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Impacts of Coated Sodium Butyrate (Butigold®) on Growth Efficacy and Intestinal Development of *Gallus Gallus Domesticus Cobb 400*

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ABSTRACT: The poultry industry has undergone significant intensification in recent decades, with production systems increasingly focused on achieving optimal growth rates, feed efficiency, and overall flock health with the Coated Sodium Butyrate (Butigold®). In such high-performance systems, the gastrointestinal tract (GIT) of broiler chickens plays a central role, functioning not only as the primary site of digestion and nutrient absorption but also as a vital component of the bird's immune defence system. The structural and functional integrity of the GIT directly influences feed conversion efficiency, nutrient utilization, and resistance to enteric pathogens. Consequently, maintaining gut health is critical for sustainable broiler production, particularly in the context of global restrictions on antibiotic growth promoters (AGPs) due to concerns over antimicrobial resistance.

KEYWORDS: Antioxidant indices, Bactericidal Assay, Cobb 400, Intestinal significance, Coated Sodium Butyrate

INTRODUCTION

One promising nutritional strategy for enhancing gut health and performance in poultry is the dietary inclusion of short-chain fatty acids (SCFAs) or their salts, particularly butyric acid and sodium butyrate. Butyric acid is a naturally occurring SCFA produced by bacterial fermentation of dietary fibre in the hindgut. It serves as a preferred energy source for enterocytes, promotes epithelial cell proliferation, stimulates villus development, and strengthens mucosal barrier function. Beyond its trophic effects on the intestinal epithelium, butyric acid has been reported to exert antimicrobial activity by reducing gut pH, thereby creating an environment favourable for beneficial micro-flora such as *Lactobacillus sp*. [1] while inhibiting opportunistic pathogens, including *Escherichia coli* and *Salmonella sp*. Furthermore, it modulates immune function and has anti-inflammatory properties, contributing to overall bird health.

Despite these well-recognized benefits, the application of free butyric acid in poultry diets is limited by its strong odour, corrosive nature, and rapid absorption in the upper gastrointestinal tract. When absorbed too early, butyric acid is unable to exert its beneficial effects in the distal small intestine [2], where its role in modulating microbiota, enhancing mucin secretion, and reinforcing immune responses is critical. This challenge has led to the development of protected or coated butyrate products. Such formulations are designed to ensure the slow release of butyric acid along the entire length of the intestine, thereby extending its bioactivity and improving its functional efficacy [3].

Coated sodium butyrate, in particular, has emerged as a commercially viable solution. By encapsulating the butyrate core in a protective lipid or polymer matrix, coated formulations can resist early dissociation in the crop and proventriculus, releasing butyric acid progressively in the duodenum, jejunum, and ileum. This targeted delivery not only optimizes nutrient absorption and intestinal morphology but also sustains antimicrobial action where pathogenic bacteria often colonize [4]. Previous research has demonstrated that coated sodium butyrate supplementation can improve feed intake, weight gain, and feed conversion ratio (FCR), while reducing mortality rates and enhancing gut microbial balance in broilers. Moreover, it has been shown to stimulate villus height (VH), increase the villus height to crypt depth ratio (VCR), and augment goblet cell counts factors that contribute to increased absorptive surface area and improved mucosal defence [5].

In addition to its direct effects on the GIT, sodium butyrate has been implicated in enhancing antioxidant status and systemic immunity. By modulating oxidative stress markers and promoting the expression of host defence peptides (HDPs), sodium

butyrate can strengthen the bird's ability to combat bacterial, fungal, and possibly viral pathogens. The antimicrobial properties of HDPs, combined with improved intestinal barrier function, may contribute to reduced pathogen colonization and shedding, thereby lowering disease transmission risk within flocks [6]. Furthermore, a healthier gut environment, characterized by a higher population of lactic acid bacteria and a reduced load of coliforms, can indirectly enhance growth performance through better nutrient utilization and reduced energy expenditure on immune challenges.

Butigold® (ARCL Organics) is a commercially available coated sodium butyrate product specifically designed for sustained release in the avian GIT. Its formulation aims to deliver the functional benefits of butyric acid throughout the small intestine while mitigating the limitations of uncoated products [7]. While studies on sodium butyrate supplementation in poultry are numerous, there is comparatively limited literature on the comprehensive evaluation of Butigold's effects encompassing growth performance, intestinal development, histomorphological indices, antioxidant capacity, gut microbiota, and immune responses in commercial broilers under standard rearing conditions. Most existing studies focus on either performance parameters or gut morphology, leaving a gap in understanding the integrated physiological and immunological impacts of coated sodium butyrate in broiler nutrition [8].

Given this background, the present study was designed to evaluate the efficacy of coated sodium butyrate (Butigold) supplementation in a maize–soybean meal-based diet for commercial broilers. The study aimed to investigate multiple dimensions of bird health and productivity, including growth performance (average body weight, body weight gain, feed intake, and FCR), intestinal morphometry (relative length and weight of intestinal segments, villus height, crypt depth, and goblet cell counts), antioxidant activity of the intestinal mucosa, gut microbial composition (beneficial and pathogenic bacteria), and the antimicrobial potential of blood serum against common poultry pathogens. By integrating these performances, morphological, microbial, and immunological assessments, the study aimed to provide a comprehensive understanding of how coated sodium butyrate supplementation affects broiler health and productivity [9].

The findings from this research are expected to contribute to the growing body of evidence supporting non-antibiotic feed additives in poultry production [10]. In particular, they may offer practical insights into the potential of coated sodium butyrate as a multifunctional growth promoter and gut health enhancer, aligning with global efforts toward sustainable, antibiotic-free poultry farming.

MATERIALS AND METHODS

Experimental birds and their grouping

Day-old commercial broiler chicks (n=100) were divided into two groups of 50 chicks in each, weighed group-wise, and randomly located to two Pens, one for control (control-C) and the other for Test (T) diet. Two dietary treatments were formulated that one of commercial maize-soybean meal-based diet (C) another of Control diet supplemented with 2.0 kg of coated butyrate per ton of feed (T). Both the dietary treatments were prepared for pre-starter, starter, and finisher phases following commercial standard (23%, 20.8% and 18.50% crude protein with 2950, 3025, and 3100 kcal ME.kg diet). Each dietary treatment was offered to one group and was fed with pre-starter (0 to 14d), starter (15 to 24d), and finisher (25 to 42 days) diets. Chicks were raised on the floor in pens under standard conditions with 22 hour light regime throughout the trial period.

Growth performance measurement

Body weights and feed consumption were recorded for each group on day 0, 14, 24, 35, and 42. Accordingly, average body weight (ABW), average body weight gain (ABWG), average feed intake (AFI), and feed conversion rate (FCR) during each period were calculated.

Intestinal sample and blood sample collection

Three healthy birds were weighed and selected from each treatment according to their average body weight after an 8 h fast at 42 d of age. Blood samples were collected from the jugular vein of each variant by using a 23-gauze needle. Then chicks were necropsies immediately followed by dissection for collection of whole GI tract. The length and weight of the small intestine, including the duodenum, jejunum and ileum, and part of the caeca were measured after excision. Meanwhile, the mucosa was gently scraped onto sterile vials and similarly stored at -18˚C for later determination of antioxidant capacity. Samples of the jejunum and ileum (approximately 1 cm obtained from the midpoint) were fixed in 4% formaldehyde solution for the intestinal histomorphologic indices.

Relative length and weight of intestine

The small intestine and caeca excised from each chick were measured to determine the average relative length (cm/kg) and relative weight (g/kg) of intestinal segments, which were reported as the ratio of intestinal tract length (cm) or weight (g) to body weight (kg) based on prior studies by [11] and [12].

Analysis of intestinal histology

A Consecutive section (5 mm) of each jejunal and ileal segments from each variant was stained with haematoxylin-eosin and morphological observations were conducted by MAGNUS CH2Oi optical microscope. Computation was done by calibrated stage and ocular micrometre, Villus height (VH) was determined from the apex of the villus until the junction of the villus and crypt, and crypt depth (CD) was defined as the depth of invagination between the villus and the basal membrane. The villus height to crypt depth ratio (VCR) was then calculated as per method described by [13]. In addition, eight intact and neat rows of intestinal villi stained with periodic acid Schiff (PAS) were selected to quantify of goblet cells (GC) between each set of 100 intestinal mucosal epithelial columnar cells (IEL) as per study of [14].

Antioxidant indices of the intestinal mucosa

Mucosa of jejunum and ileum were homogenized with nine times volume of ice-cold physiological saline and centrifugation at 2500 rpm for 10 min. The total antioxidant capacity (T-AOC) in 10% homogenate supernatant samples was determined by DPPH free radical assay. The measurement of the DPPH radical scavenging activity was performed according to methodology described by [15]. The samples were reacted with the stable DPPH radical in an ethanol solution.The reaction mixture consisted of adding 0.5 ml of sample, 3 ml of absolute ethanol and 0.3 ml of DPPH radical solution 0.5 mM in ethanol. When DPPH reacts with an antioxidant compound, it donates hydrogen and thus reduced. The changes in colour (from deep violet to light yellow) were read absorbance at 517 nm after 3100 min of reaction using a UV-VIS spectrophotometer (SHIMADZU, 1900i). The mixture of ethanol (3.3 mL) and sample (0.5 mL) served as blank. The control solution was prepared by mixing ethanol (3.5 mL) and DPPH radical solution (0.3 mL). The scavenging activity percentage (AA %) was determined as suggested by [16].

Indirect bactericidal effects of sodium butyrate

Intestinal sample was serially diluted via 10-fold dilution (from 10^{-1} to 10^{-6}) with 0.9% of sterile saline solution. The enumeration of *Lactobacillus sp.* was done by the method described by [17]. BCG Dextrose agar was used for the Lactobacilli count, while E. coli and Salmonella spp were enumerated by EMB agar and SS agar plate, respectively. The plates were incubated for 3 days at 35 °C in an inverted position.

Microbiocidal assay of blood serum:

To obtain serum, blood vials were placed on a slanted surface for 10 to 15 minutes to allow clotting, followed by centrifugation at 2000X g for 10 minutes. Transparent supernatant was then collected, and blood serum microbiocidal assay was done by taking pathogenic bacteria *E. Coli*, *Salmonella sp.* and fungi *Aspergillus sp.* pas target organisms, followed by the method described by [18}.

Serum and the microbial inoculum in the ratio of 9:1 (10^2 cfu) were aliquoted into individual sterile micro centrifuge tubes and incubated for 6h at 40°C for bacteria and 25^{o}C for fungi. Consequently, spread plates were made onto Muller-Hinton agar and Sabouraud dextrose agar, respectively, by taking 0.1ml of previously incubated aliquot, cultured without serum, taken as a positive control. Plates were incubated at 37^{o}C and 25^{o}C for 3 days, respectively, for bacterial and fungal pathogens. The microbiocidal assay was calculated by growth inhibition percentage against the positive control.

RESULTS

Growth performance

The growth performance of broilers fed diets with or without inclusion of coated butyrate (Butigold) is presented in Table 1 and 2. Average growth performance of tested birds increased by 1.02, 1.06 and 2.57 folds respectively against control during pre-starter, starter, and finisher period, with Improved FCR values. The overall growth (1.32 folds more ABW (Average body weight) of test than control) indicated that dietary coated butyrate supplementation had significant positive effects ($P<0.05$) on average body weight, average feed intake, and feed conversion ratio (FCR) during the overall period (0–42 d). Moreover, mortality of birds reduced by 2% in test variant fed diets with Butigold.

Table 1. Effects of coated butyrate on the growth performance of broilers

Parameters	Control pen	Test pen
Specimen (Nos)	48±2	48±2
ABW (g)	44.4±2	44±2
Pre-starter (0-14days)		
ABWon14d (g)	317±2	324.57±2
ABWG (g)	270.60±2	278.57±2
AFI (g)	499.49±2	470±2
FCR	0.17±2	0.32±2
Mortality (Nos.)	0.0±2	0.0±2
Starter(15-24days)		
ABW on 24d (g)	674±2	705±2
ABWG (g)	355±2	378.43±2
AFI (g)	1003±2	1018±2
FCR	0.81±2	0.68±2
Mortality (Nos.)	None	None
Finisher, (25-35days)		
ABW on 35d (g)	908±2	1308±2
ABWG (g)	232±2	601±2
AFI (g)	798±2	948±2
FCR	1.41±2	0.43±2
Mortality (Nos.)	0.0±2	None
Finisher, (36-42days)		
ABW on 42d (g)	1218±2	1613.21±2
ABWG (g)	308±2	303.21±2
AFI (g/bird)	878±2	834±2
FCR	0.83±2	0.73±2
Mortality (Nos.)	None	None

Table 2. Overall growth performance

*Total period (0-42 days)	**Control Pen	***Test Pen	****SEM[4]	*****P-Value
AFI (g/bird)	3184.49±2	3276±2	17.44±2	0.001
ABW (g/bird)	1218±2	1613.21±2	74.56±2	0.019
FCR (Feed to body weight)	0.61±2	0.03±2	0.0±2	0.005
Morality (%)	4.00	2.00	-	-

*The values are given as the means based on six birds for each treatment (n=6). **Control=basal diet; ***Test=basal diet supplemented with 2.0 kg per ton of coated butyrate. **/*** ABW, Average body weight (g); ABWG, Average body weight gain (g); AFI, Average feed intake (g); FCR, feed conversion rate. ****SEM, standard error of the mean. *****T-test: paired two-sample for mean.

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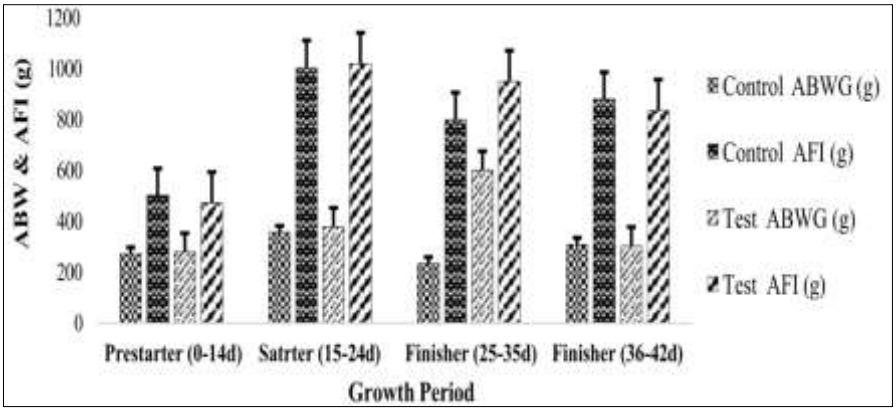

Figure 1. Average body weight and average feed intake of control and Test birds throughout the trial period

Relative length and weight of the intestine

Basal feed, incorporated with coated butyrate at 2 kg/ton of feed had significant positive effects (P<0.05) on the relative length (RL) and relative weight (RW) of the duodenum, jejunum, and ileum at 42 d of age, while no significant effects found on caeca (P>0.05) as presented in Table 3.

RL of duodenum, jejunum, and ileum increased by 1.11, 1.03, and 1.07 fold, respectively, over control, while RW of duodenum, jejunum, and ileum increased by 1.10, 1.05, and 1.12 fold, respectively.

Table 3. Effects of coated butyrate on development of the intestine and caeca in broilers

Items	*Control birds	**Test birds	***SEM	****P value
Relative length; RL(*d 42*)				
Duodenum	3.96±2	4.66±2	0.0±2	0.02±2
Jejunum	50.05±2	51.79±2	0.0±2	0.0±2
Ileum	46.90±2	50.40±2	0.1±2	0.04±2
Caeca	4.65±2	4.61±2	0.0±2	0.0±2
Relative weight; RW (*d 42)*				
Duodenum	6.36±2	7.21±2	0.0±2	0.0±2
Jejunum	13.17±2	14.08±2	0.1±2	0.0±2
Ileum	11.28±2	12.92±2	0.2±2	0.01±2
Caeca	4.73±2	4.77±2	0.03±2	0.0±2

*The values are given as the means based on six birds for each treatment (n=6).Control=basal diet; Test = basal diet supplemented with 2 kg coated butyrate per ton of feed. **RL; Relative length, RW; Relative weight.
***SEM, standard error of the mean. *****T-test: paired two-sample for means

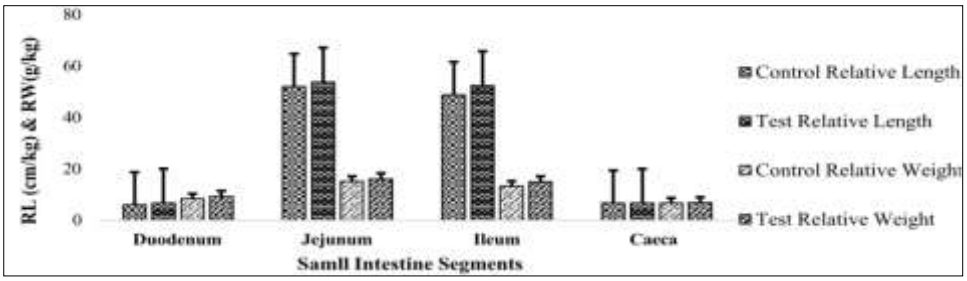

Figure 2. Relative length and weight of the small intestinal segment of the control and test birds

Intestinal histological indices:

As indicated in Figure 3, dietary coated butyrate supplementation was found to be tended to increase jejunum and ileal villus height (VH) and also demonstrated significant stimulation of goblet cells on jejunal and ileal villi. Coated butyrate-fed broilers'

jejunum and ileum showed 1.06- and 1.17-fold VH, respectively, against the control, as well as more goblet cells, i.e., 1.88 and 1.94-fold more than the control group.

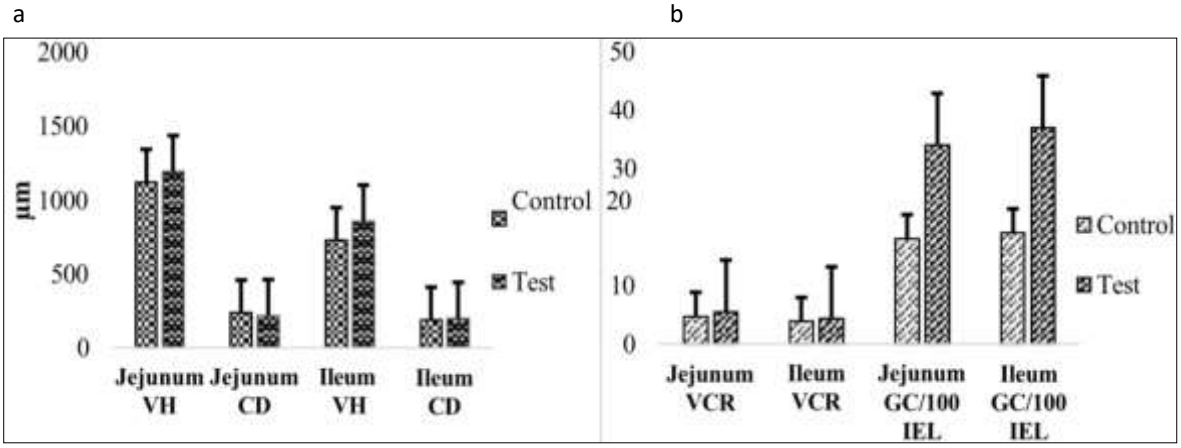

Figure 3. Intestinal morphological indices of jejunum and ileumin broilers. (a,b) VH, villus height (µm); CD, crypt depth (µm); VCR, the ratio of VH to CD; GC, goblet cell

Antioxidant indices of the intestinal mucosa:

Broilers fed a basal diet with coated butyrate (test) showed greater total intestinal antioxidant activity (T-AOC) than the basal diet-fed control. DPPH free radical scavenging activity (AA %) of test birds' intestinal extract exhibits 1.08 fold higher in comparison to the control describing in Figure. 4.

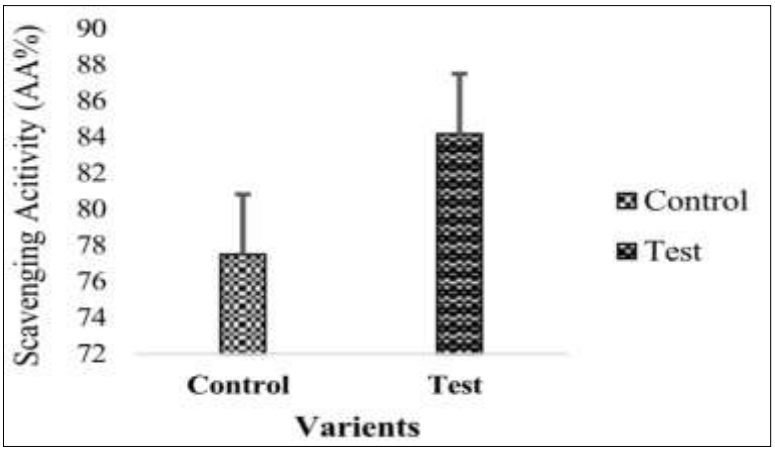

Figure 4. Total antioxidant activity (T-AOC) of the intestinal extract of control and test birds

Indirect bactericidal effect of sodium butyrate

As depicted in Table 4 and Figure 5, the Intestinal *lactobacillus sp.* community of the test variant increased by 1.45-foldagainst the control; in contrast, secondary pathogen coliform count decreased by 1.75 folds. In addition*, Salmonella sp*. was neither detected in the control nor the test.

Table 4. Effect of coated butyrate on the small intestinal microbial community o fbroilers

Small intestine microbiota*	Control birds	Test birds
Lactobacillus sp. count (cfu/g)	4.71±2	6.30±2
Coliform count (cfu/g)	0.72±2	0.55±2
Salmonella sp. count (cfu/g)	N/D	N/D

The values are given as the means based on six specimens for each treatment (n=6).

Control=basal diet; Test=basal diet supplemented with 2 kg coated butyrate per ton of feed.

*Test done by selective media for a specific organism

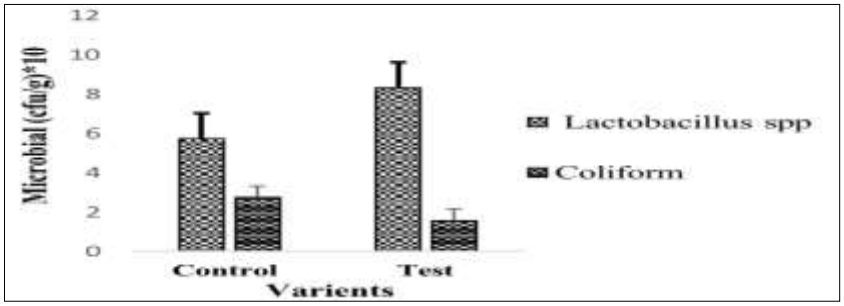

Figure 5. Effect of coated butyrate on intestinal microbial community

Microbiocidal assay of blood serum (BS)

Blood serum micro biocidal assay indicated that test birds fed with coated butyrate possess 1.61, 1.22, and 1.69-fold higher antimicrobial activity against *Escherichia coli*, *Salmonella sp*., and *Aspergillus niger*, respectively, in contrast to the control, depicted in Table 5 and Figure 6.

Table 5. Effect of Butigold on immunity against common broiler pathogen

Microbial Specimen	*Control the bird's BS (Microbiocidal assay,%)	Test bird's BS (Microbiocidal assay, %)
Escherichia coli	26.87±2	44.72±2
Salmonella sp.	13.15±2	16.61±2
Aspergillus niger	21.63±2	38.05±2

The values are given as the means based on six specimens for each treatment (n=6).Control=basal diet; Test =basal diet supplemented with2 kg coated butyrate per ton of feed. *Against positive control.

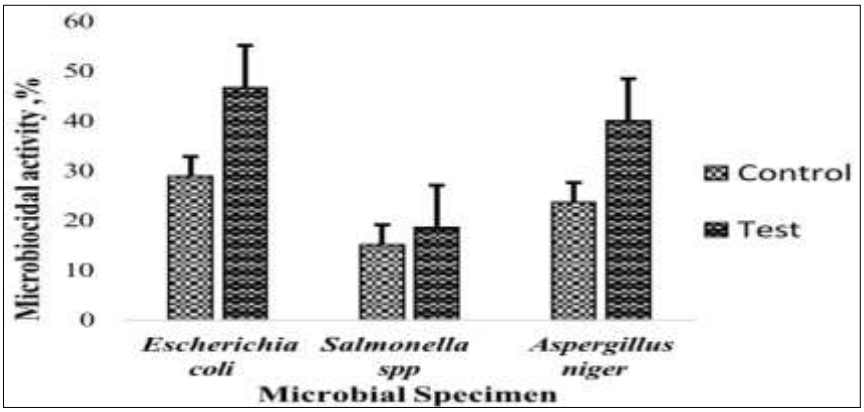

Figure 6. Coated butyrate on the enhancement of immunity against common broiler pathogens

DISCUSSION

The present study indicated that coated butyrate exhibited beneficial effects on the growth performance of broilers in terms of increased feed intake coupled with body weight gain and significantly improved FCR. [19] And [20] also reported similar observations earlier. Moreover, coated butyrate additions delivered apart of butyrate in the further distal intestinal tract because of its slow release in the intestinal tract, which caused mucosal modulation in the gut. A considerable quantity of butyric acid may therefore be preferentially applied by enterocytes to stimulate intestinal development and function in chicks that ultimately improved growth performance through increased utilization of nutrients and by promoting gut health.

Butyric acid, the active ingredient of coated butyrate, is readily absorbed by enterocytes as a source of energy to accelerate proper intestinal development and function, as well as for overall health. Long villi and shallow crypts are generally thought to support a large surface area for higher absorption capacities and healthy development of the intestine, but they also support greater tissue turnover, thereby accounting for the optimal status of the gut. The current study showed that coated butyrate tended to increase the ileal VH, although it had no influence on the CD coupled with VCR of the jejunum and ileum, which is like previous reports by [21] and [19]. (2014). Moreover, microscopic analysis of the villi also indicated that coated butyrate in the diet resulted in more

goblet cells in both the jejunum and ileum, of which the primary function is the production and stimulation of mucus. The mucus layer, which is the first line of defence in the intestinal mucosa, is composed of large quantities of mucin, which is regulated via altering the number of goblet cells or mucin gene expression, particularly MUC2. In support o fthis observation, also noted longer, flattened leaf like villi. In addition, dietary treatments had significant effects on the relative length and weight of the jejunum, ileum, and duodenum at 42d. In brief, the improved intestinal structural health and enhancement of goblet cells contributed to the absorption and reinforcement of the intestinal integrity of broilers, which might have resulted in the increased relative weight and length of the small intestine in our study.

It has been proposed that oxidative stress due to an imbalance between prooxidant and antioxidant systems caused by stressor contributes to the waste of nutrients as well as to the generation of oxidative damage in living bodies. T-AOC reflects the total antioxidant ability; increased T-AOC suggested that dietary sodium butyrate enhanced antioxidant properties and retarded damage of the mucosa.

Sodium butyrate lowers the pH of the intestine, which favours the growth of lactic acid-producing bacteria such as *Lactobacilli sp*. [22] as they require an acidic medium for their growth. It has been reported that lacticacid-producing bacteria compete for space and nutrients with pathogenic bacteria within the intestine [23]. *Lactobacilli sp*. is produced bacteriocins which moderate the pathogenic bacteria count and maintain a healthy environment in the bird's intestine. Similarly, the current study indicated that sodium butyrate favours the growth of *Lactobacilli sp* .that convert glucose to lactic acid within the intestine of birds, causing the inhibition of pathogenic bacteria such as *Salmonella sp*. and *E. coli*.

Blood serum samples of the test group yielded significant killing ability against *Escherichia coli*, *Salmonella sp*. and *Aspergillus niger* compared with those of the control group (Figure 6). Not much data is available describing the effect of sodium butyrate on the immune functions of broilers. Host defence peptides (HDPs), also known as antimicrobial peptides, exist in almost all forms of life and are an integral part of innate immunity [24]. Chicken genome encodes for14 β-defensins (AvBD1-14) and four cathelicidins (fowlicidins 1-3 and cathelicidin-B1) as a member of the HDPs [25]. HDPs exhibit their properties as broad-spectrum antimicrobials against bacteria, enveloped viruses, fungi, and protozoa by direct binding and lysis of microbial membranes [26], which prevents the development of resistance to HDPs in pathogens. Sodium butyrate induced HDP gene expression in chicken macrophage cells, monocytes, bone marrow cells, and jejunal and caecal explants. In addition, sodium butyrate enhanced the antibacterial activity of chicken monocytes and reduced the colonisation of *Salmonella sp*. Similar findings were reported in response to oral supplementation of sodium butyrate or butyric acid that reduced the colonisation and shedding of *S. enteritidisin* broilers [27].These findings may have arisen either due to the direct antimicrobial activity of butyric acid or due to the decreased invasiveness of *Salmonella sp*. (possibly due to the down regulation of genesin SPI1) through intestinal epithelium [28]. Sodium butyrate can inhibit then itricoxide production and expression of cyto-kines such as IL-1β, IL-6, IFN-γ, and IL-10 in chicken macrophage cells stimulated by the presence of *S. typhimurium lipopolysaccharides* [29].

CONCLUSION

The present study divulged that coated sodium butyrate (Butigold) supplementation at the rate of 2g per kg of feed enhanced overall growth performance, encouraged intestinal structural development and microbial health, and immune competence of commercial broiler chickens. In addition, Butigold supplementation ameliorated the intestinal microbial community.

Further study with comparatively low doses, i.e., 1g and 0.5g per kg of feed, may be obligatory for the economizing broiler production.

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