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Nutritive value and phytochemical screening of turmeric and clove as a potential phyto-additive in livestock production

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Target Audience: *Researchers, students, Animal Nutritionists, livestock farmers*

Abstract

The aim of this study is to investigate the nutritive values and medicinal potentials of Turmeric (Curcuma longa) and Clove (Syzygium aromaticum) found in the South Western Region of Nigeria as an alternative feed additive in livestock production. These analyses were examined in agreement with the standard procedures and compared. S. aromaticum showed higher presence of crude fat (2.90%), moisture (13.29%) and crude Fibre (11.07%) while C. longa had higher crude protein (7.09%), ash (6.29%) and carbonhydrate (69.66%). Mineral profile revealed that C. Longa had higher of potassium, iron, and phosporous content of 2.489, 40.96 and 0.03 mg/100g respectively compared to S. aromaticum that had the higher calcium of (0.78mg/100g). C. longa contains higher amounts of Vitamin B_1 (0.165g/100g), B_2 (0.228mg/100g) and B_3 (0.5.129mg/100g) when compared to S.aromaticum. Phytochemical screening showed predominantly higher tannin (0.019%), total Phenolic (24.00%), alkaliod (9.50%) and saponin (4.70%) contents in S. aromaticum relative to C. longa which had higher phytate (6.50%) and flavonoid (8.00%). The results presented here showed that C. longa and S. aromaticum contained varying amounts of the proximate, minerals, vitamin and phytochemicals contents. Hence, they could be explored as potential alternative phyto-additive in livestock production.

Keywords: *vitamin, proximate, minerals, phytochemicals, tannins, flavoniod, phenol, alkaloid*

Description of Problem

The persistent and increasing issues on the use of antibiotic as feed additive and growth promoter in livestock feed have prompted the interest in alternative products and have engineered the search for herbal preparations which are medicinal plants. These medicinal plants are cheap, safe, increase production, decrease mortality and production performance. They are able to maintain the optimum growth of livestock animals (1). Herbal medicines are being practiced in the form of therapy for livestock farmers because they contains phytochemicals or bioactive chemicals which have been reported to perform multiple biological activities such as antibacterial, antiviral, antifungal, antioxidant, antidiarrheal, anti-stress and anticancer (2). For many years now, plants are been used as alternative for synthetic drugs in livestock production.

Clove *(Syzygium aromaticum)* is a medium sized tree (8-12 m), aromatic flower buds from the *Mirtaceae* family native. It is considered as one of the most versatile spices. Cloves are grown in Nigeria where they are locally referred to as kanafuru. Cloves are available throughout the year owing to different harvest seasons in different countries. It contains a large number of biologically active compounds,

such as eugenol, eugenol acetate, and β-caryophyllene (3). Eugenol is the principle active ingredient and the most biologically active compound in cloves. Cloves also contain a plethora of compounds with potent antioxidant properties, namely vitamin C, vitamin E, eugenol, flavonoids. So much attention has been given to it due to the potent antioxidant and antimicrobial activities it has among other spices. Many studies have evaluated the effects of clove powder (CLP) on performance, immune response, blood parameters, and lymphoid organs in broiler chickens (1; 4).

Turmeric (*Curcuma longa*) is a flowering plant of the ginger family, zingiberaceae. It has been widely used as spice, food preservative and coloring material that has biological actions and medicinal applications (5). It is also used as antibacterial agent, antioxidant and growth stimulant for centuries. It is commonly grown in Nigeria and is popularly called "ata ile pupa" in Yoruba language. Curcumin (diferuloylmethane), a natural polyphenol, is the main active ingredient of turmeric (C. *longa*). The curcumin is known to perform a number of biological activities, like anti-inflammatory, antioxidant , antimicrobial, anticoagulant in livestock animals. It was likewise reported to improve the nutrients' digestibility, metabolism, and prevent diseases in farm animals. (5).

Turmeric and clove improve nutrient absorption and digestive secretions, reduce the pathogenic stress in the gut, exert antioxidant properties and enhance the livestock's immune status (6). They act as digestibility enhancer, balancing the gut microbial ecosystem and stimulating the secretion of endogenous digestive enzymes and thus improving growth performance in poultry (7). Clove is one of the amazing natural supplements that are used to achieve rapid growth in broiler farming (1). It is effective as a growth promoter when added to broiler feed. Many studies have reported that clove is rich in trace minerals which are essential for protein and carbohydrate metabolism which reduces the synthesis of fatty acid and cholesterol and this could improve broiler performance (1). It was reported that turmeric used as feed additive at level of 0.5% enhances the overall performance of broiler chickens (8). Aflatoxicosis is a cause of economic losses in poultry industry and it is characterized by mortality, listlessness, anorexia, decreased growth rates, negative feed conversions, fatty liver, decreased egg production, poor pigmentation, and increased susceptibility to other diseases in both meat and egg producing chickens. In an experiment conducted by (9), it was concluded that turmeric extract can decrease adverse effect of aflatoxin on liver and kidney and can be used as a supportive treatment in aflatoxicosis in chickens. A study conducted on laying birds discovered that the supplementation of garlic and turmeric powder showed an increase in double yolk egg rates especially for the turmeric diet. Turmeric also had beneficial effect on shell and albumen weight (10). The purpose of this study is to further investigate the nutritive and medicinal potentials of Turmeric and clove as a potential phyto-additive in livestock production.

Materials and Methods

source and preparation of experimental materials

Turmeric rhizome and clove buds were locally purchased at the Toll gate market, Ogere Remo, Ogun State, Southwest Nigeria. Turmeric rhizomes were manually cleaned, peeled and cut into thin pieces, they were air dried under shade at temperature between 25 – 29°C for 15days until crispy. Dried clove buds were cleaned and air dried

for 24 hours prior to milling. The dried materials were pulverized into fine powder in a blender at the Teaching and Research Farm, Department of Agriculture and Industrial Technology, Babcock University, Ilisan-Remo, Ogun State. The powdered samples were stored in a dry, clean container with tight lid for further analysis.

Statistical analysis

All data were analysed using descriptive statistics and t-tests. The mean and standard deviation were measured as statistical values. SPSS Version 22 was used to analyse the data.

Chemical analysis

Proximate analysis

The parameters determined for proximate analysis include Moisture, dry matter, crude fibre contents, total ash and fat content, protein and carbohydrate content of the sample were determined according to (11). Crude protein determination was by Kjeldahl method, Carbohydrate content was determined by calculating the difference between the sums of all the proximate compositions from 100%.

Mineral analysis

The atomic absorption spectrophotometer (AAS) was used for the analysis of the following minerals: calcium, potassium, phosphorus, and iron. One gram of the sample was weighed out with the aid of an analytical balance into an oven dried crucible (at 130^{o}C). The sample was thereafter transferred into a furnace at 550^{o}C with the aid of a laboratory tong, for 3 hours until a white or light grey ash resulted. After ashing, the crucibles were allow to cool in the desiccators After cooling 50mls of concentrated HCl was added to dissolve the ash residue, filtered and read on AAS to determine the elements in the sample.

$$Mineral\ content\ (mg/kg) = \frac{Concentration\ (AAS) x\ dilution\ factor}{Sample\ taken\ (g)}$$

Vitamin analysis

Vitamins B_1 , B_2 and B_3 analysis were performed using the method reported by Association of Official Analytical Chemists (12).

Vitamin B_1 *Thiamine* determination

1g of sample was weighed into 100ml volumetric flask; 25ml of 0.1M H2SO4 was added and mixed by careful swirling. Additional 25ml of 0.1M H2S04 was added to rinse any adhering sample particle off the flask. The flask was set in a boiling water bath to ensure a complete dissolution of the sample in the acid. The flask was shaken frequently in the first 5 minutes and subsequently every 5minutes for 3 minutes. 5ml of taka-diastase in 0.5M Sodium acetate solution was added and flask set in cold water to cool content below 50 O C. The flask was stopped and keeps at 45-50 0 C for 2 hours and thereafter made up to 100ml in ark after mixing thoroughly. The mixture was filtered through a No 42 whatman filter paper discarding the first 10ml and keeping the remaining. 10ml of the remaining mixture filtrate was pipette into a 50ml volumetric flask and 5ml of acid potassium chloride solution was added, shaking thoroughly to mix well. Standard Thiamine solution of range 10mg/ml to 50mg/ml were prepared from 100mg/ml stock and treated same way prepared from sample above. The absorbances of the sample as well as that of standards were read on a fluorescent UV spectrometer (Cecil A20model) at a wavelength of 285nm.

$$Vitamin\ B\ (mg/100g) = \quad 1$$

$$\frac{Absorbance\ x Ave.Gradient\ x\ dilution\ factor}{Weight\ of\ Sample\ (g)}$$..(12)

Vitamins B_2 *Riboflavin* determination

1g of each sample was weighed into a 250ml volumetric flask, 5mls of 5 NHCL was added, followed by the addition of 5mls of dichloroethene. The mixture was shaken and 90mls of deionized water was added. The whole mixture was thoroughly shaken and was heated on a steam bath for 30 minutes to extract all the Riboflavin. The mixture was then cooled and made up to volume with deionized water. It was then filtered, discarding the first 20ml of the aliquot. 2ml of the filterate obtained was pipetted into another 250ml volumetric flask and made up to mark with deionized water. Standard solutions was prepared by dissolving 0.05mg Riboflavin into 100mls of distilled water. Different standard solution concentration of between 0 to 5ppm was prepared from above to obtain the equivalence. The Absorbance, the standards and samples was read on the Fluorescent Spectrophotometer at 460nm wavelength. The amount of Vit.B_2 in samples was calculated using the formula:

$$Vitamin\ B_2\ (mg/100g) = \frac{Meter\ Reading\ x\ Standard\ x\ dilution\ factor}{Weight\ of\ Sample\ (g)}$$....(12)

Vitamin B_3 *Niacin or Nicotinic acid* determination

5g of sample was blended and 100ml of distilled water added to dissolve all Nicotinic acid or Niacin present. 5ml of this solution was drawn into 100ml volumetric flask and make up to mark with distilled water. 10 – 50ppm of Niacin stock solution was also prepared. The absorbencies of the diluted stock solutions and sample extract were measured at a wavelength of 385nm on a Spectrophotometer. Different concentrations of standard stock solutions were read on the Spectrophotometer for absorbances at the specified wavelength to obtain the Gradient Factor.

Amount of Niacin in sample was calculated using the formula:

$$Vitamin\ B_3\ \left(\frac{mg}{100g}\right) = Absorbance\ xDilution\ factor\ xGradient\ factor\ stock = Factor\ Solution\ 10$$.....(12)

Phytochemical analysis

The test samples were subjected to phytochemical analysis in order to find out the presence of phytochemical constituents. Saponin was determined by the method described by (11). Alkaloids and flavonoid was determined by the method described by (13). Phenol and Tannins were determined by the method described by (14).

Tannin determination

Finely grounded sample was weighed (0.2g) into a 50ml sample bottle. 10mls of 70% aqueous acetone was added and properly covered. The bottle was put in an ice bath shaker and shaken for 2hours at 300^{o}C .The solution was then centrifuge and the supernatant stored in ice, 0.2ml of the solution was pipetted into the test tube and 0.8ml of distilled water was added. Standard tannin acid solution was prepared from a 0.5mg/ml of the stock and the solution made up to 1ml with distilled water, 0.5ml of Folinciocateau reagent was added to the sample and standard followed by 2.5ml of 20% Na2CO3 the solution was then vortexed and allow to incubate for 40minutes at room temperature, its absorbance was read at 725nm against a reagent blank concentration of the same solution from a standard tannic acid curve prepared. (14)

$$Tannin\ (mg/kg) = \frac{Absorbance\ of\ sample\ x\ concentration\ of\ standard}{Absorbance\ of\ standard}$$----(17)

Alkaloid determination

Five gram of the sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added and allowed to stand for 4minutes, this was filtered and extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was alkaloid which was dried and weighed (13)

$$Alkaloid\ (\%) = \frac{W3-W2x\ 100\%}{W1} \text{----------(15)}$$

Where: W1 =initial weight of sample,
W2 =weight of the extract,
W3 = final weight of the residue

Saponin determination

The spectrophotometric method of (16). Two gram of the finely grinded sample was weighed into a 250ml beaker and 100ml of Isobutyl alcohol was added. Shaker was used to shake the mixture for 5hours to ensure uniform mixing. The mixture was filtered using No 1 Whatman filter paper into 100ml beaker containing 20ml of 40% saturated solution of magnesium carbonate. The mixture obtained again was filtered using No 1 Whatman filter paper to obtain a clean colourless solution. One (1ml) was added into 50ml volumetric flask using pipette, 2ml of 5% iron (III) chloride ($FeCl_3$) solution was added and made up to the mark with distill water. It was allowed to stand for 30min for the color to develop. The absorbance was read against the blank at 380nm.

$$Saponin\ (mg/kg) = \frac{Absorbance\ of\ sample\ x\ concentration\ of\ standard}{Absorbance\ of\ standard} \text{--(17)}$$

Total Flavonoid determination

Ten gram of the sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered using Whatman filter paper No. 42 (125mm). The filtrate was transferred into crucible and evaporated into dryness over water bath and weighed to a constant weight. (18)

Determination of phenol

The fat free sample was boiled with 50ml of ether for the extraction of the phenolic component for fifteen minutes. Five ml of the extract was pipette into a 50ml flask and then 10ml of distilled water was added. Two milliliter of ammonium hydroxide solution and 5 ml of amyl alcohol were added to the sample and made up to the mark. It was left to react for 30 minutes for colour development; the absorbance was measured at 550nm.(16)

Results and Discussions

Proximate composition

The crude protein, ash and carbohydrate contents (7.09±.04, 6.29±0.01 and 69.66±0.01) respectively in turmeric powder were significant higher compared to that of clove powder (5.87±0.02, 4.95±0.10 and 61.92±0.02). However, higher values were documented for fat, moisture as well as fibre (2.90±0.20, 13.29±0.01 and 11.07±0.01) respectively in clove powder compared to turmeric powder (1.50±0.10, 12.18±0.01 and 3.2± 8.01). The 6.29% ash content in turmeric shows that it will have a reasonable amount of mineral. The result from this study agreed with the report of (19), who reported that turmeric is an excellent source of carbohydrate and protein. The fibre content of 11.07% present is an advantage to livestock as it can contribute to the cleansing of the digestive tract thus preventing the absorption of excess cholesterol which is in

line with the report of (19) that fibre is known for its bulkiness to the food and it prevents the intake of excess starchy food, which prevent against metabolic conditions such as hypercholesterdemic.

Earlier, investigation carried out by (19) shows that turmeric contains 8.92% moisture, 2.85% ash, 4.60% crude fibre, 6.85% fat, 9.40% crude protein and 67.38% carbohydrate while (20) reported that clove contains 10.0 % moisture, 20.0% crude fibre, 5.2% ash, 12.1%fat, 51.5% carbohydrate and 1.2% crude protein. However, the results of the proximate analysis on turmeric from this study contradict the report of (19), who reported higher values of crude protein (9.40%), crude fat (6.85%) and crude fibre (4.60%) while other parameters were higher in this study when compared to the values reported by (19). The proximate content of clove buds reported by (20) showed higher percentage of ash (5.2%), carbohydrate (51.5%), and moisture (10.0%) when compared with this study. The variations could arise from soil type, soil nutrient, farming practises, geographical locations and varied environmental conditions.

Table 1: Proximate composition of the rhizomes of *Curcuma longa L*. and *Syzygium aromaticum*

Parameters	Percentage composition (%) of leaves	
	Syzygium aromaticum (Mean±SE)	***Curcuma longa*** (Mean±SE)
Crude protein	5.87±.02^{b}	7.09±.04^{a}
Crude fat	2.90±.20^{a}	1.50±.10^{b}
Moisture content	13.29±.01^{a}	12.18±.01^{b}
Crude fibre	11.07±.01^{a}	3.28±.01^{b}
Total ash	4.95±.10^{b}	6.29±.01^{a}
Carbohydrate	61.92±.02^{b}	69.66±.01^{a}

**$P<0.01

Mineral and Vitamin composition

The mineral profile showed that turmeric has high values of iron and potassium while clove plant is high in calcium. Phosphorus was found only in turmeric plant. This results further ascertained the recommendations of (21) and (22) that constant feeding on turmeric extracts could be needed in maintaining strong bone, muscle contraction and relaxation, blood clothing, reduce blood pressure, and helps in the haemoglobin formation because of its high potassium and iron.

Clove would be a good source of calcium supplements for livestock especially poultry as observed in this study and calcium plays a vital role in structural and physiological functions, are needed in a certain ratio for bone growth and repair and for other body functions of livestock animals. It functions partly in muscle contraction and relaxation, blood clotting, membrane permeability, nerve function, cardiac regulation and enzyme activation. Vitamin D, which is a precursor for calcium, is however needed for active absorption. (19)

Potassium (K) is identified to reduce blood pressure and it functions in controlling skeletal muscle contraction and nerve impulse transmission. It is vital for the maintenance of osmotic and fluid balance in the body. It is required for chemical reactions in muscles and for skeletal growth. High calcium and potassium meals are

usually recommended for animals with soft bone problems. (22). The potassium and calcium contents of the extract is necessary for livestock with soft bone problems to improve bone mineralization and reduces bone resorption (22).

In table 3, the observed values of vitamins B_1 (5.129±0.004), Vit B_2 (0.228±0.003) , Vit B_3(0.165±0.002) in turmeric plant were higher compared to clove plant Vit B_1(0.074±0.002), Vit B_2 (0.069±0.001) and Vit B_3 (1.063±0.003) Likewise the Fe(2.489±0.002) and K (40.960±0.02) content of turmeric were higher to clove Fe (0.392±0.001) and K (1.330±0.03). However, Calcium content in clove plant (0.78±0.02) was higher than that of turmeric plant (0.290±0.02) as shown in Table 2. Phosphorus was found only in turmeric plant

The results obtained on vitamins are in line with the reports of (19) for turmeric except for the value of Niacin which is slightly higher than the values reported by (19).

Table 2: Mineral composition of rhizomes of *Curcuma longa L*. and *Syzygium aromaticum*

Parameters	Percentage composition (%) of rhizomes	
	Syzygium aromaticum (Mean±SE)	***Curcuma longa*** (Mean±SE)
Fe (g/100g)	1.33±.03[b]	40.96±.02[a]
K (g/100g)	0.392±.001[b]	2.489±.002[a]
Ca^{2+} (g/100g)	0.78±.02[a]	0.29±.02[b]
P (g/100g)	BDL	0.03±.00

******P<0.01(BDL – Below Detectable level) Fe – Iron, K – Potassium, Ca – Calcium, P – Phosphorus

Table 3: Vitamin composition of rhizomes of *Curcuma longa L*. and *Syzygium aromaticum*

Parameters	Percentage composition (%) of rhizomes	
	Syzygium aromaticum (Mean±SE)	***Curcuma longa*** (Mean±SE)
Vitamin B1	0.74±.002[b]	0.165±.002[a]
Vitamin B2	0.069±.001[b]	0.228±.003[a]
Vitamin B3	1.063±.003[b]	5.129±.004[a]

******P<0.01

Phytochemical composition

Significant variations (p<0.01) were observed in phytochemicals percentage between clove and turmeric powder except for flavonoid's percentage that was not significantly different (p>0.05) between the two rhizome plants (Table 4). The percentages of total phenolic, alkaloid and saponin (24.00± 1.0, 9.50±0.0 and 4.70±0.10) respectively in clove powder were higher than that of turmeric power (4.91± 1.0, 6.64± 1.0 and 2.30±0.0). Turmeric powder had higher percentage of phytate than clove powder (6.50±0.01 vs 4.64±0.02) respectively. Tannin was only present in clove plant though the percentage was very low**.** The presence of this phytochemicals confirmed the medicinal properties of the clove and turmeric rhizomes.

Phytochemicals consist of a large group of naturally occurring non nutrient, biologically active compounds found in plants. Phytochemicals are basically produced only by plants. Phytochemicals act as natural defence system for the host plants and in addition provide colour, aroma and flavour. Plants use phytochemicals as natural protection from bacteria, fungi and viruses. Phytochemical analysis is useful to detect the presence of the bioactive principle constituents in the plant which subsequently may lead to the discovery and development of medicinal drugs (23)

Flavonoids exhibit a range of biological activities, which is their ability to scavenge for biological radicals and superoxide anions radicals and thus has ability to promote health.

Flavonoids also exhibits anti-inflammatory, antiangionic, anti - allergic effects, analgesic and antioxidant properties. The potent antioxidant activity of flavonoids is their ability to scavenge hydroxyl radicals, superoxide anions and lipid peroxy radicals may be the most important function of flavonoids (19).

Tannins are also known antimicrobial agents. Tannins are water soluble plant polyphenols that precipitate proteins (24). Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them. The growth of many fungi, yeasts, bacteria, and viruses was inhibited by tannins. Tannins are reported to have various physiological effects like anti - irritant, antisecretolytic, antiphlogistic, antimicrobial and antiparasitic effects. Phytotherapeutically tannin-containing plants such as turmeric plant, *acalypha racemosa* and *acalypha marginata* are used to treat nonspecific diarrhoea, inflammations of mouth and throat and slightly injured skins (24). Due to presence of tannins, plant develop an astringent taste. Tannins interact and precipitate with proteins which results in bitter taste of plants. Consequently, they act as feed deterrents in most of the cases.

Total phenolic acids are capable of removing free radicals, chelating metal catalysts; activate antioxidant enzymes, reducing α- tocopherol radicals, and inhibiting oxidases (25). Polyphenols are the most abundant antioxidants in diets. Alkaniod are crude extract used as medicinal agents in the form of tinctures and as fluid extracts where as some need further processing. Saponins are a special class of glycosides which have soapy characteristics. It has also been shown that saponins are active antifungal agents.

The phytochemical evaluation of turmeric rhizomes conducted by (23) indicated presence of Alkaloids and Flavonoids and the absence of saponin and tannin, as compared to our result that shows the presence of saponin at 2.3%. Tannin was below detectable level thus making turmeric an excellent feed additives/supplements for livestock especially ruminant animals. Phytochemical analysis of clove shows the presence of phenol 5.28%, flavonoid 10.59%, tannin 0.49% and absence of alkaniod and saponin (26) as compared to this result which indicated the presence of alkaniod 9.5% and saponin 4.7% and others such as phytate 4.64%, flavonoid 4.00%, tannin 0.0019% and phenol 24%. This investigation shows higher percentage compared to (23) and (26). These variations could be due to the stage of harvesting of the plants and the methods of processing.

Table 4: Phytochemical composition of rhizomes of *Curcuma longa L*. and *Syzygium aromaticum*

Parameter	Percentage composition (%) of rhizomes	
	Syzygium aromaticum (Mean±SE)	***Curcuma longa*** (Mean±SE)
Phytate	4.64±.02[b]	6.50±.01[a]
Flavonoid	4.00±1.0	8.00±0.2
Tannin	0.019±.00	BDL
Total Phenolic	24.00±1.0[a]	4.91±1.0[b]
Alkaloid	9.50±.01[a]	6.64±1.0[b]
Saponin	4.70±.10[a]	2.30±.05[b]

******P<0.01

(BDL-Below Detectable level

Conclusions and Applications

1. In conclusion, this study revealed that *Syzygium aromaticum* and *Curcuma longa* are good sources of crude proteins, crude fat and minerals, carbohydrate, ash, moisture and crude fibre which have the potentials of being combined in livestock nutrition as feed supplements/additives.
2. They showed presence of some important Phytochemicals like alkaloids, tannins, phenolic compounds, pyhtate, Alkaniods, saponins and flavonoids.
3. They contain essentials minerals like iron, potassium, phosphorus, calcium and vitamins which are very essential in the livestock nutrition.
4. Turmeric and Clove have the potentials of being used singly or can be combined in livestock nutrition for improved health and body growth.

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