Original Paper

Silage characteristics, nutrient profiles and *in vitro* digestibility of differently ensiled *Theobroma cacao* bean shell meals

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Silage technology is one of the major strategies for nutritionally improving and preserving feedstuff during seasonal shortages for ruminant animals. Thus, this study was conducted to determine the silage characteristics, nutrient profiles and *in vitro* digestibility of raw, poultry manure- and urea- ensiled cocoa bean shell meal. The mixtures of raw CBSM and poultry manure (PCBSM) in ratio 1 : 1 (w/w) and 5% urea solution (UCBSM) in ratio 1 : 1 (v/w) were ensiled for seven days under anaerobic conditions. Data generated were subjected to appropriate statistical tools. According to physicochemical properties the silages produced could be acceptable/palatable for ruminants. Nutrient compositions was best improved in poultry manure ensiled cocoa bean shell. Mineral compositions were significantly improved by the treatments while anti-nutrients decomposed and reduced significantly. Alkaloid, oxalate and theobromine content were lower in the PCBSM while the UCBSM was lower in phytate. *In vitro* digestibility study showed that the feed materials are degradable and would nutrients could be absorbed and utilized by the animals, if fed. The organic matter digestibility (OMD), short chain fatty acid (SCFA) and metabolizable energy (ME) values confirmed the nutritional potentials of PCBSM as best feed materials over all. Methane production were minimal, would not lead to bloat in animals and thus, eco-friendly. Summarily, poultry manure ensiled cocoa bean shell meal could be a suitable alternative feed material in ruminant production and is thus, recommended.

Keywords: anaerobic fermentation, alternative feed resources, non-synthetic fertilizer, small ruminant

1 Introduction

Livestock production is an important tool in the economy of developing countries. Its production is undertaken in a multitude of ways across the planet, providing a large variety of goods and services, and using different animal species and different sets of resources, in a wide spectrum of agro-ecological and socio-economic conditions (Steinfeld et al., 2006). The complex factor that affects livestock production is nutrition, as feed resources are limited in quantity and quality (Omotoso et al., 2018). The systems of goat production in Nigeria are usually characterized by limitations posed by non-availability of year-round feed resources due to prolonged dry season (Aina et al., 2002).

In Nigeria, livestock are raised extensively on natural grasses and crop residues. Studies have shown that grasses alone cannot provide adequate nutrients for optimum production of goats. Crop residues are the most abundant and readily available feed resources for livestock production (Ajeigbe et al., 2011). However, meeting the nutritional needs of the animals has been the major constraint militating against the increased production of valuable sources of animal protein (Fajemisin and Adeleye, 2005). Ruminants do better when energy and protein-rich diets are strategically combined for feeding but the expensive nature of conventional feedstuffs as a result of competition between man and livestock makes this combination difficult. Feed quality determines the performance of livestock in terms of weight gain, meat production and feed conversion efficiency. Inadequacy of quality feeds especially during the dry periods has necessitated the use of alternative feed resources. However, some of these feed resources contain antinutrients, have low nutrient digestibility and

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are even unpalatable to animals. Conversely, in improving these resources, various methods of treatment such as biological, chemical, fungal, ensiling, supplementation with urea and other high quality protein source had earlier been reported (Iyayi et al., 2001; Erika and Anuraga, 2015; Preston et al., 2019). In a bid to overcome the challenges of nutrition in Nigerian livestock industry, cocoa bean shell has been identified to be one of the potential crop residues abundant and regarded as waste in Nigeria. The shell has been reported to contain appreciable amount of protein, fibre and minerals and thus, could be a potential feedstuff for ruminant animals. However, for its optimal utilization by goats, the shell needs to be processed first. Hence, the aim of the presented study was to evaluate the silage characteristics and nutrients profile of differently processed cocoa bean shell meal.

2 Material and methods

2.1 Description of the study area

The research work was carried out in the Small Ruminants Unit of the Teaching and Research Farm and the Laboratory analysis was carried out at the Nutrition Laboratory of the Department of Animal Production and Health, Federal University of Technology, Akure (FUTA) located in the humid rainforest zone of western Nigeria which is characterized by two seasons – raining and dry season. The mean annual relative humidity is over 75% and that of temperature is about 27 °C with Latitude 7° 15′ N and Longitude 5° 15′ E (Nigerian Meteorological Agency, 2014).

2.2 Collection and processing of the cocoa bean shell, feed grade urea and poultry manure

Dried cocoa bean shell (CBS) was collected at cocoa processing industry in Akure, Ondo State. Poultry manure was collected from laying chickens raised under intensive system (battery cage) at the Teaching and Research Farm, Animal Production and Health, FUTA. Urea fertilizer was purchased from an agrochemical shop in Akure. The CBS was further sun-dried for 2–3 days based on the intensity of the sunlight; to facilitate crushing, at the FUTA feed mill to 2 mm particle size. The crushed CBS was divided into three equal portions, the first portion was untreated/raw, second portion was treated with poultry manure (1 kg poultry litter/1 kg cocoa bean shell meal (CBSM) and the third portion was treated with 5% urea solution (1 litre urea solution/1 kg CBSM) and each ensiled in plastic drums under anaerobic condition for seven days. Thereafter, dried for another three days and samples were analysed for nutrients and anti-nutrients composition.

2.3 Laboratory procedures

Dry matter (DM), crude protein (CP), crude fibre (CF), ether extract (EE), ash, nitrogen free extract (NFE), theobromine concentration, pH, temperature and antinutirents content (tannin, phytate, oxalate, saponin) of the samples were determined by standard laboratory methods and procedures (AOAC, 2002).

2.4 In vitro digestibility study

Two adult male West African Dwarf (WAD) goats of the same age and uniform conformation were selected as donors of rumen inoculum for *in vitro* studies. The animals were fed for 2 weeks with 40% concentrate and 60% *Panicum maximum* at 5% body weight. Rumen liquor was collected in the morning before feeding through a stomach tube against negative pressure created by a suction pump into the thermo-flask that had been pre-warmed to a temperature of 39 °C. The buffer solution was prepared by mixing McDougall's solution (9.8 g/l $NaHCO_3$ + 2.77 g/l Na_2HPO_4 + 0.57 g/l KCl + 0.47 g/l NaCl + 2.16 g/l $MgSO_3 \cdot 7H_2O$ + 16 g/l $CaCl_2 \cdot 2\ H_2O$) with rumen liquor at 1: 4 (v/v) under continuous flushing with CO_2 to minimize changes in microbial populations and to avoid O_2 contamination for the incubation. Incubation procedure was carried out using 120 ml calibrated transparent plastic syringes with a fitted silicon tube. The sample weighing 200 mg (n = 3) was carefully dropped into syringes and thereafter 30 ml each of the inoculum containing cheesecloth strained rumen liquor and buffer solution. The syringe was tapped and pushed upward by the piston in order to completely eliminate air in the inoculum. The silicon tube fitted to the syringe was then tightened by a metal clip so as to prevent the escape of gas. Incubation was carried out at 39 ±1 °C and the volume of gas production was measured at 3, 6, 9, 12, 15, 18, 21, and 24 h. At the end of the termination hour, 4 ml of NaOH (10M) was introduced to estimate the methane production according to Fievez et al. (2005), metabolizable energy (ME), organic matter digestibility (OMD), and short chain fatty acids (SCFA) were estimated according to the methods of Menke and Steingass (1988). The average of the volume of gas produced from the blanks was deducted from the volume of gas produced per sample.

The following were calculated as ME (MJ/KgDM) = 2.20 + 0.136GV + 0.057CP + 0.0029 CF; OMD (%) = 14.88 + 0.889GV + 0.45CP + 0.651XA; SCFA = 0.0239 V–0.0601. % IVDMD = 1 – [(residue + filter paper) – filter paper] – blank/(sample wt.) × (DM) where; blank = (blank residue + filter paper) – filter paper, GV, CP, CF, and XA are total gas volume, crude protein, crude fiber, and ash, respectively.

2.5 Statistical analysis

Data generated were subjected to analysis of variance (ANOVA), and the treatment means were compared by the methods of Duncan's Multiple Range Test using SAS (2010) version 9.3.

3 Results and discussion

The physicochemical properties of the differently ensiled CBSM were variable (Table 1). The treatment had influence on the texture; poultry manure-ensiled CBSM (PCBSM) was slightly slippery to touch while urea- ensiled CBSM (UCBSM) was slippery. This is as a result of the particle sizes, heat effect produced from anaerobic process during fermentation and the chemical reactions with the feed material. The smell of raw CBSM (RCBSM) was pleasant while that of PCBSM and UCBSM were choky or pungent smell. This could be traceable to the ammonia smell produced from the poultry manure and urea, upon reaction with water. This suggests that ammonia is being liberated to hydrolyze the chemical/physical bonds between lignin, cellulose and hemicellulose in the plant cell walls (Iyayi et al., 2001). Though, the brownish colouration of the CBSM were retained, but the fermentation process made it darker. The pH of the silages reached the level of a very weak acid, respectively, approaching a neutral value (6.5 UCBSM, 6.9 PCBSM). The values could be influenced by the moisture content and buffering capacity of the pod. This is in agreement with the results of Imoisili et al. (2013) and suggests why cocoa pod is suitable for traditional soap production. More so, effect of ensiling and the chemical reaction of urea and uric acid released from poultry manure aids the activity of lactic acid bacteria to ferment sugar to lactic acid, which decreases the pH. Temperature of the silage ranged from 32 °C (RCBSM) to 37 °C (UCBSM). The temperature recorded is believed to produce excellent silage as optimum internal temperature during fermentation is below 37.7 °C. High temperature during ensiling (over 30 °C) reduces lactic acid concentration, aerobic stability and increases pH; and will result in dark silage due to caramelization of sugar which is end product of Maillard reaction (McDonald et al., 1995; Adesogan and Newman, 2010). This probably explains the darker colouration observed in UCBSM. Thus, heat produced by fermentation raises the temperature and rate of microbial processes. The higher temperature could be attributed to the effects of fermentation and ureolysis. According Table 1, dry matter contents increased from 77.3% (UCBSM) to 90.5% (RCBSM) and crude protein content of RCBSM was the least (10.71%CP) and that of PCBSM was the highest (18.2%CP). The CP content of RCBSM obtained in this study was lower than reported by Aregheore (2002) but higher then published by Meffeja et al. (2006). This could be attributed species/variety and age at harvest, of the cocoa analyzed, and the soil on which they are grown. However, the highest CP contents obtained in PCBSM could be assigned to

Table 1 Physicochemical analysis of raw, poultry manure- and urea- ensiled cocoa bean shell meal

Parameters	RCBSM	PCBSM	UCBSM	*p*-value
Physical properties				
Texture	dry and firm	slightly slippery	slippery	–
Smell/aroma	pleasant	slightly choky/pungent	slightly choky/pungent	–
Colour	brownish	dark brown	deep brown	–
pH	6.8	6.9	6.5	–
Temperature (°C)	32.0	36.0	37.0	–
Chemical properties				
Dry matter	90.5 ±0.26^{a}	87.4 ±2.47^{a}	77.3 ±1.30^{b}	0.16
Crude protein	10.71 ±0.01^{c}	18.2 ±1.43^{a}	15.8 ±0.13^{b}	0.03
Crude fibre	9.67 ±0.03^{a}	7.83 ±0.64^{ab}	7.26 ±0.80^{b}	0.03
Ash	6.37 ±0.02^{c}	7.38 ±0.84^{b}	7.1 ±0.29^{a}	0.02
Ether extract	8.54 ±0.19^{b}	10.4 ±0.08^{a}	10.8 ±0.44^{a}	0.00
Nitrogen free extract	64.7 ±0.15^{a}	56.2 ±1.21^{c}	59.1 ±0.08^{b}	0.01
Neutral detergent fibre	57.27 ±0.01^{a}	54.43 ±0.01^{b}	50.02 ±0.01^{c}	0.02
Acid detergent fibre	48.01 ±0.01^{a}	41.71 ±0.01^{b}	37.32 ±0.01^{c}	0.01
Acid detergent lignin	24.04 ±0.01^{a}	20.39 ±0.01^{b}	18.24 ±0.01^{c}	0.01

a, b, c – means on the same row with different superscripts are significantly (P <0.05) different; RCBSM – raw cocoa bean shell meal, PCBSM – poultry litter cocoa bean shell meal, UCBSM – urea cocoa bean shell meal, n = 3; each analysis was replicated thrice the effect of poultry manure on the cocoa bean shell and the high nitrogen percentage (46.7%) compared to the most feed proteins source (16–18.87%) and the ability of urea to be easily transformed into ammonia (Panday, 2016). Ammoniation of the cocoa bean shell improved the quantity of cellulose (hemicellulose) for microbial fermentation attack, because the small NH3 molecules are able to penetrate the interfibroid spaces of the crystalline cellulose in order to break down the H-bridges. They can thus, be used by bacteria in the rumen and broken down to ammonia during the normal fermentation process in the rumen (Fajemisin et al., 2013). Moreover, the relatively high CP value obtained in poultry litter treated ensiled CPHM could be traced to the fact that poultry litter consists mainly of uric acid which can be degraded by rumen microbes to yield ammonia. In addition, proteolysis could have taken place during ensiling. The lower crude fibre value (7.26%) of the UCBSM might be attributed to the ability of ammonia from urea to hydrolyse the chemical/physical bonds between lignin and cellulose and hemicellulose in the plant cell walls (Erika and Anuraga, 2015). On the other hand, the fibre fraction values reported in this study showed a decrease after urea treatment. Eghosa et al. (2010) and Erika and Anuraga (2015) reported the same trend when urea treatment on proximate composition of cocoa pod was assessed. This suggested that urea is very effective in breaking the lignin bond and degrading fibrous feeds by fibrolytic microorganisms; and could reduce the bulkiness of feed in the rumen for effective utilization.

The mineral and anti-nutrients composition observed in this study were significantly (p <0.05) influenced by the treatments (Table 2). The different treatments on the CBSM improved the mineral composition especially by the non-protein nitrogen (NPN) sources, but best enriched in UCBSM. Though, statistically (p >0.05) similar with PCBSM. The concentration and availability of these minerals depends on the pod composition, soil type and hydrolysis during soaking in 5% urea solution, because when urea and water reacts, enzyme urease will speed up the dissociation into ammonia and carbon-dioxide, and thus more minerals will be released which would assist the rate of chemical reactions within the animal's body (Steve, 2014). These are necessary building blocks for microbial protein synthesis. More so, poultry manure contributes significant amounts of Ca, P, K, and numerous trace minerals. The high amount of potassium in CBSM implies that the crop residue will support the regulation of acid-base balance, if incorporated in ruminants' diet. Anti-nutrients factors are substances which either by themselves or through their metabolic products, interfere with feed utilization and affect the health and production of animal or which act to reduce nutrient intake, digestion, absorption and utilization and may produce other adverse effects (Akande et al., 2010). Thus, it is important to find a way to reduce the anti-nutrient contents of CBSM. In Table 2, significant reduction was observed PCBSM compared to others. The PCBSM recorded the least values (1.43% alkaloid, oxalate 0.54%, and phytate 1.16% except for saponin 0.66% and theobromine 2.43% in UCBSM). The significant decrease in all antinutrients could be as a result of the treatment and fermentation. The reduced level of alkaloid and theobromine will reduce the astringent or bitter taste of the CBSM and invariably made it palatable. However, the chemical reaction and heat generated could have decomposed the antinutrients, hence, the reduced

Table 2 Mineral and anti-nutrient composition of raw, poultry manure- and urea- ensiled cocoa bean shell meal

Parameters (%)	RCBSM	PCBSM	UCBSM	p- value
Minerals				
Calcium	0.46 ± 0.01^{b}	0.52 ± 0.01^{ab}	0.58 ± 0.01^{a}	0.001
Phosphorus	0.16 ± 0.01^{c}	0.22 ± 0.01^{b}	0.28 ± 0.01^{a}	0.001
Potassium	3.26 ± 0.01^{b}	3.82 ± 0.01^{ab}	3.93 ± 0.01^{a}	0.01
Magnesium	1.26 ± 0.01^{b}	1.30 ± 0.01^{b}	1.47 ± 0.01^{a}	0.02
Sodium	0.04 ± 0.01^{b}	0.07 ± 0.01^{ab}	0.09 ± 0.01^{a}	0.001
Anti-nutrients				
Alkaloid	2.36 ± 0.01^{c}	1.43 ± 0.02^{b}	1.83 ± 0.2^{a}	0.01
Oxalate	1.65 ± 0.02^{c}	0.54 ± 0.01^{b}	0.92 ± 0.01^{a}	0.03
Phytate	1.36 ± 0.01^{b}	1.16 ± 0.02^{a}	1.17 ± 0.01^{a}	0.03
Saponin	0.84 ± 0.24^{b}	0.66 ± 0.01^{a}	0.65 ± 0.02^{a}	0.02
Theobromine	3.64 ± 0.01^{c}	3.23 ± 0.01^{b}	2.43 ± 0.02^{a}	0.00

a, b, c – means on the same row with different superscripts are significantly (P <0.05) different; RCBSM – raw cocoa bean shell meal, PCBSM – poultry litter cocoa bean shell meal, UCBSM – urea cocoa bean shell meal, n = 3; each analysis was replicated thrice reported values. This invariably would reduce the toxicity level, increased nutrient and minerals bioavailability and increases feed utilization.

Gas production is a nutritionally wasteful product but provides a useful basis form with which ME, OMD and SCFA may be predicted. There are many factors that determine the amount of gas produced during fermentation. This would depend on the nature and level of fibre, antinutrient compositions (Babayemi and Bamikole, 2006) and potency of the rumen liquor used for the incubation. Table 3 presents the *in vitro* gas production of untreated, poultry manure- and urea- ensiled CBS meal. The result showed that gas volume produced at different incubation time differed significantly (p <0.05). The volume of gas produced increased with increasing incubation time. Though, slight decline in gas volume production were observed after 18 hrs of incubation. The study revealed that raw/ untreated CBSM produced the least gas volume (2.07 ml) at 3 hrs and highest (3.81 ml) at 18 hrs. Poultry manure ensiled CBS produced the least volume of gas (3.16 ml) at 3 hours of incubation time, while the highest volume of gas (8.15 ml) were observed at 18 and 21 hrs period of incubation. Meanwhile, gas volume production of urea ensiled CBSM at 3 hrs was the least (3.09 ml) and highest (8.06 ml) at 18 hrs. This implied that digestion would take place within the normal rumen retention time at 21 to 24 hours. Thus, if fed to animals, the feed or protein would have metabolized/digested within 21 hours and so would not result in nutritional disorder (bloat) in animals, if fed. The gas produced during fermentation can be used as an indirect measure of dry matter degradability (Kamalak et al., 2005). The gas production is a function and mirror of degradable carbohydrates and therefore, the amount of gas produced depends on the nature of the carbohydrates.

The *in vitro* characteristics of raw CBSM, PCBSM and UCBSM are presented in Table 4. The results showed a significant (p <0.05) difference for all the parameters assessed with respect to the treatments. It was noted that the least values obtained in all the parameters assessed were for raw CBSM. However, the highest volume of methane gas (3.00 ml) produced was obtained in PCBSM while the least (2.56 ml) was obtained in RCBSM. The carbon-dioxide (CO_2) gas produced from the insoluble fraction was highest in UCBSM (8.99 ml). The organic matter digestibility (OMD) ranged from 26.99% (RCBSM) to 34.97% (PCBSM). The short chain fatty acid (SCFA) value of PCBSM and UCBSM were numerically and statistically (p >0.05) similar (0.25 µm). The metabolizable energy of PCBSM was the highest (4.34 MJ/KgDM) and least in RCBSM. The same trend was observed for IVDMD. Gas volume is a good parameter from which to predict digestibility, fermentation end-product and microbial protein synthesis of the substrate by rumen microbes in the *in vitro* system (Preston et al., 2019). The methane

Table 3 *In vitro* gas production of raw, poultry manure- and urea- ensiled cocoa bean shell meal

	Gas volume (ml)							
Samples	3 hrs	6 hrs	9 hrs	12 hrs	15 hrs	18 hrs	21 hrs	24 hrs
Raw CBSM	2.07^{b}	2.33^{c}	2.33^{b}	2.41^{b}	3.03^{c}	3.81^{b}	3.80^{b}	3.55^{b}
PCBSM	3.16^{a}	4.44^{a}	5.03^{a}	6.73^{a}	8.14^{a}	8.15^{a}	8.15^{a}	7.98^{a}
UCBSM	3.09^{ab}	3.99^{b}	5.00^{a}	6.80^{a}	7.48^{b}	8.06^{a}	8.05^{a}	7.97^{a}
SEM	0.23	0.41	0.17	1.01	0.19	1.26	1.03	0.37
p-value	0.02	0.01	0.02	0.02	0.00	0.01	0.01	0.03

a, b, c – means on the same row with different superscripts are statistically (P <0.05) different; CBSM – cocoa bean shell meal; PCBSM – poultry manure ensiled CBSM; UCBSM – urea ensiled CBSM

Table 4 *In vitro* characteristics of raw, poultry manure- and urea- ensiled cocoa bean shell meal

Diets	Methane (ml)	CO_2 (ml)	OMD (%)	SCFA (µm)	ME (MJ/kgDM)	IVDMD (%)
Raw CBSM	2.56^{c}	5.33^{d}	26.99^{d}	0.14^{d}	3.32^{e}	0.05^{bc}
PCBSM	3.00^{c}	7.47^{c}	34.97^{c}	0.25^{c}	4.34^{d}	0.06^{b}
UCBSM	2.96^{c}	8.99^{b}	33.70^{c}	0.25^{b}	4.21^{c}	0.06^{b}
SEM	0.11	2.01	0.44	0.02	0.14	0.01
p-value	0.01	0.01	0.01	0.02	0.01	0.00

b, c, d, e – means on the same row with different superscript are significantly (P <0.05) different; OMD – organic matter digestibility; CO_2 (carbon-dioxide) – gas production from insoluble fraction; SCFA – short chain fatty acid; ME – metabolizable energy; IVDMD – *in vitro* dry matter disappearance; CBSM – cocoa bean shell meal; PCBSM – poultry manure ensiled CBSM; UCBSM – urea ensiled CBSM gas volume produced in this study were similar to those reported by Okoruwa and Agbonlahor (2016) in an experiment conducted to investigate the gas production characteristics of cocoa pod husk with soursop pulp meals used in replacement of napier grass in the practical diet of West African Dwarf sheep. Methane gas is an important gas among gases produced by ruminants during fermentation which have negative correlation with energy utilization in ruminants. Hence, the apparently low methane gas volume observed in this study is an indication the diets do not aid methanogenesis and if fed to animals, it will not result to accumulation of gas in the rumen (bloat). There was gradual increase in the value of organic matter digestibility and metabolizable energy reported, hence a mutual relationship exists between total methane production and ME with OMD (Babayemi and Bamikole, 2006). The low short chain fatty acids reported in this study were due to the lower methane gas production which was evident within the 24 hours incubation period. Akinfemi et al. (2009) confirmed a close association between SCFA and *in vitro* gas production. That the quantitative parameters to measure the effective degradability of the diets could be derived through the *in vitro* gas production. The IVDMD result showed that there were significant losses of DM as a result of the treatment over the RCBSM because great quantities of cell contents were dissolved in the water.

4 Conclusions

The study revealed that composite cocoa bean shell ensiled with poultry manure at 1 : 1 (w/w) had a best nutrient and mineral profiles; *in vitro* degradability study revealed that poultry ensiled cocoa bean shell meal had the best potentials to be used in ruminants' nutrition. However, *in vivo* study should be carried out to validate the potentials of these feed materials.

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