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Comparative evaluation of growth performance, gut morphology, micro-flora, haematology and immune response of broilers fed with Sodium butyrate and *Saccharomycescerevisiae* supplemented diets

Abstract

The effects of dietary sodium butyrate (SB) and *Saccharomyces cerevisiae* (SC) on some bodily parameters of chickens were evaluated. One hundred and twentyday-old broilers (CHI, Ajanla Farms,Ibadan, Nigeria) were randomly divided into four treatments A, B, C and D, consisting three replicate pens of 10 chickens. They were fed as follows: A-diet withoutsupplement (control); B-(200mg/kg SB); C-(200mg/kg SB+1.0g/kg SC), and D-(1.0g/kg SC). Growth performance, organ weights, carcass quality, and lipid profile were determined. Intestinal villus height(VH), villus width (VW), villus surface area (VSA), crypt depth (CD), villus height to crypts depth ratio (VH: CD) were assessed. Some selected bacteria population, haematology, serum biochemistry and humoral immune responses against NDV and SRBCs antigens were evaluated. Supplementation increased growth performance, bursa and thymus weights. There was significantly ($p<0.05$) higher dressing percentage and lower cholesterol in groups B and C. High density lipoprotein and triglyceride were reduced significantly in B. Triglyceride and abdominal fat were significantly higher inD. The CD was higher ($p<0.05$) in duodenum of the control. In supplemented groups, VSA and VH: CD in the threeintestinal segments, VH in duodenum and jejunum were enhanced. Supplementation lowered gut microflora, increased WBC, lymphocyte counts and immune performance. DietC stimulated more positive influence on productive parameters and could be an alternative growth promoter in broilers.

Keywords: broilers, dietary supplementation, growth performance, gut health, humoral immunity, lipid profile

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Introduction

Since the discovery of antibiotics, they have been used at therapeutic doses for the treatment of diseases and sub-therapeutic doses as growth promoters in animal feeds.[1] In the poultry industry, they have been considered as essential additive/supplements for improved growth and in maintaining gut ecosystem balance for more than 50 years.[2] However, due to increasing frequency of resistance in livestock[3] and dwindling efficacy in humans,[4] the EU in 2006 imposed a complete ban on the use of antibiotics in poultry feeds.[5] Following this ban, many researchers focused attention on finding alternative products. These alternatives should not only have the advantageous properties as the antimicrobials but must be safe to man, animals and the environment.

Sodium butyrate (SB): an organic acid is being advocated as one of the possible alternative to antibiotics.[1] It modulates the growth of symbiotic intestinal microflora.[7] Sodium butyrate is a selective bactericidal agent due to its ability to lower pH in crop, gizzard and upper part of chicken intestine.[8] It therefore improves gut health through the control of harmful bacteria such as *Salmonellaspp*., *Escherichiacoli* and *Campylobacterjejuni*.[8,9] Sodium butyrate is available either in powder or in microencapsulated (coated with fatty acid matrix) form. Variations in result of some trials with the compound were attributed to differences in presentation.[10,11] Improvement in broiler performance was however reported when it was combined with other additives.[12] Although the effects of SB dietary inclusion on various bodily parameters of chickens are well documented, there is paucity of information on its effects on humoral immunity and haematological profile of broilers.[1,11] Similarly, studies on its effects on growth performance of chickens, variable results have been reported.[8,10,13] *Saccharomycescerevisiae* (SC); a yeast, is a natural ingredient in human diets such as bread.[14] It is safe, acceptable by man and environmentally friendly.[15] It enhances growth of pigs[15] and a probiotic of choice for broiler production in the study area.[16] Besides the inconsistent results and inconclusive mechanisms, the challenges of using natural growth promoter in animal feed may also include side effects, regulatory obstacles and cost. A comprehensive study is, thus, needed to assess the effects of SB on various production indices of broilers under tropical environment. The objectives of this study were, therefore, to

(i) Evaluate the effects of SB either alone or in combination with a probiotic on the growth performance and carcass quality of broilers.

(ii) Determine effects of the additive on gut micro-architecture of broilers.

(iii) Determine if the inclusion would lead to reduced faecal shedding of some selected bacteria.

(iv) Determine if it would improve immunity or lead to some clinical concerns such as leukogram abnormalities in broilers.

Materials and methods

Experimental chicks, husbandry and diets

The seven week study was carried out using 120 day-old unsexed commercial broiler chicks (CHI, Ajanla Farms Ltd, Ibadan, Oyo State, Nigeria). The SB (Bodybio) and SC (Golden Speed) were sourced from Bodybio Incorporated, Millville New Jersey, USA and Golden Speed, Xinjiang Mauri Food Co Ltd, Yili, Xinjiang, PR China, respectively. Two experimental diets (starter and finisher) were formulated to meet the National Research Council (NRC) nutrient requirements for poultry.[17] They were formulted using basal ingredients and subjected to proximate analysis to determine their chemical composition (Table 1). The microencapsulated SB and the SC were weighed using an electronic balance (Diamond® Taiwan) and thoroughly mixed with the formulated diets (FD). This was with a view to attaining uniform dispersion of each ingredient in the feed; so that all "pecks" are uniform.

Table 1 Gross and proximate composition of broiler starter and finisher diets supplemented with sodium butyrate and *Saccharomycescerevisiae* (all ingredients expressed on as-fed basis)

Items	Starter diet	Finisher diet
Ingredient (%)		
Maize (yellow)	44.29	50.12
Guinea corn	11.6	7
Soya bean meal	15.54	9.6
Wheat offal	10	15
Fish meal	2.5	-
Palm kernel cake	5	8.6
Bone meal	2.5	4
Lime stone	5	3
Blood meal	2.34	1
Sodium chloride	0.33	0.21
Lysine	0.3	0.35
Methionine	0.1	0.12
Vitamin/mineral premix	0.5	1
Total	100	100
¥Proximate composition		
Calculated ME (kcal/kg)	3150	3180
CP (%)	22.5	19.5
DM (%)	87	88
Crude fiber (%)	6.05	6.35
Crude fat (%)	2.16	2.35
Total ash (%)	5.77	5.44

Mineral /vitamin premix provided the following per kg of diets vitamin; A, 10000 IU; vitamin D3, 2000 IU; vitamin E, 5 IU; vitamin K, 2mg; Riboflavin, 4.20mg; vitamin B12, 0.01mg; pantothenic acid 5mg; nicotinic acid, 20mg; folic acid, 0.5mg; choline, 3mg; Mg, 5mg; Fe, 20mg; Cu, 10mg; Zn, 50mg; Co, 125mg, iodine, 0.5mg, ¥While the chemical composition of the diets was determined according to AOAC (1990); metabolizable energy (ME) was calculated values

The chicks on arrival were weighed and randomly assigned into four groups A, B, C and D. Each treatment was replicated three times with ten birds in a replicate. The groups were fed as follows: A-the FD with no supplement (control); B-FD supplemented with SB at 200mg/kg; C-FD supplemented with SB and SC at 200mg/kg and 1.0 g/kg and D-FD supplemented withonly SC at 1.0g/kg. The birds in each group were offered their group specific diet without antibiotics in starter (1 to 21 days) and finisher (22 to 49 days) phases. Diets and clean drinking water were supplied *ad libitum* throughout the study period. The stocking density was approximately 4 birds /m^2.

Animal care

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.[18]

Determination of performance

The average body weight (ABW), weight gain (WG), feed intake (FI) and feed conversion ratio (FCR) were recorded weekly and used to assess the growth performance of the broilers.[19] The health status of the birds was recorded daily by visually observing possible clinical signs, morbidities and mortalities. The weekly ABW of the broilers and FI were determined by subtracting respective bird initial weights (kg) or feed intake (W1) from the final bird weights or feed intake (W2) and divided by number of weeks (n) (W2–W1/n). Feed conversion ratio was determined on as-fed basis by dividing the feed consumed in a week in kg by live weight gained (kg) within the same period. Daily weight gain, feed intake and FCR were determined by dividing their respective weekly figures by seven.

Relative weight of lymphoid organs

At 49 days of age, 3 birds from each group (one per replicate) were randomly selected, weighed and humanely sacrificed by cervical dislocation. Their gastrointestinal tracts were excised; spleen, thymus, bursa of Fabricius, caeca tonsils (immune organs), liver and gizzard, were removed and their relative weights determined as a percentage of the life weight.

Gut morphology

Portions of the three segments of their small intestine: duodenum (from gizzard outlet to the end of the pancreatic loop), jejunum (from the pancreatic loop to Meckel's diverticulum), and ileum (from Meckel's diverticulum to the caecal junction) were also isolated and preserved in 10% neutral buffered formalin and used to determine gut morphology.[20]

Histological procedures

The segments were promptly fixed in Bouins fluid for 48 hrs. The fixed tissues were subsequently dehydrated in increasing concentrations of ethanol, cleared in xylene at one hr. interval. Following infiltration and embedding with paraffin, 5µm thick sections of each intestinal segment were obtained using a rotatry microtome. The thick sections were stained routinely with hematoxylin and eosin for light microscopy. Photomicrographs were captured using a Moticam® digital camera (Motic China Group Co., Ltd., Xiamen, China).Villus height, VH; villus width, VW; villus surface area, VSA; crypt depth, CD; villus height to crypts depth ratio, "VH: C D" were the indices used to assess gut morphology.[1,19]

Determination of histomorphometry

The VHs, VWs and CDs of the intestinal segments were determined using a standardized ocular micrometer. Their VH and CD were

measured randomly by choosing several profiles that were straight or nearly straight in outline per animal. While VD was determined by measuring the basal diameter of each villus, VSA was determined following the methods of Kisielinski et al.[20]

Gut microflora

The effects of the test diets on the gut microflora of the birds were determined following the methods of Zou*et al*. (2010) but with the following modifications. On days 21 and 49, three birds per group were randomly isolated and allowed to defecate on a clean receptacle. 2g of the freshly voided faces was collected with swab sticks into a labeled sterile sample bottles and used for bacterial analyses.

Microbial populations were determined by serial dilution (10^{-1} to 10^{-10}) in anaerobic diluent before inoculation onto petri dishes of sterile agar as described by Zou et al.[21] Anaerobic bacteria was incubated at 37 ºC for 36 hr. Lactic acid bacteria was incubated in an anaerobic condition at 37 ºC for 48 hrs. Coliform and *Clostridiumperfringens* were incubated anaerobically at 37 ºC for 24 h.[22] The selected bacterial were then enumerated following standard methods.[23]

Determination of haematology

The packed cell volume (PCV) of the birds was determined by microhaematocrit method;[24] and the haemoglobin concentration was determined by the cynomethemoglobin method (Jain, 1986) using SP6-500UV spectrophotometer (PYE UNICAM, England). Similarly, their RBC count and TWBC were enumerated using the Leishman stained blood smear and the different cells of the leucocytes series counted by the longitudinal counting method.

Serum biochemical techniques

Total serum protein (TP) was determined in each sample following the Biuret method[25] using the Randox Total Protein Test kits (Randox Laboratories, Leeds, UK). Serum albumin concentration was determined following the bromocresol green method,[26] using the Randox Albumin Test Kit (Randox Laboratories, Leeds, UK). The serum globulin fraction was calculated by subtracting the value of the albumin fraction from the total serum protein.[25] The lipid profile of the experimental birds including total cholesterol, triglyceride, high density lipoprotein (HDLP), low density lipoprotein (LDLP) and very low density lipoprotein (VLDL) were determined. They were evaluated using commercially available test kits manufactured by Biosystem, S.A. Costa Brava 30 Barcelona Spain. The serum total cholesterol determination was done based on the enzymatic colorimetric method[27] and was done using the Biosystem total cholesterol working reagent and assayed using a CHEM5V3 semi-automated blood analyzer (Erba Diagnostics, Mannheim, Germany). The serum triglyceride concentration was determined based on the glycerol-phosphate oxidase method.[28] This was done using the Biosystem triglyceride working reagent and assayed with a CHEM5V3 semi-automated haemo analyzer (Erba Diagnostics, Mannhein, Germany). The serum high density lipoprotein concentration was determined by the dextran sulphate magnesium (II) precipitation method. This was done using the Biosystem HDL-C precipitation reagent and the supernatant assayed after this using CHEM5V3 semi-automated haemo analyzer (Erba Diagnostics, Mannhein, Germany). The serum low density lipoprotein was calculated using Friedewald's formula.[29,30] Very low density lipoprotein of the broilers was determined by dividing the value of triglyceride concentration by Buccolo & David.[28]

Determination of humoral immunity

The antibody titer or antibody production level is attributed to humoral immune response in poultry. Humoral immune responses against Newcastle disease virus (NDV) and sheep red blood cells (SRBCs) antigens were evaluated through serological titration as defined previously.[28,31] Briefly, all the chicks were vaccinated with Newcastle Disease vaccine (Kaniket Disease Vaccine, Lentogenic 'F' Strain P, Biomed Private Ltd, Ghaziabad- 201009 U-R, India) on day 2 and day 9 via the ocular route and boosted on day 16 and day 23 via drinking water. Rectal temperatures of the birds were recorded a day before and six days following immunization. In case of SRBCs, 2 birds per treatment replicate (6 per group) were randomly selected, identified and injected intravenously with 1ml of 5% SRBCs antigen (sheep blood collected in Alsevier's solution, washed thrice and suspended in phosphate buffer saline). Booster dose was administered on day 21. Blood samples were collected on days 0, 7, 14 and 42, respectively. Sera were separated (2,000×g for 10 min) and stored at –20°C till use. The antibody responses to NDV and SRBCs were measured using micro titer haemagglutination inhibition (HI) and hemagglutination (HA) assays, respectively as described by Kings.[31]

Statistical analysis

The replicate pens were the experimental unit for performance and excreta data. Results on immune response were converted to $\log_2$ of the antibody titre.[32] Statistical analyses were performed using the SPSS program (SPSS Inc., Chicago, IL). The normality of data distribution was checked using the Kolmogorov-Smirnov test. One-way ANOVA was performed to examine differences among the groups. The significance of mean differences between groups was determined by Duncan multiple range tests. Level of significance was taken as $P<0.05$.

Results

Growth performance

In general, birds were in a healthy condition throughout the study period. A relatively low mortality rate of 5.21% was recorded: three from group A and one each from groups B and D, respectively (Table 2). The additives did not have significant effect on FCR ($P = 0.1108$); they however, improved growth performance and showed significant ($p< 0.05$) difference in feed intake ($P = 0.0066$), daily weight gain ($P = 0.0343$) and final body weight ($P = 0.0144$) when compared to the control. Groups C and D consumed more feed and had higher final body weight than B. Supplementation did not have any significant ($p> 0.05$) effect on relative weights of the immune organs of the birds, but values obtained in bursa of Fibricious and thymus tended to favour the groups (Table 2).

Carcass quality and lipid profile

Dressing percentage was significantly ($p<0.05$) higher in groups B and C (Table 3). Birds in C and D laid down significantly higher abdominal fat ($P = 0.0238$) than B and the control. Serum cholesterol and LDL were significantly decreased by the additives ($P = 0.0001$; $P = 0.0000$). Triglyceride and HDL were significantly ($p<0.05$) decreased in group B than others (Table 3). Although there was no treatment effect of the VLDL of the broiler, values were lower in the supplemented groups.

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Table 2 Effects of broiler chickens fed diets supplemented sodium butyrate and *Saccharomycescerevisiae* on growth performance and immune organ weights

Parameters	Treatment groups				
	A	B	C	D	p-Value
Feed intake/day(g)	100.30±3.47[a]	87.12±5.46 [b]	93.96±3.46[ab]	103.87±3.09[a]	0.0066
Daily weight (g)	42.43±3.39[b]	46.77±3.33[ab]	52.49±3.43[ab]	55.39±3.32[a]	0.0343
FCR	2.17±0.21	2.55±0.20	1.84±0.21	1.94±0.21	0.1108
Initial body weight (g)	75.00±0.00	77.00±0.00	76.00±0.00	75.00±0.00	0.4411
Final body weight (kg)	2.47±0.10[a]	2.86±0.12[ab]	3.01±0.17[a]	3.18±0.02[a]	0.0144
Mortality (%)	3 (12.50)	1 (4.14)	0 (0.00)	1 (4.14)	
Relative weight of Immune organs (%)					
Spleen	0.01±0.00	0.01±0.00	0.12±0.00	0.02±0.00	0.6241
Caecal tonsil	0.04±0.00	0.04±0.00	0.03±0.00	0.04±0.00	0.7724
Bursa of Fib	0.09±0.02	0.63±0.03	0.12±0.00	0.15±0.06	0.1444
Thymus	0.13±0.07	0.22±0.04	0.26±0.08	0.18±0.06	0.2468

[a,b]Row means with different superscripts differ significantly at $p < 0.05$

Values presented in mean±standard error of the mean

Table 3 Effects of broiler chicken diets supplemented with sodium butyrate and *Saccharomycescerevisiae* on carcass quality and lipid profile

Parameters	Treatment groups				
	A	B	C	D	p-Value
Dressing (%)	71.35±4.02[b]	75.52±5.00[a]	75.24±5.22[a]	72.72±4.09[b]	0.0312
Drumstick (g)	118.17±8.07[a]	119.00±6.56[a]	108.67±1.64[a]	81.67±2.20[b]	0.0037
*Gizzard	0.02±0.00	0.03±0.01	0.02±0.00	0.02±0.00	0.0854
*Liver	0.24±0.01[b]	0.22±0.02[b]	0.25±0.01[b]	0.33±0.01[a]	0.0321
Abdominal fat (g)	22.04±1.37[b]	20.39±2.34[b]	38.57±2.42[a]	42.28±4.00[a]	0.0238
Cholesterol (mg/dL	146.11±4.23[a]	95.00±4.53[b]	84.22±3.28[b]	122±9.71[a]	0.0001
LDL (mg/dL)	29.78±5.60[a]	9.33±1.01[b]	9.78±1.47[b]	7.89±0.79[b]	0.0000
HDL (mg/dL)	116.22±7.86[ab]	89.11±4.19[c]	91.44±12.80[b]	139.33±3.32[a]	0.0013
VLDL	19.82±1.32	17.54±2.54	17.19±2.00	16.98±2.11	0.0634
Triglyceride (mg/dL)	112.11±7.02[b]	102.44±3.50[c]	139.00±31.05[ab]	178.56±10.05[a]	0.0434

[a,b,c]Row means with different superscripts differ significantly at $p < 0.05$

Values presented in mean±standard error of the mean

*Determined relative to the carcass weight

Gut morphology

Crept depth was significantly higher in the duodenum of control ($P = 0.022$) and reduced in jejunum and ileum of the treated birds (Table 4). Other mucosal histomorphometric indices did not vary statistically ($p > 0.05$) among the groups. However, VSA and VH: CD in the three segments and VH in duodenum and jejunum of the supplemented groups were enhanced (Table 4).

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Table 4 Effects of broiler chickens fed diets supplemented sodium butyrate and *Saccharomycescerevisiae* on small intestine morphology at week 7

Intestinal segment	Parameters	Treatment groups				
		A	B	C	D	p-Value
Duodenum						
	VH (µm)	550.33±33.33	631.67±70.50	575.33±32.20	714.67±67.50	1.819
	VW (µm)	118.33±7.50	104.33±0.90	91.67±6.10	95.67±13.70	1.976
	CD (µm)	169.67±18.49[a]	84.67±4.26[b]	87.00±4.05[b]	96.00±4.51[b]	0.022
	VHCD	4.53±0.56	7.87±0.86	6.16±0.13	7.03±0.61	1.645
	VSA (mm^2)	0.53±0.08	0.67±0.073	0.68±0.06	0.67±0.05	1.003
Jejunum						
	VH (µm)	308.67±76.46	381.33±65.47	418.00±55.50	410.33±40.91	0.663
	VW (µm)	101.67±7.75	104.00±4.36	103.33±6.16	86.00±18.08	0.679
	CD (µm)	105.00±18.20	84.67±11.89	90.00±5.50	104.33±8.40	0.736
	VHCD	3.03±0.37	4.48±0.44	4.58±1.04	4.00±0.13	0.637
	VSA (mm^2)	0.31±0.07	0.39±0.06	0.44±0.09	0.34±0.05	0.669
Ileum						
	VH (µm)	200.33±27.16	167.33±14.72	178.33±17.75	202.67±13.78	0.808
	VW (µm)	116.00±1.15	116.00±6.00	129.67±8.45	109.00±3.00	2.543
	CD (µm)	69.67±4.05	67.00±3.79	64.00±4.16	66.00±4.16	0.428
	VHCD	3.24±0.45	3.43±0.33	3.69±0.13	3.30±0.11	1.055
	VSA (mm^2)	0.20±0.03	0.21±0.03	0.23±0.04	0.22±0.02	0.436

[a.] Row means with same superscript does not differ significantly at $p < 0.05$

Values presented in mean±standard error of the mean

Faecalmicroflora

The faecal populations of *E coli* and *Salmonell*a were decreased ($p < 0.05$) by the dietary supplementation on days 21 and 49 respectively (Table 5). On day 21, although *C. perfringens* and *Lactobacillus* counts did not vary significantly ($p > 0.05$) among the groups, supplementation with SB alone (group B) tended to have reduced their population than SC and the control (Table 5). *Clostridiumperfringens* counts were reduced significantly ($p < 0.05$) in groups B and C than D and the control on day 49 ($P = 0.0373$). Supplementation generally reduced population of the selected bacteria (Table 5).

Table 5 Effects of broiler diets supplemented with sodium butyrate and *Saccharomycescerevisiae* on the counts (cfu/g of faeces) of selected bacteria

Parameters	Treatment groups				
	A	B	C	D	p-Value
Day 21					
E coli (x 10^7)	2.29±1.40[a]	1.03±0.30[b]	1.21±1.37[b]	1.40±09[b]	0.0412
Salmonella spp(x 10^6)	3.30±0.63[a]	1.64±0.08[b]	1.43±0.00[b]	1.60±0.43[b]	0.0371
C. perfringens (x 10^6)	1.47±0.16	1.23±0.30	1.20±0.13	1.43±1.40	0.8412
Lactobacillus (x 10^6)	1.65±0.74	1.36±0.04	1.28±0.00	1.55±0.52	0.3271
Day 49					
E coli (x 10^6)	3.43±2.23[a]	1.32±0.09[b]	1.30±0.89[b]	1.63±35[b]	0.0062
Salmonella spp(x 10^5)	2.87±0.63[a]	1.09±0.43[b]	1.43±0.22[b]	1.69±0.53[b]	0.0484
C. perfringens (x 10^5)	1.86±0.16[a]	1.23±0.30[b]	1.20±0.13[b]	2.00±0.06[a]	0.0373
Lactobacillus (x10^5)	1.54±0.08	1.41±0.01	1.42±0.00	1.59±0.52	0.4167

[a,b]Row means with different superscripts differ significantly at $p < 0.05$

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Haematology and serum biochemistry

White blood cells and lymphocyte counts were significantly ($p<0.05$) increased by the supplementation. Neutrophil to lymphocyte ratio were reduced in the supplemented groups (Table 6). Haemoglobin concentrations of group D and the control were significantly higher than B ($P = 0.0318$). Packed cell volume, RBC and neutrophil counts were not significantly ($p>0.05$) affected by the treatment. While ALT was significantly ($p<0.05$) lower in C, when compared to the control and B, AST was significantly ($p<0.05$) reduced in birds that consumed the supplemented diets (Table 6).

The dietary treatment did not significantly ($p>0.05$) affect total protein, albumin and globulin fraction of the birds. The three liver enzymes evaluated were affected by the supplementation (Table 6). Bilirubin and urea values were lower in the treated groups than the control.

Table 6 Effects of broiler chickens fed diets supplemented sodium butyrate and *Saccharomycescerevisiae* on haematology and serum biochemistry

Parameters	Treatment groups				
	A	B	C	D	p-Value
PCV (%)	29.11±0.68	31.11±1.96	29.56±0.69	28.89±0.35	0.5775
Hb (g/dL)	9.97±0.52^{a}	7.56±0.08^{b}	7.62±0.89^{ab}	9.01±0.26^{ab}	0.0318
RBC x 10^{6}/ µL	3.57±0.34	3.82±0.26	4.03±0.27	3.92±0.20	0.6763
WBC x 10^{3}/µL	11.99±0.78^{c}	16.66±0.78^{b}	19.90±0.78^{a}	19.22±0.78^{ab}	0.0001
Lymph. (%)	73.33±8.04^{b}	86.76±6.03^{a}	84.38±7.01^{a}	83.66±10.41^{a}	0.0121
Neutrophil (%)	26.33± 1.79	23.00±1.79	25.11±1.79	26.44±1.79	0.5066
Neutro lymph	0.36±0.04	0.27±0.09	0.28±0.01	0.32±0.00	0.3412
ALT (µ/L)	14.00 1.08^{a}	13.00±1.34^{a}	8.72±0.06^{b}	10.79±0.037^{ab}	0.0000
AST (µ/L)	76.00±4.13^{a}	54.11±4.32^{b}	56.67±4.13^{b}	58.33±1.61^{b}	0.0010
Protein (g/L)	2.37±0.40	2.44±0.22	2.45±0.06	2.49±0.12	0.4678
Albumin (g/L)	1.97±0.40	1.47±0.01	1.57±0.11	1.37±0.01	0.2763
Glob. (g/dL)	1.45±0.13	1.57±0.14	1.44±0.14	1.55±0.13	0.3123
Bil. (mg/dL)	0.16±0.02^{a}	0.09±0.00^{b}	0.13±0.01^{ab}	0.12±0.01^{ab}	0.0427
Urea (mg/dL)	10.04±0.92^{a}	7.19±1.20^{b}	5.80±0.27^{b}	6.02±0.84^{b}	0.0279
Creat. (mg/dL)	0.34±0.02^{a}	0.28±0.03^{ab}	0.15±0.02^{b}	0.36±0.06^{a}	0.0173
Rec. temp. (°C)	40.83±0.28	40.98±0.28	40.54±0.28	41.08±0.27	0.5526

^{a,b,c}Row means with different superscripts differ significantly at $p < 0.05$

Values presented in mean±standard error of the mean

Immune response

The effects of SC and SB on humoral immune response in broiler chickens are presented in Table 7. Immune responses on days 42 (NDV), 14 and 42 (SRBC) were non-significant ($p>0.05$) among the groups. However, the supplemented groups showed a tendency towards better response than the control. On days 7 and 14, antibody titer against NDV and SRBCs registered higher immune response ($p<0.05$) in supplemented groups (Table 7).

Table 7 Effects of broiler chickens fed diets supplemented sodium butyrate and *Saccharomycescerevisiae* on humoral immune response

Antibody titre (log_2) on periods of vaccination								
Treatment	Newcastle disease vaccine (NDV)				Sheep red blood cells (SRBC)			
	Day 0	Day 7	Day 14	Day 42	Day 0	Day 7	Day 14	Day 42
A	3.30±0.91	3.90±0.03^{b}	5.11±1.10^{b}	6.51±0.40	Nil	6.85±2.10^{b}	9.01± 2.09	7.40±1.30
B	3.90±1.00	6.16±0.12^{a}	8.58±2.19^{a}	6.52±1.24	Nil	8.69±3.11^{a}	9.49±2.00	8.20±3.10
C	2.98±0.09	5.63±1.15^{a}	8.43±1.32^{a}	7.82±1.50	Nil	9.21±3.02^{a}	9.08±3.00	7.50±2.00
D	3.00±0.00	6.41±0.09^{a}	9.23±2.07^{a}	6.92±2.16	Nil	8.80±1.20^{a}	9.82±4.00	7.54±2.17
p-Value	0.2811	0.0421	0.0281	0. 2376	-	0.0272	0.3010	0.6839

^{a,b}Column means with different superscripts differ significantly at $p < 0.05$

Values presented in mean±standard error of the mean

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Discussion

The growth performance of supplemented groups was enhanced compared to the control group. This is in line with the observations of other researchers that have shown the beneficial health effects of SB[33–36] to have positive effects on broiler production parameters such as weight gain, feed intake, and FCR. The variation in FCR between control and the treatment groups is due to unidentified factors. However, it is assumed that better performance may be due to the creation of the acidic environment in the gut after consumption of the supplemented diets which in turns decreases the load of pathogens.[11] Average weekly feed intake was noted to be lower in B and C groups compared to D and the control.

Sodium butyrate improves intraluminal digestibility of mineral and proteins.[13] This may have resulted in improved weight gain in the groups fed with SB than the control. The probiotic *S .cerevisiae* is famous for having growth promoting properties and is being encouraged in local poultry industry,[36] hence its choice in our study. The fact that group C performed better than B suggests that the actions of SB and SC may be synergistic. Contrary to our findings, other researchers Mahdavi & Torki,[33,37] Zou et al.,[38] reported that different levels of dietary sodium butyrate did not improve feed intake, weight gain and FCR in broilers. The variations could be due to the use of SB in different presentations; either coated or uncoated forms. In our study, we used the coated form (micro-capsulated). The uncoated (powder) form has low pKa value in comparison with the pH of small intestine in chickens and leads to reduced nutrient absorption, poor FCR and reduced weight gain.[39,40]

To our knowledge, no study is available in the literature that highlights the effects of combination of SB and SC on changes in immune organs of broilers to which we may compare our results. According to Sikandar et al.,[28] in healthy animals the increase in weight of immune organs is correlated with improved immune responses of the body and that bursa, thymus and spleen are the key players of the immune system. In the current study, weights of bursa and thymus increased in chickens fed with the supplemented diet. This is reminiscent of the findings of Qamaret al, that SC modulates the immune system by stimulation of IgA in response to pathogens. Eshak et al. reported that bursa weighed more in chickens treated with SB. The increased weights may be due to increased thickness of the parenchymal areas of these organs.[28] The greater size of bursa in B and spleen in C groups may be an indication that SB and SC may also have a revitalizing effect in immune organs of broilers. We noted significantly higher dressing percentage and lower cholesterol in groups B and C. Also, HDL and triglyceride values were reduced significantly in B (P=0.0434).

Onifade,[41,42] observed that addition of innocuous microorganisms including SC to the diet of rabbits and broiler chickens decreases serum cholesterol, triglycerides and phospholipids. In our study, only cholesterol was reduced; triglyceride and abdominal fat were significantly higher in group D that was fed with only SC supplemented diet. Therefore, the observed general improvement in carcass quality of the broilers when compared to the control could be attributed to SB. In poultry, the small intestine is the site for absorption in which the available nutrients are taken up through epithelial cells and drained into the general circulation. According to Sikandar, architectural modifications of the small intestine have direct relationship with production performance of animals. We observed that CD was significantly higher in the duodenum of control, VSA and VH: CD in the three segments and VH in duodenum and jejunum of the SB and SC offered groups were enhanced. In line with our finding, Ferket reported that characteristic features of a bird's digestive tract for the optimal functions include large surface area covered with long healthy villi having shallow crypts. Long villi and shallow crypts provide a larger surface area for the absorption of nutrients and low renewal rate, allowing efficient enzyme production and maturation of the intestinal cells.[43] As the ingredient status of the diets in all treatment groups did not vary, the observed enhancement in growth performance of the supplemented groups compared to the control could be as a result of the mucosal architectural modulations (reduced CD, increased VH and VSA) in the treated groups.[28,44] Our observation is in line with others who have reported that SB or SC supplementation markedly increased the intestinal absorption area by promoting villus growth in height.[28,43] We noted that supplementation with SB and or SC reduced population of the selected bacteria. The faecal populations of *E coli* and *Salmonella* were significantly reduced on days 21 and 49. This is in agreement with the report of other researchers. Although we did not determine their mechanism of action, previous reports showed that following the conversion of SB to butyric acid, it enters the bacterial cell wall through diffusion and becomes toxic to the bacterial cell. According to Van Deun et al.,[6] SB acts as a selective bactericidal agent by lowering the pH of crop, gizzard and in the upper part of the intestine, thereby controlling harmful bacteria such as *Salmonella spp*., *Escherichia* coli and *Campylobacterjejuni*. Similarly, due to the presence of mannose receptors in SC, it causes adherence of flagellate bacteria, and these pathogens are then eliminated in animals' faeces.[46] The observed reduction in the population of the selected bacteria may have contributed to absence of some common bacteria diseases, low mortality and improved growth performance recorded in the present study.

To the best of our knowledge, this study reports for the first time the effects of diets supplemented sodium butyrate and *S.cerevisiae* on haematology and serum biochemistry of broilers reared under tropical humid environment. Dietary content can affect the blood profile of healthy animals.[47] The determination of haematological indices and evaluation of liver enzymes of the birds were conducted with a view to determining any possible negative effects including leucogram abnormalities, liver synthetic activities indicated by serum total proteins and hepatoxicity indicated by activities of AST and ALT. This was also in line with the report of Isaac et al.,[47] that haematological components which consist of RBCs, WBCs or leucocytes, platelets and Hb are valuable tools in monitoring food toxicity as well as the health status in farm animals. Total protein values were noted to be slightly higher in the supplemented groups. In agreement with our finding, Payard & Mahmoudi[27] reported higher plasma protein values in pigs fed with *S. cerevisiae* supplemented diet. Neutrophil and PCV values were not affected by the dietary treatment but rather within normal range for chickens. In like manner, probiotics were reported to have no adverse effects on erythrocytes, PCV, haemoglobin concentration, MCV, MCH and leucocytes of rabbit.[48,49]

White blood cell, lymphocyte counts were higher in the supplemented groups. Though not much data is available on the effects of SB or its combination with SC on the immune functions of broilers, our observation suggests that they may enhance their immune competence. Value of neutrophil to lymphocyte ratio is used as an indicator of stress in animals. It was observed that this was reduced in the supplemented groups. This implies that SB and or SC could reduce stress in broiler chickens. According to Ihedioha & Chineme,[50] serum activities of ALT are influenced by age, muscle activity and physiological state of animals. In the present study, precautions were taken to reduce the effects these factors could have on our findings. For instance, birds selected were from the same source, provided equal

space and similarly managed. Obidike[51] reported that AST activity is more useful in assessing the severity of liver disease. According to the author, AST, being a systolic and mitochondrial enzyme is present in higher concentration in the liver than other liver enzymes and is thus released in higher quantities in cases of liver or any other major organ damage. Urea, ALT and AST were reduced in the supplemented groups. From our result, it could be inferred that dietary inclusion of SB and SC may have stabilized hepatocyte membrane in the broilers, and this subsequently reduced the serum levels of the enzymes. Sheep red blood cells act as thymus-dependent immunogens. As a result, researchers take interest in using it for antibody response evaluation in chickens;[52] hence, the rationale for its use in our study. From the results of the antibody assay, we observed on day zero, that while antibody titre against SRBCs was zero in all the groups, its mean value ranged from 2.98 in group C to 3.30 GMT in the control. The presence of this antibody in the chicks at this age might be due to maternal antibody. On days 7 and 14, antibody titer against NDV and SRBCs registered higher titres ($p<0.05$) in supplemented groups. Overall, these groups indicated better immune response throughout the periods of assay compared to the control. This observation suggests that SB and SC could modulate the function of B and T cells in later stages of the antigenic exposure and can thus regulate the host immunity.[53–59]

Conclusion

Diet C stimulated more positive influence on the production parameters we investigated by improving their gut health through modulation of intestinal mucosal, reduced bacterial load and improved antibody performance. This suggests that dietary supplementation at 200mg/kg SB+1.0g/kg feed could be an alternative to antibiotic growth promoter in broiler production. More studies are recommended to elucidate SB's appropriate inclusion level and potentials in reducing stress in broilers under the tropical environment.

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Disclosure of interest

The authors report no conflict of interest.

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