



Influence of supplementing Guinea grass with differently processed African yam bean on gas production and *in vitro* digestibility

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ABSTRACT - This study evaluated the potential utilisation of African yam bean (AYB) seed as a supplement to Guinea grass on *in vitro* gas and methane (CH₄) production, as well as the effect of processing on AYB nutritive value. In experiment 1, unprocessed AYB meal at 10, 15, 20, and 25% inclusion levels was added to Guinea grass substrate and evaluated for *in vitro* gas production, CH₄, and *in vitro* organic matter digestibility (IVOMD). In experiment 2, the effect of soaked, boiled, toasted, and fermented AYB meal at 20% inclusion on *in vitro* fermentation was evaluated. *In vitro* gas production as well as *in vitro* organic matter digestibility of Guinea grass increased with AYB supplementation. The associative effect of Guinea grass with AYB showed an increase in gas and CH₄ production. At 20% inclusion level, AYB processing methods did not affect the gas production, CH₄, and IVOMD of the substrate. Fermentation improved the crude protein, iron, and zinc contents, reduced acid detergent fibre concentration but did not reduce the concentrations of alkaloid, total phenol, saponin, and trypsin inhibitors of AYB. Seed soaking for 48 h reduced the total phenol, tannin, oxalate, and phytate content, while seed boiling reduced the concentrations of alkaloid, total phenol, tannin, and trypsin inhibitors. Compared with the raw AYB, boiling is the most effective processing method to reduce the investigated phytochemicals, followed by soaking, toasting, and fermentation, in that order.

Keywords: legume, methane, protein, ruminal fermentation, tannin, underutilised feed

1. Introduction

The utilisation of few crop species for the production of most of the world's food supply has been noted as a limitation due to excessive homogeneity, oversimplification of farming systems, and disruptions of the ecological balance (FAO, 2015). This has serious negative implications on sustainable agriculture such as decreased resilience to climate change impacts, thus necessitating crop diversification (Bhartiya et al., 2015). Restrictions to the feeding of genetically modified soybean to livestock across the European Union has also heightened the demand for alternative vegetable protein sources (Nábrádi and Popp, 2011; Johnston et al., 2019), and increased production of tropical legume seeds could help meet this demand aside from the reduction in synthetic fertilizer use, which could guarantee a more sustainable farming system (Sinclair and Vadez, 2012; FAO, 2015). Improved utilisation of tropical legume seeds also offers opportunities to further develop the crop-livestock production system.

African yam bean is an underutilized legume that produces two harvestable parts: edible seeds and tubers. Despite the good nutritional potential that is comparable to pigeon pea and cowpea, poor awareness and the presence of phytochemicals, often referred to as anti-nutritional factors, continue to limit its utilisation as food and animal feed (Uguru and Madukaife, 2001; Baiyeri et al., 2018).

Some of these phytochemicals, such as phytate, saponins, oxalate, and tannins, have been reported to reduce feed intake and nutrient absorption, besides being toxic at high concentrations (Campos-Vega et al., 2009; Laleg et al., 2016). However, these compounds are reported to exert strong antibacterial effects and hence, may be useful in modulating rumen fermentation such as in mitigating rumen methane (CH_4) production (Gerber et al., 2013; Adejoro et al., 2019). This has, therefore, generated renewed interest in their exploitation for sustainable livestock production (Bhartiya et al., 2015; Adejoro et al., 2019). However, the concentration of these phytochemicals can be significantly reduced by seed processing methods (Torres et al., 2006; Laleg et al., 2016), and this could change rumen fermentation parameters.

We hypothesise that different processing methods could affect the feeding value of AYB as a supplement to grass forage in terms of gas and CH_4 production and organic matter digestibility. Therefore, the objectives of this study were to evaluate the effect of raw and processed AYB meal in Guinea grass substrate on *in vitro* gas and CH_4 production and organic matter digestibility and evaluate the effect of processing methods on the chemical composition of AYB meal.

2. Material and Methods

The study followed the guidelines for the use and conduct of experiments with live animals and was approved by the Animal Ethics Committee with approval number TETFund/2016-2017/BATCH11/No3.

African yam bean seeds were purchased from the local market in Ikole Ekiti, Ekiti State, Nigeria. The seeds were cleaned manually by physical selection to eliminate dirt and shrivelled or damaged seeds before processing. The AYB seeds were processed in a laboratory in Ikole Ekiti, Ekiti State, Nigeria (7.7983° N, 5.5145° E, 560 m above sea level) in April 2019.

The procedure by Obatolu et al. (2007) was followed in seed processing. From the same batch of AYB seeds, different samples were prepared using soaking, boiling, fermentation, or toasting as processing methods. A seed sample was soaked in distilled water (1:10, w v⁻¹) for 24 and 48 h at room temperature, drained and dried in a forced-air oven at 60 °C as soaked AYB. A weighted sample of the AYB seeds was boiled in hot water (100 °C) for 60 min, drained, and dried in a forced-air oven. A sample of AYB was toasted at 120 °C on a hot plate using an open pan for about 15 min under continuous stirring to prevent charring of the seeds and ensure uniform toasting. On observing light brown colouration – an indication of good toast –, the seeds were removed from the heat and cooled to room temperature, and few charred seeds were removed by handpicking before storage. Weighted AYB seeds were also soaked in distilled water for 24 h, the water was drained, and the seeds were spread in a covered plastic container lined with dried jute bags. The container was filled with seeds to a depth of 7 cm, airtight-sealed, and placed in a warm (34±2 °C) place for 96 h to create the appropriate condition for fermentation. The fermented seed samples were opened and oven-dried at 60 °C for 18 h. All processed seeds were thereafter milled through 1-mm sieve and stored in a cool dry place.

In two separate experiments, graded levels of raw AYB seeds and differently processed AYB seed meals were evaluated as supplements along with Guinea grass as main roughage. The Guinea grass (*Megathyrsus maximus* Jacq.) was from an eight-week old re-growth and presented: crude protein (CP), 86.2 g kg⁻¹; neutral detergent fibre (NDF), 544.8 g kg⁻¹; acid detergent fibre (ADF), 458.3 g kg⁻¹; and acid detergent lignin (ADL), 86.4 g kg⁻¹.

Four replicate bottles per treatment were incubated, and each treatment was repeated in three incubation runs. In the first experiment, the treatments were arranged as grass only, grass + AYB meal (90:10, w w⁻¹), grass+ AYB meal (85:15, w w⁻¹), grass+ AYB meal (80:20, w w⁻¹), grass + AYB meal (75:25, w w⁻¹), and AYB meal only. In the second experiment, the effect of supplementing soaked, boiled, toasted, or fermented AYB seed meal with guinea grass was evaluated at grass + AYB meal

(80:20, w w⁻¹). The treatments included grass + soaked AYB meal₂₄ (AYB soaked for 24 h), grass + soaked AYB meal₄₈ (AYB soaked for 48 h), grass + boiled AYB meal (AYB boiled in hot water for 60 min), grass + toasted AYB meal, and grass + fermented AYB meal. In both experiments, 400 mg of diet (grass or grass plus AYB meal) was incubated with 40 mL of rumen liquor. The *in vitro* fermentation procedure of Menke et al. (1979) as modified by Theodorou et al. (1994) was followed. We used rumen liquor from four cannulated beef steers that were fed *Eragrostis* and Lucerne hay *ad libitum*. Feed samples were incubated in buffered rumen fluid, and gas production was measured at 3, 6, 12, 24, and 48 h (Adejoro and Hassen, 2018) using a pressure transducer (PX4200-015GI, Omega Engineering Inc.) and a data tracker (Tracker 220 series indicators; Omega Engineering Inc., Laval, QC, Canada). Sample of gas at each incubation sampling time was taken using a syringe and stored in vacuum glass vials. Gas samples were analysed for CH₄ in a gas chromatograph (8610C BTU Gas Analyser GC System, SRI Instruments GmbH, Bad Honnef, Germany), pre-equipped with a flame ionisation detector.

The *in vitro* organic matter digestibility (IVOMD) of AYB as affected by processing method was determined in the two incubation experiments using the two-phase digestion method of Tilley and Terry (1963) with details described in Adejoro and Hassen (2018). During the first stage, 200 mg of feed samples were incubated in four replicates of each diet with rumen liquor for 48 h at 39 °C under anaerobic conditions. Blanks and a standard substrate were included in each batch. This was followed by an acid-pepsin digestion phase at 39 °C for 48 h. The residual material was oven-dried at 105 °C for 18 h, weighed, and subsequently ashed in a muffle furnace at 550 °C for 3h. Digested *in vitro* organic matter was estimated from the weights of starting material and residuals.

Samples of raw, soaked, toasted, boiled, or fermented AYB seeds and Guinea grass were analysed according to AOAC (2000) for dry matter (DM; ID 934.01), total ash (ID 942.05), CP (ID 968.06), ether extract (ID 934.01), and starch (ID 934.01). Crude fibre (CF) and NDF were determined using the Fibertec system technique as described by Van Soest et al. (1991), with the addition of heat-stable alpha-amylase and sodium sulphite, while ADF and ADL were also analysed (non-sequential) using the Fibertec system procedure. Both NDF and ADF were expressed exclusive of residual ash. Individual amino acid concentration of raw and processed AYB was analysed according to the methods of Benitez et al. (1989) using the PTH Amino acid analyser (120A PTH, Applied Biosystems Inc., CA. 94404, USA). Sodium and potassium were determined using a flame photometer (Coming, 403, UK), while other minerals were analysed using atomic absorption spectrophotometer (Perkin-Elmer model 403, walk CT, USA). All determinations were performed in duplicate. Total phenol and total tannins were determined by the by Folin-Ciocalteu spectrophotometric method using tannic acid as standard (Porter et al., 1986). The quantitative evaluation of trypsin inhibitors and phytate was determined according to the procedure described by Mbithi-Mwikya et al. (2000), while alkaloid, oxalate, and saponin contents were equally determined using standard procedures (Adejoro et al., 2013).

Samples were analysed in duplicates in three repetitions for each processing method. For the *in vitro* gas production, the four bottles for each treatment in each incubation run served as analytical replicates, and each repeat incubation run served as a statistical replicate, making a total of 12 replicates per treatment. The associative effect between Guinea grass and AYB seed supplementation on fermentation parameters was calculated as percentage difference between the values measured for each inclusion level and the balanced median values of their components as follows:

$$\% \text{ difference} = 100 \times [(\text{observed value} - \text{calculated value}) / \text{calculated value}],$$

in which the calculated value was obtained from the observed values for the 100% Guinea grass and the 100% AYB incubated individually. Positive or negative values indicated positive or negative associative effects at the inclusion levels, respectively. Data were analysed using the general linear model of SAS (Statistical Analysis System, version 9.4). The model statement was of the form:

$$Y_{ij} = \mu + B_i + T_j + e_{ij},$$

in which Y_{ij} = mean of individual observation, μ = overall mean, B_i = block effect (incubation runs), T_j = effect of treatment, and e_{ij} = residual error. Mean separation was conducted using Tukey's test,

and significance was declared at $P < 0.05$. A linear and quadratic trend comparison of increasing AYB inclusion was done using Contrast statement of SAS.

3. Results

Increasing the inclusion levels of AYB seed meal increased 48 h gas production ($P < 0.05$) (Table 1). African yam bean alone produced a higher total gas compared with the grass alone or grass supplemented with AYB. Similarly, 48 h *in vitro* CH_4 production was affected by AYB inclusion ($P < 0.05$). The 100% AYB recorded a higher CH_4 volume compared with the 100% Guinea grass, while the grass with AYB supplementations were intermediate. The effect of AYB inclusion on IVOMD of Guinea grass showed that supplementation at 15-25% inclusion level resulted in increased IVOMD ($P < 0.01$) compared with the grass-only diet. Methane to total gas ratio was affected by AYB supplementation of Guinea grass ($P < 0.05$) with CH_4 concentration higher in AYB-supplemented diets compared with the grass-only diet. Methane per unit of organic matter digested (CH_4/IVOMD) was equally affected by AYB supplementation with grass supplemented with AYB and 100% AYB treatment producing higher CH_4 per unit of organic matter digested. The associative effects of Guinea grass and AYB inclusion on 48 h gas production, CH_4 volume, and CH_4 concentration showed that there was no linear or quadratic association between the grass and AYB across the inclusion levels (Table 2). However, there was a negative linear and quadratic associative effect ($P < 0.05$) on IVOMD. Similarly, the association between the grass and AYB inclusion levels was not different in terms of the CH_4 to IVOMD ratio across the inclusion levels.

In the second experiment, AYB seed processing methods did not affect 48 h *in vitro* gas production when Guinea grass was supplemented with 20% AYB meal (Table 3). The effect of AYB seed processing method when supplemented to Guinea grass showed that processing method did not affect CH_4 production, although fermented AYB produced numerically lower CH_4 , but methane to total gas ratio

Table 1 - *In vitro* gas production, digestibility, and methane (CH_4) production in Guinea grass supplemented with graded levels of raw African yam bean meal

Treatment/diet ¹	Grass	C+10% AYB	C+15% AYB	C+20% AYB	C+25% AYB	100% AYB	SEM	P-value
48 h Gas (mL)	70.3c	74.8b	77.0b	72.3bc	79.6b	92.9a	2.50	0.003
48 h CH_4 (mL)	10.8c	17.1b	18.4b	16.7b	18.9b	22.5a	1.06	<0.01
$\text{CH}_4/\text{Total gas}$	0.15b	0.22a	0.23a	0.23a	0.24a	0.25a	0.030	0.036
IVOMD (g kg^{-1} DM)	593c	623b	613b	620b	647b	919a	22.1	<0.011
CH_4/IVOMD (g kg^{-1} IVOMD)	81.7b	123a	134a	120a	130a	110a	5.45	0.038

DM - dry matter; IVOMD - *in vitro* organic matter digestibility; SEM - standard error of the mean.

¹ C - control substrate (Guinea grass only) or control + 10, 15, 20, or 25% African yam bean (AYB) meal.

a-c - Mean values followed by different letters in the same row are significantly different by Tukey's test at 5% probability.

Table 2 - Associative effects obtained from fermentation of Guinea grass-African yam bean

Treatment ¹	C+10% AYB	C+15% AYB	C+20% AYB	C+25% AYB	SEM	P-value ²		
						Treat	L	Q
48H Gas (mL)	+3.17	+4.53	+3.39	+4.80	0.310	0.141	0.150	0.961
48H CH_4 (mL)	+43.1	+46.5	+34.4	+37.6	2.43	0.314	0.20	0.979
$\text{CH}_4/\text{Total gas}$	+40.6	+42.5	+33.7	+38.1	2.43	0.653	0.49	0.808
IVOMD (g kg^{-1} DM)	-0.58a	-4.60ab	-5.89b	-4.14ab	0.671	0.013	0.017	0.011
CH_4/IVOMD (g kg^{-1} IVOMD)	+46.3	+57.1	+38.2	+47.7	3.13	0.211	0.58	0.912

DM - dry matter; IVOMD - *in vitro* organic matter digestibility; SEM - standard error of the mean.

¹ C - control substrate (Guinea grass only) or control + 10, 15, 20, or 25% African yam bean (AYB) meal.

² Treatment - main associative effect of inclusion levels; L - linear associative relationship of increasing levels (10, 15, 20, and 25%) of AYB;

Q - quadratic associative relationship of increasing levels (10, 15, 20, and 25%) of AYB.

Positive or negative values indicate positive or negative associative effects between Guinea grass and African yam bean mixture.

a-b - Mean values followed by different letters in the same row are significantly different by Tukey's test at 5% probability.

was not different across the treatments. Although IVOMD was not different across the treatments, CH₄ per unit of IVOMD showed differences across the treatments ($P < 0.05$). Grass with fermented AYB produced lower CH₄ per unit of organic matter digested compared with grass with soaked AYB treatment.

Seed processing method affected CP, EE, ash, CF, total carbohydrate, and NDF content of the AYB meal ($P < 0.05$) (Table 4). Processed AYB meals had higher CP compared with the unprocessed meal, with fermented seeds having the highest CP followed by toasted, boiled, and soaked seed meal. Boiling reduced EE composition of AYB meal compared with the unprocessed meal ($P < 0.05$). Fermentation reduced the total carbohydrate content of AYB seed meal ($P < 0.05$) while soaking, toasting, and boiling did not differ from the unprocessed seeds in terms of total carbohydrate concentration. Neutral detergent fibre concentration of AYB seed meal was influenced by processing methods ($P < 0.01$) with toasted > soaked > boiled > fermented > raw seed meal in descending order. Processing methods did not affect ADF and ADL concentration of AYB seeds. Seed processing methods did not affect calcium, magnesium, potassium, and manganese contents of AYB meal, while iron and zinc concentrations were affected ($P < 0.05$), with fermented or toasted AYB having higher iron concentration than soaked, boiled, and raw AYB.

Saponin concentration was not different across treatments, while concentrations of alkaloid, total phenol, total tannin, oxalate, phytate, and trypsin inhibitors were influenced ($P < 0.05$) by seed

Table 3 - *In vitro* gas production, digestibility, and methane (CH₄) production of Guinea grass supplemented with African yam bean seed subjected to soaking, toasting, boiling, or fermentation

Parameter ¹	Grass +AYB _{s24}	Grass +AYB _{s48}	Grass +AYB _{boil}	Grass + AYB _{toast}	Grass + AYB _{ferm}	SEM	P-value
48 h Gas (mL)	75.9	77.8	70.1	77.2	67.6	1.57	0.141
48 h CH ₄ (mL)	16.4	17.5	15.3	15.9	12.2	0.63	0.068
CH ₄ /Total gas	0.22	0.23	0.22	0.21	0.18	0.010	0.251
IVOMD (g kg ⁻¹ DM)	640	638	645	648	648	57.1	0.359
CH ₄ /IVOMD (g kg ⁻¹ IVOMD)	123a	122a	106ab	110ab	84.1b	83.1	0.039

DM - dry matter; IVOMD - *in vitro* organic matter digestibility; SEM - standard error of the mean.

¹ Grass - *Panicum* grass; AYB_{s24} - AYB seeds soaked for 24 h; AYB_{s48} - AYB seeds soaked for 48 h; AYB_{boil} - AYB seeds boiled for 60 min; AYB_{toast} - toasted AYB seeds; AYB_{ferm} - fermented AYB seeds.

a-b - Mean values followed by different letters in the same row are significantly different by Tukey's test at 5% probability.

Table 4 - Chemical composition of raw, soaked, boiled, toasted, and fermented African yam bean seeds

Parameter	Raw	Soaked	Boiled	Toasted	Fermented	SEM	P-value
CP (g kg ⁻¹ DM)	185c	194b	194b	196b	205a	2.20	<0.001
EE (g kg ⁻¹ DM)	11.4bc	13.3abc	16.5a	15.3ab	9.05c	0.900	0.044
Ash (g kg ⁻¹ DM)	31.3bc	27.2cd	24.3d	37.1a	31.9b	1.50	0.004
CF (g kg ⁻¹ DM)	43.2c	45.5bc	50.6ab	49.6abc	54.6a	1.50	0.036
Carbohydrates (g kg ⁻¹ DM)	633a	628a	628a	629a	612b	2.60	0.029
NDF (g kg ⁻¹ DM)	239b	360a	341ab	391a	328ab	18.3	0.001
ADF (g kg ⁻¹ DM)	324	317	313	282	255	9.70	0.065
ADL (g kg ⁻¹ DM)	67.1	65.4	66.0	64.7	62.3	1.01	0.748
Calcium (g kg ⁻¹)	0.12	0.15	0.15	0.15	0.15	0.011	0.32
Magnesium (g kg ⁻¹)	2.49	2.18	2.43	2.33	2.24	0.070	0.74
Potassium (g kg ⁻¹)	14.4	10.9	12.5	12.9	12.1	0.57	0.48
Sodium (g kg ⁻¹)	38.6	40.6	44.0	43.6	42.0	3.52	0.42
Manganese (mg kg ⁻¹)	23.5	23.3	23.9	25.9	23.8	0.35	0.09
Iron (mg kg ⁻¹)	69.7b	73.6b	73.2b	88.1a	88.1a	2.81	0.02
Zinc (mg kg ⁻¹)	62.4	59.35	63.2	61.9	70.6	2.82	0.06

CP - crude protein; DM - dry matter; EE - ether extract; CF - crude fibre; NDF - neutral detergent fibre; ADF - acid detergent fibre; ADL - acid detergent lignin; SEM - standard error of the means.

a-c - Mean values followed by different letters in the same row are significantly different by Tukey's test at 5% probability.

processing method (Table 5). Boiling reduced the alkaloid content of AYB meal, while boiling, soaking, and toasting reduced the total phenol content. Fermented and unprocessed AYB seeds were similar in alkaloid, tannin, and total phenol concentrations. Soaking, boiling, and fermentation reduced the oxalate concentration, but toasting had no effect on oxalate concentration. Boiled and unprocessed seeds were similar in phytate concentration, while soaked, toasted, and fermented seeds had lower phytate concentration. Concentration of trypsin inhibitors in AYB seeds was not affected by soaking and fermentation, but toasting and boiling reduced its concentration in descending order of magnitude.

Leucine was the most abundant amino acid in AYB seed meal. The concentration of amino acids in AYB seed meal was not affected by processing methods, except for methionine and arginine ($P < 0.05$) (Table 6). Fermentation improved the methionine and arginine concentrations in fermented AYB, while soaking and boiling reduced the concentration of these two amino acids. Fermented seed meal had higher methionine and arginine concentrations than unprocessed seeds ($P < 0.05$).

Table 5 - Phytochemical composition of raw, soaked, boiled, toasted, and fermented Africa yam bean seeds

Parameter	Raw	Soaked	Boiled	Toasted	Fermented	SEM	P-value
Alkaloid (g 100 g ⁻¹ DM)	3.31a	3.71a	0.51b	2.89a	2.66a	0.391	0.014
Total phenol (g 100 g ⁻¹ DM)	14.8a	12.0b	4.27c	11.0b	13.6ab	0.52	<0.001
Tannin (g 100 g ⁻¹ DM)	5.03a	2.06c	3.33b	4.31a	4.30a	0.382	<0.001
Saponin (g 100 g ⁻¹ DM)	4.95	6.75	3.00	6.15	6.20	0.051	0.167
Oxalate (mg 100 g ⁻¹ DM)	7.64a	4.29b	4.92b	6.32ab	4.39b	0.451	0.002
Phytate (mg g ⁻¹ DM)	2.08a	1.06bc	1.85a	1.36b	0.82c	0.160	0.005
Anti-trypsin inhibitors (mg g ⁻¹ TIU)	19.9a	18.6ab	12.9c	17.8b	18.8ab	1.12	<0.001

DM - dry matter; SEM - standard error of the means.

a-c - Mean values followed by different letters in the same row are significantly different by Tukey's test at 5% probability.

Table 6 - Amino acid composition (g 100 g⁻¹ crude protein) of raw, soaked, boiled, toasted, and fermented African yam bean seeds

Parameter	Raw	Soaked	Boiled	Toasted	Fermented	SEM	P-value
Leucine	8.39	7.24	7.56	8.35	8.55	0.180	0.248
Lysine	4.87	4.11	4.01	4.30	4.80	0.141	0.462
Isoleucine	4.36	3.97	4.24	4.55	4.62	0.091	0.101
Phenylalanine	4.22	3.55	3.60	3.95	4.57	0.173	0.141
Tryptophan	1.13	0.87	0.91	1.08	1.24	0.071	0.118
Valine	4.03	4.05	4.12b	4.1	4.30	0.082	0.063
Methionine	1.25bc	1.18c	1.18c	1.31b	1.50a	0.060	0.041
Proline	3.66	3.45	3.35	3.45	4.04	0.111	0.335
Arginine	6.63b	5.68c	5.64c	6.15b	6.93a	0.183	0.009
Tyrosine	4.04	3.43	3.44	3.70	3.96	0.102	0.238
Histidine	2.33	1.82	1.76	2.27	2.41	0.224	0.381
Cystine	1.15	0.97	0.91	1.03	1.25	0.071	0.617
Alanine	4.40	3.61	3.66	4.02	4.70	0.161	0.415
Glutamic acid	13.4	10.7	11.2	12.4	13.7	0.431	0.190
Glycine	4.33	3.27	3.58	3.67	5.05	0.250	0.183
Threonine	3.42	3.43	3.16	3.50	3.53	0.072	0.452
Serine	3.52	3.45	3.71	4.22	4.19	0.161	0.150
Aspartic acid	9.53	8.80	8.66	9.58	9.56	0.150	0.159

SEM - standard error of the means.

a-c - Mean values followed by different letters in the same row are significantly different by Tukey's test at 5% probability.

4. Discussion

The CP, NDF, and ADF contents of the Guinea grass forage reported in this study is within the range reported in literature, while lower values due to increased lignification associated with maturity and drought are common (Babayemi, 2009). However, the CP is close to the threshold of 8%, below which optimal rumen function may be hindered if fed alone (Ikhimioya, 2008). Therefore, supplementation with legume would enhance animal performance through improved digestibility and intake of the forage. Furthermore, increased use of home-grown legumes for livestock feeding justifies the inclusion of AYB because of its economic and ecological advantages such as promotion of organic farming, diversification, and adaptability to local farming systems (Campos-Vega et al., 2009; Bhartiya et al., 2015).

Previous studies on the replacement of soybean with legume seeds in the diet of sheep or dairy cows revealed the possibility for total or partial replacement with chickpea, faba bean, peas, field bean, or lupins (Bonanno et al., 2016; Ramin et al., 2017; Zagorakis et al., 2018). Studies on digestive interaction between grass and legumes (seeds or forages) revealed inconsistent results due to inclusion levels, nature of dietary components, and the possible presence of bioactive compounds. More significant associative responses on digestibility have been observed in legume supplementation of poor-quality hay when bioactive compounds are involved (Niderkorn et al., 2011), but the phytochemicals investigated in AYB did not reflect this, probably due to their low concentration in the total substrate incubated compared with the 100% AYB. However, enhanced gas production has been associated with grass-legume supplementation in ruminant animals (Niderkorn et al., 2011; Adejoro and Hassen, 2018; Zagorakis et al., 2018), and current result agrees with this.

Obatolu et al. (2007) described the proximate and functional characteristics of AYB. Little information exists in literature on AYB inclusion in the diet of ruminants either from *in vitro* or animal experimentation. In the current study, raw and processed AYB seeds improved IVOMD and gas production significantly with up to 14% increase in total gas production. This result can be related to the report of Tadele et al., (2014), who observed improved *in vivo* digestibility of Rhodes grass when supplemented with lupin seeds, which resulted in improvement of growth of Washera sheep compared with those that received only grass. An increase in nitrogen supply to microbes through non-protein nitrogen (NPN) or legume supplementation, fed as either whole seed, ground seed, or whole plant silage has been reported to improve microbial activity, thus improving organic matter digestibility and *in vitro* gas production (Mitsumori and Sun, 2008). This leads to improved efficiency of fermentative digestion in the rumen and improved efficiency of feed conversion to products such as meat or milk (Ramin et al., 2017).

Beyond removing unwanted anti-nutritional factors in legume seeds, processing also improves the nutritive value and digestibility of seeds (Khattab et al., 2009). In terms of nutritive value, toasting and soaking did not affect CP digestibility of lupin seed, and values obtained in the present study were comparable with that of soybean meal (Tadele et al., 2014). However, Mogensen et al. (2008) and Vaga et al. (2017) observed that toasting could be used to decrease effective rumen protein degradability and improve bypass protein supply, which may be useful to dairy animals (Mustafa et al., 2003). Fibre digestion could be increased when the proportion of ruminal degradable protein in the diet is lower, due to increased rumen retention time and slow release of essential growth factors to cellulolytic microbes (Mitsumori and Sun, 2008; Gerber et al., 2013). However, the kinetics of microbial degradation of AYB meals as affected by processing methods needs further evaluation.

There is adequate evidence that the presence of tannins in diets can reduce enteric CH₄ production (Patra and Saxena, 2011; Adejoro et al., 2019). The use of legumes is recommended in the tropics as a means of abating overall animal-derived CH₄ production (Gerber et al., 2013). Nevertheless, the presence of phytochemicals in AYB did not affect enteric CH₄, and this may be due to the concentration or nature of phenolic compounds present in the incubated substrates. In a similar *in vitro* study by Ramin et al. (2017), CH₄ emission was neither affected by field bean inclusion as a substitute to soybean meal nor by the seed processing methods, although processed seeds had lower phenol and tannin concentrations. Supplementing legumes or NPN in nitrogen-limited diets often results in improved fermentability of the substrate, but with increased acetate and higher ruminal CH₄ production. However, improved

growth in animals will translate into lower CH_4 intensity, expressed in volume of CH_4 per unit of animal product produced when grass is supplemented with legume (Hristov et al., 2013).

Generally, AYB meal had lower CP compared with soybean (Zagorakis et al., 2018), but the CP value is close to other legume seeds such as peas, field bean, kidney bean, and cowpea (Khatab et al., 2009; Ramin et al., 2017). Likewise, lipid content of raw and processed AYB seeds was low but with a high amount of carbohydrate content similar to many other legume seeds (Doss et al., 2011; Zagorakis et al., 2018). Generally, legume seed proteins are known to be limiting in methionine and cysteine, high in leucine and with a moderate amount of other essential amino acids (Khatab et al., 2009), and this was observed in the present study. Processing methods employed in the present study slightly increased the CP content of AYB seeds, which could be as a result of potential leaching of soluble constituents of legume seeds into water, during soaking or boiling (Campos-Vega et al., 2009). Fermented AYB meal had the highest CP content compared with other methods, which could be due to an increase in microbial biomass during fermentation mobilising soluble carbohydrates for their metabolism and increasing microbial protein (Torres et al., 2006; Hefnawy, 2011). Although a slight increase in CP was observed following toasting or boiling, a negative effect on CP quality of legume seeds associated with higher acid detergent insoluble nitrogen concentration may occur depending on the duration of heat treatment (Vaga et al., 2017).

The concentration and the type of phytochemicals in legume seeds may differ due to genetic and agronomic factors under which they are sown (Mogensen et al., 2008). Soaking, toasting, and fermentation have been shown to significantly reduce these phytochemicals to varying degrees, depending on the characteristics of the antinutritional factors present (Fernandes et al., 2010). The significant reduction in oxalate and phytate in the soaked and boiled AYB observed in this study agrees with previous reports for lentil seeds (Hefnawy, 2011). Water-soluble phytochemicals such as oxalic acids, phytate, and hydrogen cyanide are easily destroyed or removed from feedstuff through soaking or boiling (Adejoro et al., 2013). Simple sugars and other water-soluble nutrients may equally be lost in this process, but the presence and strength of seed coat may affect the extent of leaching (Fernandes et al., 2010). Similarly, heat labile phytochemicals such as antitrypsin inhibitors are easily destroyed by the high temperatures during heating or boiling (Adejoro et al., 2013).

Seed fermentation did not significantly affect tannin concentration in AYB seeds, and this observation is similar to previous reports on ground bean and pigeon pea (Torres et al., 2006). Condensed tannins, which are more widely distributed in plant species, are not readily degraded by anaerobic microbes (Makkar, 2003). However, fermentation considerably reduced the concentration of phytate in AYB seeds, and this relates to the activation of phytase enzymes hydrolysing phytate. Phytate removal often results in increased bioavailability of minerals such as phosphorus, iron, zinc, and magnesium (Nikmaram et al., 2017), but the extent of phytate removal is dependent on the type of microorganism, the fermentation conditions, and the initial concentration of phytate in the raw seeds (Egounlety and Aworth, 2003).

5. Conclusions

Supplementation of Guinea grass with African yam bean seed meal up to 20% inclusion increases gas production and digestibility of substrate, but no significant reduction in CH_4 is observed. Soaking African yam bean in water for 24-48 h or boiling it at 100 °C for 60 min is sufficient to reduce most of the antinutritional factors present, thus reducing the potential toxicity associated with its consumption by animals. The improvement in the *in vitro* organic matter digestibility of Guinea grass justifies the inclusion of soaked African yam bean seed meal in ruminant forages especially when dietary protein becomes limiting.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: O.M.A. Jesuyon, F.A. Adejoro, S.O. Baiyeri and O.V. Boluwaji. Data curation: T.A. Sanni, O.M.A. Jesuyon, F.A. Adejoro and A.M. Akanmu. Formal analysis: F.A. Adejoro, A.M. Akanmu and A. Hassen. Funding acquisition: T.A. Sanni. Investigation: F.A. Adejoro and A.M. Akanmu. Methodology: F.A. Adejoro. Project administration: T.A. Sanni and O.M.A. Jesuyon. Supervision: T.A. Sanni and A. Hassen. Validation: F.A. Adejoro. Visualization: O.M.A. Jesuyon. Writing-original draft: F.A. Adejoro, O.V. Boluwaji and A.M. Akanmu. Writing-review & editing: O.M.A. Jesuyon, F.A. Adejoro and S.O. Baiyeri.

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