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Nutritional Potentials and *In Vitro* Estimation of Composite Cocoa Pod Husk Based Diets for Ruminants

O.B. Omotoso[1], A.N. Fajemisin[1], I.O. Omotoso[2] and J.A. Alokan[1],
[1]Department of Animal Production and Health, School of Agriculture and Agricultural Technology, Federal University of Technology, Akure; [2]Department of Microbiology, Faculty of Life Sciences, University of Ilorin, Ilorin
Corresponding author: O.B. Omotoso; E-mail: tos2bod@yahoo.com; Tel: 08060186726

Abstract
The use of cocoa pod husk in livestock nutrition is being limited because of its fibrous nature and anti-nutrients, which have detrimental effect(s) on the animals. This study was conducted to evaluate the nutritive value and effects of replacing urea-treated ensiled cocoa pod husk meal(UTCPHM) with cassava peel in a complete concentrate diet (CCD)on *in vitro* digestibility and methane (CH_4) production. UTCPHM was prepared by soaking the raw milled pod in 5% urea solution under anaerobic condition for 7 days, decanted and the filtrates were further ensiled for 28 days, dried; and were incorporated in CCD at 0, 10, 15, 20, 25 and 30% on part basis. Feed samples were analyzed for chemical composition and data generated from *in vitro* study were subjected to statistical analysis in a completely randomized design experiment. Results revealed that; the treatment significantly reduced the crude fibre, fibre fraction contents, anti-nutrients and improved the CP content of the pod by 71.84%. DM and CP of the diets ranged from (89.34 – 89.71%) and (10.52 – 12.84%) respectively. The *in vitro* dry matter digestibility(IVDMD) increased with increasing levels of UTCPHM in the diets. With increasing levels of UTCPHM, CH_4production relatively reduced as compared to diet E. Metabolizable energy (5.66 Kcal/g) of diet F was the highest. Therefore, inclusion of UTCPHM at 30% in CCDs has the potential for improving digestibility and reducing CH_4production in ruminants.

Keywords: Methane,urea, cocoa pod husk, ruminants

Introduction

Livestock production is undertaken in a multitude of ways across the planet, providing a large variety of goods and services, using different animal species and different sets of resources, in a wide spectrum of agro-ecological and socio-economic conditions (Steinfeld *et al.,* 2006).In recent years, emphases have been shifted to the use of by-products of agro-industrial origin as low cost alternative carbohydrate sources for livestock nutrition. Sucharita *et al.* (1998) concluded that effective utilization of non-conventional feeds should be the major areas of research in the less developed countries due to shortage of conventional feedstuffs, ever increasing human population and to ensure their food security, is dependent on the better utilization of alternative feed resources. Though, ruminant animals can thrive very well on fibrous feeds to keep their rumen healthy, higher quantities of cellulose, hemicellulose in the cell wall and presence of anti-nutritional factors usually limit their efficient utilization by the ruminants (Makkar, 1993).

Consequently, cocoa pod husk which is an abundant residue generated on cocoa farmlands has been regarded as a "waste" in Nigeria, except for the negligible amount used in the manufacture of local soap. It has however been reported to contain about 80.00 – 88.96 % DM, 6.00 - 9.14 % CP, 24.93 - 35.74 % CF and 14.10 - 21.16 % lignin (Aregheore,2002;Ozung*et al.,* 2016). To nutritionally upgrade the crop residue for efficient utilization by ruminant animals, urea, a non-protein nitrogen, which can be easily converted to ammonia in the rumen, could be effective.

Thus, an *in vitro* study was conducted to evaluate the nutritive value and effects of incorporating graded levels of urea-treated ensiled cocoa pod husk meal in total mixed rations on digestibility and methane production.

Materials and Methods

The study was carried out at the Nutrition Laboratory of the Animal Production and Health, Federal University of Technology, Akure (FUTA) located on Latitude 7^0 15"N and Longitude 5^0 15"E (Ajibade *et al*., 2014).Cocoa pod husk was collected at various cocoa farmlands in Ilara-mokin, Ondo State, sun-dried for 8 – 12 days, crushed at the FUTA feedmill to 1mm particle size, and thensoaked in the 5% urea solution under anaerobic condition for 7 days. Thereafter, they were decanted and the filtrates further ensiled for 28 days, dried and incorporated at 0, 10, 15, 20, 25 and 30% replacement levels in a complete concentrate diets (Table 1). The feed samples were analysed for chemical composition according to AOAC (2002) method.

Table 1: Gross composition of graded inclusion levels of UTCPHM in a complete concentrate diets

	Diets / Level of UTCPHM Replacement (%)					
Ingredients (%)	0 (A)	10 (B)	15 (C)	20 (D)	25 (E)	30 (F)
Urea-ensiled CPH	0.00	5.00	7.50	10.00	12.50	15.00
Cassava peels	50.00	45.00	42.50	40.00	37.50	35.00
Brewer dried grain	27.00	27.00	27.00	27.00	27.00	27.00
Wheat offal	5.00	5.00	5.00	5.00	5.00	5.00
Palm kernel cake	15.00	15.00	15.00	15.00	15.00	15.00
Bone meal	1.00	1.00	1.00	1.00	1.00	1.00
Salt	1.00	1.00	1.00	1.00	1.00	1.00
Premix	1.00	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00	100.00

Two adult male WAD goats of same age and uniform conformation were selected as donors of rumen inoculum for *in vitro* studies. The animals were fed for two (2) weeks with 40% concentrate feed 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.Buffer solution prepared was the McDougall's solution which consist solution (g/litre) of 9.8 $NaHCO_3$ + 2.77 Na_2HPO_4 + 0.57 KCl + 0.47 NaCl + 2.16 $MgSO_3.7H_2O$ + 16 $CaCl_2.2H_2O$ and mixed 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 120ml calibrated transparent plastic syringes with fitted silicon tube.

The sample weighing 200mg (n=3) was carefully dropped into syringes and thereafter, 30ml each of the inoculum containing cheese cloth strained rumen liquor and buffer solution. The syringe were 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 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 hrs. At the end of the termination hour, 4ml 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 + 889GV + 0.45CP + 0.651XA; SCFA = 0.0239 V - 0.0601.Where; GV, CP CF and XA are total gas volume, crude protein, crude fibre and ash respectively.

Data obtained were subjected to analysis of variance using SAS(2008) and the significant means were separated using Duncan Multiple Range Test of the same package.

Results and Discussion

The 88.97% DM, 8.31% CP and 33.87% CF contents obtained for the raw cocoa pod husk meal (RCPHM) in this study (Table 2) agreed with the reported values of about 80.00 – 88.96% DM, 6.00 - 9.14% CP, and 24.93 - 35.74% CF by Aregheore (2002) and Ozung *et al.,*(2016). The dry matter of the pod before and after treatment (soaking in 5% urea solution for 7 days and ensiled for 28 days) and the formulated diets were high; this could be attributed to the dried nature of the pod and cassava peels, culminating in its high lignification. The CP content of the pod and invariably, that of the diets were improved. The CP contents (10.52 – 12.84%) of the diets could adequately meet the protein requirement by ruminant animals for growth (NRC, 2007). The reduction in fibre contents could be as a result of the fermentation process during ensiling. The reduction in the alkaloid and theobromine might be traced to decanting after been soaked in urea solution, and thus would make the feed more palatable to the animals. The amount of gas produced during fermentation is dependent on the nature, level of fibre and potency of the rumen liquor used for the incubation. The result showed that gas volume produced at different incubation time differed significantly at $p<0.05$.

The observed increase in the cumulative gas volume from 3 hours to 21 hours (Table 3), and the gradual decline after 21 hours of incubation from Diet A to F could be associated to the replacement levels of UTCPHM and thus, predicted digestibility, fermentation end-product and microbial protein synthesis of the diets by rumen microbes in the *in vitro* system. This also implied that digestion would take place within the normal rumen retention time at 21 to 24 hours and could be an indirect measure of dry matter degradability. This trend

agreed with the report of Babayemi and Bamikole (2006) when similar method was used to evaluate the nutritive value of *Tephrosia candida* DC leaf and its mixtures with Guinea grass for ruminant feeding.

Table 2: Chemical composition of raw, urea-treated ensiled cocoa pod husk and formulated diets

			Diets / Level of UTCPH Replacement (%)						
Parameters (%)	RCPHM	UTCPH	0 (A)	10 (B)	15 (C)	20 (D)	25 (E)	30 (F)	SEM
Dry matter	88.97	89.87	89.34	89.41	89.39	89.64	89.71	89.64	0.52
Crude protein	8.31	14.28	10.52^{d}	10.87^{d}	11.35^{c}	12.53^{b}	12.67^{ab}	12.84^{a}	0.26
Crude fibre	33.87	23.91	17.29	17.32	17.37	17.36	17.32	17.31	1.77
Ether extract	4.92	3.82	2.33^{d}	2.67^{c}	3.07^{b}	3.15^{a}	3.17^{a}	3.16^{a}	0.22
Ash	6.25	6.05	5.47^{a}	5.33^{ab}	5.19^{b}	5.11^{b}	5.02^{c}	5.01^{c}	0.38
NFE	35.62	41.81	53.73^{a}	53.22^{ab}	52.40^{b}	51.48^{b}	51.47^{b}	51.32^{b}	1.34
NDF	63.23	52.64	73.90^{a}	72.62^{ab}	71.91^{b}	70.68^{c}	70.58^{c}	69.13^{d}	0.41
ADF	51.40	42.21	51.66^{a}	49.62^{bc}	50.83^{ab}	49.13^{bc}	49.59^{bc}	48.42^{c}	0.33
ADL	24.05	19.23	25.14^{a}	23.37^{b}	22.42^{b}	23.17^{b}	23.19^{b}	22.48^{b}	0.26
Calcium	0.41	0.51	0.54^{d}	0.56^{cd}	0.60^{c}	0.66^{b}	0.69^{ab}	0.71^{a}	0.02
Phosphorus	0.11	0.20	0.26^{d}	0.28^{cd}	0.29^{c}	0.32^{b}	0.34^{a}	0.35^{a}	0.01
Alkaloid	6.50	3.45	0.80^{a}	1.49^{b}	1.75^{c}	2.00^{d}	2.39^{e}	2.81^{f}	0.16
Theobromine	3.89	2.05	0.00^{a}	0.77^{b}	0.95^{c}	1.12^{d}	1.33^{e}	1.47^{f}	0.12

abc: means on the same row with different superscripts are statistically (P<0.05) different

Table 3: *In vitro* gas production of raw and replacement levels of urea treated CPHM diets

	Gas volume (ml)							
Diets	3 HRS	6 HRS	9 HRS	12 HRS	15 HRS	18 HRS	21 HRS	24 HRS
Raw CPHM	1.67^{f}	1.82^{e}	1.99^{e}	2.13^{e}	2.47^{e}	3.01^{f}	2.89^{f}	2.89^{f}
A	3.33^{e}	4.33^{d}	6.33^{d}	6.33^{d}	8.33^{d}	10.33^{e}	10.67^{e}	10.67^{e}
B	4.67^{d}	5.67^{c}	6.67^{d}	7.33^{d}	9.67^{c}	11.67^{d}	11.93^{d}	12.33^{d}
C	6.00^{c}	6.00^{c}	8.00^{c}	9.00^{c}	11.00^{b}	13.00^{c}	14.00^{c}	14.07^{c}
D	8.33^{b}	9.00^{b}	11.00^{b}	13.00^{b}	16.33^{a}	14.00^{b}	17.67^{b}	17.67^{b}
E	9.67^{a}	11.67^{a}	14.33^{a}	14.33^{a}	16.33^{a}	17.67^{a}	20.00^{a}	20.03^{a}
F	9.69^{a}	11.64^{a}	14.29^{a}	14.32^{a}	16.34^{a}	17.62^{a}	20.01^{a}	20.19^{a}
SEM	0.66	0.81	1.00	1.06	1.13	1.15	1.08	0.97

abc: means on the same row with different superscripts are statistically (p<0.05) different.

From Table 4, methane gas produced ranged from 3 ml (diet A) - 5 ml (diet F) and these values were in line with the values reported by Okoruwa and Agbonlahor (2016) when they investigated the gas production characteristics of cocoa pod husk with soursop pulp meals used in replacement for napier grass in the diet of WAD sheep. Thus, the methane gas volume produced in this study could be traced to the gradual increment in protein quality of the diets.

Table 4: *In vitro* characteristics of raw and replacement levels of urea treated CPHM diets

Diets	Methane (ml)	CO_2 (ml)	OMD (%)	SCFA (µm)	ME (Kcal/g)	IVDMD (%)
Raw CPHM	1.00^{d}	1.89^{e}	25.26^{e}	0.01^{e}	3.16^{f}	0.02^{c}
A	3.00^{c}	7.67^{d}	32.56^{d}	0.19^{d}	4.28^{e}	0.05^{bc}
B	3.00^{c}	9.33^{c}	34.15^{c}	0.23^{c}	4.53^{d}	0.06^{b}
C	3.00^{c}	11.07^{b}	35.73^{c}	0.28^{b}	4.79^{c}	0.06^{b}
D	4.00^{b}	13.67^{ab}	38.98^{b}	0.36^{a}	5.29^{b}	0.07^{a}
E	5.00^{a}	15.03^{a}	41.13^{a}	0.40^{a}	5.63^{a}	0.08^{a}
F	5.00^{a}	15.19^{a}	41.37^{a}	0.42^{a}	5.66^{a}	0.08^{a}
SEM	0.29	2.09	0.94	0.02	0.14	0.01

abc: means on the same row with different superscript are significantly (P<0.05) different

Hence, the apparently low methane gas volume produced in this study is an indication of effective utilization of the diets. The gradual increase in the value of OMD and ME reported, implied a mutual relationship exists between total methane production and ME with OMD (Babayemi and Bamikole, 2006). The low SCFA

reported in this study were due to the lower methane gas production which was evident within the 24 hours incubation period. The reduced value of IVDMD of urea-treated ensiled CPHM based-diets over the raw CPH Mcould be traced to the effect of soaking in urea solution because great quantities of cell contents were dissolved in the water.

Conclusion and Recommendations

The study established that cocoa pod husk has nutritional potentials in ruminant nutrition, and could be nutritionally upgraded by soaking in 5% urea solution for 7 days, ensiled for 28 days, and incorporated up to 30% replacement level in the ruminants' diets. However, *in vivo* study should be carried out to substantiate the *in vitro* study.

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