Int.J.Curr.Microbiol.App.Sci (2020) *9*(2): 2521-2532

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 9 Number 2 (2020)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2020.902.287

Effect of Butyric Acid Supplementation on Gut Health in Broiler Chicken

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Keywords

Gut health, *E. coli*, Duodenum, Goblet cells, Gut pH

Article Info

Accepted:
18 January 2020
Available Online:
10 February 2020

A B S T R A C T

An experiment was conducted using 600 broiler chicks to investigate the effect of four levels of butyric acid (0, 0.1, 0.2 and 0.4 %) on gut health from day 1 to 42 days. Influence of butyric acid on pH in different sections of the small intestine, gut histomorphology and the microbial load in the caecum was tested on 21 days and 42 days of the trial. Gut pH was significantly higher in duodenum and ileum on day 21 and only duodenum on day 42. Caecal *E. coli*, *Salmonella spp.* and *Clostridium spp.* count was significantly lower and *Lactobacilli spp.* count was significantly higher in butyric acid supplemented groups. Intestinal villous height was significantly higher in duodenum, jejunum on day 21 and duodenum, jejunum and ileum on day 42, intestinal crypt depth was significantly higher in all sections of small intestine except in ileum on day 42 in BA supplemented groups. Except in ileum on day 21, the goblet cell count was significantly higher in all sections of the small intestine in treated groups.

Introduction

Antibiotics have been used as growth promoters in poultry to improve performance by reducing the burden of pathogens (Dibner and Richards, 2005). Currently, there is a restriction on the use of antibiotic growth promoters (AGPs), for the reason of developing antibiotic resistance (Naveen kumar *et al.*, 2018). Butyric acid (BA) is one of the important organic acids that can be used as alternative to antibiotics (Leeson *et al.*, 2005). Butyric acid being a SCFA, 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. Supplementation of butyric acid derivatives in the diets of broiler chickens could replace antibiotics while maintaining growth performance (Namkung *et al.*, 2011), decrease infection by *Salmonella enteritidis* (Fernandez-Rubio *et al.*, 2009), and increase growth performance under stress (Zhang *et al.*, 2011). Researchers have observed that butyrate is quickly absorbed in the upper digestive tract, mainly the crop, which makes it less ideal as a feed additive. They have also observed that butyrate is necessary for normal epithelial cell development; therefore, if the butyrate could bypass degradation in the crop and proven triculus, then the epithelial cells in the small intestine could utilize the butyrate. Butyric acid is a readily available energy source for intestinal villi and stimulates their differentiation and multiplication, consequently increases feed efficiency (Adil *et al.,* 2011). It also induces the production of host defense peptides when it enters bloodstream. Villi height and absorptive surface of the small intestine increases leading to better nutrient utilization (Smulikowska *et al.,* 2006; Hu and Guo, 2007; Czerwi´nski *et al.,* 2012).

A balanced gut microbiota constitutes an efficient barrier against pathogen colonization, produces metabolic substrates such as vitamins and short-chain fatty acids, and stimulates the immune system in a non-inflammatory manner. Butyric acid may also reduce hindgut protein fermentation because it suppresses protein-fermenting microbiota, especially the gram-negative population in broilers (Gunal *et al.,* 2006), by disrupting their energy metabolism (Ricke, 2003) and decreasing hindgut pH. In addition, butyric acid decreases bacterial colonization of the intestinal wall (Langhout *et al.,* 1999) and as a consequence, less toxic compounds are produced by pathogenic microbiota, resulting in less damage to the epithelial cells (Antongiovanni *et al.,* 2007). In this context, the present study was conducted to evaluate potential benefits of butyric acid on the gut health of broilers.

Materials and Methods

Dietary treatments and management

A total of Six hundred, day old straight run commercial broiler chicks were randomly divided into four treatment groups, each treatment consisted of six replicates of twenty five chicks each. Broilers were fed with diets as per NRC (1994) specifications (Table 1). Birds were fed with basal diet along with graded levels of (0.1, 0.2 and 0.4 %) butyric acid. Chicks were reared under deep litter system with all standard managemental practices till six weeks of age and standard vaccination schedule was followed for immunizing chicks. Feed and water was provided *ad libitum*.All the procedures followed during the trial were approved by the Institutional animal ethical committee, Veterinary College, Bengaluru, KVAFSU, Bidar.

Gut health

On 21st and 42nd day of the experiment, 12 birds from each treatment group were sacrificed and intestinal content from duodenum, jejunum and ileum was collected for pH estimation. The caecal contents were collected aseptically in sterile container and immediately subjected to enumeration of gut microbes viz., *Escherichia coli, Salmonella, Lactobacillus and Clostridium perfringens*as per standard protocols. On 21st and 42nd day of the experiment, from the sacrificed birds, tissue samples of duodenum, jejunum and ileum were collected, flushed with buffered saline and fixed in 10% neutral buffered formalin for histomorphological studies *viz.,* villus height, crypt depth and goblet cells count.

Statistical analysis

Experimental data was analysed using SPSS statistical software (Version 20 for windows, SPSS). One-way analysis of variance (ANOVA) was used to analyse variance. Values were expressed as mean ± SE and compared by Duncan's test. Significance of difference between treatments means was determined at the $P \leq 0.05$ levels.

Results and Discussion

Effect of butyric acid on gut health

Gut pH

The effect of dietary butyric acid on gut pH *viz;* Duodenum, jejunum and ileum in broiler chicken on day 21 and 42 of the trail is presented in Table 2. On day 21, the duodenal pH was significantly higher at 0.1 and 0.2 % butyric acid supplementation compared to the control. The jejunal pH was unaffected. The ileal pH was significantly higher in 0.4 % butyric acid supplemented group than the groups supplemented with control and 0.1 % BA. On day 42, the duodenal pH was significantly higher at 0.4 % butyric acid supplementation compared to the control group. However, the pH of jejunum and ileum was not affected by different levels of butyric acid supplementation compared to the control group. According to Bolton and Dewar (1965), the free butyrate is absorbed quickly by dissociation and absorption occurs in the upper digestive tract (crop, proventriculus) itself and only less than one per cent is recovered from the upper small intestine. Hence, in the present study the butyric acid might not have lowered the pH in small intestine and the increase in pH in few portions observed was within normal physiological range

Caecal microbial count

The effects of butyric acid supplementation on caecal microbial count of broiler chicken on day 21 and 42 of the experiment are presented in Table 3 and Fig. 1. On day 21, The *Escherichia coli* count was higher in all BA supplemented groups than that in the control group. There was no significant ($P > 0.05$) difference in *Salmonella* count among different groups. The *Clostridium perfringens* count was significantly ($P \leq 0.05$) higher in 0.1 % BA group when compared to other groups. The statistical analysis indicated significantly ($P \leq 0.05$) lower *Lactobacillus* count in control group than other groups. On day 42, non-significant ($P > 0.05$) difference in *Escherichia coli* count was recorded between different experimental groups. The *Salmonella* count was significantly ($P \leq 0.05$) higher in control group compared to BA groups. There was significant ($P \leq 0.05$) difference in *Clostridium perfringens* count among different groups. The lowest count was noticed in 0.4 % BA compared to other groups. Statistical analysis indicated significantly ($P \leq 0.05$) higher *Lactobacillus* count in 0.4 % BA group compared to other groups.Vogt *et al.,* (1982) concluded that Sodium butyrate lowers the pH of intestine that favors the growth of lactic acid producing bacteria such as *Lactobacilli spp*. as they require an acidic medium for their growth. It has been reported that lactic acid producing bacteria compete for space and nutrients with pathogenic bacteria within the intestine (Furuse and Okumura, 1994; Rolfe, 2000). *Lactobacilli spp*. produces bacteriocins (Joerger, 2003) which moderate the pathogenic bacterial count and maintain a healthy environment in the bird's intestine. Audisio *et al.,* (2000) reported that sodium butyrate favors the growth of *Lactobacilli spp*. that converts glucose to lactic acid within the intestine of birds, causing the inhibition of pathogenic bacteria such as *Salmonella spp*. and *E.coli*. Collibacillosis is very common in poultry and may be responsible for high chick mortality (Calnek *et al.,* 1991). Kwon and Ricke (2005) showed that butyrate has highest bactericidal efficacy against *E. coli* and *Salmonella.*

Gut histomorphology

The effect of butyric acid supplementation on intestinal histomorphologic indices of broilers on day 21 are presented in Table 4. Villous height of duodenum and jejunum was significantly higher in 0.4 % and 0.2 % butyric acid supplemented groups, respectively. Jejunal villous height was not affected by butyric acid supplementation Fig.2. Crypt depth of duodenum, jejunum and ileum was significantly higher in 0.1% butyric acid supplemented group. More number of goblet cells was evident in BA supplemented groups than the control group. The goblet cells in jejunum were unaffected. However, the control group showed more goblet cells compared to butyric acid supplemented groups.

The effect of butyric acid supplementation on intestinal histomorphologic indices of broilers on day 42 are presented in Table 5. Villous height and crypt depth of duodenum and jejunum was significantly higher in 0.2 % butyric acid supplemented group compared to other groups Figure 2 and 3. Villous height of ileum was significantly higher in 0.1 % butyric acid supplemented group compared to other groups, but crypt depth was unaffected. More number of goblet cells was evident in duodenum in all groups except at 0.4 % BA supplemented group, whereas jejunum and ileum showed more goblet cells in 0.2 % butyric acid supplemented group, indicating better health of the gut Figure 4. The histomorphometric parameters such as villous height and crypt depth plays a vital role in maintaining gut health and integrity as it is frequently exposed to both nutritional and pathological stress.

Table.1 Per cent ingredient and nutrient composition of basal experimental diet

Ingredients	Prestarter (1-7 days)	Starter (8-21 days)	Finisher (22-42 days)
Yellow maize	52.17	56.47	58.90
Soya bean meal	40.9	35.54	32
Vegetable oil	3.00	4.3	4.3
Dicalcium phosphate	1.50	1.00	0.70
Common salt	0.40	0.35	0.35
Mineral mixture*	1.50	1.90	1.90
Vitamin premix **	0.20	0.10	0.10
DL-Methionine	0.20	0.15	0.15
Vit.B complex with E***	0.05	0.10	0.10
Antibiotic	0.03	0.03	0.0
Coccidiostat	0.05	0.05	0.0
Nutrient composition			
Crude Protein (%) [b]	22.63	18.92	20.45
Crude Fat (%) [b]	1.47	4.23	4.95
Crude Fibre (%) [b]	3.39	3.51	3.73
Moisture (%) [b]	13.29	10.81	10.07
Ash (%) [b]	6.86	6.54	6.23
Calcium (%) [a]	1.03	1.01	0.97
Phosphorous (%) [a]	0.457	0.453	0.424

* Mineral mixture: Each 100 g contains Calcium- 30 %, Phosphorus- 9 %, Manganese- 0.4 %, Zinc-0.4 %, Iron- 2000 ppm, Copper- 500 ppm, Iodine- 100 ppm and Selenium- 23 ppm.

** Vitamin premix: Each gram contains Vitamin A - 82, 500 IU, Vitamin B_2 - 50 mg, Vitamin D_3 -12,000 IU and Vitamin K - 10 mg.

*** Vit. B complex with E: Each gram contains Vitamin B_1- 4 mg, Vitamin B_6- 8 mg, Vitamin B_{12} - 40 mcg, Vitamin E- 40 mg, Calcium D pantothenate- 40 mg and Niacin-60 mg.

[a] calculated values; [b] analyzed value

Table.2 Effect of butyric acid on Gut pH in broiler chicken

Treatment Groups	Gut pH on 21st Day			Gut pH on 42nd Day		
	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
Basal diet (Control)	5.93 ± 0.01 ^{b}	6.23 ± 0.08	6.65 ± 0.18 ^{b}	5.73 ± 0.04 ^{b}	5.61 ± 0.08	5.71 ± 0.16
Basal diet + 0.1 % butyric acid	5.99 ± 0.02 ^{a}	6.46 ± 0.16	6.49 ± 0.16 ^{b}	5.77 ± 0.03 ^{ab}	5.71 ± 0.06	5.99 ± 0.18
Basal diet + 0.2 % butyric acid	6.01 ± 0.02 ^{a}	6.19 ± 0.14	6.74 ± 0.13^{ab}	5.76 ± 0.03 ^{ab}	5.62 ± 0.10	6.16 ± 0.29
Basal diet + 0.4 % butyric acid	5.97 ± 0.01 ^{ab}	6.38 ± 0.06	7.21 ± 0.21 ^{a}	5.87 ± 0.02 ^{a}	5.66 ± 0.07	6.24 ± 0.29
P value	0.054	0.359	0.038	0.068	0.803	0.441

*Values are means ± standard error. Means within each column with different superscript differ significantly (P≤0.05)

Table.3 Effect of butyric acid on Caecal microbial count (CFU/g) in broiler chicken

Treatment Groups	Caecal Micro-flora Count (log_{10} CFU/g) on day 21				
	Escherichia coli	*Salmonella spp.*	*Clostridium perfringenes*	*Lactobacillus spp.*	P value
Basal diet (Control)	8.65 ± 0.17 ^{c}	8.92 ± 0.15	5.49 ± 0.26 ^{b}	8.79 ± 0.13 ^{c}	0.000
Basal diet + 0.1 % butyric acid	9.19 ± 0.16 ^{b}	9.19 ± 0.17	6.58 ± 0.30 ^{a}	9.56 ±0.13 ^{a}	0.126
Basal diet + 0.2 % butyric acid	9.21 ± 0.05 ^{b}	8.84 ± 0.10	5.79 ± 0.06 ^{b}	9.18 ± 0.10 ^{b}	0.010
Basal diet + 0.4 % butyric acid	9.92 ± 0.01 ^{a}	9.25 ± 0.11	5.93 ± 0.17 ^{b}	9.59 ± 0.09 ^{a}	0.000
Treatment Groups	Caecal Micro-flora Count (log_{10} CFU/g) on day 42				
	Escherichia coli	*Salmonella spp.*	*Clostridium perfringenes*	*Lactobacillus spp.*	P value
Basal diet (Control)	7.16 ± 0.13	7.35 ± 0.13 ^{a}	7.50 ± 0.03 ^{a}	8.05 ± 0.22 ^{b}	0.629
Basal diet + 0.1 % butyric acid	6.86 ± 0.21	6.77 ± 0.15 ^{b}	6.56 ± 0.10 ^{b}	7.96 ± 0.15 ^{b}	0.025
Basal diet + 0.2 % butyric acid	7.07 ± 0.26	6.99 ± 0.11 ^{ab}	6.46 ± 0.03 ^{b}	7.88 ± 0.09 ^{b}	0.000
Basal diet + 0.4 % butyric acid	6.86 ± 0.14	6.85 ± 0.14 ^{b}	5.96 ± 0.09 ^{c}	9.04 ± 0.21 ^{a}	0.000

*Values are means ± standard error. Means within each column with different superscript differ significantly (P≤0.05)

Table.4 Effect of butyric acid on Gut histomorphology on 21st day in broiler chicken

Parameter	Site	Treatment Groups				P value
		Basal diet (Control)	Basal diet + 0.1 % butyric acid	Basal diet + 0.2 % butyric acid	Basal diet + 0.4 % butyric acid	
Villous height	**Duodenum**	1550.00 ± 38.04 [c]	1705.00 ± 17.78 [b]	1774.16 ± 41.93 [b]	2090.41 ± 26.11 [a]	0.000
	Jejunum	1095.00 ± 111.22 [b]	1655.00 ± 50.16 [a]	1627.91 ± 86.42 [a]	1259.16 ± 141.83 [b]	0.000
	ileum	646.66 ± 28.47	694.16 ± 45.10	646.66 ± 43.98	601.25 ± 16.76	0.344
Crypt depth	**Duodenum**	191.25 ± 5.64 [a]	191.25 ± 8.59 [a]	122.08 ± 6.46 [c]	152.91 ± 9.01 [b]	0.000
	Jejunum	141.66 ± 8.03 [b]	181.66 ± 9.25 [a]	108.33 ± 3.03 [c]	121.66 ± 4.18 [c]	0.000
	ileum	117.50 ± 6.44 [a]	111.25 ± 5.00[a]	85.41 ± 3.34 [b]	81.25 ± 2.59 [b]	0.000
Goblet cell count	**Duodenum**	4.41 ±0.33 [b]	8.33 ±0.81 [a]	8.58 ± 0.63 [a]	7.91 ±0.55 [a]	0.000
	Jejunum	7.00 ± 0.77	7.91 ±1.22	8.33 ± 0.86	6.75 ±0.49	0.544
	ileum	7.66 ± 0.87 [a]	4.66 ± 0.33[bc]	3.50 ± 0.41 [c]	5.75 ± 0.46 [b]	0.000

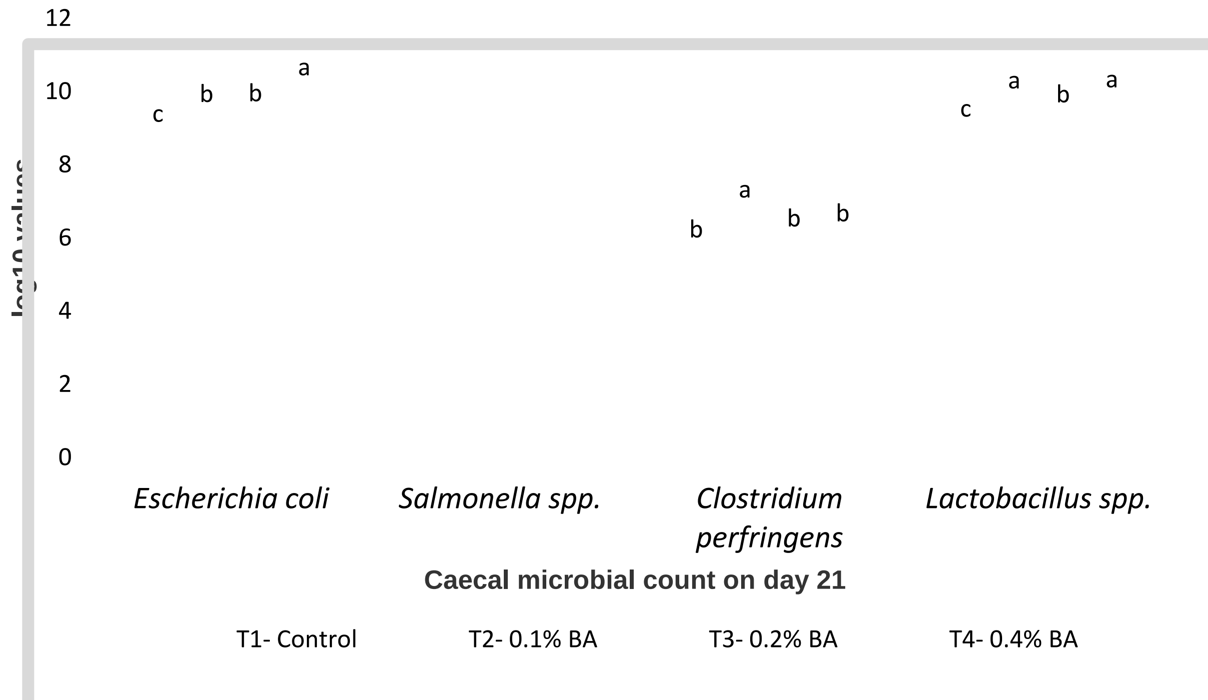
*Values are means ± standard error. Means within each column with different superscript differ significantly (P≤0.05)

Table.5 Effect of butyric acid on gut histomorphology on 42nd day in broiler chicken

Parameter	Site	Treatment Groups				P value
		Basal diet (Control)	Basal diet + 0.1 % butyric acid	Basal diet + 0.2 % butyric acid	Basal diet + 0.4 % butyric acid	
Villous height	**Duodenum**	1875.00 ±22.35 [b]	2042.50 ±93.74 [b]	2254.58 ±51.05 [a]	1889.16 ±36.98 [b]	0.000
	Jejunum	886.25 ±39.90 [c]	1437.50 ±26.36 [ab]	1565.00 ±41.85 [a]	1422.08 ±70.93 [b]	0.000
	ileum	729.16 ± 15.28 [c]	1042.08 ± 46.46 [a]	991.66 ± 26.54 [ab]	936.66 ± 15.63 [b]	0.000
Crypt depth	**Duodenum**	302.08 ± 12.46 [ab]	293.75 ± 13.04 [ab]	325.00 ± 17.07 [a]	277.91 ± 12.40 [b]	0.128
	Jejunum	223.75 ± 12.50 [b]	241.25 ± 15.03 [ab]	280.41 ± 20.20 [a]	238.33 ± 8.71 [b]	0.057
	ileum	182.50 ± 4.41	202.08 ± 9.52	185.00 ± 10.20	204.16 ± 9.80	0.195
Goblet cell count	**Duodenum**	13.41 ±1.45 [a]	13.66 ±0.64 [a]	11.75 ±0.78 [a]	8.16 ±0.82 [b]	0.001
	Jejunum	6.58 ±0.46 [b]	7.66 ±0.73[ab]	8.83 ±0.64 [a]	7.83 ±0.42 [ab]	0.071
	ileum	7.58 ±0.65 [b]	8.83 ±0.62 [b]	11.08 ±0.93 [a]	8.16 ±0.54 [b]	0.006

*Values are means ± standard error. Means within each column with different superscript differ significantly (P≤0.05)

Fig.1 Effect of butyric acid on caecalmicroflorain broiler chicken



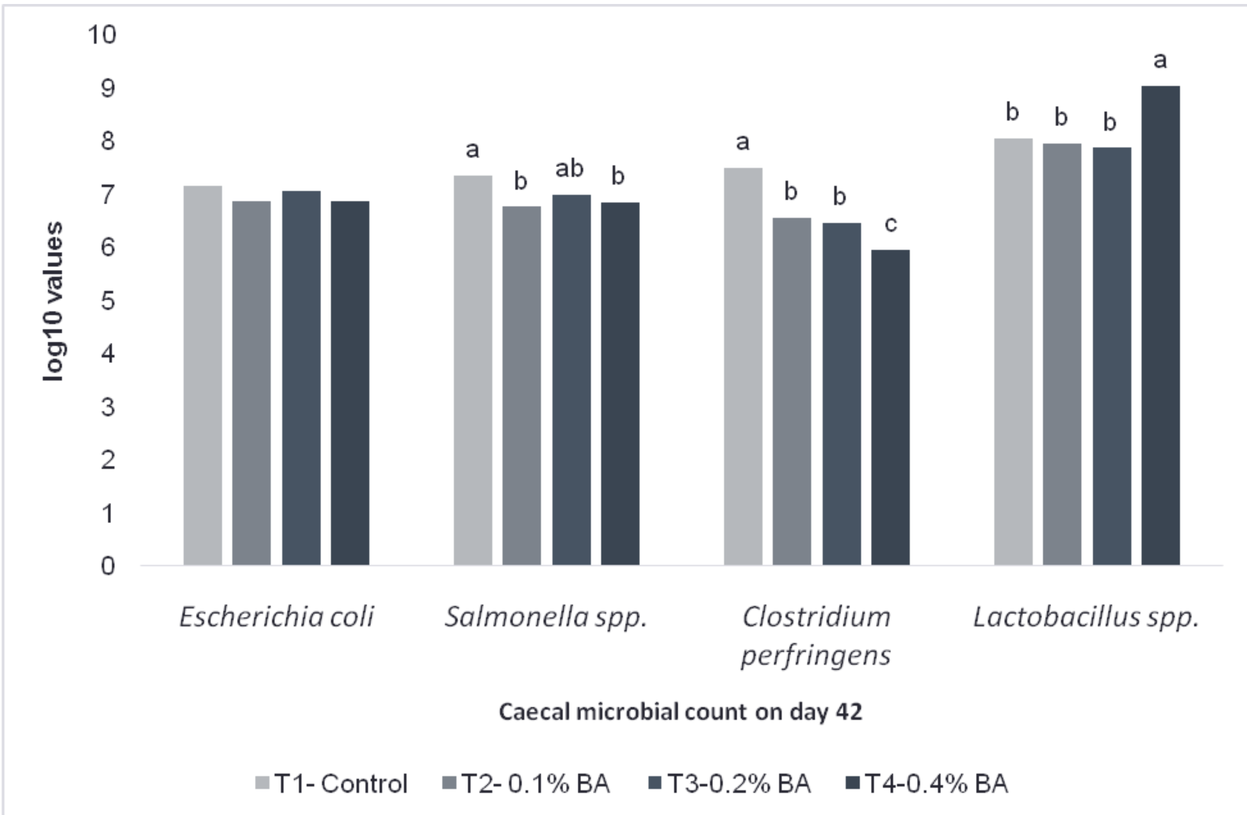
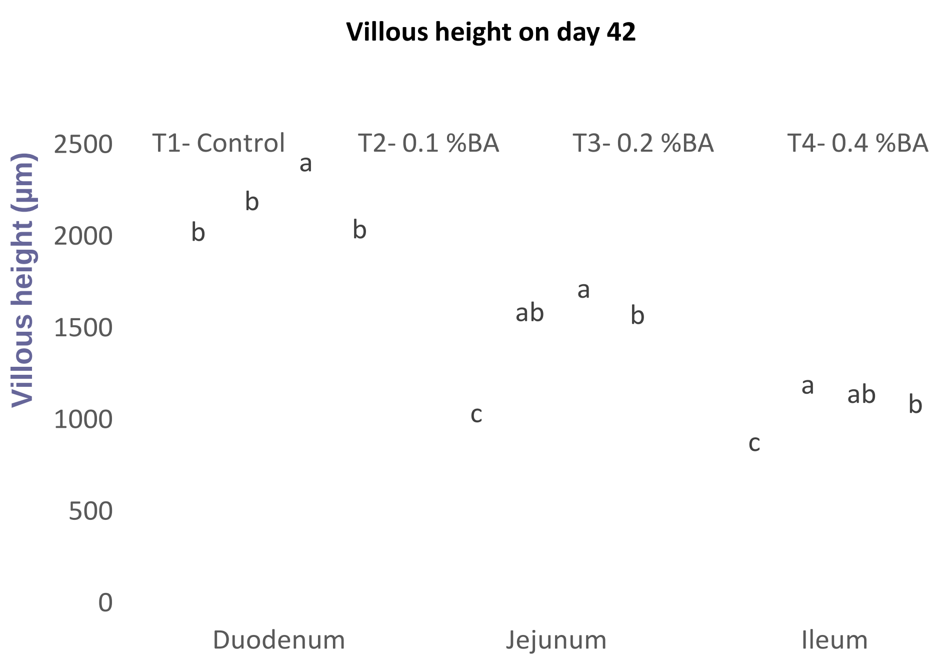


Fig.2 Effect of butyric acid on intestinal villous height in broiler chicken



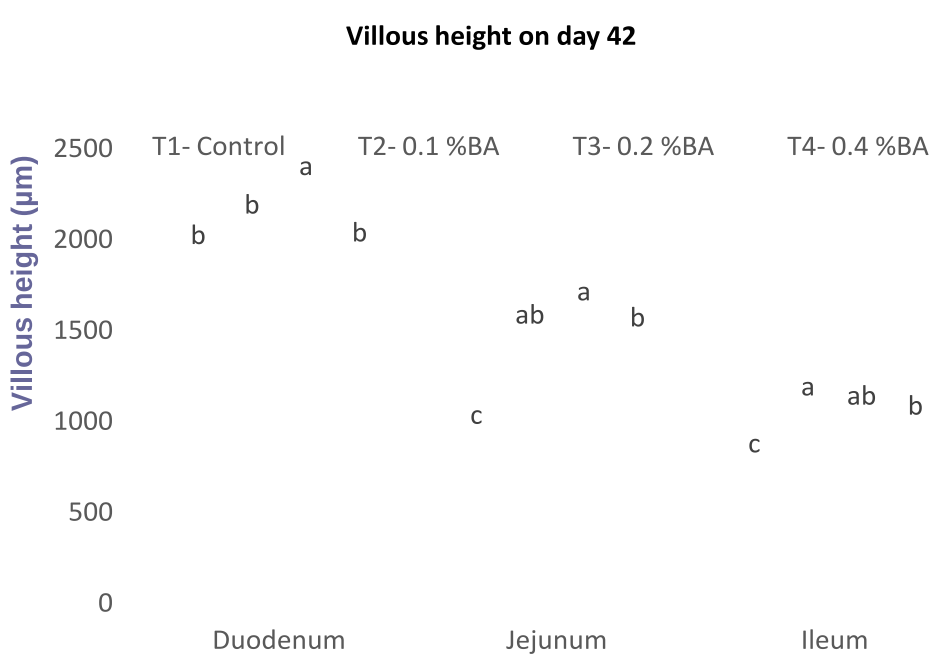


Fig.3 Effect of butyric acid on intestinal crypt depth in broiler chicken

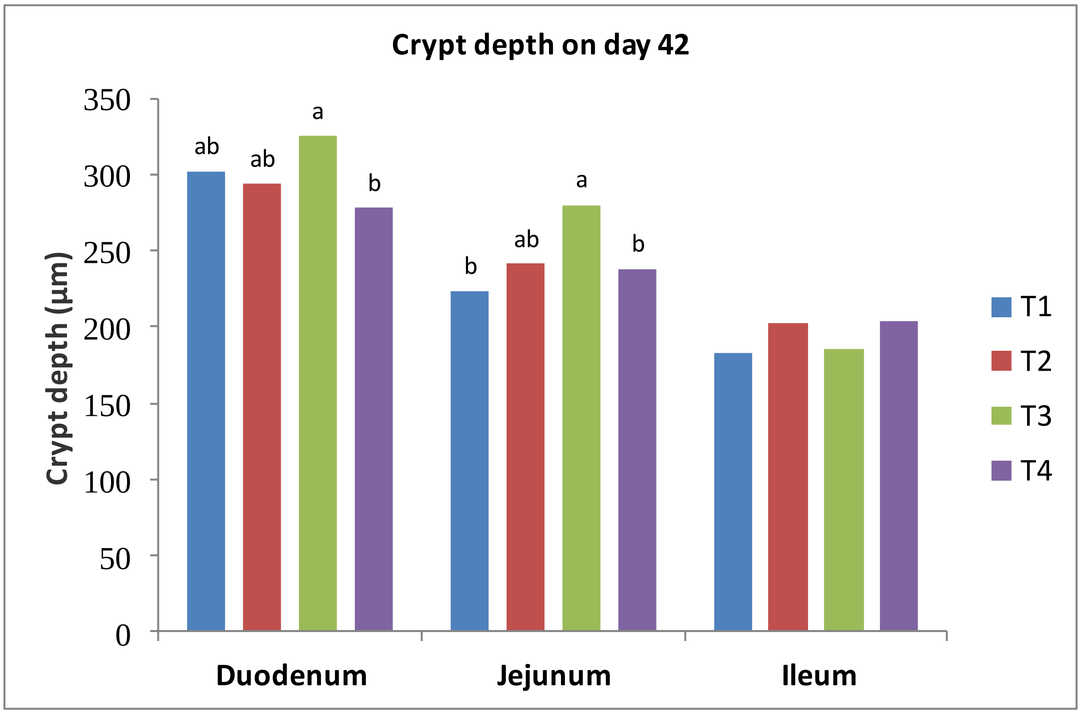
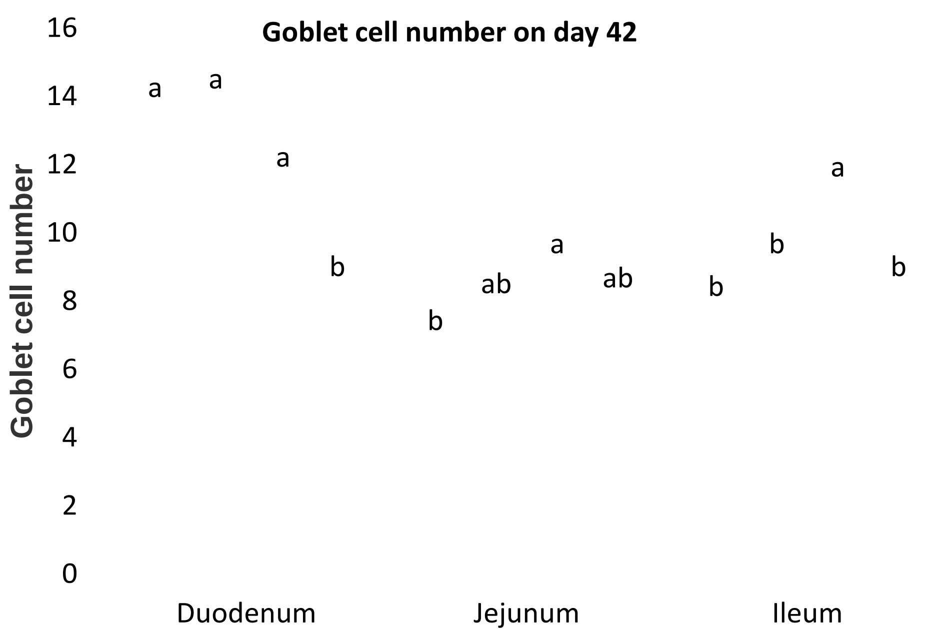


Fig.4 Effect of butyric acid on goblet cell number in broiler chicken



The butyric acid appeared to have a role in intestinal development in this study. Small intestine is the site for the absorption of nutrients through epithelial cells which has specialized structures for uptake of nutrients in the gut and subsequently drained into blood stream. The increased villous height and corresponding surface area could support better nutrient uptake and in turn the gain in body weight (Ashraf *et al.*, 2013). The deeper crypt depth indicates increased proliferation of epithelial cells in crypts which repairs the gut epithelial lining for better absorption of nutrients. Butyrate acts as a rich source of energy for enterocytes (Ahsan *et al.*, 2016) and it may possibly increase the cell mitosis in crypts. Sakata (1987) infused butyrate into fistulated rats and found increase in the proliferation of crypt cells in both small and large intestines. Gut health can also be evaluated by determining the number of goblet cells in the epithelial lining of small intestine. The increase in the goblet cell count in the present study is indicative of better protection from the pathogenic and nutritional insult to the small intestine, indicating better gut health. The mucous in the goblet cells acts as lubricant, source of nutrients for the normal commensals and protection of the gut from pathogens (Majidi-Mosieh *et al.*, 2017).

From the results of the present study, it can be concluded that the Gut health in broiler chicken could be improved by supplementation of butyric acid as evidenced by reduced ceacal pathogenic microbial load and increased intestinal morphometrics.

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How to cite this article:

Nataraja, D., V. Malathi, J. N. Sreedhara, Jayanaik and Kavitha Rani, B. 2020. Effect of Butyric Acid Supplementation on Gut Health in Broiler Chicken. *Int.J.Curr.Microbiol.App.Sci.* 9(02): 2521-2532. doi: https://doi.org/10.20546/ijcmas.2020.902.287