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Full Length Article

Protective Effect of Sodium Butyrate on Growth Performance, Immune Responses and Gut Mucosal Morphometry in *Salmonella*-Challenged Broiler Chickens

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Abstract

Effect of sodium butyrate (SB) was evlauted on gut development, immune system and performance traits in broilers challenged with *Salmonella gallinarum*. For this purpose, 240 *Salmonella*-free broiler chicks were divided into four groups with six replicates of 10 birds each. Groups NC (non-infected + non-medicated) and NC-S (*Salmonella* infected + non-medicated) served as negative controls. Group PC-S (*Salmonella* infected + medicated with enrofloxacin) was kept as positive control and birds representing SB-S (*Salmonella* infected) group were treated orally with SB at the dose of 1.0 g/kg, as a supplement to the basal feed. Better growth performance was reported in SB supplemented birds. NC-S group displayed lower (P<0.05) antibody titer against ND and higher (P<0.05) cellular immunity was recorded in SB-S as compared to NC-S and PC-S groups. Contrary to NC-S the SB-S group exhibited higher (P<0.05) relative weights of thymus and bursa. Unlike the PC-S and SB-S groups, reduced villus height and villus to crypt ratio (P<0.05) and increased (P<0.05) cecal *Salmonella* population and mortality rate were evident in NC-S group. The prophylactic use of SB diminished the stress associated with *S. gallinarum* infection in birds. © 2017 Friends Science Publishers

Keywords: Broiler; Organic acid; *Salmonella*; Immunity; Gut; Villi

Introduction

Salmonellosis is one of the leading causes of production losses in poultry (Saleem *et al.,* 2016). Antibiotics, including enrofloxacin are being used to combat Salmonellosis and enhance growth performance in poultry (Randall *et al.,* 2006). Owing to the development of resistance in humans, the use of in-feed antibiotics has been banned in many countries (Kabploy *et al.,* 2016). This ban challenged the animal performance and as a result, the poultry sector faced greater challenges to meet growth targets (Abudabos *et al.,* 2016). Subsequently, this situation stimulated extensive research on organic acid alternatives and products such as sodium butyrate are being presented as a safe alternative to antibiotic growth promoters (AGP) in animal nutrition (Khan and Iqbal, 2016).

Animal growth and production depend upon the appropriate digestion and absorption of the feed-stuff accomplished by the villi of small intestinal mucosa (Awad *et al.,* 2014). The villus height is linked with area of contact surface with the nutrients. The gut mucosa is among the earliest locations in animal body which get exposed to in-feed antigens that may alter its architecture and physiology (Awad *et al.,* 2014). The gut microstructures can be modulated by various supplements added into the diet (Sultan *et al.,* 2015a; Sikandar *et al.,* 2017).

Micro-encapsulated sodium butyrate (known as acidifier) is a valuable tool in maintaining the gut health (Sultan *et al.,* 2015b) and minimizing the pathogenic microorganism including *Salmonella* species by reducing the intestinal pH (Fernandez-Rubio *et al.,* 2009; Khan and Iqbal, 2016). Sodium butyrate contributes to the release of host defense peptides (Hancock and Sahl, 2006). These peptides have immunomodulatory properties and activate various immune related cells (Sunkara *et al.,* 2011). Sodium butyrate also stimulates the immune system via regulation of Th1, Th17 and IL-10 regulatory T cells (Park *et al.,* 2015). Butyric acid may downregulate the expression of all those genes which are participating in the invasion of *Salmonella* protein into the host cells (Van Immerseel *et al.,*

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2006). Furthermore, SB is reported to increase growth performance of healthy (Sikandar *et al.,* 2017) and *Salmonella enteritidis* infected birds (Fernandez-Rubio *et al.,* 2009). As most of the butyrate is absorbed in the upper gastrointestinal tract, therefore the micro-encapsulation technology has been established to deliver portions of the active ingredient (butyrate) slowly in the lower intestinal segments (Moquet *et al.,* 2016).

To the best of our knowledge, studies regarding the protective effect of SB on the growth performance, immunity and gut health of *S. gallinarum* infected birds have not been conducted so far. Owing to the reported beneficial properties, we hypothesized that SB may be a possible candidate to replace AGPs in broilers. This product may act as growth promoter through improvement of gut microarchitecture and modulation of systemic immune responses in addition to a local mucosal effect, which has not been fully elucidated up till now. The current research was, therefore, planned to evaluate the potential protective effect of SB on growth performance, gut morphology and immune system in broiler chickens challenged with *Salmonella gallinarum*.

Materials and Methods

Animal handling and experimental manipulation procedures were approved by the Ethical Review Committee for the use of Laboratory Animals of the University of Veterinary and Animal Sciences, Lahore, Pakistan (Notification No. DR/257 dated 13-04-15).

Selection and Rearing of Experimental Birds

A total of 240 one-day old Hubbard male chicks were purchased from a local hatchery, weighed and randomly categorized into four groups. The groups NC (non-infected, non-medicated) and NC-S (*Salmonella* infected, non-medicated) served as negative controls. Group PC-S (*Salmonella* infected, medicated orally with enrofloxacin, 10% solution, ISO 9001 certified, Symans Pharmaceuticals Pvt. Ltd.) and maintained as positive control. *Salmonella* infected group SB-S was treated orally with SB, (CM3000, Hangzhou, China) at the dose of 1 g/kg added to the basal feed (Table 1). The ration was produced as per our previous study (Sikandar *et al.,* 2017). All groups comprised of six replicates of 10 chicks each maintained as per the standard husbandry practice in an environmentally controlled shed with 0.037 m^2/bird stocking density and wood shaving bed. Birds were maintained free from natural *Salmonella* infection and were monitored for general body condition and health status thrice a day throughout the experimental period. Chicks were vaccinated with Newcastle disease (NDV, Nobilis LaSota, Intervet International B.V. Boxmeer, Holland) and other infectious diseases like infectious bronchitis, infectious bursal disease and Marek's disease following the locally recommended vaccination schedule.

Evaluation of Chicks for Salmonellosis upon Arrival

Three chicks were selected randomly upon arrival and killed in University Diagnostic Lab (UDL). Their cecal tonsils and ceca were aseptically isolated and mixed in test tubes containing 10 mL tetrathionate broth and incubated overnight at 37°C. Later on the samples were cultured on brilliant green agar plates (CM0263; Oxoid) and incubated overnight at 37°C. The plates were then analyzed for the presence or absence of *Salmonella*.

Challenge of Birds with Infective Material

Local isolates of *Salmonella gallinarium* seed were received in freeze dried culture from University Diagnostic Laboratory, UVAS, Lahore-Pakistan and the organisms were retrieved on MacConkey agar with subsequent purification by transferring to *Salmonella-Shigella* agar (SS agar). The culture was then grown overnight at 37°C in trypton soy broth (Oxoid) prior to infection of chicks. Challenge was given via oral gavage at the rate of 0.2 mL suspension of *S. Gallinarum* with 10^7 CFU/chick (approximately) and the same amount of sterile buffered peptone water (Sigma) was administered to non-infected control (NC) group.

Growth Performance

Average body weight (ABW, the initial body weight was subtracted from the final body weight) and feed conversion ratio (FCR, determined by using the formula: FCR=MFC/MW, where MFC= Mean feed consumption and MW = Mean weight) were computed to record the weekly growth performance (Sikandar *et al.,* 2017). All dead birds were weighed and removed and the mortality rate was noted as a cumulative percentage. The feed intake and weight gain ratio of the mortality was adjusted.

Humoral Immune Response

The antibody titer against NDV was evaluated for determination of the humoral response. Briefly, one apparently healthy bird from each treatment replicate (group size of n=06) was randomly selected, wing-tagged and blood samples were collected from jugular vein on d-21 and d-35 (for HI). Sera were separated through centrifugation at 2,000 x g for 10 min and were stored at -20°C till analysis. The immune response to NDV was measured using micro-titer hemagglutination inhibition (Darabighane *et al.,* 2012).

Cell-Mediated Immunity

Cell mediated immune response was evaluated in terms of the cutaneous hypersensitivity test created by Phytohemagglutinin-P (PHA-P, Sigma-Aldrich, USA) injection as reported previously by Corrier (1990) with slight modification. Briefly, one apparently healthy bird per replicate (n=06 per group) was randomly selected on d-17

and wing-tagged. The PHA-P solution (prepared in a sterile phosphate buffered saline, PBS) was intradermally injected in between the third and fourth digits of the right foot at the rate of 100 µg/100 µL/chicken). The left foot served as control and was injected with 100 µL of PBS. The net increase in thickness of the PHA-P injection site was assessed with micrometer on 24, 48 and 72 h post-injection. Foot web index (cell-mediated response) to PHA-P was evaluated by deducting the thickness of left foot from the thickness of right foot.

Sampling Protocol

One chicken from each replicate (n=6/group) was randomly selected, weighed and killed humanely by intravenous administration of overdosed barbiturates on d-21 and d-35 and samples were collected for the assessment of following parameters.

Evisceration of Lymphoid Organs

Liver, thymus, spleen and bursa of Fabricius were isolated from the carcass and weighed. The representative samples of small intestines were subsequently transferred to laboratory in labeled specimen bottles containing 10% neutral buffered formalin for subsequent histological procedure.

Light Microscopy of Small Intestine

Segments of approximately 2 cm length were excised from duodenum (10 cm distal to the duodeno - gizzard junction), jejunum (5 cm proximal to the Meckel's diverticulum) and ileum (5 cm anterior to the ileo-cecal junction) and processed using paraffin embedding technique, sectioned (5 µm) using a microtome (AMOS Scientific AEM-450, Austria) and stained with Hematoxylin and Eosin (Sikandar *et al.,* 2017) stain.

Total three sections per intestinal sample were collected (one section after every 10 sections). Five well-orientated villi per section having intact lamina propria were selected randomly for all observations. Thus an average of 15 values per sample was estimated. Conclusively, the mean values from six birds were expressed as mean values for one treatment group. Slides were examined under a microscope (Olympus CX31, Olympus USA) at 4x magnification that were operated with digitalized live image analysis program (Olympus DP20, Olympus USA). The villus height (VH) and villus height to crypt depth ratio (VH: CD) were measured for histomorphometry as per our previous report (Sikandar *et al.,* 2017).

Cecal Salmonella Quantification

Salmonella contained in the cecal contents were quantified as described by Park and Kim, (2014) with minor alterations. Briefly ceca were obtained aseptically from already mentioned eviscerated birds (n=6 per treatment) and promptly transported in sterilized bottles to Microbiology section, UDL, UVAS, Lahore. One gram digesta per cecum was taken and mixed in sterile normal saline (NS, Nacl 0.9% w/v) and 2 mL sample of the processed ceca was added to peptone water and incubated overnight at 37ºC. A 10-fold serial dilution (1:9, w/v) was made, out of which 50 µL of peptone water (collected from the 7^{th} dilution i.e., 10^7) was poured on SS agar (culture medium). Colony forming units (CFUs) were counted with the help of the colony counter in triplicate and the mean data were expressed as $\log_{10}$ cfu per gram.

Statistics

The normal distribution of the data was confirmed using Kolmogorov-Smirnov test and data were presented as mean ± SEM. The data were analyzed by one-way ANOVA using SPSS (for windows version 20, Chicago, IL, USA) software. Statistical difference among treatment means was determined through Duncan's multiple range test. In all statistical analyses $P<0.05$ was considered significant.

Results

Growth Performance

All the infected groups achieved higher ($P<0.05$) FCR during starter phase compared to the non – infected negative control group as mentioned in Table 2. The body weight, feed intake and weight gain were better ($P<0.05$) in NC group as compared to infected groups. Compared to other groups, the NC-S group exhibited lower ($P<0.05$) growth performance during grower phase.

Immune Responses

Antibody titer against NDV was noted to be higher ($P<0.05$) in SB-S group compared to negative control, NC-S and PC-S groups on d-35 as presented in Table 3. Post-PHA-P cell-mediated immune response remained lower ($P<0.05$) in NC-S compared to other groups at 24 h. The response of SB-S against PHA-P was recorded higher ($P<0.05$) compared to negative control, NC-S and PC-S groups on 48 h as mentioned in Table 4.

Relative Organs Weight

Table 5 shows that on d-21, thymus and bursa weighed lower ($P<0.05$) in NC-S group. On d-35, thymus gained more ($P<0.05$) weight in SB-S group compared to other groups. The weight of bursa decreased ($P<0.05$) in NC-S compared to negative control and SB-S groups.

Gut Mucosal Histomorphology

The villus height, villus width, villus surface area and VH:

Table 1: Control diet composition and calculated analysis

Ingredients (g/kg)	Starter phase	Grower phase
Corn	401.5	575.7
Rice broken	150	---
Soy meal	115.4	96
Sunflower meal	120	130
Canola meal	90	50
Rapeseed meal	50	76
Rice polish	---	40
Guar meal	10	---
Wheat bran	13.4	---
Molasses	20	---
Soda bicarbonate	.3	.65
Sodium chloride	2.1	2.1
Di-calcium Phosphate	17.3	19.6
Vit-Mineral Premix*	10	10
Nutrient Composition		
Calculated ME (kcal.kg)	2750	2850
CP	196	185
DM	870	880
Crude fiber	12.6	18
Crude fat	21.6	23.5
Total ash	57.7	54

Note: *Vitamin mineral premix (each kg contained): ascorbic acid, 26,000 IU ; retinol, 200,000 IU; cholecalciferol, 80,000 IU; tocopherol, 1,072 IU; thiamine, 11,666 IU ; pyridoxine, 33,333 IU; menadione, 11,333 IU; riboflavin, 54,000 IU; niacin, 5,36,000 IU; folic acid, 13,600 IU; methylcobalamin, 223 IU; biotin, 1,340 IU; Ca, 195 g; K, 70 g; Na, 18 g; Mg, 6 g; Fe, 2,000 mg; Zn, 1,200 mg; Mn, 1,200 mg; Cu, 400 mg; I, 40 mg , Co, 20 mg and Se, 8 mg

Table 2: Effect of Sodium butyrate and Enrofloxacin on performance of *Salmonella*- challenged broilers

Age at week	Parameters	Treatments				
		NC	NC-S	PC-S	SB-S	P-value
1st	Weekly weight gain(g)	94.33 ±2.60^{a}	68.00 ±2.65^{b}	72.00 ±2.52^{b}	68.00 ±2.03^{b}	0.000
	FCR-1	1.36 ±0.03^{b}	1.84 ±0.08^{a}	1.66 ±0.07^{a}	1.85 ±0.06^{a}	0.002
2nd	Weekly weight gain(g)	241.00 ±2.00^{a}	51.00 ±0.58^{c}	96.33 ±5.78^{b}	96.67 ±4.54^{b}	0.000
	FCR-2	1.29 ±0.02^{c}	4.76 ±0.04^{a}	3.07 ±0.20^{b}	2.92 ±0.26^{b}	0.000
3rd	Weekly weight gain(g)	272.00 ±6.66^{a}	71.00 ±7.55^{c}	220.33 ±4.91^{b}	216.33 ±5.78^{b}	0.000
	FCR-3	2.07 ±0.07^{b}	5.05 ±0.34^{a}	2.25 ±0.03^{b}	2.31 ±0.06^{b}	0.000
4st	Weekly weight gain(g)	314.67 ±7.51^{b}	156.00 ±17.09^{c}	494.67 ±20.85^{a}	520.33 ±19.63^{a}	0.000
	FCR-4	2.45 ±0.06^{b}	3.93 ±0.41^{a}	1.51 ±0.09^{c}	1.46 ±0.06^{c}	0.000
5th	Weekly weight gain(g)	461.66 ±60.92^{a}	219.67 ±20.41^{b}	517.67 ±26.44^{a}	526.00 ±18.52^{a}	0.001
	FCR-5	2.64 ±0.35^{b}	4.28 ±0.38^{a}	2.14 ±0.13^{b}	2.27 ±0.13^{b}	0.002

Note: Means within a row marked with different superscripts were significantly different (P<0.05). Values represent Mean ± SEM. Group NC= non-infected, non-medicated; NC-S= *S. gallinarum*-challenged, non-medicated; PC-S= *S. gallinarum*-challenged+50ppm enrofloxacin SB-S=*S. gallinarum*-challenged+ 1.0 g/kg sodium butyrate

CD ratio decreased (P<0.05) and the crypt depth increased in all the segments of small intestine in NC-S group. Specific histomorphological attributes on d-21 and d-35 in various groups were observed as reported in in Table 6.

Table 3: Effect of Sodium butyrate and Enrofloxacin on humoral immune response in *Salmonella*-challenged broilers

Parameter	Treatments				
	NC	NC-S	PC-S	SB-S	P-value
Titer against NDV on d-21	2.16± 0.07	1.84± 0.13	1.91± 0.12	1.84± 0.13	0.080
Titer against NDV on d-35	2.19± 0.14^{bc}	1.93± 0.07^{c}	2.05± 0.05^{bc}	2.21± 0.07^{ab}	0.003

Note: Means within a row marked with different superscripts were significantly different (P<0.05). Values represent Mean ± SEM. Group NC= non-infected, non-medicated; NC-S= *S. gallinarum*-challenged, non-medicated; PC-S= *S. gallinarum*-challenged+50ppm enrofloxacin and SB-S=*S. gallinarum*-challenged+ 1.0 g/kg sodium butyrate

Table 4: Effect of Sodium butyrate and Enrofloxacin on cell mediated immune response against PHA-P in *Salmonella*-challenged broilers

Time interval post injection	Skin thickness (mm) after PHA-P injection in various groups				
	NC	NC-S	PC-S	SB-S	P-value
24 hr	0.535 ±0.04^{a}	0.383 ±0.01^{b}	0.528 ±0.03^{a}	0.555 ±0.03^{a}	0.002
48hr	0.362 ±0.02^{ab}	0.295 ±0.02^{cd}	0.343 ±0.01^{bc}	0.408± 0.03^{a}	0.009
72hr	0.317 ±0.02	0.275 ±0.02	0.313 ±0.02	0.352 ±0.02	0.091

Note: Means within a row marked with different superscripts were significantly different (P<0.05). Values represent Mean ± SEM. Group NC= non-infected, non-medicated; NC-S= *S. gallinarum*-challenged, non-medicated; PC-S=*S. gallinarum*-challenged+50ppm enrofloxacin SB-S=*S. gallinarum*-challenged+ 1.0 g/kg sodium butyrate

Table 5: Effect of Sodium butyrate and Enrofloxacin on the relative organs weight in *Salmonella*-challenged broilers

Treatments	Relative organs weight[1]							
	At day-21				At day-35			
	Liver	Spleen	Thymus	Bursal	Liver	Spleen	Thymus	Bursal
NC	2.18 ±0.15	0.20 ±0.02	0.89 ±0.02^{a}	0.22 ±0.01^{ab}	2.52 ±0.05	0.11 ±0.01	0.39 ±0.01^{c}	0.10 ±0.02^{a}
NC-S	2.35 ±0.08	0.26 ±0.05	0.52 ±0.07^{b}	0.17 ±0.02^{b}	2.64 ±0.13	0.12 ±0.01	0.33 ±0.02^{d}	0.06 ±0.01^{b}
PC-S	2.36 ±0.10	0.25 ±0.02	0.99 ±0.04^{a}	0.24 ±0.00^{a}	2.61 ±0.07	0.12 ±0.01	0.53 ±0.03^{b}	0.09 ±0.01^{ab}
SB-S	2.29 ±0.09	0.21 ±0.02	0.93 ±0.03^{a}	0.27 ±0.02^{a}	2.52 ±0.02	0.13 ±0.01	0.61 ±0.01^{a}	0.11 ±0.01^{a}
P-value	0.629	0.522	0.000	0.000	0.628	0.655	0.000	0.042

Note: Means within a column marked with different superscripts were significantly different (P<0.05). Values represent Mean ± SEM. [1]Relative organs weight= organ weight/body weight x 100. Group NC= non-infected, non-medicated; NC-S= *S. gallinarum*-challenged, non-medicated; PC-S=*S. gallinarum*-challenged+50ppm enrofloxacin and SB-S=*S. gallinarum*-challenged+ 1.0 g/kg sodium butyrate

D-21: The villus height and villus: crypt ratio of duodenum decreased (P<0.05) in NC-S compared to all other treatment groups. Villus surface area in jejunum and ileum increased (P<0.05) in SB-S group compared to NC-S groups. The villus height and VH: CD ratio of all the segments were recorded comparable (P<0.05) in PC-S and SB-S groups.

Table 6: Effect of Sodium butyrate and Enrofloxacin on small intestine morphology in *Salmonella*-challenged broilers

Intestinal segments	Parameters	Treatments								
		NC		NC-S		PC-S		SB-S		P-value
		D-21	D-35	D-21	D-35	D-21	D-35	D-21	D-35	D-21, 35
Duodenum	Villus height (µm)	951.10 ±14.25^{a}	1057.20 ±23.75^{b}	549.09 ±31.49^{c}	556.98 ±30.56^{c}	872.40 ±17.35^{b}	1159.57 ±45.30^{ab}	897.52 ±24.77^{ab}	1266.32 ±112.16^{a}	0.000
	VH:CD	9.06 ±0.74^{a}	6.30 ± 0.28^{b}	4.08 ±0.23^{c}	2.94 ± 0.35^{c}	7.21 ±0.31^{b}	7.08 ±0.35^{ab}	8.09 ±0.78^{ab}	8.04 ±0.38^{a}	0.000
Jejunum	Villus height (µm)	874.19 ±17.93^{a}	917.20 ±9.90^{a}	599.75 ±19.09^{c}	582.75 ±26.44^{b}	842.18 ±39.66^{ab}	901.01 ±27.49^{a}	775.10 ±27.86^{b}	888.90 ±16.93^{a}	0.000
	VH:CD	7.21 ±0.35^{a}	6.75 ±0.19^{a}	4.06 ±0.26^{b}	3.47 ±0.20^{b}	6.48 ±0.54^{a}	7.19 ±0.47^{a}	7.38 ±0.49^{a}	6.71 ±0.53^{a}	0.000
Ileum	Villus height (µm)	766.10 ±29.48^{a}	885.03 ±15.20^{a}	492.66 ±31.82^{b}	521.22 ± 18.18^{b}	713.31 ±33.38^{a}	892.33± 11.38^{a}	750.96 ±26.54^{a}	896.23 ±12.25^{a}	0.000
	VH:CD	5.96 ±0.13^{ab}	6.23 ±0.27^{b}	3.68 ±0.11^{c}	3.59 ±0.08^{c}	5.59 ±0.42^{b}	6.42 ±0.26^{b}	5.93 ±0.20^{ab}	7.21 ±0.26^{a}	0.000

Note: Means within a row marked with different superscripts were significantly different (P<0.05). Group NC= non-infected, non-medicated; NC-S= *S. gallinarum*-challenged, non-medicated; PC-S= *S. gallinarum*-challenged+50ppm enrofloxacin and SB-S=*S. gallinarum*- challenged+ 1.0 g/kg sodium butyrate

Table 7: Effect of Sodium butyrate and Enrofloxacin on intestinal *Salmonella* quantification in *Salmonella*-challenged broilers

Treatments	Salmonella, $\log_{10}$ cfu/g of cecal material	
	D-21	D-35
NC	0.67±0.21^{c}	0.83±0.31^{c}
NC-S	5.5±0.43^{a}	6.17±0.48^{a}
PC-S	2.5±0.22^{b}	2.67±0.21^{b}
SB-S	2.67±0.21^{b}	3.17±0.17^{b}
P-value	0.000	0.000

Note: Means within a row marked with different superscripts were significantly different (P<0.05). Values represent Mean ± SEM. Group NC= non-infected, non-medicated; NC-S= *S. gallinarum*-challenged, non-medicated; PC-S=*S.* gallinarum-challenged+50ppm enrofloxacin and SB-S=*S. gallinarum*-challenged+ 1.0 g/kg sodium butyrate

D-35: Compared to NC and NC-S groups, the villus height, villus surface area and VH: CD of duodenum increased (P<0.05) in SB-S group. The histomorphological parameters of jejunum and ileum decreased (P<0.05) in NC-S compared to other groups. The villus surface area and VH:CD ratio of ileum increased (P<0.05) in sodium butyrate supplemented group compared to other groups.

Cecal Salmonella Quantification

Salmonella organisms were in greater number (P<0.05) in the cecal contents of NC-S group mentioned in Table 7. The effect of sodium butyrate supply was comparable to that of antibiotics on cecal Salmonellosis on d-21 and d-35.

Mortality

Highest (P<0.05) mortality was recorded in NC-S (46.67±8.33^{a}) compared to NC (0.00±0.00^{b}), PC-S (6.67±4.41^{b}) and SB-S (10.00±2.89^{b}) and mortality in the supplemented groups was statistically similar to NC group.

Discussion

The application of antibiotics in animal feed facing a ban since it leads to presence of residues in the muscles and subsequently to antibiotic resistance (Kabploy *et al.,* 2016). Therefore, currently it is a trend in the poultry sector to use non antibiotic feed additives for growth promotion. Among others, the sodium butyrate (SB) has been used effectively in broiler production (Fernandez-Rubio *et al.,* 2009). Only limited studies are available in literature emphasizing its protective effect, consequently the present study was designed with the objective to fill this gap and to examine the effect of SB on broiler growth performance during infection with *S. gallinarum.*

In the current study, SB supplementation in the feed improved growth performance similar to the antibiotic treated chickens, suggesting the potential role as alternative to AGP. The challenged birds showed negative growth performance in contrast with birds of the non-infected NC group. This stunted growth may be due to underdeveloped intestinal function as presented in Table 6. The FCR was lower (P<0.05) in the supplemented groups from the second week onwards, which indicates a potential underlying protective effect of the supplemented products against the challenged pathogen (Abudabos *et al.,* 2016), which resulted in better feed efficiency. Such improved growth may be associated with acidic environment created by SB in the gut or improved villus height in the small intestine (Sikandar *et al.,* 2017). The NC-S (challenged group) had the lowest villus height, which is associated with higher FCR. Feed intake in the challenged birds (NC-S) dropped (P<0.05) when compared to non-infected control (NC),

which may also be the reason of lower weight gain. This reduction in the feed intake in combination with malabsorption could add to inadequate immune responses in the infected chickens (Awad *et al.,* 2014).

Sodium butyrate has the potential to minimize the enteric pathogens load (Fernandez-Rubio *et al.,* 2009), however, the exact mechanism of action is still not fully clarified. Several studies have been executed to categorize the role of this product, one postulation being its immunomodulatory role (Darabighane *et al.,* 2012). Concerning immune system, we noted higher geometric mean HI titers in SB group compared to NC-S on d- 35. Sodium butyrate maintained gut health and balanced the gut normal microflora (Sultan *et al.,* 2015), which in turn standardized the immune-functions. The higher cell-mediated immune response post-PHA-P in SB-S group showed in Table 4 indicated the established immunomodulatory properties of organic acid in *Salmonella* challenged birds. It has already been found that the short chain fatty acids support the regulation of Th1, Th17 and IL-10 regulatory T cells, which maintain the immune system physiology (Park *et al.,* 2015). Sunkara *et al.* (2011) reported that butyrate triggered the immune system activity by inducing the production of host defense peptides (HDPs) without provoking pro-inflammatory responses. The HDPs have immunomodulatory properties and promote chemotaxis and activate various immune related cells (Hancock *et al.,* 2006). It can be concluded that SB has a potential stimulatory effect on immune system in the event of an acute infection and the stimulated humoral and cell mediated immune responses may cause reduction of *Salmonella*-induced stress in the infected chickens.

The mechanism responsible for *Salmonella*-induced lower weight of the visceral organs is not clear but it is assumed that this may be attributed to migration of cells from the organs to the overall systemic circulation or to necrosis. The relative weight of thymus was significantly higher in SB-S compared to other groups, which might support the compensatory mechanisms through which additional lymphocytes are generated based on requirement during infection (Darabighane *et al.,* 2012). Relative weights of liver and spleen did not differ among groups, reinforcing the findings of Saleem *et al.* (2016) pertaining to the treatment of *S. pullorum*-challanged broilers with acetic acid.

The gut mucosa is the primary site, which is exposed to in-feed antigens. The antigens may cause lesions in the epithelial layer of the mucosa which result in sloughing off the lining epithelium (Sikandar *et al.,* 2012). We found increases VH, VSA and VH: CD ratio of intestine in SB treated group, which revealed active defensive measures in the gut. *Salmonella* is susceptible to low pH, therefore a likely explanation may be the availability of excessive acidic environment created by the sodium butyrate in the gut. Greater villus height led to improved villus function, which in turn exhibited better growth performance as shown in Table 2 probably by increasing enzyme actions and absorptive surface area for the available nutrients in the supplemented groups. In the current study, deeper crypts were reported in the NC-S group, which indicated rapid tissue turnover for renewal of the mucosa (Awad *et al.,* 2014) in order to maintain the surface area for absorption. This enhanced proliferation of immature cells may result in the utilization of that energy which would be required for growth. The most affected area in the gut mucosa was the lining epithelium in the NC-S group. Epithelium covering most of the villi was necrosed, sloughed off and the villi were atrophied, ulcerated and looked denuded. These lesions caused a reduction of villus surface area for the uptake of available nutrients in that group. The size of villi was smaller in NC-S, which may be caused by pathogenic effect of the *Salmonella*.

Reduced number of *Salmonella* organisms was noted in cecal area in the supplemented groups (Table 7). *Salmonella* colonization was higher ($P<0.05$) in NC-S compared to other groups. Our findings are in line with the results of Saleem *et al.* (2016), who reported higher *Salmonella* colonization in the ceca in the challenged birds. Micro-capsulated sodium butyrate minimized the number of bacterial pathogens by reducing the pH of intestine (Fernandez-Rubio *et al.,* 2009). This low pH affects the DNA synthesis and arrests the cell proliferation. According to Van Immerseel *et al.* (2006) butyric acid may downregulate the expression of all those genes which are participating in the invasion of *Salmonella*. Butyrate in the intestine can influence the *Salmonella* by several ways i.e., it can change the expression of genes (SPI-1), which are responsible for pathogenicity of *Salmonella*. These genes in cluster lead the *Salmonella* to invade the epithelial cells. SPI-1 genes are regulated by a protein known as HilA (Durant *et al.,* 2000). The butyrate causes the downregulation of HilA protein and the SPI-1 gene. Sodium butyrate contributes to the release of host defense peptides comprising of a huge collection of antimicrobial components. These peptides minimize the bacterial load in the ceca (Sunkara *et al.,* 2011) and reduce the chances of subsequent mortality.

No mortality was recorded in the negative control group throughout the trial. Mortality started in the second week of infection and lasted until the end of trial in NC-S. Saleem *et al.* (2016) reported that Salmonellosis caused high mortality during the young age. Mortality peaked during 3rd week of the current study.

Conclusion

Supplementation of sodium butyrate diminished the stress associated with *S. gallinarum* infection and improved growth performance, villus microarchitecture and immunity in broilers. It can be concluded from the current study that SB may be used as alternative to antibiotics in poultry industry.

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