GSAR Journal of Agriculture and Veterinary Sciences
ISSN: 3048-9075 (Online)
Abbreviated key title: Glob.J. Agri.Vet.Sci.
Frequency: Monthly
Published By GSAR Publishers
Journal Homepage Link- https://gsarpublishers.com/journal-gjavs-home/

NUTRITIVE POTENTIALS OF LEAF PROTEIN CONCENTRATE AND BAGASSE OBTAINED FROM COCOA (*Theobroma cacao*) PRUNINGS AT IBADAN, SOUTH WEST NIGERIA

By

***[1]Orimoloye, P. O., [2]Eguaoje, S.A., [1]Atolagbe, T.P., and [1]Agbebaku, C**

[1]Department of Value Addition Cocoa Research Institute of Nigeria, Idi-Ayunre, PMB. 5244, Ibadan, Nigeria
[2]Department of Animal Health and production, Edo State College of Agriculture and Natural Resources, Iguoriakhi

Article History

Received: 25/04/2026
Accepted: 04/05/2026
Published: 06/05/2026

Vol – 3 Issue –5

PP: -01-06

Abstract

This study was carried out at the Department of Value Addition Cocoa Research Institute of Nigeria, Ibadan, Oyo State, Nigeria to investigate the nutritive potentialities of cocoa (Theobroma cacao) leaf protein concentrate and bagasse obtained from Cocoa pruning from the Cocoa Research Institute of Nigeria Mainstation. Fresh Cocoa leaf prunings were harvested early in the morning. It was washed, blended and processed into Cocoa Leaf protein concentrates and bagasse which were sundried and immediately taken to the laboratory for chemical analysis. The result showed that crude protein content was higher (39.89%) in cocoa leaf protein concentrate while lowest (10.88%) in cocoa leaf meal. Crude fibre content was higher (46.25%) in those from cocoa leaf bagasse. Nitrogen free extract was highest (35.88%) in cocoa leaf meal. Calcium value was highest 584.85mg/100g in Cocoa leaf bagasse, Sodium, potassium and iron was highest in cocoa LPC. Magnesium was highest in CLB while phosphorus was highest in CLPC. Phytochemical composition showed that higher alkaloid value of (0.53mg/100g) was obtained from Cocoa Leaf Meal. Saponin content was highest (0.32mg/100g) in those obtained from cocoa leaf meal. Oxalate content was higher (0.66mg/100g) in those obtained from cocoa leaf meal. Phytate content was also highest (0.87mg/100g) in those from cocoa leaf meal. Phenol content was higher (0.60mg/100g) in those obtained from cocoa leaf protein concentrate. Flavonoid content was also higher (13.26mg/100g) in those obtained from cocoa leaf protein concentrate. It is concluded therefore that Cocoa Leaf Protein Concentrate holds prospect as a viable substitute for Soya Bean Meal, Groundnut Meal etc in livestock feed formulation. The Bagasse yield was also high and could be used as feed for ruminants and can also ensiled and used for dry season feeding when feed is scarce.

Keywords: *Cocoa Leaf Meal, Cocoa Leaf Protein Concentrate, Cocoa Leaf Bagasse, Chemical Composition*

INTRODUCTION

The dependence on feedlot-based animal production to meet human need for meat has geometrically increased demand for cheaper, readily available and sustainable plant protein sources. This has recently led to re-energized interest in Leaf Protein Concentrate to reduce the use of human edible and expensive vegetable protein sources in animal feed (Akaeze, 2015). There is a shortfall in the supply of good quality protein for meeting the requirement of increasing animal and human population, which has necessitated the search for additional sources (Isuosuo, 2012). Leaf protein concentrate (LPC), a concentrated form of proteins derived from the foliage of plants is a cheap and most abundant source of available protein. Their protein value equals that of most animal products. Trees, shrubs and grasses have been suggested as a potential source of leaf protein concentrate and the production of proteins is advantageous over crops as they do not involve the recurring cost of cultivation (Suman et al., 2014). Leaf protein concentrate (LPC) is a concentrated form of the proteins found in the leaves of plants, leaf protein concentrates was first introduced as a human food in the 1960's but it has not archived much success despite early

*Corresponding Author: **Orimoloye, P. O**

promises because of certain palatability issues (Pirie, 1971; Signh, 1984).

Leaf protein concentrate obtained from a green crop fractionation is free from indigestible fiber and it contains high proportion of proteins which are nutritionally superior. For this reason, its use in the nutrition of human beings and other non-ruminants has been advocated. Apart from proteins, it contains reasonable amounts of vitamin A, minerals, fat and vitamin E. to enhance yield and usability. Although leaves contain proteins and other essential elements, the presence of anti-nutritional factor and the high fibre content of the leaves pose a restriction to their utilization by non-ruminant animals. So, an alternative way to fully utilize leaves in non-ruminants is to process them into Leaf Protein Concentrates (LPC) which is rich in protein, low in fibre and antinutritional factors (Olomu and Nwokoro, 2009; Aletor and Adebayo, 2012). Leaf protein concentrates on the other hand have proven to be a viable source. Akaeze (2010) reported that leaf protein concentrates (LPC) makes for a more effective utilization of the vegetables.

Theobroma cacao (cacao tree or cocoa tree) is a small (6–12 m (20–39 ft) tall) evergreen tree in the Malvaceae family. Its seeds cocoa beans are used to make chocolate liquor, cocoa solids, cocoa butter and chocolate. Although the tree is native to the tropics of the Americas, the largest producer of cocoa beans in 2022 was Ivory Coast. The plants leaves are alternate, entire, unlobed, 10–50cm long and 5–10cm broad. In 2023, world production of cocoa beans was 5.6 million tonnes, led by Ivory Coast with 42.5% of the total (table). Other major producers were Ghana (11.7%) and Indonesia (11.5%) (Hernández, 1995). The cacao bean in 80% of chocolate is made using beans of the Forastero group, the main and most ubiquitous variety being the Amenolado variety, while the Arriba variety (such as the National variety) are less commonly found in Forastero produce. Forastero trees are significantly hardier and more disease-resistant than Criollo trees, resulting in cheaper cacao beans Kongor *et al.,* (2016). Most times the leaves during pruning are discarded along with the chopped off branches which are left to dry off and used as fire wood. These leaves can be harvested and processed into leaf protein concentrate and bagasse which are rich sources of protein and fibre for livestock and can be stored for a long period to be used as dry season feed materials. Therefore, the objective of this research is to investigate the nutritive potentialities of cocoa (*Theobroma cacao*) leaf protein concentrate and bagasse obtained from Cocoa pruning from Cocoa Research Institute of Nigeria.

MATERIALS AND METHODS

Experimental Site

This study was carried out at the department of value addition Cocoa Research Institute of Nigeria, Oyo State, Nigeria.

Production of Cocoa Leaf Protein Concentrate (CLPC) Using Heat Coagulation Method:

Fresh Cocoa leaf prunings were harvested early in the morning and were immediately taken to the laboratory for processing. The leaves were washed to remove debris and sand particles, and then chopped to smaller sizes before weighing. The leaves were then processed by grinding into slurry using an electric blender. Each sample of slurry was placed on a sieve cloth and pressed strongly to separate the juice from the chaff (Bagasse). The juice was then heated and the curd separated leaving the whey fraction. Different temperatures were taken during heating for the curd formation. The curd was separated from the whey using Whitman filter paper and the sample of LPC from the processed Cocoa leaf was taken and yield recorded.

Proximate analysis of CLPC

The proximate composition of cocoa leaf protein concentrate was analyzed according to the method described by AOAC (2016).

Mineral Analysis of CLPC

The mineral content of cocoa leaf protein concentrate (CLPC) was analyzed using atomic Absorption spectrophotometer (AAS) for the following metals: P, Ca, Na, K, Fe, Zn, Mg following the procedure described by (Ahmed *et al.*, 2015).

Phytochemical analysis of CLPC:

The Cocoa leaf protein concentrate (CLPC) was tested for specific presence of certain phytochemicals according to the method of (Harbone, 1998; Tiwari *et al.* 2011).

Statistical Analysis:

Data collected from the study were subjected to analysis of variance using the GENSTAT 12th edition for windows package at 5% ($p<0.05$) and means were separated using Duncan's Multiple Range Test.

RESULTS

Proximate composition of Cocoa (*Theobroma cacao*) Leaf Meal (CLM), Cocoa Leaf Protein Concentrate (CLPC) and Cocoa Leaf Bagasse (CLB) obtained from cocoa leaf prunings.

The proximate composition of Cocoa Leaf Meal, its Protein Concentrate and Bagasse is shown in Table 1. The result obtained for the proximate composition showed they highest dry matter content of (87.15%) was obtained from cocoa leaf meal, followed by (85.42%) recorded from cocoa leaf protein concentrate while cocoa leaf bagasse had least dry matter content of (82.40%). However, they were statistically ($p>0.05$) similar. Percentage crude protein content was significantly ($p<0.05$) highest (39.89%) in those obtained from cocoa leaf protein concentrate than (18.62%) obtained from cocoa leaf bagasse while lowest crude protein value of (10.88%) was recorded for cocoa leaf meal. Percentage crude fibre content was significantly ($p<0.05$) higher (46.25%) in those obtained from cocoa leaf bagasse than (24.52%) obtained from cocoa leaf meal while lowest crude fibre value of (19.80%) was recorded for cocoa leaf protein concentrate. Ether extract content varied significantly ($p<0.05$) with higher value (9.55%) in those obtained from cocoa leaf meal than (5.10%) obtained from cocoa leaf protein concentrate while lowest ether extract value of (2.60%) was recorded for cocoa leaf bagasse. Crude ash was also significantly ($p<0.05$) highest (9.55%) in those obtained from cocoa leaf meal than

*Corresponding Author: **Orimoloye, P. O**

(5.10%) from cocoa leaf protein concentrate while least ash value of (2.60%) was recorded from cocoa leaf bagasse. Nitrogen free extract was also higher significantly ($p<0.05$) with a mean value of 35.88% in cocoa leaf meal, followed by (10.67%) obtained from cocoa leaf protein concentrate while lowest percentage NFE value of (10.12%) recorded in cocoa leaf bagasse.

Table 1: Proximate composition of Cocoa (*Theobroma cacao*) Leaf meal (CLM), Leaf protein concentrates (CLPC) and Bagasse (CLB) obtained from cocoa leaf prunings.

PARAMETERS	CLM	CLPC	CLB	SEM ±
Dry Matter Content	87.15±9.27	85.42±9.18	82.40±8.33	14.36
Crude Protein	10.88±2.85[c]	39.89±2.52[a]	18.62±2.87[b]	5.67
Crude Fibre	24.52±1.88[b]	19.80±1.92[c]	46.25±1.84[a]	3.20
Crude Fat	9.55±0.03[a]	5.10±0.05[b]	2.60±0.03[c]	0.32
Crude Ash	6.32±1.54[b]	9.96±1.48[a]	4.81±1.22[c]	0.18
Nitrogen Free Extract	35.88±3.64[a]	10.67±3.52[b]	10.12±3.66[b]	4.07

*Analyzed

Mineral constituents of Cocoa (*Theobroma cacao*) Leaf Meal (CLM), Cocoa Leaf Protein Concentrate (CLPC) and Cocoa Leaf Bagasse (CLB) obtained from cocoa leaf prunings.

The mineral constituents of Cocoa Leaf Meal, its Protein Concentrate and Bagasse are shown in Table 2. The result obtained showed a significant ($p<0.05$) variation in the calcium content with highest mean value of 584.85mg/100g from cocoa leaf bagasse, followed by (546.37mg/100g) recorded from cocoa leaf protein concentrate while cocoa leaf bagasse had least calcium content of (356.26mg/100g). Sodium content was higher significantly ($p<0.05$) (215.20mg/100g) in those obtained from cocoa leaf protein concentrate than (138.30mg/100g) obtained from cocoa leaf meal while lowest sodium value of (132.61mg/100g) was recorded for cocoa leaf bagasse. Potassium content was also higher (683.72mg/100g) in those obtained from cocoa leaf protein concentrate than (486.90mg/100g) obtained from cocoa leaf bagasse while lowest potassium value of (420.22mg/100g) was recorded for cocoa leaf meal. Significant ($p<0.05$) variation was observed in Iron content with highest value (204.65mg/100g) in those obtained from cocoa leaf protein concentrate than (138.87mg/100g) obtained from cocoa leaf bagasse while lowest iron value of (124.30mg/100g) was recorded for cocoa leaf meal. Magnesium was higher (303.41mg/100g) in those obtained from cocoa leaf bagasse than (109.93mg/100g) from cocoa leaf meal while least magnesium value of (108.92mg/100g) was recorded from cocoa leaf protein concentrate. Phosphorus was significantly ($p<0.05$) higher (1828.91mg/100g) in cocoa leaf protein concentrate, followed by (1772.85mg/100g) obtained from cocoa leaf bagasse while lowest phosphorus value of (1522.67mg/100g) recorded in cocoa leaf meal.

Table 2: Mineral constituents of Cocoa (*Theobroma cacao*) Leaf meal (CLM), Leaf protein concentrates (CLPC) and Bagasse (CLB) obtained from cocoa leaf prunings.

PARAMETERS	CLM	CLPC	CLB	SEM±
Calcium (mg/100g)	356.26±9.29[c]	546.37±9.32[b]	584.85±9.65[a]	23.24
Sodium(mg/100g)	138.30±5.43[b]	215.20±5.52[a]	132.61±5.87[c]	9.62
Potassium(mg/100g)	420.22±8.86[c]	683.72±8.92[a]	486.90±8.84[b]	11.52
Iron(mg/100g)	124.30±4.03[c]	204.65±4.05[a]	138.87±4.03[b]	5.24
Magnesium (mg/100g)	109.93±5.54[b]	108.92±5.48[b]	303.41±5.22[a]	6.03
Phosphorus(mg/100g)	1522.67±43.64[c]	1828.91±47.52[a]	1772.85±43.68[b]	38.27

*Analyzed

Phytochemical analysis of Cocoa (*Theobroma cacao*) Leaf meal (CLM), Leaf Protein Concentrate (CLPC) and Cocoa leaf Bagasse (CLB) obtained from cocoa leaf prunings.

The phytochemical analysis of cocoa (*Theobroma cacao*) Leaf meal (CLM), Leaf Protein Concentrate (CLPC) and Bagasse (CLB) Processed using heat coagulation methods is shown in Table 3. The result obtained for the phytochemical composition showed that higher alkaloid value of (0.53mg/g) was obtained from cocoa leaf meal, followed by (0.26mg/g) recorded from cocoa leaf protein concentrate while cocoa leaf bagasse had least alkaloid content of (0.07mg/g). Saponin content was higher (0.32mg/g) in those obtained from cocoa leaf meal than (0.08mg/g) from cocoa leaf protein concentrate while lowest value of (0.02mg/g) was recorded for cocoa leaf bagasse. Oxalate content was higher (0.66mg/g) in those obtained from cocoa leaf meal than (0.26mg/g) from cocoa leaf protein concentrate while lowest oxalate value of (0.02mg/g) was recorded for cocoa leaf bagasse. Phytate content was also highest (0.87mg/g) in those obtained from cocoa leaf meal than (0.39mg/g) from cocoa leaf protein concentrate while lowest phytate value of (0.11mg/g) was recorded for cocoa leaf bagasse. Phenolic content was highest (0.60mg/g) in those obtained from cocoa leaf protein concentrates than (0.53mg/g) from cocoa leaf meal while

*Corresponding Author: **Orimoloye, P. O**

lowest value of (0.07mg/g) was recorded for cocoa leaf bagasse. Flavonoid content was also higher (13.26mg/g) in those obtained from cocoa leaf protein concentrates than (8.26mg/g) recorded from cocoa leaf meal while lowest flavonoid value (2.06mg/g) was recorded for cocoa leaf bagasse.

Table 2: Phytochemical analysis of Cocoa (*Theobroma cacao*) Leaf meal (CLM), Leaf Protein Concentrate (CLPC) and Cocoa leaf Bagasse (CLB) obtained from cocoa leaf prunings.

PARAMETERS	CLM	CLPC	CLB	SEM ±
Alkaloids (mg/g)	0.53±0.00a	0.26±1.03b	0.07±0.00^{c}	0.01
Saponin (mg/g)	0.32±0.01a	0.08±0.00b	0.02±0.00^{c}	0.00
Oxalate (mg/g)	0.66±0.00a	0.26±0.00b	0.04±0.00^{c}	0.02
Phytate (mg/g)	0.87±0.03a	0.39±0.02b	0.11±0.03^{c}	0.09
Phenols (mg/g)	0.53±0.03b	0.60±0.02a	0.07±0.01^{c}	0.12
Flavonoids (mg/g)	8.26±1.25b	13.26±1.32a	2.06±1.45^{c}	0.08

*Analyzed

DISCUSSION

Proximate composition of Cocoa (*Theobroma cacao*) Leaf Meal (CLM), Cocoa Leaf Protein Concentrate (CLPC) and Bagasse (CLB) Obtained from Cocoa Leaf Prunings.

The observed significant variation in the dry matter content with higher value recorded from Cocoa Leaf Meal compared to those from Cocoa LPC and Bagasse extracted using heat coagulation method might have resulted from geographic, climatic and seasonal variations. Morton, 1987 stated that the low moisture content was indicative of high dry matter content and possible shelf life. The crude protein content obtained from Cocoa LPC extracted using heat coagulation method was significantly higher than that in LPC value (32.64%) reported by Akaeze *et al*. (2015) and as well higher than that in LPC of *Amaranthus hybridus* leaf (34.8%) reported by Adeyeye and Omolayo (2011). The crude fibre value was higher in Cocoa Leaf Bagasse compared to that of the Cocoa LPC and the Meal of the cocoa leaf pruning. The crucial role crude fibre plays in the digestive process and ultimately in the proper functioning of the physiological system cannot be overemphasized. It has been reported that it helps lower the blood pressure and thus reducing the risk of cardiovascular diseases, it also helps in proper bowel functioning and movement, thereby ensuring that waste in moved through the intestine. It is reported that inadequate fibre in the diet of rabbits and other ruminants could lead to a condition known as GI stasis (De blas et al., 1999). The crude fibre was present in appreciable amount in the bagasse of Cocoa Leaf Bagasse. The crude fat of CLPC extracted using heat coagulation method (9.55%) was lower than 9.60% in Amaranthus hybridus and 8.13% in RLPC as reported by Adeyeye and Omolayo (2011) and Odoh (2019) respectively. The bagasse (2.60%) was lower than 5.98% in RLPC reported by Odoh (2019). The reduction in the fat content of the bagasse compare to the meal could be due to the fibrous nature of the bagasse which contain mainly of lignified materials compare to the leaf protein concentrate that contain pure protein. The Ash content of Cocoa Leaf Protein Concentrate was higher compare to that in the Fresh Leaf and the Bagasse. The significant variation recorded CLPC samples compared to CLM and CLB. This is an indication to the mineral quality of the CLPC compared to the other samples. The mean value observed was higher than those reported for vegetable species (Saidu and Adunbarin, 1998) that is Water leaf (*Talium triangulare)* (0.62)%, Rosselle (*Hibiscus sabdariffa*) the reported value of ash indicated that they were good source of mineral element. The result obtained from this study corroborates the findings of Amata and Lebari (2011). The higher value observed in Nitrogen Free Extract in the Cocoa Leaf Meal than that in bagasse is a pointer to the fact that the samples were higher in carbohydrate compared to that of CLPC. NFE is a measure of the carbohydrate content in a feed calculated by subtracting protein, fat, fibre, ash, and moisture from the total weight.

Mineral constituents of Cocoa (*Theobroma cacao*) Leaf Meal (CLM), Cocoa Leaf Protein Concentrate (CLPC) and Cocoa Leaf Bagasse (CLB) obtained from cocoa leaf prunings.

Higher value of calcium recorded Cocoa leaf bagasse is a pointer to the fact that it could support optimum bone formation and development if fed to livestocks as calcium plays a significant role in bone formation (Olusanya, 2008). Sodium content in Cocoa Leaf Protein Concentrate was higher significantly than from cocoa leaf meal and cocoa leaf bagasse. These values reported for sodium was lower than 312mg/kg in *Telferia occidentalis* LPC reported by Adeyeye and Omolayo (2011). High amount of potassium in the body was reported to increase iron utilization (Adeyeye 2002) and is beneficial to people taking dulretics to control hypertension (Arinathan *et al.*, 2003). The potassium value obtained in this study is higher than 23.20 mg/100g reported for *Moringa oleifera* leaf protein concentrate (Sodamade *et al.*, 2013) and 14.55mg/100g reported for *astragalina* leaves (Gafar *et al.*, 2011). Iron is vital for animals because it's essential for oxygen transport via hemoglobin and myoglobin, energy production through the electron transport chain, and DNA synthesis. Its deficiency can lead to anemia and impaired growth, while a proper balance is maintained through complex regulatory mechanism. The micro mineral: iron with mean value (204mg/100g) recorded in Cocoa Leaf Protein Concentrate extracted using heat coagulation method was significantly higher than the value analyzed in Lucerne LPC as reported by Siebrits et al. (1986). This finding is a pointer to the fact that the diet by virtue of the iron composition will support the blood quality of farm animals. Significantly

*Corresponding Author: **Orimoloye, P. O**

higher elemental magnesium 303.41mg/100g in CLB was higher than 116mg/kg and 457mg/kg of *Amaranthus hybridus* LPC reported by Adeyeye and Omolayo (2011) compared to 109.93mg/100g from CLM and 108.92mg/100g in CLPC extracted using heat coagulation method respectively which were lower than 116mg/kg and 457mg/kg of *Amaranthus hybridus* LPC reported by Adeyeye and Omolayo (2011). The elemental phosphorus value recorded for Cocoa Leaf Protein Concentrate using heat coagulation method was higher than that in the Cocoa leaf meal and Cocoa Leaf Bagasse. This finding was higher than those reported in Lucenea LPC by Siebrits et al.(1986). Phosphorus as an element helps in maintaining balance in the body fluid; it facilitates nervous functions and maintains healthy body sugar level.

Phytochemical analysis of Cocoa Leaf Meal (CLM), Cocoa Leaf Protein Concentrate (CLPC) and Cocoa Leaf Bagasse (CLB) Obtained from Cocoa Leaf Prunings.

Alkaloids are generally toxic to other organisms. They often have pharmacological effects and are used as medications, as antimicrobial, antipyretic, local anesthetic and stimulant, psychedelic, analgesic, antibacterial, anticancer, antihypertension agent, the cholinomimetic, anticholinergic, vasodilator antiarrhythmic, antiasthma and antimalarial. Hence, the presence of alkaloids in Cocoa Leaf Meal though reduced in the Cocoa Leaf Protein Concentrate and Bagasse of the Cocoa Leaf confirms its uses as antipyretic, antimalarial and analgesic drug. Alkaloids may also contribute to the plant's effects as antimalarial, anti-diarrhoea and analgesic agents (Mir, 2013). Saponins are being used commercially as dietary supplements and nutraceuticals. Saponins are expected to lead to hydrolysis of glycoside from terpenoid and hence reduce the toxicity associated with the intact molecule. It is found to be higher in the leaf meal than in the LPC and bagasse of cocoa pruning. Its reduction could have been as a result of the heat treatment. The Cocoa leaf meal contains low level of oxalate but this was further in the Cocoa Leaf Protein concentrate and bagasse. This reduction could be accrued to the heat treatment given to the LPC and the sun drying effect on the bagasse. Oxalates are naturally occurring compounds in plant-based foods or feed that can have a detrimental effect when fed to the animals due to its level of toxicity, its ability to bind with calcium thereby reducing its absorption and potentially leading to calcium deficiency in animals. Significantly higher phytate value recorded in cocoa leaf meal compared to cocoa leaf protein concentrate and bagasse could be due to the processing method which significantly reduce the phytate value in LPC and bagasse. Total phenolic varies among the leaf meal, leaf protein concentrate and bagasse of cocoa leaf obtained from the cocoa prunings collected in the study area. It is observed that it was higher in the leaf protein concentrate compare to the leaf meal and bagasse. Phenolic compounds are secondary metabolites in plants that are involved in a number of metabolic pathways and are essential for plant growth and reproduction, and as protecting agents against pathogens. Phenolic compounds may play an important role in preventing chronic illnesses such as cardiovascular disease, certain type of cancers, neurodegenerative disease, and diabetes. The higher phenolic content recorded in the leaf protein concentrate is traceable to the heat treatment applied during the extraction. Its presence in the cocoa leaf meal proves the plant to be a viable phytobiotics which could replace the use of antibiotics in poultry production (Asl Marjan and Hossein, 2008). Flavonoids and other polyphenols such as terpenoids belong to the recently popular phytochemicals, chemicals derived from plant material with potentially beneficial effects on human health. The antioxidant activity of flavonoids is efficient in trapping superoxide anion (O_2), hydroxyl (OH), peroxyl (ROO) and alcohoxyl (RO) radicals. Flavonoid has been shown to possess many pharmacological properties such as: anti-oxidant activities, anti-inflammatory activities, anti-cancer activities and anti-microbial effects, hence, flavonoids may have a contributory effect to its fertility properties and other pharmacological effects the plant possesses (Wu *et al.*, 2003).

CONCLUSION

It is concluded therefore based on the findings from this study that Cocoa Leaf Protein Concentrate holds prospect as a viable substitute for Soya Bean Meal, Groundnut Meal etc in livestock feed formulation. The Bagasse yield was also high and could be used as feed for ruminants and can also ensiled and used for dry season feeding when feed is scarce.

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*Corresponding Author: **Orimoloye, P. O**