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Nutrient Composition and In Vitro Gas Production of False Yam (*Icacinaoliviformis*) Leaves

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ABSTRACT

*Icacinaoliviformis*is common shrub found in the Northern Regions of Ghana. It has received very little attention regarding its use as feed supplement for ruminants. This study was carried out to determine the chemical composition and potential digestibility of the leaves of *Icacinaoliviformis* (IOL) when incubated with or without ammonium hydrogen carbonate (NH_4HCO_3) in the media. The media with (NH_4HCO_3) was referred to as nitrogen sufficient (NS) and the one without (NH_4HCO3) was nitrogen deficient (ND). The results of the study showed that IOL had a dry matter content of 377.3g/Kg with a crude protein content of 173g/kg. The NDF, ADF and ADL were 439.5, 393.5 and 191.9g/kg respectively. The gross energy content was estimated to be 18.4MJ/Kg DM. There was no significant difference ($p>0.05$) between the ND and NS media for all the parameters measured except for ammonium nitrogen where the NS was significantly ($p=0.021$) higher than the ND. The potential degradability recorded for the IOL in both ND and NS media was 158.47 and 170.93 respectively. The rate of degradability of the ND was 0.055 and that of NS was 0.043. The pH and ammonium nitrogen recorded were all within the optimum range required for microbial cell synthesis and cellulolysis. The IOL could be included in the feed formulation for ruminants in Ghana.

Keywords: *Icacinaoliviformis*, *in vitro* gas, leaves, nitrogen deficient, nitrogen sufficient.

INTRODUCTION

Icacinaoliviformis is an underutilised shrub found in the savannah regions of Ghana. It is a drought tolerant plant and usually produces fresh leaves especially in the dry season when most conventional sources of forage are dry and of poor nutrient value. The plant has a huge tuber beneath and is sometimes referred to as false yam. The tuber sometimes weighs over 50kg and provides a rich source of starch in times of famine (Fay, 1987). The tuber has been

reported to contain protein and carbohydrate contents of 5.4% and 53.1% respectively (Dei *et al.*, 2011).The use of *I.oliviformis* in diet of human has been limited by the presence of a gum resin which makes up 0.9-2.8% of the tuber (NRI, 1987). The leaves of *Icacinaoliviformish* as not been investigated for its potential as feed for ruminant since the plant was discovered. The presence of plant secondary metabolites (PSM) in trees and shrubs of tropical origin makes the nitrogen in them insufficient for microbial degradation (Salem, 2005). This insufficient supply of nitrogen in the rumen has been attributed to the complex formed between PSM especially tannins and the dietary protein at a pH of 4.0. To 7.0 in the rumen (Osuga, *et al*., 2005; Min *et al*., 2005). Ansah *et al*. (2012) included the leaves in the diet of rabbits at 5% and 10 inclusion levels. The results of that study showed a better growth and digestibility from the animals on the 5% diet. The present study sought to investigate the potential digestibility and chemical composition of the *Icacinaoliviformis* leaves.

Hypothesis

Excluding nitrogen from the in vitro incubation media will not reduce the in vitro gas production (IVGP) of *Icacinaoliviformis* significantly.

MATERIALS AND METHODS

Study Area

Fresh leaves of *Icacinaoliviformis* (IOL) were harvested around the Nyankpala campus of the University for Development Studies during the early dry season (October to November, 2011). The leaves were then shade dried and milled to pass through 1mm sieve screen and packaged into plastic bags, sealed and transported to the Princes Margret laboratory of the Harper Adams University for nutrient analysis and in vitro gas studies.

Chemical Analysis

Fresh IOL samples (200.0g) were placed in an oven set to a temperature of 60^0C for a period of 48hours after the initial weight was recorded (AOAC, 2000). The new weight after drying was used to calculate the dry matter content of the samples and expressed in g/kg. The dry matter was determined at the Agriculture Sub-sector Improvement Project (AgSsIP) laboratory at the Nyankpala Campus of the University for Development Studies.

Shade dried milled IOL samples were analyzed for nitrogen and used for computing the crude protein content using the formulae Crude protein (g/kg DM) = total nitrogen (g/kg DM) x 6.25. The nitrogen content was determined by combusting 1.0g of each sample with oxygen using the Leco (FP-528-UK).

The nitrogen oxide after combustion is reduced to nitrogen and measured with a thermal conductivity detector with helium as a reference. Ash content was determined by burning 2.0g of the samples at a temperature of 550^0C for 4hours in a mufflefurnace (Carbolite, AAF 1100, Hope valley, England). The residue was weighed and used for computing the ash (AOAC, 2000). The ash content was subtracted from the dry matter content to get the organic matter content.

The ether content was extracted using petroleum ether (AOAC, 2000). The gross energy content was determined by combusting 2.0g of each sample using the bomb calorimeter (Parr 6200 Calorimeter-UK).The neutral detergent fiber, acid detergent fber and acid detergent lignin were analyzed according to the method of Goering and Van Soest (1970).

All chemical analyses were done at the nutrition laboratory of the *Princess Margaret Laboratories* of the Harper Adams University.

In vitro gas analysis and experimental design

The randomized complete block design was used with each treatment having 5 replicates. The method described by the odorou *et al*. (1991) was used for the *In vitro* gas studies. In this particular study, the nitrogen content of the media was varied in a way that in one media, ammonium hydrogen carbonate (NH_4HCO_3) was excluded to represent the nitrogen deficient media (ND) and the other media had ammonium hydrogen carbonate (NH_4HCO_3) representing the nitrogen sufficient media (NS). The NS media preparation was done according to the procedure of the odorou *et al.* (1991) and the ND was done according to the procedure of Getachew *et al.* (2000). Approximately 2.0g of IOL was weighed into a 250ml fischer and duran bottles and placed in an incubator (39^0C) overnight.

Rumen fluid was collected from 4 rams fitted with a fistula. The animals had an average weight of 95kg. They were fed *ad libitum* on wheat straw and offered 250 g/day of concentrate (Wynnstay ram master coarse mix, UK) at a rate of 1.1 x maintenance (AFRC 1993). The rams were group housed with straw as bedding and under constant light supply. Water was offered *ad libitum*.

Rumen fluid was collected 3-4hours post feeding through a suction process. The fluid was collected into a pre-warmed vacuum flask and quickly sent to the laboratory. The fluid from each of the four animals were pooled and strained using a four layer cheese cloth placed over a 5 liter conical flask with help of a funnel.

On the day of incubation the two media were placed in a water bath with the temperature set to 39^0C. Carbon dioxide was continuously flushed through each media to ensure they are anaerobic. The strained rumen fluid was mixed with media in a proportion of 1.4 liters rumen fluid to 10 liters media (Huntington *et al*., 1998). The rumen fluid formed 14% of the total incubation buffered rumen fluid.

Approximately 200ml of the buffered rumen fluid (Media + rumen fluid) was pumped into the pre-weighed samples in the vessels using a peristaltic pump, gassed for 5seconds, sealed with rubber corks and placed in the incubator set to 39^0C. The rubber corks were fitted with a needle and valve to aid in the measuring of pressure (gas accumulation).

Gas production from each vessel was measured using a pressure transducer. The pressure readings were taken at 0, 3, 6, 12, 18, 24, 36, 48, 60, 72 hours and converted from PSI to volume (ml/g DM). The pressure reading at time zero was assumed to be zero since there is no method available to measure gas production at time zero.

After each incubation period, the pH of the content of each bottle was measured. The content of each bottle was strained using a four layer cheese cloth. The liquid was stored for analysis of ammonium nitrogen.

Exactly 5ml of defrosted filtrate from the in vitro experiment was measured into digestion tube and 6ml of magnesium oxide added. The sample was steamed via a receiver solution prepared from 50g boric acid, 50ml bromocresol green and 35ml methyl red in 5L distilled water. This was back titrated using 5mM sulphuric acid to determine the color change. Ammonium nitrogen was calculated from the titer value obtained for each sample using the following formulae:

$$\textbf{Ammonium nitrogen (g/L)} = \frac{\textit{Sample titre (ml)}}{\textit{Weight of sample distilled (g)}} \times \mathbf{0.01401} \times \mathbf{0.01} \times \mathbf{1000}$$

Where 0.01401 represents the weight of nitrogen atom and 0.01 is the concentration (normality) of the acid solution.

Statistical Analysis

The gas measurements were then fitted to the exponential curve of Orskov and McDonald (1979) without an intercept using sigmaPlot 10th edition.

$$Y = b (1 - e^{-ct})$$

Where:
Y = gas volume at time t (ml)
b = asymptotic gas production (ml/g DM)
t = time (h)
c = fractional rate of gas production (ml/h)

The data was analyzed using the independent t-test in statistical package for social sciences (SPSS) 18th edition. The in vitro production curves were plotted using MS Excel, 2010.

RESULTS AND DISCUSSION

Table 1 shows the chemical composition of the *Icacinaoliviformis* leaf (IOL). The CP obtained was higher than what was reported by Dei *et al*. (2011) for *Icacinaoliviformis* tuber. The crude protein in IOL was above the 70g/kg DM required to enhance voluntary feed intake in ruminant (Nori *et al.*, 2009).

Table 1. Chemical composition of *Icacinaoliviformis* leaf

Composition	g/kg
DM	377.3±4.4
Ash	68.9±0.9
OM	308.4±0.5
CP	173.0±1.3
Ether	39.3±1.6
NDF	439.5±2.1
ADF	393.5±5.4
ADL	191.9±3.7
GE MJ/kg DM	18.4±0.2

The CP reported for the IOL was higher than what has been reported for most grasses and cereal based crop residue which serve as the main source of forage for free ranging ruminant in the dry season. Alhassan *et al*. (1999) reported that the protein content of most grasses drop from an average of 12.5% in the wet season to 3% in the dry season. Avornyo*et al*. (2000) reported a crude protein content of 3.3% for rice straw which is a common cereal based crop residue eaten by ruminant in the dry season. The neutral detergent fiber (NDF) and acid detergent fber (ADF) compared favourably with the values reported for some tropical legume shrubs (Zhou *et al*., 2011; McSweeny *et al*., 2009).

Effects of additional nitrogen on fermentation pattern

Results on the effect of additional nitrogen on the *in vitro* gas production (IVGP) are presented in Table 2. There was no significant difference between the 2 media on potential degradability (P=0.570), rate of degradability (P=0.156) and pH (P=0.764). There was a significant difference (P=0.021) between the two treatments for ammonium nitrogen.

Table 2. Effect of additional nitrogen on *in vitro* rumen degradability and ammonium nitrogen concentration of IOL

Media	C	a+b	pH	NH_4N (mg/l)
ND	0.055	158.47	6.74	268
NS	0.043	170.93	6.82	321
Sed	*0.074*	*20.77*	*0.248*	*0.174*
P.value	*0.156*	*0.570*	*0.764*	*0.021*

C=rate of degradability, a+b =potential degradability

The nitrogen sufficient (NS) media had a higher potential degradability than the nitrogen deficient (ND). Ammonium nitrogen of the incubation media was higher for the NS compared with the ND. The gas production for IOL in both ND and NS witnessed a sharp increase within the first 12hours. After 12hours, IOL in the NS media declined marginally and began to increase after 24hours (Fig1).

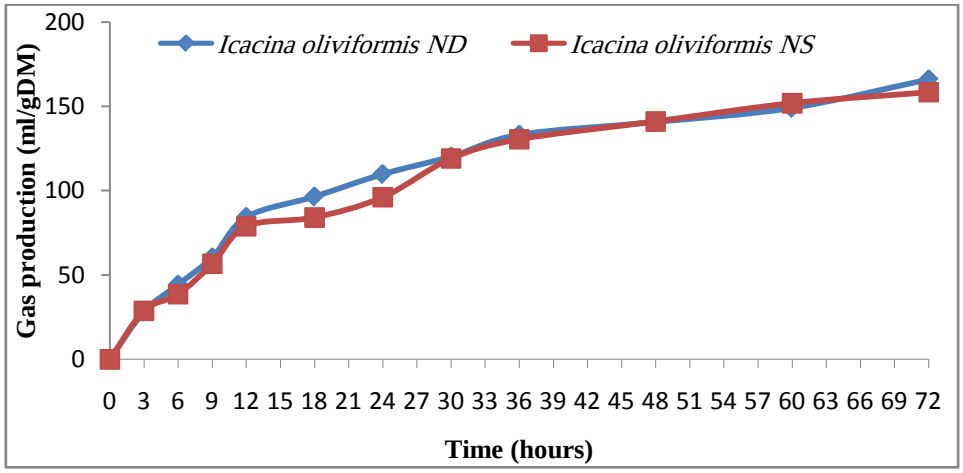

Figure 1. Cumulative *in vitro* gas production of *Icacinaoliviformis* leaf in nitrogen deficient and nitrogen sufficient media

Ammonium nitrogen concentration in the incubation media is a balance between degradation of dietary protein and the uptake of ammonium nitrogen for microbial cell synthesis (Hariadi and Santoso, 2009). The ammonium nitrogen recorded in the ND media could be due to the microbial degradation of the protein in the IOL substrate. Fiber degrading microbes in the rumen require ammonium nitrogen for cell synthesis in order to multiply and degrade the fiber to supply the volatile fatty acids for absorption (Russell *et al.*, 1992). Satter and Slyter (1974) reported that ruminal ammonia concentrations between 88 and 133mg/l of NH,-N in rumen fluid is the optimum to stimulate microbial protein synthesis and a maximum of 50mg/l is enough to stimulate microbial growth. Despite the significantly low ammonium nitrogen reported in ND, it was still within the range recommended by Satter and Slyter (1974). The high ammonium nitrogen obtained in NS could be due to the extra nitrogen added to the incubation media. The IVGP according to Lopez *et al*. (1998) and France *et al*. (2000) gives an indication of the extent to which the carbohydrate of the substrate is being fermented by anaerobic microbes from the rumen. The higher the IVGP, the higher the potential degradability of the substrate. The lack of significant difference in IVGP between the IOL in NS and ND media could suggest that the IOL has the potential to boost digestibility when fed to ruminants. It also indicates an inefficient utilization of the ammonium nitrogen in the NS media by the fiber degrading microbes. Despite the relatively high ammonium nitrogen in the

NS media which was partly due to the additional nitrogen added, it did not result in a significantly higher IVGP. The pH of the incubation media was not affected by the IOL substrate as they all fell within the optimum pH required for cellolysis and microbial protein synthesis (VanSoest, 1994; Russell *et al*., 1992).

CONCLUSION AND RECOMMENDATION

The addition of nitrogen to the incubation media did not significantly increase the in vitro gas production (IVGP) of IOL. The use of IOL as feed supplement has the potential to improve digestibility. Further study is recommended to determine the palatability and intake of IOL in ruminants.

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