

DETERMINATION OF NUTRITIONAL AND ANTI-NUTRITIONAL CHARACTERISTICS AND METABOLISABLE ENERGY OF *Mucuna sloanei* SEEDS

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ABSTRACT

An experiment was conducted to evaluate the nutritional and anti-nutritional characteristics of raw and processed *Mucuna sloanei* meal, as well as their metabolisable energy. In the experiment some nutritional and anti-nutritional factors of the raw (RMS), boiled (BMS), toasted (TMS) and soaked-and-boiled (SBMS) *Mucuna sloanei* meals and their metabolisable energy were assessed. Apparent metabolisable energy (AME) and true metabolisable energy (TME) of raw and processed meals were investigated via voluntary and force-feeding, respectively, using adult broiler birds (roosters), *Mucuna sloanei* and their respective diets were analyzed for proximate composition. The gross energy was determined using Adiabatic Bomb Calorimeter. In the experiment SBMS caused significant ($P<0.05$) reductions in the anti-nutritional factors (L-Dopa=1.17%, Tannin=0.15%, Trypsin inhibitor =0.11 Tiu/mg, phytate=0.26%). Soaked-and-boiled *Mucuna sloanei* (SBMS) gave lower crude protein value (28.58%). There were no significant ($P>0.05$) differences among the raw and processed forms for gross energy (GE) and AME while SBMS gave significantly ($P<0.05$) higher TME (2.85kcal/g) than all the others. SBMS gave the best value in terms of energy metabolism, anti-nutrient detoxification

INTRODUCTION

Nigeria, like many other developing countries, is currently faced with the shortage and high cost of conventional feeds for poultry. Presently, commercial feed firms produce broiler feed of doubtful composition of ingredients and adequacy of nutrients. Fishmeal is one of the ingredients that have been recognized as a high quality animal protein for poultry feeds. This conventional costly item (fishmeal), when added to the diet, increases poultry production cost (Okoye, 2001). There is high cost of conventional feedstuffs such as maize, guinea corn, groundnut cake, because they are competed for by man and industries. The limiting factors associated with using non-conventional feed ingredients as animal feeds include: procurement, storage and poor feed intake; others include presence of toxic substances, low digestibility and low nutrient content. All these factors subsequently lead to low animal performance (Alawa and Amadi, 1991). To achieve efficient utilization of non-conventional feed ingredients, it is therefore, necessary to subject the feedstuff to the best processing method that will harness its use in livestock production. The presence of these antinutritional factors in a feedstuff confers toxicity action to such feedstuff. These anti-nutritional factors include haemagglutinins, protease inhibitors (like trypsin inhibitor), tannins, dopamine, LDopa, antivitamins, lipoxygenase, nicotin, phytates. These factors tend to limit the use of these products in poultry rations, especially their raw seeds (Ologhobo, 1992). These anti-nutritional factors are detrimental to the health and performance of monogastric animals.

MATERIALS AND METHODS

Determination of Anti-nutritional Factors

Anti-nutritional factors were determined as shown below

Determination of L-Dopa

L-Dopa determination was by A.O.A.C. (1995) procedure.

0.5g of samples was weighed into 100ml beaker. 9.5ml of 0.1N HCl was added to it by a means of 10ml measuring cylinder. The mixtures were transferred to a homogenizer or blender for 1 minute.

1ml of aliquots of this resulting suspension was transferred into 300ml test tube and 9ml of carbon suspension added. The mixture was agitated for 15 minutes on a boiling water bath and cooled. The cooled solution was filtered into a 100ml conical flask, 3ml of EDTA diluent added, followed by the addition of 5ml of iron (ii) chloride and allowed to stand for 5minutes to develop colour, before reading on the spectrophotometer. Standard L-Dopa (3, 4- dihydroxyl phenylalanine) solution of range 5ppm to 20ppm was prepared from 100 ppm stock L-Dopa solution in 0.1N HCl to obtain a gradient factor. The absorbance of the extracted sample as well as standard L-Dopa of range 5ppm to 20ppm were read at a wavelength 530nm spectronic 210 spectrophotometer. The % L-Dopa was calculated using the absorbance of sample by gradient factor multiplied by 0.72 all over the weight of sample.

$$\%L\text{-Dopa} = \frac{\text{absorbance} \times \text{grad. factor} \times 0.72}{\text{Weight of sample}}$$

Determination of Hydrocyanic Acid (Hydrocyanides)

Knowles *et al.* (1990) method was used in determining the hydrocyanic acid during which 5g of each sample was weighed into 250ml conical flask. Each sample was soaked with 25ml of distilled water for 3hours, incubated for another 16hours at a temperature of 38°C, and after the extraction, filtration was done using double layer of hardened filter paper. The distillation was done using Markham distillation apparatus. Each sample extracted was transferred into a two-necked 500ml flask connected to a steam generator, and this was steam-distilled with saturated sodium bicarbonate solution contained in a 50ml flask for 60minutes. 1ml of starch indicator was added to 20ml of each distillate and was titrated with 0.02N of iodine solution. The colour change was from colourless to blue which was the end point. The percentage (%) hydrocyanide was calculated with the formular:

$$\% \text{ Hydrocyanic acid} = \text{Titre} \times \frac{100}{10} \times \frac{0.27}{1000} \times \frac{100}{\text{weight of sample}}$$

Determination of Tannic Acid (Tannin)

The tannin in the test feedstuffs was determined according to the method of Maga (1982). Thus 2g of each sample was weighed into a beaker. Each was soaked with solvent mixture (80ml of acetone and 20ml of glacial acetic acid) for 5hours to extract tannin. Each filtrate was in the water bath for 4hours, after which the filtrates were removed. The samples were filtered through double layer filter paper to obtain the filtrate. A set of standard solution of tannic acid was prepared ranging from 10ppm to 50ppm. The absorbencies of the standard solution as well as that of the filtrates were read at 500nm on a spectronic 20. The percentage tannin was calculated using the formula:

$$\% \text{ Tannin} = \frac{\text{Absorbance} \times \text{Average gradient} \times \text{Dilution factor}}{10,000}$$

Determination of Trypsin Inhibitors

The determination of trypsin inhibitor was carried out according to the procedure outlined by Kakade and Evans (1965). This involves weighing of 0.2g of the samples into a screw cap centrifuge tube. 10ml of 0.1M phosphate buffer was added and the contents shaken at room temperature for 1hour on a UDY shaker. The suspension obtained was centrifuged at 5000 rpm for 5minutes and filtered through Whatman No. 42 filter paper. The volume of each was adjusted to 2ml with phosphate buffer. The test tube was placed in water bath, maintained at 37°C. Six millilitres of 5% TCA solution was added to one of the tubes to serve as a blank. 2ml of casein solution was added to all the tubes, which were previously kept at 37°C. These were incubated for 20mins. The reaction was stopped after 20mins by adding 6ml of TCA solution to the experimental tubes and the tubes were shaken. The reaction was allowed to proceed for 1hour at room temperature. The mixture was filtered through Whatman No. 42 filter paper. Absorbance of filtered sample and

trypsin standard solutions was read at 280nm. The trypsin inhibitor in mg/g was calculated using the formular:

$$\text{T.I. mg/g} = \frac{\text{A standard} - \text{A sample}}{0.1\text{g} \times \text{sample wt in g}} \times \frac{\text{Dilution factor}}{1000 \times \text{sample size}}$$

Determiration of Phytic acid (Phytate)

The phytic acid was determined using the procedure described by Lucas and Markaka (1975). This entails the weighing of 2g of each sample into 250mls conical flask. 100mls of 2% concentrated hydrochloric acid was used to soak each sample in the conical flask for 3hrs. This was filtered through a double layer of hardened filter paper. 50mls of each filtrate was placed in 250mls beaker and 107mls of distilled water was added in each case to give proper acidity. Ten mls of 0.3% Ammonium thiocyanate solution was added into each solution as indicator. This was titrated with standard iron (iii) chloride solution, which contained 0.00195g iron per ml. the end-point was slightly brownish-yellow which persisted for 5mins. The percentage phytic acid was calculated using the formula:

$$\% \text{ phytic acid} = \frac{X \times 1.19 \times 100}{2}$$

Where X = Titre value x 0.00195

Determiration of True Metabolisable Energy

True Metabolisable Energy was determined according to Sibbald *et al.* (1976). A total of 100 day-old, Anak broiler chicks were purchased and reared to 15 weeks of age, fifteen (15) birds of equal live weight were selected for trial. The birds were randomly assigned to each of the feedstuffs (which were the raw, boiled for sixty minutes, soaked for 24hrs and boiled for 30 mins, and toasted *Mucuna sloanei*). The birds were placed in individual metabolic cages. There were three replicates per treatment with a bird per replicate. The birds were fasted for 24hrs and later each bird was force-fed with 13g of the relevant feedstuff in accordance with the recommendation of Ukachukwu (2005). Feeding was done through a glass tube inserted into the crop by rotating movement and removal of the glass tube was by reverse rotatory movement. The feed was worked down the tube using a glass rod. Quantitative collection of droppings was done for 24hrs and dried in a forced drought oven at 60°C. Two sets of three birds were housed in individual metabolic cages and subjected to fasting for 48hrs, and their droppings were collected and dried. All the feed and the samples were analyzed for energy. Energy was determined using Adiabatic-bomb calorimeter. True metabolisable energy of the samples was calculated using the equation described by Sibbald *et al.* (1976).

$$\text{TME (kcal/g air dry)} = \frac{(\text{G.E.f} \times \text{X}) - (\text{Yef} - \text{Yec})}{\text{X}}$$

Where:

TME = True Metabolisable Energy

G.E.f = gross energy of the feedstuff (kcal/g)

Yef = energy voided as excreta by fed birds

Yec = energy voided as excreta by unfed birds

X = weight of feedstuff fed (g)

AME Determiration

A rapid method for AME determination as proposed by Farell (1978) was used. *Mucuna sloanei* was subjected to 4 processing methods (raw, toasted, boiled and soaked-and-boiled) as described above (according to the procedure of Ukachukwu and Obioha, 2000. Twenty five adult roosters (about fifteen weeks old) were used. They were trained to consume their daily requirement in one hour.

Training Procedure: They were fed *ad libitum* for the 1st three days, fed for 8hrs each day for the 2nd three days, fed for 5hrs each day for the 3rd three days, fed for 2hrs each day for the 4th three days and fed for 1hr each day for the remaining nine days.

The Feed Samples: A practical diet was used as a basal diet (BD) (chicks mash). BD was mixed with each Test Ingredient (TI) at 50-50. Hence, there were 5 feed samples – 100% BD, 50:50 BD:raw, 50:50 BD:toasted, 50:50 BD:boiled, and 50:50 BD:soaked-and-boiled.

Experimental Procedure: At the end of training, test feed was fed to birds, each feed sample was fed to 5 roosters. This gives 5 sets x 5 roosters which gave a total of 25 roosters. After 1hr feeding, troughs were removed, feed intake was measured, access to water continued. Collection trays were placed under each cage, and excreta voided during subsequent 24hrs were collected, dried and bottled for analysis. Feed and excreta were assayed for gross energy. AME was calculated as follows:

$$\text{AME} = \text{IE} - (\text{FE} + \text{UE}),$$

Where,

IE = Ingested Energy (feed intake x GE of 1g of Ingredient)

(FE + UE) = Energy of Excreta consisting of fecal and urinary energy

AME of test materials was calculated by difference.

Data were subjected to analysis of variance (ANOVA) in CRD by the method of Steel and Torrie (1980), separation of means where necessary was by Duncan's New Multiple Range Test (DNMRT).

RESULTS AND DISCUSSION

Proximate Composition and Gross Energy Content of Raw and Processed *Mucuna sloanei* Seed Meal

Proximate composition of test material:

Values of the proximate composition of the raw and processed *Mucuna sloanei* are presented in Table 1. The crude protein values ranged from 28.07% in toasted to 31.43% in the boiled. The crude protein content was higher than that recorded (27.93%-29.37%) by Sridhar and Rajeev (2007) and Tuleun *et al.* (2010). The lower crude protein values recorded for soaked-and-boiled (SBMS) was an indication that some nitrogenous compounds were solubilized and removed in the course of the soaking and boiling.

Table 1: Proximate Composition of *Mucuna sloanei* seed Meal (DM basis)

Treatments	CP (%)	EE (%)	CF(%)	ASH (%)	NFE (%)	GE(Kcal/g)
Boiled	31.43	6.64	16.23	3.95	41.75	3.06
Raw	30.68	6.18	18.76	4.15	40.23	3.12
Toasted	28.07	6.55	17.07	4.50	43.81	3.07
Soaked-and-boiled	28.58	6.09	16.13	3.46	45.74	3.17

CP: crude protein, Kcal: kilocalorie, CF: crude fibre, NFE: nitrogen free extract, G.E.: gross energy, g: gram, EE: ether extract.

For ether extract, the values ranged from 6.09% in the soaked-and-boiled to 6.64% in the boiled. The values observed were a little bit higher than that reported (3.17%-5.78%) by Tuleun *et al.* (2010). The high crude fibre content observed could be as a result of the seed coat incorporated in the feedstuff. It does not make it unacceptable for inclusion in the diet of farm animals (Tuleun *et al.*, 2010). Fibre acts as a diluent but its absence in diets leads to incidence of wide range of diseases including diabetes mellitus (Oke *et al.*, 1996). The gross energy values were lower than that observed (3.46 kcal/g-3.67kcal/g) by Tuleun *et al.* (2010).

Anti-nutritional factors of the Raw and Processed *Mucuna sloanei*

Anti-nutrient compositions of the raw and processed *Mucuna sloanei* are presented in table 2. There were significant differences ($P<0.05$) among the treatment means for all the anti-nutritional factors measured. There was an overall reduction in the value of anti-nutritional factors which could be associated with processing though they varied in degree with the different types of processing employed as observed by Akinmutimi (2004). The level of L-Dopa (3, 4-dihydroxy-L-phenylalanine) in the raw bean was higher than the processed forms and ranged from 1.17% in the soaked-and-boiled form (SBMS) to 1.50% in the raw (RMS). Those of the boiled (BMS) and toasted (TMS) forms were 1.38% and 1.43% respectively and similar.

Table 2: Antinutrient Compositions of the Raw and Processed *Mucuna sloanei*

Treatments	L-Dopa (%)	Tannin (%)	Tryp inhibit (TIU/mg)	HCN (mg/kg)	Phytate (%)
Soaked-and-boiled	1.17 ^c	0.15 ^d	0.11 ^d	5.76 ^b	0.26 ^c
Boiled	1.38 ^b	0.35 ^c	0.29 ^c	5.45 ^b	1.26 ^b
Toasted	1.43 ^{ab}	0.46 ^b	0.35 ^b	8.10 ^b	1.32 ^b
Raw	1.50 ^a	0.59 ^a	0.46 ^a	19.76 ^a	1.48 ^a
SEM	0.038	0.026	0.023	0.027	0.015

Tryp inhibit: trypsin inhibitor, HCN: hydrocyanide.

The different treatments showed that significant differences for L-Dopa were derived as a result of processing. This is in agreement with the report of Ukachukwu and Szabo (2003) that boiled mucuna beans in ordinary water or in water with additives of $\text{Ca}(\text{OH})_2$, trona (a traditional lake salt in the region) or wood ash for 30 - 45 minutes resulted in a 64.1 – 66.0% reduction of L-Dopa to 2.2 – 2.3%. The solubility of L-Dopa in water at 25⁰C is known and is quite low (66mg/40ml of water) (Eilittä *et al.*, 2003). L-Dopa limits the utilization of mucuna as a feed (Capo-Chichi *et al.*, 2003). They further reported that the most promising results were obtained with cooked beans. The value for a safe L-Dopa level for poultry feed was placed at 0.3 to 0.4% (Ukachukwu and Szabo, 2003; Iyayi and Taiwo, 2003). There were significant differences among treatment means for tannins. The level of tannin in *Mucuna sloanei* seed meals ranged from 0.15% in the SBMS to 0.59% in the raw sample (RMS). Those of TMS and BMS were 0.46% and 0.35% respectively. The significant differences observed among the treatment means for tannins could be as a result of the type of processing used. It could also mean that the processing method had the ability to break the linkage formed by tannic acid like other phenolic compound with protein and other macro molecules and to overcome the intra-molecular force existing within the tannin structure (Batesmith, 1973) thus improving the rate of thermo-disintegration and resulting in better elimination. This points to better digestibility of protein if boiled seeds are fed. This is because tannic acid is known to adversely affect protein digestibility (Griffiths, 1991). Like other Legumes, *Mucuna sloanei* seeds contain tannins. Tannin has the capability of decreasing the digestibility and palatability of proteins because they form insoluble complexes with them (Osagie *et al.*, 1996). Tannins can also interact with dietary iron by preventing its absorption. Tannins are not easily completely destroyed by heat due to their high molecular weight (Price *et al.*, 1987; Nwaogu and Udebuani, 2010;).

There were significant differences ($P<0.05$) among treatment means for trypsin inhibitor. Trypsin inhibitor levels of the samples were between the ranges of 0.46TIU/mg in the RMS to 0.11TIU/mg in the SBMS, whereas those of TMS and BMS were 0.35TIU/mg and 0.29TIU/mg respectively. It could be observed that different processing forms influence the trypsin inhibitor content of *Mucuna sloanei* seed meal. This could probably be the effect of temperature on solubilization which wet heat treatment offers (Ukachukwu and Obioha, 2000). The reduction of trypsin inhibitor by processing is attributable to heat denaturation (Udensi *et al.*, 2005). Trypsin inhibitor activities affect protein digestibility negatively (Nwaogu and Udebuani, 2010). This implies poor digestibility of protein caused by irreversible binding of digestive enzymes by trypsin inhibitors thus making the

enzyme unavailable for the breaking down of the proteins (Leiner and Kakade, 1980). This could result in growth depression (Maynard *et al.*, 1979).

Significant differences ($P < 0.05$) were observed among the treatment means for hydrogen cyanide (HCN). It could be observed that the processed forms were better than the raw form. HCN content in the samples indicates that processing decreased significantly ($P < 0.05$) its level from 19.76 to 5.76mg/kg with the lower value in the raw sample. Cyanogenic glycosides are precursor of HCN, a well-known natural toxicant in foods. The presence of this toxicant serves a note of caution due to its toxicity. The HCN value ranged from 19.76mg/kg in the RMS sample to 5.45mg/kg in the BMS sample. However, the TMS and SBMS samples were 8.10mg/kg and 5.76mg/kg respectively. The values for the processed forms showed no significant difference ($P > 0.05$). Different processing forms (soaked-and-boiled, boiled and toasted) resulted in greater significant ($P < 0.05$) reductions in the raw seed content of HCN. This could probably be the extent of temperature and solubilization which wet heat treatment offers as reported by Ukachukwu and Obioha (2000). Oke *et al.* (1996) reported that heat as a processing technique was able to hydrolyse cyanogenic glycosides probably due to the volatile nature of HCN. The result in table 2 also agrees with the report of Tuleun *et al.* (2010) that cooking *mucuna* seeds in water or potash solution resulted in a greater significant ($P < 0.05$) reduction in the raw seed content of hydrocyanide.

Significant differences ($P < 0.05$) were observed among the treatments for phytate. Phytate in the samples ranged from 1.48% in the RMS sample to 0.26% in the SBMS sample. Those of TMS and BMS samples were 1.32% and 1.36% respectively. There were no significant difference ($P < 0.05$) between the TMS and BMS samples. Processing methods used could have probably affected the phytate content of the *Mucuna sloanei* seed meal. Some of the hydrogen bonds contained in phytate could have been broken by heat treatment to give the result we observed in Table 2 for phytate. This does not agree with the report of Nwaogu and Udebuani (2010) who observed that phytate is not easily destroyed by processing temperatures.

From Table 2, it could be observed that there were a general reduction in the content of anti-nutritional factors as a result of processing, however, they vary with different processing techniques which is in agreement with the report of Akinmutimi (2004). Combined effects of these identified anti-nutritional factors could make the mucuna bean unacceptable as feedstuff. However, the application of these processing methods could render the seed safe for consumption by detoxifying the constituent toxicants and anti-nutritional factors (Ukachukwu and Obioha, 1997).

Metabolisable Energy of Raw and Processed *Mucuna sloanei*

Table 3 shows the gross energy (GE), true metabolisable energy (TME) and apparent metabolisable energy (AME) of raw and processed *Mucuna sloanei* in broiler birds.

Table 3: Gross Energy (GE), True Metabolisable Energy (TME) and Apparent Metabolisable Energy (AME) of Raw and Processed *Mucuna sloanei* in Broiler Birds

Treatments	GE (Kcal/g)	TME(Kcal/g)	AME(Kcal/g)
Raw	3.12	2.27 ^c	2.17
Toasted	3.07	2.63 ^b	2.25
Soaked-and-Boiled	3.17	2.85 ^a	2.50
Boiled	3.06	2.56 ^b	2.23
SEM	0.37	0.074	0.071

GE: gross energy, TME: True Metabolisable Energy, Apparent Metabolisable Energy, Kcal/g: Kilocalorie per gram, SEM: standard error mean.

There were significant differences ($P < 0.05$) among the treatment for true metabolisable energy (TME). The mean true metabolisable energy (TME) for raw *Mucuna sloanei* (RMS) (2.27kcal/g) is

significantly ($p < 0.05$) lower than the values observed for various forms of processed *Mucuna sloanei* seed meal which were significantly ($P < 0.05$) different from each other. The mean TME values ranged between 2.27kcal/g for raw (RMS) and 2.85kcal/g for soaked-and-boiled (SBMS) samples whereas those of toasted (TMS) and boiled (BMS) samples were 2.63kcal/g and 2.56kcal/g respectively.

This is in agreement with the report of Ologhobo and Fetuga, (1986); and Ukachukwu *et al.*, (1999) that processing significantly ($P < 0.05$) influenced metabolisable energy (ME). This implies that low mean TME value 2.27kcal/g of the raw could be as a result of anti-nutritional factors inhibiting the expression of the nutrients in the raw beans (Ologhobo and Fetuga, 1986; Ukachukwu *et al.*, 1999; Akinmutimi *et al.*, 2002). Kakade and Evans, (1965) had reported that the trypsin inhibitor of many legumes comprises up to 30 - 40% cysteine. They argued that if these legumes are properly processed to remove the toxic effect the trypsin inhibitor content could contribute significantly to the sulphur amino acid yield of the seeds. Metabolisation of these amino acids would yield more energy. The high significant ($P < 0.05$) values observed in the BMS, TMS and SBMS forms could be as a result of the processing employed.

No significant ($P > 0.05$) difference was observed for the apparent metabolisable energy (AME) values among the treatment means. The AME value for SBMS was slightly better though not significantly than other processed forms including the raw. The AME values observed followed the same trend (though not significantly) as the TME values. The processed forms were slightly better than the raw form, which could be attributed to the high level of anti-nutritional factors in RMS. However, different processing techniques seem to have contributed to the improvement of the AME.

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