EFFECT OF DIGESTION OF COW DUNG ON THE PHYSICO-CHEMICAL AND GRAIN YIELD OF GROWN AMARANTH (*Amarranthus spinosus*)

By

Nwokeocha U.C.O, Sir Odoh F.O and Okafor C.C.
Department of Food Technology, Institute of Management and Technology (IMT), Enugu
P.M.B 1079, Enugu, Nigeria.

Abstract

This study was carried out to determine the effect of digestion of cow dung on the physico-chemical and grain yield of grown amaranth (Amarranthus spinosus). A 200 kg of cow dung was divided into two equal parts; the first portion was digested with water in the ratio of 2:1 at 40°C for 21 days, while the second portion was left undigested. The digested and undigested manures were applied to the soil and mixed thoroughly two days before seedlings were transplanted. Amaranthus spinosus seedlings were raised and transplanted to seedbeds at a spacing of 10 cm by 20 cm and depth of 3cm, one seedling per hole. The seedlings were allowed to grow for two months before harvesting. At the end of the experiment, 20 matured plants were randomly selected. The leaves were prepared for analyses. Thereafter, the laboratory result was analyzed statistically using One Way ANOVA at (P5≤5) significant level. Proximate composition results were moisture 39.70% and 40.22%, ash 8.87 and 7.38%, crude fibre 13.73 and 12.48%, protein 14.55 and 12.22%, fat 4.45 and 3.91% and carbohydrate 18.71 and 23.79%; phytochemical constituents were alkaloid 7.57 and 6.78 mg/ 100g, saponin 8.89 and 8.43 mg/100g, flavonoid 9.05 and 7.67 mg/ 100g and tannin 2.59 and 1.90 mg/100g; the minerals were iron 0.08 and 0.07 mg/ 100g, potassium 8.96 and 4.88 mg/100g then, yield 59 and 50 kg/ha for digested and undigested cow dung grown A. spinosus respectively. High amounts of phytochemicals, minerals and proximate composition recorded in this research gives preference to the use of biogas slurry in growing of garden than undigested cow dung. The results of this study further encourage the use of biogas slurry in growing A. spinosus for better nutritional quality.

Keywords: Digestion, Cow Dung, Physico-Chemical, Grain Yield, Amaranth

Introduction

Amaranthus species are a highly popular group of vegetables that belong to different species (Kahu and Umeh, 2019). *Amarranthus spinosus* (Family *Amaranthaceae*) is commonly known as the spiny, prickly or thorny amaranth. Amaranth originated in America and is one of the oldest food crops in the world with evidence of its cultivation reaching back as far as 6700 BC (Forestry and Fisheries, 2010). It is an annual vegetable that is widely distributed in the humid zone of the tropics including Nigerian (Mofunanya et al., 2015). The leaves of both the grain and vegetable types may be eaten raw or cooked. Amaranths grown principally for vegetable use have better tasting leaves than the grain types. In Nigeria especially Yoruba community all species are referred to as "tete" even though they may add a second name to indicate a particular variety or species. The Hausas refers to them as "alaiyaho while Igbos calls them "imne" (Alegbejo, 2013). The nutritional value of *Amaranthaceae* is equivalent to that of spinach, and its protein, iron and calcium contents are higher than those of cabbage and lettuce.

Amaranthaceae grains can be used in the making of bread, cookies and jam and leaves can be consumed raw or cooked in salad. Leaves have a major nutritional potential, considering the lack of economically accessible protein sources to people of poor resources (Escudero *et al.*,2000).

Greens Leaf amaranth is used as a steamed vegetable in soups and stews.
One of the reasons there has been recent interest in amaranth is because of its useful nutritional qualities. The leaves stem and head are reportedly high in protein (15 to 24 % on a dry-matter basis) (Akubugwo, et al., 2007). In Nigeria, *Amaranthus* leaves combined with condiments are used to prepare soup The leaves contain the following (nutrients, 83.48% moisture, 13.80% ash, 17.92% crude protein, 4 65%, %fat, 8.1% fibre, 52.18% carbohydrate, sodium (Na) 7.43mg/100g, %potassium (K) 541.20 mg/100g, calcium (Ca) 44.15mg/100g, magnesium (Mg) 231.22mg/100g, iron (Fe) 13.58mg/100g, Zinc (Zn) 3.80mg/100g and phosphorus (P) 34.91mg/100g (Akubugwo, *et al.*, 2007).

Production of amaranth has reduced drastically due to poor soil fertility. Commercial and subsistence farming has been and is still relying on the use of inorganic fertilizers for growing amaranth because they are easily absorbed and utilized; prices of this vegetable has skyrocketed due the continual use of inorganic fertilizers as they are expensive to purchase (Kahu and Umeh, 2019). Furthermore, low and declining soil fertility due to continuous cultivation, soil erosion and nutrients losses through runoff and leaching is a serious problem in many parts of Nigeria (Nyankanga, 2012). Nitrogen is a growth limiting nutrient and its use in crop production especially leafy vegetables is a well-known practice. Low levels of nitrogen and its losses in the soils have been associated with reduced yields with its attendant poor quality of crops, leafy vegetables included. Inhibited mineral uptake by plant roots and accumulation of anti-nutrients in plant tissues restricts bioavailability of essential mineral elements posing a risk of hidden hunger symptoms (Munene, 2017).

In recent times, attention has been directed towards organic manure because of the inability of inorganic fertilizers to give the soil the desired sound health (Oyedeji *et al.,* 2014). Presently in Nigeria, vegetable farmers always have heaps of plant wastes and animal droppings such as cow dung and orange waste around them that they need not pay money for their collection and usage. This free access is therefore being abused. The repeated use of these manures as bio- fertilizers on the same piece of land, irrespective of the type and quality of the droppings for the cultivation of amaranth is on the increase (Adewole and Dedeke, 2012). Anaerobic digestion can convert energy stored in organic matter present in manure into biogas. The digester slurry is a good fertilizer. It is claimed that its value as fertilizer could double crop yield (Ubwa *et al.,* 2013). Currently, there is a dearth of information on the yield, phytochemicals and nutritional quality of *amaranthus spinosus* after application of different biogas slurry to soil.

MATERIALS AND METHODS

Study site: The study was carried out on a farmland at the Institute of Management and Technology (IMT) Enugu, Enugu North Local Government Area of Enugu State, Nigeria. Pre-cropping chemical analysis of the experimental soil was not conducted before the land was cleared for planting. The experiment was laid out in a completely randomized design with three replicates.

Preparation of Biogas Slurry

This was done using the method as of Putri *et al.* (2012) with slight modification. The cow dung of 200 kg was divided into two equal halves. The first portion was poured into separate digesters and mixed with water in the ratio of 2:1. The sample was mixed thoroughly until all ingredients were well blended in the digesters. It was then was stored at temperature of 30°C for 21 days, while the second portions was left undigested.

Nursery Practices in the Seeds

The nursery practices were carried out using the method of Law- Ogbomo and Ajayi (2009) with slight modification. Seeds of *Amarranthus spinosus* variety Nigerian Institute of Tropical Agriculture (IITA) Ibadan were sowed in seed trays (nursery) filled with top soil bed by broadcasting the seeds. The seedlings remained in the nursery for four weeks with routine management of watering and weeding when necessary, after which they were transplanted to the field. The planting method applied was as described by Mofunanya *et al*. (2015). The land was cleared manually after which West African hoe was used to make beds with a space of 50 cm between beds. The digested and undigested manures were applied to the soil and mixed thoroughly two days before seedlings were transplanted. *Amaranthus spinosus* seedlings were raised and transplanted to seedbeds at a spacing of 10 cm by 20 cm and depth of 3cm, one seedling per hole. The seedlings were allowed to grow for two months before harvesting. Weeding was done manually at 4 and 7 weeks after sowing. Eight plants were selected per plot for the determination of herbage yield at two months after transplanting. At the end of the experiment, 20 matured plants were randomly uprooted. The leaves were separated with knife and yield was determined; Leaves were dried at temperature 60°C for 8 h whereby a constant weight was obtained and milled into powder in an electric mill. One hundred grams were taken from pulverized samples for the determination of physico-chemicals constituents of the leaves.

Analysis

Proximate composition

Moisture, Ash, Fat, Crude fibre, Protein and carbohydrate contents were determined using the method of AOAC, (2010). For the phytochemical determinations of the tannins, the method of Ojinnaka *et al*., (2013) was adopted, while the rest followed the procedures of AOAC (2010).

Statistical analysis was carried out using SPSS version 23.0.0 (SPSS, 2010).

RESULTS AND DISCUSSIONS

Table 1: Proximate composition of *Amarranthus spinosus* grown with digested (biogas slurry) and undigested cow dung

Sample	Moisture (%)	Ash (%)	Crude fibre (%)	Protein (%)	Fat (%)	Carbohydrate (%)
Digested	$39.70^{a}\pm0.107$	$8.87^{a}\pm0.118$	$13.73^{a}\pm0.070$	$14.55^{a}\pm0.321$	$4.45^{a}\pm0.014$	$18.71^{b}\pm0.389$
Undigested	$40.22^{a}\pm0.143$	$7.38^{b}\pm0.199$	$12.48^{b}\pm0.056$	$12.22^{b}\pm0.023$	$3.91^{b}\pm0.025$	$23.79^{a}\pm0.297$

Values are mean ± standard deviation of duplicate determination. Mean with different superscript are different from each other significantly ($P < 0.05$).

Proximate composition of *Amaranthus spinosus*

The result of the moisture content of *Amaranthus spinosus* samples in Table 1 ranged from 39.70 to 40.22%. The sample grown with digested cow dung had lower moisture content of 39.70% while the undigested cow dung grown sample was 40.22%. There was no significant different ($P\geq0.05$) between both samples. The moisture mean value was in agreement with 43.47% in A. hybridus grown with poultry by (Oyedeji *et al.* 2014). The value was however lower than 86.40% reported by Mofunanya *et al.* (2015) in *Amaranthus spinosus* grown with saw dust, poultry droppings and cow dung: 500 kg/ha. The reason for high moisture content in both digested and undigested cow dung was attributed to the dense organic matter in the organic fertilizers which improves the water holding capacity of the soil. Hence, the plants will be able to absorb more water for use and in turn the amount of moisture on the leaves might be higher (Kahu and Umeh, 2019).

The ash content of the vegetables was 8.87 and 7.38% for digested and undigested grown *Amaranthus spinosus* respectively. The ash content in the digested sample was significantly higher than that of the undigested sample possibly because of the balanced nutrient in the manure (Oyedeji *et al.,* 2014). The ash content of the samples was higher than a range of 2.58 to 3.06% reported by Kahu and Umeh (2019) in poultry manure at different proportions. The crude fibre content of the digested and undigested cow dung grown plant were 13.73 and 12.48% respectively. There was significant different between both samples, however, the digested sample: had higher crude fibre value than the undigested sample. The mean crude fibre content of the samples were significantly ($P\leq0.05$) higher than 1.8% obtained by Alegbejo (2013) in *A. Spinosu.* Dietary fiber in leafy vegetables contributes in the regulation of intestinal transit, increase dietary bulk and fencal consistency due to their ability to absorb water (Sossa- Vihotogbe *et al.,* 2013). Crude fibre was found to be highest for the digested cow dung grown amaranth, Mofunanya *et al.* (2014) reported similar results for *Amaranthus spinosus* L and suggested that high crude fibre may be due to the high levels of carbon containing compounds in the organic manure slurry which are easily converted to CO_2 and absorbed by the plants which readily synthesize fibre (Kahu and Umeh, 2019).

The protein content of the samples were 14.5% and 12.22% for digested and undigested cow dung grown amaranth with the digested sample having higher protein (14.55%) than undigested sample (12.22%). There was significant difference (P5005) between both samples. The protein value in this research was in line with 12.13 and 12.5% protein in *Moringa oleifera* leaf grown with digested and undigested cow dung as reported by Anamayi *et al.* (2016). Makinde *et al.* (2010) reported that organic material alone or in combination with NPK significantly increased protein and ash content of vegetables. Digested cow dung (biogas slurry) contains very high amount of Nitrogen, such 22that when applied to the soil the nitrogen is converted into ammonia by the process of nitrogen fixation by some bacteria such as *azotobacter, clostridium, rhizobium* and *azospirillum*. Also, the ammonia can be further converted to nitrate by nitrifying bacteria. The ammonia in the form of ammonium and nitrate are taken by the plants and used for synthesis of nucleotides and protein.
The fat content of the digested and undigested cow dung grown *amaranth* were 4.45 and 3.91% respectively with the digested sample having higher fat content (4.45%) than the undigested sample (3.91%).

Both samples differed significantly (P≤0.05) from each other. The fat content of the samples were significantly (P≤0.05) lower than 5.80% fat in *Amaranthus cruentus* grown with compost manure as reported by Oyeyemi *et al.* (2017). The values were however; higher than 1.50% fat reported by Anamayi *et al.* (2016) in cow dung grown *moringa leaf.* The variation in the fat composition of the samples with that of previous research findings could be due to the concentration of the manure applied to the soil and the variety of the crop.
The carbohydrate content of the digested and undigested cow dung grown vegetable were 18.71 and 23.79% respectively with undigested sample having higher mean value (23.79%) than the digested value.

However, both samples differed significantly from each other. These values were higher than 10.37% recorded in A. hybridus grown with poultry manure Oyedeji *et al.* (2014). The variation in carbohydrate could be due to the species, the age of the leaf and the concentration of the manure added. The untreated plants contained high carbohydrate because the plants harvested from the treated plots had more available nutrients like mineral elements, fat and protein, thereby having less carbohydrate than the untreated plants which doesn't contain many nutrients (Kahu and Umeh, 2019). Organic manures activate many species of living organisms which release phyto-hormones and may stimulate the plant growth and nutrients and such organisms need nitrogen for multiplication (Ouda and Mahadeen, 2008).
Table 2: Phytochemicals constituents of *Amarranthus spinosus* with digested (biogas slurry) and undigested cow dung

Sample	**Alkaloid (mg/100g)**	**Saponin (mg/100g)**	**Flavonoid (mg/100g)**	**Tannin (mg/100g)**
Digested	7.57^{a}±0.042	8.89^{a}±0.028	9.05^{a}±0.070	2.59^{a}±0.120
Undigested	6.78^{b}±0.156	8.43^{b}±0.042	7.67^{b}±0.035	1.90^{b}±0.049

Values are mean ± standard deviation of duplicate determination. Mean with different superscript are different from each other significantly (P<0.05).

The tannin content of the samples was 2.59 and 1.90 mg/l00g. The digested cow dung grown vegetable contained higher tannin content (2.59 mg/100g) than the undigested sample (1.90 mg/100g). However, the values of tannin obtained in this research were significantly higher than 0.95 mg/100g in *Amaranthus caudatus* grown with poultry manure at l0t/ ha revealed by Kahu et al. (2019). Tannins are synthesized from gallic acid with a carbohydrate usually glucose, it is found in a number of plants and stem bark (Alemika er al., 2007), and could be found present in cow dung manure due to constant use of forage as feeding, whereas gallic acid is derived from 3-dehydoshikimic acid which comes from glyceraldehydes-3-phospahte via the Carbon cycle. The significant difference observed for tannins among the test samples may be due to moderate contribution of phosphorus to its synthesis including other biomolecules like carbohydrate.

The saponin content of the digested and undigested samples was 8.89 and 8.43 mg/100g. Saponins contents were higher in plant leaf derived from digested cow dung compared to the undigested cow dung. This may be as a result of presence of considerable high levels of glycosides, triterpene and some other sugar units in the sewage sludge which are responsible for the synthesis of saponins (Yang et al., 2014). The saponin contents in this study were lower than 53 mg/ 100 g reported by Amabye (2015) in *Amaranthus spinosus* leaves. The values were also lower than the findings of Okoye (2018) with 18.4 mg/ 100g saponin in leaf and stem of *Amaranthus hybridus*. The variation could be due to the type of manure used, the part of the plant used for the analysis and the strain of the plant.

The alkaloid content of the samples was 7.57 and 6.78 mg/100g in the amaranth grown with digested and undigested cow dung respectively.
There was significant difference between both samples. The digested sample was higher in alkaloid content than undigested sample. The values recorded were significantly (P≤005) lower than 13.14 mg/ 100g reported by Amabye (2015) in *Amaranthus spinosus* Leaves.

Ahmad *et al*. (2013) reported that pure isolated plant alkaloids are used as medicinal agents for analgesic, antispasmodic, and bactericidal effects.
The flavonoid content of the samples were 9.05 and 7.67 mg/100g respectively with the digested cow dung grown amaranth having higher mean value than the undigested cow dung grown amaranth. The values obtained were significantly lower than 15.20 mg/100g obtained from *Amarcmthus spinosus* grown with cow dung (Mofunanya *et al.*, 2015). Results of this study are also in consonance with results of the; biggest and most extensive scientific study and research into the benefits of organic food by Katherine (2007) who reported that organic food is more nutritious than non-organic (ordinary produce) food and may in fact lengthen peoples' lives. It was found that they contain higher levels of antioxidants and flavonoids which help ward off heart disease and cancer as well as iron and zinc. Research that was carried out in the Newcastle University also showed that organic food contains more

antioxidants and less unhealthy fatty acids. They found that levels of antioxidants in milk from organic cattle were between 50 and 80% higher than normal milk. Organic wheat, tomatoes, potatoes, cabbage, onions and lettuce had between 20 and 47% more nutrients than non- organic foods (Mofunanya *et al.,* 2015).

Table 3: Mineral constituents and Yield of *Amarranthus spinosus* grown with digested (biogas slurry) and undigested cow dung

Sample	Iron (mg/100g)	Potassium (mg/100g)	Yield (kg/ha)
Digested	0.08^{a}±0.003	8.96^{a}±0.042	59^{a}±0.00
Undigested	0.07^{a}±0.002	4.88^{b}±0.042	50^{b}±0.00

Values are mean ± standard deviation of duplicate determination. Mean with different superscript are different from each other significantly ($P \leq 0.05$).

The iron content of the digested and undigested cow dung grown amaranth samples were 0.08 and 0.07 mg/100g with digested sample having the higher mean value (0.08 mg/l00g) than the undigested sample (0.07 mg/100g). This study has established that both samples did not significantly ($P \leq 0.05$) from each other. The iron content of the samples were significantly ($P > 0.05$) lower than 1.40 mg/100g reported by Escudero *et al*. (2000) in *Amaranthus muricatus* leaf and stem. The iron contents were however similar to the research work of Mofunanya *et al.* (2015) that had 0.09 mg/100g in *Amaranthus spinosus* grown with cow dung. Iron (Fe) is an essential micronutrient for most organisms due to its role in fundamental metabolic processes and it is deficiency is a yield-limiting factor with major implications for field crop production in many agricultural soils (Munene, 2017). Iron availability is limited by its bioavailability and soil dissolution kinetics of iron-bearing mineral phases in aerobic and neutral environment. Its involved in chlorophyll synthesis and chloroplast structure and functions (Munene, 2017). Iron plays numerous biochemical roles in the body, including oxygen binding in hemoglobin and acting as an important catalytic centre in many enzymes such as the cytochrome oxidase (Geissler and Powers, 2005).

The RDA for iron is 8mg/day indicating that the studied vegetable could be recommended in diets for reducing anemia, which affects over one million people worldwide (FAO, 2001).
The potassium content of the digested and undigested samples was 8.96 and 4.88 mg/ 100g respectively. Potassium was lower (4.88 mg/ 100g) in the amaranth grown with undigested cow dung and significantly ($P < 0.05$) higher in the digested cow dung grown amaranth. The values obtained in this work were in line with 7.60 mg/100g obtained in *Amaranthus spinosus* grown with organic manure as reported by Mofunanya *et al*. (2015). These findings were also slightly higher than 14.59 mg/100g reported by Okoye et al. (2018) in *Amaranthus hybridus* (Stem and Leaves) Potassium is the major factor in intracellular fluid and functions in the maintenance of weight, regulation of acid-base balance, conduction of nerve impulse, muscular contraction (especially of the cardiac muscle), correct functioning of the cell

membrane, regulation of the sodium-potassium adenosine triphosphatase (ATPase) system and the maintenance of fluid volume (Agbaire, 2011). It also plays a vital role in the transfer of phosphate from adenosine triphosphate to pyruvic acid. The metabolism of potassium is regulated by the hormone, aldosterone. Vegetables, fruits and nuts tend to contain many times more potassium than sodium.

The yield of the digested and undigested cow dung grown amaranth was 59 and 50 t/ha with the digested sample having higher yield (59 t/ha) than undigested sample (50 t/ha). The yield of the samples in this study was slightly similar to 63 kg/ha reported by Sanni (2016) in *Amaranthus hybridus* grown with cow dung.

The performance of the crops could be as a result of the high content of nitrogen, phosphorus and potassium contained in cow dung (Reyhan and Amisalani, 2006). Application of organic fertilizers probably increased nitrogen in the soil which positively affected leaf fresh weight land quality of the leaves because nitrogen stimulates plant vegetative growth and increases leaf area; as a result of increment in the leaf area increases the rate of plant photosynthesis and thus higher leaf quality and leaf weight. The consistently poor performance of amaranth grown in control plots revealed that when nutrients are available in adequate amount, plants tend to grow at their optimum potential. These nutrients deficiency were probably the limiting factor of plant growth and productivity in control treatment (Sanni, 2016)

CONCLUSION

Application of cow dung manure was found to improve the nutrient and yield of *A. spinosus* leaf. People eat vegetables not just because they like vegetable but also for the nutritional benefits derived from them. High amounts of phytochemicals, minerals and proximate composition recorded in this research gives preference to the use of biogas slurry in growing of garden than undigested cow dung. The results of this study, therefore, encourage the use of biogas slurry in growing *A. spinosus* for better nutritional quality.

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