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GROWTH PERFORMANCE, HAEMATOLOGICAL AND SERUM BIOCHEMICAL INDICES OF WEANER PIGS FED Carica papaya SEED AND LEAF MEAL AS DIETARY SUPPLEMENT

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Supporting Information

ABSTRACT: A 28-day feeding trial was carried out to evaluate the performance characteristics and hematobiochemical parameters of weaner pigs fed graded levels of *Carica papaya* seed and leaf meal supplementation. A total of 36 cross-bred (Large white x Landrace) strains of weaner pigs of average initial weight of 8.86 ± 0.10 kg were used for the study. Four treatment diets designated T_1 , T_2 , T_3 and T_4 replicated 3 times in a completely randomized design (CRD) were formulated to include the *Carica papaya* leaf and seed meal at 0, 1, 2 and 3% levels, respectively. Data were collected on daily feed intake (DFI), daily weight gain (DWG), feed conversion ratio (FCR), cost benefit analysis, hematological indices and serum biochemistry. The body weight gain of the weaner pigs was highest in T_4 (3%) *Carica papaya* seed and leaf meal mix (CSLM) supplementation with the value of 353 g while the least value of 228 g was recorded by T_2 which significantly differed (P<0.05) from the control group. Hemato-biochemical parameters showed significant differences (P<0.05) between treatments, indicating positive influence of CSLM on the investigated parameters. The blood urea nitrogen and creatinine, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase concentration increased as the dietary levels of CLSM increased in the diets. Based on the above findings, it is recommended that CLSM can be included at a level of 3% for optimum performance, hemato-biochemical stability and profit maximization.



Keywords: Carica papaya, Dietary supplementation, Haematology, Serum biochemistry, Weaner pigs, biochemistry, supplement

INTRODUCTION

The increasing demand for animal protein coupled with more stringent economic conditions in Nigeria have encouraged greater interest in fast growing animals like pigs with short generation interval. Janson (2018) stated that with rising demands for animal protein production and continuous challenges in availability and composition of feed raw materials, the need for advancements in sustainable animal production rapidly increases. Moreover, with no end in sight to the war in Ukraine and tight global stocks, uncertainty continues to hang over conventional feed raw materials like wheat, soybean, millet and oilseed cakes etc. (AMIS, 2023).

Pig production despite the social and religious barriers attached to it is gradually gaining ground in Nigeria though dominated by small scale pig farmers. According to the Federal Ministry of Agriculture and Rural Development (FMARD), the Nigerian pig population stand at 8.0 million (FAOSTAT, 2019) as against the population figure of 3.6 million reported by National Agricultural sample survey (National Bureau of Statistics, 2016). This represents only 1.05% of the world pig population. Consumption of pork in Nigeria is on upward spiral trend despite the Muslims and Pentecostals religious views of the meat and intensifying pig productivity will certainly bridge the animal protein deficit gap and boost economic prosperity in sub-Saharan African countries (Nkwocha et al., 2021).

Sastry and Thomas (2015) reported that there is a wide range of feedstuff on which pigs can live on. Therefore, alternative feed sources need to be investigated such as *Carica papaya* leaf along with usual feed material as *Carica papaya* leaf and seeds have been used as animal feed in many places.

Carica papaya is a multipurpose plant with useful characteristics. Scientific studies revealed the existence of considerable levels of glycosides, flavonoids, alkaloids, saponins, phenolic compounds, amino acids, lipids, carbohydrates, enzymes, vitamins, and minerals in papaya leaves (Alara et al., 2022; Ugo et al., 2019). *Carica papaya* leaves and seeds are used as livestock feed and its twigs are reported to be very palatable to ruminants and have appreciable crude protein

levels ranging from 21-25.1% (Rahmasari et al., 2021). *Carica papaya* is the power house of papain, cystatin, chymopapain A and B, tocopherol, glucosinolates, vitamin C and papaya peptidase A (Oloruntola et al., 2018; Palanisamy and Basalingappa, 2020). According to Maisarah et al. (2014), proximate analysis of dried pawpaw seeds contains 97.27% dry matter, 30.08% crude protein, 34.80% crude fat, 1.67% crude fiber, 7.11% ash and 23.67% nitrogen free extract.

The leaves of *Carica papaya* are an excellent source of the sulphur containing amino acids, methionine and cysteine, which are often limiting in most feedstuff used for feeding animals (Abdel-Halim et al., 2020). Onyimonyi and Ernest (2009) showed that dietary inclusion of 2% *Carica papaya* leaf meal in finisher diets improved carcass and organoleptic traits of swine meat and moreover, the phenolic compounds in leaf meals inhibits lipogenesis in animals. In a similar experiment on *Carica papaya* seed meal reported by Saputri et al. (2017), adding water extract of papaya seeds as much as 300 mg/kg/day was able to reduce cholesterol by 13.39% and SGPT level 31.4% in hypercholesterolemic white male rats. Calvache et al. (2016) treated papaya peel residues with ethanol and drying them in a microwave oven to generate dietary fibre concentrates (DFCs) in order to demonstrate its antioxidant activity. Carotenoids, phenolics, ascorbic acid, proteocatechuic acid, manghaslin, quercetin 3-0-rutinosides, caffeoyl hexoside, ferulic acid, lutein, zeaxanthin, and beta-carotene were detected in the chromatographic analysis of the samples. Sharma et al. (2022) investigated the antioxidant bioactives, biological activities, innovative products and safety aspects of *Carica papaya* leave extracts and demonstrated successful medical evidence for the use of papaya leaf extracts in the health care system as a supplemental herbal medication in a variety of clinical settings.

In line with enough medical and nutritional evidence of the value of papaya leaves and seed meals, it is therefore ideal to evaluate the nutritive value of *Carica papaya* leaf and seed meal so as to determine the optimal dietary inclusion level for optimum productivity of weaner pigs.

MATERIALS AND METHODS

Experimental site

This study was carried out at the Teaching and Research farm of the Imo state Polytechnic Umuagwo- Ohaji, Imo State. The site has geographical coordinates of latitudes 5° 17¹ and 5° 19¹ N and longitudes 07° 54¹ and 06° 56¹ E of the equator with temperature ranging from (26.5–32 °C) and humidity of (70–85%). The mean annual rainfall ranges between (2000–2500 mm). The soil is sandy loamy and slightly acidic (NIMET, 2015).

Experimental animals and design

36 cross-bred (Large White x Landrace) strains of weaner pigs with average initial weight of 8.86±0.10 kg were used for the study. Pigs were acclimatized in the study area by feeding control diet for one week during which time routine management practices notably deworming was carried out to keep endoparasites under check and oxytetracycline were also given to the pigs as prophylactics against bacterial infections. The pigs were divided into 4 groups of 9 weaner pigs each in a completely randomized design (CRD) and fed one of the experimental diets for 28 days. Each treatment was replicated three times (3 weaner pigs per replicate). Feed and clean water were provided *ad libitum*. Feed intake was recorded daily by computing the difference between the feed offered and the feed left over throughout the duration of the experiment.

Housing and management

The pigs were housed in pens with total area measuring 22.4 m² i.e. (7.0 x 3.2) m divided into 16 compartments, each measuring 1.4×1.0 m. The piggery house is constructed with concrete floor with dwarf wall in which the open part was covered with wire mesh. The roof was covered with corrugated iron sheets with overhang to prevent the splashing of water into the pens. The pens were washed and disinfected with germicide "Izal" one week before the commencement of the experiment.

Ethical regulation of experiment

This study was approved by the Animal Production and Health Departmental Board of the Imo State Polytechnic, Umuagwo-Ohaji, Owerri, Imo State, Nigeria. The Authors voluntarily participated and there was no deception, no risk of harm, accuracy of reporting were ensured and they complied with the ARRIVE guidelines.

Preparation of Carica papaya seeds and leaf meal

Fresh Carica papaya seeds and leaves used for this experiment were plucked at the forestry unit of Imo State Polytechnic, Umuagwo, Imo state. The leaves were chopped, spread on a clean tarpaulin under the shade to air-dry for about 10 days and then milled to *Carica papaya* leaf meal (CLM). Seeds were collected from ripped pawpaw fruits and were spread lightly on tarpaulin to air-dry for 10 days and then ground to form *Carica papaya* seed meal (CSM). The CLM

and CSM were thoroughly mixed together in a ratio of 2:2 to form *Carica papaya* leaf and seed meal composite mix (CSLM) and subjected to proximate analysis in accordance with standard methods of AOAC (2016). The *Carica papaya* seed and leaf meal mix were therefore included at the levels of 0% (T₁), 1% (T₂), 2% (T₃) and 3% (T₄) accordingly. Other conventional feed ingredients such as Maize, rice meal, fish meal, groundnut cake (GNC), wheat offal, bone meal, palm kernel cake (PKC) and salt were procured from certified raw material sales outlets at Owerri, Imo State and injected into the feed in line with their inclusion rate (Table 1).

Phytochemical analysis

The CSLM was analyzed in the Laboratory and the percentage proportions of the respective toxicants notably; Tannins, Flavonoids, alkaloids, saponins, cardiac glycosides, phytate, oxalate, phenols, steroids, terpenoids etc. were evaluated using elaborate laboratory procedures as described by Roghini and Vijayalakshmi (2018) (Table 2).

| Dietary levels of CSLM | T _{1 (0%)} | T _{2 (1%)} | T3 (2%) | T4 (3%) |
|------------------------|---------------------|---------------------|---------|---------|
| Ingredients | 11(0%) | 2 (1%) | 13 (2%) | 4 (3%) |
| Maize meal | 34.00 | 33.00 | 33.00 | 33.00 |
| Rice meal | 25.75 | 25.75 | 24.75 | 24.75 |
| CSLM | 0 | 1 | 2 | 3 |
| GNC | 18 | 18 | 18 | 18 |
| Wheat offal | 8.00 | 8.00 | 8.00 | 7.00 |
| Fish meal | 3 | 3 | 3 | 3 |
| P.K.C | 8 | 8 | 8 | 8 |
| Bone meal | 3 | 3 | 3 | 3 |
| Salt (Nacl) | 0.25 | 0.25 | 0.25 | 0.25 |
| Total | 100 | 100 | 100 | 100 |
| Calculated analysis | | | | |
| Energy (kilocal/kg) | 2810.65 | 2810.65 | 2810.65 | 2810 |
| Crude Protein (CP) | 19.045 | 19.045 | 19.03 | 19.03 |

 Table 2 - Proximate composition of Carica papaya seed and leaf meal composite mix.

| Parameter | Composition (%) |
|---|-----------------|
| Dry matter | 93.70 |
| Crude protein | 17.00 |
| Ash | 7.14 |
| Ether extract | 12.42 |
| Crude fibre | 18.49 |
| Nitrogen free extract | 24.28 |
| ME (Kcal/kg) | 2496.96 |
| ME = Metabolizable energy calculated; ME (Kcal/kg) = 37x %CP + 81 X %EE + 35.5 X %NFE (Pauzenga, 1985). | |

Haematological and serum biochemical studies

At the end of the 28 day feeding trials, three pigs were randomly selected from each treatment i.e. a pig per replicate, starved of feed overnight but was given access to water. Blood samples were collected (10 ml per pig) through the vein at the ham section with a 10 ml sterile syringe after local disinfection with methylated spirit. Five (5) ml of blood samples were collected into Bijou bottles containing ethylene diamine tetra acetic acid (EDTA) as the anti-coagulant and shaked vigorously to avoid coagulation. The remaining 5 ml of blood samples for serum biochemical indices were dispensed into labeled bottle (without EDTA) and the blood sera were separated by centrifuging for 10 minutes at 2000 revolutions per minute (rpm) at 4°C after which the sera was decanted into a well labeled bottle for further analysis. Blood samples for haematology were taken to the laboratory and analyzed for the following parameters namely haemoglobin (Hb), packed cell volume (PCV), white blood cell (WBC), red blood cell (RBC), mean cell volume (MCV), mean cell haemoglobin concentration (MCHC) and blood clotting Time (BCT) according to Merck Veterinary Manuals, (Aiello et al., 2016).

Serum biochemical indices variables taken were blood urea, serum creatinine, blood glucose, cholesterol, serum total protein, albumin, globulin, alkaline phosphatase, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and were determined according to Latimer (2003).

Statistical analysis

All data collected were subjected to analysis of variance (ANOVA) using Statistical Package for Service Solution (SPSS) version 20. The treatment means were separated by Fisher's Least Significant Difference (LSD) Test (Williams and Abdi, 2010).

RESULTS AND DISCUSSION

Tables 2 and 3 showed the proximate and phytochemical composition of *Carica papaya* seed and leaf meal mix. The nutritive assessment of CSLM showed that the mix contained 17.00% crude protein while other compositional values of 7.14, 12.42, 18.49 and 24.28% were recorded for ash, ether extract, crude fibre and nitrogen free extract respectively (Table 2). The combination of *Carica papaya* seed and leaf meal as a dietary supplement positively enhanced the crude protein content of the test ingredient. The oily nature of pawpaw seed reflected in the value obtained for ether extract of the CSLM and this agreed with the reports of Rahmasari et al. (2021) that the seed is high in lipids. The crude fibre level of CSLM is relatively high probably due to the presence of non-starchy polysaccharides existing in the leaves (Bidhendi and Geitmann, 2016; Rahmasari et al., 2021). The presence of high level of ash content indicates that the total inorganic mineral in CSLM is high (Maisarah et al., 2014).

The test additive (CSLM) significantly (P<0.05) affected the average daily feed intake, weight gain, feed conversion ratio and cost per kg live weight of weaner pigs (Table 4). T₄ recorded the highest feed intake (635 g) followed by T₁ (600 g) >T₂ (540 g) >T₃ (522 g) in that order. Moreover, there was a proportional increase in average daily weight gain (ADWG) as CSLM increased in the ration. The T₄ promoted the highest weight gain while the least was recorded by weaner pigs in group 2 (T₂). Weight gain is a function of feed utilization for the formation of adipose tissues. T₂ promoted the least weight gain of 228 g but a positive trend of weight gain was observed in T₃ and T₄ with higher inclusion levels of CSLM. The higher weight gain of T₃ (260 g) and T₄ (353 gm) may be attributed to the action of papain, a natural enzyme occurring on the leaves of *Carica papaya* plant which helps in the digestion of proteins in the digestive tract and synthesis of vitamins C and E (Singh et al., 2011; FAO, 2014; Oloruntola et al., 2018; Rahmasari et al., 2021; Sharma et al., 2022). According to Barroso et al (2016), the major active ingredient recorded in pawpaw seed such as carpine, chymopapin, and papain are enzymes capable of enhancing appetite and metabolism and this appears to be responsible for increased daily feed intake while benzyl isothiocyanate has active anthelminthic activity.

Feed conversion ratio (FCR) increased (P<0.05) as dietary CSLM increased in the ration. The best FCR was recorded by T₄ which was consistently different (P<0.05) from other treatment means. Conversely, the control diet (T₁) was significantly costlier than CSLM based diets. Nevertheless, efficiency in relationship to cost reduction is influenced by other variable notably growth rate and feed conversion ratio which as a matter of fact T₄ < T₃ were the cheapest (Table 5).

Although studies on the utilization of *Carica papaya* seed and leaf meal is limited in swine, it was shown that papaya seed meal was able to improve the production performances of poultry, including increasing growth rate, egg production, and feed efficiency of poultry. In broiler chickens, Muazu and Aliyu-Paiko (2020) showed that incorporation of 1% papaya seed powder in rations increased the final body weight and feed intake. In line with this, Rachmatika and Prijono (2015) reported that incorporation of 1.2% papaya seed in diets increased body weight gain, reduced feed intake, and improved feed efficiency of *Raja* ducks. Likewise, Soedji et al. (2017) documented that inclusion of 0.5, 1.0, and 2% papaya seed in the rations increased daily weight gain of pullet when compared with the control.

| Parameter | Qualitative Score | Quantitative |
|--------------------|-------------------|--------------------------------|
| Tannins | + | 20.14% |
| Saponins | + | 42.70 μg/ml |
| Alkaloids | + | 63.05 μg/ml |
| Flavanoids | + | 1375.40 μg/ml |
| Phytate | + | 7.95% |
| Oxalate | + | 2.8 x 10 ⁴ mg/100 g |
| Phenols | - | 0 |
| Cardiac Glycosides | ++ | 181.34% |
| Steroids | ++ | 54.17 mg/100 g |
| Terpenoids | ++ | 118.85 mg/100g |

Table 3 - Quantitative and qualitative values of phytochemical analysis of *Carica papaya* seed and leaf meal composite mix (CSLM).

| Dietary levels of CSLM | T1 (0%) | T _{2 (1%)} | T _{3 (2%)} | T4 (3%) | SEM |
|---|-----------------|---------------------|---------------------|----------------|---------------|
| Parameters | 11(0%) | 2 (1%) | 13 (2%) | 4 (3%) | JEIN |
| Initial body weight (kg) | 8.90 a | 8.96 a | 8.73 ^b | 8.83 a | 0.06 |
| Final body weight (kg) | 17.70 a | 15.33 bc | 16.00 b | 18.70 ª | 0.89 |
| Body weight change (kg) | 8.80 a | 6.37 ^b | 7.27 b | 9.87 a | 0.90 |
| Daily feed intake (gm) | 600 ª | 540 ^b | 522 ^b | 635 a | 30.32 |
| Daily body weight gain (gm) | 314 a | 228 ^b | 260 b | 353 a | 32.15 |
| Feed conversion ratio | 1.91 bc | 2.37 a | 2.01 ^b | 1.86 ° | 0.14 |
| Cost/kg live weight (\$) | 0.16 | 0.10 | 0.09 | 0.09 | 8.19 |
| $T_1\!\!:$ diet without CLSM (control), $T_2\!\!:$ diet containing 1% CLSM, $T_3\!\!:$ diet | containing 2% C | LSM and T4: die | t containing 3 | % CLSM. abc Me | ans along the |

 Table 4 - Performance characteristics of weaner pigs on graded levels of Carica papaya seed and leaf meal supplementation

Haematological values such as HB, WBC, RBC, MCV, MCH, MCHC and BCT obtained for pigs fed control diet (T₁) were superior when compared with groups fed CSLM based diets. This definite trend of reduction in the haematological parameters though in line with the recommended values reported by Latimer (2003) for normal haematological reference range for pigs did not negatively affect the performance parameters (Table 5). PCV ranged from 37.80-46.00% while haemoglobin, white blood cells and red blood cells ranged from 12.60-15.30 g/dl, 3.10-5.10x10³ μ I and 4.50-7.40x10⁶ μ I respectively.

The serum biochemistry of weaner pigs fed graded levels of *Carica papaya* seed and leaf meal mix revealed that urea, creatinine, ALP, ALT, AST concentrations significantly (P<0.05) increased as dietary levels of CSLM increased in the diets. However, the values for creatinine (1.50-2.10 mg/dl); urea (18-23 mg/dl); alkaline phosphatase (41-65.00 μ l); alanine aminotransferase (8.60-14 μ l) and aspartate aminotransferase (8-11 μ l) recorded in this study falls within the normal range of (0.8-2.30 mg/dl creatinine), (8.20-24.6 mg/dl urea), (41.0-176.10 μ l alkaline phosphatase), (21.7-46.50 μ l ALT) and (15.3-55.3 μ l AST) recommended by Merk Veterinary Manual, (2016). Nevertheless, other biochemical parameters like Glucose, cholesterol, total protein, Albumin and globulin decreased substantially (P<0.05) in CSLM based diets (Table 6). This reduction is in agreement with the report of Juárez-Rojop et al. (2012) that aqueous extract of *Carica papaya* (0.75 g and 1.5 g/100 ml) significantly decreased blood glucose levels, cholesterol, triacylglycerol and aminotransferase blood levels. The decreasing trend in blood glucose level of CLSM based diets revealed that *Carica papaya* leaf and seed possesses hypoglycemic and antidiabetic effects and could be used to stabilize blood glucose level in animals justifying the reasons for its use in traditional Ayurveda medicines for diabetes in India.

However, the combination of *Carica papaya* leaves and seed meal elevated the blood serum enzymes as observed in Alanine aminotransferase, alkaline phosphatase and Aspartate transferase. It appears from the result that the albumin values of weaner pigs on the control diet were slightly above the range of 22.60-40 g/l (Merk Veterinary Manual, 2016) recommended for healthy tone of pigs while the AST values of (8-11. µl) obtained from the result was comparatively low from the recommended reference range of (15.30-55.30 µl) (Merk Veterinary Manual, 2016).

| Dietary levels of CSLM Parameters | T _{1 (0%)} | T _{2 (1%)} | T _{3 (2%)} | T4 (3%) | SEM |
|---|---------------------|---------------------------|---------------------------|--------------------|------|
| Haemoglobin (HB) g/dl | 15.30 ª | 14.50 a | 14.00 a | 12.60 b | 0.80 |
| 0 ()0 | | | | | |
| Packed cell volume (PCV) (%) | 46.00 a | 43.00 ^a | 42.00 ^a | 37.80 ^b | 2.73 |
| White blood count (WBC) x 10 ³ | 5.10 ª | 4.80 a | 4.00 a | 3.10 b | 0.67 |
| Red blood count (RBC) x10 ⁶ µl | 7.40 a | 6.60 a | 6.00 a | 4.50 ^b | 0.77 |
| Mean cell volume (MCV) fl | 80.00 a | 78.20 a | 75.00 a | 72.40 d | 2.53 |
| Mean cell Hemoglobin (MCH) pg | 15.10 ª | 14.50 ª | 14.00 a | 13.00 b | 0.70 |
| MCHC (pg) | 26.20 ª | 25.40 ª | 25.00 ª | 23.30 ^b | 0.97 |
| Blood clothing time (BCT) | 33.00 ^b | 34.50 ^b | 36.30 ª | 40.00 a | 2.33 |

Table 5 - Haematological parameters of weaner pigs on graded levels of Carica papaya seed and leaf meal mix

T₁: diet without CLSM (control), T₂: diet containing 1% CLSM, T₃: diet containing 2% CLSM and T₄: diet containing 3% CLSM. ^{Abcd} Mean along the rows having different superscript of letters differed significantly at P < 0.05 level (LSD); MCHC = Mean cell haemoglobin concentration

| Table 6 - Serum biochemistry of weaner pigs fed graded levels of C | Carica papaya seed and leaf meal mix. |
|--|---------------------------------------|
|--|---------------------------------------|

| Dietary levels of CSLM | T _{1 (0%)} | T _{2 (1%)} | T _{3 (2%)} | T4 (3%) | SEM |
|------------------------|---------------------------|---------------------|---------------------|--------------------|------|
| Parameters | 11(0%) | 2 (1%) | 13 (276) | 14 (3%) | JEIM |
| Urea (mg/dl) | 18.00 b | 19.50 ab | 20.40 a | 23.00 a | 1.67 |
| Creatinine (mg/dl) | 1.50 b | 1.60 b | 1.70 b | 2.10 a | 0.20 |
| Glucose (mg/dl) | 79.00 ^a | 77.00 a | 76.20 a | 72.00 ^b | 2.33 |
| Cholesterol (mg/dl) | 115.40 | 110.60 | 110.00 | 108.00 | 2.47 |
| Total protein (g/l) | 80.00 | 78.00 | 76.4.60 | 73.00 | 2.33 |
| Albumin (g/l) | 42.10 a | 41.20 a | 41.00 ª | 39.00 b | 1.70 |
| Globulin (g/l) | 37.80 ª | 37.80 ª | 35.60 ^b | 34.00 ^b | 1.07 |
| Enzymes | | | | | |
| ALP (μ/Ι) | 41.00 bc | 48.00 ^b | 48.00 ^b | 65.00 ª | 5.67 |
| ALT (μ/Ι) | 8.60 b | 12.50 ª | 13.30 a | 14.00 a | 1.80 |
| AST (μ/Ι) | 8.00 b | 8.80 b | 9.50 ab | 11.00 a | 1.00 |

rows having different superscript of letters differed significantly at P < 0.05 level (LSD); ALP = alkaline phosphatase; ALT = Alanine Aminotransferase; AST = Aspartate aminotransferase.

CONCLUSION

The result of the study indicated that *Carica papaya* seed and leaf meal mix at inclusion level dietary level of 3% fully support productive performance of weaner pigs, in terms of improved nutrient utilization and gut health of weaner pigs. Moreover, the positive response of the hematological and serum biochemical parameters shows that its inclusion in pig ration poses no danger to their physiological wellbeing hence a good supplement for pigs.

For future studies, the authors recommend an in-depth study on the mineral constituents of *Carica papaya* leaf and seed meal and an examination of the carcass characteristics of weaner pig placed on this supplement.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contribution

G. Nkwocha = Conceptualization, Statistical design, data analysis, section writing and proof-read.

B. Ekenyem and Kevin Anukam = Data analysis, results editing, proof-reading and grammar checking.

A. Adeolu & R. Nwose = Results and Discussion, Conclusion/Recommendation section writing, reference sorting and editing.

F. Anosike & M. Callistus = Materials & Methods, reference sorting, data processing, data sorting and coding.

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Competing interests

The authors declare no competing interests in this research and publication.

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