Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X Oct - Dec 2016 / v.18 / n.4 / 705-710

http://dx.doi.org/10.1590/1806-9061-2016-0330

Performance, Intestinal Morphology and Microbiology of Broiler Chickens Fed Egg Powder in the Starter Diet

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■Keywords

Broilers; egg powder; intestinal microbiology; intestinal morphology; performance; starter diet.

Submitted: June/2016
Approved: August/2016

ABSTRACT

The effect of egg powder inclusion in the pre-starter diet (1-7 days of age) on the performance, and intestinal morphology and microbiology of male broiler chickens was evaluated in a completely randomized design. Starter diets with equal metabolizable energy and crude protein levels were formulated to contain 0, 20, 40, or 60 g egg powder/kg diet. Results showed that body weight, feed intake, feed conversion ratio, and European efficiency index were positively affected ($p\leq0.05$) by egg powder inclusion in the starter diet. Inclusion of 40 g egg powder/kg diet in the starter diet promoted the best performance results. Jejunal villus height and villus to crypt ratio increased ($p\leq0.05$) and intestinal length decreased ($p\leq0.05$) as a result of egg powder inclusion in the starter diet. Egg powder inclusion in the starter diet reduced (p≤0.05*E. coli* counts, but increased ($p\leq0.05$) lactic acid bacteria counts in the small intestine. The results obtained in the present study indicate that the inclusion of 40 60 g egg powder/kg of starter diet g improved the performance and intestinal health of broilers.

INTRODUCTION

At the end of incubation period and during hatching, the residual yolk is internalized into the abdominal cavity of chicks, and may supply the nutritional needs of chicks for a short period of time after hatch (Noy & Sklan, 2001). During incubation, the embryo's needs are derived from egg reserves directly by the blood stream, but after hatch, all of the nutritional needs must be supplied through intestinal tract (Noy & Sklan, 1998). In the first week after hatch, chick's digestive system is not fully developed, and the utilization of common feed ingredients, such as cereals and plant protein sources are limited (Noy & Sklan, 2001). During this period, the digestive system develops at a very fast rate, and which suffer significant size, weight, and morphology changes (Jin *et al.*, 1998; Noy *et al.*, 2001).

Digestible ingredients are required to support the fast growth rates of the digestive system and of the body of broiler chickens, and to enhance the immune system to protect their health (Murakami *et al.*, 1992). Another important aspect of the intestinal system that impacts broiler health and performance is its microbial population. Intestinal colonization with beneficial populations, like lactic acid, prevents the colonization with pathogens, such as *E. coli* (Shen *et al.*, 2006).

One of the best ways to supply broiler early nutritional requirements is offering starter diets. The starter diet must include highly digestible ingredients, because digestion capacity of newly-hatched chicks is limited. Several literature studies evaluated the inclusion of alternative ingredients for starter broiler feeds. One of them is the egg by-product, consisting of broken and unsellable eggs. Sparks (2006) demonstrated that egg by-products are rich in lipids, proteins, antibodies, and other bioactive nutrients. The beneficial effects of feeding broiler chicks with egg by-products were also reported in the studies of El-Deek & Al-Harthi (2009) and El-Deek *et al.* (2011). Those authors suggest that dried egg powder can be included in the diet of newly-hatched chicks as an alternative to antibiotics, due to its high content of antimicrobial proteins and antibodies. It was also demonstrated that dried egg powder can be fed to large broiler flocks, with no negative effects on their performance (El-Deek *et al.*, 2011).

Based on studies mentioned above, it seems that broilers are able to efficiently utilize egg by-products soon after hatch. The objective of this study was to test this hypothesis and to determine the appropriate inclusion levels of egg by-products in starter diets, according to broiler performance, and intestinal morphology and microbiology parameters.

MATERIALS AND METHODS

The egg powder used in the present study was acquired Golpodr Company (Gorgan, Iran). The egg powder is prepared from both egg white and egg yolk, which are mixed, pasteurized, and spray-dried at 64 °C. Crude protein, ether extract, lysine, methionine, threonine, calcium and available phosphorus contents of the egg powder were, respectively, 435, 387, 70, 33, 57, 46, 0.5 and 7.5 g/kg, on as-fed basis.

In total, 320 one-day-old male Ross 308 broilers were purchased from a commercial hatchery. Chicks were randomly assigned to four treatments, with four replicates of 20 chicks per each. Treatments consisted of egg powder inclusion at levels of 0, 20, 40 or 60 g/kg in the starter diet fed from hatch until day 7. All experimental groups were fed the same starter diet as the control group from day 8 to day 10. During the remaining experimental period (days 7-42), all birds were fed equal grower (days 11-24) and finisher (days 25-42) diets. Diets were formulated according to the Ross 308 management guide, and contained equal amino acids and metabolizable energy levels (Table 1). Feed and water were *ad libitum* throughout the experiment.

Birds were housed in an environmentally-controlled room on floor pens. House temperature was initially maintained at 32 ˚C until day 7 and gradually to 23-24 ˚C for the rest of experiment, and relative humidity was maintained at 65%. A continuous lighting program

Table 1 – Feedstuffs and calculated nutritional composition of the experimental diets

Feedstuffs (g/kg)	Starter (0-7 d)*				Grower (11-24 d)	Finisher (25-42 d)
	control	20 g/kg	40 g/kg	60 g/kg		
Corn grain	515	525	534	543	587	591
Egg powder	-	20.0	40.0	60.0	-	-
Corn gluten meal	50.0	50.0	50.0	50.0	50.0	50.0
Soybean meal(42% CP)	354	333	313	292	283	279
Vegetable oil	31.9	24.7	18.0	11.3	36.7	42.0
Dl-methionine	2.46	1.94	1.50	1.06	1.80	1.10
L-lysine HCl	3.04	2.23	1.42	0.61	2.60	1.00
L-threonine	0.60	-	-	-	0.30	-
Dicalciumphosphate	20.8	20.3	19.9	19.4	18.4	16.8
Caco3	13.0	13.3	13.7	14.1	10.8	10.5
Na bicarbonate	1.60	1.20	0.70	0.30	1.40	0. 70
Common salt	2.30	2.60	2.90	3.10	2.50	3.00
Vitamin and mineral[a]premix	5.00	5.00	5.00	5.00	5.00	5.00
Nutrient content (g/kg, on as-fedbasis)						
Crude protein	236	236	236	236	209	208
Metabolizable energy (kcal/kg)	3050	3050	3050	3050	3150	3200
Lysine	12.8	12.8	12.8	12.8	10.8	9.5
Methionine	5.90	5.80	5.80	5.80	5.00	4.20
Methionine+cystine	9.20	9.40	9.70	10.0	8.00	7.20
Threonine	8.10	8.10	8.70	9.30	6.90	6.50
Calcium	10.5	10.5	10.5	10.5	9.00	8.50
Available phosphorus	5.00	5.00	5.00	5.00	4.50	4.20

[a]Premix provided the following per kilogram of diet: 4.8 mg of retinol acetate, 100 µg of cholecalciferol, 20 mg of DL-α-tocopheryl acetate, 4.5 mg menadione sodium bisulfate, 4.1 mg thiamine hydrochloride, 9.5 mg of riboflavin, 15 mg of calcium-D-pantothenate, 45 mg of nicotinic acid, 9 mg of pyridoxine hydrochloride, 2.2 mg of folic acid, 0.25 mg biotin, 12 mg ascorbic acid, 550 mg of choline chloride, 80 mg of Zn, 30 mg of Fe, 100 mg of Mn, 20 mg of Cu, 0.4 mg of Co, 1.2 mg of I, 0.4 mg of Se.

* All experimental groups werefed the same starter diet as the control group from day 8 to day 10.

was used until day 3, and then a 23-h light: 1-h dark cycle was applied thereafter.

Birds were individually weighed, and feed intake was recorded on days 7, 24 and 42 of age. Body weight, feed intake, feed conversion ratio, and European efficiency index were calculated for each experimental phase.

At the end of the starter (day 7) and finishing phases (day 42 of age), one bird per pen (4 birds per treatment) were euthanized by cervical dislocation. The jejunum was collected from the midpoint between the bile duct entry and Meckel's diverticulum. Jejunal samples (approximately 2.5-cm long each) were cut and rinsed with saline solution to remove the digesta. Samples were fixed in 10% neutral-buffered formalin for histology. In the laboratory, samples were dehydrated, cleared, and embedded in paraffin, according to standard histology procedures. Six-μm thick sections were cut and placed on glass slides, stained with hematoxylin and eosin stain, and examined under light microscopy.

Morphology indices included the number of goblet cells (in 1 μm^2 of villus), total length of the small intestine, villus height (from the tip of the villus to the crypt), crypt depth (from the base of the villi to the submucosa), and villus-to-crypt ratio.

At 7 and 42 days of age, four chicks per replicate sacrificed killed by cervical dislocation and the contents of their small and large intestine were extracted. The content of both the small and large intestines of each bird were pooled for subsequent analysis of the bacterial populations by serial dilution. One g of feces was serially diluted (1:10) to 10^{-6} with a 0.85% NaCl solution, and 0.1 mL of each dilution was plated onto Mac Conkey agar plates (Difco Laboratories, Detroit, MI, USA), MRS agar (Merck, Germany), and Plate count agar (Merck, Germany) to enumerate *E. coli*, lactic acid bacteria (LAB) and total aerobic bacteria, respectively. Plates incubated for 24 h at 37 °C. The number of colony-forming units (cfu) per plate was counted to determine the total number of the selected bacterial populations per gram of feces.

Data were analyzed using GLM procedure of SAS (SAS Institute, Cary, NC) according to a completely randomized design. Means were compared by Duncan's multiple range test at $p \leq 0.05$.

RESULTS

The live performance parameters are presented in Table 2. At day 7 of age, body weight was influenced by the dietary inclusion of egg powder in their starter diet. The control birds presented lower body weight ($p \leq 0.05$) compared with those fed 40 and 60 g egg powder/kg diet, whereas those fed 20 g egg powder/kg diet presented intermediate values. Until day 7 of age, the feed intake of the chicks fed 40 g egg powder/kg diet was higher ($p \leq 0.05$) relative to the other groups. Feed intake determined during the grower, finisher, and entire experimental period was not different ($p > 0.05$) among treatments. The broilers fed egg powder presented better feed conversion ratio and European efficiency index than the control group. The best feed conversion ratios and European efficiency index values determined in each experimental phase were obtained in the broilers fed 40 g egg powder/kg diet.

Table 2 – Effect of different levels of egg powder inclusion in the starter diet on performance of male broiler chickens.

	Egg powder level				SEM	p-value
	control	20 g/kg	40 g/kg	60 g/kg		
Body weight (g)						
7 d	172^{b}	184^{ab}	199^{a}	188^{a}	3.3	0.01
24 d	1132^{c}	1191^{bc}	1266^{a}	1230^{ab}	16.0	<0.01
42 d	2704^{b}	2874^{a}	2953^{a}	2910^{a}	33.8	0.03
Feed intake (g)						
0-7 d	169^{b}	172^{b}	177^{a}	172^{b}	1.1	0.02
7-24 d	1459	1496	1534	1529	16.8	0.40
24-42 d	3202	3373	3383	3360	52.7	0.62
0-42 d	4830	5040	5094	5061	53.3	0.31
Feed conversion ratio						
0-7 d	0.98^{a}	0.93^{ab}	0.89^{b}	0.91^{b}	0.012	0.04
7-24 d	1.52^{a}	1.48^{b}	1.44^{c}	1.47^{b}	0.008	<0.01
24-42 d	2.04	2.00	2.01	2.00	0.011	0.63
0-42 d	1.78^{a}	1.75^{ab}	1.72^{b}	1.74^{b}	0.007	0.01
European efficiency index	360^{b}	390^{a}	407^{a}	399^{a}	5.6	<0.01

[ab] Means in the same row with different superscripts are significantly different ($p \leq 0.05$).

The results presented in Table 3 show the effect of dietary treatments on bacterial population counts in the jejunum. Bacterial population counts on day 7 of age were more affected by dietary egg powder inclusion than on day 42. Broilers fed egg powder in the starter diet presented lower numbers of *E. coli* (p≤0.05) both on days 7 and 42. *E. coli* count was the highest in the control group, and was reduced by as the level of egg powder inclusion in starter diet increased.

Lactic-acid bacteria and total aerobic bacteria populations presented the same trend as *E. coli*. Differences among treatments were observed only on day 7, with the highest count of lactic acid bacteria determined in the chicks fed 60 g egg powder/kg diet (p≤0.05).

The impact of the dietary treatments on intestinal morphology are shown in Table 4. On day 7, villus height increased only in birds fed 40 g egg powder/kg diet, while no differences were detected among the other groups. At the end of the experiment, on day 42, dietary egg powder inclusion promoted higher jejunal villus height compared with the control diet (p≤0.05). The broilers fed egg powder presented higher and lower crypt depth values on days 7 and 42, respectively, compared with the control group. Higher villus to crypt ratios (p≤0.05) were obtained on day 42 in the broilers fed egg powder compared with the control group, but no differences were detected on day 7. Goblet cell numbers decreased with increasing inclusion levels of egg powder in the starter diet both on days 7 and 21 (p≤0.05).

DISCUSSION

The results of this study showed that egg powder inclusion in the starter diet improved broiler live performance (Table 2). According to Tables 3 and 4, intestinal morphology and microbiology of chickens were positively affected by the inclusion of egg powder in the starter diet. These effects may be explained when considering the nutritional value of the egg for the embryos and newly-hatched chicks. During the incubation period, the egg is able to supply the nutrient requirements of the embryo and for short while after hatch. The nutritional profile of the egg powder included in the starter diet in the present experiment is similar to that consumed by the embryo, promoting the continuous growth of the chicks. There is a strong relationship between initial body weight of chicks and their subsequent performance (Jin *et al.*, 1998; Noy & Sklan, 1999; Madsen & Sorensen, 2004). Most feedstuffs commonly included in pre-starter and starter broiler diets, such as cereals and protein meals, have low digestibility and nutrient availability for chicks, as their digestive system is still immature (Madsen & Sorensen, 2004). However, young chicks have high nutritional requirements because of the fast growth rate of the digestive tract and other organs.

The nutritive value of egg and egg products was reported in the previous studies (Schaafsma *et al.*, 2000; Spark, 2006; Anton *et al.*, 2006). The egg contains high levels of good quality proteins, especially albumins, lipids, antibodies, bioactive components, and lysozyme. The high digestibility and availability of egg powder nutrients aid the development of the digestive tract of young chicks. Egg powder is able to supply the required nutrients, and foster the growth of the digestive tract and of other important organs early in life.

The results presented in Table 3 show that bacterial population in the jejunal contents was affected by the evaluated dietary treatments, particularly at 7 days of age, when *E. coli* counts were reduced when egg

Table 3 – Effect of different levels of egg powder inclusion in the starter diet on the intestinal microbiology of male broiler chickens (log 10).

	Egg powder level				SEM	p-value
	control	20 g/kg	40 g/kg	60 g/kg		
E. coli						
7 d	6.92[a]	6.45[b]	5.99[c]	5.29[d]	0.165	<0.01
42 d	7.86[a]	6.94[b]	6.91[b]	6.70[b]	0.133	<0.01
Lactic acid bacteria						
7 d	7.92[b]	8.07[b]	8.15[b]	8.80[a]	0.113	0.01
42 d	7.39	7.42	7.48	7.46	0.069	0.97
Total count						
7 d	7.45[c]	7.90[b]	8.70[a]	8.52[a]	0.135	<0.01
42 d	7.59	6.89	7.96	7.12	0.212	0.30

[ab] Means in the same row with different superscripts are significantly different (p≤0.05).

powder was included in the starter diet. On the other hand, the population of beneficial microorganisms, such as lactic-acid bacteria and the total bacteria, increased with the inclusion of egg powder in the starter diet. Eggs contain bioactive components and lysozyme, which may act as antibiotics against undesirable microorganisms (Spark, 2006; Anton *et al.*, 2006). The presence of these components in the intestinal tract of chicks reduced *E. coli* colonization, allowing the colonization of beneficial microbes, such as lactic-acid bacteria.

Table 4 shows that egg powder inclusion in the starter diet of broiler chicks affected some jejunal morphometric parameters. Villus height, villus to crypt ratio, and relative intestinal length increased when broilers were fed starter diets with egg powder. Cook & Bird (1973) reported that villus area and crypt depth linearly increase during the first seven days of the broilers' life, and Uni *et al.* (1995) and Geyra *et al.* (2001) also reported accelerated intestinal tract growth early post-hatch. All of those studies indicated that high nutrient levels are required during this period to support the growth of digestive tract. In addition, because of the limited capacity of the digestive tract of newly-hatched chicks, feedstuffs must be highly digestible. Egg powder is highly digestible and may be efficiently utilized by young chickens, as shown by the positive impact of egg powder on the intestinal morphometry of chicks in the present study.

It was concluded that that egg powder inclusion at the level of 40 g/kg in the starter diet improves the performance and intestinal health of broiler chickens.

ACKNOWLEDGMENTS

Authors are grateful to the Tehran Science and Research Branch, Islamic Azad University for the financial support.

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Table 4 – Effect of different levels of egg powder inclusion in the starter diet on the intestinal morphology of male broiler chickens.

	Egg powder level				SEM	p-value
	control	20 g/kg	40 g/kg	60 g/kg		
Villus height (μm)						
7 d	582^{b}	611^{b}	772^{a}	621^{b}	23.2	<0.01
42 d	1165^{b}	1255^{a}	1270^{a}	1263^{a}	15.3	0.02
Crypt depth (μm)						
7 d	102^{b}	122^{a}	128^{a}	128^{a}	3.5	<0.01
42 d	147^{a}	123^{b}	113^{b}	117^{b}	3.8	<0.01
Villus/crypt ratio						
7 d	5.69	5.04	6.08	4.81	0.209	0.10
42 d	7.93^{b}	10.2^{a}	11.2^{a}	10.8^{a}	0.38	<0.01
Goblet cell (n/μm of villus)						
7 d	11.1^{a}	9.07^{ab}	8.98^{ab}	7.51^{b}	0.461	0.03
42 d	13.4^{a}	12.3^{ab}	11.2^{ab}	11.0^{b}	0.38	0.10
Intestinal length, cm/100 g BW						
7 d	53.9^{a}	50.1^{b}	48.8^{b}	48.6^{b}	0.72	0.01
42 d	7.42^{a}	6.76^{ab}	6.37^{b}	6.33^{b}	0.165	0.05

[ab] Means in the same row with different superscripts are significantly different ($p \leq 0.05$).

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