were analysed in a completely randomized design, using the general linear models procedure of SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA). Means were compared by Duncan's multiple-range test. A difference with equal or less than 5% ($P \leq 0.05$) probability was considered significant.

Results and discussion

Performance measurements

The effects of SBM substitution with ISP in pre-starter diet on broiler chickens performance are shown in Tables 3 and 4. The inclusion of ISP in the pre-starter diet did not have an effect ($P > 0.05$) on FI, BWG, FCR, PER and EPEF during 1–3 days of age (data not shown). Feeding pre-starter diet having 20 or 30% SBM substituted with ISP increased ($P < 0.05$) FI during 1–24 days of age. The dietary inclusion of ISP increased ($P < 0.05$) BWG during 1–7, 1–24 days of age. Replacement of SBM with ISP at 20 or 30% levels numerically increased final body weight by about 2.7% compared with broiler chickens fed on the basal diet based on SBM only. The better ($P < 0.05$) FCR in the periods 1–7, 1–24 days of age was obtained when birds were fed pre-starter diet containing different levels of ISP. Statistical analysis of the obtained data revealed that inclusion of ISP instead of SBM at 10% significantly reduced FCR in comparison to birds fed control diet in the periods 1–7 and 1–24 days of age; the 30% substitution caused further FCR decrease in comparison to 10% substitution, but neither 10 nor 30% differ from 20% substitution. Ebling et al. (2015) reported that diets with 6% ISP increased the coefficients of total tract apparent retention of dry

Table 3. Effect of pre-starter diet containing different levels of soybean meal substitution with isolated soya protein on broiler chicken's performance: feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR)

Indices	FI, g/chick			BWG, g/chick			FCR, g/g		
	days								
	1–7	1–24	1–35	1–7	1–24	1–35	1–7	1–24	1–35
Substitution[1], %									
0	164	1297^{b}	2689^{ab}	105^{c}	904^{c}	1802	1.312^{a}	1.435^{a}	1.492
10	161	1292^{b}	2657^{b}	108^{b}	928^{b}	1820	1.247^{b}	1.392^{b}	1.460
20	161	1315^{a}	2667^{ab}	108^{b}	954^{a}	1847	1.224^{bc}	1.378^{bc}	1.444
30	162	1318^{a}	2692^{a}	111^{a}	960^{a}	1851	1.180^{c}	1.333^{c}	1.554
SEM[2]	4.98	30.54	51.35	3.69	49.90	22.18	0.0002	0.0002	0.0002
P-value	0.173	<.0001	<.0001	0.001	<.0001	0.079	0.0001	<.0001	0.076
Orthogonal polynomials									
linear	0.191	<.0001	<.0001	0.0001	<.0001	0.585	<.0001	<.0001	0.688
quadratic	0.070	0.102	<.0001	0.768	0.10	0.039	0.074	0.003	0.013

[1] soybean meal substitution with isolated soya protein in pre-starter diet; [2] SEM – standard error of mean; [ab] – means with different superscript within the column are significantly different at $P \leq 0.05$

Table 4. Effect of pre-starter diet containing different levels of soybean meal substitution with isolated soya protein on protein efficiency ratio (PER) and European Production Efficiency Factor (EPEF) in broiler chickens

Indices	PER			EPEF		
	days					
	1–7	1–24	1–35	1–7	1–24	1–35
Substitution[1], %						
0	2.78[b]	3.17[c]	3.11[b]	277	262[c]	336[c]
10	2.90[a]	3.27[b]	3.23[a]	280	278[b]	352[b]
20	2.93[a]	3.30[ab]	3.22[a]	281	288[a]	357[a]
30	2.97[a]	3.31[a]	3.24[a]	285	291[a]	359[a]
SEM[2]	0.003	0.001	0.001	7.18	8.12	12.77
P-value	0.0001	<.0001	<.0001	0.567	<.0001	0.0004
Orthogonal polynomials						
linear	<.0001	<.0001	<.0001	<.0001	<.0001	0.0003
quadratic	0.090	0.003	<.0001	0.272	0.005	0.004

[1] soybean meal substitution with isolated soya protein in pre-starter diet; [2] SEM – standard error of mean; [abc] – means with different superscript within the column are significantly different at $P \leq 0.05$

matter (DM) and gross energy (GE), but decreased FI, which negatively affected broiler performance. Longo et al. (2005) also reported that diets with 5.35% ISP decreased FI, but observed similar average daily gain (ADG) and FCR. In the present study, feeding pre-starter diet containing ISP improved broiler chickens performance. Similarly, van der Eijk (2015) showed that ISP inclusion improved ($P < 0.05$) feed utilization. It has been reported that 5% processed soya protein improved BWG and feed efficiency of 7 day-old chickens, whereas improved FCR was reported for young turkeys fed diet containing ISP (Jankowski et al., 2009). In this study ISP inclusion in pre-starter diet improved BWG and FCR in broiler chickens. The SBM is known to contain anti-nutritional compounds, such as soya antigens and lectins (Li et al., 2003) and trypsin inhibitors which have been shown to be detrimental to bird's performance (Friedman et al., 1991). Such anti-nutritional factors present in SBM, may affect small intestine characteristics and decrease digestive enzymes activities and consequently reduce broiler performance (Saki et al., 2012). These factors not only reduce the nutritional value of SBM but also reduce the utilization of nutrients from other ingredients. Processed soya products including soya protein concentrate (SPC) and ISP contain lower amount of oligosaccharides and antigenic substances. Therefore, these products have higher nutritive values than SBM (Peisker, 2001).

Immune system

Dietary ISP inclusion did not have an effect ($P > 0.05$) on relative weight of immune organs at 3 and 7 day of age (data not shown). The lack of significant effect of ISP inclusion on the relative immune organ weight could be due to the short duration (only 7 days) of pre-starter diet feeding period and too fast introduction of the common grower (days 7–24) and finisher (days 24–35) commercial diets. SBM substitution with ISP in pre-starter diet did not have an effect ($P > 0.05$) on the total anti-SRBC titer and IgG titers of broiler chickens at 28 and 35 day of age (Table 5). The IgM titer did not differ between groups at 28 day of life but it was significantly higher in groups fed pre-starter diet containing 20 or 30% SBM substitution with ISP at 35 days of age ($P = 0.003$). For all parameters (total anti-SRBC, IgG and IgM) there was stated the linear increase ($P = 0.017$, $P = 0.021$ and $P = 0.0003$, respectively) at 35 day of age (Table 5). Based on gene expression patterns in jejunal tissue, dietary protein source influences the expression of genes responsible for mucin secretion, amino acid transport and immune functionality in an age dependent manner (Cowieson et al., 2016). Soya, as a plant source of dietary proteins for animal, contains a range of phytochemicals, such as isoflavones: genistein, daidzein and glycitein. Isoflavones content in ISP was 91.05 mg per 100 g as reported by Bhagwat et al. (2017). Inhibition of pathogens (phytoalexin activity) and molecular signal in symbiotic interaction (in mycorrhizia and rhizobia) are areas where isoflavones are essential (Lozovaya et al., 2007; Mierziak et al., 2014). Soya protein can affect immune response and act as antitumor factor (Ford et al., 2001). Isoflavones influence the signal transduction process of macrophages and

Table 5. Effect of pre-starter diet containing different levels of soybean meal substitution with isolated soya protein on immune response in broiler chickens

Indices	Total anti-SRBC[1] mg/dl		IgG[2] mg/dl		IgM[3] mg/dl	
	day					
	28	35	28	35	28	35
Substitution[4], %						
0	367.8	555.1	309.2	430.8	58.6	124.3[b]
10	370.5	562.0	307.7	437.2	62.8	124.8[b]
20	370.8	571.6	310.2	442.6	60.6	129.0[a]
30	374.8	578.2	312.0	446.8	62.8	131.4[a]
SEM[5]	17.46	38.95	18.62	33.100	8.62	5.74
P-value	0.585	0.099	0.423	0.108	0.473	0.003
Orthogonal polynomials						
linear	0.479	0.017	0.229	0.021	0.224	0.0003
quadratic	0.345	0.965	0.0001	0.733	0.404	0.675

[1] total anti-SRBC – sheep red blood cells; [2] IgG – immunoglobulin G; [3] IgM – immunoglobulin M; [4] soybean meal substitution with isolated soya protein in pre-starter diet; [5] SEM – standard error of mean; [ab] – means with different superscript within the column are significantly different at $P \leq 0.05$

other phagocytic cells and the activity of cytotoxic T lymphocytes, thus affect both innate and adaptive immunity (Ford et al., 2001).

Conclusions

Soybean meal (SBM) substitution with isolated soya protein (ISP) in the pre-starter diet can improve the overall growth performance and immune response of broiler chickens. The substitution of 20 or 30% of SBM with ISP could be suggested as beneficial for the bird health.

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