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RESEARCH ARTICLE

Phytochemicals and Proximate Compositions of Cocoa-based Silage

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HIGHLIGHTS

> The fermentation was found to influence the proximate composition of the silage
> The ensiling of the combined diets was found to influence the phytochemicals present in the silage.

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ABSTRACT

Presently, agro-industrial by-products are used as livestock feeds. There is a need to determine the nutritional properties of these products prior to using them as animal feeds. The qualitative phytochemical analysis revealed the presence of tannins, Phenol, Phlobatannins and Resin. Proximate analysis showed that the fermentation had significantly increased the Protein (15.90-22.12%), Carbohydrate (16.37–45.26%), Calcium (26.09 – 35.81ppm), Phosphorus (1.14–1.18ppm), Potassium (2.79–2.98ppm), Butyric acids (2.59–3.11µg/100g), Gallic acids (0.01–0.11mg/kg) and Caloric values (1x103 to 11.5x103KJ/kg) of the silage from Diet 1 to Diet 5(0-100% cocoa pod and cassava pulp). The highest value of crude protein (22.12%) was observed for Diet 5 (50-100% cocoa pod inclusion). Diet 5(50-100% cocoa pod) also had the lowest value for crude fiber (6.08%); fat (0.88%) and highest value for Calcium (35.81ppm) and calories values (11.50 x 103KJ/kg). This result showed that the combinations were a promising source of protein in animal diet if well processed. All the concentrations of anti-nutrients were found to be within acceptable levels for human and animal consumption.

Contents

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1. Introduction

Phytochemicals are metabolites found in plant-originated feeds such as trees, forages, grasses, agro-industrial by-products, etc. They are majorly responsible for health improvement in plant-based feeds apart from the supplement of minerals and vitamins [1]. They are regarded as anti-nutrients but have some nutritional, chemical, biological and pharmacological properties [2, 3]. To preserve some of these feeds and derive the benefits of phytochemicals in them, there is a need for ensiling which supports natural acid fermentation under anaerobic conditions [4]. Browse plants are known to have high potentials in feed resources for livestock especially during the gap periods due to their phytochemical's properties, vitamins and minerals supplement [5]. There is a need to sought out for browse plants that are nutritious and available throughout all the seasons [6]. Acacia leaves (Figure 1) are rich in protein (about 13-24%); low in Nutrient detergent fiber (NDF) and condensed tannins [7]. It is one of the upper potential degradability within 48hours and in vitro organic matter (OM) was just medium [8]. Most browse species such as Acacia leaf investigated showed possession of essential ingredients which recommended as valuable browse plants [9].

Figure 1 Acacia leaf

Many agricultural by-products such as cocoa pod (Figure 2), cassava pulp (Figure 3) and peel are normally used for all seasons because they are available at the time. These agricultural by-products were avoided by farmers due to their low nutritional quality which requires necessary preparation physically or chemically before animal feeding [10]. Cocoa pod had low crude proteins (0.31%); ash (1.48%); Ether extract (0.12%); high moisture content (80.06%) and carbohydrates (11.03%) in fresh weights [11, 12]. The fresh cocoa pod contained 0.40% theobromine which can be reduced or minimized during fermentation [13].

Figure 2 Cocoa pod

Figure 3 Cassava pulp

It had also been observed that a low quantity of *Theobromine* is found in the husks [14]. The cocoa pod also contained phenolic and caffeic acids [15]. Cassava (*Manihot esculenta*) is a dietary staple feeds in the tropics, it thrives and produces stable yields under unfavorable conditions which other plants cannot tolerate [16]. Cassava pulp contained crude protein (1.2-2.8%); Dry matter (15.8-23.4%); crude fiber (17.9-24.0%); fat (0.1-2.4%); ash (1.7-2.8%) and NFE (55.0-74.4%) on dry matter basis [17].

However, Aro [18] reported that fermentation increased the crude protein and reduced the crude fibre. It also helps to detoxify the toxic substances and improved the textures and flavours of the silage [19]. Sun [20] reported that the microorganisms typically associated with cassava fermentation are mainly acid bacteria. Thus, assessing the proximate and phytochemical properties of these agro by-products and browse plants is very important to determine

their nutritional significance. Likewise, to confirm their nutritional potency before introducing them as animal feeds.

2. Materials and Methods

2.1. Study Area

The research experimented at the Ruminant section of the Teaching and Research farm, and chemical analyses were done in the nutrition laboratory of the Animal production department, Landmark University, Omu Aran, Kwara State, Nigeria.

2.2. Experimental Design

A 5 × 6 full factorial design was adopted using cocoa pods and cassava pulp at (0, 25, 50, 75, and 100%) with the inclusion of Acacia leaf at 0,10, 20, 30, 40, and 50% levels (Table 1).

Table 1 Ensiled combinations of Cocoa based diets

	Diets (as Composition of Cassava pulp/Cocoa pod/Acacia leaf)				
	Diet 1	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**
(0)	100/0/0	75/25/0	50/50/0	25/75/0	0/100/0
(10)	90/0/10	67.5/22.5/10	45/45/10	22.5/67.5/10	0/90/10
(20)	80/0/20	60/20/20	40/40/20	20/60/20	0/80/20
(30)	70/0/30	52.5/17.5/30	35/35/30	17.5/52.5/30	0/70/30
(40)	60/0/40	45/15/40	30/30/40	15/45/40	0/60/40
(50)	50/0/50	37.5/12.5/50	25.0/25.0/50	12.5/37.5/50	0/50/50

2.3. Experimental-Procedure

2.3.1. Silage Preparation

Cocoa pods were gotten from a cocoa farm in Ekiti state, sundried for some days, grounded to small particle size. The cassava pulp was collected from cassava processing industries in Ondo state. The pulp was sundried for 5hrs to reduce the cyanide and moisture content. The Acacia leaves were also gotten from the pasture plant of the farm. The combinations were mixed together with fermentable carbohydrates (banana slurry) based on the graded levels of the experimental designs. Each group was ensiled in 200 liters capacity plastic drums under anaerobic conditions for 7 weeks (Figure 4) [21].

Figure 4 Ensiled cocoa pod, cassava pulp and Acacia leaf Diet

2.4. Data Collection

2.4.1. Proximate Analysis of Coco-based Diets

2.4.1.1. Moisture Content

This was determined by using a standard procedure of AOAC [22] which is based on the mass loss of the sample till it reached a constant mass of 100 °C

2.4.1.2. Ash Content

Ash content is determined by the method described by AOAC [22], which consists of burning the silage till white ashes are got in a muffle furnace under a temperature of 550°C.

2.4.1.3. Ether Extract

The ether extract was also determined by the method of AOAC [22] using the Soxhlet extractor. This was achieved after the evaporation of the solvent and the drying of the silage in the oven at a temperature of 105°C for 28mins. The differences in the weight of the samples provided the ether extract of the silage.

2.4.1.4. Crude Protein

This was determined by assaying total nitrogen using the Kjedahl method of AOAC [22]. This is achieved using Sulphuric mineralization, the nitrogen content gotten was multiplied by the conversion-coefficient of nitrogen into protein.

2.4.1.5. Crude Fiber

The crude fiber was determined by the method of AOAC [22] in a muffle furnace at 500°C for 3hrs.

2.4.1.6. Carbohydrate

This is done by the method described by AOAC [22].

2.4.1.7. Energy Value

The energy value of the silage is calculated with the coefficient of AOAC [22] for the proteins, lipids and carbohydrates.

2.5. Minerals

The minerals were determined by the method described by AOAC [22] which was measured out in the ash with electronic scanning using an absorption spectrophotometer to obtain the mineral contents.

2.6. Qualitative Determination of Phytochemical Analysis

2.6.1. Tannin Content

The assaying of tannins was done according to the method described by Trease [23]. The absorbance is read with a spectrophotometer at 500mm. The green color observed confirmed the presence of tannins in the samples.

2.6.2. Phlobatanins Content

This was also carried out according to the method described by Trease [23]. The red color confirmed the Phlobatannins presence.

2.6.3. Phenols Content

The phenols are determined according to the method described by Trease [23]. The blue- green color confirmed the presence of phenol in the silage.

2.6.4. Resin Content

This was also determined using the method of Trease [23]. The orange color confirmed the presence of Resin in the samples.

2.7. Statistical Analysis

The comparisons of the values of the physical-chemicals parameters are subjected to SPSS, version 20 [24]. The analysis of variance followed the Duncan multiple Range test and the significance of differences of average was obtained with a risk error of $p<0.05$.

3. Results

3.1. Physicochemical Values of Cocoa-based Silage

The study of the physico-chemical composition was determined in Table 2. The results revealed that fermentation increased crude protein, carbohydrate, calcium, phosphorus, potassium, butyric acids (BA), gallic acids (GA), and caloric values of the silage from Diet 1 to Diet 5 (0-100% cocoa pod and cassava pulp), while it decreased the moisture crude fiber, ash, lipid, moisture, Silicate, Acid detergent fibre (ADF), Acid detergent lipids (ADL), Neutral detergent acids, Hemicellulose and Cellulose from Diet 1 to Diet 5.

Table 2 Physico-chemical composition of Cocoa based silage per diet

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	P value
Crude protein (%)	15.90^{d}	16.52^{c}	16.76^{c}	19.18^{b}	22.12^{a}	<0.001
Carbohydrate (%)	45.26^{a}	40.78^{b}	38.06^{c}	26.13^{d}	16.37^{e}	<0.001
Crude Fibre (%)	6.21	6.13	6.12	6.09	6.08	0.396
Crude Lipids (%)	0.88	0.75	0.38	0.35	0.29	0.501
Moisture (%)	66.69^{a}	48.38^{b}	33.11^{c}	26.88^{c}	26.01^{c}	<0.001
Ash (%)	3.23	3.10	3.10	3.09	3.09	0.221
Silicate (%)	0.02	0.03	0.03	0.02	0.02	0.856
ADF (%)	0.47	0.50	0.45	0.41	0.39	0.993
ADL (%)	0.52	0.69	0.63	0.41	0.35	0.765
NDF (%)	1.19	0.92	0.89	0.79	0.73	0.573
Hemicellulose(%)	1.74	1.67	1.21	1.15	1.07	0.578
Cellulose (%)	1.11	1.16	1.01	0.95	0.89	0.984
Lignin (%)	1.63	2.54	2.21	2.09	1.93	0.392
BA (µg/100g)	2.79	2.59	2.82	2.94	3.11	0.984
GA (mg/kg)	0.11	0.01	0.01	0.02	0.03	0.452
Phosphorus (ppm)	1.18	1.14	1.14	1.14	1.16	0.658
Calcium (ppm)	26.55^{c}	26.09^{c}	26.94^{c}	31.31^{b}	35.81^{a}	<0.001
Potassium (ppm)	2.79	2.79	2.80	2.88	2.98	0.989
Calories (KJ/kg)	4.1^{e}x103	7.2^{d}x103	9.7^{bc}x103	10.9^{b}x103	11.5^{a}x103	<0.001

^{abcde} On the same line, the averages followed by different letters are significantly different ($p<0.05$);

Diet 1: 0% CoP; Diet 2 : 12.5%-25% CoP; Diet 3= 25%-50% CoP; Diet 4: 37.5%-75% CoP; Diet 50 : 50%-100% CoP

The mean crude protein, carbohydrate, crude fiber, lipid, moisture content, ash, silicate, hemicellulose, butyric acids, gallic acids, phosphorus, calcium, potassium and calories values ranges from 15.90 - 22.12%; 16.37 – 45.26%; 6.08 – 6.21%; 0.29 – 0.88%; 26.01 – 66.69%; 3.09 – 3.23%; 0.02 – 0.03%; 0.39 – 0.52%; 1.07 – 1.74%; 0.89 – 1.11%; 1.63 – 2.54%; 2.59 – 3.11(µg/100g); 0.01 – 0.11(mg/kg); 1.14 – 1.18ppm; 26.09 – 35.81ppm; 2.79 – 2.98ppm and $4.1x10^3$ to $11.5x10^3$(KJ/kg) respectively.

The highest value of crude protein (22.12%) was observed for Diet 5 (50-100% cocoa pod) while Diet 1 (50-100% cassava pulp) had the lowest value (6.37%) for carbohydrate. Diet 5(50-100% cocoa pod) also had the lowest value of 6.08% for crude fiber; a highest value of 0.88% for lipid; a highest value of 35.81ppm for Calcium and 11.50 x 10^3(KJ/kg) for calories values. So, fermentation had a significant ($p>0.05$) effect on crude fiber, lipid, ash, silicate, Hemicellulose, lignin, Gallic acid, Butyric acid, Phosphorus and Potassium on the combinations of the cocoa pod, cassava pulp and acacia leaf.

3.2. Phytochemical Contents in the Silage

Phytochemical's screening of tannins, phenol, Phlobatannins and resin are found in all the silage under study (Table 3). All the diets contained the phytochemicals mentioned except Diet 1_0; Diet 2_{10} and Diet 4_{50} which showed the absence of these secondary metabolites.

Table 3 Phytochemicals screening of Cocoa based Silage

Samples	Cocoa pod (%)	Tannins	Phenol	Phlobatannins	Resin
Diet1_0	0%	—	—	—	—
Diet2_0	25%	—	—	+	+
Diet3_0	50%	—	—	+	+
Diet4_0	75%	—	—	+	—
Diet5_0	100%	+	—	+	—
Diet1_{10}	0%	+	—	+	—
Diet2_{10}	22.5%	—	—	—	—
Diet3_{10}	45%	+	—	+	—
Diet4_{10}	67.5%	+	—	+	—
Diet5_{10}	90%	—	—	—	+
Diet1_{20}	0%	+	—	+	+
Diet2_{20}	20%	+	—	+	—
Diet3_{20}	40%	+	—	+	—
Diet4_{20}	60%	+	—	—	+
Diet5_{20}	80%	+	+	+	+
Diet1_{30}	0%	+	—	+	—
Diet2_{30}	17.5%	+	—	—	+
Diet3_{30}	35%	+	+	+	+
Diet4_{30}	52.5%	+	—	—	—
Diet5_{30}	70%	+	+	—	+
Diet1_{40}	0%	+	—	+	—
Diet2_{40}	15%	+	—	—	+
Diet3_{40}	30%	+	+	—	+
Diet4_{40}	45%	+	—	—	—
Diet5_{40}	60%	+	+	—	—
Diet1_{50}	0%	+	—	—	—
Diet2_{50}	12.5%	+	—	—	—
Diet3_{50}	25%	+	—	+	+
Diet4_{50}	37.5%	—	—	—	—
Diet5_{50}	50%	+	+	—	—

+ : present — : absent

4. Discussion

The increase in protein content after fermentation among the groups in this study corroborates the findings of Omotoso [25] who also reported the increase in crude protein values of fermented cocoa pod. The increase in crude protein is in agreement with the reports of Okoruwa [26] in a study of a mixture of groundnut shell and rice straw which found that the microbial fermentation increased the protein content of the mixtures. This also was similar to Ozung et al. [27] who had an increase in crude protein values for a fermented cocoa-pod. Likewise, the crude fiber content of the silage in this study declined from 6.21 to 6.08%. This agreed with the

report by Olugosi [28] who indicated a decline in cocoa pod fermentation. Moreover, the decline in moisture contents in this study was in agreement with Bwai, [29] who reported a reduction in moisture content when using Acacia leaf meal. Fermentation also increased the mineral contents of the silage which is in tandem with the reports of [29] who revealed the presence of some essential minerals when using Acacia leaf. The major minerals in these combinations are calcium, phosphorus, potassium as reported by Marcel [30] and Meffeja [31]. The phytochemical investigation likewise revealed tannin, phenol, Phlobatannins, and resin. This was similar to the reports of [29] who also indicated the essential phytochemicals present in his study. Tannins as one of the phytochemicals possess antiviral, anti-parasitic and antibacterial ability. From the study, it was observed that phytochemicals such as tannins decreased after fermentation. During fermentation, there is hydrolyzation of tannins due to its sensitivity to acidic conditions, there are certain amount of acid produced by acetic acid bacteria which lowered pH and reduced tannin contents; which is in tandem with the reports of Djali [32]. In addition, Martinez-Pinilla [33] confirmed that the existence of tannin is inversely proportional to the amount of theobromine in cocoa products. Yulistiani [34] also affirmed that fermentation reduced phytochemicals contents and increased digestibility of the protein contents in the diets. So, this silage is expected to have many medicinal uses.

5. Conclusion

The Physico-chemical composition had shown that this silage has essential nutritional composition. They contained a significant quantity of proteins, minerals, fiber, and lipid contents. The presence of phytochemicals in the silage and lower moisture content provided the capacity to be preserved for a long period time. Fermentation had helped in the detoxification of toxic substances in the silage. These diets have helped to limit the anti-nutritional substances and improved the availability of protein and minerals in the silage.

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Ethics approval

The authors confirm that the ethical policies of the journal, as noted on the journal authors guidelines have been adhered to. Approval to carry out the research and use of the animal was obtained from the ethics committee of the University of Ilorin, Kwara State, Nigeria.

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Declaration of Conflict of Interest

Authors declare no conflict of interest.

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