LOJ Immunology & Infectious Disease

Research Article

# The Effect of Some Natural Fermenters on Growth, Heamatology and Escherichia Coli Infection Control in Weaned Pigs

**Karaye GP[1]*, AC Kudi[2], AKB Sackey[2] and MA Raji[3]**

[1]*Department of Parasitology and Entomology, University of Jos, Jos, PMB 2084, Plateau State, Nigeria*

[2]*Department of Veterinary Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria*

[3]*Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Ilorin*

***Corresponding author:** Karaye GP, Department of Parasitology and Entomology, University of Jos, Jos, PMB 2084, Plateau State, Nigeria

**Received:** July 25, 2019 **Published:** August 02, 2019

## Abstract

The present study was carried out to evaluate the activities of the Lactobacillus species (LAB) isolated from "burukutu" and pig hindgut with patented probiotics on body weight, hemogram and E. coli infection control in weaned pigs. Sixty (60) large white breed of weaned pigs, aged between 30-35 days old with an average weight of 5 kg. They were randomly allotted to five replicate groups with six pigs each. Group A1 were administered 0.8 ml (6x106cfu) of lactobacillus isolated from "burukutu" and inoculated into wet feed, Group A2, 0.8 ml lactobacillus from "burukutu" (6x106cfu) inoculated in dry feed. Group B1, had, 0.8 ml (6x106cfu) of lactobacillus bacteria isolated from hindgut of pigs and inoculated into wet feed; Group B2, 0.8 ml (6x106cfu) dry feed containing lactobacillus from Pig hindgut. Group C1, 0.8 ml (6x106cfu) fermented liquid feed inoculated with Bacillus subtilis and Bacillus pumilis; Group C2, 0.8 ml (6x106cfu) dry feed inoculated with Bacillus subtilis and Bacillus pumilis. Group D1, 0.8 ml (6x106cfu) fermented liquid feed inoculated with Lactobacillus acidophilus; Group D2, 0.8 ml (6x106cfu) dry feed inoculated with Lactobacillus acidophilus. Group E1, fed wet basal diet; Group E2, fed dry basal diet. Animals in the various treatment's groups were infected orally with 6 ml (1x1010cfu·ml-1) of E. coli bacteria. Weekly body weight, hematological values and faecal shedding of E. coli post infection were evaluated. WBC, lymphocytes, neutrophils and total protein values were significantly high ($p<0.01$) in the treatment groups from week 4 to week nine. The wet form of the feed in each treatment produced significantly heavier animals ($p<0.01$) with an average weight of 25 kg. All dietary treatments showed significant reduction ($p<0.05$) in E. coli counts. The present study has demonstrated that lactobacillus species isolated from "burukutu" and pig-hindgut was able to improve weight gain and performance of weaned pigs. Therefore, isolated bacteria from "burukutu" and pig hindgut can serve as potent probiotics and growth promoters.

**Keywords:** Burukutu; Pig Hindgut; Lactic Acid Bacteria; Escherichia Coli Infection Body Weight; Haematology

## Introduction

The neonatal period is a critical time in piglet ontogeny, because the gastrointestinal tract (GIT) and immune system are not yet fully developed [1]. These in efficiencies make piglets vulnerable to invasion by pathogenic microorganisms and with their low resistance to diseases affect the whole process of individual development [2]. Supplementation with Lactic Acid and Bacteria (LAB) in neonatal piglets can regulate the formation of the piglet gut microflora, thus benefiting the health of piglets [1,3] Currently, there is growing attention on fermented pig feed because it improves growth performance [4-6] and influence the bacterial ecology of the gastrointestinal tract (GIT), in particular members of the family Enterobacteriaceae, including Salmonella spp [6,7]. A considerable amount of research has been conducted to select beneficial strains of lactic acid bacteria for fermented liquid pig feed production for example, [6,8] tested 146 strains of bacteria for their ability to control Salmonella. Bacterial species often used for inoculating feed to produce fermented liquid feed are Lactobacillus plantarum and Pediococcus spp [8]. In recent years, reports have described the beneficial effects of LAB, such as regulation of the intestinal microflora, inhibition or prevention of pathogens in the gastrointestinal tract, enhancement of intestinal mucosal immunity and maintaining intestinal barrier function [9,10]. The aim of this study, therefore, was to determine the invivo effect of some selected fermenters, on weight gain and haemogram of weaned pigs.

## Materials and Methods

### Study Area

The study was carried out in Samaru Campus of A.B.U, Zaria, Sabon Gari Local Government Area of Kaduna State and Located on Latitude (11°11´N, 07, 38´E, 686m above sea level) in the Northern Guinea Savannah with temperature ranging from 13.8° to 36.7° C. and an annual rainfall of 1092.8mm [11]. Agriculture in Zaria can be divided into two types: Rainfall type (from May to October) and irrigation farming in the dry season (from November to April). Dry season farming is the second most prevalent Agricultural activity in Zaria with vegetables being the common produce, but in some cases, fruits are sandwiched among cereal crops [12].

### Ethical Approval

Approval for the conduct of this research was obtained from the ethical clearance committee for animal use and care of Ahmadu Bello University Zaria; with the approval number: ABUCAUC/2016/005.

### Source of Experimental Animals

A total of 60 Large white breed of weaned pigs aged 42-60 days old with average body weight of 5.5 kg were sourced from breeders in Hayin Mallam, a small village (settlement) in Samaru, Sabon-Gari Local Government Area of Kaduna State and popularly known as 'Pig-City'. Forty-eight (48) of them were sheltered in the piggery pen of the Division of Agricultural colleges Samaru, Zaria. Whilst the remaining 12 weaners which will serve as the control group, were housed separately in the Piggery pens of the Department of Veterinary Medicine, Faculty of Veterinary Medicine, A.B.U Samaru, Zaria, Kaduna State these separation was done, so as to avoid cross contamination of diseases. The Pigs were fed ad libitum with water and commercial Pig Weaner diet (Table 1) containing 21% crude protein and 3606 kcal ME/kg.

**Table 1:** Feed composition for weaned pigs.

| Ingredients | Weight (kg) |
|---|---|
| Maize | 50 |
| Bran (wheat and guinea corn husk mixture) | 100 |
| G.N.C | 40 |
| Methionine | 0.1 |
| Lysine | 0.1 |
| Bone meal | 3.0 |
| Crayfish | 1.0 |
| Salt | 0.1 |
| Premix | 0.25 |
| Limestone | 1.0 |

The ingredients composing the feed were mixed thoroughly and bagged in 100kg grain bags to ease transportation and stored at room temperature in the feed store of the Department of Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. Prior to the feeding with the experimental feed mixes, the following base-line parameters were determined for the experimental animals: For haematological parameters, white blood cells (WBC) especially neutrophils, lymphocytes, basophils and eosinophils were evaluated. These parameters were determined using the automatic blood analyzer (CELL-Dyn 3200, Abbott Lab, Abbott Park, IL). Blood Samples were centrifuged at 6.67g at 40C.

### Body Weight

All the pigs in each group were weighted weekly, using a salter's scale hung on a beam to support the animal. The following mix ratios of feed versus fermenters were used in mixing wet and dry feed forms for feeding of the experimental animals: 8 kilograms of the feed was weighed using a kitchen scale and poured into a pair of 10liters plastic buckets into which 0.8mls of the overnight culture of lactobacillus bacteria was added and thoroughly mixed. Then, 8 liters of distilled water was added to one of the buckets and stirred with a clean spatula while the feed-mix in the second bucket was left dry. This procedure was repeated for other fermenters. However, the control feed-mix was constituted without inclusion of any of the fermenters used for the other experimental feed-mixes.

### Experimental Design

**Table 2:** Experimental grouping of the weaned pigs based on dietry treatment.

| Fermenters used | FEED GROUPS | | | |
|---|---|---|---|---|
| | WF+Lact Group | DF+ Lact Group | Control BD (WF) Group E | Control BD (DF) Group E |
| Lac 1BKT | 6 | 6 | | |
| Lac 2 Pg | 6 | 6 | | |
| Lac 3 STAB® | 6 | 6 | | |
| Lac 4 PTAB® | 6 | 6 | | |
| Un inoculated | - | - | 6 | 6 |

The weaned pigs were tagged and randomly allocated into 5 groups of 6 pigs each with each group having a replicate (Table 2).

## Keys

WF+Lact = Wet Feed plus Lactobacillus bacteria

DF+Lact = Dried Feed plus Lactobacillus bacteria

Control BD (WF) = Basal diet in wet form

Control BD (DF) = Basal diet in dry form

Lac BKT = Lactic acid bacteria isolates from "burukutu"

Lac Pg = Lactic acid bacteria isolates from hindgut of pigs

Lac PSTAB®= Bacillus pumilis/Bacillus subtilis a (patented probiotic)

Lac PTAB®= Lactobacillus acidophilus a (patented probiotic)

## Diets and Feeding Regimen

A grower diet was formulated for the experimental weaned pigs (Table 1). Then two dietary treatments were formulated with or without bacteria inclusions. 1. Dry feed (DF) supplied as meal and 2. Liquid feed supplied as wet form (WF). The liquid feed was prepared by mixing meal and water in a 1:4 ratio. The WF was prepared by mixing the feed with water in a 20 liters well labeled tank and agitated at 27°C (room temperature) before inoculating all the four treatment groups with lactobacillus from the different sources (BKT, Pig gut, Patented bacteria PSTAB and Patented bacteria PTAB). The control group was fed only the basal diet without LAB inclusion. For the dry feed, it was prepared by mixing the basal feed in four different 20 liters well labelled tanks which were agitated at 27°C (room temperature) before inoculating all the four treatment groups with lactobacillus from different sources (BKT, Pig gut, Patented bacteria PSTAB and Patented bacteria PTAB). The same procedure was applied for feed preparation like the first, but the only difference was that water was not added to the feed. For animals in the control group, only the basal diet was offered in dry and wet forms without LAB inclusion. The animals in all the groups were initially fed for a period of three weeks without the treatments (pre inoculation feeding). Then the various bacteria, isolates were added accordingly into their various rations as described by previous researchers [11,13]. The appropriate amounts of feed were consumed within approximately 30 min once daily in the [14]. according to their body weight so that the whole ration fresh water was also provided ad lib.

## Lactobacillus Enumeration and Isolation

After three weeks treatment period, faecal samples were collected from the rectum of all the animals in each treatment groups to ascertain the level of lactobacillus shed in the faeces. Approximately five (5)g of feces was collected from the rectum of each animal in the treatment group with a sterilized spatula, and with the animal properly restrained. The fecal samples were properly labelled and immediately transported to the Microbiology Laboratory of the Department of Veterinary Medicine, Ahmadu Bello University Zaria, and processed, using the following steps as described by [15]:

a) Samples of feces were suspended in quarter-strength Ringer's solution, homogenized with a classic blender (PBI International, Milan, Italy) and poured on plates containing prepared Rogosa agar (Oxoid Ltd.) which were properly labelled according to the treatment groups for total count of lactobacilli. After anaerobic incubation at 370 C for 24 hours, 10 colonies were randomly selected from plates containing the last sample dilution (10-8).

b) Isolates were cultivated in MRS broth (Oxoid Ltd.) at 370 C for 24 hours and re-streaked into MRS agar. The total Lactobacillus counts were carried out on MRS-agar plates. Numbers of colony forming units (CFU) was expressed as log10 CFU per gram.

c) Microscopy observation and Gram staining's performed to confirm isolates.

## Source of E. Coli for Infection of Pigs

Feces were collected from a diarrhoeic weaner pig in a piggery in Hayin Mallam in Samaru, placed into a sterile polythene bag and transported to the bacterial zoonoses laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University Zaria. Ten (10g) of the feces was inoculated into 90mls of broth (Tryptone soya broth), incubated at 37ºC for 24hours for enrichment. A loop full of the broth culture was streaked on EMB agar medium and incubated for 24hours at 370 C. Colonies characteristic of E. coli (green metallic sheen) were tested using the method of characterization as mentioned in Bergey's Manual of Systematic Bacteriology [16].

## Serotyping of Pathogenic E. Coli

A loop full of sterile normal saline was placed on a clean glass slide and a colony from the plate was picked and emulsified to form a homogenous mixture then a drop of the polyvalent phase3 sera (Oxoid, UK) was added and gently rocked for about five seconds until E.coli antigen were detected by agglutination reaction.

## Infection of The Weaners with E. Coli Isolate

The fed treatment LAB groups were challenged with E. coli. Each weaner received 6 mls (1x1010CFU·ml-1) orally, of the E. coli strain solution using a 10mls syringe that was held at the back of their oral cavities at 5.00 pm on day of infection according to the method described by [17].

## Faecal Sampling and Bacteriological Analysis of Challenged (Infected) Pigs

Faecal samples were collected from each weaned pig 15 hours after the challenge at 8.00am of the following day and serially diluted (0.1 in 9.9mls). Two tubes at 10-4 and 10-6 were selected and spread plated on EMB-agar (Oxoid, UK) for Enterobacteriaceae enumeration and incubated at 37°C for 24-48 h, as described by [5]. Number of colonies forming units (CFU) was expressed as log10 CFU per gram to obtain the number of colonies shed on a daily basis and this was done for a period of two weeks and results collated. Similarly, the control groups were also infected at the same time and observed for clinical signs of colibacillosis such as diarrhea, rough hair coat and lethargy and those showing such clinical signs were treated with Vetcotrim® bolus antibiotic (Kepro company UK, 5g/50kg per os).

## Results

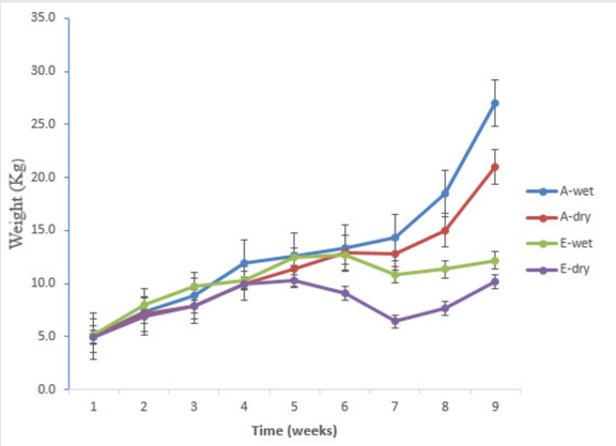


**Figure 1:** Mean weekly body weight gain of weaned pigs fed BKT lactobacillus isolates in wet and dry feeds.

A wet = wet feed + BKT bacteria Isolate

A dry = dry feed + BKT bacteria Isolate

E wet = wet feed only

E dry = dry feed only

There was a significant ($p<0.05$) progressive increase in mean weekly body weight in animals fed BKT bacteria inoculated wet and dry form of feed when compared with the control animals fed basal diet in wet and dried form. The group fed BKT in wet feed form produced significantly heavier animals compared to those fed dry feed with BKT bacteria. From week four, there was a gradual increase in weight in all the treatment group and the control group ($p<0.05$) but however, from week seven an exponential increase in weight was observed in the treatment group and a marked decrease in weight in the control group until week nine when the experiment was terminated (Figure 1). The group fed PGUT bacteria isolates showed a significant exponential ($p<0.05$) (Figure 2) increase in weight which was as compared to the control group fed with basal diet in both wet and dry form. However, the group fed PGUT bacteria in wet form produced significantly ($p<0.05$) heavier animals as compared to those fed inoculated dry feed. Also, there was a steady weight gain between week four and seven in the group fed PGUT bacteria in wet feed. And this increased significantly ($p<0.05$) from week seven to nine, while the group fed dry feed with PGUT bacteria isolate showed a gradual decrease in weight between week and six but increased rapidly from week seven to nine. The control group fed wet feed only showed a gradual increase in body weight from week four to five then plateaued between week five and six and gradually decreased till week nine while the control group fed dry feed only, group started showing a decrease in body weight from week four to seven before it increased steadily up to the ninth week (Figure 2). The group fed with PSTAB bacteria in wet and dry form of feed showed an exponential ($p<0.05$) increase in body weight as compared to the control group fed basal diet in wet and dry form. Though, animals in the treatment wet group where slightly heavier than those in the treatment dry group. There was a gradual increase in body weight in the treatment group from week four to seven which later progressed until week nine. While the control group showed a slow increase at week four which progressed steadily to week nine though much lower than the treated groups (Figure 3). From week four, a gradual increase in body weight was observed in all the treatment and the control groups but from week seven, the treatment groups showed a significant ($p<0.05$) increase in body weight as compared to the control. Also, for control animals, a decrease in weight was observed from week five to week seven with a gradual increase from seven to nine-week (Figure 4).

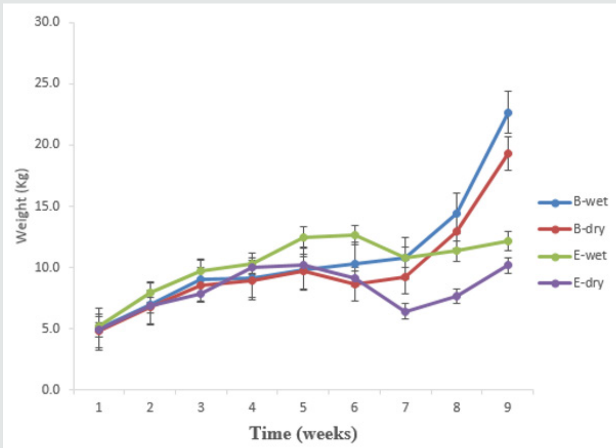


**Figure 2:** Mean weekly body weight gain of weaned pig fed lactobacillus isolates from pig gut in wet and dry feed.

B wet = wet feed + PGUT bacteria Isolate

B dry = dry feed + PGUT bacteria Isolate

E wet = wet feed only

E dry = dry feed only

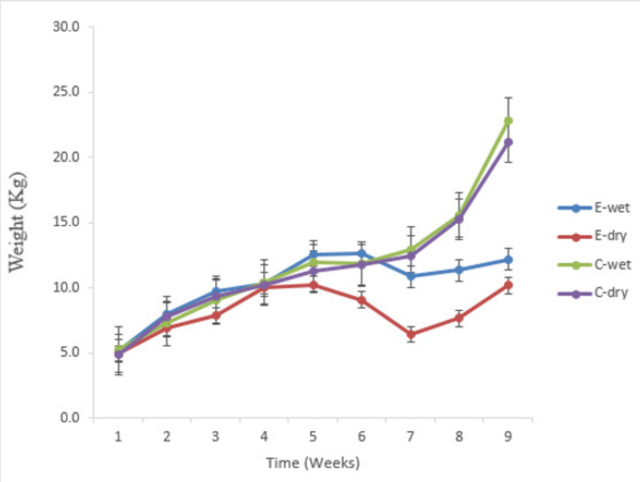


**Figure 3:** Mean weekly body weight gain of weaned pig fed lactobacillus isolates containing Bacillus subtilis and Bacillus pumilis in wet and dry feed.

C wet = wet feed + PSTAB bacterial Isolate

C dry = dry feed + PSTAB bacterial Isolate

E wet = wet feed only

E dry = dry feed only

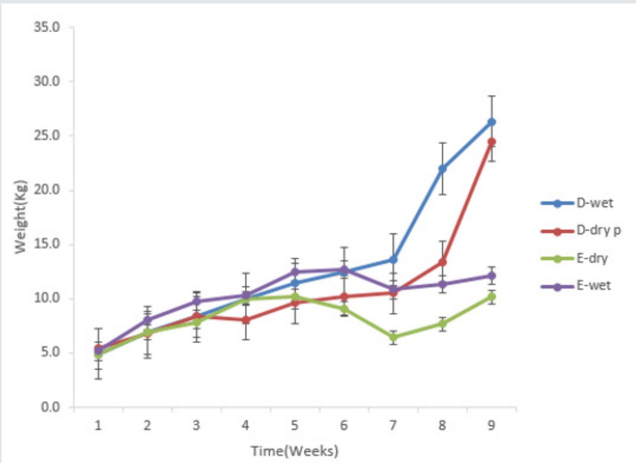


**Figure 4:** Mean weekly body weight gain of weaned pigs fed lactobacillus acidiphilus formulated in pig food supplement (tablet) in wet and dry forms.

D wet = wet feed + PTAB bacteria Isolate

D dry = dry feed + PTAB bacteria Isolate

E wet = wet feed only

E dry = dry feed only

## Mean WBC Count

Results of the effect of probiotic bacteria on weekly mean WBC counts of weaned piglets from pre feeding to nine weeks is presented in (Table 3). At week 4 WBC was significantly higher in piglets fed with BKT isolates (p<0.01) when compared to other treatments and the control groups. Also, there was no significant difference between PGUT, PSTAB and PTAB, the lowest was observed in the control group. At 5 weeks, PTAB produce high WBC count (p<0.01) when compared to other treatment and the control. The result in 6 weeks revealed that BKT, PGUT and PTAB isolates produced similar WBC counts at (p<0.05) while PSTAB gave the least WBC count that was similar to control group (p<0.01). At week 7, PGUT and BKT had similar values of WBC but significantly higher than the control (p<0.01). Similarly, there was no significant differences observed from the treatments PSTAB, PTAB and the control group. At week 8 BKT, PSTAB and PTAB significantly produce more WBC count (p<0.01), though similar WBC values were found in PTAB and control group, PGUT produced the least values. At 9-week PTAB significantly produce more WBC values (p<0.01). Then any other treatment and the control. However, BKT and PSTAB gave similar result that was significantly higher (p<0.05) than PGUT and control treatment. The feed form as presented in (Table 3). The result showed that from week 4-9 piglets fed wet feed form significantly produce more WBC counts than the dry form (p<0.001). However, WBC did not defer significantly from week 1-3.

## Effect of Probiotics on Haematological Parameters of Weaned Pigs Fed Actobacillus Fermented Feed

Table 3

## Mean Hemoglobin Value

The result for mean Hgb values for all the animals fed wet and dry feed forms in the treatment and control groups were all within the normal range for piglets (swine).The group fed with isolates from PTAB in dry feed produced the highest Hgb value which was significantly higher (p<0.05) than the other treatment group and the control group (Table 4). (Table 5) The effect of probiotic bacteria on the weekly mean neutrophil values from 1st week to 9th week of the study. From 1st, 3rd and 5th week of the study, there was no significant difference (0<0.05) among the treatment groups and the control. However, at week 4 BKT and PSTAB isolates were significantly produced higher mean neutrophil values (0<0.01) than PTAB, PGUT and the control group which was statistically similar. At week 6, BKT and PSTAB were statistically similar and produced higher (0<0.01) weekly mean neutrophil values than PTAB, PGUT and the control group. At week 7, BKT significantly produced the highest (p<0.01) neutrophil value which was similar to PSTAB and the control group. PTAB gave the lowest neutrophil value at this week. At week 8, a similar trend was observed as in the case of week 7 but the lowest neutrophil value was produced by PGUT. At 9 weeks, BKT significantly gave the highest neutrophil values (p<0.01) when compared to their treatment groups that are similar. However, the control group significantly produced a high neutrophil value (0<0.05) than PTAB which gave the last neutrophil value. The feed form as presented in Table 5 showed that from 7-9 weeks piglets fed wet form of food significantly produced more neutrophil values (p<0.05 and p<0.001). However, neutrophil values did not differ significantly from week 1-3. (Table 6) The effect of probiotic bacteria on the weekly mean lymphocyte values from 1st to 9 weeks of the study. From 1st, 2nd, 4th and 6th week of the study, there was no significant difference (0<0.05) among the treatment and control groups. However, at week 7, 8 and 9, BKT treatment group produced the highest lymphocyte values (p<0.01) which was statistically higher than PSTAB, PTAB and a PGUT treatment groups which were statistically similar. The feed form as presented in Table 6 showed that from 3-9 weeks, piglets fed wet food forms significantly produced more lymphocyte values (p<0.05 and p<0.01). However, weekly lymphocyte valued did not differ significantly at weeks 1, 2 and 5.

**Table 3:** Effect of probiotics on the WBC values of weaned pigs for a period of nine weeks of pre feeding, feeding and challenged with E. coli infection.

| Treatments / Bacteria | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| BKT | 14.767 | 17.81 | 16.28 | 27.27$^{a}$ | 17.79$^{b}$ | 21.73$^{a}$ | 24.03$^{ab}$ | 23.94$^{a}$ | 21.42$^{b}$ |
| PGUT | 16.71 | 14.86 | 21.84 | 24.27$^{bc}$ | 16.03$^{b}$ | 20.58$^{ab}$ | 25.62$^{a}$ | 15.84$^{c}$ | 18.78$^{c}$ |
| PSTAB | 15.42 | 14.98 | 17.58 | 23.02$^{c}$ | 19.28b | 18.04$^{c}$ | 22.55$^{bc}$ | 22.25$^{ab}$ | 20.67$^{b}$ |
| PTAB | 16.67 | 16.59 | 17.82 | 25.39$^{b}$ | 24.22$^{a}$ | 19.98$^{abc}$ | 22.41$^{bc}$ | 23.33$^{a}$ | 25.75$^{a}$ |
| CONTROL | 16.12 | 16.50 | 17.31 | 19.38$^{d}$ | 17.58 | 18.13$^{bc}$ | 20.71$^{c}$ | 20.62$^{b}$ | 18.08$^{c}$ |
| SED | 1.21 | 1.37 | 2.05 | 0.84 | 1.67 | 1.25 | 1.28 | 1.05 | 0.82 |
| LSD | 2.43 | 2.75 | 4.12 | 1.69 | 3.35 | 2.51 | 2.57 | 2.11 | 1.65 |
| Significance | NS | NS | NS | ** | ** | * | ** | ** | ** |
| Feed | | | | | | | | | |
| DRY | 16.13$^{a}$ | 16.46$^{a}$ | 18.25$^{a}$ | 22.32$^{b}$ | 16.52$^{b}$ | 18.20$^{b}$ | 20.94$^{b}$ | 20.31$^{b}$ | 20.04$^{b}$ |
| WET | 15.82$^{a}$ | 15.84$^{a}$ | 18.08$^{a}$ | 25.40$^{a}$ | 21.44$^{a}$ | 21.18$^{a}$ | 25.18$^{a}$ | 22.08$^{a}$ | 21.83$^{a}$ |
| SED | 0.76 | 0.87 | 1.30 | 0.53 | 1.06 | 0.79 | 0.81 | 0.67 | 0.52 |
| LSD | 1.53 | 1.75 | 2.61 | 1.06 | 2.13 | 1.59 | 1.63 | 1.35 | 1.04 |
| Significance | NS | NS | NS | ** | ** | ** | ** | ** | ** |

** = Significant at 1% and * at 5% level respectively. Mean within a column of any set of treatment followed by different letter are significantly different at 5%level while means followed by similar letters in column are not significantly different

NS = Not Significant.

SED = Standard deviation

BKT = Bacteria from burukutu

PGUT = Bacteria from pig intestine

PSTAB = Patented bacteria

PTAB, Patented bacteria

**Table 4:** Effect of probiotics on the hemogram (HgB) of weaned pigs for a period of nine weeks pre feeding, feeding and challenged with E. coli infection.

| Treatments / Bacteria | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| BKT | 13.32$^{a}$ | 13.22 | 13.13$^{a}$ | 13.58$^{b}$ | 13.92$^{a}$ | 14.25$^{ab}$ | 12.17$^{a}$ | 11.17$^{ab}$ | 10.67$^{a}$ |
| PGUT | 11.99$^{b}$ | 13.27 | 11.53$^{b}$ | 12.70$^{b}$ | 10.73$^{c}$ | 14.14$^{ab}$ | 8.79$^{c}$ | 7.83$^{c}$ | 8.92$^{b}$ |
| PSTAB | 11.69$^{b}$ | 13.69 | 11.73$^{b}$ | 14,03$^{a}$ | 12.76$^{b}$ | 12.82$^{bc}$ | 11.93$^{a}$ | 11.18$^{ab}$ | 11.29$^{a}$ |
| PTAB | 14.92$^{a}$ | 12.90 | 12.11$^{b}$ | 12.80$^{b}$ | 11.56$^{c}$ | 15.21$^{a}$ | 11.75$^{a}$ | 11.96$^{a}$ | 11.08$^{a}$ |
| CONTROL | 14.11$^{a}$ | 12.93 | 12.40$^{ab}$ | 12.59$^{b}$ | 11.57$^{c}$ | 11.87$^{c}$ | 10.52$^{b}$ | 9.67$^{bc}$ | 9.00$^{b}$ |
| SED | 0.48 | 0.57 | 0.58 | 0.52 | 0.44 | 0.74 | 0.49 | 0.92 | 0.32 |
| LSD | 0.96 | 1.14 | 0.89 | 1.06 | 0.89 | 1.49 | 0.98 | 1.84 | 0.65 |
| Significance | ** | NS | * | * | ** | ** | ** | ** | ** |
| **Feed** | | | | | | | | | |
| DRY | 13.34 | 13.26 | 11.94 | 13.12 | 11.81 | 13.92 | 10.63$^{b}$ | 10.18 | 9.85$^{b}$ |
| WET | 12.75 | 13.14 | 12.42 | 13.18 | 12.32 | 13.39 | 11.43$^{a}$ | 10.53 | 10.53$^{a}$ |
| SED | 0.30 | 0.36 | 0.81 | 0.33 | 0.28 | 5.48 | 0.28 | 0.58 | 0.20 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| LSD | 0.61 | 0.72 | 0.57 | 0.67 | 0.57 | 0.94 | 0.62 | 1.17 | 0.41 |
| Significance | NS | NS | NS | NS | NS | NS | * | * | * |

** = Significant at 1% and * at 5% level respectively. Mean within a column of any set of treatment followed by different letter are significantly different at 5%level while means followed by similar letters in column are not significantly different

NS = Not Significant

SED = Standard deviation

BKT = Bacteria from burukutu

PGUT = Bacteria from pig intestine

PSTAB = Patented bacteria

PTAB, Patented bacteria

**Table 5:** Effect of probiotics on the hemogram (Neutrophils) of weaned pigs for a period of nine weeks pre feeding, feeding and challenged with E. coli infection.

| Treatments | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Bacteria | | | | | | | | | |
| BKT | 37.58a | 35.25ab | 36.58 | 46.00a | 43.33 | 42.83a | 47.92a | 47.08a | 47.08a |
| PGUT | 36.17ab | 36.92ab | 36.50 | 40.08bc | 37.33 | 35.42b | 44.46bc | 37.33c | 42.83bc |
| PSTAB | 31.67b | 39.42ab | 32.42 | 38.92cd | 44.25 | 41.25a | 46.25abc | 45.00ab | 42.876bc |
| PTAB | 32.25b | 33.67 | 36.25 | 43.92ab | 37.33 | 41.83a | 43.92c | 42.71b | 41.17c |
| CONTROL | 33.92ab | 41.00a | 34.00 | 35.50d | 41.58 | 32.42b | 46.38ab | 45.83a | 44.00b |
| SED | 2.28 | 2.90b | 2.37 | 1.92 | 3.83 | 2.08 | 1.20 | 1.18 | 1.08 |
| LSD | 4.59 | 5.82 | 4.76 | 3.86 | 7.69 | 4.17 | 2.40 | 2.36 | 2.16 |
| Significance | NS | NS | NS | ** | NS | ** | * | ** | ** |
| Feed | | | | | | | | | |
| DRY | 33.03 | 35.50 | 36.00 | 40.77 | 40.57 | 39.77 | 44.50b | 40.38b | 41.40b |
| WET | 35.60 | 39.00 | 34.30 | 41.00 | 40.97 | 37.63 | 47.07a | 46.80a | 45.62a |
| SED | 1.45 | 1.83 | 1.50 | 1.21 | 2.42 | 1.31 | 0.76 | 0.74 | 0.68 |
| LSD | 2.90 | 3.68 | 3.01 | 2.44 | 4.86 | 2.64 | 1.52 | 1.49 | 1.37 |
| Significance | NS | NS | NS | NS | NS | NS | ** | ** | ** |

** = Significant at 1% and * at 5% level respectively. Mean within a column of any set of treatment followed by different letter are significantly different at 5%level while means followed by similar letters in column are not significantly different

NS = Not Significant

SED = Standard deviation

BKT = Bacteria from burukutu

PGUT = Bacteria from pig intestine

PSTAB = Patented bacteria

**Table 6:** Effect of probiotics on the (Lymphocytes) of weaned pigs for a period of nine weeks pre feeding, feeding and challenged with E. coli infection.

| Treatments | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Bacteria | | | | | | | | | |
| BKT | 56.33b | 58.50a | 51.42c | 61.00ab | 60.33a | 57.75a | 72.42a | 70.17a | 63.33a |
| PGUT | 56.50b | 57.58ab | 59.25ab | 61.50ab | 57.42ab | 53.17b | 57.17d | 54.83c | 50.92c |
| PSTAB | 61.17a | 58.50a | 59.25ab | 61.67a | 58.00ab | 58.42a | 62.58bc | 61.50b | 57.25b |
| PTAB | 58.67ab | 57.75ab | 60.33a | 57.92ab | 60.17a | 57.67a | 59.75cd | 58.17bc | 56.17b |
| CONTROL | 58.25ab | 53.92b | 55.83b | 51.42b | 56.00b | 51.58b | 63.58b | 61.50b | 57.42b |
| SED | 2.15 | 2.07 | 1.73 | 2.10 | 1.83 | 2.12 | 1.77 | 1.79 | 1.87 |
| LSD | 4.31 | 4.16 | 3.47 | 4.21 | 3.68 | 4.26 | 3.56 | 3.60 | 3.76 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Significance | NS | NS | ** | NS | NS | NS | ** | ** | ** |
| **Feed** | | | | | | | | | |
| DRY | 58.50 | 57.70 | 58.47[a] | 58.47[b] | 57.23 | 55.53[b] | 60.77[b] | 59.13[b] | 54.43[b] |
| WET | 51.87 | 56.80 | 55.97[b] | 61.33[a] | 59.53 | 58.30[a] | 65.43[a] | 63.33[a] | 59.60[a] |
| SED | 1.36 | 1.31 | 1.09 | 1.33 | 1.16 | 1.34 | 1.12 | 1.13 | 1.18 |
| LSD | 2.72 | 2.63 | 2.20 | 2.66 | 2.33 | 2.69 | 2.25 | 2.27 | 2.38 |
| Significance | NS | NS | * | * | NS | * | ** | ** | ** |

** = Significant at 1% and * at 5% level respectively. Mean within a column of any set of treatment followed by different letter are significantly different at 5% level while means followed by similar letters in column are not significantly different NS = Not Significant. SED = Standard deviation, BKT = Bacteria from burukutu, PGUT = Bacteria from pig intestine, PSTAB = Patented bacteria, PTAB, Patented bacteria

## Discussion

This study has shown that there was a significant increase in weight of animals in the treatment groups fed probiotic bacteria in the wet and dried form of the feed as compared to the control wet and dried treatment groups. The observed increase was more pronounced in groups fed the wet form of the feed than those fed the dried form. This finding underscores the benefit associated with feeding diets in a liquid form, and the need for pigs to be provided with water and feed simultaneously [14,18] and in this way, the weaned pigs do not need separate learning for feeding and drinking behavior's [7,19] Again, [19], demonstrated that the dry matter intake of the newly weaned pig can be increased by providing fermented liquid feed. All the groups that received wet forms of the diet with inclusion of probiotics from locally brewed drink (burukutu) showed a better growth as compared to those that received patented bacteria (PSTAB, PTAB) and that from pig (PGUT). The observed overall increase in weight in the BKT wet fed and PTAB wet groups as compared to the other groups (PSTAB and PGUT) indicates that, the probiotic strain used in this two treatment groups produce good acidity which is required to ferment liquid feed as reported by [6].that the fermentation of a nutritionally balanced feed will improve performance by increasing feed intake and gut health with resultant nutrient utilization. [6], had also reported a 22.3% improvement in weight gain and a 10.9% improvement in feed efficiency in the use of fermented liquid feed as compared to dry feed. This study has also shown that, probiotic inclusions in the dry form of feed in the BKT dry and PSTAB dry treatment groups also produced heavier animals than PTAB and PGUT groups, while the control as well as the PGUT inoculated dry feeds produced low weights. This observation agrees with reports that probiotics may not have positive effects on average daily weight gain and feed conversion of pigs [11,19]. As well as the reports of [8] that indicated that Lactobacillus fermentum and Lactobacillus ingluviei were associated with weight gain in animals while Lactobacillus plantarum was associated with weight loss in murines similarly, Lactobacillus gasseri was associated with weight loss both in obese humans and in pigs. The serum profile data indicated that there was no significant difference in white blood cell count, neutrophil, lymphocyte and hemoglobin concentration at the pre feeding period. No bacteria and feed form interaction were noted for white blood cell count, neutrophil, lymphocyte and hemoglobin concentration. This could be due to good hygiene provided for the piglets so that these blood parameters which are related to immune system were unaffected. There was an increase in WBC count only when probiotics was fed to the treatment groups and during challenge with pathogenic E.coli strain from week four of this study, This observation agrees with the report of [12] which states that microbial infection or the presence of foreign bodies or antigens in the circulatory system increases WBC counts. On the contrary, [12] observed an increase in WBC counts in a study of unchallenged postweaning piglets (weaned at 21 days of age) indicating increased total WBC following weaning (and age) respectively. Therefore, the increased total WBC in our weaned pigs could be attributed to the effects of the stress induced by weaning and challenge with E. coli. Lymphocyte counts of the pigs increased at weeks 4 and weeks 7 as observed in the groups fed probiotics inoculated feed and challenged with E. coli infection, which was significantly different from the control Groups challenged with E. coli infection. [13,20] and [13] had reported that the exact mechanisms of immune modulation by probiotics have not been fully explained but they may stimulate different subsets of immune system cells of which macrophages and lymphocytes are inclusive [3,21,22]. Mentioned that probiotics containing lactic acid producing bacteria enhance immune responses and defense activities against undesirable microorganisms and such protection has been partially attributed to increase innate immune response. These could have been the reason for the increase in lymphocyte counts in this study. Neutrophils are majorly responsible for phagocytosis of pathogenic microorganisms during the first few hours after their entry into tissues. There was however a significant increase in the neutrophil values in all the treatment groups and the control indicating the possible response of the neutrophils to the foreign bodies. The significant increase in total protein as observed in this study in all the treatment groups may be due to improvement in appetite and feed utilization by the animals. This concurs with the findings of [23], that increase in total protein indicate a high conversion of nutrients that can be correlated with immune stimulator through the action of probiotic flora. However, our results were parallel with that reported by [9] who reported no significant difference in the levels of serum albumin and globulin in probiotic treated calves, however, they observed a significant increase in the levels of serum total proteins. The findings in this study are also in harmony with that recorded by [14], in probiotic treated kids [24]. reported that there was no effect of complex probiotic feeding on total protein and albumin levels [1,4] also didn't observe significant effect on total protein and albumin levels with supplementation of rations with probiotics in Broiler chickens.

## Conclusion

The findings from this study have shown that: The addition of probiotics basal diets has promising effects on the performance of weaned piglets in terms of increasing body weight gain and improving the immune system thus reducing the severity of infection by pathogenic microorganisms. BKT inoculated feeds both in the wet and dried form, were found to be better than the patented bacteria as a probiotic as it increased the body weight as well as improved the bodies defence by increasing the immune system cells like WBC. Thus, locally sourced probiotics have like BKT and PGUT the potential of being patented for use in the Pig Industry in Nigeria.

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