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Study on Usage Period of Dietary Protected Butyric Acid on Performance, Carcass Characteristics, Serum Metabolite Levels and Humoral Immune Response of Broiler Chickens

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Abstract: This study was undertaken to investigate the effect of dietary inclusion of protected Butyric Acid (BA) glycerides on growth performance, gastrointestinal tract parameters, carcass traits, blood metabolites and humoral immune response of broiler chicks. Four hundred and eighty days-old unsexed Arbor-acres broiler chicks were randomly distributed between 48 battery pens. Four dietary inclusion of BA (0-2 or 3 g kg^{-1}) on different (starting, growing and finishing) periods was tested. There was no significant difference in Body Weight (BW), Body Weight Gain (BWG), Feed Intake (FI), Feed Conversion Ratio (FCR) and mortality among treatments ($p>0.05$). Chicks fed diets included 2 g BA kg^{-1} showed higher BWG during 0-21 days of age ($p>0.05$). The relative weights of breast, thighs, abdominal fat, liver, pancreases, gall bladder, spleen, bursa of fabricius, thymus and cecum to BW were not affected by experimental treatments ($p>0.05$). Dietary inclusion of BA significantly increased the relative weight of intestine segments as a percentage of body weight ($p<0.05$). The length of small intestine was affected by dietary inclusion of BA ($p<0.05$). Birds fed on diets included 3 g BA kg^{-1} during the whole rearing period (SGF3) presented longer small intestine as compared to the control group ($p<0.05$). The ileal pH as well as serum metabolites except calcium were not significantly affected by BA ($p>0.05$). Dietary butyric acid did not have a clear positive effect on performance of broilers reared under good hygiene conditions.

Key words: Butyric acid, performance, humoral immune response, serum metabolites, broiler chickens, SDF3

INTRODUCTION

Antibiotics have been common feed additives in poultry rations as growth promoters to improve performance via reducing the burden of pathogens (Leitner *et al*., 2001). These growth promoters have been frequently used therapeutically and prophylactically to treat poultry diseases. The first recorded research indicated the positive effect of antibiotics on chicken growth was on 1940s (Moore *et al*., 1946). However, it has been increasing pressure to reduce or even eliminate antibiotic usage in poultry feed due to the negative human health issue of antibiotic resistance (Dibner and Buttin, 2002; Derebas and Demir, 2004; Gunal *et al*., 2006).

Strategies to reduce antibiotic usage in poultry diets include improved biosecurity, vaccination, genetic selection and antibiotic replacements such as prebiotics, probiotics, essential oils and organic acids. Organic Acids (OA) have been used as feed and food preservatives to prevent microbial spoilage. Inclusion of OA to farm animal diets has been widely accepted to control the microbial balance in the gut (Piva *et al*., 2007). It has been reported that acidification of diets with various weak organic acids such as fumaric, propionic, lactic and sorbic decreased pathogen colonization and production of toxic metabolites (Alp *et al*., 1999; Chaveerach *et al*., 2004) and improved digestibility of protein and of Ca, P, Mg, Zn and served as substrates during metabolism (Brenes *et al*., 2003; García *et al*., 2007). The mentioned week acids mostly act on the microflora either by decreasing the pH of gastrointestinal tract that in turn inhibit growth of pathogenic bacteria and via direct action against microflora (Hernández *et al*., 2006; Lippens *et al*., 2006). One of the proposed action mechanisms of organic acids against bacteria is that undissociated short chain organic acids are lipophillic penetrating the cell wall of bacteria. Within the cells, the acids produce H^+ ions, which in turn disrupt the normal physiology of the bacteria. The inherent limitation of the effective dose of OA in modulating intestinal flora may reside in the prompt absorption, metabolisation, or both that they undergo upon entering the duodenum. This could be overcome by

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microencapsulating the active compounds in a matrix to dissolve and release active forms (Mroz, 2005; Hernández *et al*., 2006; Piva *et al*., 2007).

Specific microbial populations degrade dietary structural carbohydrates in the gut producing Short Chain Fatty Acids (SCFA) of these, butyric acid is considered as the most efficient one (Friedman and Bar-Shira, 2005). Butyric acid has been widely reported as the major development promoter of the gut wall tissues and an important growth modulator of symbiotic intestinal microflora (Van Immerseel *et al*., 2004, 2005; Friedman and Bar-Shira, 2005; Leeson *et al*., 2005). Also, butyric acid is known the main enterocytes energy source. It is essential for the correct development of the Gut Associated Lymphoid Tissue (GALT) as well (Friedman and Bar-Shira, 2005). There is very limited information about effects of butyric acid in poultry. In testing, the survival of *Salmonella enterica* serovar typhimurium during exposure to short-chain fatty acids, Kwon and Ricke (1998) showed butyrate and valerate to have the greatest efficacy. Moreover, Leeson *et al.* (2005) and Antongiovanni *et al.* (2007a) reported positive beneficial effects of butyric acid on performance traits of broilers.

The overall objectives of this study were to assess the effects of different usage periods of butyric acid glycerides and different levels of butyric acid glycerides in broiler diets on performance, relative organ weight, serum metabolites and humoral immune response.

MATERIALS AND METHODS

Birds, feeding and management: All procedures were approved by the Institutional Animal Care and Use Committee of Razi University. A total number of 480 unsexed day-old Arbor acres broiler chicks were distributed between 48 cage pens (battery) in a completely randomized experimental design with eight treatments and six replications of ten chicks each. The temperature was maintained at 32±1°C in the 1st week and reduced by 2.5°C week^{-1} to 21°C. From day 1 until day 4 the lighting schedule was 24 h light. During days 5-49, the dark periods were increased to 1 h. Birds were fed diets (starter: 1-21, grower: 22-42 and finisher: 43-49 days) based on NRC. The height of used nipple drinkers were adjusted twice weekly by visual inspection. The composition of experimental diets is shown in Table 1. Experimental diets were: without (BA) butyric acid glycerides (Silo company, Italy) as Control (C), or with 2.0 g BA kg^{-1} in starter diet (S2), 2 g BA kg^{-1} in starter and grower diets (SG2), 2 g BA kg^{-1} in starter, grower and finisher diets (SGF2), 3.0 g BA kg^{-1} in starter diet (S3), 3 g BA kg^{-1} in starter and grower diets (SG3), 3 g BA kg^{-1} in starter, grower and finisher diets (SGF3) and descending concentration, 3.0, 2.0 and 1.0 g BA kg^{-1} in starter, grower and finisher (D), respectively (Table 2). The used BA (product of BABY-C4) contained 25-30% monoglycerides in the 1 or 3 positions, 50-55%

Table 1: Composition of feed mixtures (%)

	Butyric acid[1]									
	Starter			Grower			Finisher			
Ingredients (%)	0	0.20	0.30	0	0.20	0.30	0	0.10	0.20	0.30
Maize	59.00	58.78	58.66	67.37	67.14	67.03	72.01	71.89	71.78	71.67
Soybean meal	35.54	35.58	35.61	28.43	28.48	28.50	24.26	24.28	24.31	24.33
Sunflower oil	1.56	1.54	1.53	0.65	0.63	0.62	0.56	0.55	0.54	0.53
DCP	1.41	1.41	1.41	1.02	1.02	1.02	0.84	0.84	0.84	0.84
Oyster shell	1.26	1.26	1.26	1.34	1.34	1.34	1.26	1.26	1.26	1.26
Common salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[3]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-methionine	0.13	0.13	0.13	0.06	0.06	0.06	0.02	0.02	0.02	0.02
HCL-lysine	0.09	0.09	0.09	0.14	0.14	0.14	0.05	0.05	0.05	0.05
Calculated analyses										
ME (kcal kg^{-1})	290000	29000	29000	295000	29500	29500	300000	30000	30000	30000
Crude protein (%)	20.840	20.840	20.840	18.440	18.440	18.440	16.870	16.870	16.870	16.870
Ether extract (%)	3.930	3.905	3.893	3.372	3.347	3.335	3.432	3.420	3.407	3.395
Lysine (%)	1.793	1.797	1.800	1.044	1.044	1.045	0.880	0.880	0.880	0.880
Methionine (%)	0.453	0.453	0.453	0.350	0.350	0.350	0.300	0.300	0.300	0.300
Met + Cys (%)	0.794	0.794	0.794	0.659	0.659	0.659	0.589	0.589	0.589	0.589
Calcium (%)	0.906	0.906	0.906	0.829	0.829	0.829	0.750	0.750	0.750	0.750
Available P (%)	0.408	0.408	0.408	0.322	0.322	0.322	0.281	0.281	0.281	0.281

[1]The composition of butyric glycerides was: 25-30% monobutyrine, 50-55% dibutyrine and 15-25% tributyrine [2]Vitamin premix provided 1 kg of diet with: vitamin A, 10,800 IU; vitamin D3, 2160 IU; vitamin E, 15 IU; vitamin K3, 1.0 mg; vitamin B1, 4 mg; riboflavin, 5 mg; pantothenic acid 10 mg; niacin, 25 mg; vitamin B6, 8 mg; folic acid, 0.4 mg; vitamin B12, 0.08 mg; biotin, 0.15 mg; [3]Mineral premix provided 1 kg of diet with: I, 0.35 mg; Se, 0.15 mg; Zn, 40 mg; Cu, 8 mg; Fe, 80 mg; Mn, 100 mg

Table 2: Dietary treatments

	Concentration of butyric acid in each rearing period (g kg^{-1})		
Treatments	Starter	Grower	Finisher
C	0	0	0
S2	2	0	0
SG2	2	2	0
SGF	2	2	2
S3	3	0	0
SG3	3	3	0
SGF3	3	3	3
D	3	2	1

diglycerides in the 1 or 3 positions and 15-25% triglyceride. The ME of BABY-C4 was assumed to be 8 MJ kg^{-1}. Feed and water were available *ad libitum*.

Collection of samples, measurements and chemical analysis: Body Weights (BW) were recorded for each replicate on days 0, 21, 42 and 49 of age and Feed Intake (FI) was measured over these periods in order to calculate Feed Conversion Ratio (FCR) for each feeding period. Mortality ratio was recorded daily and FCR was corrected for mortality by adding body weights to the total pen weight at the end of each period.

Blood samples were collected from brachial vein of three randomly selected chicks per each treatment at 21, 42 and 49 days of age and transferred into tubes containing potassium EDTA. After providing the blood smear and staining by Giemsa, differential counting of lymphocytes was done using light microscope.

At 49 days of age, 6 chicks treatment^{-1} (1 chick pen^{-1}) were killed by cervical dislocation and the weights of thighs and breast (all with skin), abdominal fat, liver, gallbladder, pancreas, spleen, bursa of fabricius and thymus were measured. Relative organ weights were calculated as (organ weight (g)/BW (kg)).

Before evisceration, blood samples (1 chick pen^{-1}) were taken via wing vein of chicks. The blood samples were transferred into tubes and then centrifuged (10 min and 3000 rpm). The sera was removed and stored at 20°C for further analysis. The serum levels of glucose, cholesterol, total protein, uric acid, calcium and phosphorus were measured with spectrophotometer by using commercial kits (Pars Azmon, Iran).

Small intestine of birds was opened immediately after killing and length and empty weight of various sections were measured. Then approximately 1 g of ileal content per chicken was collected and transferred to 2 mL of distilled water and pH of ileal content was measured using pH meter (Chaveerach *et al*., 2004).

Statistical analysis: Data were analyzed by GLM procedure of SAS (SAS Institute Inc., Cary, NC). Probability values <0.05 were taken to indicate statistical significance. The treatment means were compared using Duncan's multiple range test.

RESULTS

Growth performance: Performance data of chicks fed on experimental diets are detailed in Table 3. Although, the BW and FCR were not statistically affected by dietary inclusion of butyric acid, chicks fed on diets included 0.2% BA showed higher BWG and improved FCR comparing with birds within other experimental groups. Mortality was not affected by the dietary inclusion of BA ($p>0.05$).

Ileal pH, relative organ weights and intestine length: The data of ileal pH, relative organ weights and intestinal length are summarized in Table 4. The weights of thighs, breast, abdominal fat, liver, pancreas and gallbladder expressed as a percentage of BW were not statistically affected by the BA addition. Length and weight of intestinal sections were increased by dietary inclusion of BA ($p<0.05$). The length of jejunum in D treatment and the length of small intestine in D and SGF3 treatments were significantly higher than other groups ($p<0.05$). The relative weights of jejunum, ileum and small intestine were higher in S3, SGF3 and SGF3, D and SGF3 comparing with other experimental groups, respectively ($p<0.05$). Dietary treatments had no statistically significant effect on ileal pH ($p>0.05$); however, the ileal pH value tended to decrease due to butyric acid supplementation. The relative weight of cecum did not statistically differ among treatments ($p>0.05$).

Serum metabolites: The effects of dietary BA inclusion on serum metabolite contents are shown in Table 5. Serum level of glucose, cholesterol, total protein and uric acid were not affected by BA addition ($p>0.05$). Statistically analyzed data showed that BA addition to diets caused an increase in the serum levels of Ca ($p<0.05$) and P ($p>0.05$). The serum Ca level of chicks fed on diet SGF3 comparing with other experimental diets was higher ($p<0.05$).

Immune response: The effects of dietary BA inclusion on the numbers of white blood cells, relative weights of thymus, bursa of fabricius and spleen are shown in Table 6. The relative weight of spleen, bursa and thymus did not statistically differ among the treatments ($p>0.05$). Dietary BA inclusion did not affect the counts of lymphocytes, heterophils, monocytes, basophils and eosinophils at 21, 42 and 49 days of age ($p>0.05$).

Table 3: Performance traits of broiler chickens fed on BA included diets in different rearing periods

	Treatments†								
Variables	C	S2	SG2	SGF2	S3	SG3	SGF3	D	p-value
Weight gain (g/chick/d)									
01-21	34.2	35.1	34.7	36.1	32.6	32.5	33.6	31.7	NS
22-42	68.4	72.1	70.8	71.2	71.3	69.1	70.9	69.0	NS
43-49	94.1	91.6	95.8	90.6	93.6	102.3	98.1	100.5	NS
01-49	56.2	58.2	58.0	58.1	57.0	56.9	57.7	56.1	NS
Feed intake (g chick^{-1})									
01-21	1179	1157	1152	1178	1145	1149	1145	1139	NS
22-42	3115	3076	3067	3120	3123	3059	3129	3070	NS
43-49	1219	1284	1262	1234	1272	1241	1303	1226	NS
01-49	5433	5494	5468	5506	5519	5416	5546	5356	NS
FCR (g g^{-1})									
01-21	1.61	1.58	1.58	1.54	1.68	1.68	1.61	1.68	NS
22-42	2.13	2.00	2.00	2.07	2.07	2.11	2.10	2.03	NS
43-49	2.17	2.16	2.22	2.32	2.32	2.04	2.25	2.07	NS
01-49	1.99	1.92	1.94	1.97	2.01	1.97	1.99	1.95	NS
Mortality (%)									
01-21	1.67	0.00	0.00	1.67	1.67	1.67	5.00	5.00	NS
22-42	5.00	1.67	1.67	1.67	1.67	1.67	1.67	3.33	NS
43-49	0.00	1.67	0.00	0.00	0.00	0.00	0.00	0.00	NS
01-49	6.67	3.33	1.67	3.33	3.33	3.33	6.67	8.33	NS

†C = 0%; S2 = 0.2% BA in starter diet; SG2 = 0.2% BA in Starter and Grower diets; SGF2 = 0.2% BA in Starter, Grower and finisher diets; S3 = 0.3% BA in starter diet; SG3 = 0.3% BA in Starter and Grower diets; SGF3 = 0.3% BA in starter, grower and finisher diets; D (Descending concentrations) = 0.3% BA in starter diet, 0.2% BA in grower diet and 0.1% BA in finisher diet

Table 4: Carcass yield, intestinal parameters and ileal pH of broiler chickens fed on BA-included diets in different rearing periods

	Treatments†								
Variables	C	S2	SG2	SGF2	S3	SG3	SGF3	D	p-value
Breast (%)	23.60	21.40	22.30	23.30	21.60	23.30	23.10	22.80	NS
Thigh (%)	21.30	21.70	20.60	19.70	21.80	22.10	21.40	20.60	NS
Abdominal fat (%)	2.11	2.52	2.57	1.66	2.61	2.67	2.36	2.56	NS
Liver (%)	2.10	2.04	1.94	1.84	1.75	1.83	1.94	2.04	NS
Pancreas (%)	0.17	0.19	0.20	0.20	0.17	0.19	0.19	0.19	NS
Gallbladder (%)	0.10	0.10	0.09	0.10	0.09	0.09	0.10	0.10	NS
Small intestine weight (%)	2.19^{a}	2.34^{ab}	2.27^{a}	2.33^{ab}	2.54^{ab}	2.44^{ab}	2.66^{b}	2.53^{ab}	*
Duodenum weight (%)	0.43	0.43	0.44	0.45	0.44	0.45	0.45	0.45	NS
Jejunum weight (%)	0.86^{a}	0.97^{ab}	0.89^{ab}	0.92^{ab}	1.06^{b}	1.02^{ab}	1.08^{b}	0.96^{ab}	*
Ileum weight (%)	0.90^{a}	0.94^{ab}	0.94^{ab}	0.95^{ab}	1.02^{ab}	0.97^{ab}	1.12^{b}	1.13^{b}	*
Cecum weight (%)	0.36	0.41	0.36	0.40	0.39	0.33	0.33	0.36	NS
Small intestine length (cm)	174.57^{a}	182.28^{ab}	183.42^{ab}	182.67^{ab}	184.00^{ab}	184.75^{ab}	190.45^{b}	191.41^{b}	*
Duodenum length (cm)	28.82	30.53	30.67	30.08	31.08	30.58	31.37	30.91	NS
Jejunum length (cm)	68.92^{a}	72.00^{ab}	73.67^{ab}	70.25^{ab}	72.67^{ab}	74.50^{ab}	74.25^{ab}	75.83^{b}	*
Ileum length (cm)	76.83	79.75	79.08	82.33	80.25	79.67	84.83	84.67	NS
Ileal pH	7.10	7.01	7.02	6.78	7.24	6.8	6.97	6.84	NS

†C = 0%; S2 = 0.2% BA in starter diet; SG2 = 0.2% BA in starter and grower diets; SGF2 = 0.2% BA in starter, grower and finisher diets; S3 = 0.3% BA in starter diet; SG3 = 0.3% BA in starter and grower diets; SGF3 = 0.3% BA in starter, grower and finisher diets; D (Descending concentrations) = 0.3% BA in starter diet, 0.2% BA in grower diet and 0.1% BA in finisher diet

Table 5: Serum metabolites levels of broiler chickens fed on BA included diets in different rearing periods

	Treatments†								
Diets	C	S2	SG2	SGF2	S3	SG3	SGF3	D	p-value
Glucose (mg dL^{-1})	223.33	226.57	229.77	239.71	222.15	229.78	222.80	234.62	NS
Cholesterol (mg dL^{-1})	114.07	128.88	131.08	122.01	115.15	126.30	122.98	111.40	NS
Uric acid (mg dL^{-1})	9.88	11.43	9.84	12.07	12.83	11.68	8.71	10.72	NS
Total protein (g dL^{-1})	3.50	3.20	3.20	3.30	3.60	3.40	3.60	4.10	NS
Calcium (mg dL^{-1})	9.80^{a}	9.90^{a}	11.10^{ab}	10.50^{ab}	10.60^{ab}	10.50^{ab}	11.90^{b}	11.30^{ab}	*
Phosphorus (mg dL^{-1})	7.30	7.80	7.50	7.40	8.00	7.90	8.10	8.90	NS

†C = 0%; S2 = 0.2% BA in starter diet; SG2 = 0.2% BA in starter and grower diets; SGF2 = 0.2% BA in starter, grower and Finisher diets; S3 = 0.3% BA in starter diet; SG3 = 0.3% BA in starter and grower diets; SGF3 = 0.3% BA in starter, grower and finisher diets; D (Descending concentrations) = 0.3% BA in starter diet, 0.2% BA in grower diet and 0.1% BA in finisher diet

Table 6: Percentage myeloid, mononuclear cells and lymphoid organ relative weights of broiler chickens fed on BA-included diets in different rearing periods

	Treatments†								
Variables (%)	C	S2	SG2	SGF2	S3	SG3	SGF3	D	p-value
Thymus	0.27	0.28	0.25	0.25	0.27	0.25	0.29	0.29	NS
Bursa of fabricius	0.19	0.18	0.19	0.19	0.18	0.15	0.12	0.18	NS
Spleen	0.11	0.14	0.12	0.12	0.11	0.11	0.12	0.12	NS
21 day									NS
Lymphocyte	62.67	65.33	64.67	59.00	61.33	58.33	66.67	65.33	NS
Heterophil	33.00	30.33	29.33	34.33	35.33	37.67	28.67	31.00	NS
Monocyte	1.33	0.67	1.33	1.00	0.67	0.67	1.00	0.67	NS
Basophil	2.67	3.00	4.00	4.67	2.00	2.68	3.67	2.67	NS
Eosinophil	0.33	0.67	0.67	0.33	0.67	0.67	0.00	0.33	NS
42 day									NS
Lymphocyte	62.33	61.67	61.67	60.00	60.33	59.67	60.33	62.33	NS
Heterophil	31.33	32.67	32.67	33.33	33.33	33.33	33.33	31.67	NS
Monocyte	3.00	3.33	3.33	3.33	3.67	4.00	3.33	2.67	NS
Basophil	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	NS
Eosinophil	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	NS
49 day									NS
Lymphocyte	57.67	55.33	55.33	55.33	52.33	61.33	55.33	56.00	NS
Heterophil	36.00	37.33	37.33	37.67	39.33	32.33	36.33	36.67	NS
Monocyte	5.33	6.00	6.00	6.33	7.33	5.33	7.00	6.67	NS
Basophil	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	NS
Eosinophil	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	NS

†C = 0%; S2 = 0.2% BA in starter diet; SG2 = 0.2% BA in starter and grower diets; SGF2 = 0.2% BA in starter, grower and finisher diets; S3 = 0.3% BA in starter diet; SG3 = 0.3% BA in starter and grower diets; SGF3 = 0.3% BA in starter, grower and finisher diets; D (Descending concentrations) = 0.3% BA in starter diet, 0.2% BA in grower diet and 0.1% BA in finisher diet

DISCUSSION

Growth performance of birds and mortality were not affected by dietary treatments throughout the experimental period; however, FCR and BWG in the groups fed on 0.2% butyric glycerides diets were numerically better than other treatments. These results are in an agreement with those of Denli *et al.* (2003), Leeson *et al.* (2005), Hernández *et al.* (2006), Gunal *et al.* (2006) and Antongiovanni *et al.* (2007a), who reported that the dietary addition of organic acids did not have any significant effect on BWG, FI or FCR. The beneficial effects of adding organic acids to diets on BWG, FI or FCR were reported by Ünsal and Rüstü (2004), Rafacz-Livingston *et al.* (2005b) and Józefiak *et al.* (2007). It has been suggested that in case of well-nourished healthy chicks housing at a moderate stoking density and hygienic condition, dietary inclusion of organic acids were ineffective on bird's performance. Organic acids, like antibiotics are more growth permitting than growth promoting in the sense that they can only permit the animal to grow to its genetic potential given the diet it is fed (Alp *et al.*, 1999; Dibner and Buttin, 2002; Gunal *et al.*, 2006).

Dietary treatments had no significant effect on the relative weights of abdominal fat, breast muscle, thighs, liver, gallbladder and pancreases. Other experiments indicated that the addition of organic acids to diet had no effect on the relative weight of abdominal fat (Denli *et al.*, 2003; Derebas and Demir, 2004; Antongiovanni *et al.*, 2007a), breast muscle and thighs (Leeson *et al.*, 2005; Antongiovanni *et al.*, 2007a), liver and gallbladder (Denli *et al.*, 2003; Brenes *et al.*, 2003; Antongiovanni *et al.*, 2007a) pancreases (Derebas and Demir, 2004). The length of jejunum and small intestine were significantly increased by dietary administration of butyric acid. The results of this experiment are in agreement with other reports by Reilly *et al.* (1995) and Ichikawa *et al.* (2002); however, Ünsal and Rüstü (2004) did not observe any beneficial effect of dietary inclusion of propionic acid on small intestine length. Butyric acid is considered the prime enterocytes energy source and it is also necessary for the suitable development of the GALT (Friedman and Bar-Shira, 2005; Mroz, 2005). In addition, the greater colonic blood flow has been observed with administration of organic acids. These actions are important for the maintaining function of the whole gastrointestinal system, not just the colon. Greater blood flow enhances tissue oxygenation and transport of absorbed nutrients. The mechanisms of action may involve local neural networks as well as chemo-receptors together with direct effects on smooth muscle cells (Mroz, 2005; Tellez *et al.*, 2006). Autonomic nervous system presumably transmits the short chain fatty acids induced nervous signal from the colon to the central nervous system, which then releases a secondary neural or hormonal signal. These signals may motivate production of growth factor or gastrin from liver and enteroendocrine cells, respectively that stimulates jejunal growth (Reilly *et al.*, 1995).

The highest relative weights of jejunum, ileum and small intestine were observed in SGF3, D and SGF3 treatments, respectively. These results are in agreement with study of Furuse *et al.* (1991), who found that acetic acid addition to feed of chicks significantly increased weight of jejunum and ileum. This increase in relative small intestine weight may be partly due to the higher length of small intestine of chicks fed on BA included diets. Dietary BA did not have any significant effect on the relative weight of cecum that is in agreement with the results reported by Ünsal and Rüstü (2004). Ileal pH was not affected by dietary treatments ($p>0.05$) that is in agreement with the previously reported data Denli *et al.* (2003), Hernández *et al.* (2006) and Józefiak *et al.* (2007). Alp *et al.* (1999) found that the addition of 3 g kg^{-1} mixture of lactic, fumaric, propionic, citric and formic acid to the diet significantly decreased pH level of ileum content.

The reason for the lack of effect of ileal pH on broiler is unknown, but it may be partly associated with the capacity of the chicks to maintain its gastrointestinal tract environment homeostasis or insufficient amount of BA in studied diets.

There was no significant difference in the serum levels of glucose, cholesterol, uric acid, total protein between control and BA treated chicks. It has been also reported that dietary inclusion by organic acids had no significant effect on serum levels of cholesterol and glucose (Hernández *et al.*, 2006), uric acid (Bowering *et al.*, 1969; Hernández *et al.*, 2006), total protein (Brenes *et al.*, 2003; Hernández *et al.*, 2006). Dietary inclusion of BA increased serum levels of Ca and P. The results obtained in the present study are in consistence to previous observations in chickens (Brenes *et al.*, 2003; Rafacz-Livingston *et al.*, 2005b). In contrast, Hernández *et al.* (2006) reported no significant effect of dietary formic acid on serum levels of Ca and P. Several mechanisms for the enhanced mineral serum concentration following the feeding of BA could be proposed. First, this organic acid might decrease the pH of the digesta in the small intestine that in turn inhibit phytic acid to chelate minerals and form insoluble phytate salts which are resistant to hydrolysis by endogenous phytase (Applegate *et al.*, 2003; Rafacz-Livingston *et al.*, 2005a). Another possible mode of action may be associated with chelating effects of organic acids reducing the binding of Ca to phytate that in turn prevent the formation of insoluble Ca-phytate complexes (Boling *et al.*, 2000; Rafacz-Livingston *et al.*, 2005a). Third, the addition of BA to diet in the present study increased the intestine length, which might cause extending the absorption site of minerals.

The relative weights of thymus, bursa of fabricius and spleen were not significantly affected by the dietary BA inclusion. These organs have been widely chosen as rough indicators for potential effects of the butyric acid on the immune system. Similarly, Shaiful Islam (2005) observed that absolute weights of bursa of fabricius and spleen in broilers were not affected by humic acid addition to diets.

The proportional counts of lymphocytes, heterophils, monocytes, basophils and eosinophils in blood on days 21, 42 and 49 of age were not affected by BA addition. Antongiovanni *et al.* (2007b) also reported that butyric glycerides did not have any significant effect on IgG and IgM concentrations in broilers. These initial results need to be confirmed and extended by future investigations.

CONCLUSION

Based on results of the present study it can be concluded that dietary butyric acid did not have a clear positive effect on performance, plasma metabolite levels of broiler chickens reared under appropriate hygiene conditions; however, there was a positive effect of BA on the length and relative weight of small intestine segments.

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