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Effect of dietary supplementation of Holarrhena pubescens leaf powder on the growth performance, carcass characteristics, microbial count and haemato-biochemical parameters of piglets

Olujimi John Alagbe

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

***Corresponding Author** **Olujimi John Alagbe**

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Abstract: This 60 days experiment was carried out to ascertain the effect of dietary supplementation of Holarrhena pubescens leaf powder (HPLP) on the growth performance, carcass characteristics, intestinal microbial population and some heamato-biochemical parameter of piglets. A total of 50 cross bred male pig with an initial body weight 9.90 ± 0.28 kg were randomly divided into five groups (GA, GB, GC, GD and GE) with five replicates (1 animal per replicate). Feed (Standard) used in this study was Corn-soya based diet compounded with Nutritional Research Council (2012) standard. The first week was a pre-feeding period where piglets were acclimatized and dewormed against parasites and provided unlimited accesses to clean water throughout the experimental period. A completely randomized design method was adopted and piglets in GA was fed standard diet without HPLP while those in GB, GC, GD and GE received same diet supplemented with HPLP at 30 g, 60 g, 90 g and 120 g in that order. Phytochemical screening of HPLP showed that it contained phenols, terpenoids and flavonoids as the most prominent compounds while others include, alkaloids, tannins, saponins and glycosides. Increase in the supplementation significantly improved ($P<0.05$) average daily weight gain, average daily feed intake, dressing percentage and reduced feed conversion ratio and mortality among animals. Staphylococcus aureus, Escherichia coli, Proteus spp, Pseudomonas spp and Staphyllococus spp count were significantly suppressed by HPLC, conversely, Lacobacillus spp increased across the group. Pack cell volume, haemoglobin, red blood cell, white blood cell, total protein, glucose, cholesterol, triglycerides, high density lipoprotein, low density lipoprotein were significantly influenced ($P<0.05$) except for monocytes, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase ($P>0.05$). Supplementation of HPLP at 120 g/kg diet gave the best result and the performance of animals were not compromised.

Keywords: *Antimicrobial, Blood, Carcass, Piglets, Phyto-constituents, Performance*.

Introduction

Globally, there is clear demand to reduce antibiotic use in animals and in this way reduce the risk of multi-drug resistance. The use of medicinal plants or herbs have been gaining increasing attention in the livestock industry due to their beneficial effect on feed intake and performance (Alagbe et al., 2020). They have also been reported to be non-toxic, environmental friendly and does not have any withdrawal period (Adewale et al., 2021; Ojediran et al., 2024). Herbs contain phyto-constitutents or metabolites (terpenoids, tannins, flavonoids, phenolic compounds etc.), that have been investigated as an effective nutritional strategy to improve the overall health of animals (Musa et al., 2020). Among the potential herbal plant with therapeutic property is *Holarrhena pubescens*.

Holarrhena pubescens is a multipurpose deciduous tree that belongs to the Apocynaceae. It is native to South central china and widely distributed in most parts of Philippines, India, Pakistan, Bangladesh, Thailand, Taiwan and tropical Africa including, Zambia, Zimbabwe, Madagascar, Kenya, Tanzania amongst others (Maroyi et al., 2008). The leaves are oblong and elliptic in shape and the tree can grow up to 1300 m in altitude (Guar et al., 2010).

Phytochemical screening of the leaves, roots and stem bark has shown that it contains, alkaloids, phenolic compounds, flavonoids, terpenoids, steroids and tannins, glycosides in various concentrations (Dua et al., 2013). Reports have shown that Holarrhena pubescens possess numerous pharmacological properties including, anti-inflammatory, antioxidant, antidiarrheal, anti-diabetic, analgesic, anti-urolithic, anti-helminthic, antimicrobial and gastro-protective (Ali et al., 2011).

Ethno-medically, Holarrhena pubescens is used in traditional medicine in the treatment of piles, stomach ache, fever, body pain, intestinal parasites, tooth ache, diabetes, dysentery, leprosy, skin infection, gonorrhea, rheumatism and arthritis (Jamadagni et al., 2017). Aqueous extract from the stem bark of Holarrhena pubescens are also used to treat snake bite, indigestion, respiratory disorder, malaria, stomach cramps and dysentery (Devi et al., 2013). Ethanolic extract from the roots and stem bark of Holarrhena pubescens have been proven to inhibit the activities of pathogenic organisms such as, Plasmodium falciparum, Pseudomonas aeruginosa, Escherichia coli and Candida albicans (Dubey et al., 2012)

Previous studies have shown that the use of medicinal plants in piglets have shown that it has growth promoting effects (Maenner et al., 2011; Jacela et al., 2010), influence the intestinal microbiota population (Yan and Kim, 2012), modulation of immune system (Li et al., 2012), enhance the activities of enzymes in the gastro intestinal tract (Yan et al., 2010), scavenge free radicals and thus prevents diseases ((Labaque et al., 2013). Most of these result showed that herbs pose positive effect on the general performance of animals. However, research on the impact of Holarrhena pubescens leaf powder on piglets are scanty. This research is important because herbs needs to be supplemented at right dose to get the required result and it will also help to address the elevated reports on antimicrobial resistance.

Therefore, the purpose of this research was to determine the effect of dietary supplementation of *Holarrhena pubescens* leaf powder on the growth performance, carcass characteristics, microbial count and haemato-biochemical parameters of piglets.

Materials and methods

Description of experimental location and ethical approval

The study was carried out at the Piggery section, Sumitra Research Institute, Gujarat, India located between 16o 20N East India between the months of September to November, 2024. All procedures were approved by the ethics committee on Animal Use at the Department of Animal Production in the Institute under protocol number ER/H1D/2-2024.

Holarrhena pubescens leaf collection and preparation

Freshly harvested Holarrhena pubescens leaves were collected from Sumitra Research Institute, Gujarat in August 2023. The identity of the leaves was confirmed at the Taxonomy department of the institute where a specimen was deposited with voucher number TDH/2023TG. The leaves were cleaned and air dried for 10 days in an open shade. The dried leaves were grinded using an electric blending machine and the powdered samples were stored in an air tight plastic container for further laboratory evaluation.

Care of experimental animals and design

A total of 50 cross bred male pigs with an initial body weight of 9.90 ± 0.28 kg and weaned on 28 days were sourced from a reputable breeding farm in Gujarat. Animals were individually housed in pens measuring 1m × 2m × 1m. Prior to the arrival of pigs, pens were thoroughly washed with disinfectants and also disinfected with Morigad Plus®, feeding and watering troughs were properly washed and subsequently cleaned daily. The first week was considered as a pre-feeding stage and pigs were treated against parasites using Oramectin Plus ® dose was administered according to the package insert before they were randomly divided into five groups (A, B, C, D and E) with five replicates (1 animal per replicate). Feed used in this study was Corn-soya based diet compounded with Nutritional Research Council (2012) standard. Feed was provided thrice a day (6:30, 12:00 and 16:30 H). Pigs also had unlimited access to fresh clean water throughout the 60 days trial. A completely randomized design was adopted, animals in group A (control) received standard diet (corn-soya diet) without Holarrhena pubescens leaf powder while those in group B, C, D and E were fed same diet supplemented Holarrhena pubescens leaf powder with at 30 g, 60 g, 90 g and 120 g per kilogram diet.

Data collection

Growth performance parameters such as; average weight gain, average daily weight gain, total feed consumption, average daily feed consumption, feed conversion ratio and percentage mortality was estimated according to methods recently published by John (2024).

Calculations

Average weight gain (kg) = average final body weight - average initial body weight

Average daily weight gain (kg) = average weight gain ÷ 60 (number of experimental period)

Total feed consumption (kg) = feed served - feed refused

Average daily feed consumed (kg) = total feed consumed ÷ 60 (number of experimental period)

Feed conversion ratio = average daily feed consumed ÷ average daily weight gain.

Qualitative phytochemical analysis of Holarrhena pubescens leaf powder

Screening for alkaloids

One gram of Holarrhena pubescens leaf powder was mixed with 10 mL of 1 percent dilute hydrochroloric acid on water bath and filtered while hot. Two mL of the filtrate was treated with a few drops of Dragendorff's reagent. An orange brown precipitate was taken as evidence for the presence of alkaloids in the sample (Evans, 2002).

Test for flavonoids

Five millilitres of dilute ammonia solution was added to one gram of Holarrhena pubescens leaf powder followed by addition of concentrated Tetraoxosulphate (VI) acid. A yellow colouration observed indicated the presence of flavonoids. The yellow colouration disappeared on standing (Edeoga et al., 2005).

Test for tannins

One gram of Holarrhena pubescens leaf powder was added to few drops of 1% lead acetate. A yellowish precipitate suggested the presence of tannins (Savithramma et al., 2011).

Test for saponins

One gram of *Holarrhena pubescens* leaf powder was mixed with 20 mL of distilled water and then agitated for 15 minutes. Formation of foam shows the presence of saponins (Shin et al., 2001).

Test for glycosides

One gram of Holarrhena pubescens leaf powder were treated with 2 mL of glacial acetic acid containing two drops of ferric chloride solution followed by the addition of concentrated sulphuric acid. A reddish brown colouration at the junction of the two layers and bluish green colour in the upper layer indicated the presence of glycosides (Shin et al., 2001).

Test for Anthraquinones

One gram of Holarrhena pubescens leaf powder was added to 5 mL benzene in a test tube and stirred, mixture is allowed to stay for 2

minutes before 10 percent ammonia solution was added. The mixture was shaken and the presence of a red colour suggested the presence of anthraquinones (Evans, 2002).

Test for Anthocyanins

Two grams of Holarrhena pubescens leaf powder was added to 5 mL of hydrochloric acid and 2 mL of ammonia. The appearance of pink-red to blue-violet suggest the presence of anthocyanins (Savithramma et al., 2011).

Test for Terpenoids

To 1 g of Holarrhena pubescens leaf powder, 1 ml of concentrated sulfuric acid was added and heated to 70 ℃ for 2 min. A visual check for the development of a grey colouration indicated the presence of terpenoids (Edeoga et al., 2005).

Test for Phenols

To 1 g of Holarrhena pubescens leaf powder, 1 ml of 1 % of sodium hydroxide was added and mixed for 1 min. No incubation condition as the colour development is instantaneous. A visual check observing the development of a red colouration indicated the presence of phenols (Edeoga et al., 2005).

Carcass and organ weight evaluation

At the end of the experiment (day 60), five pigs were randomly selected per treatment and feed starved over the night and given access to only clean water. Weights of animals were taken before taking animals to the slaughter slab. Slaughtering procedures followed the method recently published by John (2024). Pigs were first stunned to make them unconscious followed by bleeding, scalding, removal of hair and eviscerated before dissecting into separate parts for carcass and organ measurements. Measurement was carried out on the cut parts which were the head, trotters, back, belly, hams, loins and shoulder. The internal organs such as heart, liver, spleen, kidney, lungs and the large/small intestine were measured with the aid of a digital sensitive scale.

Intestinal gut microbial analysis

At the end of the trial, five pigs were randomly selected per treatment (from dose used for carcass evaluation). One gram of intestinal content was collected into a sterile labeled sample bottles and mixed with 0.1 % peptone saline solution. Collected samples were placed in an ice pack before they were taken immediately to the microbiology laboratory of Sumitra Research Institute, Gujarat, India. Enumeration of each bacterial count was carried out according to recent procedure by Williams et al. (2022) and expressed in Log 10 (Cfu).

Blood collection and evaluation at the end of the trial

On the 60th day of the experiment, 5 mL of blood samples were collected from the Jugular vein of five randomly selected rabbits per treatment for blood analysis. 2.5 mL of blood was collected into bottles with anticoagulant for haematological studies while those for serum evaluation was transferred into bottles without anticoagulant before all samples were placed in a prepared ice pack to protect samples before getting to the laboratory. Haematological evaluation was carried out according to methods outlined by Daniel et al. (2024). Commercial diagnostic kit (Kermat automated haematology analyzer; model- LKI -400K, Netherlands). Red blood cell, heamoglobin, pack cell volume and white blood cell counts was determined using flow cytometry differentiation technique (Alagbe, 2023). Same kit was also used to ascertain total albumin, globulin, creatinine, urea, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase were determined using triangle laser scattering and cyanide free technique. Plasma lipid composition (cholesterol, triglycerides, low density lipoprotein and high density lipoprotein) were accessed using Mac automatic biochemistry analyzer (Model XVB/208D, Netherlands). The machine was calibrated using buffer solutions, thereafter it was adjusted to a sample volume (3.5 - 50.0 µL), reagent volume (35.0 – 400.0 µL), wavelength of about 1200 nm and absorbance (0 to 5 Abs).

Laboratory analysis of experimental diet

Analysis of experimental diet was done using commercial kit, NIRTM DA1800 near infra-red feed analyzer which possess the following technical specifications; wavelength range (1200 to 1800 nm), ambient temperature (4 - 45 ℃), ambient humidity (< 90 percent relative humidity), and results was generated at an analysis time of less than 1 minutes. Calcium and phosphorus was analyzed using Trace Series Atomic Absorption Spectrometer (Model 12GH/08, Netherlands) following all instructions of the manufacturer to ensure accuracy.

Data analysis

All the data on growth performance, carcass characteristics, microbial population and blood parameters were subjected to one-way Analysis of Variance using Statistical Package for Social Sciences (version 21). The differences among the treatment means were determined ($P<0.05$) by Duncan multiple range test of the same statistical package.

Table 1: Gross composition of experimental diet fed to piglets for 60 days

Feed items or Ingredients	Content (%)
Corn	53.00
Rice bran	4.00
Soya meal	33.5
Fish meal	2.00
Stone powder (Calcium carbonate)	2.50
Bone meal	4.00
Lysine	0.20
Methionine	0.20
*Premix	0.25
Toxin quarsh binder®	0.01
Common salt	0.34
Total	100.0
Determined analysis	
Crude protein (%)	22.81
Crude fibre (%)	3.61
Crude fat (%)	4.04
Calcium (%)	1.95
Phosphorus (%)	0.68
Metabolizable energy (Kcal/kg)	2700.8

Phytochemical screening of Holarrhena pubescens leaf powder (Table 2) revealed that phenols, terpenoids and flavonoids are the most prominent phyto-constituents followed by alkaloids, glycosides, tannins and saponins. Anthracyanins and anthraquinones were absent in the analysis

Table 2: Phytochemical screening of Holarrhena pubescens leaf powder

Components	Results
Alkaloids	+++
Flavonoids	++++
Terpenoids	++++
Saponins	+
Phenols	++++
Anthracyanins	-
Anthraquinones	-
Tannins	+
Glycosides	+++

+ Present, +++moderately present, ++++highly present-absent

The effect of Holarrhena pubescens leaf powder (HPLP) on the growth performance of weaned pigs (Table 3). Average daily body weight was higher ($P<0.05$) for pigs in GE (120 g HPLP/kg diet), intermediate in GB (30 g HPLP/kg diet), GC (60 g HPLP per kg diet) and GD (90 g HPLP per kg diet) as compared to the lowest recorded for GA (control). Average daily feed intake in GB and GC were similar ($P>0.05$) to those pigs which received GD and GE but significantly higher ($P<0.05$) than GA (control). Mortality was higher ($P<0.05$) for GA than the rest of the treatments, with no differences ($P>0.05$) between the other treatments.

Table 3: The effect of Holarrhena pubescens leaf powder on the growth performance of weaned pigs.

Variables	G_A	G_B	G_C	G_D	G_E	SEM	p-value
Number of pigs	15.00	15.00	15.00	15.00	15.00	-	-
Duration of experiment in days	60.00	60.00	60.00	60.00	60.00	-	-
Initial body weight (kg)	10.02	10.10	10.20	10.00	9.99	0.10	0.27
Final body weight (kg)	29.82^{c}	34.03^{b}	34.05^{b}	34.11^{b}	38.92^{a}	0.57	0.34
Body weight gain (kg)	19.80^{c}	23.93^{b}	23.85^{b}	24.11^{b}	28.93^{a}	0.41	0.30
Daily body weight gain (kg)	0.33^{c}	0.40^{b}	0.40^{b}	0.40^{b}	0.48^{a}	0.001	0.0029
Total feed intake (kg)	72.51^{b}	81.23^{a}	82.03^{a}	82.11^{a}	83.04^{a}	1.56	0.61
Daily feed intake (kg)	1.21^{b}	1.36^{a}	1.37^{a}	1.37^{a}	1.38^{a}	0.01	0.28
Feed conversion ratio	3.66^{a}	3.40^{b}	3.41^{b}	3.40^{b}	3.00^{c}	0.01	0.14
Mortality (%)	1.20	-	-	-	-	0.001	0.002

Different letters in the same column (a, b, c) suggests significant differences at $P<0.05$; SEM, standard error of means; each value represents the mean of five replicates (10 pigs per replicate); GA: basal diet without Holarrhena pubescens leaf powder (control; HPLP); GB: basal diet with 30 g HPLP per kg diet; GC: basal diet with 60 g HPLP per kg diet; GD: basal diet with 90 g HPLP per kg diet; GE: basal diet with 120 g HPLP per kg diet.

The effect of Holarrhena pubescens leaf powder on the carcass characteristics of weaned pigs (Table 4). Carcass weight was higher ($P<0.05$) in GE (120 g HPLP per kg diet) than GB (30 g HPLP/kg diet), GC (60 g HPLP per kg diet) and GD (90 g HPLP per kg diet) which had similar ($P>0.05$) values while the lowest value was recorded in GA (control; no HPLP). Dressing percentage values which varied from 62.98 to 70.93 % was higher in GE than other groups. Weights of fore limb, hind limb, belly, back and lungs were higher ($P<0.05$) for GE than GA, but was same ($P>0.05$) with that for the rest of the treatments (GB, GC and GD). Conversely, weights of head, kidney, heart, gastro intestinal tract and liver were not influenced ($P>0.05$) by the treatment.

Table 4: The effect of Holarrhena pubescens leaf powder on the carcass characteristics of weaned pigs

Variables	G_A	G_B	G_C	G_D	G_E	SEM	p-value
Live weight	29.45^c	34.98^b	37.12^b	37.55^b	41.02^a	0.67	0.39
Carcass weight	18.55^c	23.56^b	25.11^b	25.04^b	29.09^a	0.54	0.22
Dressing percentage	62.98^c	67.35^b	67.65^b	66.68^b	70.93^a	1.75	0.08
Head	2.9	2.91	2.93	2.95	2.99	0.21	0.01
Hind limb	5.88^c	7.56^b	7.95^b	7.98^b	8.22^a	0.28	0.77
Fore limb	6.94^c	8.03^b	8.11^b	8.13^b	8.40^a	0.12	0.52
Belly	1.88^c	2.14^b	2.35^b	2.71^b	3.00^a	0.05	1.26
Back	2.91^c	3.09^b	3.11^b	3.18^b	4.01^a	0.09	1.40
Liver	0.81	0.85	0.92	0.94	0.97	0.02	0.95
Lungs	0.25^c	0.26^b	0.27^b	0.30^b	0.41^a	0.01	1.00
Kidney	0.04	0.06	0.07	0.09	0.09	0.02	1.01
Spleen	0.10	0.11	0.11	0.11	0.12	0.01	0.09
Heart	0.15	0.17	0.18	0.20	0.20	0.02	0.08
GIT weight	3.10	3.13	3.17	3.3	3.41	0.74	0.05

Different letters in the same column ($^{a, b, c}$) suggests significant differences at $P<0.05$; SEM, standard error of means; each value represents the mean of five replicates (10 pigs per replicate); G_A: basal diet without *Holarrhena pubescens* leaf powder (control; HPLP); G_B: basal diet with 30 g HPLP per kg diet; G_C: basal diet with 60 g HPLP per kg diet; G_D: basal diet with 90 g HPLP per kg diet; G_E: basal diet with 120 g HPLP per kg diet

The effect of *Holarrhena pubescens* leaf powder on intestinal microbial population of weaned pigs (Table 5). Microbial count of *Staphylococcus aureus*, *Escherichia coli*, *Proteus spp*, *Pseudomonas spp* and *Streptococcus spp* was higher ($P<0.05$) for G_A but was same ($P>0.05$) with that for the rest of the treatments. Conversely, *Lactobacillus spp* count was similar ($P>0.05$) among pigs which received G_C, G_D and G_C, intermediate in G_B and lowest in G_A.

Table 5: The effect of Holarrhena pubescens leaf powder on intestinal microbial population of weaned pigs.

Microbial population (log10 Cfu)	G_A	G_B	G_C	G_D	G_E	SEM	p-value
Staphylococcus aureus	4.86^a	2.92^b	2.81^b	2.50^b	2.31^b	0.87	1.12
Escherichia coli	5.77^a	3.35^b	3.27^b	3.16^b	3.10^b	0.95	1.35
Proteus spp	2.65^a	1.95^b	1.80^b	1.52^b	1.41^b	0.02	0.46
Pseudomonas spp	2.00^a	1.03^b	1.00^b	0.86^c	0.81^c	0.01	1.10
Streptococcus spp	3.15^a	2.06^b	1.95^c	1.81^c	1.60^c	0.03	0.80
Lactobacillus spp	2.07^c	3.45^b	4.98^a	5.02^a	5.11^a	0.07	1.03

Different letters in the same column (a, b, c) suggests significant differences at $P<0.05$; SEM, standard error of means; each value represents the mean of five replicates (10 pigs per replicate); GA: basal diet without Holarrhena pubescens leaf powder (control; HPLP); GB: basal diet with 30 g HPLP per kg diet; GC: basal diet with 60 g HPLP per kg diet; GD: basal diet with 90 g HPLP per kg diet; GE: basal diet with 120 g HPLP per kg diet

The effect of Holarrhena pubescens leaf powder on some haematological indices of weaned pigs

(Table 6). Dietary supplementation of Holarrhena pubescens leaf powder significantly ($P<0.05$) influenced the hemoglobin, packed cell volume, platelets, red blood cell, white blood cell, lymphocytes, and neutrophil count. On the other hand, there were no significant differences ($P>0.05$) in monocyte count. The pack cell volume percentage, red blood cell, haemoglobin, white blood cell, neutrophils and lymphocytes count was greater ($P<0.05$) for GB, GC, GD and GE as compared to the control (GA).

Variables	G_A	G_B	G_C	G_D	G_E	SEM	p-value
Pack cell volume (%)	29.08^b	33.56^a	34.01^a	35.11^a	37.09^a	1.72	0.09
Haemoglobin (g/L)	87.56^c	99.56^b	102.7^a	109.8^a	110.3^a	10.94	1.74

Platelet ($\times10^9$/L)	110.6^{c}	134.7^{b}	139.5^{a}	140.2^{a}	143.9^{a}	11.56	1.11
Red blood cell ($\times10^{12}$/L)	7.94^{b}	10.65^{a}	10.94^{a}	11.4^{a}	11.6^{a}	0.08	0.03
White blood cell ($\times10^9$/L)	11.57^{b}	14.87^{a}	15.71^{a}	15.87^{a}	15.76^{a}	0.09	0.02
Neutrophils ($\times10^9$/L)	6.67^{ab}	8.00^{a}	8.05^{a}	9.04^{a}	9.12^{a}	0.43	0.10
Monocytes ($\times10^9$/L)	0.86	0.81	0.83	0.92	0.95	0.01	0.14
Lymphocytes ($\times10^9$/L)	9.11^{b}	11.65^{ab}	13.86^{a}	14.89^{a}	14.91^{a}	1.20	0.01

Different letters in the same column (a, b, c) suggests significant differences at P<0.05; SEM, standard error of means; each value represents the mean of five replicates (10 pigs per replicate); GA: basal diet without Holarrhena pubescens leaf powder (control; HPLP); GB: basal diet with 30 g HPLP per kg diet; GC: basal diet with 60 g HPLP per kg diet; GD: basal diet with 90 g HPLP per kg diet; GE: basal diet with 120 g HPLP per kg diet.

The effect of Holarrhena pubescens leaf powder on the serum biochemical indices of weaned pigs (Table 7). Pigs fed diet supplemented with 60 g HPLP per kg diet (GC), 90 g HPLP per kg diet (GD), and 120 g HPLP per kg diet (GE) exhibited higher (P<0.05) total protein, albumin, globulin and glucose levels than the other groups. Albumin/globulin ratio (A/G ratio) were similar (P>0.05) for pigs which received GB, GC, GD and GE but significantly higher than those fed GA (control). On the other hand, activities of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase were not influenced (P>0.05) by the treatments.

Table 7: The effect of Holarrhena pubescens leaf powder on the serum biochemical indices of weaned pigs

Variables	**G_A**	**G_B**	**G_C**	**G_D**	**G_E**	**SEM**	**p-value**
Total protein (g/dL)	5.12^{c}	6.99^{b}	7.09^{a}	7.26^{a}	7.41^{a}	0.04	1.12
Albumin (g/dL)	3.09^{b}	4.12^{a}	4.18^{a}	4.30^{a}	4.42^{a}	0.01	0.03
Globulin (g/dL)	2.03^{b}	2.87^{a}	2.91^{a}	2.96^{a}	2.99^{a}	0.02	0.01
A/G ratio	1.52^{a}	1.43^{b}	1.43^{b}	1.45^{b}	1.47^{b}	0.09	0.07
Glucose (mmol/L)	4.06^{b}	4.56^{b}	6.15^{a}	6.49^{a}	6.59^{a}	2.88	0.05
Alanine aminotransferase (IU/L)	76.87	78.94	79.12	79.15	79.88	9.52	1.00
Aspartate aminotransferase (U/L)	52.75	55.06	55.11	56.03	56.97	4.48	1.06
Alkaline phosphatase (U/L)	102.5	109.6	113.8	115.7	115.9	25.6	7.41

Different letters in the same column (a, b, c) suggests significant differences at P<0.05; SEM, standard error of means; each value represents the mean of five replicates (10 pigs per replicate); GA: basal diet without Holarrhena pubescens leaf powder (control; HPLP); GB: basal diet with 30 g HPLP per kg diet; GC: basal diet with 60 g HPLP per kg diet; GD: basal diet with 90 g HPLP per kg diet; GE: basal diet with 120 g HPLP per kg diet.

The effect of Holarrhena pubescens leaf powder on the lipid composition of weaned pigs (Table 8). Cholesterol, triglycerides, low density lipoprotein and high density lipoprotein values were influenced (P<0.05) by the treatments. Pigs fed GB (30 g HPLP/kg diet), GC (60 g HPLP per kg diet), GD (90 g HPLP per kg diet) and GE (120 g HPLP per kg diet) had a lower cholesterol, triglycerides and low density lipoproteins than the birds fed the control diet (GA; 0 g HPLP). Conversely, Pigs which received HPLP (GA, GB, GC, GD and GE) had a higher values of high density lipoprotein relative to GA (control).

Table 8: The effect of Holarrhena pubescens leaf powder on the lipid composition of weaned pigs

Variables (mg/dL)	**G_A**	**G_B**	**G_C**	**G_D**	**G_E**	**SEM**	**p-value**
Cholesterol	131.6^{a}	110.7^{b}	103.8^{b}	101.7^{b}	100.1^{b}	9.61	1.75
Triglycerides	77.81^{a}	62.17^{b}	60.98^{b}	55.12^{c}	52.09^{c}	3.82	1.20
High density lipoprotein	45.88^{c}	55.71^{b}	58.32^{b}	65.07^{a}	67.05^{a}	4.04	0.53
Low density lipoprotein	110.4^{a}	98.44^{b}	90.31^{b}	87.92^{c}	85.03^{c}	4.75	0.97

Different letters in the same column (a, b, c) suggests significant differences at P<0.05; SEM, standard error of means; each value represents the mean of five replicates (10 pigs per replicate); GA: basal diet without Holarrhena pubescens leaf powder (control; HPLP); GB: basal diet with 30 g HPLP per kg diet; GC: basal diet with 60 g HPLP per kg diet; GD: basal diet with 90 g HPLP per kg diet; GE: basal diet with 120 g HPLP per kg diet.

Discussion

Phytochemical analysis of Holarrhena pubescens leaf powder proves the presence of phyto-components or metabolites such as, flavonoids, tannins, alkaloids, phenols, glycosides and terpenoids.

Result obtained in this study is in agreement with the study carried out by (Kalimuthu, and Arunprasath, 2018; Kabir et al., 2018). Several of these phyto-components have therapeutic properties and their concentration in the plant tissues is considered as the main factor to evaluate the medicinal value and quality of a given herb (Wills, 2000; Alagbe, 2024). Phenolic compounds in Holarrhena pubescens possess a wide array of pharmacological properties, such as antioxidant (Ojediran et al., 2024a), antiulcer, cytoprotective, chemo-preventive, anti-inflammatory, anti-diabetic, antimicrobial, immune-stimulatory, heptoprotective and free-radical scavenging activity (Ojediran et al., 2024b; Daniel et al., 2023). These attributes are desirable for use of this plant as an agent against the effects that could arise from mycotoxin exposure in humans and animals (Singh et al., 2022). Flavonoids and terpenoids have been suggested to possess gastro-protective and antimicrobial properties, thus it possess excellent inhibitory effects against Pseudomonas aeruginosa, Candida albicans, Brevibacillus agri, Propionibacterium acnes, Trichophyton mentagrophytes, Microsporum canis and Staphyllococcus spp (Adewale et al., 2021). According to Shittu et al. (2021), tannins performs anti-inflammatory activities and analgesic roles making it a useful therapy in the treatment of yellow fever, malaria, diarrhea, headache, stomach and tooth ache. Medicinally, steroids have been shown to exhibit hormonal, antidiarrohea and anti-Inflammatory activities. They have also been suggested exhibit antifungal, antibacterial, antiviral and hypolipidemic activities (Musa et al., 2020). Saponins can be used as adjuvants during vaccine production and possess a strong gastro-protective property (Muritala et al., 2022). Glycosides have been reported to exhibit wide range of pharmacological activities including, anti-inflammatory and anti-helminthic (Wang et al., 2006; Alagbe, 2021). A synergy in all the phyto-constituents recorded in this experiment could enhance immune response, have strong antibacterial properties, stimulate pancreatic enzyme production and overcome loss of appetite and improve pig's growth performance (Ugbogu and Akukwe, 2009; Alagbe et al., 2021).

Piglets receiving HPLP had an improved average daily weight gain compared to the control, though those fed diet supplemented with HPLP at 120 g per kg diet (GE) was significantly higher than those which received GB (30 g HPLP per kg diet), GC (60 g HPLP per kg diet) and GD (90 g HPLP per kg diet). This result suggests that higher levels of HPLP may improve nutrient digestion and support the growth of beneficial bacteria as it has an antimicrobial effect and may help protect gut function against the colonization of pathogenic bacteria (John, 2024a; John, 2024b). The average daily weight gain recorded in this study which varied from 0.33 - 0.48 kg was also similar to the result of a study by (Oetting et al., 2006) who found that average daily weight gain of piglets fed diet supplemented with herbal extracts ranged from 0.21 - 0.55 kg.

This result was lower than those presented by (Pastorelli et al., 2006) who recorded an average daily weight gain of 0.21 - 0.32 kg. This variation could be attributed to levels of dietary supplementation, processing method of plants and phyto-constituents in the test material (John, 2024c). Overall, piglets receiving the supplemented feed had a similar feed intake to piglets on the control diet this outcome suggests that HPLP has flavoring effect due to the presence of phyto-constituents, which could arouse an animal's appetite. According to (Best, 2000), Tannins are reported to increase feed palatability, guide the mucous membrane and protect against the risk of having diarrhea or watery fecal droppings in pigs. Flavonoids and phenolic compounds have also shown to demonstrate both antimicrobial and antioxidant functions in the animal and play a role in appetite enhancement (John, 2024d; Tepe et al., 2005). The result obtained on feed intake is in line with the reports of [30] when Lippia citriodora was supplemented in the diet of piglets. Feed conversion in the control was 3.66 and decreased to 3.00 in the GE (120 g HPLP per kg diet). This result indicates that HPLP can promote the transportation of metabolic products and improve feed conversion ratio in pigs resulting in rapid growth promoting (Bartos et al., 2006; Hanezakowska et al., 2012). The result obtained in this study is in agreement with the report of (Hanezakowska et al., 2012) who recorded a feed conversion ratio range of 2.91 - 3.70 when herbal extracts were fed to piglets. Mortality was recorded only among piglets given GA (control) while none was recorded in the other groups. This result shows that HPLP exhibit antimicrobial, antioxidant, anti-inflammatory properties amongst others (John, 2024e; Taivini et al., 2001). Outcome of this study is in line with the report of (Bartos et al., 2006) when phytogenics were fed to piglets.

Piglets fed a diet containing HPLP increased ($p<0.05$) dressing percentage, fore limb, hind limb, belly, back and lungs compared to those that received the control diet (GA). Values of dressing percentage (62.98 to 70.93 %), fore limbs (6.94 to 8.13 kg) and hind limbs (5.88 to 8.22 kg) range observed in this experiment was similar to the outcome of a study by [34] who discovered that values obtained varied from 61.00 - 70.04 %, 5.03 - 8.77 kg and 5.11 - 9.00 kg respectively. Weight of lungs was higher in piglets fed GE compared to the other group, which is likely due to the activities of phyto-constitutents in HPLP. The significant increase allows more supply of oxygen needed to drive nutrient round the body of pigs. Weights of head, liver, kidney, spleen, heart and gastro intestinal tract were not influenced ($P>0.05$) by the treatment. This result suggests that the phyto-components in HPLP were not toxic to the pigs (Bartos et al., 2006). Findings of the present experiment were similar to those recorded by (Czech et al., 2009; Bruno et al., 2013).

Intestinal microbial population of Staphylococcus aureus which varied from 2.31 to 4.86 (Log 10 Cfu), Escherichia coli [(log 10 3.10 to 5.77 Cfu)], Proteus spp [(log 10 1.41 to 5.77 Cfu), Pseudomonas spp [(log 10 0.81 to 2.00 Cfu)] and Streptococcus spp [(log 10 1.60 to 3.15 Cfu)] decreased in groups fed HLPL compared to that in the control group (GA). Lactobacillus spp population values which ranged from [(log 10 2.07 to 5.11 Cfu)] elevated as the supplementation of HLPL increased in the diet. The outcome of this result indicates that the mode of action of HLPL is based on the principle of competitive exclusion, including eradicating harmful bacteria's (Staphylococcus aureus, Escherichia coli, Proteus spp, Pseudomonas spp and Streptococcus spp) by producing strong antimicrobial constituents, thus increasing the activities of beneficial bacteria (Lactobacillus spp) to mitigate gut health challenges (Bartos et al., 2006). Dietary supplementation of HLPL could also ensure easy absorption of nutrients at an optimum rate and provide efficient protection against pathogens through its own immune system (De Lange et al., 2010).

The present study showed that pack cell volume, platelets, red blood cell and haemoglobin values which varied from 29.08 to 37.09 %, [(110.6 to 143.9 (×109/L)], [(7.96 to 11.6 (×1012/L)] and [(87.56 to 110.3 g/dL)] was influenced by HPLP supplementation in the diet of piglets. The values obtained in this study is within the baseline values; pack cell volume (20.88 to 40.09 %), platelets [(102.3 to 159.80 (×109/L)], red blood cell ([6.60 - 15.00 (×1012/L)] and haemoglobin ([95.00 to 152.44 g/dL)] cited by (Winnicka, 2011; Mayengbam and Tolenkhomba, 2015). Haemoglobin is an iron containing protein found in red cells that allows it to carry oxygen (Egeli et al., 1998; Alagbe, 2022). Decrease in pack cell volume and haemoglobin concentration could result in anemia (Faustini et al., 2000; Friendship and Henry, 1996). Though pack cell volume, haemoglobin and red blood cells were higher in groups which received HPLP relative to the control, this result gives room for sufficient oxygen to drive absorbed nutrients round the body of pigs (Chmielowiec et al., 2008; Cooper et al., 2014). Chronic inflammation is one of the causes of low platelet count in pigs, this could affect the overall health of animals (Czech and Grela, 2002; Czech et al., 2009). White blood cell is saddled with the responsibility of producing antibodies to fight against infections and disease in the body (Czech et al., 2009). The lowest white blood cell count was recorded among pigs fed control diet relative to the other groups which is likely to be associated with the increase HPLP supplementation in the diet. The result also suggest that HPLP possess immune-stimulatory and hepato-protective properties (Shittu et al., 2024). Result in this current study is in agreement with the findings of Chmielowiec et al. (2008). White blood cell recorded [(11.57 to 15.76 (×109/L)] is within the baseline values for piglets cited by Egeli et al. (1998). Lymphocytes, neutrophils and monocytes values varied from [(9.11 - 14.91 (×109/L)], [(6.67 - 9.12)] and [(0.86 - 0.92 (×109/L)] were within the standard values [(8.77 - 15.80)], 6.00 - 15.00(×109/L)] and 0.50 - 1.00 (×109/L)] reported by Radostits et al. (2000). Lymphocytes and neutrophil values were higher in groups fed diet supplemented with HPLP compared to the control. Lymphocytes and neutrophils are associated with viral and bacterial infections (Radostits et al., 2000). Monocytes play a vital role in maintaining the immune system of pigs (Alagbe, 2024). Macrophages and neutrophils are the leucocytes most used during an inflammation. These blood cells moves to pathogens' entry sites and carry out phagocytosis, eliminating infectious agents. Therefore, HPLP modify the metabolism of these phagocytic cells in order to make them more efficient in fighting pathogenic organisms (Radostits et al., 2000).

It was also observed that dietary supplementation of HPLP resulted in a higher total protein (5.12 - 7.41 g/dL), albumin (3.09 - 4.42 g/dL) and 2.03 - 2.99 g/dL and values were within the bench mark reported by Mairbäurl (2003). Decrease in total protein concentration could be as a result of malnutrition, renal and liver dysfunction (Radostits et al., 2000). Albumin/globulin ratio range observed in this experiment (1.47 - 1.52 g/dL) was similar to the result of Alagbe (2024); Muhl and Liebert (2007a) who discovered that albumin/globulin ratio of piglets fed novel phytogenic feed additive varied from 1.20 - 1.72 g/dL. This result was higher than those presented by Muhl and Liebert (2007b) who found that albumin/globulin ratio of piglets fed phytogenics varied from 1.56 - 2.00 g/dL. Glucose values varied from 4.06 - 6.59 mmol/L was within the standard values recorded by Wilson et al. (1972). Glucose levels are mostly elevated during periods of malnutrition, stress due to environment/housing or poor management (Alagbe et al., 2013). Values of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase which varied from 76.87 - 79.88 (IU/L), 52.75 - 56.97 (IU/L) and 102.5 - 115.9 (IU/L) were all similar (P>0.05) in all the groups. Result obtained was in agreement with the baseline values [(alanine aminotransferase; 71.00 - 110.6 IU/L)], ([aspartate aminotransferase; 40.50 - 95.00 IU/L)] and ([alkaline phosphatase; 100.8 - 150.0 IU/L)] reported by Ventrella et al. (2017). This result suggests that dietary supplementation of HPLP at 120 g (GE) did not compromise the integrity of the liver, kidney and other vital organs in the body of piglets.

Value of triglycerides, cholesterol, low density lipoprotein and high density lipoprotein took the form of 52.09 - 77.81 mg/dL, 100.1 to 131.6 mg/dL, 85.03 - 110.4 mg/dL and 45.88 to 67.05 mg/dL. However, values obtained within reference values; [(triglycerides; 47.11 - 80.90 mg/dL)], [(cholesterol; 100.1 to 131.6 mg/dL)], [(high density lipoprotein; 45.88 to 67.05 mg/dL) and ([low density lipoprotein; 85.03 to 110.4 mg/dL)] reported by Dubreuil and Lapierre (1997); Friedrichs et al. (2012). Cholesterol, triglycerides and low density lipoprotein were higher among piglets which received control diet while HPLC groups recorded low values. This result suggests that HPLP possess hypolipidemic properties, thus preventing coronary diseases in piglets (Singh et al., 2021). Conversely, high density lipoprotein was higher in GD (90 g HPLP/kg diet) and GE (120 g HPLP/kg diet), intermediate GB (30 g HPLP/kg diet), GC (60 g HPLP/kg diet) and lowest in GA (control). High density lipoprotein is responsible for carrying cholesterol from the tissue to the liver where they are removed from the body of animals, thus preventing cardiovascular disease (Musa et al., 2020).

Conclusion

In conclusion, Holarrhena pubescens leaf powder contains numerous phyto-constituents which has medicinal properties. Supplementation of HPLP at 120 g per kg revealed the best result on growth performance. It was also able to suppress the activities of pathogenic organism and promote the profileration of beneficial organisms in the gastrointestinal tract and strengthen the immune system without compromising the health status of piglets.

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