Growth performance and physiological response of weaned pigs fed diet supplemented with novel a phytogenics

John Olujimi Alagbe[1], Daniel Nnadozie Anorue[2], Muritala Daniel Shittu[3], Sadiq Muhammad Ramalan[4], Tolulope Oreoluwa Faniyi[5] & Adekunle David Ajagbe[6]

[1] Department of Animal Nutrition and Biochemistry, Sumitra Research Institute, Gujarat, India

[2] Department of Animal Science, University of Abuja, Nigeria

[3] Department of Animal Production and Health, Ladoke Akintola University of Technology, Ogbomosho, Oyo State, Nigeria

[4] Veterinary Teaching Hospital, Faculty of Veterinary Medicine, University of Abuja, Nigeria

[5] Department of Crop and Animal Science, Animal Production Management Unit, Ajayi Crowther University, Oyo State, Nigeria

[6] Department of Animal Production, Prince Abubakar Audu University, Anyigba, Kogi State, Nigeria

Correspondence: John Olujimi Alagbe, Department of Animal Nutrition and Biochemistry, Sumitra Research Institute, Gujarat, India. E-mail: dralagbe@outlook.com

Received: June 11, 2023 DOI: 10.14295/bjs.v3i1.444

Accepted: July 13, 2023 URL: https://doi.org/10.14295/bjs.v3i1.444

Abstract

The aim of this experiment was to investigate the growth performance and physiological response of weaned pigs fed diets supplemented with a novel phytogenics (FCTNC). A total of 100 cross bred male pigs (Landrace × Duroc) with an initial weight of 6.31 ± 0.25 kg and weaned at 21 days were individually housed in a semi-open sided pens. Pigs were distributed into five treatments groups with five replicates, each replicates comprises of 20 pigs. Experimental diets were adequate in all nutrients recommended by NRC (2012). Treatment one (T1): basal diet without antibiotics; T2 (basal diet with neomycin at 1.5 g/kg^{-1}); T3, T4 and T5 were fed basal diet with 5 g/kg^{-1}, 10 g/kg^{-1} and 15 g/kg^{-1} respectively. Feed and water were offered unrestricted throughout the 56 days trial. Result revealed that there was effect of treatments ($P < 0.05$) in average body weight gain (ADG), daily feed intake (ADFI), mortality and feed conversion ratio (FCR). Pigs in T4 (24.54 kg) and T5 (24.22 kg) had the highest weight gain, intermediate in T2 (21.55 kg) and T3 (22.51 kg) and lowest in T1 (12.83 kg) ($P < 0.05$). There was a remarkable improvement in all the immune parameters examined among pigs fed FCTNC ($P < 0.05$). Microbial population of Escherichia coli and Salmonella spp decreased as the level of FCTNC increases ($P < 0.05$). Conversely, Lactobacillus spp count were highest in T4 and T5 relative to the other treatments. Haematological parameters were significantly influenced ($P < 0.05$) by the treatments. However, all values were within the normal range for a clinically healthy pigs. The study established that FCTNC could be fed to weaned pigs up to 15 g/kg^{-1} without having any negative effect on the health of animals.

Keywords: growth, phytogenics, immune, blood, weaned pigs, microbial count.

Desempenho de crescimento e resposta fisiológica de leitões desmamados alimentados com dieta suplementada com novos fitogênicos.

Resumo

O objetivo deste experimento foi investigar o desempenho zootécnico e a resposta fisiológica de leitões desmamados alimentados com dietas suplementadas com um novo fitogênico (FCTNC). Um total de 100 suínos machos mestiços (Landrace × Duroc) com peso inicial de 6,31 ± 0,25 kg e desmamados aos 21 dias foram alojados individualmente em baias semiabertas. Os porcos foram distribuídos em cinco grupos de tratamentos com cinco réplicas, cada réplica composta por 20 porcos. As dietas experimentais foram adequadas em todos os nutrientes recomendados pelo NRC (2012). Tratamento um (T1): dieta basal sem antibióticos; T2 (dieta basal

com neomicina a 1,5 g/kg^{-1}); T3, T4 e T5 receberam dieta basal com 5 g/kg^{-1}, 10 g/kg^{-1} e 15 g/kg^{-1}, respectivamente. A ração e a água foram oferecidas sem restrições durante os 56 dias de experiência. Os resultados revelaram que houve efeito dos tratamentos (P < 0,05) no ganho médio de peso corporal (GMD), consumo diário de ração (ADFI), mortalidade e conversão alimentar (CA). Suínos em T4 (24,54 kg) e T5 (24,22 kg) tiveram o maior ganho de peso, intermediário em T2 (21,55 kg) e T3 (22,51 kg) e menor em T1 (12,83 kg) (P < 0,05). Houve uma melhoria notável em todos os parâmetros imunológicos examinados entre os porcos alimentados com FCTNC (P < 0,05). A população microbiana de *Escherichia coli* e *Salmonella* spp diminuiu com o aumento do nível de FCTNC (P < 0,05). Por outro lado, a contagem de *Lactobacillus* spp foi maior em T4 e T5 em relação aos outros tratamentos. Os parâmetros hematológicos foram significativamente influenciados (P < 0,05) pelos tratamentos. No entanto, todos os valores estavam dentro do intervalo normal para porcos clinicamente saudáveis. O estudo estabeleceu que a FCTNC pode ser fornecida a leitões desmamados até 15 g/kg^{-1} sem ter qualquer efeito negativo na saúde dos animais.

Palavras-chave: crescimento, fitogênicos, imune, sangue, leitões desmamados, contagem microbiana.

1. Introduction

The utilization of alternative feed additives is on the rise in the swine industry recently to support most effective production, especially since the use of antibiotic growth promoters was banned due to antimicrobial resistance and the presence of toxic residue in animal products, which is the primary cause of several human illnesses (Andreas, 2021). Phytogenic feed additives are a class of organic compounds made from extracts of herbs, spices, and related plants. These naturally occurring bioactive chemicals are made up of a variety of distinct constituent types, including tannins, essential oils, saponins, flavonoids, and alkaloids (Singh et al., 2021; Vera, 2020). By serving as defense mechanisms against diseases, predators, and environmental stress, these substances give plants a competitive edge (Liliana; Melina, 2019).

Owing to the presence of phytochemicals, phytogenic feed additives are known to have the ability to enhance the growth performance, nutrient digestibility, and gut health of livestock species, including pigs (Musa et al., 2021; Adewale et al., 2021). Additionally, plant-derived compounds have a variety of qualities, including flavoring, antibacterial, antifungal, antiviral, hepatoprotective, immunological modulating, and physiological effects, all of which are critical to their ability to improve performance in animals (Ines, 2020).

Tests of active substances derived from plants are currently the more promising branch of preparations that can be efficient and used on a large scale while maintaining relatively reasonable economic relations. This is due to the increasing bacterial resistance and more frequent notation of multi-drug resistance strains along with the lack of work on the development of new antimicrobial agents. Numerous research have demonstrated the immunostimulatory properties of phytogenic feed additives and their ability to reduce the activity of pathogenic bacteria such *Escherichia coli*, *Staphylococcus* spp., *Salmonella* spp., and others (Ahmed; Tobias, 2020). Due to the presence of phytochemicals, they also possess special and potent instruments for reducing oxidative stress (Roberto, 2018), which has been shown to improve animal health and performance (Ahmed; Tobias, 2020).

Despite this, there is lack of information on the phytochemical properties of FCTNC as a novel feed additive. Therefore, this study was carried out to evaluate the quantitative phytochemical properties of the test ingredient on the growth performance, haematological parameters, intestinal microbiota and immune response of weaned pigs this will further promote food safety and add to our knowledge of its ethno-medicinal use of FCTNC.

2. Materials and Methods

2.1 Experimental ground, ethical guidelines, collection and processing of phytogenic additive

At the Sumitra Research Institute in Gujarat, India, that is located at (23º13'N and 72º41'E) and boasts a 1600 km beachfront, the investigation was carried out in the Division of Animal Nutrition and Biochemistry. It was done in accordance with the AD/08A/2023 ethical standards for managing livestock.

Fresh Fenugreek, clove, thyme, nutmeg and cumin leaves were harvested from Sumitra botanical gardens, Gujarat, India in the month of January 2023. It was identified by a qualified taxonomist and assigned a voucher number DF/003A, DF/004B, DF/005C, DF/006D and DF/006E respectively, washed with water to remove sand and placed separately in a plastic sieve to allow excess water to drain before spreading in a flat aluminum tray to air dry for 11 days. Leaves were grinded into powder using a mortar and pestle and stored separately in a clean transparent labeled container. Thousand (1000) grams of each sample was measured from the stored grinded

sample and mixed together form a novel phytogenic feed additive (FCTNC) as specified below;

FCTNC = 1000 g (DF/003A) + 1000 g (DF/004B) + 1000 g (DF/005C) + 1000 g (DF/006D) + 1000 g (DF/006E)

2.2 Reagents/materials needed for phytochemical evaluation

Magnesium carbonate solution, acetic acid, ethanol, ammonium hydroxide, ammonia, iron chloride, Folin-Denis reagent, tannic acid solutions, whatman No. 1 filter paper, volumetric flask, beaker, gallic acid, hydrochloric acid, sulphuric acid, beaker, ethyl acetate, permanganate, volumetric jar, water bath, sodium chloride and aluminum chloride.

2.3 Animal rearing/management and experimental layout

In India, 100 cross bred male pigs (Landrace × Duroc) with an initial weight of 6.31 ± 0.25 kilograms weaned at 21 days were used for this experiment. Pigs were quarantined for 14 days on arrival to Sumitra Research Farm, Gujarat. They were fed basal diet and given prophylactic treatments of Ivermectin® (against parasites, according to manufacturer's prescription) at (0.2 mL per kilogram). Weights of the animals were balanced before they were allotted into 5 pens (20 pigs/pen) (semi open sided pens each measuring 25 m × 16 m: length × breath) during a 56 –day trial. Completely randomized experimental design was adopted and pigs were distributed into five treatments groups with five replicates, each replicates comprises of 20 pigs. Experimental diets were adequate in all nutrients recommended by NRC (2012).

Dietary treatments were;

• Control diet: corn-soya meal based diets (basal diet: without therapeutic levels of antibiotics or phytogenic feed additives) – treatment one (T1)

• Treatment two (T2) – The basal diet with neomycin (1.5 grams/kilogram) (positive control)

• Treatment three (T3) – The basal diet supplemented with 5 grams/kilogram FCTNC (negative control)

• Treatment four (T4) - The basal diet supplemented with 10 grams/kilogram FCTNC (negative control)

• Treatment five (T5) - The basal diet supplemented with 15 grams/kilogram FCTNC (negative control)

2.4 Information obtained

2.4.1 Traits associated with performance

The total quantity of feed taken was calculated by deducting the quantity of feed discarded from the quantity of feed provided. The proportion of average feed intake to average body weight growth was used to determine the feed conversion ratio, or the amount of feed required to create one unit of gain. The difference between the subject's initial and final body weights was used to compute the weight increase. By splitting the weight acquired for each course of treatment by the overall number of trial days, the average daily body weight was calculated.

2.5 Proximate investigation of experimental diet

Investigation on experimental diet was carried out using Automated Phoenix 5000 NIR feed analyzer, USA to determine for fat, moisture, protein, crude fibre and metabolizable energy. The machine has the following technical features; wave length range (1100-2500 nm), wavelength selection (scanning diffraction grating monochromator with nominal bandwidth of 10 nm, configurable data presented in 1 nm or 2 nm increments), temperature and humidity (35-105 oF < 85 percent), lamp life (10,000 hours) and power requirements (100-240 V).

2.6 Haematological investigation

Before the commencement of the study and end (56th day), 2 mL of blood was collected very early in the morning from the culinary vein of 10 randomly selected pigs for haemtological studies. Blood samples were collected into an ethylene diaamine tetraacetic acid using OM – 2206 Auto Haematology Analyzer. The machine has the following technical specifications; operating environment (temperature 15-35 ºC; humidity 10-90%), principles (electrical resistance for counting WBC, RBC, and PLT), sample volume (pre-diluted 20 µL),

throughput (up to 60 samples per hour), input/output (RS-232, USB, LAN, keyboard and mouse interface).

2.7 Caecal microbial investigation

Microbial investigation in the caecum was examined using MALDI Biotyper® System, USA. The procedure goes through the following process;

• Adding target plate to a MALDI Biotyper project list,

• Selecting the isolated colony,

• Transferring sample onto the target plate and add matrix,

• MALDI – TOF spectrum automatically generated by the software,

• Spectrum instantly matched against the reference library to give identification,

• Final review and result validation.

The machine has the following features: speed of analysis (95 isolates + 1 QC sample, identification of 600 samples/hour), laser (200 Hz repetition rate), mass range [2.000 – 20.000 Da, microorganism identification], and temperature range (61-86 ºC), operating humidity (20-75%).

2.8 Analysis of immune parameters

Blood samples from animals collected from haematological test was used to determine the immune response of analysis via Microlisa plus versatile elisa plate reader (India) with 96-well reader control and data analysis for raw data ABS OD, Cutoff and multi standard up to 10 calibrators. Absorbance (dynamic range: 0 to 3.5 OD and resolution: 0.001 OD), light source (tungsten halogen), filters (405, 450, 492 and 630 nm), temperature (10 to 40 ºC) and memory (100 user programmable tests).

2.9 Identifying the alkaloid

Alkaloid measurement was carried out using the procedure outlined by Harborne (1973). Gravimetric analysis was used to figure out the alkaloid concentration. A total of five grams of the sample were weighed and mixed in a 1:10 (10%, *v/v*) ratio with 10 percent acetic acid solution in ethanol. At 28 °C, the blend was let to stand for 4 h. Subsequently, it underwent filtering using Whatman No. 42 filter paper. By using evaporation to reduce the filtrate to a quarter of its initial volume, concentrated aqueous NH_4OH was then added drop by drop until the alkaloid crystallized. The alkaloid residue was collected in weighted filter paper, cleaned with a solution of 1percent ammonia, and dried at 80 °C in the oven.

2.10 Measurement of saponins

According to Brunner (1984), the spectrophotometric approach was utilized for saponin analysis. A 250 mL beaker was filled with one teaspoon of the test substance and 100 mL of isobutyl alcohol. To ensure even mixing, the liquid was shaken on a UDY shaker (UDY Corporation, Fort Collins, CO) for 5 h. A Whatman No. 1 filter paper was used to filter the resulting mixture onto a 100 mL beaker, and 20 milliliters of a forty percent saturated magnesium carbonate solution was then added. To create a transparent, neutral solution, the resultant mixture was then passed using Whatman No. 1 filter paper.

A 50 mL volumetric jar containing a single milliliter of the solution that was colorless were standardized, to which two milliliters of 5% FeCl3 solution were added. The mixture was then brought to the proper volume with purified water, and let sit for 30 minutes so that the solution could turn scarlet red. Using a saponin baseline solution, standardized saponin solutions (0-10 ppm) were produced and then diluted in 2 milliliters of five percent FeCl solution, just like the samples being studied were. Upon the creation of color, the sample's absorbance and that of reference saponin solutions were measured using a Spectronic 2D spectrophotometer (Milton Roy, Houston, TX) at a wavelength of 380 nm.

2.11 Measurement of tannins

The amount of tannin in the samples was measured employing the techniques outlined by Swain (1979). The sample (two grams) was put into a 50 mL container, to which twenty milliliters of 50 percent methanol was

applied. The mixture was subsequently coated with a homogenizer and heated in a water bath at 77-80 °C for one hour while being agitated with a glass rod to avoid lumping. Using a double-layered Whatman No. 1 filter paper and 50 percent methanol as a rinse, the blend was passed through into a volumetric flask measuring 100 milliliters.

With distilled water, this was precisely prepared and well-combined. A milliliter of the sample gather was combined with 20 mL of distilled water, 2.5 mL of the *Folin-Denis* reagent, and a total of 10 mL of 17 per cent Na_2CO_3 in a volumetric flask with a capacity of 50 m after being homogenized. Distilled water was added to the proper measurements, the mixture was well-mixed, and it was let to stand for 20 minutes until a bluish-green hue appeared. The one milliliters sample mentioned was treated the same way as standard tannic acid solutions in the 0-10 ppm range. After the creation of colors, collections and standard solutions of tannic acid's absorbances were examined using a Spectronic 21D spectrophotometer at 760 nm. Tannin concentration was determined.

2.12 Measurement of total phenols

One hundred grams of the materials were extracted by stirring in 250 mL of methanol for three hours. After filtering the extracted materials using Whatman No. 1 filter paper and cleaning the remainder with one hundred milliliters of methanol, the extracts were chilled. Using a rotary evaporator and vacuum, the extracts were evaporated to dry.

To measure the entire amount of phenolic chemicals, the leftovers were dissolved in 10 milliliters of methanol. This measurement was made using the *Folin-Ciocalteau* phenol reagent and expressed as mg/100 g^{-1} of gallic acid analogues. In the specified sequence, from 0.2 milliliters of the concentrated methanol extracts, 0.8 milliliters of the *Folin-Ciocalteau* phenol reagent, and 2.0 milliliters of sodium carbonate (7.5%) were administered. After being thoroughly vortex-mixed, the blends had been diluted with 7 milliliters of deionized water. The process was permitted to finish for two hours at room temperature and in the dark before being spun up for five minutes at a force of 125 g. A spectrophotometer was used to gauge the supernatant at 756 nm. By using methanol in place of the sample, a control was applied. The outcomes were computed as gallic acid equivalents (mg/100 g^{-1}) of the sample using gallic acid as the standard. The reaction was performed three times, and the outcomes were averaged.

2.13 Measurement of total flavonoid

The above was likewise established using the procedure described by Harborne (1973). A total of five grams of the sample were cooked for 30 minutes over reflux in fifty milliliters of a 2 mol/L^{-1} HCl solution. After allowing the mixture to cool, Whatman No. 42 filter paper was used to remove the impurities. Beginning with a drop, an equal volume of ethyl acetate was added to the determined volume of the extract. Using weighted filter paper, the precipitated flavonoid was retrieved. The total weight of the flavonoid in the sample was determined by the ensuing weight discrepancy.

2.14 Analyzing the oxalate content

Oxalate was measured using AOAC's (2005) methodology. A 100 mL conical flask weighed one gram of the sample. 75 mL of 3 mol/L^{-1} H_2SO_4 was added, and the mixture was sporadically agitated with a magnetic stirring device for around one hour before it was passed using Whatman No. 1 filter paper. A twenty-five milliliter sample of the sample filtrate (extract) was taken, and it was titrated against a hot (80-90 °C) 0.1 N $KMnO_4$ solution until a light pink color developed and remained for at least 30 seconds. The formula one milliliter of 0.1 permanganate = 0.006303 g oxalate yielded the amount of oxalate present in each sample.

2.15 Statistical evaluation

Applying the Statistical Analysis System Software (SAS), all collected data underwent a one-way analysis of variance. The SAS Turkey test was used to separate the means, and significant differences were identified at $P < 0.05$.

3. Results and Discussion

3.1 Diets' gross composition

Table 1 describes the results of the composition of the diet offered to the pigs during the experiment.

Table 1. Experimental diets' gross composition.

Materials	Quantity used (kilogram)
Yellow maize	50.10
Wheat offal	12.00
Soya meal	25.00
Fish meal (72 percent)	6.00
Bone meal	4.00
Oyster shell	2.00
Lysine	0.15
Methionine	0.15
Mineral/Vitamin Premix	0.25
Salt	0.35
Total	100.00
Calculated analysis (%)	
Crude protein	17.17
Crude fibre	6.05
Ether extract	3.51
Calcium	1.30
Phosphorus	0.45
Energy (kcal/kg^{-1})	2772.9
Determined analysis (%)	
Crude protein	18.16
Crude fibre	5.90
Ether extract	3.85
Calcium	1.49
Phosphorus	0.51
Energy (kcal/kg^{-1})	2805.7

Note: **Mineral/Vitamin premix** supplied per kg diet: - vitamin A, 8,500 I.U; vitamin E, 8.66 mg; vitamin D 3,000I.U, vitamin K, 5.88 mg; vitamin B2, 5.0 mg; Niacin, 40 mg; vitamin B12, 25 mg; choline chloride, 100 mg; Manganese, 5.0 mg; Zinc, 35.1 mg; Copper, 2.0 g; folic acid, 2.5 mg; Iron, 5.8g; pantothenic acid, 10 mg; biotin, 30.5 g; antioxidant, 56 mg. Source: Authors, 2023.

3.2 Composition of secondary metabolites (bioactive compounds) in FCTNC

Table 2 shows the composition of secondary metabolites (bioactive compounds) in novel phytogenic feed additive (FCTNC). The compounds contains substantial amounts of flavonoids (1018.10 mg/100 g^{-1}), tannins (405.55 mg/100 g^{-1}), phenols (196.71 mg/100 g^{-1}), saponins (50.49 mg/100 g^{-1}), alkaloids (100.68 mg/100 g^{-1}) and oxalates (16.81 mg/100 g^{-1}).

Table 2. Composition of secondary metabolites (bioactive compounds) in FCTNC.

Constituents	Unit	Concentrations
Total flavonoids	mg/100 g^{-1}	1018.10
Total tannins	mg/100 g^{-1}	406.55
Total phenols	mg/100 g^{-1}	196.71
Total saponins	mg/100 g^{-1}	50.49
Alkaloids	mg/100 g^{-1}	100.68
Oxalates	mg/100 g^{-1}	16.81

Source: Authors, 2023.

3.3 Growth performance of weaned pigs fed novel phytogenic feed additive (FCTNC)

Table 3 revealed growth performance of weaned pigs fed novel phytogenic feed additive (FCTNC). Final body weight and weight gain values varies from 12.83 to 24.54 kg and 12.83 to 24.54 kg/pig^{-1}. Average daily weight gain (0.23 to 0.44 kg), total feed intake (38.04 to 48.25 kg), average daily feed intake (0.70 to 0.86 kg), feed conversion ratio (2.00 to 2.96) and mortality (1.00 to 2.7%). Average daily weight gain of pigs fed FCTNC significantly ($P < 0.05$) against a positive control group. Likewise, average daily feed intake increased numerically. As a result, feed conversion ratio was better for FCTNC supplemented animals relative to the other groups ($P < 0.05$).

Table 3. Growth performance of weaned pigs fed novel phytogenic feed additive FCTNC.

Parameters (Kilogram/pig^{-1})	Control (T_1)	T_2: 1.5 g/kg^{-1} Neomycin	T_3:5 g/kg^{-1} FCTNC	T_4: 10 g/kg^{-1} FCTNC	T_5: 15 g/kg^{-1} FCTNC	SEM
Initial body weight	6.31	6.30	6.29	6.28	6.26	0.15
Final body weight	19.14^{c}	27.85^{b}	28.80^{b}	30.50^{a}	30.80^{a}	0.73
Weight gain	12.83^{c}	21.55^{b}	22.51^{b}	24.22^{a}	24.54^{a}	0.62
Average daily weight gain	0.23^{c}	0.38^{b}	0.40^{b}	0.43a	0.44^{a}	0.01
Total feed intake	38.04^{b}	39.58^{b}	47.08^{a}	48.14^{a}	48.25^{a}	0.73
Average daily feed intake	0.70^{b}	0.71^{b}	0.84^{a}	0.86^{a}	0.86^{a}	0.01
Feed conversion ratio	2.96^{a}	2.66^{b}	2.09^{c}	2.00^{c}	2.00^{c}	0.01
Mortality	2.70	1.00	-	-	-	0.01

Note: Values in cells with various characters vary markedly ($P < 0.05$); SEM: standard error of mean. Source: Authors, 2023.

3.4 Haematological indices of weaned pigs fed FCTNC before the commencement of the experiment

Table 4 represents the haematological indices of weaned pigs before the commencement of the experiment. There was no effect of treatments in pack cell volume (PCVL), red blood cell (RBCL), platelets (PLT), mean platelet volume, mean corpuscular volume (MCVL), mean corpuscular haemoglobin (MCHL), mean corpuscular haemoglobin concentration (MCBC), white blood cell (WBCL), neutrophils, basophils, monocytes and lymphocytes values which varies from 27.18 to 29.10 percent, 90.80 to 91.45 g/L^{-1}, 121.3 to 128.5 (×109/L), 5.11 to 5.85 (fl), 6.60 to 7.12 (×1012/L), 34.08 to 37.00 (fl), 18.09 to 18.50 (pg), 40.80 to 45.00 g/L^{-1}, 10.08 to 10.71 (×109/L), 7.08 to 8.89 (×109/L), 0.10 to 0.14 (×109/L), 0.18 to 0.22 (×109/L) and 8.05-8.55 (×109/L) respectively.

Table 4: Haematological indices of weaned pigs fed FCTNC before the commencement of the experiment.

Parameters	Control (T_1)	T_2: 1.5 g/kg^{-1} Neomycin	T_3: 5g/kg^{-1} FCTNC	T_4: 10g/kg^{-1} FCTNC	T_5: 15g/kg^{-1} FCTNC	SEM
Pack cell volume (%)	27.18	28.02	29.10	28.65	28.70	0.03
Haemoglobin (g/L)	91.45	90.88	91.28	92.03	90.80	0.88
Platelet ($\times10^9$/L)	128.5	121.3	125.9	121.7	123.8	1.21
Mean platelet volume (fl)	5.11	5.70	5.51	5.72	5.85	0.01
Red blood cell ($\times10^{12}$/L)	6.60	6.84	7.00	7.10	7.12	0.01
Mean corpuscular volume (fl)	34.08	35.68	36.10	36.07	37.00	0.04
MCHL (pg)	18.09	18.11	18.50	18.23	18.17	0.01
MCBC (g/L^{-1})	41.60	40.80	43.10	45.17	45.00	0.02
White blood cell ($\times10^9$/L)	10.71	10.89	10.10	10.08	10.11	0.01
Neutrophils ($\times10^9$/L)	7.10	7.08	7.11	8.71	8.89	0.01
Basophils ($\times10^9$/L)	0.12	0.10	0.11	0.14	0.11	0.001
Monocytes ($\times10^9$/L)	0.18	0.16	0.21	0.22	0.20	0.001
Lymphocytes ($\times10^9$/L)	8.55	8.06	8.11	8.05	8.09	0.001

Note: MCHL = mean corpuscular haemoglobin. MCBC = mean corpuscular haemoglobin concentration. Source: Authors, 2023.

3.5 Heamatological indices of weaned pigs fed novel phytogenic feed additive (FCTNC)

Data on the heamatological indices of weaned pigs fed novel phytogenic feed additive (FCTNC) is presented in Table 5. There was statistically significant ($P < 0.05$) differences were observed in all the treatment groups except for basophils. Pack cell volume, haemoglobin, red blood cell, platelets follow similar pattern and the values varied from 28.10-34.81 %, 90.10-115.9 g/L^{-1}, 129.86-144.50 ($\times10^9$/L) and 7.71-9.67 ($\times10^{12}$/L) respectively. White blood cell 12.03-16.07 ($\times10^9$/L), neutrophils [9.00-10.89 ($\times10^9$/L)], basophils [0.1-0.18 ($\times10^9$/L)], monocytes [0.20-0.35 ($\times10^9$/L)] and lymphocytes [10.51-11.91 ($\times10^9$/L)].

Table 5. Heamatological indices of weaned pigs fed novel phytogenic feed additive (FCTNC).

Parameters	Control (T_1)	T_2: 1.5 g/kg Neomycin	T_3:5 g/kg FCTNC	T_4: 10 g/kg FCTNC	T_5: 15 g/kg FCTNC	SEM
Pack cell volume (%)	28.10^b	31.73^a	33.80^a	34.02^a	34.81^a	0.01
Haemoglobin (g/L^{-1})	90.10^b	99.56^b	107.2^a	112.8^a	115.9^a	1.07
Platelet ($\times10^9$/L)	129.86^b	135.90^a	137.10^a	142.12^a	144.50^a	1.20
Mean platelet volume (fl)	6.67^b	6.92^b	8.08^a	8.12^a	8.15^a	0.01
Red blood cell ($\times10^{12}$/L)	7.71^b	7.00^b	9.16^a	9.50^a	9.67^a	0.01
Mean corpuscular volume (fl)	50.80^b	51.84^b	60.80^a	61.22^a	63.00^a	0.03
MCHB (pg)	20.40^b	26.80^b	30.40^a	31.02^a	32.07^a	0.02
MCHBC (g/L^{-1})	45.60^b	48.77^b	50.90^a	55.67^a	56.18^a	0.03
White blood cell ($\times10^9$/L)	12.03^c	12.55^c	14.16^b	14.18^b	16.07^a	0.01
Neutrophils ($\times10^9$/L)	9.00^b	9.11^b	9.40^b	9.87^b	10.89^a	0.01
Basophils ($\times10^9$/L)	0.18	0.15	0.10	0.15	0.13	0.001
Monocytes ($\times10^9$/L)	0.20^b	0.22^b	0.25^b	0.30^a	0.35^a	0.001
Lymphocytes ($\times10^9$/L)	10.51^b	10.06^b	11.14^a	11.77^a	11.91^a	0.01

Note: Values in cells with various characters vary markedly ($P < 0.05$); SEM: standard error of mean. Source:

Authors, 2023.

3.6 Caecal microbial population of weaned pigs fed FCTNC

There was effect of treatments on Escherichia coli, Salmonella spp and Lactobacillus spp count ($P < 0.05$) as presented in (Table 6). *Escherichia coli* and *Salmonella* spp count were highest in T1 (control) relatively to the other treatments ($P < 0.05$). Conversely, *Lactobacillus* spp count were highest in T4 and T5, intermediate in T2 and T3, lowest in T1 ($P < 0.05$).

Table 6: Caecal microbial population of weaned pigs fed FCTNC.

Parameters (CFU/g^{-1})	Control (T_1)	T_2: 1.5 g/kg^{-1} Neomycin	T_3:5 g/kg^{-1} FCTNC	T_4: 10 g/kg^{-1} FCTNC	T_5: 15 g/kg^{-1} FCTNC	SEM
Escherichia coli	4.71^a	3.97^b	2.00^c	1.82^d	1.50^d	0.03
Lactobacillus spp	5.00^c	6.60^b	6.93^b	7.94^a	8.00^a	0.10
Salmonella spp	2.81^a	1.56^b	1.21^b	1.10^b	1.00^b	0.01

Note: CFU = Colony Formation Unit. Values in cells with various characters vary markedly ($P < 0.05$); SEM: standard error of mean. Source: Authors, 2023.

3.7 Immune response of weaned pigs fed FCTNC

Statistical differences ($P < 0.05$) were observed during the study in immunoglobulin A (IgA), G (IgG) and M (Ig M). Pigs fed T4 (10 g/kg^{-1} FCTNC) and T5 (15 g/kg^{-1} FCTNC) had the highest value relative to the other treatment groups ($P < 0.05$) in (Table 7). IgA values varied from 1.00-2.83 (µg/mL^{-1}), IgG [2.85 to 4.40 (µg/mL^{-1})] and IgM [2.00 to 3.96 (µg/mL^{-1})].

Table 7. Immune response of weaned pigs fed FCTNC.

Parameters (µg/mL^{-1})	Control (T_1)	T_2: 1.5 g/kg^{-1} Neomycin	T_3:5 g/kg^{-1} FCTNC	T_4: 10 g/kg^{-1} FCTNC	T_5: 15 g/kg^{-1} FCTNC	SEM
Immunoglobulin A (IgA)	1.00^c	1.88^b	2.33^a	2.42^a	2.83^a	0.11
Immunoglobulin G (IgG)	2.85^c	3.56^b	3.91^b	4.15^a	4.40^a	0.22
Immunoglobulin M (IgM)	2.00^b	2.64^b	3.88^a	3.90^a	3.96^a	0.12

Note: Values in cells with various characters vary markedly (P<0.05); SEM: standard error of mean. Source: Authors, 2023.

4. Discussion

The practice of caring for, treating, and managing animals using local or indigenous knowledge and techniques is known as ethno veterinary medicine. Ethno veterinary medicine encompasses social behaviors and methods for managing animals in farming systems in addition to the use of plants as medicines (Chris and Abel, 2019). Animal defense mechanisms are aided by the high amounts of flavonoids, which function as pharmacological inhibitors of illnesses (Oluwafemi et al., 2021).

Additionally, they are known to have a variety of pharmacological and biological effects that lower the risk of cardiovascular illnesses, such as anti-inflammatory, immune-modulatory, and anti-allergic qualities (Musa et al., 2021). Phenols lower the incidence of cardiovascular infections (Lan, 2009) and enhance blood circulation by strengthening capillaries (Mbabie et al., 2012). Alkaloids function as substances that have anti-plasmodic and analgesic characteristics (Koncić et al., 2012; Edeoga et al., 2005). Saponins may increase the permeability of the gut wall, resulting in better vitamin and mineral absorption (Manu, 2019). Oxalates present in high concentrations in the diet can prevent the body from absorbing calcium (Alagbe, 2023).

The increase in weight gain recorded among pigs in T4 (10 g/kg^{-1} FCTNC) and T5 (15 g/kg^{-1} FCTNC) along with increased feed consumption reveals better feed utilization by the animals. The presence of some bioactive

compounds in FCTNC, fenugreek (trigonelline), clove (eugenol), thyme (thymol), nutmeg (sabinene) and cumin (cuminaldehyde) could improve palatability (organoleptic stimulation), reduce retention time of feed and nutrient competition by reducing microbial pressure in the gut which would translate to better weight gain (Alagbe et al., 2022; Chen et al., 2018).

These bioactive compounds are also capable of rejuvenating intestinal epithelial cells and helps to increase the surface area of the intestinal villi to promote absorption (Franz et al., 2010) as well as perfect liver protection and other organs to reduce mortality in animals (Gabler et al., 2019). Bioactive substances have antioxidant, anti-inflammatory, antibacterial, amongst others (Gogoi et al., 2018; Agubosi et al., 2021). The outcome of this investigation agrees with the findings of Gois et al. (2016); Yang et al. (2019) when essential oil was supplemented in the diet of weaned pigs.

For disease prognosis, treatment monitoring, and feed stress monitoring, haematological tests have been found to be helpful (Togun; Oseni, 2005). According to Khan and Zafar (2005), hematological indices are reliable markers of an animal's physiological state. It was observed from this experiment that there the blood values before the commencement of the experiment were not significantly ($P > 0.05$) affected. However, all values were within the reference values for healthy weaned pig recorded by Research Animal Resource (2009).

Result after the dietary supplementation of FCTNC showed that there was a remarkable increase in pack cell volume, haemoglobin, mean platelet volume, red blood cell, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration among birds in T2 to T5 relative to the other treatment groups ($P < 0.05$). Similarly, white blood cells, neutrophils, monocytes and lymphocytes values recorded a numerical increase in T2 to T5. The pack cell volume (PCV), haemoglobin and red blood cell range recorded among pigs fed FCTNC; 28.10-34.81%, 90.10-115.9 g/L and 7.71-9.67 ($\times 10^{12}$/L) were within the values reported by Friedrichs et al. (2012).

Etiology of morphologic anaemia is assessed based on the PCV values (Alagbe et al., 2023). PCV is elevated in dehydration and polycythemia (Oluwafemi et al., 2021). Red blood cells helps to carry oxygen through the body and filter carbon dioxide (Singh et al., 2021). Platelets or thrombocytes are component of the blood whose function is to stop bleeding by clumping and clotting blood vessels (Shittu et al., 2021). In iron deficiency, mean corpuscular volume, mean corpuscular heamoglobin and mean corpuscular haemoglobin concentration are low (Adewale et al., 2021; Egeli et al., 1998). Monocytes helps to kill microorganisms, ingest foreign material and remove dead cells (Singh et al., 2021; Alagbe et al., 2019). Basophils are triggered during allergic reactions, excrete heparin for blood clotting and histamine to induce inflammation (Olafadehan et al., 2021). Neutrophils are produced in the bone marrow and are responsible in fighting microorganisms by releasing enzymes to kill pathogens (Olafadehan et al., 2021). Basophils release cytokines and also functions in signaling tissues (Adewale et al., 2021). However, all values were within the normal physiological range for a healthy weaned pig reported by Ventrella et al. (2017) and Klem et al. (2010).

The digestion and absorption of food, host metabolism, energy production, a balanced gut microbiota, mucus layer, barrier function, and mucosal immunity are all physiological and functional aspects of a healthy gut (Anna, 2021). The results of this study demonstrated that adding FCTNC to the diet considerably (P 0.05) decreased the number of harmful microorganisms, including Escherichia coli and Salmonella spp. Through the upregulation of antioxidant enzymes and the direct scavenging of reactive oxygen species, this demonstrated that the test material may both prevent the adherence of pathogens and provide significant antioxidant benefits (Anna, 2021; Alagbe, 2017).

It takes more than just the presence of disease-causing microbes in the stomach to bring about a disease. Once the gut defense is compromised by microorganisms, disease develops (Anna, 2021). Despite the incorporation of conventional antibiotics in diet of pigs in T2, the population of pathogenic bacteria were higher compared to other treatments fed FCTNC. Beneficial bacteria's (Lactobacillus spp) significantly ($P < 0.05$) increased from T1 through T5. A protective barrier between the host and the bacteria is created by commensal microbiota, which also stimulates the immune system's growth, including the mucus layer (Cremonesi et al., 2022; Li et al., 2021; Li et al., 2022). Since the gastro intestinal tract is the immune system's largest organ and serves as a vital physiological function as a barrier against viruses and antigens, maintaining its integrity is essential for immune system performance (Hu et al., 2019). The findings of Zhe et al. (2023), who fed weaned pigs Litsea cubeba essential oil, are consistent with the results of this investigation.

Immunity is a collection of responses to various aggressors that the body has identified as antigens (Marie, 2020). Pigs fed FCTNC (T3 to T5) had significantly ($P < 0.05$) increased immunological parameters, indicating that FCTNC may be able to alter immune responses through diverse pathways through interactions with the immune

system. The control of cytokine expression is one of these ways, and it is essential for both the development of the innate immune system and the adaptive immune system (Andreas, 2021).

Due to the inclusion of phytochemicals or other bioactive substances in the test materials, they may also control the expression of pro-inflammatory mediators and enzymes implicated in the NF-kB and mitogen activated protein kinase pathways (Marie, 2020). Animals bred without the use of antibiotics can be more productive and have healthier immune systems with the help of dietary immunomodulation (Alagbe, 2023).

Immunoglobulin A (IgA) is the first line of defense in the resistance against infection through inhibiting bacteria adhesion to epithelial cells and by neutralization of bacteria toxins both intracellular and extracellular (Neeray, 2021). Immunoglobulin M (IgM) and IgG aids in the excretion of pathogens and neutralization of toxins respectively (Adreas, 2021). This study's findings is in agreement with the reports of Wang et al. (2021) when Macleaya cordata extract was supplemented in the diet of weaned pigs. Chen et al. (2019) also recorded a numerical increase ($P < 0.05$) in immune parameters of pigs fed phytogenic feed additive.

5. Conclusions

Phytogenic compounds are a promising class of additions in the antibiotic alternatives. This viewpoint is supported by the existence of an unlimited number of plants that contain extremely potent compounds. Numerous antibacterial components and maybe new modes of action can be found in phytogens. Weaned pigs' growth performance, gut microbiota, immunological response, and blood profile were all improved by adding up to 15 g/kg^{-1} of FCTNC to their diet without having a negative impact on the animals' overall health.

6. Acknowledgments

We thank the institutions: Sumitra Research Institute, India; University of Abuja, Nigeria; Ladoke Akintola University of Technology, Nigeria, and Prince Abubakar Audu University, Nigeria.

7. Authors' Contributions

Alagbe Olujimi John: experimental design, data collection, data analysis, study writing, corrections and publication. *Daniel Nnadozie Anorue*: experimental design, animal experimentation, biological analysis, writing and corrections. *Muritala Daniel Shittu*: Hematological analysis, data analysis and corrections. *Sadiq Muhammad Ramalan*: experimental analysis, data collection, study writing and scientific reading. *Tolulope Oreoluwa Faniyi*: data analysis, writing, corrections and publication. *Adekunle David Ajagbe*: experimental analysis, data analysis, verification of results, scientific writing and corrections.

8. Conflicts of Interest

The author declare that there was no conflict of interest.

9. Ethics Approval

Yes applicable.

10. References

Alagbe, O. J., Oluchi, C., Precious, A., Rufus, A. O., Taiwo, O. A., Adegoke, E. A., & Emiola, I. A. (2023). Haemato-biochemical indices and intestinal microbial population of broiler chickens fed diet supplemented with *Prosopis africana* (African mesquite) essential oil. *Brazilian Journal of Science*, 2(9), 98-110. https://doi.org/10.14295/bjs.v2i9.390

Alagbe, O. J., Agubosi, O. C. P., & Oluwafemi, R. A. (2023). Histopathology of broiler chickens fed diet supplemented with *Prosopis africana* (African mesquite) essential oil. *Brazilian Journal of Science*, 2(9), 49-59. https://doi.org/10.14295/bjs.v2i9.385

Alagbe, J. O., Kadiri, M. C., Oluwafemi, R. A., Agubosi, O. C. P., & Anorue, D. N. (2023). Analysis of bioactive compounds in ethanolic extracts of *Xylopia aethiopica* leaves using gas chromatography and mass spectrometry technique. *European Science Methodical Journal*, 1(1), 1-10.

https://europeanscience.org/index.php/3/article/view/7

Alagbe, J. O. (2023). Bioactive compounds in ethanolic extract of *Strychnos innocua* root using gas chromatography and mass spectrometry (GC-MS). *Drug Discovery*, 2023, 17, e4dd1005. https://www.discoveryjournals.org/drugdiscovery

Alagbe, J. O., Ramalan, S. M., Shittu, M. D., & Olagoke, O. C. (2022). Effect of *Trichilia monadelpha* stem bark extract on the fatty acid composition of rabbit's thigh meat. *Journal of Environmental Issues and Climate Change*, 1(1), 63-71. https://doi.org/10.59110/jeicc.v1i1.76

Alagbe, J. O., & Adegbite, M. B. (2019). Haematological and serum biochemical indices of starter broiler chicks fed aqueous extract of *Balanites aegyptiaca* and *Alchornea cordifolia* bark mixture. *International Journal of Biological, Physical and Chemical Studies*, 1(1), 8-15. https://www.al-kindipublisher.com/index.php/ijbpcs/article/view/415

Alagbe, J. O. (2017). Effect of dietary inclusion of *Polyalthia longifolia* leaf meal as phytobiotic compared with antibiotics on performance, carcass characteristics and haematology of broiler chicken. *Scholarly Journal of Agricultural Science*, 7(3), 68-74.

Alagbe, J. O. (2017). Effect of dietary inclusion of *Polyalthia longifolia* leaf meal as phytobiotic compared with antibiotics on the nutrient retention, immune response and serum biochemistry of broiler chicken. *Greener Journal of Agricultural Sciences*, 7(3), 74-81. http://doi.org/10.15580/GJAS.2017.3.052217067

Adewale, A. O., Alagbe, J. O., & Adeoye, A. O. (2021). Dietary supplementation of *Rauvolfia vomitoria* Root extract as a phytogenic feed additive in growing rabbit diets: Haematology and serum biochemical indices. *International Journal of Orange Technologies*, 3(3), 1-12. https://doi.org/10.31149/ijot.v3i3.1328

Andreas, S. M. (2021). Effect of phytogenic feed additives on bird health. *International Poultry Production*, 22(6), 2-3.

Anna, C. B. (2021). Gut health is the key to reducing antimicrobial use. *International Pig Tropics*, 32(3), 4-6.

Aufy, A., Steiner, T., & Zentner, E. (2011). Phytogenic feed additives and ammonia emissions – thinking outside the box. *International Pig Tropics*, 26(7), 13.

Brunner, J. H. 1984. Direct spectrophotometric determination of saponins. *Analytical Chemistry*, 34, 1314-1326.

Chen, J. S., Kang, B. J., Yao, K., Fu, C. X., & Zhao, Y. R. (2019) Effects of dietary *Macleaya cordata* extract on growth performance, immune responses, antioxidant capacity, and intestinal development in weaned piglets. *Journal of Applied Animal Research*, 47(1), 349-356. https://doi.org/10.1080/09712119.2019.1636800

Chen, J. S., Kang, B. J., Zhao, Y. R., Yao, K., & Fu, C. X. (2018). Effects of natural dietary supplementation with *Macleaya cordata* extract containing sanguinarine on growth performance and gut health of early-weaned piglets. *Journal of Animal Physiology Animal Nutrition*, 102(6), 1666-16774. https://doi.org/10.1111/jpn.12976

Chris, K., & Abel, S. (2019). Gut flora and immune modulation by plant extracts. International Pig Magazine, 2(3), 6-8.

Cremonesi, P., Biscarini, F., Castiglioni, B., Sgoifo, C.A, Compiani, R., & Moroni, P. (2022). Gut microbiome modifications over time when removing in-feed antibiotics from the prophylaxis of post-weaning diarrhea in piglets. *PLoS One*, 17(3), e0262199. https://doi.org/10.1371/journal.pone.0262199

Edeoga, H. O, Okwu, D. E., & Mbaebie B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal Biotechnology*, 4(7), 685-688. https://doi.org/10.5897/AJB2005.000-3127

Egeli, A. K., Framstad, T., & Morberg, H. (1998). Clinical biochemistry, hematology and body weights in piglets. *Acta Veterinaria Scandinavica*, 39, 381-393. https://doi.org/10.1186/BF03547786

Freidrichs, K. R., Harr, K. E., & Freeman, K. P. (2012). ASVCP reference interval guidelines: Determination of de novo reference interval in veterinary species and other related topics. *Veterinary Clinical Pathology*, 41(4), 441-453. https://doi.org/10.1111/vcp.12006

Gabler, N., Helm, E., De Mille, C., Gabler, N. K, Helm, E.T., & De Mille, C. (2019). Impact of weaning stress, disease, and diet on pig performance, intestinal function and integrity. *Journal of Animal Science*, 97(2), 31-32. https://doi.org/10.1093%2Fjas%2Fskz122.058

Gois, F. D, Cairo, P. L. G, Cantarelli, V. D., Costa, L. C. D., Fontana, R., Allaman, I. B., Sbardella, M., de Carvalho, F. M., & Costa L. B. (2016). Effect of brazilian red pepper (*Schinus terebinthifolius* Raddi)

essential oil on performance, diarrhea and gut health of weanling pigs. *Livestock Science*, 183, 24-27. https://doi.org/10.1016/j.livsci.2015.11.009

Harborne, J. B. (1973). Phytochemical methods: a guide to modern techniques of plant analysis. Chapman and Hall Ltd, London, U.K.

Hu, W., Li, C. Z, Dai, J. M, Cui, H. Y., & Lin, L. (2019). Antibacterial activity and mechanism of *Litsea cubeba* essential oil against methicillin-resistant *Staphylococcus aureus* (MRSA). *Indian Crops and Products*, 130, 34-41. https://doi.org/10.1016/j.indcrop.2018.12.078

Ines, G. (2020). Phytogenics: successful AGP replacements in swine diets. *International Pig Magazine*, 28(1), 4-6. http://www.positiveaction.info/pdfs/articles/pt28.1p7.pdf

Khan, T. A., & Zafar, F. (2005). Haematological study in response to various doses of estrogen in broiler production. *International Journal of Poultry Science*, 40(10), 748-751.

Klem, T. B., Bleken, E., Morberg, H., Thoresen, S. I., & Framstad, T. (2010). Hematologic and biochemical reference intervals for Norwegian pigs. *Veterinary Clinical Pathology*, 39(2), 221-226. https://doi.org/10.1111/j.1939-165X.2009.00199.x

Koncić, M. Z., Kremer, D., Gruz, J., Strnad, M., G. Biševac, G., Kosalec, I., Šamec, D., Piljac-Žegarac, J., & Karlović, K. (2012). Antioxidant and antimicrobial properties of *Moltkia petraea* (tratt.) Griseb. flower, leaf and stem infusions. *Food Chemistry and Toxicology*, 48(6), 1537-1542. https://doi.org/10.1016/j.fct.2010.03.021

Li, H., Ma, L. T., Li, Z. Q, Yin, J., Tan, B., Chen, J. S, Jiang, Q., & Ma, X. K. (2021). Evolution of the gut microbiota and its fermentation characteristics of Ningxiang pigs at the young stage. *Animals*, 11(3), 638. https://doi.org/10.3390/ani11030638

Li, Q., Yu, C. N., Chen, Y. H, Liu, S. X, Azevedo, P., Gong, J., Karmin, O., & Yang, C. B. (2022). *Citral alleviates* peptidoglycan-induced inflammation and disruption of barrier functions in porcine intestinal epithelial cells. *Journal of Cellula Physiology*, 237(3), 1768-1779. https://doi.org/10.1002/jcp.30640

Liliana, L. B., & Melina, B. (2019). Producing quality poultry with antibiotic free programs. *International Poultry Production*, 26(6), 4-6.

Manu, D. L. (2019). Support gut health with a unique combination of fatty acid and phytogenics. *International Pig Magazine*, 4(2), 1-2.

Marie, L. L. (2021). Immunity – an underestimated consumer of nutrients. *International Pig Production*, 1(8), 4-6.

Mbaebie, B. O., Edeoga, H. O., & Afolayan, A. J. (2012). Phytochemical analysis and antioxidants activities of aqueous stem bark extract of *Schotia latifolia* Jacq. *Asian Pacific Journal of Tropical Biomedicine*, 2(2), 118-124. https://doi.org/10.1016/S2221-1691(11)60204-9

Muritala, D. S., Alagbe, J. O., Ojebiyi, O. O., Ojediran, T. K., & Rafiu, T. A. (2022). Growth performance and haematological and serum biochemical parameters of broiler chickens given varied concentrations of *Polyalthia longifolia* leaf extract in place of conventional antibiotics. *Animal Science and Genetics*, 18(2), 57-71.

National Research Council. (2012). Nutrient Requirements of Pigs: Ninth Revised Edition, 2012. Washington, DC: The National Academies Press. https://doi.org/10.17226/2114

Neeray, K. K. (2021). Immunoglobulin types, structure and functions. Materials for Nurses Preparation, p 22.

Olafadehan, O. A., Oluwafemi, R. A., & Alagbe, J. O. (2020). Performance, haemato-biochemical parameters of broiler chicks administered Rolfe (*Daniellia oliveri*) leaf extract as an antibiotic alternative. Drug Discovery, 14(33), 135-145. http://discoveryjournals.org/drugdiscovery/current_issue/2020/v14/n33/A19.pdf

Oluwafemi, R. A., Uankhoba, I. P., & Alagbe, J. O. (2021). Effects of turmeric oil as a dietary supplements on the growth performance and carcass characteristics of broiler chicken. *International Journal of Orange Technologies*, 3(4), 1-9. https://doi.org/10.31149/ijot.v3i4.1602

Oluwafemi, R. A., Uankhoba, I. P., & Alagbe, J. O. (2021). Effects of turmeric oil as a dietary supplement on the haematology and serum biochemical indices of broiler chickens. Concepts of Daury & Veterinary Sciences, 4(4), 447-451. https://doi.org/10.32474/CDVS.2021.04.000193

Oluwafemi, R. A., Daniel, S. E., & Alagbe, J. O. (2021). Haematological and serum biochemical indices of

broiler chicks fed different inclusion levels of ginger (*Zingiber officinale*) and garlic (*Allium sativum*) oil mixture. *Asian Journal of Advances in Research*, 1(4), 1068-1074. https://www.mbimph.com/index.php/AJOAIR/article/view/2519

Oluwafemi, R. A., Abdullahi, H., & Alagbe, J. O. (2021). Effect of dietary inclusion of ginger (*Zingiber officinale*) and garlic (*Allium sativum*) oil mixture on the growth performance and caecal microbial population of broiler chickens. *International Journal of Clinical Ccase Reports and Reviews*, 8(5), 1-5. https://doi.org/10.31579/2690-4861/161.

Research Animal Resource (RAR). (2009). Reference values for laboratory animals. Normal haematological values. RAR Values. RAR University of Minnesota.

Roberto, M. N. (2018). Phytogenic compounds in the management of male poultry infertility. *International Hatchery Practice*, 35(7), 7-8.

Singh, A. S., Alagbe, J. O., Sharma, S., Oluwafemi, R. A., & Agubosi, O. C. P. (2021). Effect of dietary supplementation of melon (*Citrallus linatus*) seed oil on the growth performance and antioxidant status of growing rabbits. *Journal of Multidimensional Research and Reviews*, 2(1), 78-95. https://doi.org/10.47540/ijias.v1i2.175

Shittu, M. D., Alagbe, J. O., Adejumo, D. O., Ademola, S. G., Abiola, A. O., Samson, B. O., & Ushie, F. T. (2021). Productive performance, caeca microbial population and immune-modulatory activity of broiler chicks fed different levels *Sida acuta* leaf extract in replacement of antibiotics. *Journal of Multidimensional Research & Review*, 2(4), 34-42. https://jmrr.org/V2I4/V2I4P05.pdf

Swain, T. (1979). Tannins and lignins. *In*: Rosenthal, G. A., & Janzen, D. H. eds. Herbivores: their interactions with plant metabolites. Academic Press, New York, NY, p. 657-682.

Togun, V. A., & Oseni, B. S. A. (2005). Effect of low level inclusion of biscuit dust in broiler finisher diet apparently healthy red Sokoto goats. Proceeding of the 27th Annual Conference of Nigeria Society for Animal Production, 50-53.

Ventrella, D., Dondi, F., & Barone, F. (2017). The biomedical piglet: Establishing reference intervals for haematology and clinical chemistry parameters of two age group with or without iron supplementation. *BMC Veterinary Research*, 13. https://doi.org/10.1186/s12917-017-0946-2

Vera, B. K. (2021). Essential oils knowledge: from facts to farm or from farm facts. *International Poultry Production*, 25(3), 2-3.

Wang, F., Yin, Y. Y., Yang, M., Chen, J. S., Fu, C. X., & Huang, K. (2021). Effects of combined supplementation of Macleaya cordata extract and benzoic acid on the growth performance, immune responses, antioxidant capacity, intestinal morphology, and microbial composition in weaned piglets. *Frontiers Veterinary Science*, 8. https://doi.org/10.3389/fvets.2021.708597

Yang, J. J., Wang, C. L., Huang, K. H, Zhang, M. H., Wang, J., & Pan, X. C. (2020). Compound *Lactobacillus* sp. administration ameliorates stress and body growth through gut microbiota optimization on weaning piglets. *Applied Microbiology Biotechnology*, 104, 6749-6765. https://doi.org/10.1007/s00253-020-10727-4

Zhe, Y., Fang, W., Yexin, Y., Peng, H., Qian, J., Zhimou, L., Yulong, Y., & Jiashun, C. (2023). Dietary *Litsea cubeba* essential oil supplementation improves growth performance and intestinal health of weaned piglets. *Animal Nutrition*, 13, 9-18. https://doi.org/10.1016/j.aninu.2022.11.004

Funding

The study has not received any external funding.

Intitutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.