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K Deepa
Department of Animal Nutrition, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

MR Purushothaman
Department of Animal Nutrition, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

P Vasanthakumar
Department of Animal Nutrition, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

K Sivakumar
Department of Animal Nutrition, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

Corresponding Author:
K Deepa
Department of Animal Nutrition, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

Effect of dietary supplementation of sodium butyrate on ceacal microflora and villi morphology in broiler chicken

K Deepa, MR Purushothaman, P Vasanthakumar and K Sivakumar

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Abstract
Objective: A feeding trial was conducted to assess the effect of supplementation of sodium butyrate on the gut health in broiler chicken.
Methods: The trial consisted of six experimental groups with 12 replicates, each containing 6 chicks with a total of 432 birds. The experimental groups consisted of a control group fed the diet without antibiotic (CON), another group fed the diet with antibiotic oxytetracycline (50 ppm; AB), and groups fed the diet with two levels each of coated-sodium butyrate (CSB) at 0.09 and 0.18%, and uncoated sodium butyrate (UCSB) at 0.03 and 0.06% without antibiotic. The trial was carried out in deep litter pen for 42 days. After 42 days, the birds were slaughtered for collection of jejunum and contents of ceacum for the study of villi morphology and microflora count respectively.
Results: The caecal *Escherichia coli* and *Clostridium perfringens* count were reduced (P<0.01) on addition of coated SB when compared to other treatment groups while the *Lactobacillus sp.* count in caecum was not influenced due to supplementation of CSB, UCSB or AB. The jejunum villi height (P<0.01), villus height to crypt depth ratio (P<0.05) and villi height to villi width ratio (P<0.01) were significantly increased on addition of CSB at 0.18 per cent when compared to antibiotic free diet fed group.
Conclusion: Dietary Sodium butyrate supplementation in broiler chicken reduces the pathogenic bacterial count and improves the villi morphology thereby increasing the overall gut health.

Keywords: Broiler chicken, sodium butyrate, oxytetracycline, ceacal microflora, villi morphology

Introduction
In recent days, commercial broiler birds are certainly exposed to physiological stress due to rapid growth, intensive rearing, high stock density resulting in diminishing immune competence, gut health etc. Today, the ban of antibiotic growth promoters in many countries increases the need to find an alternate microbial load suppressing agents agents like probiotics, prebiotics or organic acids. This restriction in the use of antimicrobial growth promoters increases the incidences of enteric diseases in commercial flocks paves the way to greater susceptibility of the birds to illness, infection and mortality [1]. To overcome these losses, several antibiotics are being used in feed but, the usages have possible lead to the emergence and dissemination of multiple antibiotic resistant pathogens and reduction in response to human and animal infections. Of which, prebiotics are costlier affecting economics in poultry production, while probiotics have different degrees of survivability in feed and in the gut environment.
Organic acids could be the best possible choice for securing the supply of safe food. Organic acids penetrate the lipophilic bacterial cell wall, dissociates at neutral cytosolic pH and releases anions and protons causing lethal accumulation of anions, affects purine bases [2], denatures essential enzymes [3] and results in bacterial cell death. The dissociation of the organic acid in gut is pH dependent. Generally, short chain fatty acids (formic acid, acetic acid, and butyric acid) are preferred acidifiers, among which, butyric acid (BA) is considered as the prime enterocyte energy source, necessary for development of Gut Associated Lymphoid Tissue (GALT) [4] and has the highest bactericidal efficacy against the acid-intolerant species such as *Escherichia coli* and *Salmonella sp* [5].

The additional effects of BA are its stimulation on growth of intestinal villi, inhibition of coccidial oocyst, enhancement of pancreas and gastrointestinal hormones secretion and nutrient utilization, improvement in immunity, antioxidant properties and increase in carcass yield with low abdominal fat content. BA has a pKa (4.81) that dissociates in crop. Hence, encapsulation of BA or salt form of BA is done for stronger and wider intestinal bactericidal effect. To attain maximum stability with little odour, highly soluble sodium salt of butyric acid is used in experimental diet. Further, encapsulated sodium butyrate allows more BA to reach the distal sections of gastrointestinal tract than monoglyceride of BA [6]. Based on the above back ground, the present study was undertaken to evaluate the response of butyric acid as sodium salt in coated and uncoated forms both at two different levels to improve the gut health in broiler chicken.

Materials and Methods

The biological experiment was conducted from January to February 2016 in Poultry farm at Veterinary College and Research Institute, Namakkal. The experimental groups consisted of a control group fed the diet without antibiotic (CON), another group fed the diet with antibiotic oxytetracycline (50 ppm; AB), and groups fed the diet with two levels each of coated-sodium butyrate (CSB) at 0.09 and 0.18%, and uncoated sodium butyrate (UCSB) at 0.03 and 0.06% without antibiotic. The SB used in the study was laboratory grade chemical with 98 per cent purity. The coated SB was encapsulated with vegetable fatty acid containing 30 per cent SB. The pre-starter, starter and finisher diets were fed to birds from 1 to 8, 9 to 28 and 29 to 42 days of age respectively. The trial consisted of six experimental groups with 12 replicates, each containing 6 chicks with a total of 432 Cobb 400 broiler birds. Completely randomized design was followed. The birds were housed in deep litter pens and reared under uniform standard management practices. The chicks were fed with weighed quantity of experimental diets and had free access to water. The chicks were vaccinated against Ranikhet Disease ($RDVF_1$) on seventh and twenty first days, and Infectious Bursal Disease on fourteenth day of age.

After 42 days, the birds were slaughtered and the intestinal content was removed and transferred to sterile test tubes. About 1g of intestinal content from each experimental group was diluted with 9 ml of sterilized physiological saline solution and thoroughly mixed. A serial dilution up to 10^{-9} was prepared. For each dilution, 1 ml of aliquot was spread on the appropriate selective agar plates and incubated at 37 ^{o}C for 24 hours. Mc Conkey agar, Perfringens agar and MRS agar were used as medium for *Escherichia coli, Clostridium perfringens* and *Lactobacillus* count respectively by pour plate method. After incubation, the colonies were counted and expressed as the numbers of colony forming units (cfu) per gram of ingesta content [7].

From the slaughtered birds the small intestine was removed and milked out. The intestine samples were taken from jejunum portion and preserved in neutral buffered formaldehyde for histological studies. The tissue was fixed in paraffin. The paraffin embedded tissue was sectioned to 3 to 4 µm thickness and stained with hematoxylin and eosin for histo-morphological studies [8]. By using ocular micrometer (Erma), each specimen was scanned at least in 10 fields and the mean value for 5 observations was considered to be a measurable unit for each specimen during subsequent calculations. Intestinal villi parameters *viz*., villi length, villi width and crypt depth were measured and expressed in micro meter (µm).

Results and Discussion

Caecal microbial load

The effect of SB on caecal microbial colonization of *Escherichia coli, Clostridium perfringens* and *Lactobacillus sp.* are presented in Table 1. Caecal *Escherichia coli* count was found to be reduced in group fed CSB at 0.09 per cent (6.57 $\log_{10}$ cfu/g) and 0.18 per cent (6.59 $\log_{10}$ cfu/g) when compared to group supplemented with UCSB and control diet with or without AB (7.13 – 7.40 $\log_{10}$ cfu/g). The *E. coli* count in group supplemented with both levels of UCSB was found to be equivalent to group fed with or without AB. This result was found to align with the work of Panda *et al* [9] when 0.6 per cent of BA was used whereas, comparable *E.coli count* was observed by addition of 0.07 per cent protected SB [10] and 0.2 per cent of SB [11].

In the present study, caecal *Clostridium perfringens* count was found to be significantly reduced by supplementation of AB (oxytetracycline) in feed while there was a further reduction in count by addition of CSB at 0.09 or 0.18 per cent when compared to group fed AB free diet. This result matched with the work carried out by Timbermont *et al* [12]. On the other hand, caecal *Lactobacillus* count does not show any significant effect by supplementation of either AB or CSB and UCSB. The Lactobacillus count was higher ($P>0.05$) in birds fed with both the levels of CSB and 0.06 per cent UCSB when compared to birds offered control diet with or without AB.

The presence of *Clostridium perfringens* in the intestine compete with other gut microorganisms and causes the change in the microbiota. *Clostridium perfringens* proliferate and produce toxin and increase the severity of disease [13]. Butyrate has been found to have direct effect on mucin secretion in poultry, which support antibacterial activity on *E. coli, Salmonella sp. and Clostridium sp.* [14]. Sodium butyrate supplementation contributed to changes in the diversity, composition and predicted functions of the cecal microbiota in broiler birds [15]. Thus, CSB was found to be better than AB (oxytetracycline) in reducing caecal *E.coli* and *Clostridium sp.* and increasing *Lactobacillus sp.*

Jejunum villi morphology

The effect of supplementation of SB on jejunum villi morphology is presented in Table 2. Supplementation of CSB at higher level significantly increased ($P<0.01$) villi height (1279.16 *vs.* 978.12 µm), villi height to crypt depth ratio (4.82 *vs.* 3.66) ($P<0.05$) and villi height to villi width ratio (11.85 *vs.* 7.15) ($P<0.01$) of jejunum when compared to group fed control diet with or without AB (Figure 1). However, the difference on these parameters due to low levels of CSB was not significant. The groups supplemented with UCSB, control diets with or without AB on the above parameters were comparable. The study highlights increased villi height, villi height to crypt depth ratio and villi height to villi width ratio of jejunum increased absorptive surface and hence, it suggests that CSB at 0.18 per cent level might have increased nutrient absorption and would have resulted in better weight gain.

Similar to the present study, previous studies showed increased jejunum villi height [10, 16, 17], villi height to crypt depth ratio [11, 18] and comparable crypt depth [10, 11, 16, 19-21] by supplementation of different forms of butyric acid.

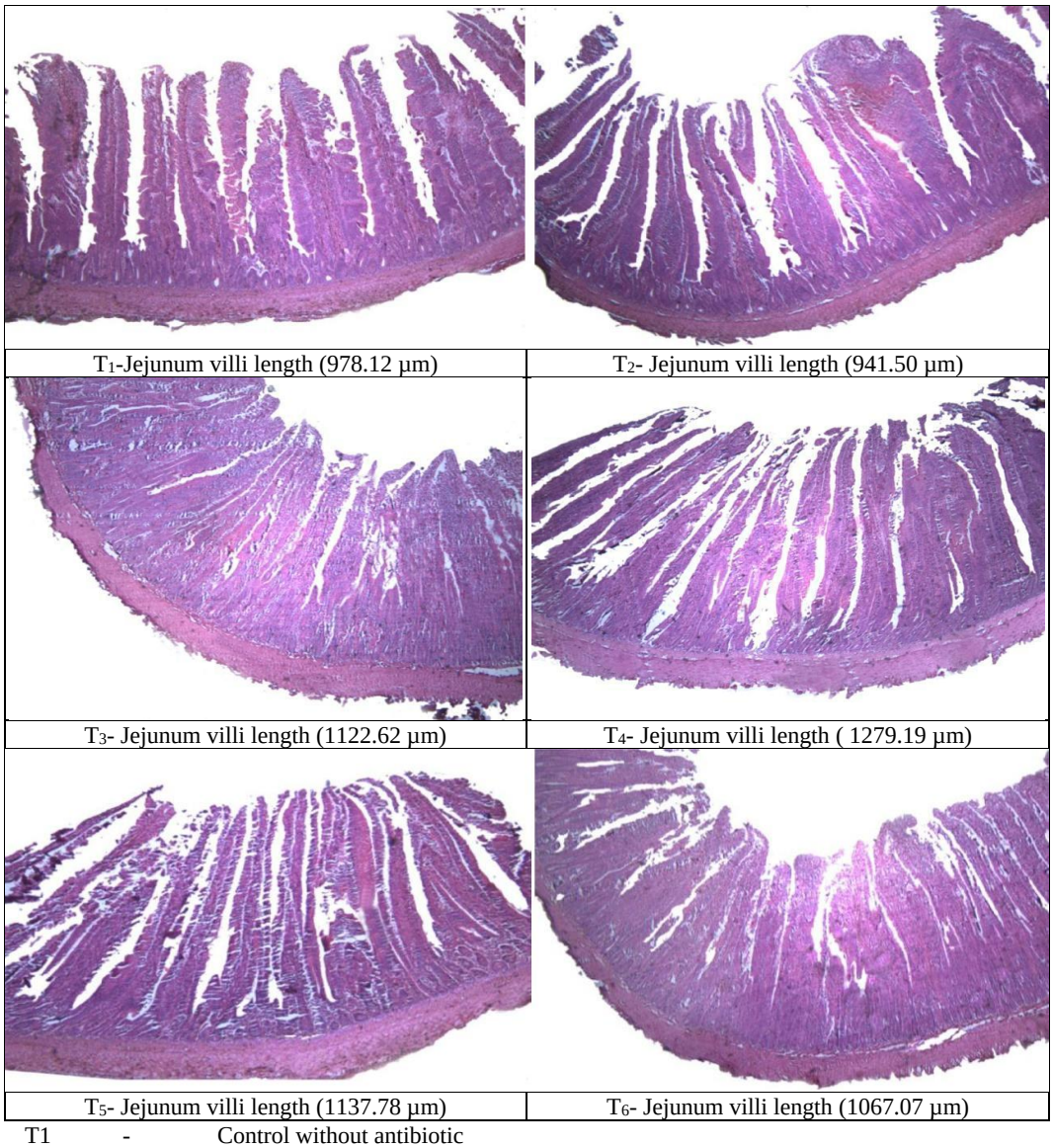
Frankiel *et al*. [22] have shown that short chain fatty acid mixture infusions into the rat isolated caecum caused trophic

effects in the jejunum mucosa. Further, an increase in the villus height and villus height to crypt depth ratio are directly correlated with increased epithelial cell turnover [23] and an indicator of activated intestinal villi function [24, 25].

Table 1: Effect of sodium butyrate supplementation at different levels and forms on caecal microbial load (log_{10} cfu/g) in broiler chicken

Treatment group	*Escherichia coli* *	*Clostridium perfringens* *	*Lactobacillus* species
T1 – Control without AB	$7.40^{b} \pm 0.02$	$7.11^{c} \pm 0.02$	5.93 ± 0.20
T2 – Control with AB (50 ppm)	$7.22^{b} \pm 0.07$	$6.27^{ab} \pm 0.16$	6.17 ± 0.22
T3 – T1 + 0.09 % CSB	$6.57^{a} \pm 0.22$	$6.04^{a} \pm 0.19$	6.51 ± 0.15
T4 – T1 + 0.18% CSB	$6.59^{a} \pm 0.24$	$6.04^{a} \pm 0.24$	6.50 ± 0.21
T5 – T1 + 0.03 % UCSB	$7.13^{b} \pm 0.11$	$6.74^{bc} \pm 0.20$	6.11 ± 0.20
T6 – T1 + 0.06 % UCSB	$7.32^{b} \pm 0.03$	$6.70^{bc} \pm 0.27$	6.47 ± 0.20
P value	0.001	0.003	0.231
Pooled SE	0.079	0.101	0.085

Each value is a mean of six observations
Means with at least one common superscript in a column do not differ significantly at *P>0.01
AB = Antibiotic (Antibiotic used was oxytetracycline)
CSB = Coated Sodium Butyrate
UCSB = Uncoated Sodium Butyrate



T_1-Jejunum villi length (978.12 µm) | T_2- Jejunum villi length (941.50 µm)
T_3- Jejunum villi length (1122.62 µm) | T_4- Jejunum villi length (1279.19 µm)
T_5- Jejunum villi length (1137.78 µm) | T_6- Jejunum villi length (1067.07 µm)

T1 - Control without antibiotic
T2 - Control with antibiotic
T3 - T1 + 0.09 % coated sodium butyrate
T4 - T1 + 0.18 % coated sodium butyrate
T5 - T1 + 0.03 % uncoated sodium butyrate
T6 - T1 + 0.06 % uncoated sodium butyrate

Fig 1: Jejunum villi length (µm) of broilers birds at 42 days of age as influenced by different treatment groups (H&E, x40)

Table 2: Effect of sodium butyrate supplementation at different levels and forms on villi morphology of jejunum

Treatment groups	Villi height** (µm)	Villi width (µm)	Crypt depth (µm)	Villi height: Crypt depth ratio*	Villi height: Villi width ratio**
T1 – Control without AB	$978.12^{a} \pm 28.4$	143.90 ± 8.82	240.54 ± 10.02	$3.66^{a} \pm 0.16$	$7.15^{b} \pm 0.58$
T2 – Control with AB (50 ppm)	$941.50^{a} \pm 44.7$	136.05 ± 8.66	260.62 ± 20.79	$3.80^{a} \pm 0.28$	$7.19^{b} \pm 0.52$
T3 – T1 + 0.09 % CSB	$1122.61^{ab} \pm 140.7$	126.49 ± 11.66	243.01 ± 16.14	$4.76^{ab} \pm 0.55$	$9.85^{ab} \pm 1.06$
T4 – T1 + 0.18% CSB	$1279.19^{b} \pm 85.46$	124.18 ± 11.65	277.32 ± 11.76	$4.82^{b} \pm 0.50$	$11.85^{a} \pm 1.62$
T5 – T1 + 0.03 % UCSB	$1137.78^{ab} \pm 24.32$	138.77 ± 6.81	242.61 ± 10.41	$4.77^{a} \pm 0.21$	$8.49^{b} \pm 0.52$
T6 – T1 + 0.06 % UCSB	$1067.07^{ab} \pm 31.29$	139.62 ± 5.60	259.11 ± 16.46	$4.42^{a} \pm 0.44$	$7.75^{b} \pm 0.35$
P value	0.000	0.604	0.474	0.044	0.002
Pooled SE	22.665	3.700	6.016	0.148	0.406

Each value is a mean of twelve observations

Means with at least one common superscript in a column do not differ significantly at * $P>0.05$, ** $P>0.01$.

AB = Antibiotic (Antibiotic used was oxytetracycline)

CSB = Coated Sodium Butyrate

UCSB = Uncoated Sodium Butyrate

Conclusion

Dietary addition of coated sodium butyrate reduced caecal *Escherichia coli* and *Clostridium perfringens* count and increased jejunum villi height, villi height to crypt depth ratio, villi height to villi width ratio, which proved to improve the overall gut health in commercial broiler chicken. Therefore, sodium butyrate may be considered as a potential replacement for the antimicrobial growth promoters in diets of broiler chicken.

Conflict of Interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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