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Effect of Butyric Acid on Performance, Gastrointestinal Tract Health and Carcass Characteristics in Broiler Chickens

A. K. Panda*, S. V. Rama Rao, M. V. L. N. Raju and G. Shyam Sunder
Project Directorate on Poultry, Rajendranagar, Hyderabad, 500 030, India

ABSTRACT : An experiment was conducted to study the effect of graded levels of butyric acid (butyrate) on performance, gastrointestinal tract health and carcass characteristics in young broiler chickens. Control starter (0-3 wk) and finisher (4-5 wk) diets were formulated to contain 2,900 kcal ME/kg and 22% CP, and 3,000 kcal ME/kg and 20% CP, respectively. Subsequently, four other experimental diets were formulated to contain 0.05% antibiotic (furazolidone) or 0.2, 0.4 and 0.6% butyric acid. Each diet was fed at random to 8 replicates of 6 chicks each throughout the experimental period (0-5 wk). The results showed that 0.4% butyrate in the diet was similar to antibiotic in maintaining body weight gain and reducing *E. coli* numbers but superior for feed conversion ratio. No added advantage on these parameters was obtained by enhancing the concentration of butyrate from 0.4 to 0.6% in the diet. Feed intake and mortality were not influenced by the dietary treatments. A reduction in pH of the upper GI tract (crop, proventiculus and gizzard) was observed by inclusion of butyrate in the diets of broilers compared to either control or antibiotic-fed group. Butyrate at 0.4% was more effective in reducing the pH than 0.2% butyrate. Within the lower GI tract, 0.4 and 0.6% butyrate was effective in lowering pH in the duodenum, but no effect was found in either the jejunum or ileum. The villus length and crypt depth in the duodenum increased significantly in all the butyrate treated diets irrespective of the level tested. Carcass yield was higher and abdominal fat content was lower significantly in all the butyrate treatment groups compared to the control or antibiotic group. From these findings, it is concluded that 0.4% butyric acid supplementation maintained performance, intestinal tract health, and villi development and carcass quality in broiler chickens. (**Key Words :** Butyric Acid, Performance, GI Tract Health, Carcass Characteristics, Broiler Chickens)

INTRODUCTION

Under modern system of poultry production, birds are inevitably exposed to considerable stress during their productive lifetime. The time immediately after hatching is also a period of stress. The gastrointestinal (GI) tract of newly hatched chicks is immature and sterile. It begins to develop function and its microflora when it starts to ingest feed. At this time, the chick is very susceptible to pathogenic microorganisms (Adams, 2004). Under such circumstances, anti-microbial feed additives such as antibiotics are often used to suppress or eliminate harmful organisms in the intestine, and to improve growth and feed efficiency (Jin et al., 1997). However, the use of antibiotics as routine feed additives has been banned in the recent years because of the public concern over possible antibiotic residual effects and the development of drug resistant bacteria (Leeson, 2007). This has led to the application of non-antibiotics chemical substances (Yang et al., 2007) and among the candidate replacement are organic acids (both individual as well as blends of several acids). However, organic acids have not gained much attention in poultry production till today.

Amongst the organic acids, short chain fatty acids (SCFA) are considered as potential alternative to antibiotic growth promoter (Van Immerseel et al., 2005). Butyric acid is one such SCFA, which has higher bactericidal activity when the acid is undissociated (Leeson, 2007). Bacterial cell take up undissociated fatty acids and once these acids dissociate, there is change in the intracellular pH leading to death of bacterial cells. Butyrate also appears to play a role in development of the intestinal epithelium. It is reported that butyrate derived from the fermentation of non-starch polysaccharides is considered to be important for normal development of epithelial cells with improved gastrointestinal health and reduced incidence of colon cancer in humans (Brons et al., 2002). However, the levels of SCFA are quite low in the intestine and caeca of young chicks (van der Wielen, 2000) and so the young may be the

* Corresponding Author: A. K. Panda. Tel: +91-40-24015651, Fax: +91-40-24017002, E-mail: akpanda59@rediffmail.com
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Table 1. Composition of experimental diets (as fed basis)

Ingredients (% of the diet)	Starter (0-3 wk)	Finisher (4-6 wk)
Maize	56.22	58.42
Soybean meal	38.60	35.80
Dicalcium phosphate	1.88	1.62
Shell grit	0.72	0.68
Salt	0.30	0.40
DL-methionine	0.21	0.20
Choline chloride, 50%	0.10	0.06
Vegetable oil	1.62	2.66
Vitamin premix[1]	0.05	0.04
Mineral premix[2]	0.10	0.10
Toxin binder	0.20	0.02
Nutrient composition (calculated)		
ME (kcal/kg)	2,916	3,013
Crude protein (%)	22.13	20.32
Lysine (%)	1.21	1.07
Methionine (%)	0.53	0.50
Available phosphorous (%)	0.45	0.40
Calcium (%)	0.90	0.85

[1] Supplies per kg diet: Vitamin A, 16,500 IU; vitamin D_3, 3,200 ICU; vitamin E, 12 mg; vitamin K, 2 mg; vitamin B_1, 1.2 mg; vitamin B_2 10 mg; vitamin B_6, 2.4 mg; vitamin B_{12}, 12 μg; niacin, 18 mg; pantothenic acid, 12 mg.

[2] Mn, 90 mg; Zn, 72 mg; Fe, 60 mg; Cu, 10 mg; I, 1.2 mg.

best candidates for dietary supplementation.

Although, SCFA such as acetate and propionate have been successfully used as water sanitizers, there is little information available on butyrate in poultry. Sakata (1987) reported that infusion of butyrate into fistulated rats increased the proliferation of crypt cells in both the small and large intestines. Sharma et al. (1995) suggested that the effect on crypt cell growth might reflect changes in the gut microflora, which is known to be a major modulator of epithelial cell activity. However, no such information is available in poultry. Hence, further studies are needed on the effects of butyrate supplementation in young broilers. The present study was therefore; conducted to study the graded levels of butyrate supplementation on performance, GI tract health and carcass characteristics in young broiler chickens.

MATERIALS AND METHODS

Birds and management

Day old, commercial broiler chicks (240) were wing banded and randomly distributed into 40 raised-floored stainless steel battery brooder pens with 6 birds per pen. The brooder temperature was maintained at 34±1°C up to 7 days of age and gradually reduced to 26±1°C by 21 days of age after which, chicks were maintained at room temperature (25-27°C). Uniform management and vaccination schedules were followed for all the birds. The experiment was conducted following the guidelines of the Institute Animal Ethics Committee (Project Directorate on Poultry, Hyderabad, India).

Diets

Control starter (0-3 wk) and finisher (4-5 wk) diet was formulated to contain 2,900 kcal ME/kg and 22% CP, and 3,000 kcal ME/kg and 20% CP, respectively (Table 1). Subsequently four other experimental diets were formulated by adding either 0.05% antibiotic or 0.2, 0.4 and 0.6% butyric acid. Butyric acid and antibiotic was added to the basal diet at the expense of maize. Each diet was fed at random to 8 replicates of 6 chicks each throughout the experimental period.

Parameters studied

Individual body weight of chicks and replicate-wise feed intake were recorded at weekly interval. Feed conversion ratio was calculated as the ratio between feed consumed and weight gained.

On 22nd day, five birds from each dietary treatment were killed and the pH of the crop, proventiculus, gizzard, duodenum, jejunum and ileum were measured by using a digital pH meter (E. Merck India Limited, Mumbai). On the same day another three birds from each dietary group were sacrificed and crops, small intestine and caecum were removed quickly and stored at 4°C for *Escherchia coli* (*E. coli*) count. The crop was incised to expose inner lining, which was thoroughly washed with normal physiological saline to make the volume to 10 ml. The *E. coli* counts were made in aliquots drawn from the saline extract. Intestinal contents (dudeno-jejunal junction) and caecal contents, approximately 1 g from each bird were collected and suspended in 9 ml of nutrient broth. Serial diluents of each sample were made in nutrient broth and *E. coli* counts were made on MacConkey's agar and EMB agar (American Public Health Association 1984) by surface spread method.

Duodenal samples (of about 5 mm thickness) were collected from all the above eight birds of each dietary treatment on the same day and fixed in 10% formal saline (10 ml normal saline in 90 ml formalin solution). After fixation the tissues were washed in running water, dehydrated in ascending grade of alcohol, cleared in benzene and finally embedded in melted paraffin. Sections of 4-5 μ thickness were prepared from tissue embedded paraffin blocks with the help of microtome, deparaffinized in xyalin and stained with haematoxylin and eosin (H and E) stain (Culling, 1963) to measure villus length and crypt depth. Villus height was measured by the distance from crypt opening to the tip of villus whereas crypt depth was determined from the base of the crypt to the level of opening (Kik et al., 1990).

Table 2. Performance of broiler chickens fed diets containing butyric acid or antibiotic

Treatment	0-3 wk			0-5 wk		
	Body weight gain (g)	Feed intake (g)	Feed conversion ratio	Body weight gain (g)	Feed intake (g)	Feed conversion ratio
Control	604[b]	898	1.49[a]	1,340[b]	2,488	1.86[a]
Furazolidone	642[a]	904	1.41[b]	1,388[a]	2,492	1.80[b]
0.2% butyrate	614[b]	872	1.42[b]	1,354[b]	2,454	1.81[b]
0.4% butyrate	646[a]	880	1.36[c]	1,394[a]	2,440	1.75[c]
0.6% butyrate	638[a]	876	1.37[c]	1,386[a]	2,460	1.77[bc]
SEM	4.8	8.4	0.01	5.4	10.2	0.01

[a, b, c] Means with different superscripts in a column differ significantly ($p<0.05$).

One bird representing the mean body weight of each replicate (eight birds per treatment) was selected and sacrificed by cervical dislocation on 35 d of age. The data on weight of edible carcass, liver, gizzard, abdominal fat and breast meat were recorded and all the data were expressed as percentage of the pre-slaughter weight of the same bird.

Statistical analysis

Data were subjected to statistical analysis under completely randomized design employing one-way analysis of variance (Snedecor and Cochran, 1989). The means of different treatments were compared with Duncan's multiple range tests (Duncan, 1955). Significance was considered at $p<0.05$ levels.

RESULTS

The performance of the birds fed furazolidone or butyric acid (butyrate) are presented in Table 2. Body weight gain and feed conversion ratio was influenced by dietary treatments during both the starter and finisher period. The body weight gain of birds fed control diet was comparable with that of 0.2% butyrate supplemented group. However, significantly higher body weight gain was observed in furazolidone and 0.4-0.6% butyric acid groups. Significantly improved feed conversion ratio was observed in all the dietary treatments compared to control. Among the treatment groups, feed conversion ratio was better in 0.4% butyrate group compared to furazolidone or 0.2% butyrate group. Feed intake and mortality (data not given) was not influenced by the dietary treatments. Only 5 chicks (control-2; furazolidone-1; 0.2% butyrate-1 and 0.6% butyrate-1) died during the whole experimental period.

Table 4. Effect of butyric acid and antibiotic on duodenal morphology of the broilers

Treatment	Duodenum (nm)	
	Villus length	Crypt depth
Control	1,324[b]	232[b]
Furazolidone	1,349[b]	204[c]
0.2% butyrate	1,420[a]	256[a]
0.4% butyrate	1,442[a]	274[a]
0.6% butyrate	1,432[a]	268[a]
SEM	10.20	5.24

[a, b, c] Means with different superscripts in a column differ significantly ($p<0.05$).

The pH of the upper but not lower gastro-intestinal tract (except duodenum) was influenced by the butyric acid treatment in the present study (Table 3). The pH of crop, proventiculus and gizzard reduced significantly in the entire butyrate treatment groups compared to control and furazolidone group. Amongst the butyrate groups, pH of crop, proventiculus and gizzard was further reduced in 0.4 and 0.6% butyrate compared to 0.2% butyrate. Duodenal pH was comparatively lower in the 0.4 and 0.6% butyrate group compared to control, antibiotic or 0.2% butyrate group. However, no such effect was found in subsequent lower tract. The pH of jejunum and ileum was comparable among all the dietary treatments.

The result on *E. coli* counts in response to dietary treatments are shown in Table 4. *E. coli* counts in the crop reduced significantly in all the butyrate treated diets and antibiotic group compared to control (Figure 1). However, in small intestine and caecum, 0.2% butyrate failed to produce the same effect. The *E. coli* counts were significantly lower in small intestine and caecum in both the

Table 3. pH of the gastrointestinal segments of broiler chickens fed diets containing butyric acid or antibiotic

Treatment	Crop	Proventiculus	Gizzard	Duodenum	Jejunum	Ileum
Control	4.84[a]	3.48[a]	2.56[a]	5.46[a]	6.00	6.24
Furazolidone	4.92[a]	3.42[a]	2.48[a]	5.40[ab]	6.09	6.27
0.2% butyrate	4.27[b]	3.18[b]	2.32[b]	5.34[b]	5.87	6.06
0.4% butyrate	4.02[c]	3.04[c]	2.16[c]	5.22[c]	5.78	6.02
0.6% butyrate	4.01[c]	3.02[c]	2.14[c]	5.19[c]	5.82	6.16
SEM	0.04	0.03	0.03	0.02	0.08	0.07

[a, b, c] Means with different superscripts in a column differ significantly ($p<0.05$).

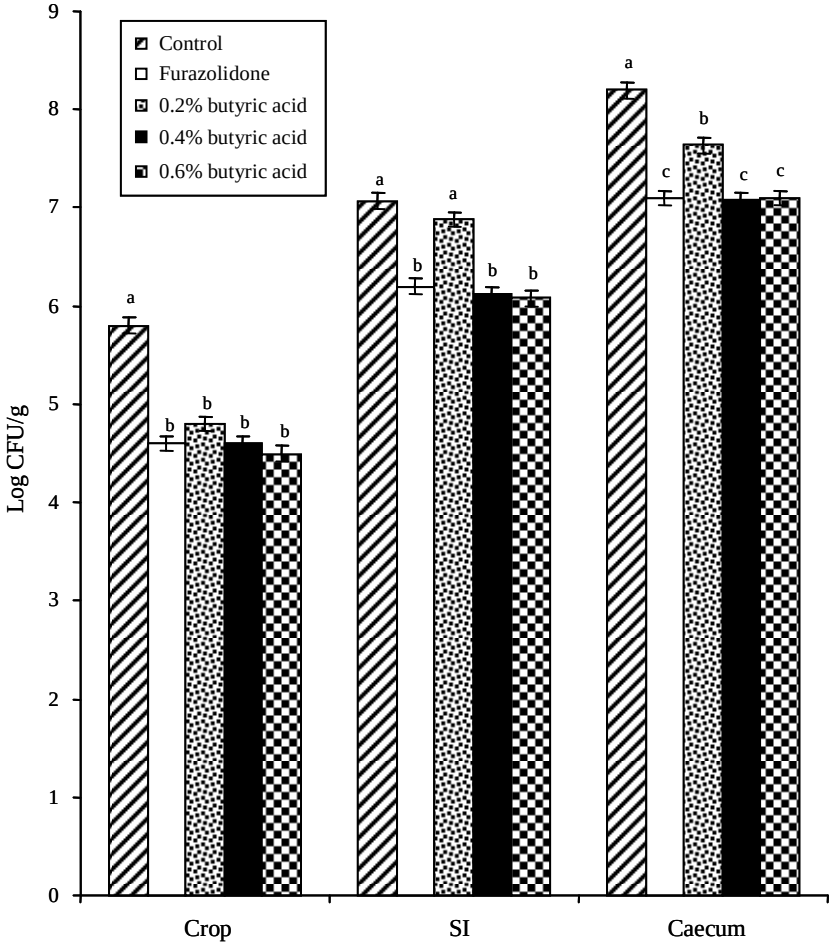

Figure 1. Effect of diets containing butyric acid or antibiotic on *E. coli* counts.

antibiotic and butyrate (0.4 or 0.6%) diet compared to either control or 0.2% butyrate. Though no difference in villus length was found between control and antibiotic fed group, crypt depth was reduced in the antibiotic fed group compared to control (Table 4). The villi length and crypth depth in duodenum increased significantly in all the butyrate treated diets irrespective of the levels tested. No difference on the above parameters could be noticed due to the concentrations of butyrate (0.2-0.6%) in the diet.

The dressing percentage and abdominal fat content was influenced by the butyric acid treatments employed in the present study (Table 5). Dressing percentage increased and abdominal fat content decreased significantly in all the butyrate treatment groups compared to either control or furazolidone group. The relative weights of liver, gizzard and breast meat were not influenced by the dietary treatments.

DISCUSSION

The results of the present study suggested that organic acid could replace antibiotics in broiler chicken's diet for realizing optimum performance. Butyric acid at 0.2% was not sufficient to maintain the performance. Higher

Table 5. Carcass characteristics of broiler chickens fed diets containing butyric acid or antibiotic

Treatment	% Pre-slaughter live weight				
	Dressed weight	Liver	Gizzard	Abdominal fat	Breast meat
Control	70.28[b]	2.48	2.64	1.80[b]	16.24
Furazolidone	70.36[b]	2.52	2.60	1.84[b]	16.20
0.2% butyrate	71.88[a]	2.50	2.66	1.64[a]	16.28
0.4% butyrate	72.04[a]	2.46	2.62	1.60[a]	16.32
0.6% butyrate	71.98[a]	2.48	2.64	1.58[a]	16.26
SEM	0.12	0.03	0.04	0.02	0.04

[a, b] Means with different superscripts in a column differ significantly ($p<0.05$).

concentration of butyrate i.e. 0.4% in the diet was adequate for optimum body weight gain and feed conversion ratio. Contrary to the findings of the present study, Leeson et al. (2005) and Antongiovanni et al. (2007) suggested a lower level i.e. 0.2% butyrate to maintain performance of broiler chickens. It is noteworthy to mention here that both the above workers used butyrate, which is composed of mono and diglycerides with approximately 75% by weight of butyrate. However, in the present study laboratory grade butyric acid (SISCO Research Laboratory, Mumbai, India) was used. From this, it could be inferred that the concentration of butyrate in the diet depends on the form in which it is to be used. In the current study, butyrate up to 0.6% had no adverse effect on feed intake. Similar findings are also available in literature (Pinchasov and Jensen, 1989; Antongiovanni et al., 2007). Dibner and Putin (2002) suggested that organic acids improve protein and energy digestibility by reducing microbial competition with the host for nutrients and endogenous nitrogen losses, by lowering the incidence of sub-clinical infections and secretion of immune mediators, by reducing the production of ammonia and other growth depressing microbial metabolites. Probably these could be the reasons that butyrate improved feed utilization leading to better performance in the birds.

A few studied are available in literature with respect to the effect of butyrate in broiler chickens (Leeson et al., 2005; Van Immerseel et al., 2005; Antongiovanni et al., 2007) but none of the study has reported the pH of the individual segments of the GI tract as in the present study. In our study, a reduction in pH of the upper GI tract (crop, proventiculus and gizzard) was observed by inclusion of butyrate in the diets of broilers. 0.4% butyrate was more effective in reducing the pH than 0.2% butyrate. Amongst the lower GI tract, 0.4% butyrate was only effective in lowering the pH in duodenum, but no effect was found in either jejunum or ileum. Bolton and Dewar (1965) indicated that free butyrate absorbed quickly in the upper digestive tract, and while almost 60% of the feed source was intact in the crop, less than 1% is recovered from the upper small intestine. This could be the reason that butyrate was more effective in reducing the pH in the upper GI tract and only in duodenum in lower GI tract.

Butyrate at 0.4% was equally effective as antibiotic in reducing the *E. coli* numbers in the present study. Many reports have suggested that chicken in the first 2 weeks of post hatch lack adequate immune responsiveness (Seto, 1981; Mast and Goddeeris, 1999) and therefore highly susceptible to infections. Colibacillosis is very common in the poultry and may be responsible for high chick mortality. Pathogenic coliforms are more frequently occur in the gut of newly hatched chickens (Calnek et al., 1991) and the incidence of infection increases shortly after hatching. The incidence starts to decrease after 6 d but the losses continue until 3 weeks of age (Calnek et al., 1991). One of the strategies to eliminate the cloiforms from the gastrointestinal tract is by maintaining a lower pH, which is unsuitable for the growth of the organism. Kwan and Ricke (2005) showed that amongst the SCFA, butyrate has the highest bactericidal efficacy against the acid-intolerant species such as *E. coli* and *Salmonella*. In the present study dietary inclusion of organic acid such as butyrate not only reduced the pH of crop and small intestine but also reduced the *E. coli* count in crop, small intestine and caecum. Thus, it can be suggested that butyrate could replace antibiotic totally in practical broiler diets. Though we have not studied the effect of butyrate on *Salmonella* colonization, Van Immerseel et al. (2004) reported that butyrate reduces virulence gene expression and invasiveness in *Salmonella enteritidis* leaded to decrease caecal colonization.

In addition to bactericidal activity, butyrate appeared to have a role in development of the intestinal epithelium in this study. Butyrate, irrespective of the concentrations (0.2, 0.4, 0.6%) in the diet improved the villus length and crypt depth in the duodenum. Thus, butyrate supplementation will be much helpful to young birds for intestinal development, especially when there is no protection from antibiotics. Similar to the findings of the present study, Leeson et al. (2005) reported higher crypt depth in duodenum of broiler chicks fed 0.2% butyrate compared to those fed bacitracin in the diet. 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.

Another two important findings of the present study were the improvement in dressing percentage and reduction in abdominal fat content by supplementation of butyrate to broilers diet. Similarly, Leeson et al. (2005) reported higher carcass yield in broilers fed 0.2% butyrate in the diet. Though no information on literature is available on the role of butyrate on abdominal fat content of broilers, Izat et al. (1990) reported significant reduction in abdominal fat content in male broiler chickens by dietary supplementation of propionic acid. Thus it can inferred that organic acid supplementation in broiler diet not only maintains performance but also higher carcass yield.

In the present study, 0.4% butyric acid was on par with antibiotic in maintaining body weight gain, and reducing *E. coli* numbers and found superior for feed conversion ratio. Several additional effects that go beyond those of antibiotics such as stimulating the villi growth of intestine, higher carcass yield and low abdominal fat content were also observed by dietary addition of butyrate. From the findings of the present study, it is concluded that 0.4% butyric acid could totally replace antibiotics in broiler chicken diet.

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