



ABSTRACT

The objective of this study was to determine the effect of different levels of chicory root and stem powder (CRSP) and butyric acid (BA) combination on the performance, carcass traits, relative weight of internal organs and some blood parameters in broiler chickens. Two hundred and forty one-day-old broilers (Ross 308) were used in a completely randomized design with four treatments with six replicates of 10 birds each. The treatments were: 1) basal diet without CRSP and BA (control group), 2) basal diet + 15 g/kg CRSP + 0.3% BA (CRSP15), 3) basal diet + 30 g/kg CRSP + 0.3% BA (CRSP30) and 4) basal diet + 45 g/kg CRSP + 0.3% BA (CRSP45). At day forty six, two birds per replicate (pen) were randomly selected and slaugh-tered for carcass traits and some blood parameter measurements. The results showed that body weight gain of birds fed CRSP45 diet was significantly (P<0.05) higher than those fed on the other treatment diets during starter (1-21 d), grower (22-46 d) and whole experimental period (1-46 d). The drumstick and breast yield as a percentage of live body weight were significantly (P<0.05) higher in birds fed CRSP45 diet compared with those fed the control diet. There were not significant effect (P>0.05) of dietary treatments on serum biochemical parameters and relative weights of abdominal organs excluding the liver.

KEY WORDS body weight gain, broiler chickens, butyric acid, carcass yield, chicory.

INTRODUCTION

The risk of antibiotic resistance by continuous administration of antibiotic growth promoters (AGPs) in animal feed led to a ban on sub-therapeutic use of AGPs in poultry diets in European Union since January 2006. One of the consequences of excluding AGPs from broiler diets will be inevitably change in the microbial ecology of intestinal tract in chicks (Knarreborg *et al.* 2002). Different alternatives for antibiotics are proposed such as organic acids, probiotics, prebiotics and phytogenic products (Dahiya *et al.* 2006). Amongst the organic acids, short chain fatty acids (SCFA) such as butyric acid (BA) are considered as potential alternative to AGPs. Butyrate which is derived from the fermentation of non starch polysaccharides (NSP) is considered to be important for normal development of epithelial cells (Pryde *et al.* 2002). However, the levels of SCFA are quite low in the distal gastrointestinal (GI) tract and caeca of young broiler chickens (Van der Wielen, 2006) and so the young chicks may be the best candidates for dietary supplementation. It was shown that dietary inclusion of BA had no effect on body weight or weight gain, but birds consumed less feed when diets were supplemented with butyrate compared to the control birds (Lesson *et al.* 2005).

Prebiotics are certain substrates, such as dietary fiber that escape digestion in the foregut and reach the distal parts of animal intestine and are now a central issue in nutrition application. Recent studies have indicated that chicks require fiber as a natural prebiotic in the diet to stimulate the development of the upper GI tract (Gonzalez-Alvarado *et al.* 2008). However, it is also known that inclusion of fiber in poultry diet is associated with negative effects on performance and digestibility. The adverse effects of fibers are mainly related to the soluble NSP components in the dietary fiber fraction and increased digesta viscosity in the intestine (Bach knudsen, 2001).

A prebiotic effect of dietary fiber will help to reduce the antibiotic usage in livestock and this will reduce the risk of transferring the antibiotic resistance gene to human pathogens (Looft *et al.* 2012). Furthermore, dietary fiber has been associated with gut disorder management such as *Salmonella* infection in chickens and post-weaning diarrhea in pigs (Montagne *et al.* 2003).

Chicory (Cichorium intybus) is a perennial phytogenic herb that can produce nutritious and is a potential feed resource that could partly replace cereal grain fiber in animal feed and reduce feed cost (Ivarsson et al. 2011). The dietary fiber in chicory forage has high content of pectin (80-90 g/kg dry DM), a type of NSP with uronic acid which is highly soluble in comparison with other pectin sources (Ivarsson et al. 2011). Moreover, chicory root has a high content of oligofructose and inulin, which as a prebiotic can be used to manipulate the composition of microflora in the gut (Flickinger et al. 2003). Inulin content of whole chicory plant ranges from 150 to 200 g/kg (Flickinger et al. 2003). Inulin is a chain of fructans with non-soluble protein which has minimal side effects, and is a good source of energy in an animal's diet (Lunn and Buttriss, 2007). It also regulates appetite and lipid-to-glucose metabolism. The β (2-1) glycosidic bond is responsible for the inluin resistence against the digestive enzymes of the host. Previous studies have shown that inulin can promote the growth of beneficial microbes, such as lactic acid bacteria and Bifidobacteria (Patterson et al. 2010) and inhibit growth of pathogenic bacteria like Escherichia coli and Salmonella spp. (Xu et al. 2003) along the digestive tract. The addition of chicory root fructans either inulin or oligo fructose to broiler feed improved body weight gain, feed conversion, carcass yield and increased the small intestine length of female broilers (Yusrizal and Chen, 2003a). Furthermore, supplementation of inulin to broiler diets has been found to increase the concentrations of jejunal lactate and caecal butyrate (Rehman et al. 2006).

According to some studies, supplementation of only commercial prebiotic to broiler diet did not increase body weight gain (BWG) of broiler at 42 d, but in combination with BA the effect on BWG and serum chemical parameters get significant improvement compared to control group. In addition, SCFAs such as butyrate are also considered as potential alternatives to AGPs (Lesson *et al.* 2005). Chicory in addition to be as a fiber source and prebiotic, contains a number of medicinally important compounds, such as inulin, bitter sesquiterpene lactones, coumarins, flavonoids, and vitamins (Varotto *et al.* 2000). Therefore, the aim of this study was to investigate the impact of dietary inclusion of dried chicory root and stem powder and butyric acid on performance, carcass characteristics and blood parameters in broiler chickens.

MATERIALS AND METHODS

Birds and diets

The whole chicory plant were purchased from grocery, dried in the oven at 50 °C for 48 hr and then powdered. The chemical compositions of CRSP were determined in laboratory. The obtained proportion of DM, CP, CF, Ash, EE and NFE were 30.63, 6.60, 28.18, 11.85, 3.50 and 49.87 percent, respectively. Metabolizable energy (ME) of this powder was calculated by fallowing equation: $(8.62 \times CP) + (50.12 \times EE) + (37.67 \times NFE) = 2111 \text{ kcal/kg. This value was used in diet formulation by UFFDA software. The encapsulated calcium butyrate (Greencab 70 Coated®) was provided by Nutri Concept Company, Fougeres, France.$

Total of 240 one-day-old broiler chicks (Ross 308) with an initial BW of 45 ± 0.15 g were randomly divided into 24 experimental units of 10 birds each with six replicates per treatment for a total of four different treatments. Temperature and relative humidity was maintained within the optimum range. Lighting was 23 h light and 1 h darkness. The chicks were housed in floor pens (1.25×1.25 m). Starter diets were offered *ad libitum* from 1 to 21 days of age. Then finisher diets were offered *ad libitum* from 22 to 46 days of age.

These dietary treatments were formulated according to the Ross Broiler Nutrient Specifications manual to provide a similar nutrient profile with the exception of using two feed additives or a combined addition of these additives. The diets were: 1) basal diet as a control with no supplementation, 2) basal diet + 0.3% coated butyric acid + 15 g/kg chicory root and stem powder (CRSP15), basal diet + 0.3% coated butyric acid + 30 g/kg chicory powder (CRSP30) and basal diet + 0.3% coated butyric acid + 45 g/kg chicory powder (CRSP45). Ingredients and the composition of the experimental diets are shown in Table 1. Table 1 Ingredients and composition of the dietary treatments

	Starter period				Grower period			
Diet ingredients	CON	CRSP15	CRSP30	CRSP45	CON	CRSP15	CRSP30	CRSP45
Corn grain (8% CP)	55.7	54.00	52.29	49.95	59.8	56.82	52.75	53.73
Soybean meal (43 % CP)	38.30	36.20	35.31	35.72	33.30	33.82	31.62	31.37
Corn gluten meal	0	2	3	3	0	0	2.00	2.50
Sunflower oil	1.56	1.52	1.62	2.07	3.08	3.76	3.50	3.77
Chicory powder	0	1.5	3.0	4.5	0	1.50	3.00	4.50
Dicalcium phosphate ¹	1.8	1.8	1.79	1.79	1.28	1.27	1.27	1.27
Calcium carbonate	1.31	1.31	1.31	1.30	1.38	1.37	1.37	1.36
Salt	0.37	0.36	0.36	0.36	0.32	0.32	0.32	0.32
Mineral and vitamin premix ²	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
DL-methionine	0.32	0.31	0.30	0.31	0.18	0.19	0.17	0.17
L-lysine	0.05	0.10	0.11	0.10	0.07	0.05	0.10	0.11
L-threonine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Coated butyric acid	0	0.30	0.30	0.30	0	0.30	0.30	0.30
Calculated composition								
Metabolizable energy (kcal/kg)	2900	2900	2900	2900	3050	3050	3050	3050
Crude protein (%)	21.90	22.13	22.29	22.39	19.90	19.99	20.22	20.35
Ca (g/kg)	1.00	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Available phosphorous (g/kg)	0.45	045	0.45	0.45	0.35	0.35	0.35	0.35
Methionine + cystine (g/kg)	0.99	0.92	0.99	0.99	0.80	0.80	0.80	0.80
Lysine (g/kg)	1.23	1.10	1.22	1.22	1.12	1.11	1.11	1.11

CON: basal diet without chicory root and stem powder (CRSP) and butyric acid (BA) (control group); CRSP15: basal diet + 15 g/kg CRSP + 0.3% BA; CRSP30: basal diet + 30 g/kg CRSP + 0.3% BA and CRSP45: basal diet + 45 g/kg CRSP + 0.3% BA.

¹ Dicalcium phosphate contained: 16% phosphorous and 23% calcium.

² Vitamin and mineral premix per kg of diet: vitamin A (retinol): 1500 IU; vitamin D₃ (cholecalciferol): 10 IU; vitamin E (tocopheryl acetate): 1 mg; vitamin K₃: 1.5 mg; Thiamine 4 mg; Riboflavin: 5 mg; Panthothenic acid: 10 mg; Folic acid: 0.015 mg; Cyanocobalamin: 20 mg; Niacin: 30 mg; Biotin: 80 mg; Choline chloride: 0.65 mg; Antioxidant: 100 mg; Co: 0.1 mg; Mn (MnSO₄.H₂O, 32.49% Mn): 4 mg; Cu (CuSO₄.5H₂O): 0.5 mg; I (KI, 58% I): 0.1 mg; Se (NaSeO3, 45.56% Se): 1520 mg and Ca: 100 mg.

Growth performance and carcass measurements

Body weight and feed intake (FI) were registered by pen at arrival and on d 21 and 46. Feed conversion ratio (FCR) was calculated and corrected for mortality. The performance is presented for the each rearing phase (1-21 d and 22 to 46 d) and for the overall experiment (1-46 d). After 12 h of fasting, forty-eight broilers (two birds from each group or pen) were selected randomly for carcass evaluations at 46 days of age. After slaughtering by a sharp knife for complete bleeding, their feathers were plucked mechanically and then eviscerated by hand. Whole carcase, abdominal fat pad (excluding the gizzard fat), empty gizzard, liver, heart and spleen were excised and weighed individually. Lengths of total gut (duodenum, jejunum, ileum and cecum) were recorded after ingesta were removed. The carcass yields and the weights of internal organs were calculated as a percentage of the preslaughter live body weights of broiler chickens.

Serum biochemical analysis

Blood samples were collected during slaughtering from birds in non heparinised tubes and then the blood was centrifuged at 3000×g for 10 min. to obtain serum (SIGMA 4-15 Lab Centrifuge, Germany). The Serum was collected and stored at -20 °C until analyzed for glucose, triglyceride, total cholesterol, high density lipoprotein (HDL) and lowdensity lipoprotein (LDL) cholesterol, using the Hitachi 911 autoanalyzer (Roche Diagnostics, Division of Hoffman-La Roche Limited, Quebec, Canada) according to the procedures recommended by the manufacturer of the kits (Pars-Azmoon Company, Tehran).

Statistical analysis

The experiment was carried out in a complete randomized design (CRD) and the data were subjected to one-way analysis of variance (ANOVA) according to the General liner Model (GLM) procedure of SAS version 9.1 (SAS, 2004). Differences between treatments were determined using the Duncan's multiple range test and reported as means \pm SEM and (P<0.05) was considered the significant level.

RESULTS AND DISCUSSION

Growth performance

Body weight gain, FI and FCR of chicks during the experimental period are summarized in Table 2. Chicken BWG was affected markedly by supplementing 45 g/kg of chicory powder plus coated butyric acid during the first, second and overall period (d 1-21, d 22-46 and d 1-46, P<0.05). Other treatments did not have any significant effect on BWG in comparison with control group at second feeding phase (P>0.05), but in total period (1-46 days) lower level of herbal supplement plus butyric acid

(CRSP15) also have a significant difference from control group. No significant (P>0.05) differences due to dietary treatment effects were observed on feed intake in any of the feeding periods. According to Table 2, birds receiving diet supplemented with 45 g/kg chicory powder plus butyric acid (CRSP45) at first feeding phase (1-21 days) had a significantly lower FCR compared to the control group (P<0.05). But as broilers aged, treatments did not induce any significant impact on FCR in the second and whole period (1-46 days) although it was calculated to be higher in the control (P>0.05).

Carcass traits and internal organs weight

Relative weights of organs and body parts are given in Table 3. It shows that carcass percentage were higher in broilers fed supplemented diets than those given the control diet (P<0.05). The proportions (% of live body weight) of drumstick and breast were higher in the birds fed CRSP45 diet in comparison with the control and other groups. Apart from liver relative weight, none of dietary treatments produced significant differences in any of the measured abdominal parameters (P>0.05).

Blood parameters

Table 4 summarizes the impact of treatments on serum constituents at day 46 of age. Treatments did not induce any significant effect on the serum concentration of glucose, triglyceride, total cholesterol, LDL-c and HDL-c (P<0.05).

Recent researches focus on chicory powder that has major fiber components and acts as a potential prebiotic due to inulin-type fructans and oligofructose (Izadi et al. 2013). Addition of chicory root to diet improves the growth performance, egg production and the length of small intestine in poultry (Yusrizal and Chen, 2003b; Rehman et al. 2007). In the present study, addition of 1.5% and 4.5% chicory powder increased body weight gain of broilers compared to the control group. Results are in agreement with Yusrizal and Chen (2003a) who reported that birds received 1% oligofructose were heavier, especially female broilers (10%), compared to control. Another study demonstrated that adding fructooligosaccharide and inulin to the basal diet of broiler chickens had positive effects on performance. Although, a previous report showed that feeding moderate levels of inulin (5-10%) did not speed up growth rate (Ammerman et al. 1989).

Our results showed that dietary addition of 45 g/kg CRSP improved FCR only during the first feeding period in broilers (P<0.05). According to Liu *et al.* (2011), supplementing 6% chicory root powder to broiler diet improved BWG and FCR only in first phase (1-13 days) not during the overall period (d 1-27), that is in agreement with the present study. The profound effects of chicory powder plus BA supple-

mentation in the first feeding period (1-21 d) can be attributed to over sensitivity of broiler chickens to colibacillosis infection that are more frequently occur in the gut of newly hatched chickens (Calnek *et al.* 1991). One of the strategies to eliminate the coliforms from the gastrointestinal tract is by maintaining a lower pH, which is unsuitable for the growth of this organism. This aim could be achieved by supplementation of BA and prebiotics such as chicory to broiler diets.

Prebiotics and organic acids maintained a better microbial environment in digestive tract of birds by reducing the number of pathogenic microbes. It is accepted that the main direct beneficial effect attributable to prebiotics is to induce changes in the intestinal microbiota by selective stimulation of health promoting bacteria (Gibson et al. 1995). Van Immerseel et al. (2004) have indicated significantly reduced levels of Salmonella in the ceca of birds fed organic acids. Kwan and Ricke (1998) demonstrated that amongst the short chain fatty acids, butyrate has the highest bactericidal efficacy against the acid-intolerant species such as E. coli and Salmonella. Rebole et al. (2010) have shown increases in the cecal number of Bifidobacteria and Lactobacilli due to the effect of the dietary inulin, but this prebiotic caused neither an increase in the concentration of total SCFAs (acetic plus propionic and n-butyric acids) nor a decrease in the pH of digesta. However, they suggested that dietary inulin altered the fermentation patterns of cecal content as evidenced by the significant increase in the concentration of n-butyric acid and the n-butyric acid:acetic acid molar ratio in parallel with the increase in the concentration of d-lactic acid. This positive effect of inulin on the content of nbutyric acid in the cecal digesta was also observed by Rehman et al. (2007).

The n-butyrate provides energy for the growth of mucosal epithelium and can be involved directly or indirectly in various mechanisms regulating cellular differentiation, intestinal permeability, and gene expression (Mroz *et al.* 2005). Sharma *et al.* (1995) suggested the beneficial effects of BA on crypt cell growth in rats that might reflect changes in the gut microflora, which is known to be a major modulator of epithelial cell activity.

Chicory as a source of inulin in industry contributes to animal well-being in various ways. Inulin has been reported as a highly fermentable fiber (Franck and Bosscher, 2009), highly fermentable dietary fibers are characterized by being readily fermented by enteric bacteria, producing SCFAs especially butyrate, which are the end products of fermentation of polysaccharides by the colonic flora. These fatty acids are used as an energy source by intestinal epithelial cells.

The levels of SCFAs are quite low in the intestine and caeca of young chicks (Van der Wielen, 2006).

Table 2	Effects of different	dietary treatments	on performance	of broiler chickens
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Item	CON	CRSP15	CRSP30	CRSP45	SEM	P-value
Body weight gain (g)						
1-21 days	379.2 ^b	324.8 ^b	321.3 ^b	452.0 ^a	8.02	0.0002
22-46 days	981.5 ^b	1215.9 ^b	1054.7 ^b	1375.3ª	19.32	0.0001
1-46 days	1360.67 ^b	1540.67 ^a	1375.33 ^b	1612.83ª	25.24	0.0001
Feed intake (g/bird)						
1-21 days	707.2	757.0	708.0	810.3	0.16	0.5203
22-46 days	3097.8	3645.0	3653.0	4027.7	59.68	0.8915
1-46 days	3805.0	4402.0	4361.0	4838.0	65.90	0.9522
Feed conversion ratio						
1-21 days	2.86 ^a	2.32 ^{ab}	2.20 ^{ab}	1.79 ^b	0.17	0.0011
22-46 days	3.10	2.90	2.50	2.40	0.12	0.4268
1-46 days	2.80	2.70	2.10	2.00	0.89	0.5622

CON: basal diet without chicory root and stem powder (CRSP) and butyric acid (BA) (control group); CRSP15: basal diet + 15 g/kg CRSP + 0.3% BA; CRSP30: basal diet + 30 g/kg CRSP + 0.3% BA and CRSP45: basal diet + 45 g/kg CRSP + 0.3% BA.

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

SEM: standard error of the means.

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Item	CON	CRSP15	CRSP30	CRSP45	SEM	P-value
Carcass yield ¹	52.58°	59.69 ^b	56.13 ^b	65.97 ^a	1.87	0.0004
Drumstick	14.17 ^b	15.88 ^{ab}	14.21 ^b	17.32 ^a	0.57	0.0003
Breast	14.67 ^b	14.52 ^b	13.99 ^b	16.76 ^a	0.64	0.0001
Liver	1.99 ^b	1.98 ^b	2.45 ^a	2.16 ^a	0.04	0.0001
Heart	0.54	0.51	0.52	0.52	0.01	0.6501
Spleen	0.12	0.10	0.11	0.11	0.06	0.9223
Gizzard	1.77	1.92	1.96	2.06	0.08	0.3528
Abdominal fat	1.52	1.45	1.51	1.26	0.30	0.0512
Gastric intestinal (GIT) (cm)	7.50	6.60	6.70	8.20	0.52	0.1180

¹ Carcass yield, without head either feet.

CON: basal diet without chicory root and stem powder (CRSP) and butyric acid (BA) (control group); CRSP15: basal diet + 15 g/kg CRSP + 0.3% BA; CRSP30: basal diet + 30 g/kg CRSP + 0.3% BA and CRSP45: basal diet + 45 g/kg CRSP + 0.3% BA.

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

SEM: standard error of the means

Table 4	Some blood	metabolite	(mg/dL)	at day	46 of bro	iler chickens	fed different	dietary treatments
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Item	CON	CRSP15	CRSP30	CRSP45	SEM	P-value
Glucose	187.9	176.7	196.2	193.9	6.16	0.1464
cholesterol	118.33	117.33	118.33	121.33	5.86	0.9801
Triglyceride	71.75	82.08	81.92	60.25	7.35	0.1340
HDL-c	50.33	49.46	52.43	52.80	2.30	0.4387
LDL-c	54.33	52.23	50.86	51.15	0.30	0.9569

CON: basal diet without chicory root and stem powder (CRSP) and butyric acid (BA) (control group); CRSP15: basal diet + 15 g/kg CRSP + 0.3% BA; CRSP30: basal diet + 30 g/kg CRSP + 0.3% BA and CRSP45: basal diet + 45 g/kg CRSP + 0.3% BA.

HDL: high density lipoprotein and LDL: low density lipoprotein.

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

SEM: standard error of the means.

Thus it could be suggested here that young chicks are therefore the best candidate for diet supplementation of organic acid especially butyric acid because of its both bactericidal and stimulant of villi growth property. Liu *et al.* (2011) demonstrated that dietary supplementation of CRSP at 1% and 3% levels promoted digestion and absorption through the histomorphological changes of villi and jejunum parameters. Izadi *et al.* (2013) attributed improved performance of broiler fed diet supplemented with chicory root powder to the enhancements of length, number and surface area of intestinal villi that are paralleled with an increased digestive and absorptive capacity of the jejunum. Notably, several studies have shown significant enlargement of villus height or crypt depth in the small intestine in monogastric animals that have ingested various dietary NSP sources (Rehman *et al.* 2007). Moreover, beneficial effects of CRSP can be attributed to reduced activity of urease producing bacteria in the gut. Yeo *et al.* (1997) have indicated that dietary prebiotic suppressed the growth of bacteria that produce urease. They suggested that this effect may be responsible for the increased weight gain during the first 3 wk of feeding in chicks fed the diet supplemented with prebiotic. Suppressing urease activity and ammonia production can be beneficial for improving animal health and enhancing growth, because ammonia locally produced by ureolysis in the intestinal mucosa can exert a significant damage to the surface cells.

Another important finding of the present study was the improvement in dressing percentage by supplementation of chicory powder plus butyrate to broilers diet. This result is in consistency with Panda *et al.* (2009), Yusrizal and Chen (2003b), Ammerman *et al.* (1989). Abdominal fat weight was lower numerically in the group supplemented with CRSP45 in comparison with the control group. It was shown that abdominal fat content in broiler chickens is reduced statistically by dietary supplementation of BA (Panda *et al.* 2009). Generally, it can inferred that CRSP45 diet by improving intestinal microenvironment, reducing endogenous nitrogen losses, and lowering the secretion of immune mediators, yields higher carcass percentages in broiler chickens (Panda *et al.* 2009; Yusrizal and Chen, 2003b).

The length of the GI tract was not statistically different (P>0.05) among the trial groups but it was numerically higher in the CRSP45 diet (P>0.05). This result is in disagreement with Elrayeh *et al.* (2012) who indicated that supplementation of 0.7% inulin to broiler diet increased significantly length value for total intestine. Some studies support the idea of using prebiotics for increasing the length of the intestinal villus, which affects the length of the intestine, as well. It has been reported that the longer the intestine is better for nutrient absorption, which in turn causes an increase in BWG (Yusrizal and Chen, 2003b).

Blood serum biochemical values were not significantly different among the groups that are in agreement with the results of Safamehr *et al.* (2013). Moreover, the results of the current study are in disagreement with the findings of Yusrizal and Chen (2003b) and Elrayeh and Yildiz (2012) and Mirza agazadehh *et al.* (2015) who observed that adding chicory-based fructans to feeds decreased the serum cholesterol, VLDA and TG level in broilers.

CONCLUSION

In conclusion, supplementing CRSP plus BA in the broiler diet enhanced BWG in all feeding periods and FCR in the rearing period (1-21 d). Improving digestion and absorption during the rearing period is detrimental to the health of broilers. In this study serum biochemical parameters and slaughter characteristic were not affected by dietary treatment excluding carcass yield and relative weight of liver.

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