

## Gut balance booster as a viable alternative to antibiotic growth promoter in swine production: evaluation of the effects on growth and health parameters

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### Abstract

The use of antimicrobials as growth promoters (AGPs) in food-producing animals (FPAs) may facilitate the development and spread of antimicrobial-resistance bacteria (ARB), which threatens food safety and public health. Gut balance booster (GBB) improves gut health and growth/weight gain in FPAs without aiding the emergence of ARB. This 10-week study evaluated the effects of graded levels of GBB on growth and health parameters of weanling pigs following standard procedures. Thirty-six Large White X Landrace piglets, aged six weeks, were randomly assigned into four treatment groups (A-D) of nine piglets. Each treatment was replicated thrice with three piglets per replicate. They were fed diets A-D respectively. While diet-A was the control (no GBB), diets B, C and D were supplemented at 1.00 kg/ton, 2.00 kg/ton and 3 kg/ton, respectively. Results showed that daily feed intake and weight gain were significantly ( $p < 0.05$ ) higher in supplemented groups and group-B had highest final weight gain. The GBB supplementation had no significant ( $p > 0.05$ ) effect on total lipid profile but low-density-lipoprotein was significantly ( $p < 0.05$ ) lower in group-B. The supplementation increased packed cell volume, total leucocyte and lymphocyte counts and humoral immune performance. The treatment diets also significantly reduced serum levels of Alanine aminotransferase and Aspartate aminotransferase, suggesting the hepatoprotective effect. Diet-B had more positive effects on production and health parameters assessed, indicating that GBB supplementation at 1.00 kg/ton could safely and effectively replace AGPs in pig production; and hence limits dissemination of ARB and its associated public health problems.

**Keywords:** antibiotic growth promoters; gut balance booster; immune performance; production and haematological parameters; swine production

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## Introduction

Globally, pork has continued to gain popularity as a healthy alternative to red meat and other protein sources. In Africa, pig production over the last two decades maintained increased trend and remains one of the major animal protein sources in Nigeria (Thornton, 2010; FAO, 2017; Bernard *et al.*, 2021). Since the discovery of antibiotics, they have been used at therapeutic doses to treat diseases and sub-therapeutic doses as growth promoters in animal feeds (Njoga *et al.*, 2018). They are also used in animal feed to maintain gut health, improve animal performance and prevent/control the spread of enteric zoonotic pathogens (Ajibo *et al.*, 2020; Njoga *et al.*, 2021). The intensification of pig production to meet the increasing demand for pork has exacerbated the use of antimicrobials for prophylactic and growth promotion purposes in the swine industry. However, growing concern over the emergence, proliferation and dissemination of antimicrobial-resistant bacteria (ARB) via the food chain; and dwindling efficacy of antimicrobials in veterinary and medical practices have led to the prohibition or restriction on the use of antibiotics as growth promoters in animal agriculture in some countries (Maron *et al.*, 2013; Chattopadhyay, 2014; Hao *et al.*, 2014; Okocha *et al.*, 2018).

Following the ban or restriction of non-medical use of antimicrobials in food-producing animals (FPAs), research focus shifted to the development of viable alternatives to antimicrobial growth promoters (AGPs) that could enhance livestock productivity without causing adverse public health or food safety effects. Consequently, new commercial feed additives derived from natural sources have been provided as part of alternative feed strategies to enhance productivity in livestock production. Feed additives such as organic acids, enzymes, probiotics, prebiotics, antimicrobial peptide and phytochemical compounds have been recognized as potential alternatives to AGPs (Yanh *et al.*, 2015; Lillehoj *et al.*, 2018; Bajagai *et al.*, 2020).

However, strong perceivable odour in meats, unpredictable side effects, low bioavailability and stability in the gastrointestinal tract have limited the use of most of these feed additives singly (Stevanović *et al.*, 2018; Abonyi *et al.*, 2020a). Since inclusion of a mixture of feed additives were as effective as AGPs (Hassan *et al.*, 2018); the use of combinations rather than single feed additive could boost the production of safe (residue free) pork for human consumption. As a synergetic blend of different additives/ingredients (zinc, benzoic acid, calcium, sodium butyrate and a mixture of essential oils) for improving weight gain and gut health in animals, the gut balance booster (GBB) may be used as an effective alternative for AGPs in pig production. The GBB exerts its antibacterial effect by lysing the cell wall of harmful bacteria and subsequently invading and destroying the organism (INTRACO 2021). The GBB also has immune-stimulatory and growth promotion effects by stimulating the development of gut microvilli, as well as the release of enzymes which enhances nutrient absorption and utilization (INTRACO, 2021)

The GBB have been used extensively to enhance broiler production (Hassan *et al.*, 2018), but dearth of information exists on the use in pig farming. Consequently, the study determined the effects of GBB on growth performance and health indices in weaning pigs. Specifically, the study ascertained the optimal inclusion level of GBB in weaning pigs' diet as well as the effects of graded levels of the additives on growth performance, haematology, serum biochemistry, lipid profile and humoral immune response in the animal. If found effective, the GBB could substitute AGPs in pig production and hence reduce the risk of development and spread of ARB, especially zoonotic bacteria.

## Materials and Methods

### *Experimental pigs*

The study was carried out at the Piggery Unit of the Department of Animal Health and Production, University of Nigeria, Nsukka. A total of 36 Large White X Landrace weanling pigs of mixed sexes were used. They were the progeny of five sows and one boar, born within one week, weighed between nine and 10 kg and aged six weeks. The pigs were acclimatized for two weeks prior to the study. During the acclimatisation, they

were identified by ear notching, screened for blood and endoparasites and treated prophylactically against coccidiosis and gastrointestinal worms. After the two weeks of stabilization, they were transferred to an open, well ventilated fly proof grower house with concrete floor. The mean age and weight of the pigs were  $42 \pm 4$  days and  $9.74 \pm 0.01$ kg, respectively. They were balanced for initial body weight and sex across the four treatment groups and the stocking density was approximately 1 m<sup>2</sup>/pig.

#### *Gut balance booster and the experimental diets*

The GBB used in this study was a product of Intraco Limited, Belgium marketed in Nigeria. Four starter diets (A, B, C and D) were formulated to meet NRC (2011) nutritional requirements for swine using basal ingredients, as shown in Table 1. The four diets were formulated with similar basal ingredients. However, diet A contained no GBB, diet-B had GBB incorporated at 1.00 kg/ton of feed, diet-C contained GBB at the manufacturer's inclusion rate of 2.00 kg/ton of feed, and diet-D contained GBB at 3.00 kg/ton of feed. After incorporating the GBB, the diets were thoroughly mixed to ensure homogeneity. Thereafter, the diets were analysed for proximate composition using the methods of AOAC (1990).

**Table 1.** Ingredients and proximate composition of pig starter diet supplemented with graded levels of gut balance booster

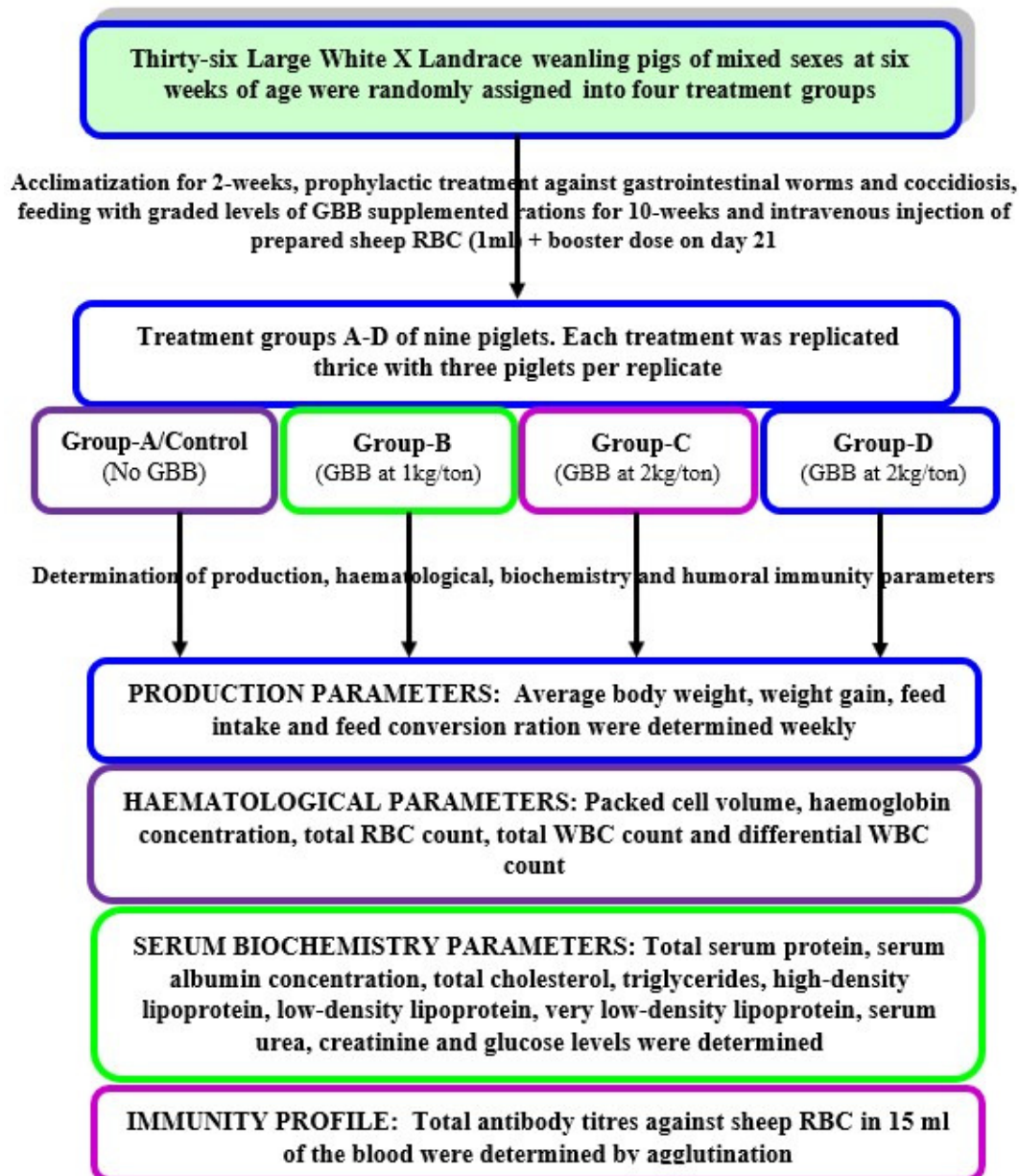
Feed ingredients (%)	Diet A	Diet B	Diet C	Diet D
Yellow maize	44.29	44.29	44.29	44.29
Guinea corn	11.60	11.50	11.40	11.30
Soya meal	15.54	15.54	15.54	15.54
Wheat offal	10.00	10.00	10.00	10.00
Fish meal	2.50	2.50	2.50	2.50
Palm kernel cake	5.00	5.00	5.00	5.00
Bone meal	2.50	2.50	2.50	2.50
Lime stone	5.00	5.00	5.00	5.00
Blood meal	2.34	2.34	2.34	2.34
Sodium chloride	0.33	0.33	0.33	0.33
Lysine	0.30	0.30	0.30	0.30
Methionine	0.10	0.10	0.10	0.10
Vita/min. premix	0.50	0.50	0.50	0.50
GBB	0.00	0.10	0.20	0.30
<b>Total (kg)</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>
<b>Proximate composition</b>				
Cal. ME (Kcal/kg)	3000.00	3000.00	3000.00	3000.00
CP (%)	21.30	21.30	21.30	21.30
DM (%)	87.00	87.00	87.00	87.00
Crude fiber (%)	5.67	5.67	5.67	5.67
Crude fat (%)	2.30	2.30	2.30	2.30
Total ash (%)	5.65	5.65	5.65	5.65

Proximate composition of the diets was determined according to AOAC (1990) while metabolizable energy (ME) was calculated values

#### *Ethical clearance and experimental design*

The Institutional Animal Care, and Use Committee of the Faculty of Veterinary Medicine, University of Nigeria approved the animal protocol for this study (FVM-UNN-IACUC-2019-056). The animals were used in accordance with the regulations and guidelines of this committee. A complete randomized experimental design of four treatments replicated three times was adopted for the research. In this model, pen was used as an experimental unit to test the main effect of diet. Each of the thrice replicated treatments had

three piglets in each replicate (pen). The groups were fed as follows: group-A, starter diet without GBB (control); group-B, starter diet supplemented with GBB at 1.00 kg/ton of feed (low dose); group-C, starter diet supplemented with GBB at 2.00 kg/ton of feed (recommended dose in broilers) and group-D, starter diet supplemented with GBB at 3.00 kg/ton of feed (high dose). The pigs were not given antibiotics and had free access to feed and clean drinking water throughout the study period of ten weeks. An over view of the experimental design and procedures for determination of the effects of gut balance booster on production, haematological, biochemistry and immunity parameters of the weanling pigs used in this study are schematically presented in Figure 1.



**Figure 1.** A schematic overview of the experimental design and procedures for determination of the effects of gut balance booster on production, haematological, biochemistry and immunity parameters of weanling pigs in Nigeria

GBB = Gut balance booster, RBC = Red blood cell, WBC = White blood cell

### *Data collection*

#### Determination of growth 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 pigs (Kiczorowska *et al.*, 2016). Their health status was recorded daily by visually observing possible clinical signs, morbidities and mortalities. The weekly ABW of the animals and FI were determined by subtracting respective pig initial weights (Kg) or feed intake (W1) from the final pig weights or feed intake (W2) and divided by number of weeks (n)  $(W2 - W1/n)$ . Their FCR was determined by dividing the feed consumed in a week in Kg by live weight gained (Kg) within the same period. Daily weight gain, FI and FCR were determined by dividing their respective weekly figures by seven. Daily water consumption of the piglets was also determined.

#### Determination of haematology parameters

The packed cell volume (PCV) of the pigs was determined by microhaematocrit method (Oluwole and Omitogun, 2016), using a Haematosporin 1400, microhaematocrit centrifuge and a Hawksley Microhaematocrit Reader (Hawksley and Sons Limited, West Sussex, UK). Haemoglobin concentration was determined by the cyanmethemoglobin method (Nkrumah *et al.*, 2011) using CHEM5V3 semiautomated blood analyzer (Erba Diagnostics, Mannheim, Germany). The red blood cell (RBC) and white blood cell (WBC) counts were enumerated manually following the haemocytometer method, using formal-citrate and Natt and Herrick's solution as diluting fluids (Dacie and Lewis, 1995), improved Neubauer counting chamber (Hawksley and Sons Limited, West Sussex, UK) and a light microscope (Leica Gallen, New York, USA).

#### Determination of biochemical parameters

Total serum protein (TSP) was determined in each sample following the Biuret method (Henok *et al.*, 2020) using the Randox Total Protein Test Kit (Randox Laboratories, Leeds, UK). Serum albumin concentration was determined following the bromocresol green method (Anonymous, 2020), 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 (Henok *et al.*, 2020).

The serum total cholesterol was determined based on the enzymatic colorimetric method (Li-Hua *et al.*, 2019) 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 (Kawano *et al.*, 2019). This was done using the Biosystem triglyceride working reagent and assayed with a CHEM5V3 semi-automated haemo analyzer (Erba Diagnostics, Mannheim, Germany). The serum high density lipoprotein cholesterol concentration was determined by the dextran sulphate magnesium (II) precipitation method (CDC, 2020). This was done using the Biosystem HDL-C precipitation reagent and the supernatant assayed with CHEM5V3 semi-automated haemo analyzer (Erba Diagnostics, Mannheim, Germany). The serum low density lipoprotein was calculated using Friedewald's formula (Kapoor *et al.*, 2015). Very low-density lipoprotein cholesterol of the pigs was determined by dividing the value of triglyceride concentration by 5 (Kawano *et al.*, 2019).

Serum urea, creatinine and glucose profiles were determined by di-methyl monoxide method as described by Gounden *et al.* (2021), Jaffe reaction method as described by Delanghe *et al.* (2011) and enzymatic colorimetric method (Zhu *et al.*, 2011), respectively. Each was done using their respective Biosystem working reagent and assayed with a CHEM5V3 semi-automated blood analyzer (Erba Diagnostics, Mannheim, Germany).

#### Determination of antibody response against sheep red blood cell

During the preparation of sheep red blood cells (SRBC) suspension, blood was obtained from healthy sheep. About 15 ml of sheep blood was collected under aseptic conditions from the jugular vein in

anticoagulant Alsever solution. The cells were washed three times with equal volume of Phosphate Buffered Saline (PBS). After the final wash, the packed cells were brought to 10% and 1% vol/vol solution in PBS (Maghsoudi *et al.*, 2020).

One piglet per treatment replicate (3 per group) were randomly selected, identified and injected intravenously with 1ml of the prepared SRBCs antigen in PBS. Booster dose was administered on day 21. Blood samples were collected on days 0, 7, 14 and 42. Sera were separated by centrifugation at 2000 revolutions per minute for 10 min) and stored at  $-20^{\circ}\text{C}$  till use. Total antibody titres to SRBC were determined by agglutination with routine procedure (Abonyi *et al.*, 2018). Antibody titres measure against SRBC was expressed as  $\log_2$  of the reciprocal of highest plasma dilution giving complete agglutination. All titrations were assessed the same day in 96-microtitre plates, using erythrocytes from the same sheep to immunize the pigs.

### Data 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. Statistical analyses were performed using the SPSS program version 23 (SPSS Inc., Chicago, IL, USA). 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 and lipid profile

A relatively low post weaning mortality rate of 2.70% was recorded: one piglet in group C (Table 2). The additives did not have significant effect on FCR ( $P = 0.1210$ ); it however, improved growth performance and showed significant ( $p < 0.05$ ) difference in feed intake ( $P = 0.0022$ ), daily weight gain ( $P = 0.0331$ ) and final body weight ( $P = 0.0240$ ) when compared to the control. The additive did not have any significant ( $p > 0.05$ ) effect on the rate of water consumption of the pigs (Table 2). Similarly, the GBB had no significant effect on the lipid profiles investigated except that low density lipoprotein (LDL) was significantly ( $p < 0.05$ ) lower in group B (Table 2).

**Table 2.** Growth performance and lipid profile of weanling pigs fed with graded levels of gut balance booster supplemented diet

Parameters	Treatment groups				
	A	B	C	D	P-value
<b>Growth Performance</b>					
Feed intake/day(g)	690.30 $\pm$ 8.47 <sup>b</sup>	850.12 $\pm$ 9.46 <sup>a</sup>	826 $\pm$ 3.46 <sup>a</sup>	900.87 $\pm$ 3.09 <sup>a</sup>	0.0022
Daily weight (g)	300.43 $\pm$ 3.39 <sup>b</sup>	510.77 $\pm$ 3.33 <sup>a</sup>	442.49 $\pm$ 3.43 <sup>a</sup>	440.39 $\pm$ 3.32 <sup>a</sup>	0.0331
FCR	3.17 $\pm$ 0.21 <sup>a</sup>	2.51 $\pm$ 0.20 <sup>a</sup>	2.84 $\pm$ 0.21 <sup>a</sup>	2.94 $\pm$ 0.21 <sup>a</sup>	0.1210
Initial body weight (kg)	9.76 $\pm$ 0.03 <sup>a</sup>	9.70 $\pm$ 0.34 <sup>a</sup>	9.80 $\pm$ 0.12 <sup>a</sup>	9.75 $\pm$ 0.08 <sup>a</sup>	0.3442
Final body weight (kg)	28.63 $\pm$ 1.10 <sup>b</sup>	36.86 $\pm$ 0.12 <sup>a</sup>	30.01 $\pm$ 2.17 <sup>b</sup>	31.10 $\pm$ 0.02 <sup>b</sup>	0.0240
Mortality rate (%)	0 (0.00)	0 (0.00)	1 (11.11)	0 (0.00)	
Water intake/da (L)	1.76 $\pm$ 0.00 <sup>a</sup>	1.98 $\pm$ 0.09 <sup>a</sup>	1.84 $\pm$ 0.07 <sup>a</sup>	1.90 $\pm$ 0.10 <sup>a</sup>	0.6241
<b>Lipid Profile</b>					
Cholesterol (mg/dL)	108.43 $\pm$ 4.73 <sup>a</sup>	106.81 $\pm$ 8.20 <sup>a</sup>	102.97 $\pm$ 10.26 <sup>a</sup>	99.86 $\pm$ 6.97 <sup>a</sup>	0.8647
Triglyceride (mg/dL)	97.33 $\pm$ 6.54 <sup>a</sup>	82.87 $\pm$ 9.08 <sup>a</sup>	87.00 $\pm$ 5.53 <sup>a</sup>	79.79 $\pm$ 8.90 <sup>a</sup>	0.3984
LDL (mg/dL)	50.98 $\pm$ 3.87 <sup>a</sup>	27.63 $\pm$ 7.43 <sup>b</sup>	48.90 $\pm$ 4.09 <sup>a</sup>	49.00 $\pm$ 2.76 <sup>a</sup>	0.0443
HDL (mg/dL)	52.27 $\pm$ 6.87 <sup>a</sup>	50.66 $\pm$ 3.98 <sup>a</sup>	53.54 $\pm$ 7.08 <sup>a</sup>	49.88 $\pm$ 5.43 <sup>a</sup>	0.8953
VLDL (mg/dL)	18.97 $\pm$ 2.01 <sup>a</sup>	17.69 $\pm$ 5.22 <sup>a</sup>	16.48 $\pm$ 3.52 <sup>a</sup>	17.60 $\pm$ 3.08 <sup>a</sup>	0.5438

<sup>ab</sup>Row means with different superscripts differ significantly at  $p < 0.05$ , Values presented in mean  $\pm$  standard error of the mean

*Haematology and serum biochemistry*

Haemoglobin concentrations (Hb), WBC, PCV and lymphocyte counts were significantly ( $p < 0.05$ ) increased by GBB supplementation (Table 3). Although RBC and neutrophil counts were not significantly ( $p > 0.05$ ) affected, neutrophil to lymphocyte ratio were slightly reduced in the supplemented groups ( $0.30 \pm 0.04$  vs.  $0.20 \pm 0.03$ ,  $0.27 \pm 0.01$  and  $0.21 \pm 0.08$ ). Result showed that ALT and AST concentrations were significantly ( $p < 0.05$ ) reduced in the treated pigs (Table 3). The treatment did not significantly ( $p > 0.05$ ) affect the pigs' total protein, albumin, and globulin fraction, but globulin and creatinine values were numerically higher among the piglets on GBB diets. Bilirubin and urea values were, however lower in the treated groups than control. Results also showed that serum glucose levels were significantly ( $p < 0.05$ ) higher in the control and group-D than in the B and C groups (Table 3).

**Table 3.** Haematology and serum biochemistry of weanling piglets fed diets supplemented with graded levels of gut balance booster

Parameters	Treatment groups				p-Value
	A	B	C	D	
PCV (%)	29.21 ± 0.60 <sup>a</sup>	30.00 ± 1.94 <sup>a</sup>	28.59 ± 0.68 <sup>a</sup>	29.80 ± 0.35 <sup>a</sup>	0.4458
Hb (g/dL)	7.97 ± 0.52 <sup>b</sup>	9.98 ± 0.08 <sup>a</sup>	9.62 ± 0.89 <sup>a</sup>	9.00 ± 0.36 <sup>a</sup>	0.0216
RBC x 10 <sup>6</sup> /μL	3.50 ± 0.34 <sup>a</sup>	4.82 ± 0.26 <sup>a</sup>	4.03 ± 0.27 <sup>a</sup>	3.92 ± 0.20 <sup>a</sup>	0.5783
WBC x 10 <sup>3</sup> /μL	12.23 ± 0.78 <sup>c</sup>	17.89 ± 0.64 <sup>b</sup>	19.80 ± 1.08 <sup>a</sup>	21.54 ± 0.76 <sup>ab</sup>	0.0032
Lymph. (%)	65.34 ± 8.04 <sup>b</sup>	85.71 ± 2.00 <sup>a</sup>	79.40 ± 7.01 <sup>a</sup>	80.32 ± 2.21 <sup>a</sup>	0.0500
Neutrophil (%)	25.00 ± 0.67 <sup>a</sup>	28.07 ± 1.08 <sup>a</sup>	25.42 ± 1.62 <sup>a</sup>	24.31 ± 0.10 <sup>a</sup>	0.4326
Neutro: lymph	0.30 ± 0.04 <sup>a</sup>	0.20 ± 0.03 <sup>a</sup>	0.27 ± 0.01 <sup>a</sup>	0.21 ± 0.08 <sup>a</sup>	0.4412
ALT (μ/L)	15.54 ± 1.06 <sup>a</sup>	13.23 ± 1.30 <sup>a</sup>	9.01 ± 0.11 <sup>b</sup>	11.80 ± 0.02 <sup>ab</sup>	0.0054
AST (μ/L)	78.00 ± 2.13 <sup>a</sup>	58.17 ± 2.36 <sup>b</sup>	66.65 ± 2.21 <sup>b</sup>	58.56 ± 1.22 <sup>b</sup>	0.0030
Protein (g/L)	3.43 ± 0.60 <sup>a</sup>	2.76 ± 0.04 <sup>a</sup>	2.74 ± 1.03 <sup>a</sup>	2.88 ± 0.03 <sup>a</sup>	0.6610
Albumin (g/L)	1.96 ± 0.21 <sup>a</sup>	1.88 ± 0.09 <sup>a</sup>	1.68 ± 0.14 <sup>a</sup>	1.57 ± 0.06 <sup>a</sup>	0.2876
Glob. (g/dL)	1.64 ± 0.08 <sup>a</sup>	1.72 ± 0.10 <sup>a</sup>	1.53 ± 0.05 <sup>a</sup>	1.56 ± 0.20 <sup>a</sup>	0.2543
<sup>β</sup> Bil. (mg/dL)	0.19 ± 0.02 <sup>a</sup>	0.15 ± 0.00 <sup>a</sup>	0.13 ± 0.01 <sup>a</sup>	0.14 ± 0.01 <sup>a</sup>	0.1427
Urea (mg/dL)	9.04 ± 0.92 <sup>a</sup>	7.19 ± 1.20 <sup>a</sup>	7.80 ± 0.27 <sup>a</sup>	7.43 ± 0.84 <sup>a</sup>	0.1279
Glucose (mg/dL)	0.54 ± 0.02 <sup>a</sup>	0.28 ± 0.03 <sup>bc</sup>	0.15 ± 0.02 <sup>c</sup>	0.56 ± 0.06 <sup>a</sup>	0.0173
<sup>ε</sup> Creat. (mg/dL)	0.75 ± 0.01 <sup>a</sup>	0.78 ± 0.02 <sup>a</sup>	0.85 ± 0.00 <sup>a</sup>	0.89 ± 0.02 <sup>a</sup>	0.5438

<sup>a,b,c</sup> Row means with different superscripts differ significantly at  $p < 0.05$ .

Values presented in mean ± standard error of the mean. <sup>β</sup>Bilirubin; <sup>ε</sup>Creatinine

*Immune response*

The effects of GBB on humoral immune performance of the weanling pigs are presented in Table 4. Immune response on day 7 was non-significant ( $p > 0.05$ ) in all the groups; however, the supplemented groups showed a tendency towards better response than the control. On day 14, antibody titre against SRBCs registered higher immune response ( $p < 0.05$ ) in GBB treated groups when compared with the control, and by day 42, groups C and D showed significantly ( $p < 0.05$ ) higher immune responses than others (Table 4).

**Table 4.** Geometric mean antibody titres (GMT) of pigs fed with graded levels of gut balance booster supplemented diet

Treatment groups	Sheep red blood cells (SRBCs)			
	Day 0	Day 7	Day 14	Day 42
A	0.00	6.03 ± 0.02 <sup>a</sup>	12.87 ± 2.00 <sup>c</sup>	15.09 ± 0.09 <sup>b</sup>
B	0.00	7.09 ± 0.08 <sup>a</sup>	20.09 ± 1.09 <sup>a</sup>	22.43 ± 0.00 <sup>a</sup>
C	0.00	8.00 ± 0.10 <sup>a</sup>	17.30 ± 3.07 <sup>b</sup>	20.00 ± 1.23 <sup>a</sup>
D	0.00	7.65 ± 2.00 <sup>a</sup>	15.45 ± 1.08 <sup>b</sup>	16.97 ± 1.48 <sup>b</sup>
P-value	--	0.4418	0.0301	0.0412

<sup>a,b</sup> Column means with different superscripts differ significantly at  $p < 0.05$ .

Values presented in mean ± standard error of the mean.

## Discussion

Our result showed that performance indices determined, including FI, FCR, daily and final weight gains of the supplemented groups were superior to the control. Mean FI, WG, and final body weight were significantly ( $p < 0.05$ ) higher in group-B. This observation agrees with the report of a study by Abonyi *et al.* (2020b) which showed that GBB improves gut health, growth performance and is cost beneficial in pigs. Similarly, Bühler (2009) reported that dietary benzoic acid (a component of GBB) had a positive influence on fattening pigs and this is in tandem with the findings of this study. However, the comparative analysis of these production parameters in earlier trial by Abonyi *et al.* (2020b) using only manufacturer's inclusion rate (2.00kg/ton of feed) for broilers did not result in statistical significance difference and group-B pigs (1.00kg/ton of feed) grew faster and heavier than the rest. According to INTRACO (2021), GBB contains benzoic acid, essential oils (EOs), zinc and sodium butyrate. The EOs contain growth-promoting plants' bioactive substances such as carvacrol, eugenol, thymol, capsaicin, and cineole (Fraga *et al.*, 2015; Patil and Patil, 2017). The growth-promoting effects of EOs, which was evident in this present study, is also consistent with the report of El-Hack *et al.* (2016) who made similar observations (El-Hack *et al.*, 2016). Cognizant that GBB contains zinc in sulphate monohydrate form, the present finding corroborates the result of a previous study which demonstrated that zinc oxide stimulated growth performance in weanling pigs (Abonyi *et al.*, 2015).

Sodium butyrate has been shown to improve the intraluminal digestibility of minerals and proteins in animals (Zhang *et al.*, 2011). The GBB may have enhanced nutrient digestibility that resulted in comparative improvement in WG in the supplemented groups. Contrary to our findings, other researchers (Mahdavi and Torki, 2009; Sikandar *et al.*, 2017) reported that different dietary sodium butyrate levels did not improve feed intake and weight gain. The variations could be due to the use of sodium butyrate in different presentations, either in coated or uncoated forms. It has been reported that the powder or uncoated form has low pKa value in comparison with the pH of small intestine and this leads to reduced nutrient absorption, poor FCR and reduced weight gain (Lesson and Summers, 2001). Therefore, the improved feed conversion and WG found in this work suggest that all the components of GBB acted synergistically to neutralize the negative effects of uncoated sodium butyrate, resulting in improved productive indices reported in the weanling pigs. Although no significant difference in water consumption was recorded, it increased linearly with feed intake across the groups.

The result showed a general reduction in lipid profile of the treatment groups compared to the control which is similar to the finding by Markowiak and Śliżewska, (2018), that combining feed additives containing prebiotics and or probiotics exert cholesterol-lowering effects. Similarly, Tang *et al.* (2017) reported that supplementation of a combination of prebiotics, probiotics, and symbiotics improved hens' performance and serum total cholesterol. Researchers have carried out many in vitro and in vivo studies to understand mechanisms that lead to the cholesterol-lowering effects, but their findings have been inconclusive (Ooi and Liong, 2010). It is important to note that only cholesterol was reduced in the other study in which GBB was used at the manufacturer's recommended rate (Abonyi *et al.*, 2020b). In both trials, the inclusion of GBB, however maintained the lipid profiles within the normal range for pigs as outlined by Khan and Line (2010).

To the best of our knowledge, this study reports for the first time the effects of graded levels of GBB supplemented diet on haematology and serum biochemistry of weanling pigs reared under humid tropical environment. Haematological parameters of animals are affected by sex, age, geographical location, dietary contents, and experimental procedures (Isaac *et al.*, 2013). Determination of haematological parameters such as the PCV can be used to assess presence and level of anaemia in the pigs (Oluwole and Omitogun, 2016) which is a common health problem in piglets reared on concrete floors in the tropics (as was the case in this study). Although PCV values of all the groups were within the normal range for pigs for the age group (Khan and Line, 2010), increased figures among the treatment groups (B, C, and D) points to the haematopoietic potentials of GBB at various inclusion levels. Sodium butyrate was reported to have no adverse effect on the erythrocytes, PCV, haemoglobin concentration, total white blood count and leucocytes of broilers (Abonyi *et*



*al.*, 2020b). In agreement with our observation, Upadhaya *et al.* (2015) also reported an increase in lymphocyte count without any significant change in blood profile in weanling pigs fed gel-based phytogetic feed supplement.

According to Ladokun *et al.* (2008), serum biochemistry and haematological parameters are important for proper maintenance of osmotic pressure between the extracellular and intracellular fluids to facilitate waste excretion and movement of biomolecules within the body. The significantly reduced serum levels of ALT and AST, suggests that GBB may have no or little toxicity effects, including leucogram abnormalities, but may be hepatoprotective.

According to Pošiváková *et al.* (2019), serum activities of ALT are influenced by age, muscle activity, and physiological state of animals. In the present study, precautions were also taken to reduce the factors' effects on the results. For instance, piglets were provided equal space and similarly managed. Mirmiran *et al.* (2019) 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. Since GBB is a relatively new product in Nigeria and is not officially indicated for use in pigs' diet formulation, it was necessary to determine if it will cause liver damage in piglets. From our result, Urea, ALT and AST were reduced in the supplemented groups, suggesting that dietary inclusion of GBB may have stabilized hepatocyte membrane of piglets, and this subsequently reduced the serum levels of these enzymes. From these results, GBB may be safe in terms of the possibility of causing organ damage in weanling piglets and could therefore be used in formulating pig starter diets.

Total protein values were slightly lower in the supplemented groups, but figures for all the groups were within the normal range (Aoyama *et al.*, 2021). Creatinine, which is a waste product formed in the muscle from high energy compounds and also an indication of high muscle mass (Ladokun *et al.*, 2008), was higher in the heavier supplemented groups. The fact that serum lipid profiles of the GBB fed groups were lower implies that this higher muscle mass is also leaner than the control. This is significant because lean meats, particularly white meat (pork), is in high demand because of their low cholesterol contents that may be cardio-protective.

The significant higher ( $p < 0.05$ ) total WBC and differential lymphocyte counts in the supplemented groups; indicate that GBB may enhance immune function and competence in piglets. The value of neutrophil to lymphocyte ratio is used to indicate stress in animals. The ratio was reduced in the supplemented groups implying that GBB could reduce stress in weanling piglets. From the antibody assay results, we observed that on day zero, antibody titre in all the groups was zero. This indicated absence of maternal antibodies to SRBCs and none prior to exposure of the piglets to the antigens. On days 14 and 42, antibody titter against SRBCs registered significantly ( $p < 0.05$ ) higher titres in the supplemented groups especially in group-B. This indicated better immune responses throughout the assay periods compared to the control. This observation suggests that GBB could modulate the function of B and T cells in later stages of the antigenic exposure and can thus regulate the host immunity. Similarly, higher globulin level in group-B is an indication that they may have a better cell mediated immune response than others (Ladokun *et al.*, 2008).

## Conclusions

Supplementation of pig diet with GBB enhanced productivity and immune response without any negative leucogram abnormalities. Specifically, the inclusion of GBB at 1.00 kg/ton in pig diet improved their growth/weight gain, PCV, Hb concentration, RBC count, total WBC count but reduced low-density lipoprotein ("bad cholesterol") content of the blood. Therefore, these findings suggested that GBB, rather than antibiotic growth promoters, could be used to enhance productivity in swine production to reduce the emergence and spread of antimicrobial-resistant organisms associated with the use of antibiotics as growth promoters in animal agriculture.

### Authors' Contributions

Conceptualization: FOA; Investigation and Methodology: FOA, UJN, IEO, CJA, SNB, CUU; Data curation and Formal analysis: EON, UJN, IEO, CJA, SNB, CUU; Visualization: EON  
Writing - original draft: FOA, EON; Writing - review and editing: EON;  
Supervision: FOA.

All authors read and approved the final manuscript.

### Ethical approval (for researches involving animals or humans)

The Institutional Animal Care, and Use Committee of the Faculty of Veterinary Medicine, University of Nigeria approved the animal protocol for this study (FVM-UNN-IACUC-2019-056). The animals were used in accordance with the regulations and guidelines of this committee.

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### Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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