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Proximate, mineral and pytochemical screening of ethanolic leaf extract of *Senna occidentalis* as phytobiotic additive in poultry rations.

Cribado próximo, mineral y fitoquímico del extracto etanólico de hoja de *Senna occidentalis* como aditivo fitobiótico en raciones de aves.

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ABSTRACT

The proximate, mineral and phytochemical components of Senna occidentalis leaves ethanolic extract were determined in triplicates using standard laboratory procedures. The results of the proximate analyses revealed that Senna occidentalis leaves ethanolic extract had 8.40% moisture content (MC), 20.49% crude protein (CP), 18.31% crude fibre (CF), 27.36% ether extract (EE), 9.20% ash, 16.24% nitrogen free extract (NFE) and 3550.81 Kcal/kg metabolizable energy (ME). It was observed that the leaves contained 190.80, 28.20, 1930.00, 18.00, 7.15, 8.26, 4.30 and 8.66mg/l of potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn) respectively. The phytochemical screening showed that cardiac glycosides and phenols were absent, flavols were present, while saponins, favonols and alkaloids were largely present. The analyses revealed that Senna occidentalis leaves ethanolic extract has a potent nutritional and phytobiotic attributes in poultry feeding.

Key Words: Coffee weed, minerals, proximate, phytochemicals, phytobiotics.

RESUMEN

Los componentes próximos, minerales y fitoquímicos del extracto etanólico de hojas de Senna occidentalis se determinaron por triplicado utilizando procedimientos estándar de laboratorio. Los resultados de los análisis aproximados revelaron que el extracto etanólico

de hojas de Senna occidentalis tenía 8.40% de contenido de humedad (MC), 20.49% de proteína cruda (CP), 18.31% de fibra cruda (CF), 27.36% de extracto de éter (EE), 9.20% de ceniza, 16,24% de extracto libre de nitrógeno (NFE) y 3550,81 Kcal / kg de energía metabolizable (ME). Se observó que las hojas contenían 190,80, 28,20, 1930,00, 18,00, 7,15, 8,26, 4,30 y 8,66 mg / l de potasio (K), sodio (Na), calcio (Ca), magnesio (Mg), manganeso (Mn). , hierro (Fe), cobre (Cu) y zinc (Zn) respectivamente. El examen fitoquímico mostró que los glucósidos cardíacos y los fenoles estaban ausentes, los flavoles estaban presentes, mientras que las saponinas, los favonoles y los alcaloides estaban presentes en gran medida. Los análisis revelaron que el extracto etanólico de hojas de Senna occidentalis tiene potentes atributos nutricionales y fitobióticos en la alimentación avícola.

Palabras clave: hierba de café, minerales, próximos, fitoquímicos, fitobióticos.

INTRODUCTION

In livestock industry, the poultry sector remains the most widely spread of all and constitutes an important pillar of food security improvement, socio-cultural and economic development in most countries (Alders, 2005; Dieye et al., 2010) and in this sector, broiler production is a veritable source of income, protein and quick returns on investment (Kekocha, 1994). However, the industry in the developing countries had been facing some challenges such as high feed to gain ratio and increase in the cost of feed because of high prices of feed ingredients (Abbas, 2013). Although, several efforts have been made to overcome these challenges amongst which is the use of antibiotics in feed as growth promoters and to prevent outbreak of diseases (Thomke and Elwinger, 1998; Phillips et al., 2004). Furthermore, medication in water using antibiotics helps birds to recover from diseases (Khalafalla et al., 2010) but its benefit as growth promoters has some disadvantages such as drug toxicity, residual effects and development of bacteria resistance (Ogbe and John, 2012).Studies have shown that the usage of chloramphenicol resulted into bacteria of the genus Salmonella developing resistance to the drug (Gassner and Wuethrich, 1994). The use of Avilamycin as a growth promoter resulted in an occurrence of avilamycin resistant Enterococcus faecium in broiler farms (Aarestrup et al., 2000). These problems have led to the ban on the use of antibiotics as growth promoters by the European Union (Butaye et al., 2000; Catala-Gregori et al., 2008). Thus, attention to safe and natural alternatives such as plants to replace antibiotics (phytobiotics) is on the increase. In this present day poultry production, feed additives or phytobiotics are now added to broiler diets to improve its productive performance by increasing growth rate, better feed conversion

ratio, carcass quality and greater livability in poultry birds. Consequently, the use of spices, herbs and leaves as additives in the diet of chickens are now being encouraged because of their health benefits and functions such as anti oxidative ability (Hui, 1996), antimicrobial activity (Dorman and Deans, 2000) and enhancement of digestion by stimulating endogenous enzymes (Brugalli, 2003) in poultry birds. In broilers, the use of garlic as a natural feed additive, improved their growth and feed conversion ratio with decreased mortality rate (Tollba and Hassan, 2003). Ginger had being reported to stimulate digestive enzymes, affect the microbial activity and impact anti-oxidative activity (Dieumou et al., 2009). Osho et al., (2014) opined that bitter leaf could be included in broiler ration and its extract could be used as medicinal application in water for poultry without adverse effect on the performance and blood quality of broiler chickens. Carlina et al., (2012) reported that up to 30% of commercial feed can be substituted with mullberry leaf powder without adversely affecting growth performance and mortality. Odoemelam et al. (2013) asserted that the inclusion of scent leaf (Occimum gratissimum) at 1.00% level in broiler diets generally improved body weight gain, dressing percentage and significantly promoted higher dressed weight and carcass quality. Omoikhoje et al. (2018) reported that 50mls/litres of water of Senna occidentalis leaf aqueous extract had no detrimental effect on the growth performance, carcass traits and internal organs of broiler chickens.

In view of the fore going, the alternative plant with phytobiotic potentials considered in this study is Coffee weed (Senna occidentalis). Senna occidentalisis a leguminous plant that mostly grows in the wild and it is commonly called sickle pod, Sickle senna, Senna obtusifolia, coffee weed and arsenic weed. It's mostly found in the northern part of Nigeria in areas that have not been cultivated during the rainy season. The ground fresh leaf is a special delicacy in making "Black" soup in Esan land in Edo State of Nigeria. Recently, Omoikhoje et al. (2018a) reported moisture content, crude protein, crude fibre, crude fat, ash and nitrogen free extract values of 9.32, 21.88, 19.27, 16.88, 9.70 and 22.4% respectively in dried aqueous leaf extract of Senna occidentalis. The authors also reported the presence of cardiac glycosides, saponins, phenols, flavone, flavonol and alkaloids in the sample. In the same vein, Omoikhoje et al. (2018b) opined that Senna occidentalis aqueous extract can be used as probiotic additive at 50mls/litre of water for improved performance, carcass traits and better cost and returns in broiler chickens. The thrust of this study is to evaluate the proximate, mineral and phytochemical components of Senna occidentalis leaf ethanolic extract.

MATERIALS AND METHODS

Experimental location and climate: The experiment was carried out at the Poultry Unit of the Teaching and Research Farm, Ambrose Alli University, Ekpoma. The farm lies between latitude 6.44°N and longitude 6.80°E in Esan West Local Government Area, Ekpoma, Edo State, Nigeria. Ekpoma is within the South – South geo-political zone of Nigeria and has a prevailing tropical climate with a mean rainfall of about 1556mm. The mean ambient temperature ranges from 26°C in December to 34^0C in February, relative humidity ranges from 61% in January to 92% in August with yearly average of about 82%. The vegetation represents an interface between the tropical rainforest and derived savannah.

Source and processing of fresh coffee weed leaves: Fresh coffee weed (Senna occidentalis) leaves were purchased from Ekpoma main market in Esan West Local Government Area, Edo State, Nigeria. The fresh leaves were thoroughly rinsed, sparsely spread on jute mat and dried at room temperature for 6 – 7 days until they became crispy. The leaves were turned regularly to avoid uneven drying and decay to ensure that the greenish colour of the leaves was maintained. Thereafter, dried crispy leaves were hammer milled through a 2mm sieve and stored in airtight containers to avoid the absorption of moisture till they were used for laboratory analyses. A measured quantity (100g) of the ground leaves was infused in 400ml of ethanol overnight (12hours). Thereafter, the solution was filtered through a fibre sieve clothe. The filtrate was concentrated to dried form using a water bath at 60^0C.

Proximate analysis: The proximate composition of the leaf meal were determined according to standard procedures of AOAC (1990).

Mineral Analysis: The minerals: calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), copper (Cu), zinc (Zn), iron (Fe) and manganese (Mn) were determined with the atomic absorption spectrophotometer model 420. Phosphorus in the digest was estimated with vanadomolybdate solution and the colour so developed was read with spectrophotometer at 420 m/u. The concentration of K was estimated with a flame photometer using the methods of AOAC (1990).

Phytochemical screening of Senna occidentalis leaves: The phytochemical screening were performed on the extracts from the leaf meal using standard procedures of Sofowora (1993), Trease and Evans (1989) as well as Odebiyi and Sofowora (1978).

Test for glycosides: One millilitre (1ml) of the leaf extract was dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution. This was under-layered

with 1ml of conc. H_2SO_4 and the appearance of a brown ring indicated the presence of glycosides.

Test for saponins: The leaf extract of 0.5g was shaken with water in a test tube and was observed for frothing, while saponin rein weiss supplied by Merck was used as standard.

Test for flavanoids: A quantity (2ml) of the leaf extract was boiled in 10ml of distilled water and filtered. The filtrate was divided into two different portions A and B of 5ml each. To portion A, 10% Lead acetate solution was added in few drops and the presence of a yellowish precipitate indicated a positive result. While, 5ml of 20% NaOH and few drops of dilute HCl were added to portion B, the formation of colourless solution indicated a positive test.

Test for phenolic compounds: The leaf extract (1ml) was added to 5ml of 90% ethanol plus one drop of 10% $FeCl_3$, a pale yellow colouration indicated a positive test.

Test for alkaloids: Dragendoff Wagner's reagent and Picric acid were used to test for alkaloids. About 1ml each of the leaf extract was transferred into three labelled test tubes A, B and C. To portion A, 2ml of Dragendoff's reagent (made up of a mixture of Potassium Bismuth Iodide salt) was added. The presence of a reddish brown precipitate indicated a positive test. To portions B and C, 2mls each of Wagner's reagent and Picric acid were respectively added and the presence of reddish brown and yellowish precipitates indicated positive tests.

RESULTS AND DISCUSSION

The results on the proximate composition (Table 1) showed that Senna occidentalis leaf ethanolic extract contained 8.40% of moisture content (MC), crude protein (CP) of 20.40%, crude fibre (CF) 18.31%, ether extract (EE) 27.36%, ash (9.30%) and 16.24% of nitrogen free extract (NFE). The percentage MC (8.40%) of S. occidentalis leaf ethanolic extract was in accordance with that obtained (8.70%) by Augustine et al. (2018) for raw S. occidentalis leaves but higher (6.49%) than that of Yakubu et al. (2017). The low moisture content of the leaves portends the fact that the dried leaves can be conserved for a long period. The CP value (20.40%) in this study coincided with 21.40% reported by Adjoudji et al. (2005) but higher than 11.63 and 17.25% obtained by Kubmarawa et al. (2011) and Augustine et al. (2018) respectively. However, this value almost fell within the range of 21.30 to 30.00% for many leafy vegetables (Lucas, 1988; Falade et al., 2004). The percentage CP in the sample indicated that it is a potential protein source for feeding farm animals. The crude fibre (18.31%) of S. occidentalis leaf ethanolic extract was higher than

13.90 and 15.03% recorded by Adjoudji et al. (2005) and Augustine et al. (2018) respectively but lower than 27.07% recorded by Kubmarawa et al. (2011). This value however coincided with 18.52% obtained by Augustine et al. (2014). This variation may be ascribed to the stages at which the leaves were harvested and the moderate value recorded in the sample may be an advantage in monogastric nutrition. The ether extract content (27.36%) of the sample was higher than 4.80, 3.32 and 3.45% recorded by Adjoudji et al. (2005), Missa Mohammed et al. (2015) and Augustine et al. (2018) respectively. Ash value (9.20%) almost coincided with 9.86 and 10.30% respectively obtained by Adjoudji et al. (2005) and Kubmarawa et al. (2011) but higher than 8.20 and 7.80% (Augustine et al. (2018). The relatively high ash content of the leaves points to the fact that the leaves might be a reasonable source of mineral such as calcium, magnesium, iron potassium etc. Moreover, percentage ash had been adjudged to be a veritable tool for assessing the amount of mineral present in plant samples (Micheal and David, 2003). NFE value (16.24%) obtained in this study was relatively lower than 38.19 and 39.60% (Yakubu et al., 2017; Augustine et al., 2018). However, the higher metabolizable energy (3550.81 Kcal/kg) of the sample compared to 2323.50 Kcal/kg reported by Augustine et al. (2018) is an evidence that the leaves might contribute to the energy content of feeds when incorporated into the diet of farm animals.

Table 1: Proximate composition of coffee weed leaf ethanolic extract

Proximate components	%
Dry matter	91.60
Crude protein	20.49
Crude fibre	18.31
Ether Extract	27.36
Ash	9.20
Nitrogen free extract (NFE)	16.24
Metabolizable Energy (ME Kcal/kg)	3550.81

The results of S. occidentalis leaves ethanolic extract for K, Na, Ca, Mg, Mn, Fe, Cu and Zn were 190.80, 28.20, 1930.00, 18.00, 7.15, 8.26, 4.30 and 8.66mg/l respectively (Table 2). The Ca content of the sample was observed to be highest (1930.00 mg/l), followed by that of K (190.80mg/l) and lowest in Cu (4.30mg/l). From the data, it was observed that S. occidentalis leaves ethanolic extract is rich in major minerals needed for the maintenance of fluid balance, nerve transmission and a healthy blood pressure. Besides,

Mg helps to transport both Ca and K across cell membranes and serves as a co-factor in many enzyme reactions (Stacey, 2015).

As shown in Table 3, the phytochemical screening of the investigated S. occidentalis leaves ethanolic extract revealed the presence of saponnins, flavols, flavonols and alkaloids but absence of cardiac glycosides and phenols. Saponnins, flavonols and alkaloids were largely present in the sample. These phytochemicals are non- nutritive chemicals produced by plants through primary or secondary metabolism and they have that protective or disease preventive properties. Meanwhile, it had been alluded that plants produce these chemicals to protect themselves but recent research investigations had shown that they can also protect humans/ animals against diseases . For instance, saponins found in beans interfere with replication of DNA cells thereby preventing the multiplication of cancer cells (Harbone et al., 1999; Molyneux et al., 2007). Besides, the consumption of saponins have been encouraged because of their hypocholesterolemic activity (Oakenful and Sidhu, 1989), while flavols and flavonols are antioxidants and antimicrobial agents that fight against a wide range of microorganisms by inhibiting their membrane bound enzymes (Chowen,1999).

Table 2: Mineral composition of coffee weed leaf ethanolic extract

Mineral components	Mg/l
Potassium	190.80
Sodium	28.20
Calcium	1930.00
Magnesium	18.00
Manganese	7.15
Iron	8.26
Copper	4.30
Zinc	8.66

Table 3: Phytochemical screening of coffee weed leaf ethanolic extract

Parameters	Results
Cardiac glycosides	- -
Saponnins	++
Phenols	- -
Flavols	+
Flavanols	++
Alkaloids	++

Key: Absent = - -, Present = +, Largely present = ++

As conclusion, the data revealed that Senna occidentalis leaves ethanolic extract is rich in energy and protein needed for cellular functions when consumed. The mineral

content of the leaves suggest that the plant can contribute to the nutrient requirement of humans due to the high concentration of major minerals such as calcium needed for strong bones and teeth, potassium and sodium for the maintenance of fluid balance, nerve transmission and a healthy blood pressure. Besides, the relatively high level of magnesium which helps to transport both Ca and K across cell membranes and serves as a co-factor in many enzyme reactions is also of paramount interest. The use of the leaf meal and its extracts as phytobiotic additive in livestock feeding particularly monogastrics to evaluate their biological and medicinal values is recommended.

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