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In vitro Gas Production and Fermentative Characteristics of Cottonseed and Cottonseed By-products

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Abstract
A study was conducted to evaluate the *in vitro* gas production and post-incubation parameters of cotton seed (*Gossypium spp*) and cotton seed by-products. Cotton seed was processed into four different sample types regarded as treatments i.e. raw cotton seed(A), parboiled cotton seed (B), roasted (C) and cotton seed cake (D). All the chemical constituents investigated were all similar for all treatments ($p>0.05$). Highest DM (90.07% DM) and CP (24.72% DM) results were obtained from cottonseed cake (D). *In vitro* gas production characteristics of cotton seed and cotton seed by-products were determined at 24hrs of incubation. The gas production results were significantly different ($p<0.05$) in all the cottonseed by-products. The result revealed after 24hrs, that cotton seed cake had the highest value of *in vitro* gas production from soluble and insoluble fraction, potential extent gas production as well as the volume of gas produced at incubation time. It can be concluded from the chemical constituent findings of this research, that cottonseed cake had highest crude protein and digestibility (or possess good fibre content) when compared to feeds consisting of other cottonseed by-products.
Keywords: Proximate analysis; Fibre fractions; Post-incubation parameters

1-INTRODUCTION

A major problem facing ruminant livestock producers in tropical areas is poor nutrition for their animals during the dry season when pastures and crop residues are limiting in nutritional quality (Murphy and Colucci 1999). Normally, it is during this period when problems such as sickness and weight losses due to poor dietary profiles arise (Ravhuhali et al., 2010). One of the ultimate objectives of livestock farmers is the efficient conversion of feeds which are either inedible by man or surplus to his requirements into animal products. However, conventional animal feeds are rapidly becoming more expensive due to the continual pressure on world feed resources by the ever increasing human population (Raji, 2009). Tropical plants and agricultural by-products represent an important fodder reserve for livestock in harsh conditions that can be used by grazing ruminant in periods of feed scarcity or drought. Many agricultural residues are poorly digested by ruminants because of their high content of cell wall materials. Physical and chemical methods are often used to break down the lignocellulosic materials of some agricultural residues and thereby improve the feeding value of crop residues such as stalks or straws.

Cotton is of the Gossypium genus that is grown on every major continent and on West Indies and Pacific Basin islands. Cotton is cultivated in areas of intense heat. In the dryer climates irrigation produces high quality cotton. Cotton is considered the most prominent source of textile fibre in the world. It makes up over 40% of the total fibre used in the world (USDA ERS, 2002). Fuzzy or whole cottonseed is the linted cottonseed remaining after the ginning process to remove cotton fibres for textile production (NCPA, 2002). Whole cottonseed has been reported as a very important dairy feed, and a lesser important beef and sheep feed. It is added to dairy feed as a concentrated source of protein, fat and energy at levels of up to 15% of the total diet or at a total dietary amount of 1.8 - 3.2 kg per head per day. Higher levels usually decrease feed intake (OECD, 2009). However, cottonseed is sometimes delinted and not further processed. For ruminant animals, proximate analysis, fibre constituents and *in vitro* gas production is important to delineate its nutrient value. The objective of this experiment was to determine the proximate analysis, fibre constituents and *in vitro* gas production of cottonseed and its by-products.

2-MATERIALS AND METHODS

2.1-Experimental sites

The experiment was conducted in the Ruminant Nutrition Laboratory, Department of Animal Science, University of Ibadan, Nigeria. The location was 7º 27' N and 3º 45' E at an altitude of 200-300 m above sea level. The average annual rainfall was about 1250 mm with a mean temperature of 25 - 29ºC.

2.2-Experimental design and sample preparation

The study was designed in a randomized complete block design in which cottonseed was processed into four different treatments namely raw cottonseed (as control) (A), parboiled cottonseed (B), roasted (C) and cottonseed cake (D). Parboiled cottonseed were obtained when raw cottonseeds were soaked in boiling water for

10 minutes while raw cottonseed was roasted using frying pan. The samples (A-D) were thereafter oven dried at 100°C, and they were ground separately, using hand mill.

2.3-Chemical analysis

Proximate composition was determined according to A.O.A.C (2000). Fibre fractions; Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL) were determined using the method of Van Soest *et al* (1991). Cellulose was taken as the difference between ADF and ADL while hemicellulose was calculated as the difference between NDF and ADF. The *in vitro* gas production was determined following the procedure of Menke and Steingass (1988). The volume of gas produced at intervals of 3 hours was determined by the measurement of the piston displacement level during the period of incubation. The gas volume of each sample was obtained from the difference between the volume of gas produced by the blank syringes and the volume of gas produced in each of the treatment samples syringe. Organic matter digestibility (OMD) and Metabolizable energy (ME) were calculated following the procedure of Menke and Steingass (1988), while SCFA was calculated as described by Getachew *et al* (2002). At the end of 24 hours of incubation, 4 ml NaOH was added to the substrate in each syringe to determine the methane production. Rates and extent of gas production were determined for each substrate from the linear equation:

$Y = a + b\,(1 - e^{-ct})$ described by Ǿrskov and McDonald (1979), where:

Y = volume of gas produced at time 't',
a = intercept (gas produced from the soluble fraction),
b = Potential gas production (ml/ g DM) from the insoluble fraction,
c = gas production rate constant (h^{-1}) for the insoluble fraction (b),
t = incubation time.

2.4-Statistical Analysis

All data were subjected to analysis of variance (ANOVA) using SAS model (1999), and means were separated using Duncan's Multiple Range Test (Duncan, 1955).

3-RESULTS AND DISCUSSION

Chemical compositions of cottonseed and cottonseed by-products are presented in Table 1. All the chemical constituents investigated were all similar for all treatments (p>0.05). Highest DM (90.07% DM) and CP (24.72% DM) results were obtained from cottonseed cake (D). The highest CP content (24.72%) obtained for the cottonseed cake was lower than the results reported by Jagadi *et al* (1987) for untoasted red beans; cowpea seeds; kapok expeller cake; sunflower expeller cake and Soya bean meal. The CP contents was somewhat higher than that reported for wheat straw (5.2 % DM; Chriyaa, 1997) and the CP contents of the by-products were similar to that reported for tall fescue and soybean stover and hulls (National Research Council (NRC), 1989). The CP of cottonseed cake (26.01%) obtained in this study was somewhat close to that reported for cottonseed short linter residue (CLR) (30.20 % DM) (Bo et al, 2012), and lies within the range reported for different varieties of cottonseed hulls (23.00 – 30.30 % DM)(Fadel, 1999; Robinson et al., 2001). Highest CF (27.00%) of roasted Cottonseed obtained was higher than that of the kapok expeller cake and at the same time lesser than sunflower cake as reported by Jagadi *et al* (1987). The EE content of the cottonseed by-products obtained in this study were lower than the value reported for cottonseed short linter residue (Bo et al, 2012) and delinted cottonseeds reviewed by Coppock et al. (1987) and closer that of whole cottonseeds reported by Sullivan et al. (1993) and NRC (1989). Parboiled cottonseed (B) had highest contents of NDF (67.00% DM), ADF (43.00 % DM) and ADL (16.00% DM).

Table 2 (Figure 1) presents the sequential gas production of the cottonseed and cottonseed by-products. The gas production results were significantly different (p<0.05) in all the cottonseed by-products. Digestibility has been reported to be synonymous to *in vitro* gas production (Fievez *et al*., 2005) so that the higher the gas production the higher the digestibility. Gas production has been reported to be more affected by digestible energy content (e.g., the presence of starch and sugars) than by protein or fat content (Abate, 1980), and high degradable N compounds have been reported to decrease gas production to some extent (Krishnamoorthy et al., 1995). In the present study, a higher degradation or digestibility was observed in cottonseed cake than for other by-products. It was speculated that cottonseed hull-associated protein of raw cottonseed (A), parboiled cottonseed (B) or roasted cottonseed (C) might not be a readily degraded form for rumen microorganisms.

In vitro gas production and post-incubation characteristics or gas production kinetics of cottonseed and cottonseed by-products for 24 hours are shown in Table 3. The results revealed that there were significant differences (p<0.05) in the post-incubation characteristics except the rate of gas production rate (C) of cottonseed and cottonseed by-products. Methane production in the rumen is an energetically wasteful process. Approximately 6% of dietary gross energy intake is lost into the atmosphere as CH_4 (Holter and Young, 1992; DeRamus et al., 2003). This CH_4 in turn contributes to climatic change and global warming (Johnson and

Johnson, 1995), as CH_4 traps outgoing terrestrial infrared radiation 20 times more effectively than does CO_2. Comparatively highest and lowest CH_4 proportions were observed in treatment D and C respectively. Long-chain fatty acids have been shown to decrease fibre digestibility and acetate production and to suppress methane production (Getachew et al., 2004; Getachew et al., 2005). The supplementation of whole cottonseed to dairy cows that are offered a forage and cereal diet resulted in a long term reduction in CH_4 emissions, which might be caused by an overall increase in dietary fat content (Dohme et al., 2001; Grainger, 2010). Thus, the very high fat content of 17.00 % DM (Table 1) could explain why a comparatively high depression of CH_4 would be obtained should the roasted cottonseed is used as a feed. The cottonseed cake produced the highest ME, OMD and SCFA contents of this study (Table 3).

4-CONCLUSIONS

There were similarities among the chemical compositions of the raw cottonseed and its by-products. It can be concluded from the chemical constituent findings of this research, that cottonseed cake had highest crude protein and digestible or good fibre content, when compared to feeds consisting of other cottonseed by-products. It can also be concluded that roasted cottonseed can be defined as a feed with a high crude protein and low digestible fibre contents, when compared to other cottonseed by-products of this study. However, *In vitro* gas characteristics result indicated that roasted cottonseed could be regarded and/or defined as a high energy and protein concentrate for ruminant animals that would be expected to generate comparatively less greenhouse gas due to its high fat content.

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Table 1: Chemical compositions of cottonseed and cottonseed by-products.

Constituents (%)	A	B	C	D	SEM
DM	89.35	89.08	90.02	90.07	0.16
CP	18.38	24.07	24.72	26.91	0.15
EE	16.00	13.00	17.00	14.00	0.24
CF	24.00	25.00	27.00	14.00	0.25
ASH	6.00	5.00	6.00	8.00	0.16
NDF	58.00	67.00	55.00	38.00	0.10
ADF	28.00	43.00	27.00	22.00	0.07
ADL	12.00	16.00	10.00	6.00	0.01
HEM	30.00	24.00	28.00	18.00	0.18
CELL	16.00	27.00	17.00	16.00	0.27

[a, b, c]: means with the same row with the same superscripts are not significantly different (p>0.05); A = Raw Cottonseed; B = Parboiled Cottonseed; C = Roasted Cottonseed; D = Cottonseed cake; SEM = Standard Error of Mean; DM = Dry matter; CP = Crude protein; NDF = Neutral detergent fibre; ADF = Acid detergent fibre; ADL = Acid detergent lignin; CELL = Cellulose; HEM = Hemicellulose.

Table 2: *In vitro* gas production of cottonseed and cottonseed by-products after 24 hours of incubation

Time (hr)	A	B	C	D	SEM
3	2.67^b	3.00^b	2.33^b	6.00^a	0.46
6	4.00^b	3.67^b	3.33^b	7.67^a	0.55
9	5.33^b	5.33^b	3.67^{bc}	9.67^a	0.44
12	5.33^b	5.33^b	4.33^b	11.00^a	0.55
15	6.00^b	7.67^b	5.67^b	18.00^a	0.46
18	6.33^b	8.33^b	5.67^b	20.33^a	0.50
21	8.67^b	8.33^b	5.67^b	23.00^a	0.47
24	8.67^b	8.33^b	6.00^c	23.33^a	0.37

$^{a, b, c}$: means with the same row with the same superscripts are not significantly different (p>0.05); A = Raw Cottonseed; B = Parboiled Cottonseed; C = Roasted Cottonseed; D = Cottonseed cake; SEM = Standard Error of Mean;

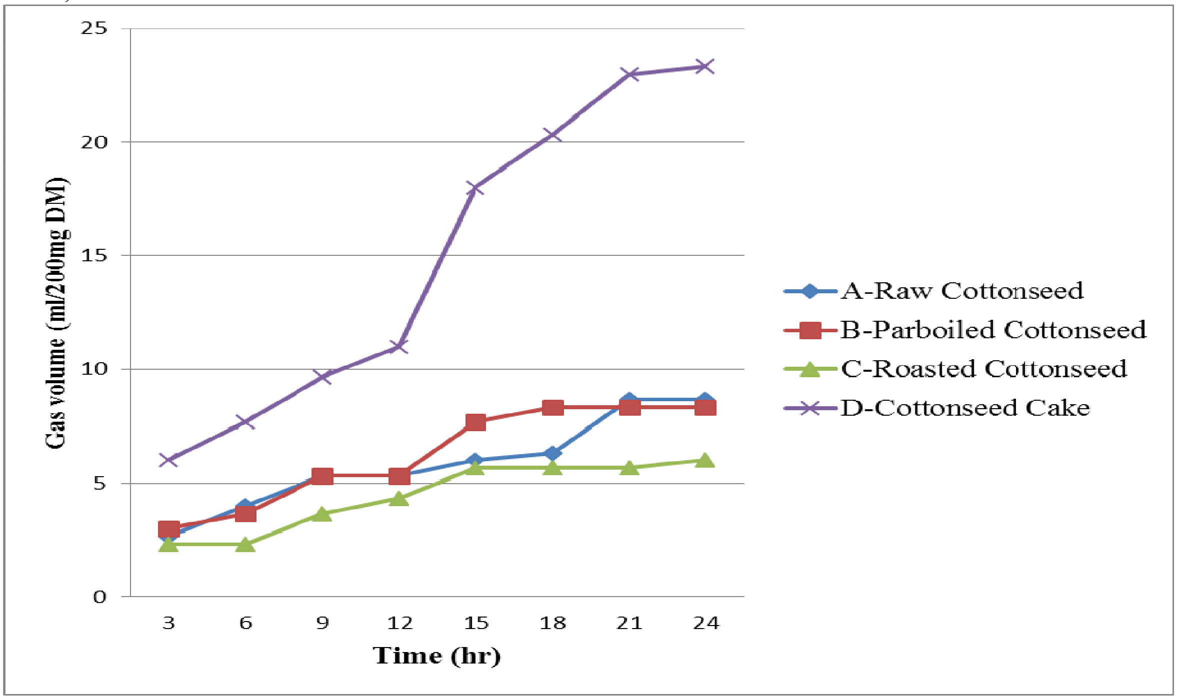

FIGURE 1: Trend of the sequential *in vitro* gas production of the cottonseed and its by-products

Table 3: *In vitro* gas production and post-incubation characteristics of cottonseed and cottonseed by- products for 24 hours

Parameter	A	B	C	D	SEM
a (ml)	2.67^b	3.00^b	2.33^b	6.00^a	0.46
b (ml)	7.00^b	5.33^{bc}	3.67^c	17.33^a	0.41
a + b (ml)	9.67^b	8.33^b	6.00^c	23.33^a	0.37
c (hr^{-1})	0.07	0.09	0.09	0.08	0.01
t (hr)	12.00	11.00	9.00	15.00	1.53
Y (ml)	6.67^b	6.33^b	4.33^b	18.00^a	0.65
METHANE	5.00^b	5.00^b	0.00^b	12.00^a	0.41
ME (MJ/Kg DM)	4.63^b	4.78^b	4.50^b	6.95^a	0.09
OMD (%)	35.65^b	36.37^b	35.24^b	52.94^a	0.57
SCFA (μ mol)	0.29^b	0.26^b	0.20^c	0.62^a	0.02

$^{a, b, c}$, means with the same row with the same superscripts are not significantly different (p>0.05); A = Raw Cottonseed; B = Parboiled Cottonseed; C = Roasted Cottonseed; D = Cottonseed cake; SEM = Standard Error of Mean; a = Intercept (gas production from the soluble fraction); b = the gas production from the insoluble fraction; c = the gas production rate constant per insoluble fraction (b); t = incubation time; (a + b) = the potential extent of gas production; Y = volume of gas produced at time 't'.