

Chemical constituents and in-vitro rumen fermentation for dietary inclusion of *Delonix regia* seed meal for sustainable ruminant production

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Abstract

This study was conducted to assess the chemical composition and in-vitro digestibility for dietary inclusion of *Delonix regia* seeds meal (DSM) for sustainable ruminant production. Four experimental treatments were formulated and DSM was included and varied as T1 (35/0%), T2 (20/15%), T3 (15/20%) and T4 (0/35%), respectively, each treatment were replicated and analyzed for the proximate composition and in-vitro analysis. There were significant differences ($P < 0.05$) in the nutrients and fiber fractions (CP, EE, ash, OM, NFE, NDF, ADF and hemicellulose) across the experimental treatments as CP ranged between 10.50 – 13.60% and decreased with inclusion of DSM. The NFE, NDF and hemicellulose were similar ($P > 0.05$) in T1 and T4. In-vitro analysis indicated that the methane gas volume was significantly ($P < 0.05$) influenced with the dietary inclusion of DSM across the experimental treatments as T2 had the highest volume of CH_4 (0.61mL) while the least was recorded for T1 (0.47 mL). There was no significant difference ($P > 0.05$) for 24hrs gas production, CH_4 percentage, CH_4 reduction percentage, in-vitro dry matter digestibility (IDMD), in-vitro organic matter digestibility (IOMD), metabolizable energy and fermentation efficiency across the treatments. Conclusively, the nutrients profile of the experimental treatments justifies the utilization of DSM for sustainable ruminant production. Similarity in post in-vitro parameters across treatments is an indication that inclusion of DSM will go a long way to resolve the challenges of feed and feeding for ruminant production.

Keywords: Chemical constituents, *Delonix-regia*-seed-meal, in-vitro, production, ruminant and sustainable

Introduction

Ruminant animal has played a crucial role in the livelihood of man. Due to its relevant in the sociocultural benefits coupled with economic gains of these animals, ruminant production is still facing a serious challenge as most of its production has been left with peasant or small scale farmers (sheep and goat) and pastoral nomads (cattle). Natural pasture which has been characterized with seasonal decline of nutrients has been the major feed resources for these animals. Lamidi *et al.* (2009) reported that available

forage for most part of the year is low in crude protein which leads to marked decreases in voluntary intake and digestibility which subsequent marked weight loss of the animal body, and economic return of the venture.

The quest for alternative feed ingredients for sustainable ruminant animal production has been the priority of the farmers and ruminant nutritionist in recent years. Most of these alternative feedstuffs has to be the one that will meet the nutritional requirement of the animals and at the same

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time environmental friendly.

Delonix regia seed has been reported to have a potential as a feed resources for farm animals especially ruminant animal. Nutritional profile of Delonix regia seeds subjected to different processing methods has been reported by Evien (2017). Nutrients composition alone cannot be used to adjudge the nutritional potential or value of a particular feed ingredient. 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 (Fajemisin, 2002). Despite the nutritional values of this neglected seed, its dietary inclusion with different feed ingredients has not been exhausted. In lieu of these, this study intend to examine the chemical compositions and *in-vitro* rumen fermentation for dietary inclusion of *Delonix regia* seed meal for sustainable ruminant animal production

Materials and method

Location

The experiment was conducted at Department of Animal Science, Faculty of Agriculture, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria. Port Harcourt is a coastal city located in the Niger Delta region of Nigeria within latitudes 6° 58' – 7° 60'E and longitudes 4°

40' – 4° 55'N. The monthly rainfall in Port Harcourt follows a sequence of increase from March to October before decreasing in the dry season months of November to February (Uko and Tamunobereton Ari, 2013 and Lamidi and Ogunkunle, 2016).

Matured Delonix regia fruits were harvested from Delonix regia trees and the seeds were collected, discarded seeds was also being gathered within the University of Port Harcourt community, sundried.

Roasting process and formation of Delonix regia seeds meal (DSM)

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 was considered roasted when change in seed colour from brown to dark brown is observed and change in aroma. The seeds were removed from the sand and spread out to cool, ground and milled to have Delonix regia seeds meal DSM.

Collection of experimental materials and composition of experimental treatments

Citrus pulp was gathered within the university community sun dried and milled to have orange pulp meal. Palm kernel cake, wheat offal, bone meal and salt were purchased from feed mill within the Port Harcourt metropolis. Four different experimental treatments were composed on percentage level as shown in Table 1.

Table 1: Percentage composition (%) of dietary inclusion of *Delonix regia* seed meal

Feed ingredients	Experimental treatments			
	T1	T2	T3	T4
Wheat offal	42	42	42	42
Orange pulp meal	20	20	20	20
Palm kernel cake	35	20	15	-
<i>Delonix regia</i> seed meal	-	15	20	35
Bone meal	2	2	2	2
Salt	1	1	1	1

Three (3) replicates of each samples of the roasted DSM and the experimental treatments were grounded with a Thomas Willey Laboratory Mill-Model 4 and passed through 1-mm sieve. Proximate composition of the experimental treatments was determined according to AOAC (2000), while neutral detergent fibre (NDF) was determined according to Van Soest et al. (1991). $NFC = 100 - (CP + Ash + EE + NDF)$ (Ojo et al., 2016); Hemicellulose = $NDF - ADF$.

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 Bamikole et al. (2019). The modified in vitro fermentation procedure of Navarro-Villa et al. (2011) was adopted as described by. A phosphate – bicarbonate buffer (Mould et al., 2005) used (g/L) were: 1.98 $Na_2HPO_4 \cdot 12H_2O$, 1.302 KH_2PO_4 , 0.105 $MgCl_2 \cdot 6H_2O$, 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°C 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, 4ml of 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°C for 24 hrs and then to constant weight to determine in vitro dry matter disappearance (IDMD).

Calculation for post incubation parameters

Organic matter digestibility (OMD) was estimated as: $OMD = 14.88 + 0.889 GV + 0.45 CP + 0.651 ash$ (Menke and Steingass, 1988). Short-chain fatty acids (SCFA) were estimated as: $SCFA = 0.0239 GV - 0.0601$ (Getachew et al., 1999). Metabolizable energy (ME) was calculated as: $ME = 2.20 + 0.136 GV + 0.057 CP + 0.00029 CF$ (Menke and Steingass, 1988). The Fermentation Efficiency (FE) was calculated as: $Fermentation Efficiency (FE) = Dry matter Digestibility (g/kg) / Total Gas Volume (mL/g)$

Statistical analysis

The study was conducted using completely randomized design (CRD). All data obtained were subjected to the analysis of variance (ANOVA). Means were separated using Duncan's Multiple Range Test SAS (1999) package.

Results and discussion

Table 2 shows the proximate composition and cell wall fractions of roasted *Delonix regia* seed meal. The DM, CP, EE, CF, ash, NFE, NDF, ADF and HEM contents were 89.03, 21.28, 2.59, 3.47, 4.36, 58.33, 39.78, 25.27 and 14.57%DM, respectively. The 21.28% CP recorded for roasted DSM in this study was similar to 20.50% CP reported for *Delonix regia* seeds by Almede et al. (2010) and Kaga (2011). The DM and other chemical constituents of DSM are within the chemical components catalogued by Lamidi and Ogunkunle (2015) for ruminant feedstuffs in South-west Nigeria.

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Table 2: Proximate composition and cell wall fraction (%DM) of roasted *Delonix regia* seeds meal

Parameters	Values
Dry matter	89.03
Crude protein	21.28
Ether extract	2.59
Crude fibre	3.47
Ash	4.36
Nitrogen free extract	58.33
Neutral detergent fibre	39.78
Acid detergent fibre	25.27
Hemicellulose	14.57

The nutrients profile and fiber fractions (DM%) of dietary inclusion of *Delonix regia* seeds meal was showed in Table 3. There was significant difference ($P<0.05$) in the nutrients and fiber fractions (CP, EE, ash, OM, NFE, NDF, ADF and hemicellulose) across the experimental treatments. Similar ($P>0.05$) DM was recorded across the experimental treatments and ranged between 92.00 – 92.50%. The CP decreases ($P<0.05$) as the inclusion of *Delonix regia* seeds meal increases, CP ranged between 10.50 – 13.60%.

T4 had highest ($P<0.05$) content of EE followed by $T3 > T2 > T1$ in that order, the value ranges between 7.01 – 10.30%. The ash content was significantly ($P<0.05$) higher in T2 (12.50%), similar ($P>0.05$) ash content was recorded for T1, T3 and T4. The NFE, NDF and hemicellulose were similar ($P>0.05$) in T1 and T4. The CP contents of the experimental treatments (10.50 - 13.60%) were above the critical lower limit (7% CP) which forage intake by ruminants and rumen microbial activity could be negatively affected (Van Soest, 1994 and Norton, 2003). The CP content recorded for this experimental treatments was far above the 7 to 8 % CP suggested as threshold for sufficient utilization of feed by MacDonald *et al.* (1995). Gaterby (2002) indicated 10 - 12% CP as a moderate level for ruminant production. Therefore, the experimental treatments 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 acids (Trevaskis *et al.* 2001; Lamidi and Ogunkunle, 2016) which in turn would facilitate microbial protein synthesis (Lamidi and Aina, 2013) and improve the production of meat, milk, skin and hide for man uses.

Similar content of Nitrogen free extract (NFE) was recorded for T1(35%PKC) and T4 (35%DSM) it is an indicator that DSM can be substitute for PKC. The NFE is an indicator of carbohydrate content of feedstuff or ingredient that is soluble or easily digestible and available for animal. It implies that the soluble carbohydrate could support the production of volatile fatty acids in the rumen during fermentation (Blummel *et al.*, 1997). Higher levels of NDF and lignin have been reported to have negative effect on DM intake and DM digestibility (Bakshi and Wadhwa, 2004). However, the NDF (21.11 – 22.70%) values observed for the experimental treatments were less than 60% which is considered safe for acceptable intakes of forage in ruminants (Meissner *et al.*, 1991). The level of ADF gives an indication of digestibility and energy content of forage or feed ingredient. It has been established that the energy level of forages declines as the ADF level increases (Iyere, *et al.*, 2019). Shroeder (2004) states that as the ADF content increases, forage digestibility decreases. Van Soest (1967) also stated that

as NDF level increases there is a decrease in the utilization of fibre. Ball *et al.* (2007) classified forages with ADF values greater than 43.00 – 45.00% as low quality forages. This attributes can also be used to describe other feed stuffs, ration or concentrate for livestock production, in lieu of this dietary inclusion of *Delonix regia* seeds for ruminant animal is in order. The higher level of ash recorded in the experimental treatments was an indication of high mineral content in the treatments, which

will be in line for body metabolism and other functions. Animashaun *et al.* (2006) reported that higher level of ash content inherent in the forages is an indication that the forage is rich in minerals which are essential in the formation and function of bloods and bones.

The 24hrs. gas production for dietary inclusion of *Delonix regia* seeds meal is indicated in Fig. 1. The highest gas volume (49.33ml) was recorded in T4, followed by T1, T3 and T2 with 45.33, 44.00 and 41.33, respectively.

Table 3: Nutrients profile and fiber fractions (%) of dietary inclusion of *Delonix regia* seeds meal

Parameters	Experimental treatments				SEM
	T1	T2	T3	T4	
Dry matter	92.00	92.50	92.01	92.04	0.21
Crude protein	13.60 ^a	13.00 ^b	12.75 ^b	10.50 ^c	0.72
Ether extract	7.01 ^c	7.54 ^{bc}	8.85 ^b	10.30 ^a	0.63
Ash	5.00 ^c	12.50 ^a	7.50 ^b	7.50 ^b	0.96
Organic matter	95.00 ^a	87.50 ^b	92.50 ^b	95.00 ^a	0.93
Nitrogen free extract	58.74 ^a	49.45 ^c	55.40 ^b	60.99 ^a	1.62
Neutral detergent fibre	21.14 ^{ab}	22.66 ^a	22.70 ^a	21.11 ^b	0.60
Acid detergent fibre	19.77 ^a	18.62 ^b	18.74 ^b	18.89 ^b	0.36
Hemicellulose	2.34 ^c	4.03 ^a	3.96 ^b	2.21 ^c	0.41

a, b, c Means on the same row with different superscripts differ significantly ($P < 0.05$). T1= Palm kernel cake 35 %/ *Delonix regia* seeds meal 0%; T2= Palm kernel cake 20 % / *Delonix regia* seeds meal 15%; T3= Palm kernel cake 15 % / *Delonix regia* seeds meal 20% and T4 = Palm kernel cake 0 % / *Delonix regia* seeds meal 35 %. SEM = Standard error of mean

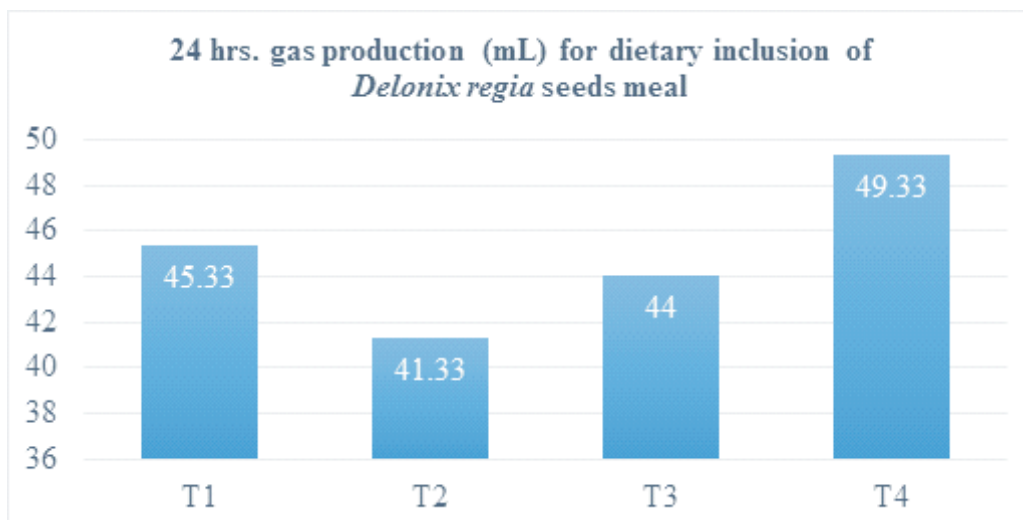


Fig 1: The 24 hrs. gas production for dietary inclusion of *Delonix regia* seeds meal

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Table 4 indicated the *in-vitro* fermentation characteristics of dietary inclusion of *Delonix regia* seeds meal. The methane gas volume was significantly influenced with the dietary inclusion of *Delonix regia* seeds meal across the experimental treatments, T2 had the highest volume of CH₄ (0.61mL, least was recorded for T1 (0.47 mL). There was no significant difference (P>0.05) in the CH₄ percentage, CH₄ reduction percentage, *in-vitro* dry matter digestibility (IDMD), *in-vitro* organic matter digestibility (IOMD), metabolizable energy and fermentation efficiency across the experimental treatments, the values ranges between 21.33 – 26.67%, 84.85 – 87.88%, 0.93 – 1.12Mml, 61.00 – 82.30%, 62.48 – 69.53%, 9.03 – 10.12 and 1.48 – 1.81, respectively. The significant results observed in methane gas volume might be due to the fact that different quantity of DSM and PKC in the experimental treatments provides different protein to meet microbial need in the rumen. However, the volume of CH₄ observed in the present study was lower than the report

of Isah (2012) for urea-molasses feed-block and what catalogued for some spices and medicinal plants in Edo and Rivers State for reducing enteric methane production in ruminant by Bamikole *et al.* (2019). The gas production is a nutritionally wasteful product (Mauricio *et al.*, 1999) but provides a useful basis from which ME, OMD and SCFA may be predicted (Fajemisin *et al.*, 2015). The range of the IDMD (61.00 – 82.30%) and IOMD (62.48 – 69.53%) recorded in this study was higher than IDMD (16.33 – 23.54g/100g) and IOMD (41.15 – 62.30%) reported for guinea grass and sweet potato peels silage by Lamidi and Akhigbe (2018), also higher than IOMD (16.33 – 24.37%) observed for Panicum and *Delonix regia* seeds silage reported by Lamidi and Ojo (2018). Meanwhile, similar value of SCFA in the experimental treatments probably showed an increased proportion of acetate and butyrate but may mean a decrease in propionate production (Babayemi *et al.*, 2006). Ruminant production of VFA contributes about 70% of the ruminant animals (Medjekal *et al.*, 2017).

Table 4: In-vitro fermentation characteristics of dietary inclusion of *Delonix regia* seeds meal

Parameters	Experimental treatments				
	T1	T2	T3	T4	SEM
Methane gas volume (mL)	0.47 ^c	0.61 ^a	0.53 ^{ab}	0.54 ^{ab}	0.02
Methane percentage (%)	21.33	24.67	23.33	26.67	0.51
Methane reduction percentage (%)	87.88	85.98	86.74	84.85	0.96
Short chain fatty acid (Mmol)	1.02	0.93	0.99	1.12	0.04
Dry matter digestibility (%)	66.93	61.00	78.03	82.30	1.63
Organic matter digestibility (%)	66.01	62.48	64.84	69.53	1.40
Metabolizable energy (MJ/kg DM)	9.58	9.03	9.40	10.12	0.22
Fermentation efficiency	1.48	1.48	1.81	1.67	0.05

a, b Means on the same row with different superscripts differ significantly (P<0.05). T1= Palm kernel cake 35/ *Delonix regia* seeds meal 0%; T2= Palm kernel cake 20/ *Delonix regia* seeds meal 15%; T3= Palm kernel cake 15/ *Delonix regia* seeds meal 20% and T4 = Palm kernel cake 0/ *Delonix regia* seeds meal 35%. SEM = Standard error of mean

Conclusion and recommendations

Conclusively, the nutrients profile of the experimental treatments justifies utilization of *Delonix regia* seeds meal for sustainable ruminant animal production considering the CP (10.50 – 13.60%). The similar value

observed for the *in-vitro* fermentation characteristics is an indication that inclusion of *Delonix regia* seeds meal (DSM) will be a perfect feed resources for ruminant production compared to other conversional feed resources such as palm

kernel cake (PKC). Furthermore, considering the higher value of *in-vitro* dry matter digestibility (IDMD), *in-vitro* organic matter digestibility (IOMD) and metabolizable energy recorded for T4 35% inclusion of *Delonix regia* seeds meal is recommended for sustainable ruminant production.

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Received: 4th August, 2019

Accepted: 18th December, 2019