Nutritional contents of *Panicum maximum* incubated in vitro with processed *Enterolobium cyclocapum.*

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ABSTRACT

The study was carried out to determine the proximate composition, anti-nutritional factors, mineral content, and in vitro gas production characteristics and parameters of processed Enterolobium cyclocapum seeds and Panicum maximum at ratio 50:50. Enterolobium cyclocapum seeds were harvested, unshelled and subjected to five treatments which include (T1 = 100% P. maximum; T2 = 50% P. maximum and 50% Raw E. cyclocapum; T3 = 50% Panicum maximum and 50% Boiled Enterolobium cyclocapum ; T4 = 50% Panicum maximum and 50% Roasted Enterolobium cyclocapum ; T5 = 50% Panicum maximum and 50% Soaked Enterolobium cyclocapum were allowed. In vitro gas production technique for 24 hours were used to determine the nutritive values of 50% Panicum maximum and 50% processed Enterolobium cyclocapum seed. All data collected were subjected to analysis of variance. The crude protein ranged from 15.82 - 24.31g/100g DM among the treatments while crude fibre was between 2.88 and 15.39g/100g DM, ether extract ranged from 1.60 to 3.33g/100g DM and ash 4.35 to 9.35g/100g DM. Calcium ranged from (8.90 – 20.86%), magnesium ranged (5.03 – 5.45%), sodium (1.99 – 2.47%) and phosphorus (1.36 – 2.12%). It was observed generally that T2 recorded the highest among the treatments while the values obtained for the heat treated seeds were lower than T2. Oxalates ranged between (0.12% - 0.19%), phytates (0.24 – 0.33%), tannin (0.03 – 0.05%) and saponnin (0.35 – 0.41%). Methane (15.50 ml/200mg DM) production indicated T2 (50% Panicum maximum and 50% Raw Enterolobium cyclocapum) was highest. The potential gas production 'a+b' ranged from 11.3 - 24.0mL/200mg DM. The highest potential gas production 'a+b' value of 24.0mL/200mg DM was obtained in T2 compared to other dietary treatment. Dry matter digestibility (DMD) ranged between 51 – 91% while the rate of fermentation was between 0.67 and 1.06ml/hr. The rate of fermentation was directly proportional to dry matter digestibility (DMD); (T5) recorded the highest value. Processing enhanced the nutritional contents of Enterolobium cyclocapum seeds. Result obtained shows that there were significant differences ($p<0.05$) among treatments which indicated that T4 (50% Panicum maximum and 50% Roasted Enterolobium cyclocapum) had the highest value (18.01) of Metabolisable Energy (ME), T1 (100% Panicum maximum) had the highest value (54.86%) of Organic Matter Digestibility (OMD) and T2 (0.51umol) Short chain fatty acids (SCFA) have the potential benefit which can be used as supplement for ruminant feeding during dry season.

Keyword: Nutritional contents, *Panicum maximum*, incubated, in vitro, processed *Enterolobium cyclocapum*.

INTRODUCTION

Poor nutrition has long been identified as one of the major constraints to livestock productivity in developing countries (Osuji *et al.*, 1993) this is due to fact that animals are fed predominantly on low quantity and quality feeds, which are deficient in nutrient essential for efficient microbial fermentation (Bamikole and Babayemi, 2004) especially during dry season resulting to low animal performance in terms of growth and reproduction. Period of dry season is always a stressful period for livestock as the environment is characterized by insufficient feed due to the minimal amount of rainfall, the negative effect of this period is obvious in the loss of weight, reduced milk production and high mortality rate of animals, incidence of disease outbreak is rampant as a result of low immunity arising from malnutrition. This situation causes nomadic farmers to travel long distance in search for greener pasture for their animals (Iyayi *et al*., 2003) and in the process, losing their animals to snake bite and exposure to harsh weather condition and great competition between human and animals in consumption of some concentrate seed like soyabean, maize e.tc. Studies have shown that the use of grasses such as *Panicum maximum* in the dry season alone is not adequate to allow optimum production. Seeds of *Enterolobium cyclocapum* have been successfully used for small ruminant production system which contain anti-nutritional secondary compounds with potential adverse effect such as inhibition of rumen microbial fermentation as well as decreased feed digestibility and animal performance (Min *et al*., 2003; Mneller-Harvey, 2006). Furthermore, the prohibitive cost of concentrate diets for ruminants in the tropics during the dry season necessitates continuous search for less expensive and high nutritive feedstuff. *Enterolobium cyclocapum* is a native legume tree found mainly in the deciduous lowland forest (Janzen, 1981). The legume is easily established and fast growing to maturity over a short period of time. Higher percentage of the tropical browse and shrub legume are seed and fruit bearing and had been reported to be high in crude protein and other nutrients (Babayemi *et al*., 2004) when degraded by the rumen microbial organisms for the production of volatile fatty acids. In vitro gas production technique is commonly used to determine the relationship between chemical composition, incubation time and digestibility of forage feed. It is less expensive, time consuming and allows for proper control of experimental condition which has been used to predict dry matter intake (Blummel and Orskov, 1993), digestibility (Khazaal *et al*., 1993) and metabolisable energy (Babayemi and Bamikole, 2006). This study therefore determined the nutritional contents of *Panicum maximum* incubated with processed *Enterolobium cyclocapum* seeds.

Materials and method

Collection of samples and experimental diets preparation.

The guinea grass (*Panicum maximum*) was cut from existing paddock at stem level and allow to re-grow for 4 weeks, harvested, chopped into smaller pieces and was air-dried and grounded into powdery form. *Enterolobium cyclocapum* seeds were harvested, unshell and subjected to four treatments; T1= 100% *Panicum maximum*, T2 = 50% *Panicum maximum* and 50% Raw *Enterolobium cyclocapum*, T3 = 50% *Panicum maximum* and 50% Boiled *Enterolobium cyclocapum*, T4 = 50% *Panicum maximum* and 50% Roasted *Enterolobium cyclocapum*, T5 = 50% *Panicum maximum* and 50% Soaked *Enterolobium cyclocapum*. T5 – 1kg of *Enterolobium cyclocapum* seeds was soaked into 2 litres of water for 24 hours, drained and sun dried for 3 days. T3 – 1kg of *Enterolobium cyclocapum* was poured into boiling water at 100^{o}c for 20 minutes. T4 – 1kg of *Enterolobium cyclocapum* seeds was roasted for 40 minutes at 60^{o}c and allowed to cool. It was then ground to powdery form individually according to the treatments. Samples of the grass and processed seeds

were mixed at ratio 50:50 and then taken to the laboratory for proximate composition, mineral content, in vitro analysis and anti- nutritional factors.

Chemical analysis

Known weight of each air-dried sample was oven dried at 65^0C to a constant weight for dry matter determination (DM). Proximate analysis; crude protein, crude fibre, ether extract and ash were analysed according to the standard methods of (AOAC, 1995).

In vitro gas production and statistical analysis

Rumen fluid was obtained from four West African dwarf goats using suctions tube before the morning feed. The animals were fed with concentrate diets (40% corn, 10% wheat offal, 10% palm kernel cake, 20% groundnut cake, 5% soya bean meal, 10% dried brewers grain, 1% common salt, 3.75% oyster shell and 0.25% fish meal). The use of rumen liquor and buffer (9.8g $NaHCO_3$+2.77g Na_2HPO4+0.57g KCl+0.47g NaCl+0.12g $MgSO4.7H_2O$+0.16g/litre $CaCl_2.H_2O$) (1:4, v/v) under continuous flushing with CO_2 for incubation was as reported by Menke and Steingass, (1988). The gas production was measured at 3, 6,9,12,15,18,21 and 24 hour. After 24 hour post incubation 4ml of 10M NaOH solution was introduced as described (Fievez *et al*., 2005) to estimate methane. Metabolizable energy (ME,MJ/KgDM) 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. Data obtained were subjected to analysis of variance (ANOVA) in a completely randomized design and means were compared where significant using Duncan Multiple Range F- test (SAS, 2002).

Results and discussion

Table 1: Proximate composition (g/100g DM) of *Panicum maximum* and processed *Enterolobium cyclocapum* mixture at ratio 50:50.

Treatment	Dry matter	Crude protein	Ether extract	Crude fibre	Ash	Nitrogen free extract
T1	92.13	19.33^c	2.30^b	15.39^a	4.35^c	47.36^d
T2	93.61	15.82^d	1.60^d	9.36^b	9.35^a	66.75^a
T3	93.36	24.31^a	3.33^a	2.88^d	8.93^b	56.71^c
T4	93.58	22.78^b	2.40^b	6.33^c	8.92^b	59.24^b
T5	93.47	23.42^b	2.06^c	3.17^d	9.10^a	58.81^b

T1 = 100% Panicum maximum, T2= 50% Panicum maximum + 50% Raw Enterolobium cyclocapum, , T3 = 50% P.maximum + 50% Boiled Enterolobium cyclocapum, T4 = 50% P.maximum + 50% Roasted Enterolobium cyclocapum, T5 = 50% P.maximum + 50% Soaked Enterolobium cyclocapum . $^{a, b,c}$ means with different superscripts on the same row are significantly different $p > 0.05$

The result of proximate composition is presented in Table 1. It was observed that there were significant variations in the values of determined nutrients with the exception of dry matter (DM). The dry matter DM of the experimental diets ranged from 92.13 in T1 to 93.61g/100g in T2 while crude protein CP of the diets ranged from 15.82 to 24.31g/100g. It was observed that crude protein increased in mixture of *Panicum maximum* and processed *Enterolobium cyclocapum* which implied

that the diets could be good sources of protein that would meet the requirement of small ruminants for growth and production. The improved crude protein CP values observed agreed with the findings of Ezenwa and Aken'ova (1988) that the effect of legume components in a grass-legume mixture would increase the protein quality of the mixture. The crude fibre values observed in this study ranged from 2.88 (T3) – 15.39g/100g (T1) and these values were lower than the values (47.80 – 51.20%) reported for other forage leguminous plants (Ifut, 1987). T2 had the highest ash value (9.35g/100g) which was lower than the value reported by Fajemisin *et al*, (2015). The lowest nitrogen free extract (NFE) value (47.36g/100g) was observed in T1 (100% *Panicum maximum)* which was significantly ($P < 0.05$) different from the values recorded for other treatments, this implied that the soluble carbohydrates could support the production of volatile fatty acids in the rumen during fermentation (Blummel *et al*, 1997b).

Table 2: **Anti-nutritional contents of processed *Enterolobium cyclocapum* and *Panicum maximum* at ratio 50: 50.**

Treatments	Oxalate	Phytate	Tanin	Saponin
T1	0.19^{a}	0.33^{a}	0.05^{a}	0.41^{a}
T2	0.16^{ab}	0.31^{ab}	0.04^{ab}	0.39^{b}
T3	0.17^{ab}	0.25^{b}	0.03^{bc}	0.36^{bc}
T4	0.18^{ab}	0.24_{bc}	0.03^{bc}	0.35^{bc}
T5	0.12^{b}	0.24^{bc}	0.03^{bc}	0.36^{bc}
±SEM	0.06	0.081	0.02	0.11

T1 = 100% Panicum maximum, T2= 50% Panicum maximum + 50% Raw Enterolobium cyclocapum, , T3 = 50% P.maximum + 50% Boiled Enterolobium cyclocapum, T4 = 50% P.maximum + 50% Roasted Enterolobium cyclocapum, T5 = 50% P.maximum + 50% Soaked Enterolobium cyclocapum . SEM means standard error of means, $^{a,\ b,c}$ means with the same superscripts on the same column are not significantly different $p > 0.05$

The concentrations of some of the anti-nutrients in *Enterolobium cyclocapum* and *Panicum maximum* mixture at ratio 50:50 were presented in Table 2. Oxalates ranged between 0.12% (T5) - 0.19% (T1), phytates ranged between 0.24% (T4 & T5) – 0.33% (T1), tannin ranged from 0.03% -0.05% and saponin ranged between 0.35% (T4) – 0.41% (T1). The processing on the treatments bring about significant decreased in the composition of anti-nutritive factors. T1(100% Panicum maximum) had the highest values among the treatments. All the processed treatment (T3,T4, and T5) had a low anti-nitritive factors compared to T1(100% Panicum maximum). Francis *et al*. (2001) reported that heat treatment substantially reduces and inactivates levels of secondary compounds in the seed. Saponins reduce intake of feed, and uptake of certain nutrients including glucose and cholesterol. From the level obtained in this study, it is not likely that the saponin content of mixture of processed *Enterolobium cyclocapum* and *Panicum maximum* 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 0.12mg/100g to 0.19mg/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 3 shows the mineral content (mg/100g) of processed *Enterolobium cyclocapum* and *Panicum maximum* mixture at 50:50. The values obtained for calcium, phosphorus, sodium and potassium declined with processing. (McDonald *et al.,* 1995 and Babayemi *et al.,*2006).
The values obtained for Calcium (8.90-20.86%) 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 (1.36-2.12%) obtained for Phosphorus were above minimum range of 0.15% recommended for ruminant (NRC, 1981). Sodium Na content ranged from (1.99-2.47%) and was within the recommended requirement for ruminants (NRC, 1981). Values (5.03-5.45%) 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 T1 and T2 recorded the highest values among the treatments while the values obtained for the heat treated seeds were lower than T2 , this signified that heating had reducing effects on the mineral contents.

Table 4: In vitro gas production (200mg/DM) parameters of *Panicum maximum* incubated with processed *Enterolobium cyclocapum* seed at ratio 50:50

Treatments	ME(Kg/DM)	SCFA(µmol)	OMD%	CH4(ml)
1	17.61^{b}	0.21^{b}	54.86^{a}	8.00^{c}
2	14.51^{c}	0.51^{a}	49.42^{b}	15.50^{a}
3	13.46^{d}	0.33^{ab}	46.15^{c}	10.50^{c}
4	18.01^{a}	0.43^{ab}	49.30^{ab}	10.00^{d}
5	13.46^{d}	0.32^{ab}	45.57^{d}	12.00^{b}
S.E.M	4.65	0.30	9.29	7.50

T1 = 100% Panicum maximum, T2= 50% Panicum maximum + 50% Raw Enterolobium cyclocapum, , T3 = 50% P.maximum + 50% Boiled Enterolobium cyclocapum, T4 = 50% P.maximum + 50% Roasted Enterolobium cyclocapum, T5 = 50% P.maximum + 50% Soaked Enterolobium cyclocapum . SEM means standard error of means, $^{a,\ b,c,d}$ means with the same superscripts on the same column are not significantly different $p > 0.05$

Table 4 showed the in vitro gas production parameters of *Panicum maximum* incubated with processed *Enterolobium cyclocapum* seed at ratio 50:50. The metabolizable energy (ME) ranged between 13.46 – 18.01, highest value of metabolizable energy was observed in T4 (18.01) and the least were in T3 and T5 (13.46). The values of ME obtained among treatments were significant. The Organic Matter Digestibility ranged from (45.57 – 54.86%) with T5 (45.57) having the least value and T1 (54.86) observed the highest value. Short chain fatty acid ranged between 0.21-0.51, methane (ml 200mg/DM) production in this study ranged between 8.00-15.50, the highest value was obtained in T2 (15.50) followed by T5 (12.00) and the least value was T1 (8.00). There were significant difference ($p<0.05$) among the treatments. Highest value of ME (18.01Mj/kg DM), OMD (54.86%) and SCFA (0.51mol) are recorded for in T4, T1 and T2 respectively. This suggests the potential of the treatments to make energy available to ruminants.

Table 5: In vitro parameters (200mg/DM) of *Panicum maximum* and processed *Enterolobium cyclocapum* seed at ratio 50:50

Treatments	A	B	c	a+b
T1	3.60^{c}	7.73^{c}	0.67^{bc}	11.30^{d}
T2	6.30^{ab}	17.70^{a}	0.68^{b}	24.00^{a}
T3	4.33^{c}	12.00^{c}	1.00^{ab}	16.33^{c}
T4	6.66^{a}	14.00^{b}	0.86^{bc}	20.66^{b}
T5	5.00^{b}	11.00^{d}	1.06^{a}	16.00^{cd}
±SEM	3.06	9.97	0.39	12.66

a= intercept (gas produced from immediately soluble fraction). b= extent of gas production from insoluble but degradable fraction, c= rate of gas production at time (t), a+b= potential extent of gas production. *T1 = 100% Panicum maximum, T2= 50% Panicum maximum + 50% Raw Enterolobium cyclocapum, , T3 = 50% P.maximum + 50% Boiled Enterolobium cyclocapum, T4 = 50% P.maximum + 50% Roasted Enterolobium cyclocapum, T5 = 50% P.maximum + 50% Soaked Enterolobium cyclocapum* . SEM means standard error of means, $^{a,\ b,c,d}$ means with the same superscripts on the same column are not significantly different $p > 0.05$

Table 5 showed the soluble 'a' fraction of the sample which ranged from 3.60-6.66, 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 7.73-17.70 and 11.30-24.00 respectively.

CONCLUSION: *Panicum maximum* and processed *Enterolobium cyclocapum* seeds improved the nutritional composition by increasing the crude protein, reducing the fibre and anti- nutritional contents of the seeds, thus it can be used as supplement in livestock feed during dry season.

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