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Original Article

Effect of ash-rumen liquor treatments on the chemical composition of cocoa (*Theobroma cacao*) pod husk

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Abstract

Many of the agricultural by-products considered as wastes have great potentials as animal feed ingredients if properly handled, processed and incorporated into animal diets. Pods of cocoa (*Theobroma cacao* L.) were washed to remove residual mucilage, chopped to pieces, sun-dried for 7-14 days and milled as cocoa pod husk (CPH) meal. The CPH were thereafter fermented with ash solution for 168 hours followed by fermentation with rumen liquor for another 168 hours. The ash and rumen liquor fermented samples were sun-dried and chemically analyzed for the proximate composition, anti-nutritional factors, mineral components and fibre fractions. The crude protein content of the raw CPH increased by 50.90-62.50% while the crude fibre content reduced by 16.86-44.86% when subjected to the combined treatments of ash solution and rumen liquor fermentation. The caffeine and theobromine contents decreased by 54.48-97.13 and 59.03-74.30%, respectively while the fibre fractions (acid detergent fibre, neutral detergent fibre, acid detergent lignin cellulose and hemicellulose) decreased in the treated CPH with a Coefficient of Variation of 2.40-121.03%. The concentrations of all the minerals: Ca, P, K, Na, Mg, Zn, Fe, Co and Mn measured were enhanced in the treated CPH samples. It could be concluded that combining ash solution treatment and fermentation with rumen liquor for 168 hours would help to enhance the nutritive quality of CPH for inclusion in livestock feed in the region where cocoa pod husk are being produced in large quantities.

INTRODUCTION

In Nigeria, large quantities of different types of agro-wastes are dumped in the environment (Oloruntola *et al*., 2017). Nigeria, which recorded a 5.2% fall in cocoa production was said to be the 17th largest producer of cocoa and produced about 0.255 million metric tonnes of cocoa in 2018 (Statista, 2019). Cocoa pod husk represents between 70 and 75% of the whole cocoa fruit weight where each ton of cocoa fruits will produce between 700 and 750 kg of cocoa pod husk (Cruz *et al.,* 2012).

An earlier report revealed that about 6.7 million metric tonnes of cocoa pod husk (CPH) were discarded as waste annually in Nigeria (Olugosi *et al*., 2019) and about 0.8 to 1.0 million tons of cocoa pod husk is generated annually in cocoa farms in Nigeria (Ojeniyi, 2006). Of recent, substantial efforts have been directed to finding alternative feed resources in livestock feeds to enhance livestock production in Nigeria (Oloruntola, 2018). Previous studies showed that untreated CPH has been used as a component of animal feeds with little success (Olubamiwa *et al.,* 2002; Oddoye *et al.,* 2010). This is attributed to the high contents of anti-nutritional factors, notably theobromine, and also high fibre contents. It is therefore of utmost importance to seek cheap and farmers' friendly biotechnology that can help enhance the utilization of CPH, an agro-waste in livestock feeding. Achieving this will go a long way to reducing feed costs, increasing animal protein production and consumption which will help to stem the menace of disease morbidity in countries where huge CPH is being generated as waste. It is therefore envisaged that if cocoa pod husk is subjected to adoptable biotechnology such as ash and rumen liquor fermentation treatments, it may help to increase its utilization with a concomitant reduction in the cost of finished feeds. Thus, this study sought to provide basal information on cocoa pod husk subjected to ash and rumen liquor fermentation to enhance the quality of CPH for inclusion in livestock finished feeds.

MATERIALS AND METHODS

This study was approved by the Research Ethics Committee of the Department of Animal

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Production and Health, the Federal University of Technology, Akure (FUTA), Nigeria as part of a doctoral research study. The study was thereafter carried out at the Central Research Laboratory, FUTA.

Collection and Preparation of Cocoa pod husk and Cornstalk ash

Pods of cocoa were collected from reputable cocoa farms in Oda, Ondo State, Nigeria and identified by the Department of Crop, Soil and Pest Management, FUTA. The pods were cut into two parts to remove the pulpy seeds and thoroughly washed with water to remove residual mucilage of the pods. Thereafter, the pods were chopped to pieces with the help of a sharp stainless steel knife after which they were sun-dried for 7-14 days depending on the intensity of the sun. The sun-dried pods were milled and kept at a moisture level of less than 10% in a cool, dry place until used. Dry maize (*Zea mays*) stalks were collected after harvesting from the Teaching and Research Farm of Federal College of Agriculture, Akure (FECA), Nigeria, and burnt to ashes. The ashes were collected in a plastic container and kept in a dry place until used.

Preparation of alkaline solution and treatment of Cocoa pod husk (CPH)

Cornstalk ash extract was prepared as described by Adamafio *et al*. (2004) and Adeyeye *et al*. (2016). Based on an earlier report by Adeyeye *et al*. (2016), 1500g of corn stalk ash was mixed with 7 L of distilled water, stirred homogeneously, and then covered for forty-eight (48) hours, decanted into a plastic bucket and stored. A measure of 0.8 L of the alkaline solution was mixed with 200g of CPH, left anaerobically for 168 hours. Thereafter, squeezed and moist CPH were sun-dried immediately for 4-5 days and stored in a dry container for chemical analyses.

Rumen liquor fermentation of ash cocoa pod husk (ACPH) and chemical analyses

The method of rumen liquor fermentation used was as described by Oloruntola *et al*. (2017). One kilogram of ash cocoa pod husk (ACPH) was successively thoroughly mixed with 100g dried autoclaved layer dropping meal and 50ml of undiluted molasses. Thereafter, the ACPH was sprayed with 260ml of freshly collected bovine rumen liquor and allowed to ferment for 168 hours (7 days) in an airtight black plastic container. The rumen liquor fermented ash cocoa pod meal was thereafter sundried for 7 days, analysed for proximate composition (AOAC, 2000), tannin (Makkar and Goodchild, 1996), phytate (Lorenz *et al.*, 2007), oxalate (Adeniyi *et al*., 2009), caffeine (Wanyika *et al*., 2010), theobromine (Erickson, 2011) and saponin contents (He *et al*., 2014). The fibre fractions: neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin, hemicellulose and cellulose of the samples were determined according to the procedure of Van Soest *et al*. (1991). The Acid Detergent Lignin (ADL) was obtained by subtracting the weight of the ash from the weight of the dried residue. The hemicellulose was estimated by subtracting ADF from NDF while cellulose was obtained by subtracting ADL from ADF. Thereafter, the processed cocoa pod husk was kept in an airtight container and named ash and rumen liquor fermented cocoa pod husk (ARFCPH). The macro minerals (sodium, potassium, calcium, magnesium, and phosphorus) and micro minerals (zinc, iron, copper and manganese) of the processed cocoa pod husk were determined as earlier described by Amabye and Gebrehiwot (2015).

Statistical Analysis

All data collected were subjected to ANOVA from General Linear Model Procedures using the software package SPSS version 20 for windows (SPSS, 2011).

RESULTS

Table 1 shows that the crude protein (CP) of ACPH was higher when compared to the untreated cocoa pod husk (19.87g/100gDM vs. 7.45g/100gDM). The crude fibre decreased by 16.86% in ash treated cocoa pod husk (ACPH) and 44.86% in ARFCPH, compared to the untreated cocoa pod husk (UCPH). The ether extract decreased by 38.89% ACPH and 38.99% in ARFCPH, compared to the UCPH; while the CHO decreased by 14.44% in ACPH and 19.14% in ARFCPH, compared to the UCPH. However, the ash content increased from 7.19g/100g by 41.21% and 61.20% in ash and ash-rumen liquor fermented CPH, respectively.

The anti-nutritional factors typified as phytate, tannin, theobromine, caffeine and oxalate of the untreated and treated CPH are presented in Table 2. The phytate contents of the samples decreased by 37.54% in ACPH and 41.11% in ARFCPH, compared to UCPH. In the same trend, the tannin concentration in the sample decreased by 50.70% in ACPH and 59.09% in ARFCPH, compared to UCPH; while the theobromine concentration

Table 1: Proximate composition (g/100g DM) of untreated, ash-treated and ash-rumen liquor fermented cocoa pod husk

	Crude protein	Crude fibre	Ether extract	Ash	CHO
UCPH	7.45	36.98	10.08	7.19	38.30
ACPH	15.19 (50.95%)*	30.75 (16.86%)**	6.16 (38.89%)**	12.23 (41.21%)*	32.67 (14.44%)**
ARFCPH	19.87 (62.51%)*	20.53 (44.86%)**	6.15 (38.99%)**	18.53 (61.20%)*	30.97 (19.14%)**
Mean	14.17	29.42	7.46	12.65	33.98
SEM	3.62	4.79	1.31	3.28	2.21
CV%	6.27	8.30	2.26	5.68	3.83

DM: Dry matter; UCPH: Untreated cocoa pod husk; ACPH: Ash treated cocoa pod husk; ARFCPH: Ash and rumen liquor fermented cocoa pod husk; Values in brackets are percent increase (*), decrease (**) of treated pods.

Table 2: Anti-nutritional factors (g/100g) of untreated, ash-treated and ash-rumen liquor fermented Cocoa pod husk

	Phytate	Tannin	Theobromine	Caffeine	Oxalate	Saponin
UCPH	11.48	2.86	3.93	3.14	1.30	1.28
ACPH	7.17(37.54%)**	1.41 (50.70%)**	1.61 (59.03%)**	1.43(54.48%)**	1.01 (22.31%)**	0.85(33.59%)**
ARFCPH	1.65(41.11%)**	1.17 (59.09%)**	1.01 (74.30%)**	0.09 (97.13%)**	0.44 (66.15%)**	0.61(52.34%)**
Mean	6.76	1.81	2.18	1.55	0.92	0.91
SEM	2.84	0.53	0.89	0.88	0.25	0.20
CV%	4.92	0.91	1.54	1.53	0.44	0.34

DM: Dry matter; UCPH: Untreated cocoa pod husk; ACPH: Ash treated cocoa pod husk;
ARFCPH: ash and rumen liquor fermented cocoa pod husk; Values in brackets are percent, decrease (**) of treated pods.

decreased by 59.03% in ACPH and 74.30% in ARFCPH, compared to UCPH. The caffeine concentration reduced in ACPH and ARFCPH by 54.48% and 97.13%, respectively, compared to the UCPH. The oxalate content in ACPH reduced by 22.31% and in ARFCPH by 66.15%, compared to UCPH; while the saponin concentration of ACPH and ARCPH reduced by 33.59%, and 52.34%, respectively, compared to UCPH.

Table 3 shows that subjecting the CPH to the combined effects of ash treatment and rumen liquor fermentation led to a decrease in the acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL), cellulose and hemicellulose with a CV% ranging from 2.40 for hemicellulose to 121.03 for acid detergent fibre.

Tables 4 shows that sodium, potassium, calcium, magnesium and phosphorus increased by 44, 79.31, 75.44, 95.32 and 77.30 %, respectively post ash and rumen liquor fermentation; while the zinc, iron, copper and manganese content of cocoa pod husk increased by 56.62, 52.70, 47.23 and 67.84 %, respectively after processing (Table 5).

Table 3: Fibre fractions (g/100g DM) of untreated, ash-treated and ash-rumen liquor fermented Cocoa pod husk

	Acid detergent fibre	Neutral detergent fibre	Acid detergent lignin	Cellulose	Hemicellulose
UCPH	42.89	57.19	30.92	15.90	14.30
ACPH	39.56 (7.76%) **	50.87 (11.05%) **	28.73 (7.08%) **	14.80 (6.92%) **	13.51 (5.52%) **
ARFCPH	22.39 (47.80%) **	35.90 (37.24%) **	26.49 (14.33%) **	11.97 (24.72%) **	11.31 (20.91%) **
Mean	34.95	47.00	28.71	14.22	13.04
SEM	6.35	6.31	1.28	1.17	0.89
CV%	121.03	119.55	4.91	4.11	2.40

DM: Dry matter; UCPH: Untreated cocoa pod husk; ACPH: Ash treated cocoa pod husk;
ARFCPH: ash and rumen liquor fermented cocoa pod husk; Values in brackets are percent decrease (**) of treated pods.

Table 4: Macro mineral contents (g/100g DM) of untreated, ash-treated and ash-rumen liquor fermented Cocoa pod husk

	Sodium	Potassium	Calcium	Magnesium	Phosphorus
UCPH	0.014	0.006	0.014	0.014	0.006
ACPH	0.041 (65.85%)*	0.018 (66.66%)*	0.050 (72.00%)*	0.039(64.10%)*	0.024 (75.00%)*
ARFCPH	0.025 (44.00%)*	0.029 (79.31%)*	0.057 (75.44%)*	0.299 (95.32%)*	0.024 (77.30%)*
Mean	0.03	0.02	0.04	0.12	0.02
SEM	0.01	0.01	0.01	0.09	0.01
CV%	0.00	0.00	0.00	0.02	0.00

DM: Dry matter; UCPH: Untreated cocoa pod husk; ACPH: Ash treated cocoa pod husk;
ARFCPH: ash and rumen liquor fermented cocoa pod husk; Values in brackets are percent increase (*).

Table 5: Micro mineral contents (*ppm*) of untreated, ash-treated and ash-rumen liquor fermented Cocoa pod husk

	Zinc	Iron	Copper	Manganese
UCPH	44.73	312.28	15.79	46.71
ACPH	97.08 (53.92%)*	536.69 (41.81%)*	23.19 (31.91%)*	55.92 (16.47%)*
ARFCPH	103.11(56.62%)*	660.22 (52.70%)*	29.92 (47.23%)*	67.84 (30.67%)*
Mean	148.31	503.06	22.97	56.82
SEM	51.82	101.84	4.08	6.12
CV%	55.18	113.63	49.95	112.23

DM: Dry matter; UCPH: Untreated cocoa pod husk; ACPH: ash treated cocoa pod husk;
ARFCPH: ash and rumen liquor fermented cocoa pod husk;
Values in brackets are percent increase (*) of treated pods.

DISCUSSION

Agricultural by-products such as cocoa pod husk (CPH) could be a good substitute for conventional feedstuffs provided appropriate measure is adopted to improve its nutritive quality and subsequent utilization in monogastric nutrition (Hamzat and Adeola, 2011). Such measures must be cost-effective and farmer's friendly. This explains why ash-rumen liquor was used to ferment this agro-waste to improve its nutritive quality.

In this study, fermenting CPH with 1.5kg/l corn stalk ash solution (CSAS) at 168 hours, the CP increased by 50.95% and by 62.51% when the ash-treated sample was further subjected to rumen liquor solid fermentation for another 168 hours. Conversely, the crude fibre decreased by 16.86% and 44.86%, respectively. This suggests that the combined treatment methods adopted have the potential to improve the nutritive quality of CPH for animal feeding with respect to crude protein and crude fibre contents. The observed findings could be due to an increase in microbial biomass, especially in the rumen liquor (*Streptococcus bovis* and *Ruminicoccus albus*) as well as an increase in extra-cellular microbial enzymes concentration and trap of nitrogen sources for microbial synthesis (Adamafio *et al.*, 2010). This is further corroborated by the reports of Ezeronye (2004) and Oboh (2006) that an increase in the CP content might be as a result of the increase in the growth of single-cell proteins during fermentation. Proteins are macromolecules that serve as an energy source when metabolized and also have structural and mechanical functions such as actin and myosin in muscle and cytoskeleton formation (Schwarzer and Cole, 2005). Therefore, the observed increase in the protein content following rumen liquor fermentation might contribute positively to its being utilized as an animal feed ingredient as energy and or protein where CPH is being produced in large quantities.

There was a reduction in caffeine and theobromine contents of CPH when fermented with 1.5kg/L CSAS at 168 hours by 54.48% (3.14-1.43g/100g) and 59.03% (3.93-1.61g/100g), respectively while the ash-treated sample subjected to rumen liquor solid-state fermentation for another 168 hours was by 97.13% (caffeine) and 74.30% (theobromine). The reduction of caffeine and theobromine contents makes CPH safe for use as an animal feed ingredient. This was supported by Adamafio *et al.* (2011) who reported a 71.8% detheobromination in cocoa pod husk fermented with *Aspergillus niger* over the same period. The decrease in the fibre fractions contents -acid detergent fibre (ADF) and neutral detergent fibre (NDF) of CPH fermented with 1.5kg/litre CSAS at 168 hours might be due to the biodegradation by the fungus secreting enzymes such as cellulase secreted by rumen microbes, and this is in agreement with previous reports by Adeyemi *et al.* (2007) and (Aderemi and Nworgu, 2007) that the degradation of the complex carbohydrates into soluble components is the synergistic actions of several extracellular enzymes such as glucosidases which reduce oligosaccharides to their monometric units.

The enhanced mineral contents (K, Ca, P and Zn) of CPH fermented with 1.50kg/litre CSAS at 168 hours suggested that the processed CPH could be a source of these minerals in animal feed. Calcium, the most abundant mineral in the body was enhanced as observed in the study. More than 99% of total body calcium is stored in bones and teeth where it functions to support their structure (Heaney *et al.,* 2000). Calcium is needed for muscle contraction, blood vessel contraction and expansion, secretion of hormones and enzymes, and sending of messages through the nervous system (Heaney *et al.,* 2000). The present study further suggests that if the processed CPH is used in animal feed formulation, it will help to promote mineralization and consequently abate the mineral deficiencies often affecting livestock. Of interest are the Zn and Fe improvements brought about by the method of processing. Zinc and iron are known to be major elements needed to boost the immunity of animals. Thus, by extension, animals placed on feed containing the processed CPH will have the advantage of being in good health with respect to enhanced immunity.

CONCLUSION

The nutritional value of cocoa pod husk was improved when it was fermented with ash-rumen liquor which is evident by the increase in the crude protein and mineral contents, and reduction of the crude fibre and the anti-nutritional contents of the processed cocoa pod husk.

Conflict of interest: All authors indicate that no actual or potential conflict of interest could inappropriately or possibly influence this work after publication.

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