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Nutrient Profile and *in vitro* Fermentation Characteristics of Flamboyant *(Delonix regia)* Seeds Subjected to Different Processing Methods for Sustainable Ruminant Production in Humid Tropic

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Abstract

This study assessed the nutrient profile and *in vitro* fermentation characteristics of flamboyant *(Delonix regia)* seeds subjected to different processing methods for sustainable ruminant production in humid tropic. Eight (8) different processing methods formed the experimental treatments, namely T_1 (raw/control); T_2 (roasted); T_3 (soaked in water for 24 hours); T_4 (soaked in water for 48 hours); T_5 (soaked in rumen liquor for 24 hours); T_6 (soaked in rumen liquor for 48 hours); T_7 (parboiled for 45 minutes.) and T_8 (cooked 45 minutes. The treatments were replicated three times in a Completely Randomized Design (CRD). Data obtained on the nutrient profile and *in vitro* fermentation characteristics was subjected to a One-way Analysis of Variance (ANOVA) using statistical analysis system (SAS) version 9. The mean was separated using Duncan's New multiple Range Test (DNMRT). The results showed that there was a significant ($P<0.05$) effects of processing methods on crude fibre (CF) and calcium (Ca) contents of the processed seeds with the values ranges from 2.97 % in T_4 to 4.59% in T_8 for crude fibre (CF). Similar ($P>0.05$) contents was recorded for dry matter (DM), crude protein (CP), ether extract (EE), ash, neutral detergent fibre (NDF), acid detergent fibre (ADF), phosphorous (P) and iron (Fe) content across the experimental treatments, the contents ranges from 88.41 – 89.51%, 20.59 – 21.45%, 2.24 – 3.05%, 4.25 – 4.89% 38.89 – 39.78%, 25.14 – 25.47%, 3.7 – 4.0%, 62.90 – 66.50mg/kg for DM, CP, EE, ash, NDF, ADF, P and Fe content respectively. Significant ($P<0.05$) different was observed for methane gas (CH_4) production, with values ranges from 15.33 – 29.33 (ml). Similar value was observed for fermentation efficiency (FE), short chain fatty acids (SCFA), in vitro organic matter digestibility (IVOMD) and metabolisible energy (ME) across the experimental treatments and the values ranges from 0.37 – 0.67, 0.75 – 1.02 Mmol, 61.92 – 68.61% and 7.38 – 9.55 MJ/kg DM, respectively. Conclusively, irrespective of the processing methods flamboyant seeds had adequate nutrients to meet the nutritional requirements of ruminants except crude fibre (CF) which can be fortified with roughages or fibrous feedstuffs. The *in vitro* fermentation characteristics showed that the seed has potentials as a feed resource to support ruminant animal production especially in the dry season.

Keywords: Fermentation-characteristics; *In vitro*; Nutrient-profile; Processing-methods and ruminant animals.

1. Introduction

Ruminant animals form a major group of the important livestock species in the animal industry in the tropics [1]. Ruminant livestock in most parts of the tropics graze intensively on naturally growing forages, Nigerian ruminant industry is facing the problem of forage scarcity with high cost of feeds precipitated by inadequate supply of feed ingredients. This constraint has affected ruminant animal production negatively and created a wide gap between the demand and supply of animal protein in Nigeria [2].

The major constraint to ruminant livestock production in the tropics is the availability of cheap and quality feedstuffs especially in the dry season [3]. Majority of the farmers supplemented the feed with available feed resources such as crop residues, browse plants and agro-industrial by products (AIBP) [4].

Most of the alternative feedstuffs have been intensively utilized by the poultry, pig and rabbit farmers putting the ruminant farmers in tight corner of using neglected or underutilized seeds such as flamboyant seeds. Nutrients composition of a feedstuff does not guarantee the nutritional value of the feedstuff, different methods of assessing nutritive values of plants or feed resources which include chemical or proximate composition evaluation, *in vitro* digestibility and feeding trial.

The *in vitro* degradability method is a laboratory estimation of assessing the potential nutritive value of the feed. It is also a method that is reproducible and parameters obtained correlate well with in-vivo trials [5]. *In vitro* gas degradability method has the advantage of not only being less expensive and less time consuming but it allows for more precision in experimental conditions than the in vivo method [6]. It is convenient, fast and allows a large number of samples to be handled at a time [7]. It is based on the quantification of substrate degraded and of gas or

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short chain fatty acid produced in rumen fermentation system [8]. Thus, this study was carried to assess the nutrient profile and *in vitro* digestibility of flamboyant (*Delonix regia)* seeds subjected to different processing methods for sustainable ruminant production in humid tropic.

2. Materials and Method

2.1. Collection of Experimental Materials

The study was carried out in the Faculty of Agriculture Teaching and Research Farm, University of Port Harcourt, Port Harcourt, Rivers State. Port Harcourt is a coastal city located in the Niger Delta region of Nigeria within latitudes 6^o 58' – 7^o 60'E and longitudes 4^o 40' – 4^o55'N [9].

Matured flamboyant (*Delonix regia)* pods were harvested from the trees within the University of Port Harcourt community, Port Harcourt, Rivers State. The harvested pods were sun-cured for three days and dehulled to obtain the seeds.

2.2. Experimental Treatments

Eight (8) different processing methods were used and they are;

Treatment 1 (T_1): 'Raw' : *Delonix regia* seeds (Untreated seeds).

Treatment 2 (T_2) 'Roasted': Dry sand (1kg) was heated to 100-110°C in a pan using a kerosene stove as source of heat. Thereafter, 1kg of the seed was poured into the pan and the content was stirred repeatedly to ensure uniform roasting and prevent charring. The seeds were considered roasted when change in seed colour from brown to dark brown was observed and change in aroma was perceived. The seeds were removed from the sand and spread out to cool, grinded, stored and labeled in an air-tight container until required for laboratory analyses.

Treatment 3 and 4 (T_3 and T_4) 'Soaked in water': 1kg of seed was soaked in 1 .5litre of distilled water at varying durations 24hrs and 48hrs, respectively. Thereafter, seeds were removed from the water and air-dried until no appreciable weight difference was observed.

Treatment 5 and 6 (T_5 and T_6) 'Soaked in rumen liquor': 1kg of seed was soaked in 1 .5litre of rumen liquor for 24hrs and 48hrs, respectively. Thereafter, seeds were removed from the rumen liquor and air- dried.

Treatment (T_7) 'Parboiled': Water was boiled and later poured into the seeds and was allowed to cool and air dry.

Treatment 8 (T_8) 'Cooked': The seeds were put in a pot and cooked with water with the use of kerosene stove 45 minutes. It was then allowed to cool and then air dried for three days, ground and sieved into 2mm.

2.3. Chemical Analysis

The proximate composition of the seeds was determined according to Association of Analytical Chemist AOAC (2000) [10], while neutral detergent fibre (NDF) was determined according to Van Soest, *et al.* [10]. Non-fibre carbohydrate (NFC) was calculated as NFC = 100 – (CP + Ash + EE +NDF). Mineral composition of the samples was carried out using the Atomic Absorption Spectrophotometer of AOAC. [11].

2.4. *In Vitro* Digestibility Trial

The *in vitro* trial was carried out at the laboratory of Department of Animal Science, University of Benin, Benin City, Edo State, Nigeria. The method adopted was as described by [12].

The modified *in vitro* fermentation procedure of Navarro, *et al.* [13] was adopted as described by Bamikole, *et al.* [12]. A phosphate – bicarbonate buffer [14] used (g/L) were: 1.98 $Na_2HPO4.12H_2O$, 1.302 KH_2PO_4, 0.105 $MgCl_2.6H_2O$, 1.407 NH_4HCO_3, 5.418 NaOH. Rumen fluid was obtained from three fistulated goats before morning feeding into a thermos flask and taken to the laboratory where it was strained through four layer of Cheesecloth under continuous flushing with CO_2.

Inoculum for incubation was prepared using ratio of rumen fluid to buffer of 1:2. 190 mg of substrate (mixture of equal proportion ground maize, Guinea grass and *Centrosema* molle) was weighed with 10 mg of the test material (experimental treatments) into nylon bags, sealed and incubated using 30ml of inoculum in 100 Ml graduated syringes at 30^oC for 24hrs. Syringes containing only the substrate (i.e. without the experimental treatments) and those containing only the inoculum (i.e. without sample) represent the control and blank respectively.

Gas production (i.e. accumulated gas in the head space of each fermentation syringe) was read 3 hourly for 24 hours to know the volume of gas produced. At the end of incubation, 4mLof 10M NaOH was introduced into the headspace of the syringes for methane determination. The bags with the residue were removed from the syringes, rinsed thoroughly with water and dried at 100^0C for 24 hours, and then to constant weight to determine *in vitro* dry matter disappearance (IDMD).

2.5. Calculation for Post Incubation Parameters

2.5.1. Organic Matter Digestibility (OMD)

This was estimated as: OMD = 14.88 + 0.889 GV + 0.45 CP + 0.651 ash [15].

2.5.2. Short-Chain Fatty Acids (SCFA)

Short-chain fatty acids (SCFA) were estimated as: SCFA = 0.0239 GV − 0.0601 [16].

2.5.3. Metabolizable Energy (ME)

This was calculated as: ME = 2.20 + 0.136 GV + 0.057 CP + 0.00029 CF [15]

2.5.4. Fermentation Efficiency (FE)

It was calculated as: Fermentation Efficiency (FE) = Dry matter Digestibility (g/kg)/ Total Gas Volume (mL/g)

2.6. Experimental Design Statistical Analysis

The study was conducted using a completely randomize design (CRD) replicated trice. All the data collected were subjected to a One-way Analysis of Variance (ANOVA) using [17].statistical package. Significant treatment means were compared and separated using Duncan's New Multiple Range Test (DNMRT).

3. Results and Discussion

Table 1 shows the effect of processing methods on the chemical composition of flamboyant (*Delonix regia)* seeds. There was a significant (P<0.05) effects of processing methods on crude protein (CF) and calcium (Ca) contents of the seeds. The values ranges from 2.97 % in T4 to 4.59% in T8 for CF. The Ca 0.7 – 1.0% for T1 and T2 respectively. Similar (P>0.05) contents was recorded for DM, CP, EE, ash, NDF, ADF, P and Fe content across the experimental treatments the contents ranges from 88.41 – 89.51%, 20.59 – 21.45%, 2.24 – 3.05%, 4.25 – 4.89% 38.89 – 39.78%, 25.14 – 25.47%, 3.7 – 4.0%, 62.90 – 66.50mg/kg for DM, CP, EE, ash, NDF, ADF, P and Fe content respectively.

Table-1. Effect of processing methods on the chemical composition of flamboyant (*Delonix regia)* seeds

Treatments									
Indices	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	SEM
DM	88.41	89.03	89.22	86.82	89.14	89.41	89.35	89.51	0.63
CP	20.59	21.28	20.78	20.27	20.59	21.09	21.17	21.45	0.83
EE	2.24	2,59	2.46	3.05	2.37	2.79	2.75	2.84	0.11
CF	3.38^{a}	3.47^{a}	3.42^{a}	2.97^{b}	3.29^{a}	4.48^{a}	4.54^{a}	4.59^{a}	1.81
Ash	4.25	4.36	4.29	4.54	4.33	4.67	4.73	4.89	0.26
NDF	39.78	39.85	39.43	39.67	39.72	38.89	39.69	39.64	0.76
ADF	25.18	25.27	25.14	25.38	25.33	25.47	25.41	25.29	0.37
Ca	0.7^{c}	1.0^{a}	0.8^{ab}	0.8^{ab}	0.9^{ab}	0.9^{ab}	0.8^{bc}	0.8^{bc}	0.01
P	3.7	4.0	3.9	3.9	3.8	3.7	3.8	3.8	0.01
Fe(mg/kg)	63.40	66.20	63.70	64.30	62.90	64.60	65.40	66.50	0.63

$^{a, b, c, d}$ Means value with different superscripts within a row are significantly different at 0.05 level using Duncan's New Multiple Range Test (DNMRT). SEM= Standard error of mean; T_1 = (Raw seeds/control); T_2 = Roasted; T_3 = water soaked for 24hrs; T_4 = water soaked for 48hrs; T_5 =Rumen liquor soaked for 24hrs; T_6 = Rumen liquor soaked for 48hrs; T_7 = parboiled; T_8 = cooked

The range of CP (20.59 – 21.45%) recorded for the experimental treatments was far above the 7 to 8 % CP suggested as threshold for sufficient utilization of feed by MacDonald, *et al.* [8]. Therefore, flamboyant seeds would provide the adequate nitrogen requirement for the rumen microorganisms to maximally digest the main components of dietary fibre leading to the production of volatile fatty acid [18]; [9] which in turn facilitate microbial protein synthesis [19].

The range of CF (2.97 – 4.59 % DM) recorded for the seeds in this study was lower compared to what [4] catalogued for feedstuff of small ruminant animal in South-west. High level of fibre has been acknowledged by Odedire and Babayemi [20] to be inversely related to feed digestibility and nutrient availability. With these, flamboyant seeds needs to be mixed with a feed stuff with high content of fiber or roughages.

The nitrogen free extract (NFE) is an indicator of carbohydrate content of feedstuff or ingredient that is soluble or easily digested and available for animal. It implies that the soluble carbohydrate could support the production of volatile fatty acid in the rumen during fermentation [21]. The higher NDF value obtained in this study is an indication that it will be a useful feeding stuff as good energy source in ruminant animal production as NDF is preferred measure for ruminant feeds and dietary balancing programs [10].

The Ca contents (0.7 – 1.0) in the experimental treatments was above the recommended range (0.20-0.26g/100g) for maintenance of growing and lactating sheep [22] while P content (3.7 – 4.0) was also above the recommended range (0.15-0.48g/100g) required by ruminants [23].

Methane (ml), fermentation efficiency, short chain fatty acid, organic matter digestibility was indicated in Table 2. There was a significant (P<0.05) different in the CH_4, with values ranges from 15.33 – 29.33 (ml). Similar value was observed for FE, SCFA, IVOMD and ME across the experimental treatments and the values ranges from 0.37 – 0.67, 0.75 – 1.02 mmol/200 mg, 61.92 – 68.61% and 7.38 – 9.55 MJ/kg DM, respectively.

Table-2. Effect of processing methods of flamboyant (*Delonix regia)* seeds on *in vitro* fermentation characteristics

Parameters					
Treatments	**Methane gas (CH_4)**	**Fermentation Efficiency (FE)**	**Short-chain Fatty acid (SCFA)**	**In-vitro Organic matter Digestibility (IVOMD)%**	**Metabolizable Energy (ME) MJ/kg/DM**
T_1	16.33^{b}	0.49	0.90	61.92	8.79
T_2	16.67^{b}	0.42	0.99	66.01	9.40
T_3	29.33^{ab}	0.43	1.02	66.92	9.55
T_4	17.33^{b}	0.37	0.91	61.70	8.27
T_5	16.67^{b}	0.58	1.07	68.62	9.81
T_6	16.67^{b}	0.67	0.88	62.02	8.75
T_7	18.00^{b}	0.51	1.01	66.79	9.48
T_8	15.33^{b}	0.59	0.75	68.61	7.38
SEM	1.04	0.04	0.38	1.39	0.28

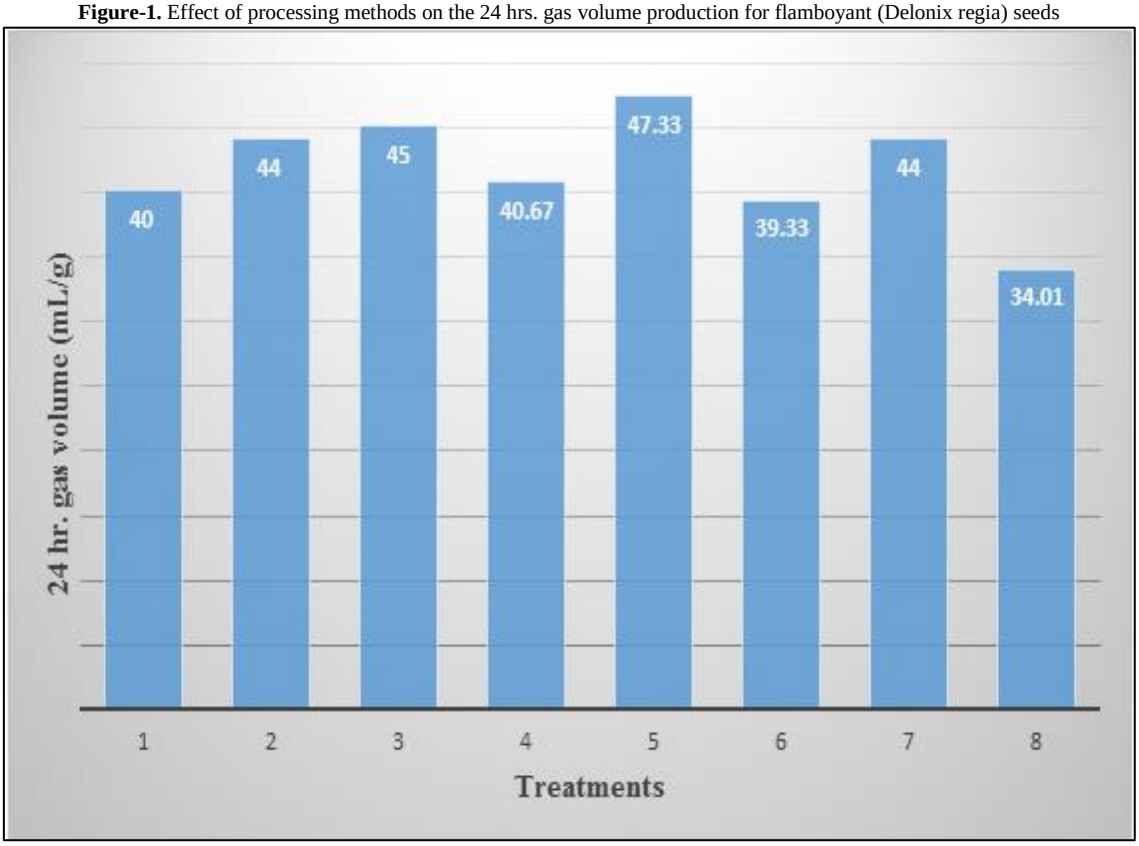
$^{a, b, c, d}$ Means value with different superscripts within a column are significantly different at 0.05 level using Duncan's New Multiple Range Test (DNMRT). SEM= Standard error of mean; T_1 = (Raw seeds/control); T_2 = Roasted; T_3 = water soaked for 24hrs; T_4 = water soaked for 48hrs; T_5 = Rumen liquor soaked for 24hrs; T_6 = Rumen liquor soaked for 48hrs; T_7 = parboiled; T_8 = cooked.

The insignificant effect of processing methods on the SCFA and ME is an indication that irrespective of the processing methods, energy content of Delonix regia seeds are not affected or compromised. The range of SCFA (0.75 – 1.02 mmol/200mg) and ME (7.38 – 9.55 MJ/kg DM) recorded in this study was higher than what [24] reported for 50:50 *Delonix regia* and *Pennisetum purpureum*, the disparity might be as a result of inclusion or mixture of the grass with the *Delonix regia* seeds. Meanwhile, the higher SCFA and ME recorded in this study is an indication that *D. regia* seeds have a potential to make energy available to ruminant. This observation correlate with the report of Ajayi [25], that higher SCFA or VFA such as butyrate and acetate suggest a potential to make energy available to ruminants.

The SCFA are essentially volatile fatty acid (VFA) of three to six carbon atoms and the major one concerned in *in vitro* and *in vivo* rumen studies are the acetic, propionic and butyric acids. Methane formation is an avenue to prevent H_2 accumulation and acidic condition in the rumen [12]. This is dependent on VFA production, which determines the amount of excess H_2 in the rumen that is converted to CH_4 by the methanogens [26]. Babayemi, *et al.* [27], reported a close relationship between the production of methane and VFA as well as acetate-to-propionate ratio has been reported. Hydrogen accumulation hinders the pathways for acetic acids synthesis and favours propionic acid production [28] thereby resulting in C_2: C_3 ratios. Ruminal production of VTA contributes about 70% of energy needs of the ruminant animals [29]

The 24hrs gas volume production for *Delonix regia* seeds treated with different processing methods was depicted in (Figure 1). Highest volume of gas at 24hrs (47.33) was noticed in T5 followed by T_3 (45) T_2 and T_7 had 44 while T_1 and T_6 had 40.67 and 39.33, respectively.

Figure-1. Effect of processing methods on the 24 hrs. gas volume production for flamboyant (Delonix regia) seeds



The gas production is a nutritionally wasteful product [30] but provides a useful basis from which ME, OMD and SCFA may be predicted [7]. High cumulative 24h gas production for the experimental treatments is an indication of efficient fermentation and high organic matter digestibility [31] this was corroborated with IVOMD recorded and as shown in Table 2. Carbohydrate digestion is central to gas production in an *in vitro* fermentation study while the contribution from protein is very small [12].

4. Conclusion and Recommendation

Conclusively, the processing methods adopted for the experiment does not affect the nutrient profile of the flamboyant (*Delonix regia)* seeds significantly, the nutrients were in line with the ruminant requirements except the crude fibre (CF) which can be fortified with roughages or fibrous feedstuffs. The *in vitro* fermentation characteristics have shown that flamboyant seeds have a potential to be use as a feed resources for sustainable ruminant animal production especially in the dry season.

Conflict of Interests

The authors declare that there is no conflict of interest regarding development and publication of this article.

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