



Effect of *Macleaya cordata* Preparation as Phytobiotic Feed Additive on the Performance and Blood Profiles of Weaned Pigs

¹Adesehinwa A. O. K., ¹Makanjuola B. A., ²Ajayi, E., ³Abiola J. O.,
¹Boladuro B. A., ¹Sorunke A. O., and ¹Afolabi O. O.

¹Swine Research Unit, Institute of Agricultural Research and Training,
Obafemi Awolowo University, Moor Plantation, Ibadan, Nigeria.

²Nigeria Institute of Animal Science (NIAS), NCRI Premises, Moor Plantation, Ibadan, Nigeria.

³Department of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria.

*Corresponding Author: aokadesehinwa@yahoo.com

Abstract

This study was designed to investigate the effect of sanguinarine the active ingredient of *Macleaya cordata* preparation as a phytobiotic (feed additive) on the growth performance and blood parameter of weaned pig. Ninety weaned cross bred (Large white × Landrace) pigs of average initial live weight of 8.82±0.21 kg were used for the study. Animals were randomized completely into three dietary treatments and each treatment was replicated three times and housed in groups of 10 (same sex) based on body weight in a concrete floor pen. Treatment 1 (control) consisted of an un-supplemented basal diet while treatments 2 and 3 were supplemented with phytobiotic at 0.2 and 0.3 g/kg diet respectively. At the end of the feeding trial, blood was collected from 6 pigs (2/replicate) per treatment. 6 ml of blood was collected through jugular vein puncture into two sample bottles using a sterile needle and syringe very early in the morning before feeding. Growth performance results showed that final body weight, daily weight gain, average daily feed intake and feed conversion ratio were not significantly ($P>0.05$) different across the treatments. The highest final body weight (27.25 kg) was recorded for treatment 3 while treatment 2 recorded the lowest final body weight (26.86kg). The treatment that received highest level of 0.3g/kg of diet inclusion had the highest total cost of feeding (? 6315.20) and average cost of feed per day (? 112.77) respectively. No significant ($P>0.05$) differences were observed in all haematology and serum biochemical parameter in this study. Conclusively, *Macleaya cordata* preparation cannot be recommended as a feed additive for non-diseased weaned pig, since it showed no enhancing effect on both performance and blood biochemical parameters.

Introduction

The change of piglets from sows' milk to a solid diet of different composition and form undeniably plays a major role in the predisposition to diarrhoea after weaning, both of microbial and dietary origin. Poor post-weaning performance has been attributed to low feed intake that is inadequate to meet the nutritional requirements of weaned piglets (Lalles *et al.*, 2007). The low feed intake may be due to weaning-related stress and appetite suppression as a result of undesirable changes in the small intestine, including

enzyme activity, decrease in the villus height/crypt depth (V/C) ratio, abrupt separation from their mother, mixing with other litters in new environment and turning over to a less digestible solid feed from highly digestible liquid milk and changes in intestinal permeability, all of which may influence nutrient digestibility and absorption, increase the incidence of post-weaning diarrhoea (PWD) and compromise the growth performance of weaned piglet (Flis *et al.*, 2017; John *et al.*, 2018).

Dritz (2004) stated that diet formulation and ingredients selection are critical factors

in the successful implementation of weaner feeding programmes, although the age and weight of pigs at weaning are major determinants of performance in the first four weeks following weaning, and subsequently through to slaughter. Therefore, weaned pig diets must be manipulated predominately to overcome the limitations of immaturity in digestive function so as to maximize the growth of the whole animal. According to Tokach *et al.* (2003), major concepts when formulating diets for newly weaned pigs were; adjusting pigs to the simplest and relatively lowest cost diets as quickly as possible after weaning, maximizing feed intake to ensure that the pig consumes sufficient energy and nutrients at a time when excess mobilization of body reserves (primarily lipid) can occur and formulating the initial diets with highly digestible ingredients that complement the pattern of digestive enzymes and digestive enzyme development in the gastrointestinal tract.

Apart from the above concepts, another means of enhancing weaned pig performance is by supplementing their diet with exogenous digestive enzyme or other products that can improve their nutrient digestion, absorption and utilization. Other alternatives that have been used in the place of exogenous enzymes include direct fed microbial (Adesehinwa *et al.*, 2018), essential oils (Pengfei *et al.*, 2012), zinc oxide (Roselli *et al.*, 2005, Ou *et al.*, 2007; Wang *et al.*, 2009); and organic acids (Partanen and Mroz, 1999; De Freitas *et al.*, 2006; Piva *et al.*, 2005).

Phytobiotics or phytogenics which include herbs, spices and plant extracts (mainly essential oils) are well known for their pharmacological effects and are thus widely used in human traditional and

alternative medicine (Grashorn, 2010). Phytogenic feed additives are plant-derived compounds incorporated into diets to improve the productivity of livestock through amelioration of feed properties, promotion of the animals' production performance, and improving the quality of food derived from those animals (Windisch *et al.*, 2008). This definition is driven by the purpose of use. Other terms such as herbs (flowering, non-woody and non-persistent plants), spices (herbs with an intensive smell or taste commonly added to human food), essential oils (volatile lipophilic compounds derived by cold expression or by steam or alcohol distillation), or oleoresins (extracts derived by non-aqueous solvents) (Windisch *et al.*, 2008) are commonly used to classify the vast varieties of phytogenic compounds with respect to origin and processing. A huge number of *in vitro* and *in vivo* studies have confirmed a wide range of activities of phytobiotics in animal nutrition like stimulation of feed intake, antimicrobial, coccidiostatic, anthelmintic and immune-stimulation (Panda *et al.*, 2006).

Herbal preparations obtained from *Macleaya cordata* extract (MCE), which contain the alkaloids sanguinarine (predominating) and chelerythrine as biologically active substances is the phytobiotic used in this study. *Macleaya cordata* extract and powdered *Macleaya cordata* aerial parts is a natural feed additive for rearing pigs, broilers, and dairy cattle. According to Franz *et al.* (2005), sanguinarine possesses various biological activities and stimulates the secretion of enzymes in the intestine. These are some of the characteristic properties of herbs and spices (Lee *et al.*, 2003). However, a major disadvantage of plant extracts lies in the

fact that their compositions are unstable (Bomba *et al.*, 2006). This is due to the influence of many factors, such as climate, season, harvesting method and others (Borovan, 2004). The multiplicity of controversial results obtained in different scientific studies examining the effect of *Macleaya cordata* extract in animal nutrition may be ascribed to the above reasons (Khadem *et al.*, 2014, Kantas *et al.*, 2015). The aim of this study therefore was to investigate the effect of Sanguinarine as a phytobiotic feed additive on the growth performance and blood parameters of weaned pigs.

Materials and methods

Experimental location, animals, design and diet

The experiment lasted for 56 days and it was carried out at AK Research Farms, Off Eleyele-Eruwa road, Ibadan, Oyo state, Nigeria. Ninety weaned cross bred (Large white \times Landrace) pigs of average initial live weight of 8.82 ± 0.21 kg were used for the study. Animals were randomized completely into three dietary treatments, each treatment was replicated three times and housed in groups of 10 (same sex) based on body weight in a concrete floor pen. Treatment 1 (control) consisted of an un-supplemented basal diet, while Treatments 2 and 3 contained 0.20 g and 0.30 g of the phytobiotic (*Macleaya cordata*) per kilogram diet respectively. *Macleaya cordata* extract has been included in the European Feed Additive Register and approved for use as a flavouring compound for farm animals. The experimental diets were formulated using the nutrient requirement recommendations for weaned pigs of National Research Council (2012) as a guide. The ingredient,

chemical composition, calculated energy and nutrient content are shown in Table 1. The diets based on maize, wheat offal and groundnut cake meal were analyzed for the content of crude nutrients by the Association of Official Analytical Chemists method (AOAC, 1990). Pigs were allowed *ad libitum* access to the diets and water served in concrete feeding and watering troughs respectively.

At the end of the feeding trial, blood was collected from 6 pigs (2/replicate) per treatment. 6 ml of blood was collected through jugular vein puncture into two sample bottles using a sterile needle and syringe very early in the morning before feeding. One set of blood samples were collected in ethylene diamine tetra-acetic acid (EDTA) bottles for haematological indices and another set of blood samples was collected without the use of anti-coagulant for serum metabolites evaluation. Data obtained from the study were verified by subjecting to one way analysis of variance (ANOVA), significant means were separated using Duncan Multiple range Test, using Statistical Analysis System (SAS) software for Windows (2003).

Results and Discussion

Proximate compositions of the experimental diet are shown in Table 2. The analysed and calculated values were in close range and in agreement with the nutrient requirements of weaned pig, as recommended by NRC (2012). This implied that the diet was nutritionally adequate.

Growth performance of the pigs fed the experimental diets

The growth performance characteristics of the pigs fed the experimental diets are

Table1. Ingredient composition of the experimental diet

Ingredients %	T1 (0 g/kg diet)	T2 (0.2 g/kg diet)	T3 (0.3 g/kg diet)
Ground nut cake	24	24	24
Palm kernel cake	10.6	10.6	10.6
Maize	42.25	42.25	42.25
Wheat offal	10	10	10
Lime stone	0.8	0.8	0.8
High quality cassava peel	5	5	5
Bone meal	1.6	1.6	1.6
Fish meal (72%)	3	3	3
Premix*	0.25	0.25	0.25
Salt	0.5	0.5	0.5
Methionine	0.03	0.03	0.03
Lysine	0.47	0.47	0.47
Palm oil	1.5	1.5	1.5
Phytobiotic	-	20g	30g
Total	100	100	100
Calculated analysis			
Crude fibre (%)	4.60	4.60	4.60
Crude protein (%)	21.16	21.16	21.16
Metabolizable energy (kcal/kg)	2875.30	2875.30	2875.30
Calcium	1.15	1.15	1.15
Phosphorus	0.62	0.62	0.62
Lysine	1.15	1.15	1.15
Methionine	0.34	0.34	0.34

*Premix supplied the following per kg diet: vitamin A 10,000,000 IU; vitamin D 32,000,000 IU; vitamin E 8,000 IU; vitamin K 2,000 mg; vitamin B1 2,000 mg; vitamin B2 5,500 mg; vitamin B6 1,200 mg; vitamin B12 12 mg; biotin 30 mg; folic acid 600 mg; niacin 10,000 mg; pantothenic acid 7,000 mg; choline chloride 500,000 mg; vitamin C 10,000 mg; iron 60,000 mg; Mn 80,000 mg; Cu 800 mg; Zn 50,000 mg; iodine 2,000 mg; cobalt 450 mg; selenium 100 mg; Mg 100,000 mg;

shown in Table 3. The results showed that final body weight and daily weight gain, average daily feed intake and feed conversion ratio were not significantly ($P>0.05$) different among the treatments. The highest final body weight (27.25 kg) was recorded in pigs fed diet supplemented with 0.3 g/kg diet of *Macleaya cordata* preparation while the lowest final body weight (26.86 kg) was recorded in pigs fed diet containing 0.2 g/kg diet of *Macleaya cordata* preparation. The values for average daily feed intake and feed conversion ratio followed similar trend with that of final

body weight and daily weight gain. The highest numerical values (897.35 g) for average daily feed intake was observed in pigs fed diet supplemented with 0.2 g/kg diet of *Macleaya cordata* preparation while lowest numerical value (855.95 g) was recorded in pig fed control diet. Inclusion of *Macleaya cordata* preparation increased the average daily feed intake when compared with the control.

Among the proposed alternatives to antibiotics are phytobiotics such as *Macleaya cordata* preparation that contain isoquinoline alkaloids, such as

Table 2: Proximate composition and fibre fraction of experimental diet

Parameters (%)	Diet
Dry matter	88.96
Crude Protein	19.93
Crude Fibre	5.59
Crude fat	3.87
Ash	6.31
NFE	53.26
NDF	45.67
ADF	21.95
ADL	5.92
Hemi cellulose	23.73
Cellulose	16.03
Gross energy (kcal/kg)	4100

NFE - Nitrogen Free Extract, NDF- Neutral Detergent Fibre, ADF- Acid Detergent Fibre,

ADL- Acid Detergent Lignin, % - percent

sanguinarine and chelerythrine, with anti-inflammatory activities. According to Pluske *et al.* (1997), there are evidences from numerous studies showing that the addition of phytochemical ingredients to the diet of pigs had beneficial outcomes, particularly in the treatment against growth retardation and disease, also as antimicrobial agents and their efficacy is influenced by the concentration of additives and the pH in the animal's intestine. The result of this study corroborated the outcome of the work of Zhao *et al.* (2017). The authors reported no significant difference in final live weight, feed intake, and average daily weight gain between the treatment and control groups of weaned pigs fed graded levels of *Macleaya cordata* preparation (0.1, 0.5 and 1 g/kg diet) throughout the three growing stages of the study. In contrast to the findings of this study, Kantas *et al.* (2015) found that supplementation of 0.75 mg/kg of sanguinarine in the feed significantly increased feed consumption and reduced feed conversion ratio in six-week-old

piglets.

Macleaya cordata (Willd) R. Br. had also showed up in the European Food Safety Authority (EFSA) database as a feed additive in intensive livestock farming, in an effort to elevate daily food consumption and growth performance (Franz *et al.*, 2005). Non significant effect of the additive on all the performance parameters recorded in this study could be attributed to differences in climatic condition, its dosages and breed of animals used for the experiment (Borovan, 2004). As well, the fact that the composition of the control diet to which the test diets were compared could be adjudged to have met the requirements of the pigs. Hence, the inclusion of the additive (*Macleaya cordata* preparation) might be of no significant effect on the utilization of the diet by the pigs.

Economics of production of the pigs fed the experimental diets

Economics of production of weaned pigs fed control and phytobiotic-supplemented diets is shown in Table 4. The result of the

Table 3: Performance of weaned pigs fed the experimental diets

Parameters	T1(0)	T2(0.2g)	T3(0.3g)	P (ANOVA)	SEM
Initial weight (kg)	8.93	8.54	8.98	0.644	0.210
Final weight (kg)	27.17	26.86	27.25	0.966	0.640
Daily weight gain (g)	325.75	327.09	326.31	0.998	8.170
Average daily feed intake (g)	855.95	897.35	895.83	0.271	11.900
Feed conversion ratio	2.73	2.91	2.88	0.538	0.071

SEM - Standard error of mean.

Table 4: Economics of production of weaned pigs fed the experimental diets

Parameters	T1(0)	T2(0.2g)	T3(0.3g)	P (ANOVA)	SEM
Cost of feed (₦/Kg)	111.40 ^c	111.80 ^b	112.00 ^a	<.0001	0.03
Total cost of feeding (₦)	6001.70	6314.50	6315.20	0.2094	83.96
Average cost of feed per day (₦)	107.17	112.76	112.77	0.2094	1.50
Feed Cost/kg Weight Gain (₦/Kg)	303.73	324.84	322.80	0.4919	7.98

SEM - Standard error of mean.

study showed significant ($P < 0.05$) linear increment in the cost of feed per kilogram of the diet as the inclusion level of the additive increased. There was no significant ($P > 0.05$) influence of the *Macleaya cordata* inclusion on the total cost of feeding and average cost of feed per day. The pigs that received highest level (0.3 g/kg of diet) of phytobiotics had the highest numerical values, in terms of total cost of feeding (? 6315.20) and average cost of feed per day (? 112.77) respectively. The least numerical cost of feed per kilogram weight gained (? 303.73/Kg) was recorded with the control treatment.

It is important to note that the economics of production is one of the factors needed to be considered in determining the importance of the inclusion of any additive in the diets of livestock in a production system. The significant difference ($P < 0.001$) in the cost of a

kilogram of the feed observed in this study was due to the inclusion of the additive in the diet. The increment in the cost of feed of the pigs fed phytobiotic supplemented diets (T2 and T3) was not justified by their feed cost per kilogram weight gained. The use of *Macleaya cordata* increased the total cost of feeding without corresponding increase in the weight gain. According to Micheal *et al.* (2010), the use of non-medicinal additives or medication is justified if there is a nutritional or pig health necessity and the value of the improved performance outweighs the cost of their inclusion. Therefore, the addition of the phytobiotic to the diet of weaned pigs could not be said to be economically worthwhile.

Haematological and Serum Biochemical parameters of the pigs fed the experimental diets

Table 5 showed effect of *Macleaya cordata* preparation on the haematological

Table 5: Effect of *Macleaya cordata* on the haematological parameters of weaned pigs fed experimental diets

Parameters	T1 (0)	T2 (0.2g)	T3 (0.3g)	P (ANOVA)	SEM
PCV (%)	38.83	35.83	32.33	0.2735	1.617
Haemoglobin (g/dl)	12.95	11.87	10.75	0.2808	0.552
RBC(10^6 ul)	6.33	5.98	5.40	0.3770	0.265
WBC (10^3 ul)	11.33	10.08	10.99	0.8044	0.757
Neutrophils (%)	41.67	45.17	43.67	0.4815	1.141
Eosinophils (%)	3.33	3.50	4.17	0.4313	1.141
Monocytes (%)	3.83	3.00	3.00	0.3563	0.266
Lymphocytes (%)	50.50	48.33	49.17	0.7153	1.035
Platelet	206500	232000	199833	0.2223	7887.508

PCV - packed cell volume, RBC - red blood cells, WBC – white blood cells

parameters of weaned pigs fed experimental diets. There was no significant ($P>0.05$) difference in all the treatments for all the haematological parameters tested. The haematological parameters constitute the first blood indices applied for in the assessment of the health status of the animal. Apart from white blood cell (WBC) and packed cell volume (PCV), majority of the haematological indices determined in the study were within the normal range of reference values specified for normal healthy pigs (Merck Manual, 2012), which proves good health and proper condition of the tested animals. Packed cell volume of the treatment 3 that received 0.3 g/kg diet of *Macleaya cordata* preparation was however lower than the value in the normal range for healthy pigs. As well, white blood cell value of pigs on the control treatment was within the normal range for healthy pigs while that of those that received doses of *Macleaya cordata* preparation were lower than the value recommended for healthy pigs. The trend observed in PCV and haemoglobin of the treatments showed that as the test ingredient increased, there was a reduction

in PCV and haemoglobin content. Similar observation was observed in red blood cells. According to Isaac *et al.* (2013), Packed Cell Volume participates in the transport of oxygen and absorption of nutrients. This implies that the use of the *Macleaya cordata* preparation reduces transportation of oxygen and nutrient absorption. The result of WBC suggests that the test ingredient did not challenge the immune system of the animal. The blood platelets were higher in pigs fed 0.2 g/kg diet of the test ingredient and this implies minimal loss of blood in case of injury (Merck Manual, 2012). Although no mortality or morbidity was observed throughout the period of the study, likewise, there was no sign of toxicity. But, it is important to note the lowered values of WBC and PCV in the *Macleaya cordata* treatment groups (T2 and T3) were observed, as this had not been reported in previous studies. Non-significant ($P>0.05$) effect in all haematological parameters observed in this study was contrary to the result obtained by Zhao *et al.* (2017), where significant ($P<0.05$) effect of the addition of *Macleaya cordata* preparation was

Table 6: Effect of *Macleaya cordata* on the serum biochemical parameters of pigs fed the experimental diets

Parameters	T1	T2	T3	P (ANOVA)	SEM
ALT (IU/L)	34.500	31.833	39.500	0.2227	1.809
ALP (IU/L)	53.62	54.00	59.67	0.8214	4.170
HDL	78.83	72.17	87.83	0.5356	5.509
T.P (g/dl)	7.8667	7.5667	7.8667	0.3911	0.100
Albumin (g/dl)	3.2000	2.8500	3.1167	0.4171	0.109
Globulin (g/dl)	4.6667	4.7167	4.6833	0.9821	0.103
BUN (mg/dL)	12.933	13.150	14.300	0.8503	0.995
Cholesterol (mg/dl)	142.50	124.50	138.50	0.5757	7.026
Creatinine (mg/dl)	1.37	1.22	1.42	0.3439	0.057
Triglyceride	53.500	54.167	54.333	0.9840	1.882

TP - Total Protein, HDL-High density lipoproteinb, ALT - Alanine Amino transferase, ALP – Alkaline Phosphatase, AST - Aspartate Amino transferase, BUN- Blood Urea Nitrogen

observed in some haematology parameters, such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and platelets.

Effect of *Macleaya cordata* on the serum biochemical parameters of the pigs fed experimental diets is presented in Table 6. No significant ($P>0.05$) difference was observed between all groups fed *Macleaya cordata* preparation and control treatment at the end of the study. Only cholesterol value for all the treatments was higher than the highest value of reference range for healthy pigs (Merck Manual, 2012), while, alkaline phosphatase, total protein and globulin were lower than the lowest value of reference range for normal healthy pigs. By implication, the use of *Macleaya cordata* as feed additive did not promote gut health of the pigs. All other serum biochemical parameters were within the normal range specified for normal healthy pigs.

High levels of cholesterol observed could not be attributed to addition of the additive to the feed of the experimental animals since observed blood cholesterol of the treatment groups were comparable to the control, although, the values across all the groups were higher when compared to the reference range (Merck Manual, 2012). Hanczakowski *et al.* (2009), had reported that blood cholesterol levels is determined by genetic factors, nutrition and environmental conditions. Moreover, high density lipoprotein (HDL) for all the treatments were not above the upper limit set for healthy pigs and the percentage share of HDL of the total cholesterol for each of the treatments (T1=55.32%; T2=62.78%; T3=63.42%) was higher than 40%. Winnicka (2011), had stated that levels of HDL cholesterol in pig serum should account for at least 40% of total cholesterol concentration. This is because a decrease in the HDL fraction below 40% was said to be

undesirable, particularly for animal reared for meat production.

Non significance ($P > 0.05$) of differences observed in all the serum biochemical parameters in this study was not in agreement with earlier investigations of Zhao *et al.* (2017), which proved that addition of *Macleaya cordata* had significant effect on some blood biochemistry parameters between treatments, including bile acids, haptoglobin, cholesterol, creatinine kinase (CK), gamma glutamyl transferase (GGT), potassium and total protein.

Conclusion

It could be concluded that *Macleaya cordata* showed no enhancing effect on the non-diseased weaned pig performance and health status (as indicated by the measured serological and haematological parameters) compared with pigs fed diet without *Macleaya cordata* supplementation. In addition, based on this research, *Macleaya cordata* preparation cannot be recommended as a feed additive for non-diseased weaned pigs fed complete diets that meet their nutrient requirements.

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