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THE EFFECT OF DIFFERENT SOURCES AND LEVELS OF INCLUSION OF INDIGENOUS MICROORGANISMS ON THE GROWTH PERFORMANCE, DAYS-TO-FIRST-OESTRUS AND HAEMATOLOGICAL PROFILE IN CROSSBRED PIGS

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Abstract

This study investigated the effect of different sources and levels of inclusion of indigenous microorganisms (IMOs) on the growth performance, puberty attainment and hematology in crossbred pigs. The experimental design was 2 × 3 factorial arrangements of treatments in a randomized complete block design (RCBD). The IMOs were cultivated using mixture of fruits and rice sources. A total of twenty-four (24) female weaner pigs (aged 10 weeks) with an average initial weight of 9.35 ± 0.23kg were randomly assigned to six (6) experimental treatments (4 pigs per treatment and 1 pig per replicate). Factor A was two sources of IMOs (fruit and rice) and factor B was levels of IMOs inclusion (0ml/L, 5ml/L and 10ml/L of IMOs) in a litre of water. The results showed that feed intake (inclusion levels and interaction), growth rate, body weight at first oestrous (sources and inclusion levels), feed efficiency and age at first oestrous (sources, inclusion levels and interaction) were significantly affected ($p < 0.05$). Results on the haematological profile showed

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higher ($p < 0.05$) mean concentrations of PCV, Hb, and RBC for gilts fed 10ml/L of Rice IMOs. The WBC count was not affected ($p > 0.05$) by levels of dietary IMOs inclusions. The study concluded that the incorporation of IMOs from fruit sources at 5ml/L concentration led to reduced feed intake, enhanced feed efficiency, lowered days-to-first-oestrus and improved health status in crossbred gilts.

Keywords: Estrous, Gilts, Haematology, Indigenous Microorganism, Probiotics, Puberty.

INTRODUCTION

With the growing global population, the demand for food has surged, leading to a significant increase in intensive livestock production over recent decades (Sharma et al., 2020). In Nigeria, pig farming holds a prominent position among livestock enterprises. This prominence is attributed to the intrinsic characteristics of pigs, such as their high survival rates, prolific reproduction, efficient feed conversion, and the ability to reach slaughter weights of 80 to 90 kg within 7 to 9 months under optimal management conditions (Ume et al., 2023). For emphasis, pigs possess natural genetic advantages that contribute to rapid growth and exceptional reproductive performance, including their litter-bearing nature (Nguyen and Nagy, 2024). Pig production, therefore, presents a substantial opportunity to address the protein deficiency in developing countries such as Nigeria. However, these attributes remain underutilized in most developing countries, resulting in a significant gap between the supply and demand for pork in these countries compared to global supply levels.

Pig production among smallholder farmers is bedeviled with significant challenges, including poor feed utilization, offensive odours from farms, and low herd productivity and reproductive performance (Mamawi et al., 2020). In gilts for examples, the onset of puberty marked by the first standing estrus in the presence of a mature boar can be delayed due to inadequate nutrition. Although this event appears spontaneous, puberty is a complex developmental process involving both chronological and physiological maturation. The early onset of puberty in gilts is crucial for achieving optimal economic performance in commercial pig farms. Notably, nearly 10% of gilts are culled before their first breeding, primarily due to reproductive issues. As documented in the literature, gilts typically reach puberty between 150 and 220 days of age and the timing of puberty is a reliable predictor of a gilt's reproductive potential (Patterson et al., 2010; Rohrer et al., 2017). The occurrence of the first estrus reflects both the efficiency of the gilt's genetic potential and the physiological mechanisms influencing sexual maturation and reproductive management (Patterson and Foxcroft, 2019) Quality nutrition is essential for an increased growth and reproduction of any pig farm enterprise. Unfortunately, many pigs are bereft of quality feeding due to the high cost of feed ingredients and non-availability of feed materials.

This scenario has propelled farmers to engage in the use of antibiotics and other synthetic products to enhance growth and reproduction irrespective of their obvious negative consequences on humans and the environment. In view of the aforementioned, there is dire need to develop natural products that are efficient in enhancing growth and reproductive performance of pigs while maintaining the integrity of the environments.

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The beneficial role of indigenous microorganisms (IMOs) in pig production has continued to gain serious attention in recent years as a sustainable alternative to conventional livestock management practices. IMOs are naturally occurring important microbes (e.g. bacteria, fungi, and yeast) that are usually harvested from the local environment and incorporated into animal nutrition to enhance animal health, feed efficiency, overall growth and reproduction of farm animals. According to Kang et al. (2019), these microorganisms impact positively nutrient cycling, soil fertility and gut health in animals and they offer a natural and eco-friendly approach to livestock agriculture. In pig production, the application of IMOs is highly emphasized due to the potential benefits in enhancing growth performance, improving immune function and optimizing reproductive outcomes necessary for economic sustainability of the enterprise. IMOs protects the normal host from invasion of pathogenic microorganisms causing disease or infections through production of bacteriocins and other inhibitory substances which prevent colonization of pathogens. Additionally, they compete with the pathogens for essential nutrients and receptors on host cells (Yadav et al., 2020). Even though previous studies have demonstrated the potential of IMOs to improve feed efficiency, enhance immune responses, promote better growth rates and reproduction in various livestock species (Kang et al., 2019; Kober et al., 2022), there is, however, a scarcity of empirical research focused on the specific effects of different sources of IMOs and their levels of inclusion in pig diets, particularly in relation to their impact on growth performance, the timing of puberty onset and haematological parameters in crossbred pigs. Hence, this issue is very important and requires further study. The research aimed to study the effect of different sources of indigenous microorganisms and their levels of inclusion on the growth performance, days-to-first-oestrus and haematological profile of crossbred pigs.

MATERIALS AND METHODS

Ethical Statement

The experimental procedure followed the regulations of the Animal Welfare and Ethics Committee, Department of Animal Science, University of Nigeria, Nsukka (UNN/C025ARO12.14.06.2024) in ensuring minimal pain and discomfort to the specimen used in this study.

Geo-Location and Duration of the Study

The study was carried out at the Piggery Unit of the Department of Animal Science Teaching and Research Farm, University of Nigeria, Nsukka. Nsukka lies in the Derived Savanna region, and is located on longitude 6° 25 N and latitude 7° 24 E and at an altitude of 430m above the sea level. The climate of the study area is a typical humid tropical type, with a relative humidity range of 56.01 to 103.83 %. Average diurnal minimum temperature ranges from 22 °C to 24.7 °C while the average maximum temperature ranges from 33 °C to 37 °C. Annual rainfall ranges from 1567.05mm to 1846.98mm (Onyenucheya and Nnamchi, 2018). The research lasted for forty (40) weeks.

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Collection and Preparation of Experimental Materials

Fruits (oranges, lime, lemon, mangoes and pineapples), rice and brown sugar were procured from a local market (Ogige Main Market) in Nsukka Local Government Area, Enugu State Nigeria.

Preparation of Fruit IMO

Fruit IMO (Figure 1), was prepared using known quantities of fruits mixture namely mango (17.86 kg), orange (43.38 kg), pineapple (17.95 kg), lime (3.99 kg) and lemon (14.84 kg). These fruits were washed in clean water and sliced into smaller pieces/sizes using a knife. Cut pieces of the fruits were placed into a 100-litre volume plastic container and mixed thoroughly with 2.00 kg of white sugar. Water (45 litres) was later poured into the container to the brim. Thereafter, the surface was covered with 0.45 kg of a mixture of rice bran and ground maize in a ratio of 2:1. The whole treatment/plastic container was then covered with cloth mesh/sieve to prevent any rodent or insect intrusion and allowed to undergo the process of fermentation under room temperature for a 14-day period. On day 14, the fermented product was made free from the solid elements (seeds, and fruit chaffs). Thereafter, the fermented fruit solution which served as the Fruit IMOs was sieved into 25 litre gallons and stored under room temperature until use in the experiment.

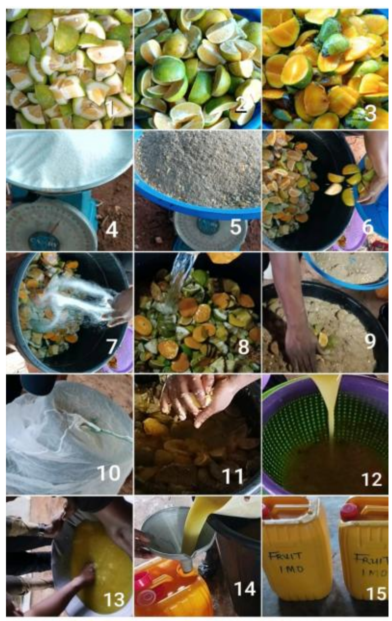

Figure 1: Preparation of fruit-based IMO. (1) Cut pieces of lemon, (2) Cut pieces of orange, (3) Cut pieces of mango, (4) Weighing of white sugar, (5) Weighing of rice bran (6) Mixing the fruits, (7) White sugar addition, (8) Water addition, (9) Surface covering with rice bran, (10) Container covering with cloth sieve, (11) Hand pressing to separate IMO solution from the chaff after 14 days, (12) Initial sieving, (13) Final sieving, (14) IMO transfer into gallons, (15) IMO storage in gallons

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Preparation of Rice IMOS

A 2.5 kg Nigerian local rice variety was parboiled allowed to cool down and placed in a properly labeled plastic sieve up to ⅔ volume. The remaining ⅓ volume of the sieve was kept empty to create an aerobic environment to the indigenous microbes and, thereafter, the sieve was covered with a sheet of brown paper held in place with a masking tape. The plastic sieve containing the cooled parboiled rice was buried 30 cm deep into the soil located under a tree shade, covered with leaves and topsoil collected from the nearby area to maintain optimum moisture for microbial growth and allowed for 7 days. All possible measures were taken to protect the materials from direct sunlight and rainwater. After 7 days, the sieve was carefully exhumed and the inoculated rice indigenous microorganisms (rice covered by white film as a result of the activities of indigenous microbes and free from red, blue and green layers and is called inoculated rice) was harvested. Afterwards, the inoculated rice was removed, weighed, mixed with brown sugar in the ratio of 1:1 and placed in a 20-litre container with lid, covered with dark polythene sheet and under room temperature for another 7 days. The enrichment of the inoculated rice with an energy source (brown sugar) further helped in growth of microbes and fermentation of the substrate. At the end of the seven days, the composite and fermented mixture of the inoculated rice and brown sugar was harvested, stored and used as rice indigenous microorganisms (Rice-based IMOs) solution (Figure 2).

Figure 2: Preparation of rice-based IMO. (A) Steamed rice covered with paper sheet, (B) Covered rice in a sieve buried, (C) Exhumed content covered in white film, (D) Weighing of the decomposed rice, (E) Weighing of brown sugar, (F) Homogenization of rice and brown sugar, (G) Homogenized product, (H) Product air-tightly covered, (I) Rice-based IMO

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Management of Experimental Animals and Treatments

A total of 24 weaner (10 weeks old) crossbred (Landrace x Large White) female pigs with an average weight of 9.81 ± 0.23kg were used for this study. The pigs were purchased from a nearby pig farm in Nsukka located close to the Piggery Unit of the Department of Animal Science Teaching and Research Farm, University of Nigeria, Nsukka, Enugu State, Nigeria where the experiment was hosted.

The experimental layout was a 2×3 factorial arrangement of treatments in a randomized complete block design (RCBD). Factor A comprised of the two (2) IMO sources (fruit and rice) while Factor B was made up of the three (3) levels of IMOs inclusion (0ml, 5ml and 10mls/liter of water). Replicates were considered as blocking variables in the analysis. The pigs were weighed using a 50 kg weighing balance, tagged and randomly assigned into six treatment groups having different inclusion levels of IMOs per litre of water. The study also consisted of 4 pigs per treatment and each pig were housed individually in a pen serving as a replicate/block.

The treatment groups were namely: T_1: 0ml of fruit IMO/litre of water, T_2: 5mls of fruit IMO/litre of water, T_3: 10mls of fruit IMO/litre of water, T_4: 0ml of rice IMO/litre of water, T_5: 5mls of rice IMO/litre of water, T_6: 10mls of rice IMO/litre of water. The animals were housed in a concrete floor, well ventilated and fly roof pig house with dwarf walls. They were quarantined for 10 days and exposed to preventive doses of Ivomecplus® and Berenyl® injection against ecto- and endo-parasites and trypanosomal infection, respectively.

The pigs were fed 5% of their body weight as daily ration comprising 18.24 % crude protein and 2874.21 kcal/kg metabolizable energy (Table 1). The experimental diet was tested through a series of AOAC analytical method for proximate analysis (AOAC, 2006). Cool, clean drinking water was provided *ad-libitum.* Pigs were weighed weekly to determine their body weight between 08:00 and 10:00 hours before feeding. All the management practices, ethical and welfare considerations associated with the use of animal for experimentation in the University of Nigeria were strictly adhered to as reported under ethical statement section.

Table 1: Percentage and calculated composition of experimental diet.

Ingredient compositions (%)	
Maize	5.00
Cassava chips	16.0
Cassava peels	20.0
PKC	18.0
Bambara chaff	22.0
Soya bean meal	12.0
Dried blood meal	5.50
Vitamin mineral premix	0.25
Lysine	0.50
Methionine	0.50

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Salt	0.25
Calculated compositions	
Crude protein (%)	18.24
Crude fibre (%)	6.19.
Metabolizable Energy (kcal/kg)	2874.21
Calcium (%)	0.89
Phosphorus (%)	0.86
Proximate compositions (%)	
Dry matter	90.64
Crude protein	18.61
Crude fibre	4.00
Ash	7.22
Ether extract	5.18
Nitrogen free extract	64.00

Data Collection

Data on weekly body weight and feed intake were determined by means of a weighting scale. The weekly weight gain of pigs was determined using a hoist scale (Diamond®, Taiwan) by subtracting weight (kg) at the previous week from the weight (kg) at the present week. Feed conversion ratio was calculated as feed consumed per body weight gain. However, body weight of pigs at oestrus and age at oestrus were also determined in kg and days, respectively.

Blood Collection and Analysis

At the 32 week of the experiment, two sows from each treatment group were randomly selected, restrained and blood samples (2ml each) collected by ear vein puncture using 5ml sterile syringe attached with a 20-gauge needle.

This activity was done after cleaning the ear region with xylene solution. The blood sample were emptied directly into bottles containing ethylene diamine tetra-acetate (EDTA) for the determination of haematological indices.

The haemoglobin concentration was determined by the cyanmethaemoglobin method (Kahar et al., 2022), Packed cell volume (PCV) was estimated by the micro haematocrit method (Farooq et al., 2023), Red blood cell (RBC) were measured by the method described by Walczak et al. (2021) and WBC counts were determined using the haemocytometer.

Thin blood smears stained with Giemsa stain were used for the determination of the differential white blood cell (WBC) counts.

Statistical Analysis

Data generated from the experiment were subjected to analysis of variance (ANOVA) using Statistical Product and Service Solutions (IBM SPSS Statistics version 23) version 20. Significantly different means were separated using Duncan's New Multiple Range Test and accepted at 5% level of probability.

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RESULTS

The effect of fruit and rice IMOs on growth performance and days-to-first-estrous of crossbred weaned female pig

Results in Table 2 showed that with the exception of daily feed intake (DFI), only growth rate, feed conversion ratio, body weight at first oestrus and age at first oestrus were significantly ($p < 0.05$) affected by IMO sources. Pigs on fruit IMO recorded significantly higher growth rate and body weight at first oestrus compared to those on rice IMOs. Also, the same pigs on fruit IMO had better feed conversion and attained age at first oestrus at a lower number of days than those fed rice IMOs.

It was also recorded that DFI, growth rate, FCR, body weight at first oestrus and age at first oestrus were significantly ($p < 0.05$) affected by changes in the levels of IMO inclusions. Feed intake and feed conversion ratio were lower at 5ml/L inclusion, however, significantly ($p < 0.05$) higher growth rate and body weight at first oestrus was recorded for the same group of pig compared to those of pigs fed 10ml/L and 0ml/L (control) IMO levels. Age at first oestrus were similar ($p > 0.05$) in the IMO treated pig.

However, the age at first oestrus was higher ($p < 0.05$) in the control group of pigs than in the IMO treated pigs. The results also indicated that daily feed intake, FCR and days-to-first-estrous were significantly ($p < 0.05$) impacted by the interaction between the two IMO sources (fruit or rice) and their dietary levels of inclusion (0, 5, or 10ml/L). These parameters changed significantly according to the either fruit or rice IMO source and their inclusion levels in the pigs. For instance, the findings in Table 2 indicates that pigs on 10ml/L fruit IMOs consumed significantly ($p < 0.05$) more feed than those on other combinations of the diet.

The lowest amount of feed consumed was recorded in pigs fed 5ml/L of either fruit or rice IMOs. A similar pattern was also shown in the feed conversion ratio for the same group of pigs. The lowest age at first oestrus was significantly ($p < 0.05$) lowest for pigs on 5 and 10ml/L fruit IMO compared with those on rice IMO and the control group of pigs. The age at first oestrus for pigs on 5 and 10ml/L fruit IMO (173.50 vs. 169.00 days) did not vary significantly ($p > 0.05$).

Table 2: Means (± SEM) of the effect of fruit and rice IMO on growth performance and age at first oestrus of crossbred weaned female pigs.

Parameters	IBW (kg)	DFI (kg)	Growth rate (kg/day)	FCR	BW at first oestrus (kg)	Age at first oestrus (days)
IMO Sources						
Fruit	8.67	1.29	0.27[a]	4.71[b]	49.73[a]	184.33[b]
Rice	10.04	1.30	0.24[b]	4.91[a]	46.81[b]	188.28[a]
SEM	0.23	0.04	0.03	0.01	0.09	1.08
P-value	0.11[NS]	0.15[NS]	0.05*	0.04*	0.05*	0.01**
Inclusion Levels						
0ml/L	9.81	1.35[a]	0.25[b]	5.51[a]	48.13[b]	189.50[a]

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5ml/L	9.31	1.12^{b}	0.27^{a}	4.22^{b}	52.76^{a}	180.92^{b}
10ml/L	8.94	1.40^{a}	0.25^{b}	5.17^{ab}	48.15^{b}	178.00^{b}
SEM	0.43	0.03	0.01	0.18	0.81	2.17
P-value	0.69^{NS}	0.05*	0.05*	0.00**	0.03*	0.00**
Interaction						
Fruit control	11.50	1.31^{b}	0.22	5.92^{a}	48.22	189.50^{a}
5ml/L/Fruit IMO	9.50	1.10^{c}	0.27	4.07^{b}	47.06	173.50^{b}
10ml/L/Fruit IMO	9.13	1.47^{a}	0.29	5.05^{a}	48.16	169.00^{b}
Rice control	8.13	1.38^{ab}	0.27	5.10^{a}	50.59	189.40^{a}
5ml/L Rice IMO	9.13	1.15^{c}	0.26	4.43^{b}	46.47	188.33^{a}
10ml/L Rice IMO	8.75	1.33^{b}	0.25	5.32^{a}	48.13	187.00^{a}
SEM	0.29	0.07	0.01	0.14	1.15	3.01
P-value	0.25^{NS}	0.04*	0.22^{NS}	0.05*	0.85^{NS}	0.02*

$^{abc:}$Means with different superscripts within columns differ ($P < 0.05$), IBW – Initial body weight, DFI – Daily feed intake, FCR – Feed conversion ratio, BW – Body weight, NS – Not significant, SEM – Standard error of the mean.

The effect of fruit and rice IMOs on the haematological indices of crossbred weaned female pigs

The results on the effect of fruit and rice IMO sources, levels of dietary inclusions and their combinations on the haematological profile of crossbred pigs are shown in Table 3. The sources of IMOs (fruit or rice-based IMO) and their interactions (IMO sources x inclusion levels) showed significant ($p < 0.05$) effects on the mean concentration of packed cell volume (PCV), haemoglobin (Hb), and the red blood cells (RBC) of crossbred pigs. The mean concentration of white blood cells (WBC) between treatment groups of pigs were not different ($p > 0.05$) at the various factor levels and their interactions. When compared with fruit IMOs, consumption of rice based IMOs resulted in higher ($p < 0.05$) indices in PCV, HB and RBC.

Furthermore, the inclusion levels of IMOs resulted in a significant ($p < 0.05$) variations in the concentrations of haematological indices of the pigs. At 10ml/L dietary inclusion, highest mean concentrations in PCV, Hb, and WBC were recorded in the pigs compared to those in other treatment groups (0 and 5ml/L IMOs). A significantly lowest average PCV, Hb, RBC and TWB concentrations in the pigs were observed in the control groups. Results in Table 3 equally showed that the mean PCV, Hb and RBC concentrations in the blood varied ($p < 0.05$) in response to the various combination of IMO sources and their levels of dietary inclusion.

The highest mean blood concentrations of PCV, Hb and RBC were reported in pigs fed 10ml/L rice-based IMOs. This was followed by those of pigs fed 10ml/L of fruit-based IMOs. In all, the lowest mean blood concentration of PCV, Hb and RBC was shown in the control group of pigs (0ml/L IMO). The total white blood (TWB) counts were similar ($p > 0.05$) in all treatment's combinations. The observed mean haematological indices in this study were within normal ranges for pigs.

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Table 3: The effect of fruit and rice IMO on haematological indices of weaner pigs.

Parameters	PCV (%)	Hb. conc. (g/dl)	RBC count (10^6/µl)	TWB count (10^3/µl)
IMO Sources				
Fruit	37.03^{b}	14.52^{b}	8.78^{b}	18.40
Rice	38.60^{a}	14.93^{a}	10.77^{a}	18.70
SEM	1.50	0.21	0.19	0.02
P-value	0.00**	0.04*	0.00**	0.09^{NS}
Inclusion Levels				
0ml/L	35.25^{c}	13.84^{c}	7.68^{c}	17.80^{c}
5ml/L	37.50^{b}	14.46^{b}	9.20^{b}	18.25^{b}
10ml/L	40.70^{a}	15.88^{a}	12.45^{a}	19.60^{a}
SEM	0.50	0.48	0.53	0.18
P-value	0.00**	0.00**	0.00**	0.00**
Interaction				
Fruit control	35.00^{f}	13.82^{d}	7.45^{d}	17.80
5ml/L/Fruit IMO	36.50^{d}	14.57^{bc}	8.50^{c}	18.00
10ml/L/Fruit IMO	39.60^{b}	15.17^{b}	10.40^{b}	19.40
Rice control	35.50^{e}	13.86^{cd}	7.90^{d}	17.80
5ml/L Rice IMO	38.50^{c}	14.35^{cd}	9.90^{b}	18.50
10ml/L Rice IMO	41.80^{a}	16.59^{a}	14.50^{a}	19.80
SEM	1.10	0.27	0.07	0.09
P-value	0.00**	0.00**	0.00**	0.47^{NS}

$^{abcdef:}$Means with different superscripts within columns differ (P < 0.05), PCV – Packed cell volume, Hb. Conc. – Haemoglobin concentration, RBC – Red blood cells, TWB – Total white blood cells, NS – Not significant, SEM – Standard error of the mean.

DISCUSSION

This research demonstrated that dietary fruit IMOs source improved growth rate, feed conversion ratio, BW at first oestrus and age at first oestrus. These parameters were also impacted positively by IMOs inclusion levels especially at 5 ml/L dietary inclusion. These treatment factors caused a reduced feed intake compared to those in the non-IMO treated control group. As evidenced in our finding, a recent study by Bolibrukh and Rublenko (2023) emphasized that indigenous microorganisms like *Bacteroides*, *Prevotella*, and *Lactobacillus* play a crucial role in shaping the gut microbiome of pigs, impacting their health and productivity by influencing physiological processes and disease resistance. As earlier mentioned, IMOs being naturally occurring microbes often harvested from various local environmental sources such as from fruits or carbohydrate substrates are known to improved feed intake and enhance growth and reproduction in many farm animals especially in pig farming.

Depending on the source, most IMOs contain some beneficial bacteria, fungi and yeast populations that aid in enhancement of an animal gut microbiome when consumed. For instance, Doriya et al*.* (2022) noted that fruit-based fermented foods offer a diverse range of microorganisms, contributing to the production of functional beverages rich in bioactive

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compounds, probiotics, and phytochemicals necessary for better growth and development of animals. The observed improvement in the feed conversion ratio of pigs fed IMOs irrespective of the sources could be attributed to the presence of beneficial microorganisms, vitamins and minerals, etc. in the products. As shown in this study, Galli et al. (2024) reported an increase in feed intake of pigs from probiotic-fed sows compared to the control ($p = 0.035$) which is not in consonance with this study when compared to the control. McCormack et al. (2017) observed that pigs with reduced residual feed intake, a sign of improved feed efficiency, had an enrichment of gut microorganisms linked to a leaner but healthier host.

Physiologically, Fleming et al. (2019) noted that peripheral concentrations of butyrate and serotonin, as well as the generation of colonic short-chain fatty acids (SCFAs), are individual mechanisms by which the microbiome affects feeding behavior in pigs. According to Jiao et al. (2018), another mechanism that might contribute to a reduction in feed intake as shown in the present study is an increase in plasma leptin concentrations induced by SCFA. Preliminary findings on the amino acid content in the fruit and rice-based IMOs used in this study indicated the presence of both long and short chain fatty acids and these may have impacted the gut microorganisms of the pigs leading to a reduction in feed intake and improved feed efficiency. Results of the interactions between IMO sources (fruit or rice) and inclusion levels (0, 5 and 10 ml/L) showed that gilts fed 5 ml/L fruit or rice IMO had significantly lower feed intake and better feed conversion ratio. Also, 5 or 10 ml/L fruit IMO resulted in lower days-to-first oestrus (173.50 and 169.00 days) compared to other combinations.

This finding is in agreement with the report of Hanim et al. (2012) which indicated the effects of indigenous microorganisms (IMOs) on animal feeding can vary significantly based on their sources. A valuable indicator of the health of the hypothalamic-pituitary-ovarian axis and the reproductive system's functionality is the evaluation of the estrous cycle in farm animals (Ali et al., 2022). The reported days-to-first-estrous in this study is similar to the range (150 - 180 days) reported by Flowers (2020). Higher days-to-first-oestrus (195.2 and 200 days, respectively) were reported by Am-In et al. (2020) and Graves et al. (2020). It is generally accepted that shorter days-to-first-estrus in gilts is advantageous for animal reproduction. Early puberty enables earlier breeding, which can increase the herd's total reproductive efficiency.

This may result in more litters produced per year per sow, thereby increasing production (Almond and Mahan-Riggs, 2018). Certain native microorganisms become early or pioneer colonists in a specific portion of the gut because they are acquired earlier on before or during parturition or from the surrounding environment (Yeoman et al., 2018). Numerous researches have demonstrated the beneficial effects of probiotic-based substances on animal reproductive performance models (Gu et al., 2019; Quereda et al., 2020). Indigenous microbes support the reproductive performance and fertility of animals as a result of their antioxidative, anti-inflammatory, antibacterial and immunomodulatory properties (Hashem and Gonzalez-Bulnes, 2022). The physiological mechanisms by

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which indigenous microorganisms influence the age at puberty in pigs may involve a complex interaction between gut microbiota, dietary components, and hormonal signaling pathways. These microorganisms can modulate gut health and immune responses, which are critical for reproductive development. According to Zhaoyue et al. (2022), dietary composition can influence the gut microbiome and, consequently, puberty. Thus, it may be inferred that the IMOs consumption during early stage of the pig growth may have exerted a benefit on the reproductive processes necessary for growth and development of the vital organs required for early attainment of puberty. The secretion of some important reproductive hormones in female animals have been linked to the consumption of probiotics. For example, Wang et al. (2022) revealed that follicle stimulating hormone (FSH) secretion was improved when animals were exposed to the consumption of the bacterium *Bacillus subtilis* with an elevated amount of oestrogen. FSH plays a key role in controlling reproduction in both male and female animals. In female animals, its activities are crucial in the maturation of granulosa cells and the formation of follicles, as well as ovulation (Long et al., 2017).

Even though the role of indigenous microorganisms, particularly from fruit or fermented rice, in modulating reproductive hormones remains underexplored, their potential influence on gut health and nutrient absorption could indirectly affect hormone levels and reproductive outcomes as evidenced in the present study. Haematological profile indices in farm animals are a valuable procedure that define the physiological health status and productivity of an animal. Umar et al*.* (2024) further emphasized that farm animals exposed to stressful conditions or foreign materials are bound to experience a shift in the concentrations of different haematological indices. Our findings showed varying significant changes in the PCV, Hb and RBC concentrations in the blood of pig at various interactions of IMO sources and level of inclusions. These blood parameters were lowest in the control groups of pigs, but peaked, however, when pigs were given rice IMOs at 10ml/L inclusion compared to those given fruit IMOs at the same amount.

Collectively, our present findings in respect of PCV, Hb, RBC and WBC blood concentrations in pigs were within ranges reported in the literature. In pigs, Suardana et al. (2024) observed that the mean concentrations of PCV, Hb, RBC and WBC ranged, respectively, from 30.57 to 41.81%, 11.40 to 14.80 g/dL, 5.98 to 8.14×10^6/μL and 13.83 to 28.53×10^3/μL. These results indicate that beneficial indigenous microbes generated from different local environmental sources are capable of exerting different physiological effects in pigs when consumed. In support of our study, Kolodina and Artemyeva (2022) found that the inclusion of microbial supplements in pig diets resulted in a significant increase in hemoglobin (12.67 g/dL) and leukocyte counts (25.03%) compared to control groups. Also, Kidega et al. (2022) indicated that pigs raised on deep litter treated with IMOs exhibited a different fatty acid profile, with a notable reduction in saturated fatty acids, potentially impacting overall health and meat quality. In piglets, the use of feed additives containing IMOs has been reported to facilitate an increased erythrocyte counts and improved oxygen transport in blood, leading to a better growth rate (Prudyus and Broda, 2024). It may be inferred that these microorganisms stimulate the immune system

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of pigs by increasing the production of antibodies and activating immune cells (Yue et al., 2023). While the benefits of IMOs are evident, the physiological mechanisms behind these changes and their long-term effects on pig health and productivity are still unclear necessitating further research.

CONCLUSION

The findings of the present study showed that 5 ml/L IMO inclusion of fruit or rice indigenous microorganisms caused reduced feed intake, better feed efficiency, improved growth rate and enhanced BW at first oestrus in gilts. In terms of reproductive processes, our findings indicated that gilts on fruit IMO source fed at 5 or 10 ml/L concentration had significantly lower days-to-first-estrous (173.50 and 169.00 days) compared to those on rice IMO and the control at various levels without compromise to the physiological health status of the gilts.

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Conflict of Interest

The authors declared an absence of conflict of interest.

Author's Contribution

NS Machebe, BC Amaefule and NE Ikeh: Conceived and designed the experiment.

LC Ali, AL Obinna and PI Umeugokwe: Collected the data

BC Amaefule and OJ Odo: Carried out the labouratory analysis.

NS Machebe and NE Ikeh: Analyzed the data.

NS Machebe and LC Ali: Wrote the manuscript.

NS Machebe: Supervised the experiment.

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