



Synergetic Influence of Some Tropical Leaf Meals and Garlic on the Haemato-biochemical Parameters and Antioxidant Activities of Weaner Pigs

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MA, AOA and JOA designed the study. All authors managed the activities of the experiment and interpreted the data collectively.

Authors MA, AOA, MOO, OOO, FEA, TOO, OJO and JOA prepared the proposal for the study. Authors MOO, OOO, FEA, TOO and OJO prepared the first draft of the Manuscript. Authors MA and AOA reviewed the second draft and author JOA reviewed the third draft. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This experiment was designed to study the effects of using garlic-composite leaf meals produced from four (4) different leaves and garlic: bitter leaf (*Vernonia amygdalina*), scent leaf (*Ocimum gatissimum*), Neem leaf (*Azadirachta indica*), Moringa leaf (*Moringa oleifera*) and Garlic (*Allium sativum*) as a premix in the diets of growing pigs. The leaves and garlic were air dried,

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milled and sieved separately. Thereafter the leaves and garlic were mixed in the ratio of 4 (*Vernonia amygdalina*): 3 (*Moringa oleifera*): 1 (*Ocimum gatissimum*): 1 (*Azadirachta indica*) and 1 (*Allium sativum*) to produce the garlic-composite tropical leaf meals. Individual leaves 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 eight weeks were allocated in a completely randomized design for this experiment comprising three treatments and three replicates with two pigs per replicate. The average weight of the pigs were 13 kg. Basal diet were formulated and subdivided into three portions in which garlic-composite leaf meals were 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 (%), Mean Corpuscular Volume (fl) 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 garlic-composite leaf meals had high nutrient potentials for pigs and could completely help growing pigs to improve in body weight as the composite leaf meals increases in pig diets.

Keywords: Pig; garlic-composite leaf meal diets; haemato-biochemical indices; antioxidants.

1. 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 phytogetic 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 *Cajanus cajan* [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 garlic-composite leaf meals on the performance, carcass characteristics and blood parameters of weaner pigs.

2. MATERIALS AND METHODS

2.1 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° 18" North of Equator and Longitude 5° 10" East of Greenwich Meridian with annual rainfall ranging between 1300 and 1650mm and annual daily temperature ranging between 27 and 38°C [14].

2.2 Source and Processing of Tropical Leaf Meals and Garlic

Fresh tropical leaves (*Vernonia amygdalina*, *Ocimum gatissimum*, *Azadirachta indica* and *Moringa oleifera*) 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 reputable market around the area.

2.3 Composition of Leaf Meals and Garlic

The blend of garlic-composite meals 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 garlic-composite leaf meals.

2.4 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 garlic-composite meals. Treatment one (I) was the control without garlic-composite leaf meals while treatments II and III contained 10g/kg feed and 20g/kg blend of garlic-composite leaf meals, respectively. The

basal composition of the experimental diets is presented in Table 1.

2.5 Experimental Animals and Management

Eighteen (18) weaner pigs of 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.

2.6 Data Collection

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

2.7 Haematological Measurements

At the end of the experimental period, blood samples were collected from jugular vein of the experimental animals for haematology. Blood samples for haematology were collected into sterile tubes containing 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 vials and determined as described by [16].

2.8 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°C prior to use. Serum parameters analyzed include aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol, Creatinine, total protein (TP), albumin and globulin.

2.9 Antioxidants Properties

Blood samples were collected from 6 hours fasted pigs 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).

2.10 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 were separated using Duncan Multiple Range Test [17] of the same statistical package.

3. RESULTS

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

Table 4 shows the effects of using different levels of garlic-composite leaf meals as premix on haematological indices of swine. Among the parameters measured red blood cell, mean corpuscular volume, granulocytes and lymphocytes were significantly ($P < 0.05$) affected by the dietary treatments. Numerically, highest packed cell volume ($44.00 \pm 0.01\%$), red blood cell ($8.73 \pm 0.03 \times 10^6/L$), mean corpuscular haemoglobin concentration (33.22 ± 0.53 g/dl), mean corpuscular volume (50.89 ± 0.11 fl), haemoglobin (14.65 ± 0.14 Hbg/dl), white blood cell ($21.30 \pm 0.57 \times 10^9/L$), granulocytes ($37.33 \pm 0.33\%$), and lymphocytes ($56.33 \pm 0.88\%$) were recorded in animals fed diet III, while lowest packed cell volume ($41.67 \pm 0.33\%$), red blood cell ($8.50 \pm 0.12 \times 10^6/L$), mean corpuscular haemoglobin concentration (32.96 ± 0.26 g/dl), mean corpuscular volume (48.74 ± 0.44 fl), haemoglobin (14.11 ± 0.40 Hbg/dl), white blood cell ($20.90 \pm 0.40 \times 10^9/L$), granulocytes ($35.00 \pm 1.00\%$), and lymphocytes ($52.66 \pm 0.33\%$) were recorded in animals fed diet I. Highest monocytes ($1.67 \pm 0.33\%$) and mean corpuscular haemoglobin (16.84 ± 0.14 pg/cell) were recorded in animals fed diet I and III respectively while lowest monocytes ($1.33 \pm 0.67\%$) and mean corpuscular haemoglobin (15.77 ± 0.50 pg/cell) were recorded in animals fed diet II.

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

Table 4 shows the effects of using different levels of garlic-composite leaf meals as premix on serum biochemistry of swine. The table shows that among all parameters measured aspartate aminotransferase, alanine aminotransferase and total protein were significantly ($P < 0.05$) influenced by the dietary treatments. The serum biochemistry of growing pigs fed graded levels of GCLM are presented in Table 4. Numerically, highest aspartate aminotransferase (54.70 ± 0.81 IU/L), cholesterol (3.80 ± 0.20 mmol/L) and Creatinine (186.30 ± 5.30 mmol/L) were recorded in animals fed diet I, while the lowest aspartate aminotransferase (42.100 ± 0.81 IU/L), cholesterol (3.63 ± 0.88 mmol/L) and creatinine (177.47 ± 1.77 mmol/L) were recorded in animals fed diet II. Highest Total Protein (73.43 ± 0.73 g/l) and Albumin (61.27 ± 1.92 g/L) were recorded in animals fed diet II, while the least Total Protein (71.97 ± 0.23 g/l) and Albumin (57.33 ± 3.70 g/L) were recorded in animals fed diet I. Highest Globulin (13.77 ± 4.66 g/L) and alanine aminotransferase (39.07 ± 2.28 IU/L) were recorded in animals fed diet I, while the lowest Globulin (12.17 ± 2.24 g/L) and alanine aminotransferase (29.90 ± 0.51 IU/L) were recorded in animals fed diet II and III respectively.

3.3 Effects of Garlic composite Leaf Mix on Antioxidants of Growing Pigs

Table 4 shows the effects of using different levels of garlic-composite leaf meals as premix on antioxidants of swine. The table shows that among all parameters measured catalase was significantly ($P < 0.05$) influenced by the dietary treatments. Numerically, highest catalase (53.01 ± 7.70 ku/ml) was recorded in animals fed diet III, while lowest catalase (28.72 ± 4.28 ku/ml) was recorded in animals fed diet I. Highest superoxide dismutase (86.10 ± 6.90 %) and glutathione (229.59 ± 4.76 %) were recorded in animals fed diet I, while lowest superoxide dismutase (76.10 ± 7.60 %) and glutathione (207.79 ± 7.72 %) were recorded in animals fed diet III.

4. DISCUSSION

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

Diet has been found to influence hematological parameters [18]. According to [19],

haematological indices like RBC, WBC, PCV, and Hb have been found helpful for disease prognosis as well as for therapeutic and feed stress monitoring. Blood is a method for evaluating the clinical and nutritional health

status of animals in feeding trials. Packed cell volume can be used to detect the increase or decrease in the red blood cell in an animal. The result of the packed cell volume of this study revealed that increase in garlic-composite leaf

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 |

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 (%) | 41.67±0.33 ^c | 42.67±0.33 ^b | 44.00±0.01 ^a | 0.01 |
| Red blood cell (x10 ⁶ /L) | 8.50±0.12 | 8.70±0.07 | 8.73±0.03 | 0.13 |
| Mean corpuscular haemoglobin concentration (g/dl) | 32.96±0.26 | 33.00±0.25 | 33.22±0.53 | 0.66 |
| Mean corpuscular volume (fL) | 48.74±0.44 ^b | 50.47±0.29 ^a | 50.89±0.11 ^a | 0.01 |
| Mean corpuscular haemoglobin (pg/cell) | 16.60±0.28 | 15.77±0.50 | 16.84±0.14 | 0.36 |
| Haemoglobin (Hbg/dl) | 14.11±0.40 | 14.45±0.22 | 14.65±0.14 | 0.43 |
| White blood cell (x 10 ⁹ /L) | 20.90±0.38 | 21.26±0.15 | 21.30±0.57 | 0.75 |
| Granulocytes (%) | 35.00±1.00 ^b | 36.33±0.33 ^{ab} | 37.33±0.33 ^a | 0.11 |
| Lymphocytes (%) | 52.66±0.33 ^b | 56.00±0.058 ^a | 56.33±0.88 ^a | 0.01 |
| Monocytes | 1.67±0.33 | 1.33±0.67 | 1.56±0.24 | 0.85 |

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 |

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 |

meals resulted in increase in packed cell volume. Packed cell volume of this study was in accordance with the result of [13] who stated that there were no deleterious effect of dietary composite leaf meals on haematology values of growing pigs due to the mineral content in the composite leaf meals. The values of the packed cell volume of pigs fed composite leaf meal was not in agreement with the study by [20].

Red blood cells facilitate transportation of oxygen to all body tissues while haemoglobin is the oxygen-carrying protein that is found within all red blood cells. Composite leaf meals in diet improved the red blood cell counts and hemoglobin (Hb) concentration in blood. The results of this study were similar to those of [21] who recorded higher levels of red blood cell and Haemoglobin in growing pigs fed on Moringa leaf meal. This could be attributed to higher levels of protein and minerals mostly iron in the composite leaf meals which is responsible for the formation of haemoglobin [22]. The higher the haemoglobin concentration the better the oxygen circulation in the body, hence, better performance of the animal [23]. Red blood cell reflect the physiological responsiveness of pigs to its internal and external environment. The values of red blood cell and haemoglobin of this study coincide with standard range reviewed by [24], but did not correlate with the results presented by [25] that garlic and ginger do not cause deleterious effect on haematology parameters of weaner pigs.

Mean corpuscular volume (MCV) helps to determine presence of anemia and liver disease. The result of the MCV of this study was in accordance with standard range reviewed by [24]. This showed that composite leaf meal had significant influence on MCV.

White blood cells are part of the body's immune system that fight against infection. Lymphocytes helps to fight against antigens. The result of the white blood cell of this study corresponded with the result by [25] who revealed that garlic and ginger do not cause deleterious effect on white blood cell of weaner pigs. The result of white

blood cell and lymphocytes of this study is in accordance with the standard range reported by [24]. This could be due to the beneficial phytochemical present in the composite leaf meals that protected the pigs against infection. The values of white blood cells of this study did not correlate with the results presented by [26].

4.2 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 [27]. This study revealed that the inclusion of garlic-composite leaf meal did not affect the serum biochemical indices of the pigs except for the Aspartate aminotransterase (AST), Alanine aminotransterase (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. [28]. 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 [28]. An increase in the concentration of AST and ALT may be because of damaged or diseased 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) than that of pigs fed diets mixed with 10g/kg and 20g/kg of garlic-composite leaf meal. This is an indication that addition of composite leaf meal in the diets of 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 [13,29,30]

According to [13] 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 garlic-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 [31].

4.3 Antioxidants Characteristics of the Growing Pigs Fed with Composite Leaf Mix

Natural compounds present in plants have been reported to exhibit antioxidant activities [32]. Catalase (CAT) is a part of the peroxidation system in animal red blood cells and certain other tissues. Catalyzing the degradation of hydrogen peroxide to water and molecular oxygen is the main function of CAT, which prevents hydrogen peroxide from forming an iron chelate with molecular oxygen. 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 and induce pain, 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 study shows that the levels of CAT in the pigs increase with increasing levels of composite leaf meals. The value obtained in pigs fed 20g/kg GCLM coincide with the result of [35,36]. Garlic-composite leaf meals such as garlic, scent leaves, neem leaves, bitter leaves and Moringa leaves are rich in catalase, including garlic-composite leaf meals in pig diets also stimulate production of catalase which might be one of the reasons for higher catalase, but did not correspond with the findings of [37] who obtained higher CAT values in weaner pigs fed with *Broussonetia papyrifera* 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 (Bratrovic, 2020). Glutathione peroxidase is an antioxidant enzyme class with the capacity to scavenge free radicals. This in turn helps to prevent lipid peroxidation and maintain intracellular homeostasis as well as redox balances [38]. The result of this study shows that the levels of SOD and GPx in the pigs decreased with increasing levels of composite leaf meals. This could be as a result of

antioxidant present in the composite leaf meals. The values SOD of this study coincide with the result of [39]. The values of SOD and GPx obtained in this study did not correlate with the result of [37,40].

5. CONCLUSIONS

Grower pigs exposed to supplementary feeding of garlic-composite leaf meal responded favorably as the garlic-composite leaf meals increases. Pigs fed 20g/kg garlic-composite leaf meals had best blood parameters. The AST and ALT was low in the treatment 20g/kg which revealed the absence of liver disease Therefore, feeding pigs on dietary additives of garlic-composite leaf meals can be the best production option for farmers to achieve the best results.

AVAILABILITY OF DATA AND MATERIALS

It is available from the corresponding author on reasonable request.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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