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Nutritional Contents of Processed *Delonix regia* Seeds Incubated In Vitro with *Pennisetum purpureum*.

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ABSTRACT

The effect of processing on nutritional values of Delonix regia seeds incubated with Pennisetum purpureum in terms of proximate, mineral, anti-nutritional compositions, gas production characteristics, methane production, predicted metabolizable energy, organic matter digestibility and short chain fatty acids production were determined. Five treatments (T1, mixture of soaked Delonix regia seed and Pennisetum purpureum; T2, mixture of raw Delonix regia seed and Pennisetum purpureum; T3, mixture of roasted Delonix regia seed and Pennisetum purpureum; T4, mixture of boiled Delonix regia seed and Pennisetum purpureum, T5 (100% Pennisetum purpureum which serves as control) were allowed. In vitro gas production techniques for 24 hours were used to determine the nutritive value of processed 50% Delonix regia seed and 50% Pennisetum purpureum. The crude protein ranged from 13.38 to 15.71g/100g DM among the treatments while crude fibre was between 20.10 and 21.40g/100g DM, ether extract ranged from 1.90 to 3.12g/100g DM and ash 7.77 to 12.34g/100g DM. Calcium ranged from (2.34 - 3.61%), magnesium ranged (2.90 – 3.48%), sodium (2.05 – 2.91%) and phosphorus (0.88 – 1.12%). It was observed generally that the raw recorded the highest among the treatments while the values obtained for the heat treated seeds were lower than the raw. Oxalates ranged between (0.23% - 0.32%), phytates (0.29 – 0.43%), tannin (0.03 – 0.08%) and saponnin (0.36 – 0.49%). Methane (ml/200mg DM) production indicated T3 (50% roasted Delonix regia seed and 50% Pennisetum purpureum) was highest. The potential gas production 'a+b' ranged from 12.33 to 28.33mL/200mg DM). The highest potential gas production 'a+b' value of 28.33mL/200mg DM was obtained in T1 compared to other dietary treatment. Dry matter digestibility (DMD) ranged between 70.23 – 91.02% while the rate of fermentation was between 0.51 and 1.18ml/hr. The rate of fermentation was directly proportional to dry matter digestibility (DMD); the soaked recorded the highest value. Processing enhanced the nutritional contents of Delonix regia seeds. The result obtained shows that there were significant differences (p<0.05) among the treatments which indicated that T1 (50% soaked Delonix regia seed and 50% Pennisetum purpureum) has the highest value of Metabolisable Energy (ME), Organic Matter Digestibility (OMD), Short chain fatty acids (SCFA) have the potential benefit which can be used as supplement for ruminant feeding during dry season.

Key words: Processing, Nutritional Contents, *Delonix regia* seeds, *Pennisetum purpureum*.

INTRODUCTION

The conventional protein feedstuffs for livestock are scarce and not readily available, because the feedstuffs are competed for by varieties of animals and human. These feedstuffs could be substituted for by seeds of multipurpose trees that are high in crude protein. Ruminants are good at converting grasses, legumes and browse plants that are totally inedible to man to flesh. Limited and inadequate supply of forage with low quality feed in dry season eventually results in retarded growth of animals and reduction in milk and meat production (Babayemi *et al.*, 2004). Feeding animals with grass / legumes mixtures have been reported to give better animal performance and productivity than feeding grasses or legumes alone because the nutrient intake is balanced. Producing and properly preserving high quality grasses and legumes can reduce the costs, thereby making feed readily available and improving productivity. (Schroeder, 2004)

Elephant grass is available in abundance in almost all ecological zones in Nigeria and can be conserved for dry season feeding. *Delonix regia* (Caesalpinidae) wild plant otherwise called flame of the forest originated from America (Madagascar) but found wild or as ornamental plants in various parts of the world including Nigeria (Purseglove, 1994). It is an ornamental, leguminous plant, which produces tones of pods containing seed in the fruiting season (Keay *et al.*, 1964). These seeds are left unutilized since they are neither consumed by any animal nor utilized for any other medical purposes. Seeding is abundant and germination is free. The flowers are usually red but sometimes bear a paler orange red flower and flowering is spontaneous, it is one of the most spectacular flowering trees of the tropics. It is widely grown ornamental leguminous plant, which produces 25-40cm long pods containing seed which laboratory analysis has reported to contain crude protein 36.92%, crude lipid 4.17%, and crude fibre 11.39% (Grant *et al.*, 1991). Report also showed that the seed extracted exhibited low haemagglutinin activity and contains non-toxic lectins. Flamboyant seed meal compare favourably with mechanically extracted groundnut cake meal in term of crude protein content (NRC, 1993). Heat treatment is employed to dry certain plant product and to improve quality of pelletized feed. Hence, heat treatment can improve the nutritional value of *Delonix regia* seed by reducing the anti-nutritional factors. The nutritive value of ruminant feed is determined by the concentration of its chemical component as well as rate and extent of digestion. In vitro gas methods used in estimating the digestibility and metabolisable energy (ME) of ruminant feeds (Menke and Steingass, 1988) measuring digestion of insoluble carbohydrate based on the assumption that the amount of gas produced from a feed incubation reflects the production of short chain fatty acids (SCFA) which are the major sources of energy for ruminants. This study therefore determined the effect of processing on the nutritional value of mixture of *Delonix regia* seed meal and *Pennisetum purpureum* in terms of proximate, mineral, anti-nutritional compositions and in vitro gas production.

MATERIALS AND METHOD

The in vitro study was carried out at the Central Laboratory of the Department of Animal Science, University of Ibadan, Nigeria. *Delonix regia* pods were harvested within and outside Moor- Plantation Ibadan and the seeds were removed from the pods manually. Also, four weeks re-growth of elephant grass (*Pennisetum purpureum*) was obtained from existing pastures at Moor Plantation Ibadan and mixed at ratio 50:50 with *Delonix regia* seed meal. *Delonix regia* seeds and *Pennisetum purpureum* were divided into five treatments: T1 (50% soaked *Delonix regia* seeds and 50% *Pennisetum purpureum*) 1kg of seeds was soaked in 2 litres of water for 24 hours, drained and sundried for 3 days. T2 (raw) which did not undergo any processing. T3 (roasted) 1kg of seeds was roasted in dry frying pan on a hot plate at 60°C for 45 minutes. T4 (boiled) 1kg of the seeds was poured into boiling water for 40 minutes, drained and sundried for 3 days. T5 four weeks re-growth of elephant grass were chopped and air dried.

Chemical Analysis: Each treatments samples (T1-T5) were ground in hammer mill separately and subjected to proximate analysis according to the standard method of (AOAC, 2001) to determine the percentage Crude Protein (CP), Ether Extract (EE), Crude Fibre (CF), Moisture content, Ash content and Nitrogen Free Extract (NFE). The mineral and anti-nutritional contents were also analysed using the standard procedure.

In Vitro Gas Production: Rumen fluid was obtained from five Red Sokoto goats using stomach tube before the morning feed. The use of rumen liquor and buffer (9.8g NaHCO₃+2.77g Na₂HPO₄+0.57g KCl+0.47g NaCl+0.12g MgSO₄.7H₂O+0.16g/litre CaCl₂.H₂O) (1:4, v/v) under continuous flushing with CO₂ for incubation was as reported by (Stephen, 2006). The gas production was measured at 3, 6, 9, 12,15,18,21 and 24 hour. After 24 hour post incubation, 6ml of 10M NaOH solution was introduced as described by Fievez *et al.*, (2005) to estimate methane. Metabolizable energy (ME,MJ/Kg DM) and Organic Matter Digestibility (OMD %) were estimated as established by Menke and Steingass, (1988) and short chain fatty acids (SCFA) was calculated as reported by Getachew *et al.*, (1998) using 24 h post incubation. $ME = 2.20 + 0.136^*Gv + 0.057^*CP + 0.0029^*CF$, $OMD = 14.88 + 0.889Gv + 0.45CP + 0.651XA$; $SCFA = 0.0239^*Gv - 0.0601$, where Gv, CP, CF, and XA are net gas production (ml/200mgDM), crude protein, crude fibre and ash of the incubated samples respectively.

Statistical Analysis. Data obtained was subjected to one way analysis of variance (ANOVA) using SAS, 1998. Means were compared using Duncan (1955) option of same software.

RESULTS AND DISCUSSIONS

Table 1 showed the proximate composition of mixture of processed *Delonix regia* seeds and *Pennisetum purpureum* at ratio 50:50. The dry matter ranged between 93.91- 94.86, the highest value was observed in T1 (94.86%) followed by T5 (94.72%), T4 (94.38%), T2 (94.09%) and the least was T3 (93.91%). Crude fibre ranged from 20.10-21.40% among the treatment with T2 recording the highest value (21.40%), T5 (21.20%) observed in this experiment were similar to 22.00% reported by Bake *et al.*,(2013). High level of crude fibre has been acknowledged by Odedire and Babayemi, (2007) to be inversely related to feed

digestibility and nutrient availability. Crude protein ranged from 13.38% - 15.71% with T4 (15.71%) recording the highest value and least value recorded in T2 (13.38%). 13.98% obtained for *Pennisetum purpureum* was higher than 8.60% reported by Ajayi, (2007). This could be as a result of age since samples used for this experiment were harvested at 4 weeks of re-growth. Grasses get lignified with age which has been shown to reduce their digestibility (Duke,1983). Ash content ranged between 7.77%-12.34%, the highest value observed in T5 (12.34%) and lowest value was T3 (7.77%).

Table1: Proximate composition (%) of processed *Delonix regia* seed and *Pennisetum purpureum* mixture at 50:50

Parameters	T1(soaked)	T2(raw)	T3(roasted)	T4(boiled)	T5(<i>P.purpureum</i>)
Dry matter	94.86	94.09	93.91	94.38	94.72
Crude fibre	20.40	21.40	20.29	20.10	21.20
Crude protein	14.01	13.38	13.76	15.71	13.98
Ash	7.87	7.93	7.77	8.09	12.34
Ether extract	2.38	3.12	2.11	2.83	1.90
Nitrogen Free Extract	55.34	54.17	56.07	53.27	50.58

Table 2 showed the in vitro gas production parameters of mixture of processed *Delonix regia* seeds and *Pennisetum purpureum* at ratio 50:50. The metabolizable energy (ME) ranged between 4.75 - 6.85, highest values of metabolizable energy was observed in T1 (6.85) and the least was T5 (4.75). The values of ME obtained among treatments were significant. The Organic Matter Digestibility ranged from (40.16 - 51.49%) with T5 (40.16) having the least value and T1 (51.49) observed the highest value. Short chain fatty acid ranged between 0.23-0.62, methane (ml 200mg/DM) production in this study ranged between 5.00-14.00, the highest value was obtained in T3 (14.00) followed by T2 (12.50) and the least value was T5 (5.00). There were significant difference ($p < 0.05$) among the treatments. Highest value of ME (6.85Mj/kg DM), OMD (51.49%) and SCFA (0.62mol) are consistently recorded for in T1 (50% soaked *Delonix regia* and 50% *Pennisetum purpureum*). This showed that mixture of soaked *Delonix regia* seeds and *Pennisetum purpureum* could have the highest potential to make energy available to ruminant which correlate with report of Ajayi, (2007) that higher short chain fatty acid (SCFA) or volatile fatty acid (VFA) such as butyrate and acetate suggest a potential to make energy available to ruminants.

Table 2: In vitro gas production (ml/200mg/DM) parameters of processed *Delonix regia* seeds incubated with *Pennisetum purpureum*.

Parameters	T1	T2	T3	T4	T5	SEM
ME(Mj/kg/DM)	6.85 ^a	5.47 ^{bc}	5.49 ^{bc}	6.24 ^b	4.75 ^c	2.10
SCFA(μ mol)	0.62 ^a	0.37 ^{bc}	0.37 ^{bc}	0.48 ^b	0.23 ^c	0.39
OMD(%)	51.49 ^a	42.06 ^{bc}	42.13 ^{bc}	47.36 ^b	40.16 ^c	0.33
CH ₄ (ml)	10.00 ^{bc}	12.50 ^b	14.00 ^a	11.00 ^{bc}	5.00 ^c	0.50

+SEM= standard error of mean,^{a,b,c} mean on the same row with different superscript are significantly different($p < 0.05$)

Table 3 showed the soluble 'a' fraction of the sample which ranged from 3.70-8.33, the value for the absolute 'a' used ideally reflects the fermentation of the soluble fraction; the extent of gas production 'b' and 'a+b' described the fermentation of the insoluble but degradable fraction of the sample. The values obtained in this study ranged between 8.63-20.00 and 12.33-28.33 respectively.

Table 3: In vitro gas production characteristics (ml/200mg) of processed *Delonix regia* seeds incubated with *Pennisetum purpureum*.

Parameters	T1	T2	T3	T4	T5	SEM
A	8.33 ^a	6.30 ^b	4.00 ^{bc}	8.30 ^{ba}	3.70 ^c	0.33
B	20.00 ^a	11.70 ^{bc}	14.00 ^b	14.36 ^{ab}	8.63 ^c	0.37
a+b	28.33 ^a	18.00 ^{bc}	18.00 ^{bc}	22.66 ^b	12.33 ^c	0.16

a= intercept (gas produced from the soluble fraction). b= gas production from the insoluble fraction. a+b= potential extent of gas production. SEM= Standard error of mean. ^{abc} mean on the same row with different superscript are significantly different (p<0.05). T1=50% soaked *Delonix regia* and 50% *Pennisetum purpureum*, T2=50% raw *Delonix regia* and 50% *Pennisetum purpureum*, T3=50% roasted *Delonix regia* and 50% *Pennisetum purpureum*, T4=50% boiled *Delonix regia* and 50% *Pennisetum purpureum*, T5=100% *Pennisetum purpureum*.

Table 4 showed the mineral content of the raw and the processed seeds. The values obtained for calcium, phosphorus, sodium and potassium declined with processing. Mineral decline in age has been reported in tropical grasses. (Babayemi *et al.*, 2006 and McDonald *et al.*, 1995). This decline in age may be due to the effect of dilution of these elements in a great quantity of dry matter that is produced and accumulated with advancing age. (Gomide, 1978) associated the high concentration of Phosphorus and potassium in young tissues to their being mobile and thus easily translocate from the oldest tissue to the young ones.

The values obtained for Calcium (2.34-3.61%) in this study were higher than the range (0.40-1.5%) recommended for goats and 1.6% recommended for lactating goats (NRC, 1981). The values (0.88-1.12%) obtained for Phosphorus were above minimum range of 0.15% recommended for ruminant (NRC, 1981). Sodium Na content ranged from (2.05-2.91%) and was within the recommended requirement for ruminants (NRC, 1981). Values (2.90-3.48%) obtained for Magnesium Mg was above the 0.80% recommended for lactating goats and 0.50% recommended for maintenance and non-lactating goats (NRC, 1981). It was observed generally that the raw recorded the highest values among the treatments while the values obtained for the heat treated seeds were lower than the raw this signified that heating had reducing effects on the mineral contents.

Table 4: Mineral contents (g/100g) of processed *Delonix regia* seeds and *Pennisetum purpureum* mixture at 50:50.

Treatments	T1	T2	T3	T4	T5	±sem
Calcium	3.58 ^b	3.61 ^a	3.28 ^{bc}	3.33 ^{bc}	2.34 ^c	0.84
Magnesium	3.35 ^b	3.48 ^a	3.25 ^c	3.32 ^b	2.90 ^c	1.02
Sodium	2.28 ^b	2.91 ^a	2.12 ^{bc}	2.05 ^c	2.06 ^c	0.37
Phosphorus	0.95 ^{bc}	1.12 ^a	0.88 ^c	0.92 ^{bc}	1.09 ^b	0.24

SEM=standard errors of mean, a,b,c mean same superscript on the same row are not significantly different. p>0.05

The concentrations of some of the anti-nutrients were given in Table 5. Oxalates ranged between (0.23-0.32%), phytates (0.29-0.43%), tannin (0.03 – 0.08%) and saponnin (0.36 – 0.49%). The raw recorded the highest values among the treatments. Francis *et al.*, (2001) reported that heat treatment substantially reduces and inactivates levels of secondary compounds in the seed. Results obtained in this present study tend to agree with their report. All the anti-nutritive factors parameters measured were lower in the treated seeds compared to raw.

The concentrations were decreasing with processing, the levels of saponnins were lower than values (0.85%) reported for *Pennisetum purpureum* (Okaraonye and Ikewuchi, 2009) and higher than values reported for *Panicum maximum* (Ajayi, 2007). Saponnins reduce intake of the feed, and uptake of certain nutrients including glucose and cholesterol. From the level obtained in this study it is not likely that the saponnin content of mixture of processed *Delonix regia* and *Pennisetum purpureum* will affect its nutritional potentials to any significant extent. The levels of tannins reported here were lower than values (28.64%) reported for *Pennisetum purpureum* (Okaraonye and Ikewuchi, 2009) and *Panicum maximum* (Ajayi, 2007). The level of tannin which adversely affect digestibility in sheep and cattle is between 2% and 5% (Diagayete and Huss; 1981). Goats are known to have threshold capacity of about 9% dietary tannin (Natis and Malachek, 1981). The levels of oxalates observed here ranged from 0.23mg/100g to 0.32mg/100g and is unlikely to pose toxicity problems, since it is below 2-5g (Oke, 1969). Oxalate affects Calcium and Magnesium metabolism (Onwuka,1983) but ruminants can consume considerable amounts of high oxalate plants without adverse effects due principally to microbial decomposition in the rumen (Oke,1969). The phytate levels observed were higher than (0.16%) reported for *Pennisetum purpureum*. The knowledge of the phytate level in feeds is necessary because high concentration can cause adverse effects on the digestibility of minerals. (Nwokolo and Bragg, 1977). Phytate forms stable complexes with Cu^{2+} , Zn^{2+} , Co^{2+} , Mn^{2+} , Fe^{2+} , and Ca^{2+} .

Table 5: Anti-nutritional contents (g/100g) of processed *Delonix regia* seeds and *Pennisetum purpureum* mixture at 50:50.

Treatments	T1	T2	T3	T4	T5	±sem
Oxalate	0.26 ^b	0.32 ^a	0.26 ^b	0.26 ^b	0.23 ^c	0.03
Phytate	0.38 ^b	0.43 ^a	0.34 ^{bc}	0.38 ^b	0.29 ^c	0.03
Tannin	0.05 ^b	0.08 ^a	0.04 ^b	0.03 ^c	0.04 ^b	0.01
Saponnin	0.36 ^b	0.49 ^a	0.38 ^b	0.36 ^b	0.37 ^b	0.02

SEM=standard errors of mean, a,b,c d mean same superscript on the same row are not significantly different.p>0.05

Figure.1 showed the rate of fermentation and the dry matter digestibility (DMD%) of the mixture of the processed seeds and the grass. The values obtained for the DMD% ranged from 70.23 – 91.02%, the soaked had the highest digestibility and rate. It was observed that the rate is directly proportional to the dry matter digestibility.

The rate of gas production ‘c’ ranged from 0.51-1.18. Babayemi *et al.*,(2004) reported that factors that determine the rate of gas production during fermentation depends on the nature and level of fibre, the presence of secondary metabolites and potency of rumen liquor for incubation; the mixture of *Delonix regia* seeds and *Pennisetum purpureum* will be higher than that of 100% *Pennisetum purpureum* grass in gas production.

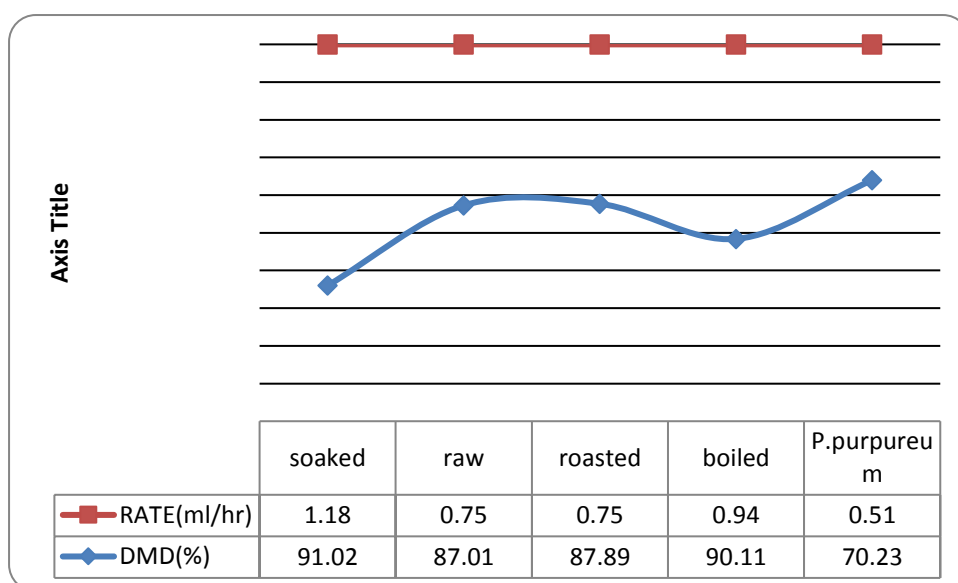


Figure 1. showing the rate of fermentation and dry matter digestibility (DMD%) of the mixture of processed *Delonix regia* seeds with *Pennisetum purpureum*.

CONCLUSION: The processing of *Delonix regia* seeds improved the nutritional composition by increasing the crude protein, reducing the fibre and anti-nutritional contents of the seeds. The rate of fermentation and dry matter digestibility were also enhanced by the processing techniques, most especially the soaked. *Delonix regia* seeds with *Pennisetum purpureum* can be processed and use as supplement in livestock feed. The result obtained T1 (50% soaked seeds and 50% *Pennisetum purpureum*) showed the highest potential gas production including the metabolizable energy (ME), organic matter digestibility (OMD) and short chain fatty acid (SCFA) compared to other treatment.

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