GROWTH PERFORMANCE AND IMMUNE RESPONSE OF WEANED PIGS FED DIETS SUPPLEMENTED WITH VARYING LEVELS OF *Citrus aurantifolia* FRUIT JUICE

Adebiyi, F.G.[1]*, Adebiyi O.A.[2] and Khidir, M.A.[1]
[1]Agricultural Biochemistry and Nutrition Unit, Department of Animal Science, University of Ibadan, Ibadan, Nigeria.
[2]Animal Production and Management Unit, Department of Animal Science, University of Ibadan, Ibadan, Nigeria
*Corresponding Author: fgadebiyi@gmail.com; +2348099118283

ABSTRACT
This study evaluated the effect of Citrus aurantifolia (CA) fruit juice supplementation on the growth performance and immune response of weaned pigs. Forty crossbred (Large White × Landrace) weaned pigs were randomly assigned to four dietary treatments in a completely randomised design. Each treatment was further subdivided into five replicates of two pigs each. The treatments included a control diet (T1) with no CA juice and three experimental diets supplemented with 15 ml/kg (T2), 20 ml/kg (T3), and 25 ml/kg (T4) of CA juice. Growth performance parameters, including average weekly weight gain (AWWG), average weekly feed intake (AWFI), and feed conversion ratio (FCR), were measured, along with differential white blood cell counts to assess the immune response of the pigs. Results showed no significant differences (P > 0.05) in AWWG, AWFI, and FCR among the treatments. However, significant differences (P < 0.05) were observed in monocyte, neutrophil, and eosinophil counts. The highest monocyte count (4.33%) was recorded in pigs fed T4 diet, while the highest neutrophil count (44%) was observed in T3, both significantly different from T2. The eosinophil count was highest in T3 (4.67%), significantly (P<0.05) higher than the control but similar to T2 and T4. In conclusion, CA fruit juice supplementation did not impair growth performance but enhanced immune parameters, particularly at 25 ml/kg. Further research is recommended to determine optimal inclusion levels and assess long-term effects on health and productivity.
Keywords: *Citrus aurantifolia*, lime juice, weaning pigs, immune response, white blood cells.

INTRODUCTION

The post-weaning period in pigs is a critical stage characterised by significant physiological, nutritional, and immunological challenges, often resulting in poor growth performance and increased susceptibility to infections (Campbell *et al*., 2013; Adebiyi *et al*., 2023). Dietary interventions during this stage aim to enhance nutrient digestibility, immune response, and overall health. Recently, attention has shifted toward natural bioactive compounds as sustainable alternatives to synthetic additives in livestock production.

Citrus aurantifolia (lime) is a sour, round, bright green, nutritional powerhouse fruit, widely recognised for its rich content of bioactive compounds. It has a high content of vitamin C, citric, and malic acids (Krismiyanto *et al*. (2021) as well as flavonoids, and essential oils, which exhibit antimicrobial, antioxidant, and immunomodulatory properties (Guimarães *et al*., 2010). They also contain small amounts of iron, calcium, vitamin B6, thiamine, and potassium (Pennistine et al., 2008). Studies have demonstrated that citrus extracts can improve growth performance and modulate immune responses in broiler chickens (Ndelekwute and Enyenihi, 2017; Aikpitanyi and Imasuen, 2020). However, there is limited information on the impact of *Citrus aurantifolia* fruit juice as a dietary supplement in swine nutrition, particularly its potential effects on growth performance and immune function during the weaning phase. Hence, this study investigated the effects of *Citrus aurantifolia* (CA) fruit juice supplementation on growth performance and immune response of weaned pigs.

MATERIALS AND METHODS

Source and preparation of *Citrus aurantifolia* fruit juice

The lime fruits used in this study were sourced from Oje Market, Ibadan, Oyo State, Nigeria. They were thoroughly washed with clean water to remove potential contaminants and then halved with a sharp knife. The juice was mechanically extracted using a 2.5 mm sieve juice extractor, poured into an airtight glass bottle, and stored at 4°C in a refrigerator to maintain freshness throughout the experimental period.

Experiment Site
The experiment was conducted at the Piggery Unit of the Teaching and Research Farm, University of Ibadan, located in Ibadan, Oyo State, Nigeria.

Management of Experimental Animals (Weaned Pigs)
A total of 40 cross-breed (Landrace X Large White) 6-7-week-old weaned pigs with an average weight 6.5 ± 0.5kg were used in this study. The animals were kept in the housing unit for 7 days to acclimatise them to the housing conditions before the commencement of the experiment.

Experimental Design
The experimental animals were randomly assigned to four treatment groups (T1, T2, T3, and T4), with each group consisting of ten pigs in a completely randomised design (CRD). Each treatment was replicated five times, with two pigs per replicate. The experiment lasted six weeks.

Experimental Diet
The experimental diet consisted of four treatments: T1 (control) contained no *Citrus aurantifolia* (CA) fruit juice, while T2, T3, and T4 were supplemented with 15, 20, and 25 ml of CA per kilogram of feed respectively. Before mixing, CA juice was diluted with 600 ml of water per kilogram of feed. Throughout the experiment, pigs had *ad-libitum* access to both feed and water. The feed composition is presented in Table 1, and diets were formulated according to the recommendations of the National Research Council (NRC, 2012) for weaning pigs.

Table 1: Nutrient Composition and Ingredient of Basal Diet

Ingredient	**%**
Maize	55.5
Soybean Meal	26.0
Groundnut Cake	8.4
Palm Kernel Cake	5.0
Soya Oil	1.5
Limestone	1.62
Dicalcium Phosphate	0.8
Salt	0.3
DL-Methionine	0.18
L-Lysine-HCL	0.30
Toxin Binder	0.15
Pig Grower Premix	0.25
Total	**100**
Calculated Analysis	
Crude Protein (%)	20.24
Crude Fibre (%)	4.68
Crude Fat (%)	5.05
Metabolised Energy (Kcal/Kg)	2937

Data Collection
The total feed intake and weight gain for each group were assessed weekly. The feed conversion ratio (FCR) was computed by dividing feed intake by weight gain.

Blood Collection for analysis of differential white blood cell count
At the end of the experiment, the weaned pigs were restricted from feeding for twelve hours but allowed access to water. Blood samples were collected from five pigs (i.e. one pig/replicate) through the anterior vena cava using sterilised disposable syringes and needles and stored in EDTA bottles for evaluation of total white blood cells, lymphocytes, neutrophils, monocytes and eosinophils count. Blood samples were analysed in duplicate

Statistical Analysis

All data collected were subjected to one-way analysis of variance (ANOVA) using SPSS (statistical software platform, vs 25) and Duncan's Multiple Range Test was used to separate significant ($\alpha = 0.05$) means.

RESULTS AND DISCUSSION

Growth Performance of Weaned Pigs Fed *Citrus aurantifolia* fruit juice

The growth performance parameters of weaned pigs fed diets supplemented with CA fruit juice are presented in Table 2. There were no significant differences ($P > 0.05$) among the treatment groups for average weekly weight gain (AWWG), average weekly feed intake (AWFI), and feed conversion ratio (FCR). This suggests that the inclusion of lime fruit juice in the diet does not adversely affect the growth performance of pigs. However, this finding contrasts with the reports of Ndelekwute and Enyenihi (2017), who observed significant improvements in growth performance when *Citrus aurantifolia* (CA) juice was used as a feed additive in broiler chickens. This discrepancy may be due to species-specific differences in physiology and metabolism. The lack of significant differences in growth performance parameters may also be attributed to the doses of lime juice used, which may not have been sufficient to elicit measurable effects. Abdel-Moneim et al. (2020) reported minimal or no significant effects of phytogenic feed additives on growth performance when used at low inclusion levels.

Table 2: Growth performance of weaned pigs fed *Citrus aurantifolia* fruit juice

Parameters	Treatments				SEM	P Value
	T1 (0ml/Kg)	T2 (15ml/Kg)	T3 (20ml/Kg)	T4 (25ml/Kg)		
AWWG (Kg)	1.44	1.39	1.41	1.40	0.05	0.992
AWFI (Kg)	3.84	3.55	3.90	3.6	0.12	0.761
FCR	2.66	2.58	2.76	2.54	0.05	0.588

AWWG=Average Weekly Weight Gain per Pig, AWFI=Average Weekly Feed Intake per Pig, FCR=Feed Conversion Ratio, SEM = Standard Error of Mean.

Differential white blood cell count of weaned pigs fed *Citrus aurantifolia* fruit juice

Table 3 shows the white blood cell count and differential parameters of pigs fed *Citrus aurantifolia* fruit juice-supplemented diets. The values obtained in this study are within the physiological range for pigs (Merck Veterinary Manual, 2021). The total white blood cell (WBC) count was highest in T4 (25 ml/kg) at 9483.33µL, while the lowest count was observed in T2 (15 ml/kg) at 8433.33µL. Lymphocyte percentage was highest in T4 (56.67%) and lowest in T2 (49.33%), though not significantly different from the control group (52%). Significant differences were observed in neutrophil, monocyte, and eosinophil counts across the various treatments. Treatment T3 (20ml/kg CA fruit juice) showed the highest neutrophil count, significantly different from T2 (15ml/kg CA fruit juice). Pigs in T4 (25 ml/kg lime juice) exhibited the highest monocyte count (4.33%), while those in T2 (15 ml/kg) had the lowest (2.73%), with the observed differences being statistically significant ($P<0.05$). The eosinophil count (Eos) varied across treatments, with the highest count observed in T3 (20 ml/kg), followed by T4 (25 ml/kg) and T2 (15 ml/kg), while T1 had the lowest value.

Table 3: White blood differential count parameters of pigs fed *Citrus aurantifolia* fruit juice supplemented diets

Parameters	Treatments				SEM	P Value
	T1 (0ml/Kg)	T2 (15ml/Kg)	T3 (20ml/Kg)	T4 (25ml/Kg)		
WBC(µL)	9383.33	8433.33	8933.33	9483.33	249.46	0.48
Lym (%)	52	49.33	54	56.67	1.42	0.34
Neut (%)	41.33^{ab}	37.67^{b}	44.00^{a}	42.00^{ab}	1.50	0.02
Mon (%)	3.33^{ab}	2.73^{b}	4.00^{a}	4.33^{a}	0.31	0.03
Eos (%)	3.33^{b}	4.31^{ab}	4.67^{a}	4.33^{ab}	0.21	0.04

WBC=White Blood Cell count, Lym=Lymphocytes, Neut=Neutrophils, Mon=Monocytes, Eos=Eosinophils, SEM = Standard Error of Mean. Values in the same row with different superscripts are significantly different ($P<0.05$)

The highest count of WBC observed in T4 suggests a possible immunostimulatory effect of *CA* supplementation. White blood cells are vital for immune defence, and an optimal count is essential for maintaining overall health in livestock (Adebiyi *et al.,* 2017). The numerically higher values of lymphocytes in T4 may indicate enhanced immune function due to the bioactive compounds in CA juice, such as flavonoids and vitamin C (Guimarães *et al*., 2010). The result observed in this study is consistent with the findings of Kadam *et al*. (2009), who also reported no significant differences in lymphocyte levels among broiler chickens treated with lemon juice.

Neutrophils and monocytes are key components of the WBC population, employing both oxygen-dependent and oxygen-independent mechanisms to combat viral infections, kill bacteria, and engulf pathogens. Monocytes play a crucial role in immune defence, acting as precursors to macrophages and dendritic cells, which are involved in antigen presentation and pathogen clearance (Abdel-Moneim *et al*., 2020). A moderate monocyte count is beneficial for immune health and contributes greatly to the host defence mechanisms. However, excessively high monocyte levels can indicate underlying inflammatory conditions or immune stress. Though the monocyte counts recorded in this study are within the normal range, the variations observed reflected that *CA* supplementation may influence monocyte production. The increase in monocyte count in T4 aligns with research by Ndelekwute and Enyenihi (2017), who reported that the inclusion of lime juice in broiler chicken diets led to significant increases in monocyte counts. The elevated eosinophil levels observed in the CA juice-supplemented groups (T2, T3, and T4) may suggest enhanced immune modulation due to the bioactive compounds present in *Citrus aurantifolia*. Citrus fruits are rich in flavonoids and other phytochemicals with immunomodulatory properties, which can influence the activity and proliferation of immune cells, including eosinophils (Guimarães et al., 2010; Abdel-Moneim et al., 2020).

CONCLUSION

The results obtained from this study showed that the inclusion of *Citrus aurantifolia* fruit juice in the diet of weaned pigs had no adverse effect on growth performance but influenced immune response, particularly monocyte, neutrophil, and eosinophil counts. Further studies are recommended to determine the optimal inclusion levels and explore its long-term effects on livestock health and productivity.

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