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NUTRIENT VALUE AND *IN VITRO* GAS PRODUCTION OF AFRICAN WILD COCOYAM (*COLOCASIA ESCULENTUM*)

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ABSTRACT

Cost of feeding conventional feedstuffs to ruminants in Nigeria is prohibitive. African wild cocoyam (*Colocasia esculentum)* is an unconventional energy feed source and non human edible food. The study determined the chemical composition and energy content, secondary metabolites and *in vitro* gas production characteristics of the African wild cocoyam (AWC). There were two main treatments which comprised soaking in boiled water or cold water for 0 (raw as control) 3, 6 and 9 days. Dry matter (DM) of boiled water treated wild cocoyam (BWTAWC) and DM in cold water treated wild cocoyam (CWTAWC) decreased from 64 % to 43 % in 9 day BWTAWC and 64 % to 52 % in 9 day CWTAWC. Crude fibre, ether extract, neutral detergent fibre and gross energy contents decreased with increasing days of soaking and ranged from 11 – 21 % (BWTAWC) and 18 – 21 % (CWTAWC), 3.9 - 6.5 % (BWTAWC) and 5 - 6.5 % (CWTAWC), 32.5 - 56.1 % (BWTAWC) and 38 - 56.1 % (CWTAWC) and 3.66 - 4.29 % (BWTAWC) and 4.10 - 4.29 % (CWTAWC), respectively. Method of soaking and length of soaking marginally increased contents of crude protein (range = 4.7 - 5.8 %) and ash (range = 8 – 13 %). Secondary metabolites present were saponin and steroids, with no tannin. Medium saponin content was detected and it decreased by soaking method and duration of soaking. There were significant ($P < 0.05$) differences in the metabolisable energy (ME) (Range = 4.33 - 9.62 MJ/Kg DM and 4.33 - 8.59 MJ/Kg DM for CWTAWC and BWTAWC respectively), organic matter digestibility (OMD) (Range = 32.26 - 56.6 % and 32.26 - 43.55 % for CWTAWC and BWTAWC, respectively) and short chain fatty acids (Range 0.65 - 0.97 for both CWTAWC and BWTAWC) among the treatment means. Methane production increased with increasing days of soaking in water but were not significantly ($P > 0.05$) different. Results showed that with high energy value, medium saponin, enhanced organic matter digestibility and relatively low methane production, African wild cocoyam has potential to be used as energy feed for ruminants. Treatment by soaking in water reduced the secondary metabolites and, therefore, safe for livestock feeding.

Key words: Cocoyam, nutrients, metabolites, gas production.

INTRODUCTION

Energy and protein are vital nutrients for ruminant productivity and the two contribute more than three quarters of the total cost of formulated feed in Nigeria [1]. Unconventional protein sources from browse trees, legumes and seeds of trees were reported to be sustainable for ruminants in the tropics [2, 3, 4]. These feed resources are rich in protein but low in energy for high performance ruminants [5]. Cereal (maize, wheat and sorghum) and root crops (cassava and potato) are also staple food for the average Nigerians. Because of the keen competition between man and livestock for these foods, the cost of procurement becomes exorbitant. As a result of this, what many of the livestock farmers do is to alternatively feed their animals with agro-industrial by-products (AIBs) [6]. The AIBs are of low nutritive value and some of them contain anti-nutritional factors [7]. The cost is becoming prohibitive due to increase in ruminant population in the recent times. The AIBs are now being biodegraded and therefore used extensively for other classes of livestock and poultry. There are a number of unconventional feed resources such as the African wild cocoyam yet to be fully explored for ruminant production.

The African wild cocoyam (*Colocasia esculentum*) can be fed to ruminants as a source of energy. Presently, it is a non human edible plant that grows along river banks, lakes, streams, brooks, oases and shady areas in Nigeria. Unlike the edible cocoyam, it has a prominent and non-hardy cocoyam corm. It is self sustaining as it has broader leaves, which enable it to suppress other weeds under and around it. There is paucity of information on the utilization of African wild cocoyam. One of the species of African wild cocoyam (*Caladium bicolor*) was described as ornamental and perennial and of dual purpose as additive for polymer [8]. Literature is scanty on the nutrient composition and potential of *Colocasia esculentum* for livestock production and management. An undocumented information showed that the plant is not usually consumed by wild animals and that it causes skin and throat irritation in human beings. This may, therefore, suggest the presence of anti-nutritional factors in the plant. Such secondary metabolites in the wild cocoyam could be detrimental or beneficial to ruminants. Since the feedstuff is relatively novel as feed for animals, there is a need for its proper and complete nutritional evaluation, to ascertain its usefulness for animal feeding. Qualitative method is a spot technique [3] to determine the presence of secondary metabolites such as saponin, hydrolysable and condensed tannin, alkaloids and steroids in plants. An insight into the presence of specific secondary metabolite is of particular interest when formulating ruminant diets. It was established that mutual reaction of metabolites might provoke significant modification to their behaviour in the digestive tract of the ruminant and invariably transform the nutritive value of the feedstuff [3]. *In vitro* gas production technique using syringes is quick and where sophisticated equipment is lacking, it is a cheaper means of evaluating the nutritive value of feedstuffs for ruminants [9]. The method has been used for the estimation of the direct and the indirect gas production in syringes for the determination of short chain fatty acids [10]. The present study was undertaken to determine the proximate composition, the toxic substances and *in vitro* gas production characteristics of African wild cocoyam as an unconventional feedstuff for ruminants.

MATERIALS AND METHODS

Site of the study

The African wild cocoyam was obtained along a stream within the University of Ibadan campus, Ibadan, Nigeria. The location of the study is 7º27'N and 3º45'E at altitude 200 - 300 m above sea level; mean temperature of 25 - 29 ºC and the average annual rainfall of about 1250 mm.

Processing and treatment

The corm of the wild cocoyam was harvested in the month of February, 2006. Since it was dug up from muddy water, the corm was thoroughly washed. The cocoyam was then sliced into size of 100 - 150 g pieces and without peeling, using sharp knife. The sliced corm was divided into two sets. Each set was subdivided into three and each was replicated five times with 4 kg of the material in a covered plastic container. The first sets in different containers were immersed in boiled water of 100 °C while the second group was immersed in the normal cold water. The two sets were left soaking for 0 (control), 3, 6 and 9 days. Soaking was terminated consecutively by withdrawing the samples from the water. Representative samples for further laboratory analysis were drained for two hours in four layer cheese cloths to ensure that the external moisture was not measured with the imbibed water. Thereafter, a known weight was taken for oven drying at 105 °C for the determination of dry matter.

Secondary metabolites

Qualitative analysis for saponin, phenols and steroids was according to Babayemi *et al*. [7]. Briefly, two g of the cocoyam samples were extracted with 30 ml of petroleum ether and 25 ml of methanol water (9/1, v/v). The mixture was made to shake for 1½ hours at 250 revolutions per minute, filtered, rinsed and separated into 50 ml volumetric flasks. From the methanol water fraction, 1.67 ml was dispensed in 9 ml distilled water and from it 1 ml was taken into a test tube. The test tube was shaken for 30 seconds and left to stand for 15 minutes. Saponin content was evaluated from the height of the foam layer as negative (< 5 mm), low (5 – 9 mm), medium (10 – 14 mm) and high (> 15 mm). For phenol analysis, 1 ml from the methanol water fraction was dispensed into a test tube with 1 % ferric chloride ($FeCl_3$) (w/v) added at 1 ml. Phenols form complexes with ferric iron, resulting in a blue solution and hence, their presence was scored as: no phenols (no visible colour change), hydrolysable (dark blue) and condensed tannins (dark green). For steroids, 10 ml from petroleum ether fraction was evaporated in a water bath at 45 ºC and 0.5 ml chloroform, 0.25 ml acetic anhydride and 0.125 ml concentrated tetra oxo sulphate six acid (H_2SO_4) were added. The mixture was agitated briefly while the colour reaction was then accessed, being steroids (blue or green), triterpenoids (read, pink or purple) or saturated steroids (light yellow).

In vitro gas production

Rumen fluid was obtained from three multiparous West African dwarf goats through suction tube before morning feeding. The goats were previously fed 60 % concentrate (40 % corn, 10 % wheat offal, 10 % palm kernel cake, 20 % groundnut cake, 5 % soybean meal,10% dried brewers grain, 1 % common salt, 3.75 % oyster shell and 0.25 % fish meal) and 40 % Guinea grass (*Panicum maximum*). The incubation procedure was as reported [10]. The 120 ml calibrated syringes fitted with silicon tube at the mouth were used while the incubation was in three batch incubation. The incubation temperature was maintained at 39 ± 1 ºC. The buffer containing $NaHCO_3$ + Na_2HPO_4 + KCl + NaCl + $MgSO_4.7H_2O$ + $CaCl_2.2H_2O$ was used and kept in the incubator for warming. About 200 mg of the feed sample (substrate) was measured and introduced into the syringe after removing the plunger. The plunger was returned by pushing the substrate upward the syringe. The rumen liquor was strained through a four layer cheese cloth. Rumen liquor and buffer were mixed together (1:4, v/v) as inoculums, all under continuous flushing with streams of CO_2. Using 50 ml capacity syringe, 30 ml of inoculums was dispensed into the substrate through the silicon tube. The plunger was pushed upward by pushing the inoculums to the tip of the syringe. Thereafter, the silicon was tightened with a metal clip. The gas production was measured on the calibration of the syringe at 3, 6, 9, 12, 15, 18, 21 and 24 hour. In order to estimate methane production by the substrate and immediately after evacuation from the incubator, 4 ml of NaOH (10 M) was introduced using 5 ml capacity syringe. The content was inserted into the silicon tube, which was fastened to the 120 ml capacity syringe. The clip was then opened while the NaOH was gradually released. The content was agitated while the plunger began to shift position to occupy the vacuum created by the absorption of CO_2. The volume of methane was read on the calibration. To determine the actual gas produced, the average of the volume of gas produced from the blanks was deducted from the volume of gas produced per sample.

Chemical and energy analysis

Crude protein, crude fibre, ether extract and ash contents of the samples were carried out in triplicates as described [11] and the amount of CP was calculated (N x 6.25). Neutral detergent fibre was assessed according to Van Soest [12]. Energy content was analysed as gross energy, which was evaluated by means of the Parr adiabatic bomb calorimetry.

Statistical analysis

Metabolisable energy (ME) was calculated as ME= 2.20 + 0.136GV + 0.057 CP + 0.0029 CF [10]. Organic matter digestibility (OMD %) was assessed as OMD = 14.88 + 0.889 GV + 0.45 CP + 0.651 XA [10]. Short chain fatty acids (SCFA) as 0.0239 GV – 0.0601 [13] was also obtained, 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 in a 2 x 4 x 3 factorial arrangement. Where significant differences occurred, the means were separated using Duncan multiple range F-test of the SAS [14] options.

RESULTS

Chemical composition

The chemical composition of the boiled water and cold water treated African wild cocoyam (AWC) is presented in Table 1. Dry matter, crude protein, crude fibre, ether extract, neutral detergent fibre and gross energy contents were affected by the treatments imposed on the wild cocoyam. Dry matter in the boiled water treated wild cocoyam (BWTAWC) and cold water treated wild cocoyam (CWTAWC) decreased progressively from 64 % (control) to 43 % (9 days soaking) in BWTAWC and from 64 % (control) to 52 % in CWTAWC. Crude fibre, ether extract, neutral detergent fibre and gross energy constituents followed similar trends. Crude protein and ash components were enhanced marginally. Crude protein in the 6 and 9 days soaking after BWTAWC was increased by 0.3 % and 0.6 %, respectively while it increased in the 3, 6 and 9 days soaking after CWTAWC by 0.1 %, 0.5 % and 1.1 %, respectively.

Secondary metabolites

Table 2 is showing the secondary metabolites of the boiled water and cold water treated wild cocoyam. The qualitatively determined secondary metabolites were saponin, phenols (condensed and hydrolysable tannins) and steroids. Saponin and steroids were detected in the wild cocoyam and no presence of phenol was noticed. Saponin content was medium (11 mm) in untreated sample and decreased to negligible (1 mm) amount after BWTAWC. The effect of cold water treatment was not immediate on the cocoyam. After 3 days on CWTAWC, the amount of saponin was still medium but decreased geometrically to 1.5 mm after 9 days soaking.

In vitro gas production characteristics

Figures 1 and 2 shows the *in vitro* gas fermentation of CWTAWC and BWTAWC, respectively. Net gas production was improved upon in both CWTAWC and BWTAWC. Gas production increased with increasing day of soaking after either with CWTAWC or BWTAWC. It was, however, observed that gas production patterns of CWTAWC and BWTAWC were the same for control and 3 day soaking.

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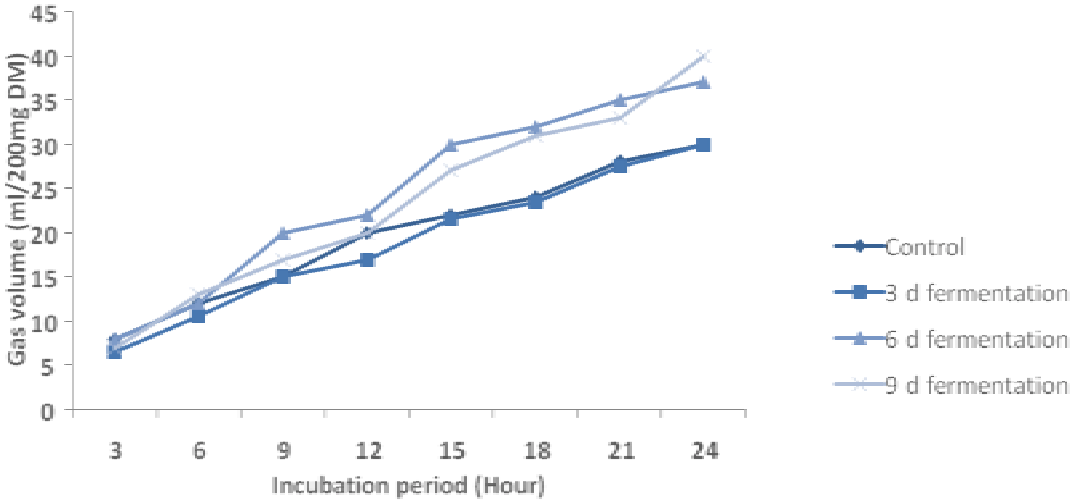

Fig. 1: In vitro gas production pattern of cold water treated wild cocoyam

Metabolisable energy (ME), organic matter digestibility (OMD), short chain fatty acids (SCFA) and methane of fresh and CWTAWC are in Table 3. The ME ranged between 4.33 MJ/Kg DM in untreated and 9.62 MJ/Kg DM in the 9 day soaking. There were significant ($P < 0.05$) differences in the ME among the treatment means. The CWTAWC were significantly ($P < 0.05$) higher in ME than in the control. The ME in the 6 and 9 day soaking of the CWTAWC was similar ($P > 0.05$) but both were significantly ($P < 0.05$) enhanced than that of the control. The OMD ranged from 43.6 % in control to 56.6 % in the 9 day soaked with cold water. The OMD increased significantly ($P < 0.05$) with increasing day of soaking. There was no apparent ($P > 0.05$) difference in the ME of control and the 3 day fermented cocoyam but significantly ($P < 0.05$) lower than those of the 6 and 9 day soaking. The highest OMD was obtained in the 9 day soaked CWTAWC. The highest (0.97) and lowest (0.65) SCFA were found in the 9 day CWTAWC and control respectively. Methane production ranged from 15 ml in the control to 20.3 ml in the 9 day CWTAWC. There were no significant ($P > 0.05$) differences between the control and the treatment means. Similar observations were noticed for the cocoyam treated with boiled water (Table 4), although with lower values when compared to the cold water treated cocoyam.

DISCUSSION

The content of crude protein in the treated and the untreated wild cocoyam was lower than 7 % CP recommended, being minimum requirement for ruminants in the tropics [15]. The objective of the present study was not intended to enhance the crude protein but in one way to depress the possible presence of toxic factors in the wild cocoyam so as to make it a readily available feedstuff for ruminant feeding without any deleterious effects. Cocoyam is not a leguminous crop and therefore, high protein was

not expected since it belongs to the class of root and tuber crops. The amount of protein in the present study was higher than 2 % CP in cassava peels [16]. The amount of energy observed in the wild cocoyam was comparable to the conventional maize and expensive ago-industrial waste (palm kernel cake).

The fact that saponin and steroids were detected in cocoyam was not surprising as most of the tropical feedstuffs always contain one anti-nutritional factor or the other [2, 3]. A medium content of saponin as it was qualitatively determined in the cocoyam was advantageous in the nutrition of ruminants. Saponin was established to reduce bacteria population in the rumen [17], thereby reducing the amount of methane production [18]. The effect of treatment was evident in the reduction of saponin content. Boiling process drastically reduced the saponin than soaking in ordinary water. Ordinary water may be recommended as a treatment to reduce the toxic factors, given that it is easy to obtain and does not involve technicality.

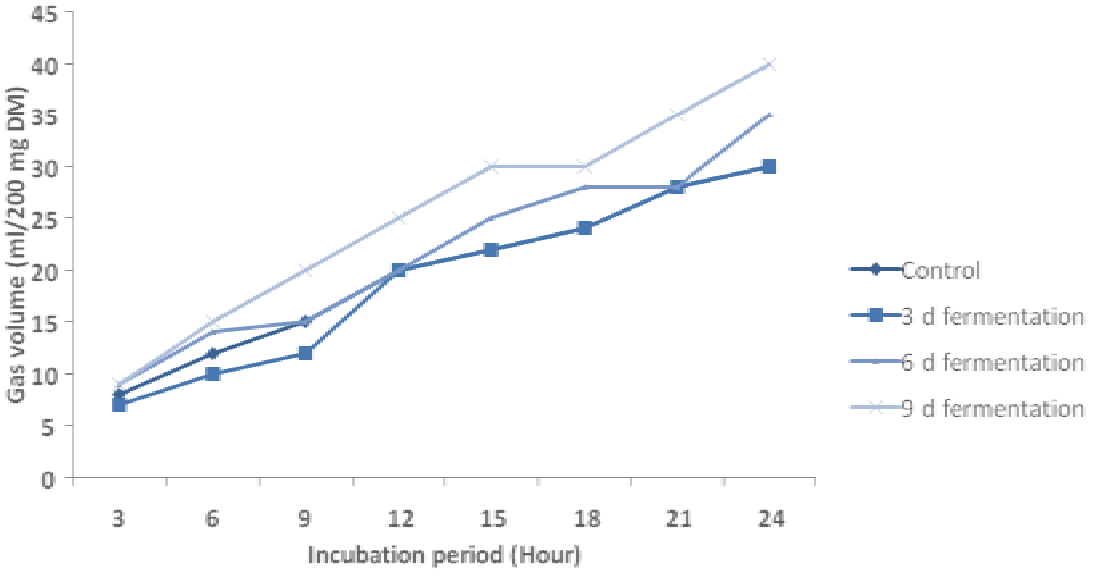

Fig. 2: In vitro gas production pattern of boiled water cocoyam

Net gas production was lowest in the untreated but was improved in both hot and cold water treated wild cocoyam (Figures 1 and 2), suggesting the effect of the anti-nutritional factors. Although gases produced during rumen soaking are waste products and of no nutritive value to the ruminant, gas production tests are used routinely in feed research as gas volumes are related to both extent and rate of substrate degradation [19]. The increase in the metabolizable energy (ME), organic matter digestibility (OMD) and short chain fatty acids (SCFA) might be due to the overwhelming effect of the toxic factors that had been removed by the treatment. The values of ME, OMD and SCFA in the present study were lower than those obtained for the different parts of *Enterolobium cyclocarpum* [20] but higher than those of tea and spent tea leaf [7] and comparable to that of the mixtures of *Tephrosia candida* and Guinea grass [4]. Although treatment had no effect on the methane production, the

trend showed that the amount of methane increased with increasing days of soaking. From the present study, It can be inferred that the lower the content of saponin in the wild cocoyam sample, the higher the methane production. This was in consonance with earlier reports [4]. Saponin reduced methanogenesis, potentially acting as defaunating agent [17] against some microorganisms in the rumen. Since a medium amount of saponin was present in the raw form of the cocoyam, it may be beneficial for ruminants without subjection to any further processing.

CONCLUSION

Proximate composition, energy content and metabolisable energy through *in vitro* gas production show that the wild cocoyam might be useful energy supplements in ruminant feeding. Raw and processed samples further indicate the presence of saponin which showed the ability to reduce rumen methane. The study also provides an insight into the total short chain fatty acids (SCFA) being potent in the wild cocoyam. The limitation of this study was that the technique does not permit accurate estimation of the proportion of individual SCFA.

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Table 1: Proximate composition (g/100g DM) of boiled and cold water treated African wild cocoyam (n=5)

Component	Treatment (T)	Period (P) 0 day	3 days	6 days	9 days	SEM	Significance T	P	T×P
Dry matter	Hot water	64.00^{1}_{a}	56.00^{2}_{a}	49.00^{3}_{b}	43.00^{4}_{b}	1.23	***	***	**
	Cold water	64.00^{1}_{a}	60.00^{2}_{a}	57.00^{2}_{a}	52.00^{3}_{a}	1.15			
	SEM	1.15	1.15	0.91	1.47				
Crude protein	Hot water	4.70^{2}_{a}	4.70^{2}_{a}	$5.00^{1,2}_{a}$	5.30^{1}_{a}	0.15	NS	***	NS
	Cold water	4.70^{2}_{a}	4.80^{2}_{a}	5.00^{2}_{a}	5.80^{1}_{a}	0.17			
	SEM	0.17	1.15	0.12	0.20				
Crude fibre	Hot water	20.67^{1}_{a}	14.00^{2}_{b}	13.00^{2}_{b}	11.00^{3}_{b}	0.51	***	***	***
	Cold water	20.67^{1}_{a}	20.87^{1}_{a}	18.50^{2}_{a}	18.20^{2}_{a}	0.47			
	SEM	0.88	0.26	0.29	0.21				
Ether extract	Hot water	6.50^{1}_{a}	4.90^{2}_{b}	$4.20^{2,3}_{b}$	3.93^{3}_{b}	0.23	***	***	*
	Cold water	6.50^{1}_{a}	$6.10^{1,2}_{a}$	5.60^{2}_{a}	5.00^{3}_{a}	0.18			
	SEM	0.23	0.21	0.18	0.20				
NDF	Hot water	56.10^{1}_{a}	53.50^{2}_{a}	42.00^{3}_{a}	32.50^{4}_{b}	0.52	*	***	***
	Cold water	56.10^{1}_{a}	43.50^{2}_{b}	43.50^{2}_{a}	38.00^{3}_{a}	0.93			
	SEM	0.46	0.58	0.44	1.24				
Ash	Hot water	9.00^{3}_{a}	13.00^{1}_{a}	$12.00^{1,2}_{a}$	11.00^{2}_{a}	0.35	***	***	***
	Cold water	9.00^{3}_{a}	12.00^{1}_{a}	10.00^{2}_{b}	8.00^{4}_{b}	0.20			
	SEM	0.28	1.73	0.20	0.21				
Gross energy (mcal/kg)	Hot water	4.28^{1}_{a}	4.11^{1}_{a}	3.85^{1}_{b}	3.66^{1}_{a}	0.18			
	Cold water	4.28^{1}_{a}	4.23^{1}_{a}	$4.15^{1,2}_{a}$	4.03^{2}_{a}	0.05	*	*	NS
	SEM	0.04	0.05	0.06	0.04				

Column (a,b) and row (1,2,3,4) means with common scripts for each variable do not differ significantly (P > 0.05). *P < 0.05, **P < 0.01, ***P < 0.001, NS = Not significant. SEM = Standard error of the mean difference. NDF = Neutral detergent fibre.

Table 2: Saponin, phenols and steroids of boiled water and cold water treated African wild cocoyam (n=5) as feedstuff for ruminants

Treatment	Saponin		Phenols	Steroids	
	Foam height	Comment		Colour	Comment
Control	11 mm	medium	negative	Green	steroids
Cold water					
3 days	6 mm	Medium	negative	Green	steroids
6 days	2.5 mm	Negligible	negative	Green	steroids
9 days	1.5 mm	Negligible	negative	Green	steroids
Hot water					
3 days	1.5 mm	Negligible	negative	Green	steroids
6 days	1 mm	Negligible	negative	Green	steroids
9 days	1 mm	Negligible	negative	Green	steroids

Table 3: Metabolisable energy (ME, MJ/kg), organic matter digestibility (OMD, %), short chain fatty acids (SCFA, µmol) and methane (CH_4, ml) of boiled and cold water treated African wild cocoyam (n=5).

Parameter	Treatment (T)	Period (P) 0 day	3 days	6 days	9 days	SEM	Significance T	P	T×P
ME	Hot water	4.33^{3}_{a}	7.40^{2}_{a}	7.78^{2}_{a}	8.59^{1}_{b}	0.13	*	***	***
	Cold water	4.33^{3}_{a}	7.04^{2}_{b}	7.35^{2}_{b}	9.62^{1}_{a}	0.09			
	SEM	0.19	0.08	0.08	0.04				
OMD	Hot water	32.26^{4}_{a}	35.11^{3}_{b}	40.28^{2}_{b}	43.55^{1}_{b}	0.59	***	***	***
	Cold water	32.26^{4}_{a}	44.30^{3}_{a}	49.90^{2}_{a}	56.60^{1}_{a}	0.61			
	SEM	1.23	0.13	0.25	0.12				
SCFA	Hot water	0.65^{3}_{a}	0.77^{2}_{a}	0.78^{2}_{a}	0.97^{1}_{a}	0.02	NS	***	*
	Cold water	0.65^{3}_{a}	0.68^{3}_{b}	0.79^{2}_{a}	0.97^{1}_{a}	0.02			
	SEM	0.03	0.02	0.02	0.01				
CH_4	Hot water	15.00^{3}_{a}	15.00^{3}_{b}	17.50^{2}_{b}	22.50^{1}_{a}	0.38	*	***	***
	Cold water	15.00^{4}_{a}	17.50^{3}_{a}	18.9^{2}_{a}	20.30^{1}_{b}	0.34			
	SEM	0.57	0.29	0.21	0.24				

Column (a, b) and row (1,2,3,4) means with common scripts for each variable do not differ significantly (P > 0.05). *P < 0.05, ***P < 0.001, NS = Not significant. SEM = Standard error of the mean difference.

Table 4: Metabolisable energy (ME) (MJ/Kg DM), organic matter digestibility (OMD) (%), short chain fatty acids (SCFA) (µmol) and methane (CH_4) (ml) of boiled water treated African wild cocoyam (n=5) as feedstuff for ruminants

	In vitro fermentation parameters			
Treatment	ME	OMD	SCFA	CH_4
Control	4.33[c]	32.26[a]	0.65[a]	15.0
3 days	7.4[b]	35.11[a]	0.77[b]	15.0
6 days	7.78[ab]	40.7[a]	0.78[ab]	17.5
9 days	8.59[a]	43.55[a]	0.97[a]	22.5
SEM	0.26	3.14	0.06	2.28

[a,b,c] Means in the same column with the same superscripts are not significantly ($P < 0.05$) different

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