Original Research Article

Synergetic Influence of Some Tropical Leaf Meals and Garlic on the haemato-biochemical parameters and antioxidant activities of weaner Pigs

Abstract

Aim: This experiment was designed to study the effects of using composite leaf meal and blend of garlic produced from five (4) different leaves and garlic: Neem, Moringa, Scent, bitter leaves and garlic as a premix in the diets of growing pigs. The leaves and garlic were air dried, milled and sieved separately. Thereafter the leaves and garlic were mixed in the ratio of 4 (Bitter leaf): 3 (Moringa leaf): 1 (Scent leaf): 1 (Neemleaf) and 1 (Garlic) to produce the blend of garlic and composite tropical leaf meal. Individual leaf and their composite mix were analyzed for proximate, mineral, antioxidant and the phytochemical components of the leaves were determined using GCMS and other standard methods.

Methodology: Eighteen large white weaner-pigs of six to eight weeks were allocated in a completely randomized design for this experiment comprising three treatments and three replicates with two pigs per replicate. A basal diets were formulated, mixed and subdivided into three portions in which composite leaf meal and blend of garlic was fed at 0g/kg, 10g/kg, and 20g/kg were used as an additives to the diets of weaner pigs and the diets were designated as I, II and III respectively. The pigs were then assigned to these 3 dietary treatments which were fed to the pigs at 5% of their body weight for 12 weeks experimental period. Water was supplied ad libitum throughout the experimental period. All data were subjected to analysis of variance.

Results: Dietary inclusion of GCLM on haematology, serum biochemistry indices and antioxidants significantly ($P<0.05$) affected the Packed Cell Volume(%), Lymphocytes (%), Granulocytes (%), Alanine aminotransferase (IU/L), Aspartate aminotransferase (IU/L), Total Protein (g/l) and catalase (Ku) of the experimental pigs.

Conclusions: It could be concluded within the limit of this study, that composite leaf meal had high nutrient potentials for pigs and could completely help growing pigs to improve in body weight when increased to the level of 20g/kg and above in pig diets.

Key words: Pig, Garlic-composite leaf meal diets, Haemato-biochemical indices, Antioxidants.

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Comment [H2]: leaves

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Comment [H4]: leaves

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Comment [H6]: ????????????? either A basal diet or basal diet is the correct one

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Introduction

The main barrier to raising enough livestock to supply human and other industrial needs for animal protein has been a lack of feed resources. Due to serious problems posed by the stiff competition for energy and protein feed stuffs, between humans and livestock, other available but neglected cheaper and novel feed resources have been focused areas of recent researches [1,2]. The conventional cereal and vegetable protein sources used in animal feeds are under pressure of competition through their use in human diets [3] hence the hike in prices of these ingredients. Thus, it is necessary to look for alternative and easily accessible cheaper feed materials that are not directly used by humans in order to generate balanced and inexpensive feeds to complement and possibly replace the scarce supply from the expensive conventional sources. Current studies have indicated that the cost of feed alone makes up between 60 and 80 percent of the entire production costs [4]. There is therefore an urgent need for alternative locally and cheap sources of feed ingredients particularly those that do not attract competition in consumption between humans and livestock or have no direct relevance in human food chain. Some of such possible sources of cheap feed stuff are phytogenic feed additives.

The need to harness the potentials of the numerous agro-industrial by-products and green vegetable plants as replacements for the more expensive conventional feed ingredients have been variously expressed [5,6]. Leaf meals not only serve as a protein source but also provide some necessary vitamins such as vitamin A and C, minerals and also oxycarotenoids, which causes yellow colour of broiler skin, shank and egg yolk [7]. Considerable attention has been focused on leaf meals from *Cajanuscajan* [8].

Phytogenic feed additives are defined as herbal substances included in the feed for the purpose of enhancing production and quality of animal products [9]. Phytogenic feed additives comprise wide variety of herbs, spices and essential oils. These feed additives help in enhancing the taste and improving the flavour of feed. Phytogenic feed additives are believed to have positive effects on digestion and intestinal health. Some of the beneficial effects of phytogenic feed additives are their ability to prevent digestive disturbances, improve feed utilization and enhance animal performance [10].

Many studies investigating the effects of various feed on the haematology and serum biochemistry of livestock concluded that feed ingredients including alternative sources affect the physiology of animal [11]. An animal's health state is often determined using the results of serum and haematology investigations. According to [12], haematological and serum parameters are reliable indicators of an animal's physiological health, and variations in these parameters are crucial in determining how well the animal will react to various physiological conditions.

A study carried out on growing pigs by [13] reported that composite leaf meal produced from five leaves do not cause any deleterious effects on the haematology and serum biochemistry. This study is therefore seeks to evaluate the effect of feeding varying levels of composite leaf meal and garlic on the performance, carcass characteristics and blood parameters of weaner pigs.

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Materials and methods

Experimental Location

The study was carried out at the Piggery Unit of the Teaching and Research Farm of the Federal University of Technology Akure, Ondo State, located between Latitude 7^0 18" North of Equator and Longitude 5^0 10" East of Greenwich Meridian with annual rainfall ranging between 1300 and 1650mm and annual daily temperature ranging between 27 and 38^{o}C [14].

Source and Processing of Tropical Leaf Meals and Garlic

Fresh tropical leaves (Bitter leaf, scent leaf, neem leaf and moringa leaf) were harvested within the Federal University of Technology, Akure, Nigeria and its environment. The leaves were air dried separately under a shade until they became crispy. The dried leaves were milled separately and sieved using a hammer mill. Powdered garlic (Allium sativum) sample were bought from Shasha Market, Akure, Nigeria.

Comment [H9]: Mention the scientific names of each plant

Composition of Leaf Meals and Garlic

The blend of garlic and composite meal were made up of Bitter leaf, Scent leaf, Neam leaf, Moringa leaf and garlic. These test ingredients were mixed in the ratio of 4 (Bitter leaf): 3 (Moringa leaf): 1 (Scent leaf): 1 (Neam leaf) and 1 (Garlic) to produce the blend of garlic and composite leaf meal.

Experimental Diets

Diets were formulated to meet the nutrient requirements of the weaner pigs [15]. Three (3) experimental diets were formulated to contain varying levels of blend of garlic and composite meal. Treatment one (I) was the control without blend of garlic and composite mix while treatments II and III contained 10g/kg feed and 20g/kg blend of garlic and composite mix, respectively. The basal composition of the experimental diets is presented in Table 1.

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Table 1: Composition of Basal Experimental Diets

Ingredients	**Quantity (Kg)**		
Maize	33.0		
Wheat Offal	9.00		
Soybean Meal	6.50		
Groundnut Cake	12.75		
Palm Kernel Cake	35.0		
Bone Meal	2.00		
Limestone	1.00		
Premix*	0.25		
Methionine	0.10		
Lysine	0.10		
Salt	0.30		
Total	**100**		
Chemical Composition	**T1- 0g/kg**	**T2- 10g/kg**	**T3- 20g/kg**
Metabolizable Energy (kcal/kg)	3011.80	3111.31	3184.58
Crude Protein (%)	16.46	22.82	19.48
Crude Fibre (%)	6.04	6.63	6.85
Moisture Content	8.19	8.84	7.91
Ash (%)	13.10	11.88	9.30
Ether Extract (%)	5.16	6.34	6.46
NFE	59.25	52.34	57.92

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Experimental Animals and Management

Eighteen (18) weaner pigs of six to eight weeks of age were purchased from a reputable piggery farm in Akure, Nigeria for this study. The weaners were randomly allotted to three (3) dietary treatments and was replicated three times with two (2) pigs per replicate. The weaners were weighed and their initial weight were recorded. The weaned pigs were subjected to standard piggery routine practices such as deworming, medication and vaccination throughout the experimental period. The weaners were offered feed (i.e 5% of their body weight), while water was offered *ad-libitum* throughout the experiment which was lasted for twelve (12) weeks.

Data Collection

Data were collected for haematology, serum biochemistry and antioxidants properties:

Haematological Measurements

At the end of the experimental period, blood samples were collected from jugular veinof the experimental animals for haematology. Blood samples for haematology were collected into sterile tubes containing EDTA Ethylene-Diamine Tetra-Acetic acid (EDTA) which was used to determine the haematological parameters such as packed cell volume (PCV), red blood cell (RBC), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume(MCV), mean corpuscular haemoglobin(MCH), haemoglobin (Hb), white blood cell (WBC) count, granulocytes, lymphocytes and monocytes.All haematological parameters were obtained from the blood samples collected in EDTA sample bottles and determined as described by [16].

Comment [H10]: Delete

Comment [H11]: Vials

Serum Analysis

For the serum analysis, blood samples were collected in test tubes and allowed to stand in slanting position for about 1 day. The blood was then centrifuged to separate the serum from whole blood. The sera were harvested (using a pipette) into cryopreservation containers and then stored in the freezer at -20^{o}C prior to use. Serum parameters analyzed include aspartate aminotransferase (AST),alanine aminotransferase (ALT),cholesterol, Creatinine, total protein (TP), albumin and globulin.

Antioxidants properties

Blood samples were collected from 6 hours fasted birds at the end of the experiment. Blood (10 ml) was centrifuged at 3000 g for 15 min. Serum was frozen at-10 °C and later thawed for analysis of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx).

Comment [H12]: Birds?????????????

Statistical analysis

The experimental design was Completely Randomized Design (CRD). All data collected will be subjected to one-way analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS) version 22 to determine treatment effects. Mean with significant differences will be separated using Duncan Multiple Range Test [17] of the same statistical package.

Comment [H13]: were

Results

Effects of Composite Leaf Meal and Garlic on Haematology of Growing Pigs

The haematology of growing pigs fed graded levels of GCLM is presented in Table 2. The GCLM effect was significantly varied ($p<0.05$) for the RBC, WBC, GRA% and LYMPH %. The RBC increases with increase in levels of GCLM which was in the range of (8.70 ± 0.12 – 13.35 ± 0.38). The highest values for WBC (44.27 ± 2.15 x 10^ 9/L) and LYMPH (73.67 ± 3.67 %) were recorded at 10g/kg of GCLM, while the lowest values for WBC (19.20 ± 1.62 x 10^ 9/L) and LYMPH (56.00 ± 1.15 %) were recorded at treatment fed with 0g/kg. The highest values for

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GRA (37.00 ± 0.58 %) was recorded at the diet fed 0g/kg of GCLM, while the lowest value for GRA (29.00±0.58 %) was recorded at the diet fed 10g/kg of GCLM. The PCV, MCHC, MCV, MCH, Haemoglobin and MON were not significantly influenced (P<0.05) by the dietary treatment. The highest values for PCV (43.33 ± 2.33 %), Haemoglobin (14.45 ± 0.78 Hbg/dl), MCHC (33.22 ± 0.05 g/dl) and MON (2.67±1.33 %) recorded at the diets fed with 0g/kg of GCLM, while the lowest values for PCV (41.00±0.58 %), Haemoglobin (13.67 ± 0.19 %), MCHC (32.96±0.26 g/dl) and MON (1.33 ± 0.88%) were recorded at the treatment with 20g/kg of GCLM. The highest values for MCV (48.60±2.55 fL) and MCH (16.20 ± 0.85 pg/cell), while the lowest values for MCV (35.84±5.52) and MCH (11.95±1.84) were recorded at the treatment fed with 20g/kg of GCLM.

Effects of Composite Leaf Meal and Garlic on Serum Biochemistry of Growing Pigs

The serum biochemistry of growing pigs fed graded levels of GCLM are presented in Table 3. Dietary inclusion of GCLM significantly (P<0.05) affected the AST, ALT and Total Protein of the experimental pigs. The obtained results showed highest AST (54.70 ± 0.81 IU/L) and ALT (39.07 ± 2.28 IU/L)were recorded in pigs fed diet with 0g/kg GCLM, while the lowest AST (42.100 ± 0.81 IU/L) and ALT (29.90 ± 0.51 IU/L) were recorded at the treatment fed with 10g/kg and 20g/kg GCLM, respectively. Highest Total Protein (73.43 ± 0.73 g/l) and Albumin (61.27 ± 1.92 g/L) were recorded in pigs fed diet with 10g/kg GCLM, while the least Total Protein (71.97 ± 0.23 g/l) and Albumin (57.33 ± 3.70 g/L) were recorded at treatment fed with 0g/kg GCLM.The highest cholesterol (3.80 ± 0.20 mmol/L), Creatinine (186.30 ± 5.30 mmol/L) and Globulin (13.73 ± 3.68 g/L) were recorded at the treatment fed with 0g/kg of GCLM, while the lowest cholesterol (3.63 ± 0.88 mmol/L) and Globulin (12.17 ± 2.24 g/L) were recorded at the treatment fed with 10g/kg of GCLM.

Comment [H14]: delete

Effects of Garlic composite leaf mix on Antioxidants of Growing Pigs

The antioxidant of growing pigs fed graded levels of GCLM is presented in Table 4. The GCLM effect was not significantly varied (P<0.05) for all the parameters except for catalase with highest mean of (53.01±7.70 ku/ml) recorded at 20g/kg treatment while the lowest mean value (28.72 ± 4.28 ku/ml) was recorded at 0g/kg treatment. The superoxide dismutase and glutathione were decreasing with increasing levels of GCLM.

Table 2: Haematology of Pigs Fed with Varying Levels of Composite Leaf Meals

Parameters	0g/kg	10g/kg	20g/kg	P-value
Packed cell volume (%)	43.33±2.33	42.33±2.73	41.00±0.58	0.74
Red blood cell (x10^ 6/L)	8.70±0.12^{b}	9.40±1.15^{b}	13.35±0.38^{a}	0.01
Mean corpuscular haemoglobin concentration (g/dl)	33.22±0.05	33.00±0.25	32.96±0.26	0.66
Mean corpuscular volume(fL)	46.81±2.90	48.60±2.55	35.84±5.52	0.12
Mean corpuscular haemoglobin (pg/cell)	15.61±0.97	16.20±0.85	11.95±1.84	0.12
Haemoglobin (Hbg/dl)	14.45±0.78	14.11±0.91	13.67±0.19	0.74
White blood cell (x 10^ 9/L)	19.20±1.62^{c}	44.27±2.15^{a}	25.87±0.27^{b}	0.01

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Granulocytes (%)	37.00±0.58[a]	29.00±0.58[b]	30.00±3.46[ab]	0.06
Lymphocytes (%	56.00±1.15[b]	73.67±3.67[a]	69.00±4.04[a]	0.02
Monocytes	2.67±1.33	1.67±0.88	1.33±0.88	0.67

Table 3: Biochemical Indices of Pigs Fed with Varying Levels of Composite Leaf Meals

Parameters	0g/kg	10g/kg	20g/kg	P-value
Aspartate Aminotransferase (IU/L)	54.70±0.81[a]	42.100±0.81[c]	48.45±1.41[b]	0.01
Alanine Aminotransferase (IU/L)	39.07±2.28[a]	32.27±1.47[b]	29.90±0.51[b]	0.02
Cholesterol(mmol/L)	3.80±0.20	3.63±0.88	3.77±0.88	0.68
Creatinine(mmol/L)	186.30±5.30	177.47±1.77	182.77±1.77	0.26
Total Protein(g/l)	71.10±0.46[b]	73.43±0.73[a]	71.97±0.23[a]	0.05
Albumin(g/L)	57.33±3.70	61.27±1.92	58.23±3.75	0.68
Globulin(g/L)	13.77±4.16	12.17±2.24	13.73±3.68	0.93

Comment [H15]: No significant change in protein values of control and treatment III group

Table 4: Blood Antioxidants of Pigs Fed with Varying Levels of Composite Leaf Meals

Parameters	0g/kg	10g/kg	20g/kg	P-value
Superoxide dismutase (%)	86.10±6.90	76.35±6.34	76.10±7.60	0.55
Catalase (ku)	28.72±4.28[b]	39.10±3.33[ab]	53.01±7.70[a]	0.05
Glutathione peroxidase (%)	229.59±4.76	212.84±7.51	207.79±7.72	0.14

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Discussion

Effects of Supplemental Composite Leaf Meals on the Haematology of Growing Pigs

Animal physiological status can be accurately predicted by haematological markers [18]. In order to monitor feed toxicity, haematological components, which include red blood cells, white blood cells, or leucocytes, mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration, are useful [19]. This is especially true for feed constituents that affect both the blood and the health status of farm animals. The results from this present study show that there were significant difference in the red blood cell, white blood cell, granulocytes percentage and lymphocytes percentage. Erythrocytes, which make up red blood cells, transport hemoglobin. When respiration, this hemoglobin combines with the oxygen carried by the blood to create oxyhaemoglobin [20].According to [21] red blood cells are crucial in the movement of oxygen and carbon dioxide throughout the body, Thus, a lower red blood cell count means a lower amount of oxygen being delivered to the tissues as well as a lower amount of carbon dioxide being exhaled back into the lungs [21,22]. The primary roles of white blood cells and their differentials are to combat infections, protect the body from invasion by foreign organisms through phagocytosis, and create or at the very least transport and disseminate antibodies in response to an immunological stimulus. Animals with low white blood cell counts are therefore at a higher risk of disease infection, whereas those with high counts are able to

Comment [H16]: In whole discussion, there is no explanation why the values were varied due to composite leaf meal. What were the reason of varied hematological values and what were the impact of feeding the composite leaf meal. The discussion here mentioned is well established facts that RBCs main role is the transportation of all substances and role of WBCs are to protect the body but question here is why the only RBCs raised in second treatment group and other hematological parameters showed decrement and insignificant results!!!!!
In nut shell whether the treatments had significant impact and if yes than justify with significant reason that which content affect the hematological values

Comment [H17]: showed

Comment [H18]: What does it mean??????

produce antibodies during the phagocytosis process, have a high level of disease resistance, and are better able to adapt to their local environment and the prevalence of diseases [23]The values obtained for the red blood cell (8.70 - 13.35x10^6/L) and white blood cell (19.20 – 44.27x10^9/L) in this study were higher than 5 – 8x10^6/L and 11 – 22x10^9/L reported carried by [24]. The abnormally increase in the red blood cell value may not certainly be attached to the presence of anti-nutrients in the feed because the value for red blood cell at the 0g/kg of composite leaf meal addition (8.70) was also outside the normal range. This shows that other variables, like adaptation to unfavorable environmental conditions and low levels of oxygen, may be responsible for the abnormally high value of red blood.

The value obtained for the granulocytes (29 - 37%) and lymphocytes (56 – 73%) in this study were higher than 5 – 8x10^6/L and 5 – 13.90 reported carried by [24,25]. Granulocytes, specifically neutrophils, help the body fight bacterial infections [26]. Lymphocytes secrete antibodies that bind to foreign micro-organisms in body tissues and mediate their destruction [27]. It cannot be concluded from this study that the GCLM used had a negative impact on the health status of the pigs since the pigs fed with 0g/kg of GCLM granulocyte and lymphocyte counts were also greater than the reference values, GCLM.

Comment [H19]: Reframe with proper and to the point justification relasted to your treatment group impact on each hematological parameters

Effects of Supplemental Composite Leaf Meals on the Biochemical Indices of Growing Pigs

Biochemical markers are helpful diagnostic tools. Serum contains a variety of components, such as proteins, enzymes, lipids, hormones, etc. Testing for these different compounds reveals details about the body's organs and tissues as well as the animal's metabolic status [28]. This study revealed that the inclusion of composite leaf meal did not affect the serum biochemical indices of the pigs fed diet containing except for the Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Total protein. The Cholesterol, Creatinine, Albumin and Globulin were not statistically influenced by the dietary treatments. AST catalyzes a reaction between the amino acids aspartate and glutamate and is an important enzyme in amino acid metabolism. AST is found in the liver, heart, skeletal muscle, kidneys, brain, and red blood cells [29]. ALT is an enzyme found in the liver that helps convert proteins into energy for the liver cells. When the liver is damaged, ALT is released into the bloodstream and levels increase [29]. An increase in the concentration of AST and ALT may be because of damaged or diseases cells which denote the status of liver function.Increased levels of AST and ALT may indicate faulty or damaged liver cells, which indicate the state of liver function. AST and ALT was significantly higher in the pigs fed control diet (54.70 and 39.07IU/L respectively) while than that of pigs fed diets mixed with 10g/kg and 20g/kg of composite leaf meal. This is an indication that addition of composite leaf meal in the diets pigs may not cause any toxic effect on liver of pigs not pose any serious deleterious health challenges to the animals, especially as it relates to liver, as increased activities of these enzymes in the serum are well-known diagnostic indicators of liver injury [11,30,31].

Comment [H20]: AST and ALT more or less same in TI and TII group in comparison to control group
Total protein more or less same in control and TII group in comparison to TI group
Discussion needs thorough churning

According to [11]decrease in total protein and albumin is an indication of poor quality of the experimental diets. This present study revealed that the total protein was significantly higher in the pigs fed with composite leaf meals than the pigs fed with control diets. It is obvious from this that adding this composite leaf meal to pigs' meals will enhance the health of the animals [32].

Antioxidants characteristics of the growing pigs fed with composite leaf mix

One of the most significant antioxidant enzymes, catalase, converts two molecules of hydrogen peroxide into one oxygen molecule and two molecules of water in a two-step process [33]. High catalase levels damage cell membranes, induce pain, cause graying of the hair, and peroxide lipids, which result in high levels of bad cholesterol, diabetes, and heart attacks. The steady-state concentration of hydrogen peroxide may rise in situations of catalase impairment, causing oxidative damage to DNA, proteins, and cell structures [34]. The result of this present study shows that the activity of CAT in the pigs fed with diets mixed with GCLM was significantly higher than that in the control group. Supplementation with adequate doses of GCLM may thus be beneficial to the antioxidant capacity of weaned piglets and promote their growth and development. The result of this present study was in accordance with the result of [35] who discovered significantly higher catalase activity in weaner pigs fed with *Broussonetiapapyrifera* leaf extract. Superoxide dismutases (SODs) constitute a very important antioxidant defense against oxidative stress in the body. The enzyme acts as a good therapeutic agent against reactive oxygen species-mediated diseases [36]. Glutathione peroxidase is an antioxidant enzyme class with the capacity to scavenge free radicals. This is in turn helps to prevent lipid peroxidation and maintain intracellular homeostasis as well as redox balances [37]

Conclusions

Grower pigs exposed to supplementary feeding of composite leaf meal and blend of garlic responded favorably, especially when added in adequate amount. Pigs fed 20g/kg composite leaf meal and blend of garlic had a marginal increase on growth performance, carcass characteristics and it also improved the blood parameters. Therefore, feeding pigs on dietary additives of composite leaf meal and blend of garlic can be the best production option for farmers to achieve the best results when these feed supplements is increased in the diets of growing pigs.

Comment [H21]: After reframing the discussion again reframe the conclusion portion.

Statements and Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

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It is available from the corresponding author on reasonable request.

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