GUT BALANCE BOOSTER AS A PROSPECTIVE ALTERNATIVE TO ANTIBIOTIC GROWTH PROMOTER IN SWINE DIET

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ABSTRACT

This study accessed the effects of dietary inclusion of Gut Balance Booster (GBB) on performance of weanling pigs. Twenty four six week old weaned Large White x Landrace pigs of mixed sexes were randomly assigned into two groups of 12 piglets each. Each treatment was replicated three times with four pigs in each replicate. They were fed diet A (normal starter diet without GBB) and diet B (starter diet with GBB at 2.00 Kg/ton). Feed intake, weight gain, feed conversion ratio (FCR), haematology, serum lipid profile, faecal egg count, shedding of selected bacteria and cost-benefit of the additive were determined. Results showed that there was no significant difference (p≤0.05) in daily feed intake, FCR and weight gain. However, the values favoured the supplemented group (1.16 ± 0.10 vs. 1.06 ± 0.13 Kg, 2.61 ± 0.34 vs. 3.04 ± 0.23, 485.29 ± 59.12 vs. 355.00 ± 40.30 g). Haemoglobin, PCV, RBC, WBC and lymphocyte counts were higher in group B. Lipid profile showed no significant difference (p≤0.05) in the two groups. The GBB reduced faecal strongyle eggs output, salmonella species, Escherichia coli and significantly increased Bacillus subtilis. Net revenue due to GBB supplementation significantly improved within the experimental period. It was concluded that in pigs, dietary inclusion of GBB at starter phase improves gut health, growth performance and is cost beneficial.

Keywords: Swine, Antibiotics, Feed additives, Growth performance, Cost benefits

INTRODUCTION

Antibiotics are used in animal feed to maintain and improve animal performance and prevent/control enteric pathogens (Bajagai *et al*., 2016). However, consumption of the animal products can result in bacterial pathogens build-up in humans and this led to its ban in 2006 by the European Union (Castanon, 2007). Following this ban, researches focused on developing other alternatives that could also enhance livestock production (Thacker, 2013). Organic acids, enzymes, probiotics, prebiotics, antimicrobial peptide and phytogenic compounds have widely been recognized as potential alternatives (Owens *et al*., 2008; Yang *et al*., 2015; Hassan *et al.,* 2018). Strong odour, poorly understood mode of action, complex chemical composition, unpredictable side effects, low availability and stability in gastro-intestinal tract limit the use of feed additives singly (Stevanović *et al*., 2018). Minimum inhibitory concentration (MIC) which may vary from bacterium to bacterium and in case of a single species, from strain to strain that they require in controlling enteric pathogens may not guarantee improved feed intake, immune competence and cost-effectiveness (Yang *et al*., 2015).

Owens *et al*. (2008) reported that inclusion of different sources of feed additives were as effective as antibiotic growth promoter in broilers. Therefore, combining these alternatives could hold the most promising solution to the identified short comings.

Gut Balance Booster (GBB) contains zinc, benzoic acid, calcium, sodium butyrate and mixture of essential oils (INTRACO, 2020). According to the manufacturer, in broilers, where it is mainly recommended to be used within the first 35 days of life, it stimulates the growth of gut microvilli and excretion of enzymes that improve nutrient absorption.

The present study focused on the effects of feeding weanling pigs starter diet supplemented with GBB on their health parameters and sought to verify some claims made by earlier workers. Specifically, the study assessed if dietary inclusion of GBB will improve growth performance and possibly serve as an alternative to antibiotics growth promoter in swine diet.

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 24 Large White x Landrace weanling pigs of mixed sexes were used. They were progeny of three sows and one boar, weighed between 8.50 to 10.00 Kg and aged six weeks. The pigs were acclimatized for two weeks prior to commencement of the study. During this period, they were identified by ear notching, screened for blood and faecal parasites and treated prophylactically against coccidiosis using Intracox (Interchemie Veterinary Services, Venray, Netherlands).

Gut Balance Booster: The GBB used in this study is a product from Intraco Limited, Antwerp, Belgium but marketed in Nigeria by Animal Care Services Konsult, Nigeria Limited.

Experimental Diets: Two starter diets (A and B) were formulated with basal ingredients following NRC (2011) nutritional requirements for swine (Table 1).

Table 1: Gross and proximate composition of pig starter diet supplemented with gut balance booster

Items	Diet A	Diet B
Ingredient		
Maize (yellow)	44.29	44.29
Guinea corn	11.60	11.60
Soya bean meal	15.54	15.52
Wheat offal	10.00	10.00
Fish meal	2.50	2.50
Palm kernel cake	5.00	5.00
Bone meal	2.50	2.50
Lime stone	5.00	5.00
Blood meal	2.34	2.34
Sodium chloride	0.33	0.33
Lysine	0.30	0.30
Methionine	0.10	0.10
Vitamin/mineral premix	0.50	0.50
Gut balance booster	0.00	0.20
Total (Kg)	100.00	100.00
¥Proximate composition		
Calculated ME (kcal/ Kg)	3000	3000
CP (%)	22.10	22.10
DM (%)	87.0	87.0
Crude fiber (%)	6.05	6.05
Crude fat (%)	2.16	2.16
Total ash (%)	5.77	5.77

¥While the chemical composition of the diets was determined according to AOAC (1990), metabolizable energy (ME) was calculated values

The two diets were formulated with similar ingredients except that diet B contained GBB incorporated at the manufacturer's inclusion rate of 2.00 Kg/ton of feed and thoroughly mixed to ensure even dispersion. Thereafter, the diets were analyzed for proximate composition using the methods of AOAC (1990).

Ethics: Animal study was supervised by the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine, University of Nigeria (FVM-UNN-IACUC-2019-056) and the animals were used in accordance with the regulations and guidelines of this committee.

Experimental Design: After the two weeks of stabilization, the 24 pigs were transferred to an open well ventilated fly proof grower house with concrete floor. A factorial experimental design of two treatments replicated thrice was adopted for the research. They were weighed and allotted to two treatments (A and B) on weight bases. Each treatment was replicated three times with four piglets in each replicate (pen).

The stocking density was approximately 0.53 m^2/pig. The groups were fed as follows: Group A-starter diet without GBB (control) and Group B-starter diet supplemented with GBB. The pigs in both groups were not offered antibiotics. Diets and clean drinking water were supplied *ad libitum* throughout the study period of seven weeks.

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 pigs (Kiczorowska *et al*., 2016). Their health status was recorded daily by visually observing possible clinical signs, morbidities and mortalities.

The weekly average body weight (ABW) of the animals and feed intake (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 on 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.

Determination of Haematology and Serum Biochemistry: The packed cell volume (PCV) of the pigs was determined by microhaematocrit method (Thrall and Weiser, 2002), using a Haematosporin 1400, microhaematocript centrifuge and a Hawksley Microhaematocript Reader (Hawksley and Sons Limited, West Sussex, UK). Haemoglobin concentration was determined by the cynomethemoglobin method (Higgins *et al*., 2007) using CHEM5V3 semi-automated blood analyzer (Erba Diagnostics, Mannheim, Germany). RBC and 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).

Biochemical Techniques: Total serum protein (TSP) was determined in each sample following the Biuret method (Weichselbaum, 1946) using the Randox Total Protein Test Kit (Randox Laboratories, Leeds, UK). Serum albumin concentration was determined following the bromocresol green method (Doumas *et al.,* 1971), 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 (Weichselbaum, 1946).

The serum total cholesterol determination was done based on the enzymatic colorimetric method (Allain *et al*., 1974) 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 (Bucolo and David, 1973). 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 (Albers *et al*., 1978). 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 (Friedewald *et al.,* 1972; Warnick *et al*., 1990). Very low density lipoprotein cholesterol of the pigs was determined by dividing the value of triglyceride concentration by 5 (Bucolo and David, 1973).

Faecal Egg Counts: Faecal samples were collected per rectum from the 24 pigs weekly. The samples were put in clean plastic containers with caps, labeled and taken to the laboratory for parasitological examination. Faecal egg counts (FEC), expressed as eggs/g (epg) of faeces was carried out within one hour of arrival at the laboratory, using a modified centrifugal

salt flotation techniques as described by MAFF (1977) and Behnke *et al*. (2010).

Determining the Selected Bacteria Population: One pig from each replicate i.e. three pigs per group were randomly isolated and allowed to defecate on a clean receptacle. Two (2) g of the freshly voided faeces was collected with swab sticks into a labeled sterile sample bottles and used for bacterial analyses. The cell population of the samples was determined via pour plate method (SGM, 2006). One (1) g of the faecal sample was suspended in 10 ml of sterile water under aseptic condition. The suspension was allowed for 10 minutes with constant shake. A 10^{-4} dilution was obtained from the suspension, using 10 fold serial dilution techniques. One (1) ml of 10^{-4} dilution was seeded in 19 ml of sterile molten nutrient agar kept at 45^{o}C. The culture plates were allowed to gel before incubation. The culture plate was incubated in inverted position at 37^{o}C for 48 hours. After the due period of incubation, the plates were observed for growth, and the colonies were counted, and the original cell population was calculated using the methods of Okore and Attama (2008), as follows: Original cell population (OCP) (cfu/ml) = [mean colony count x 1/dilution] ÷ [volume/drop], where dilution factor = 10^{-4} = 1/dilution factor = 10^{4} and volume per drop = 1 ml.

Determination of Cost Benefit: The cost benefit analysis (CBA) of inclusion of GBB in the pig starter diet within the experimental period was carried out using the cost benefit ratio (Sraders, 2019) and the benefit cost ratio (BCR) of Hayes (2019).

Data Analysis: Statistical analyses were done using the SPSS program (SPSS Incorporated, Chicago, IL). Data from the study was analyzed with independent t-test between the groups. Level of significance was taken as $p < 0.05$.

RESULTS

There was no significant difference ($p>0.05$) in the performance indices of the weanling pigs fed with the supplemented diet compared to the control. However, the values from feed intake, weight gain, FCR and final body weight were numerically higher in Group B pigs (Table 2).

There was no significant difference ($p>0.05$) in the haematological profile of the pigs. However, the values obtained in packed cell volume, RBC, haemoglobin, WBC and lymphocyte counts were numerically higher in Group B pigs (Table 3).

The biochemical profile followed similar trend as haematology and showed no significant difference among the two groups. However, triglycerides, low density lipoprotein, very low density lipoprotein and total protein were numerically lower in pigs in Group A than B (Table 4).

Gut booster balance supplementation reduced faecal strongyle eggs output noted in week 2 (106.00 ± 64.99 epg) to zero by the fourth week (Table 5).

The GBB reduced faecal shedding of *salmonella species* from 4.9 x 10^{4} to 2.9 x 10^{4} cfu/ml, *Escherichia coli* from 3.1 x 10^{4} to 2.7 x 10^{4} cfu/ml and significantly ($p<0.05$) increased *Bacillus subtilis* from 1.40 x 10^{4} to 2.8 x 10^{4} cfu/ml) (Table 6).

The result of cost benefit analysis of GBB supplementation showed a significant increase in net revenue within the experimental period (Figure 1).

DISCUSSION

This study revealed that performance indices including feed intake, FCR daily weight gain and final weight of the supplemented group were numerically superior to the control group. This was in line with the observation of Bühler (2009) who reported that dietary benzoic acid (a component of GBB) had positive influence on fattening pigs. Comparative analysis of these production parameters did not result in statistical significance as noted in this study. Similarly, the essential oils (EOs) present in GBB contain most of the bioactive substances of the plant such as carvacrol, eugenol, thymol, capsaicin and cineole (Fraga *et al*., 2015; Patil and Patil, 2017). The antimicrobial, immunomodulatory and growth-promoting effects of EOs in animals as observed in the present trial, have been reported by other

Table 2: Performance of weanling pigs fed with gut balance booster supplemented diet

Parameter	Group	Number	Mean	T-value	P-value
Adjusted Dietary Intake (Kg)					
	Group A	12	1.06 ± 0.13	-0.573	0.58
	Group B	12	1.16 ± 0.10		
Weight Gain (Kg)					
	Group A	12	355.00 ± 40.30	-1.821	0.09
	Group B	12	485.29 ± 59.12		
Feed Conversion Ratio					
	Group A	12	3.04 ± 0.23	1.037	0.32
	Group B	12	2.61 ± 0.34		
Initial Weight (Kg)					
	Group A	12	9.83 ± 0.76	-0.056	0.96
	Group B	12	9.89 ± 0.64		
Final Weight (Kg)					
	Group A	12	29.89 ± 1.59	-1.44	0.17
	Group B	12	33.72 ± 2.14		

Table 3: Haematological profile of weanling pigs fed with gut balance booster supplemented diet

Parameter	Group	Number	Mean	T-value	P -value
Haemoglobin (mg/dL)					
	Group A	12	10.10 ± 0.47	-1.57	0.16
	Group B	12	11.24 ± 0.56		
Packed Cell Volume (%)					
	Group A	12	35.80 ± 0.80	-2.06	0.07
	Group B	12	38.00 ± 0.71		
Red Blood Cell ($\times 10^6$/µl)					
	Group A	12	4.41 ± 0.17	-1.57	0.16
	Group B	12	4.90 ± 0.24		
White Blood Cell (/mm^3)					
	Group A	12	20760 ± 72.07	-0.51	0.96
	Group B	12	20980 ± 71.87		
Neutrophil (%)					
	Group A	12	43.20 ± 3.82	1.28	0.24
	Group B	12	37.20 ± 2.73		
Lymphocyte (%)					
	Group A	12	56.80 ± 3.83	-1.28	0.24
	Group B	12	62.80 ± 2.73		

Significance taken at p≤0.05

Table 4: Biochemical profile of weanling pigs fed with gut balance booster supplemented diet

Parameter	Group	Number	Mean	T - value	P - value
Cholesterol (mg/dl)					
	Group A	12	109.40 ± 7.18	0.09	0.93
	Group B	12	100.60 ± 5.56		
Triglycerides (mg/dL)					
	Group A	12	84.00 ± 10.30	-0.95	0.37
	Group B	12	96.00 ± 7.31		
Low density lipoprotein cholesterol (mg/dL)					
	Group A	12	47.80 ± 4.91	-0.72	0.49
	Group B	12	52.60 ± 4.55		
High density lipoprotein cholesterol (mg/dL)					
	Group A	12	61.60 ± 8.04	0.47	0.65
	Group B	12	56.00 ± 8.69		
Very low density lipoprotein cholesterol (mg/dL)					
	Group A	12	16.80 ± 2.06	-0.95	0.37

	Group B	12	19.20 ± 1.46		
Protein (g/L)					
	Group A	12	60.53 ± 4.21	1.05	0.21
	Group B	12	64.43 ± 3.07		
Albumin (g/L)					
	Group A	12	26.67 ± 4.12	1.32	0.34
	Group B	12	24.07 ± 3.00		
Globulin (g/L)					
	Group A	12	43.52 ± 5.50	1.21	0.63
	Group B	12	41.42 ± 3.10		

Significance taken at p≤0.05

Table 5: Faecal egg output (epg) of weanling pigs fed with gut balance booster supplemented diet

Weeks	Group	Number	Mean	T - value	P -value
0	Group A	12	1.00 ± 0.40	0.78	0.46
	Group B	12	0.60 ± 0.24		
1	Group A	12	1.00 ± 0.55	-1.62	0.14
	Group B	12	106.00 ± 64.99		
2	Group A	12	0.40 ± 0.24	-1	0.35
	Group B	12	1.00 ± 0.55		
3	Group A	12	1.20 ± 0.49	-1.13	0.29
	Group B	12	3.00 ± 1.52		
4	Group A	12	0.00 ± 0.00	-2.45	0.04
	Group B	12	0.60 ± 0.25		
5	Group A	12	0.20 ± 0.00	1	0.35
	Group B	12	0.00 ± 0.00		
6	Group A	12	0.00 ± 0.00	0	0.00
	Group B	12	0.00 ± 0.00		

Significance taken at p≤0.05; ¥ = Egg per gramme of faeces

Table 6: Microflora (cfu) of weanling pigs fed with gut balance booster supplemented diet

Microflora (cfu)	Group	Number	Mean	T-value	P-value
Week 0					
Escherichia coli (x 10^4)	Group A	12	2.56 x 10^4 ± 0.07	1.34	0.66
	Group B	12	3.10 x 10^4 ± 0.00		
Salmonella spp. (x10^4)	Group A	12	3.67 x 10^4 ± 1.09	1.21	1.33
	Group B	12	4.90 x 10^4 ± 2.55		
Bacillus subtilis (x10^4)	Group A	12	2.00 x 10^4 ± 1.00	1.60	1.42
	Group B	12	1.40 x 10^4 ± 0.00		
Week 6					
Escherichia coli (x 10^4)	Group A	12	2.43 x 10^4 ± 1.08	1.43	0.11
	Group B	12	2.70 x 10^4 ± 1.00		
Salmonella spp. (x10^4)	Group A	12	3.07 x 10^4 ± 1.04	-1.23	0.06
	Group B	12	2.90 x 10^4 ± 2.00		
Bacillus subtilis (x10^4)	Group A	12	1.40 x 10^4 ± 0.98	1.43	0.03
	Group B	12	2.80 x 10^4 ± 1.11		

Significance taken at p ≤ 0.05

researchers (Jacela *et al*., 2009; Abd El-Hack *et al*., 2016). Although the GBB used in this study contained zinc inform of zinc sulphate monohydrate, the result of this experiment was reminiscent with previous studies that have demonstrated that zinc oxide stimulated growth performance in weanling pigs (Hill *et al*., 2000; Han and Thacker, 2010; Abonyi *et al*., 2015).

In this study, supplementation with GBB reduced the population of two bacteria. Whereas the faecal populations of *Escherichia coli* and *Salmonell*a were reduced, *Bacillus subtilis* was significantly increased. The reduction in fecal *E. coli* count has also been reported when 1 g/Kg of eugenol (an essential

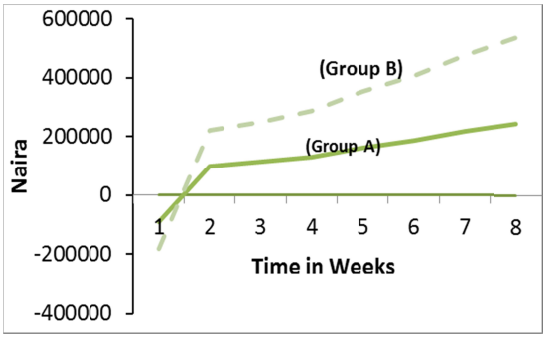

Figure 1: Cost benefit of weanling pigs fed with gut balance booster supplemented diet

oil) was supplemented in growing pigs' diet (Yan and Kim, 2012) and a reduction in the proliferation of *Salmonella* and *E. coli* was also detected in the faeces of broilers fed combined feed additive; sodium butyrate (also a component of GBB) and *Saccharomyces cerevisiae* supplemented diets (Chamba *et al*., 2014). Sodium butyrate 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., *E. coli* and *Campylobacter jejuni* in broilers (Van Deun *et al*., 2008).

The reduction in proliferation of these organisms may have been due to increase in *Bacillus subtilis.* According to Du *et al*. (2019), *Bacillus* is a beneficial bacterium and has been found in a variety of environments and organisms such as ponds, soil and gastrointestinal tract of pigs. Currently, several studies have shown that it has great application in animal production. Liu *et al*. (2017) demonstrated that dietary corn bran fermented with *B. subtilis* MA139 decreased gut cellulotic bacteria and microbiodata in finishing pigs. Optimal performance is generally possible when enough nutrients are absorbed, that is, the digestion process is not disturbed. Thus the fewer the microorganisms scavenging on diet of an animal, the more nutrients are available for it. Therefore, reduction in the population of some bacteria and faecal egg count may have contributed to improved growth performance of the GBB supplemented group. This observation also supports the manufacturer's claim that GBB has strong antibacterial effects.

Studies have shown that dietary content affects blood profile of healthy animals and its determination reflects the physiological responsiveness of the animals to its internal and external environment (Esonu *et al*., 2001; Kurtoğlu *et al*., 2005). As this is a pioneer work with GBB on swine, determination of haematological indices is no doubt necessary in order to identify possible negative effects it could have on the pigs including leucograme abnormalities. This was in line with the report of Isaac *et al*. (2013) that hematological profiles are valuable in monitoring food toxicity as well as the health status of farm animals. Neutrophil and PCV values were not affected by inclusion of GBB, but rather were within normal range for pigs (Kahn and Line, 2010). Sodium butyrate had no adverse effect on the erythrocytes, PCV, haemoglobin concentration, total white blood count and leucocytes of broilers (Makled *et al*., 2019). In agreement with this study, Upadhaya *et al.* (2016) reported an increase in lymphocyte count without any significant change in blood profile of weanling pigs fed gel-based phytogenic feed supplement.

Though no data is available describing the effect of GBB on the immune functions of pigs, in this study the white blood cell and lymphocyte counts were higher in the supplemented groups suggesting that GBB may enhance immune competence of weanling pigs.

The result showed a reduction in values of cholesterol and HDL in the treatment group compared to the control which was similar to the finding by Ooi and Liong (2010) that probiotics and prebiotics feed additives exerts cholesterol-lowering effects. Similarly, Tang *et al.* (2017) reported that dietary supplementation with prebiotics, probiotics and synbiotics improved laying performances and total serum cholesterol in hens. To understand the mechanisms behind cholesterol-lowering effects, researchers have carried out several *in vitro* and *in vivo* studies, but their findings have been inconclusive (Ooi and Liong, 2010). In this study, the inclusion of GBB in the diet however maintained the lipid profile within normal range for pigs as outlined by Kahn and Line (2010).

GBB reduced the helminth burden in pigs fed. There was no information on the anthelmintic property of GBB by the manufacturer. However, the findings of this study is in agreement with Dubey and Kashyap (2015) that reported anthelmintic effects when essential oil was added to pig's diet.

The cost benefit analysis showed significant increase in the profit margin within the trial period. This implied that its inclusion in pig starter diet is cost beneficial even to small scale pig farmers in Nigeria.

Conclusion: The packaging of GBB eliminated some problems normally encountered when handling its individual component particularly EOs. Diet B stimulated more positive influence on the production parameters investigated by improving the gut health through reducing harmful and increasing beneficial bacterial load and anthelmintic effect. This suggests that GBB could be an alternative to antibiotic growth promoter in weanling pigs. More studies are recommended to elucidate GBB's appropriate inclusion level and potentials in reducing worm burden in swine.

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