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Preliminary studies on the nutritive potentials of some aquatic weeds as feedstuffs for small ruminants

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ABSTRACT: This study assessed the nutritive potential of *Eichhornia crassipes, Nymphaea lotus* and *Nymphaea maculata* as feeds for ruminants. These plants are menace to water ways and fishing activities along rivers: Niger-Benue located at Lokoja (Kogi State), Igbokoda riverine area of Ondo State and Agunla stream in Ipetu-Ijesa, Osun State, Nigeria respectively. The methodology involved are chemical characterization and *in vitro* gas production. The results indicated that species difference was significant ($p<0.05$) with regard to dry matter (DM), crude protein (CP), crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF) and cellulose compositions. Dry matter values ranged from 88.46% in *N. lotus* to 94.15% in *E. crassipes,* while gross energy (GE) values ranged between 15,14 KJ/100gDM *(N.lotus)* and 16.88 KJ/100gDM *(N. maculata).* Also, CF (15.08%) and NFE (55.61%) values were higher in *N. lotus* and *N. maculata* respectively. NDF, ADF and cellulose contents varied among the plants. Species difference was significant ($p<0.05$) for all minerals measured except calcium and magnesium. Values of alkaloid, saponin, oxalate, tannin and phenol were least in *E. crassipes* ($p<0.05$). The total gas and methane production, metabolizable energy (ME), short chain fatty acids (SCFA) and organic matter digestibility (OMD) values were similar ($p>0.05$). However, least values of methane (6.00ml), OMD (44.88%) and total gas production (11.00ml) were observed in *E. crassipes*, *N. maculata* and *E. crassipes* respectively, while the highest value of SCFA (0.18µmol) was observed in *N. maculata*. It could be concluded that from the basic data that the selected aquatic plants have potential as alternative feed resources for ruminants.

Key words: Aquatic weeds; degradability; feedstuff; nutritive potential; ruminants

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INTRODUCTION

The livestock industry plays a critical role in the economy of any nation especially a developing country like Nigeria. It contribute immensely to the agricultural sector and plays a vital role in the supply of animal protein to the populace (Ademosun, 1994). In addition, several studies have documented the importance of small ruminants (sheep and goats) as sources of food and fibre producers in the developing countries (Akinlade *et al.,* 2001; Alokan, 2008;

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Fajemisin *et al.,* 2012). However, they are constrained by scarcity and fluctuating quantity and quality of year-round feed supply particularly during dry season. The natural pastures drop in quality and quantity, especially energy and nitrogen content. As a consequence, feed intake declines and animal productivity decreases (Kritapon *et al*., 2005). It becomes imperative to source for alternative feed resources which are more efficient, economical and can be incorporated into ruminant diet with a view to reducing feed costs and hence, achieving a greater profit margin for the producer.

Aquatic weeds such as *Eichhornia crassipes, Nymphaea lotus* (linn) and *Nymphaea maculata schum* (thonn) could be obnoxious to the environment but they are rich sources of cellulose and other nutrients (Dairo, 1997). They have large leaves that float on water. *E. crassipes* is a member of pickerelweed (Pontederiacea), while *N. lotus* and *N. maculate* belong to the family Nymphaeceae (Joyce, 1990; Okezie and Aqyakwa, 1998).

These aquatic weeds could be used as a renewable source of energy and purification of sewage. Research has indicated that due to their high moisture content, they could be converted to silage by placing the chopped plants in a closed container and allowing them to undergo microbial fermentation for about a month. It could be fed fresh to ruminant animals like buffalo, cattle, goat, and sheep (Ozah, 2011). The chemical contents of aquatic plants appears not to be widely studied and the information on inclusion of these plants in livestock feeding systems and their replacement value for conventional feedstuff in ruminants feeding in Nigeria appears scanty. Therefore, the present study was designed to evaluate the nutritive potential of *Eichhornia crassipes, Nymphaea lotus* and *Nymphaea maculata* as possible alternative sources of feed and nutrients for small ruminants.

MATERIALS AND METHODS

Collection of aquatic weeds

Samples of *E. crassipes, N. lotus* and *N. maculata* were collected fresh from the confluence of rivers Niger and Benue (Lokoja, Kogi State), Ofara at Igbokoda (Ondo State) and Agunla stream at Ipetu-Ijesa (Osun State) The plants were washed, leaves were separated from the stem and roots were cut away. The plant samples were air-dried in the laboratory at room temperature for seven days, milled to pass through 1mm mesh and stored in different labeled containers.

Chemical analysis

Samples of the aquatic plants were oven dried at 105^0C to a constant weight for dry matter determination; crude protein, crude fibre, ether extract and ash were analyzed according to AOAC (1990). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined as described by Van Soest *et al*. (1991). The samples were also analyzed for minerals after wet digestion of samples with a mixture of perchloric acid and concentrated nitric acid (1:4 v/v) and composition of some minerals (calcium, phosphorus, magnesium, potassium, sodium, manganese, iron and zinc) in the digest were determined by Atomic Absorption Spectrophotometer (AAS) model 490 Gallenkamp, London, while phosphorus was determined by the phosphovanadomolybdate method (AOAC, 1990). Anti-nutrients determined were oxalate, phytate, saponin, alkaloid, tannin and phenol.

Determination of Oxalate

1g each of milled sample of *Eichhornia crassipes*, *Nymphaea lotus* and *Nymphaea maculata* was weighed into 100ml conical flask and 75ml of 1.5% H_2SO_4 was added and the solution was carefully stirred intermittently with a magnetic stirer and allowed to stand for 1 hour. It was then filtered using whatman No. 1 filter paper. 25ml of sample filtrate (extract) was collected and titrated (80°C) against 0.1N$KMnO_4$ solution to the point when faint pink colour appeared and persisted for at least 30 second (Day and Underwood, 1986) **Note:** Samples(filterate) was titrated at 80°C.

Det ermination of Phytate

Phytate is determined according to the method of Wheeler and Ferrel (1971). 4g each of milled sample of *Eichhornia crassipes*, *Nymphaea lotus* and *Nymphaea maculata* was soaked in 100ml of 2% HCl for three hours and then filtered through muslin cloth and the filterate (extract) was collected in a conical flask. Then 5ml of 0.3% ammonium thyiocyanate solution was added as indicator and 53.5ml of distill water was added to give proper acidity and this was titrated against a standard.

Phytate = Titre value x 1.19x 1.95x 3.55m/g

Determination of Saponin

The method used was that of Obadoni and Ochuko (2001). The samples were ground and 2.5g of each was poured into a conical flask and 100cm^3 of 20% aqueous ethanol was added. The samples were heated inside a hot water bath for four hours with continous stirring at about 55°C. The mixture was filtered and the residue re - extracted with another 200ml 20% ethanol. The extracts were evaporated to 40ml inside water bath at 90°C. The extract was transferred into a 250ml seperating funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered, while the other layer was discarded, the purification process was repeated. 60ml of n-butanol was added; the combined extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponin was calculated as stated:- % Saponin= $\frac{W_3\text{-}W_1}{W} \times \frac{100}{1}$

Where W_1 = Weight of sample

W_2 = Weight of petri-dish

W_3 = Weight of filterate dried (i.e oven weight)

Determination of Alkaloid

The method used was that of Harborne (1973). 5g of each sample was weighed into 200ml beaker and 200ml of 10% acetic acid in ethanol was added and allowed to stand for 4 hours . This was filtered and the extract was concentrated on a water bath. Concentrated ammonium hydroxide was added drop wise to the extraction until the precipitation was completed.The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and filtered. The residue is the alkaloid, which was dried and weighed.

$$\% \text{ Alkaloid} = \frac{W_3 - W_1}{W_2 - W_1} \times \frac{100}{1}$$

Where

W_1 = Weight of sample

W_2 = Weight of petri-dish

W_3 = Weight of filterate dried (i.e oven weight)

Determination of Tannin

200mg each of finely milled samples was poured into a 50ml sample bottle. 10ml of 70% aqueous acetone was added and properly covered. The bottles with their content were put in an ice bath shaker and shaked for 2 hours at 30°C.

Each solution was centrifuged and the supernatant stored in ice. 0.2ml of each solution was pipette into testubes and 0.8ml of distilled water was added. Standard tannic acid solutions were prepared from 0.5mg/ml stock and the solution made to 1ml with distilled water. 0.5ml folic reagent was added to samples and standard was followed by 2.5ml of 20% Na_2CO_3. The concentration of each solution was determined by Atomic Absorption Spectrophotometer at 725mm against a reagent blank concentration of the samples from a standard tannic acid curve (Makkar and Goodchild, 1996).

$$\text{Tannic} = 1\text{ml extract} = \frac{R \times 1000}{\text{ml sampled used}} = \mu gTA$$

Where R = result read from the standard curve

Determination of Total Phenol

The fat free sample was boiled with 50ml of ether for the extraction of the phenolic component for 15min. 5ml of distilled water was added. 2ml of ammonium hydroxide solution and 5ml of concentrated amylacohol were also added. The samples were made up to mark and left to react for 30 minutes for colour development. This was measured at 505mm on atomic absorption spectophotometer. (Barry *et al*.,2001).

$$\text{Phenol} = 1\text{ml extract} = \frac{R \times 1000}{\text{ml of sampled used}} = \mu gTA$$

Where R = result read from the standard curve

***In vitro* gas production study**

The *in vitro* gas production study was carried out at the department of Animal Science, University of Ibadan, Nigeria. Rumen fluid was obtained from three West African Dwarf rams through suction tube before the morning feed. The animals were fed sufficient quantity of *Panicum maximum* and 300 g of cotton seed cake as supplement. They also had free access to water and salt lick. Incubation was as reported (Menke and Steingass, 1988) using 100 ml calibrated syringes in three batches at 39°C. To 200 mg sample in the syringe was added 30 ml inoculums containing cheese cloth-strained rumen liquor and buffer (9.8 g $NaHCO_3$ + 2.77 g Na_2HPO_4 + 0.57 g KCl + 0.47 g NaCl + 0.12 g $MgSO_4.7H_2O$ + 0.16 g/litre $CaCl_2$ $2H_2O$) (1:4,v/v) under continuous flushing with CO_2. The gas production was measured at 3, 6, 9, 12, 15, 18, 21 and 24h. After 24h of incubation, 4 ml of NaOH (10 M) was introduced to estimate the amount of methane produced. The average volume of gas produced from the blanks was deducted from the volume of gas produced per sample. Metabolizable energy (ME, MJ/kg DM) and organic matter digestibility (OMD %) were estimated as established (Menke and Steingass, 1988) and short chain fatty acids (SCFA) was calculated as reported (Getachew *et al.,* 1999): ME = 2.20 + 0.136*Gv + 0.057*CP + 0.0029*CF: OMD = 14.88 + 889Gv + 0.45CP + 0.651 XA; SCFA = 0.0239*Gv – 0.0601; where Gv, CP, CF and XA are net gas production (ml/200 mg DM), crude protein, crude fibre and ash of the incubated samples respectively.

Calculation and statistical analysis

Gross energy content was estimated using the equation GE (KJ/100gm DM) = (%CP x 16.7) + (% EE x 37.7) + (% Carbohydrates x 16.7) of Ekanayake *et al*. (1999). All data obtained were subjected to analysis of variance and mean separations where there were significant differences was by Duncan (1955) multiple range F-test (SAS, 1999).

RESULTS AND DISCUSSION

Table 1 shows significant (P<0.05) variation in the values of dry matter (DM), crude protein (CP), crude fibre (CF), ether extract (EE) and nitrogen free extract (NFE). The DM of the aquatic weeds ranged from 88.46% in *N. lotus* to 94.15% in *Eichhornia crassipes*. The CP of *E. crassipes* (18.95 %) and *N. lotus* (16.95%) were comparable to those values reported for some multipurpose trees in Nigeria (Arigbede *et al.,* 2003; Getachew *et al.,* 2004). Where the growing of browse plants are not favoured possibly as a result of the prevailing climatic conditions in some parts of Nigeria and where these plants are available abundantly, *E. crassipes* and *N. lotus* leaves and stem in particular could be used as dry season feedstuffs if properly ensiled or made into hay. Crude fibre (CF) values recorded for *E. crassipes and N. lotus* in this study were comparable to value reported for *E. crassipes* by Dairo (1997), thus the two plants could be rich sources of fibre that could enhance rumination in sheep and goats. *E. crassipes* had the highest ash value (21.82%) than the value reported by Kwashima *et al,* (2002). NFE value was least in *E. crassipes* and was significantly lower (P<0.05) than the values recorded for *N. lotus* (40.50%) and *N. maculata* (55.61%). However, the values obtained for the three plants compared favourably with the reported value by Dairo (1997) and were sufficient enough to support the production of volatile fatty acids in the rumen during fermentation (Blummel *et al.*, 1997).

The neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), hemi-cellulose and cellulose contents of the three plants are presented in Table 2. The NDF, ADF, hemicellulose and cellulose values were observed to be significantly (P<0.05) higher in *E. crassipes* and *N. lotus* than *N. maculata.* The reason for the variation might be due to differences in maturity, stage of growth and specie of the weeds and sites where the weeds were harvested (Promkot and Wanapat, 2004). The NDF values however, were within the range of 25-71% reported for some valuable multipurpose trees (*Pterocarpus erinaeus*, 47.05%; *Erythrina abyssinia* 27.08%) that are fed to sheep and goats in Nigeria (Bayer 1990, Larbi *et al.,* 1993). ADF contents in the weeds, compared favourably with the value reported for *Alchornea cordifolia* (24.01 %) by Akinlade *et al,*(2001).

Table 1: Proximate Composition (%) and energy content (KJ/100gDM) of *Eichhornia crassipes, Nymphaea lotus and Nymphae maculata*

Components	*Eichhornia crassipes*	*Nymphaea lotus*	*Nymphaea maculata*	SEM
Dry matter	94.15[a]	88.46[b]	88.61[b]	0.95
Ogarnic matter	74.48[c]	80.58[b]	87.24[a]	1.88
Crude protein	18.95[a]	16.95[a]	12.45[b]	1.00
Crude fibre	12.43[a]	15.08[a]	8.17[b]	1.13
Ether extract	16.00[a]	8.05[c]	11.01[b]	1.16
Ash	21.82[a]	19.41[b]	13.09[c]	1.30
Nitrogen free extract	31.10[c]	40.50[b]	55.61[a]	3.66
Gross energy (KJ/100gDM)	16.36	15.14	16.88	0.41

abc= Means on the same row with different superscripts are significantly varied (P<0.05)

Table 2: Fibre Fractions (%) of *Eichhornia crassipes, Nymphaea lotus and Nymphaea maculata* (n = 3)

Components (%)	Eichhornia crassipes	Nymphaea lotus	Nymphaea maculata	SEM
Neutral detergent fibre	48.46[a]	43.65[b]	29.43[c]	2.89
Acid detergent fibre	24.24[a]	24.34[a]	17.62[b]	1.40
Acid detergent lignin	8.58[a]	9.30[a]	7.66[a]	0.37
Hemicellulose	24.23[a]	19.32[b]	11.80[c]	1.93
Cellulose	15.87[a]	15.04[a]	9.96[a]	1.30

abc = Means on the same row with different superscripts are significantly varied (P<0.05)

Table 3: Mineral contents of *Eichhornia crassipes, Nymphaea lotus and Nymphaea maculata* (n = 3)

Components	*Eichhornia crassipes*	*Nymphaea lotus*	*Nymphaea maculata*	SEM
Calcium (%)	0.06	0.06	ND	0.01
Phosphorus(%)	0.91[a]	0.32[b]	0.31[b]	0.11
Magnesium(%)	0.07	0.08	0.06	0.01
Potassium(%)	8.60[a]	7.11[b]	3.74[c]	0.74
Sodium(%)	2.51[b]	2.86[a]	2.64[b]	0.07
Manganese(ppm)	124.23[a]	46.87[b]	42.23[c]	13.30
Iron(ppm)	0.18[a]	0.12[b]	ND	0.03
Zinc(ppm)	0.40[a]	0.24[b]	0.16[b]	0.04

abc = Means on the same row with different superscripts are significantly varied (P<0.05)
ND = Not determined.

Table 3 shows the macro and micro mineral concentrations of the aquatic weeds. There were significant (P<0.05) differences among the aquatic weeds in phosphorus (P), sodium (Na), potassium (K), manganese (Mn), iron (Fe) and zinc (Zn) concentrations but no significant (P>0.05) variation in calcium (Ca) and magnesium (Mg) concentrations.. However, Na (2.80%) and K (8.60%) values were apparently higher in *N. lotus* and *E. crassipes* respectively than the concentration in *N. maculata*. The high contents of P (0.91%), K (8.6%), and Mn (124.23ppm) in *E. crassipes* and Na (2.86%) in *N. lotus* indicate that the requirements of sheep and goats for these minerals could be met (McDowell, 1992) if the plants are fed *ad libitum.*

Table 4 shows anti-nutrients of the aquatic weeds and the observed values varied significantly (P<0.05) among the species. The highest value of oxalate (3.29%) was recorded in N.*lotus*, while saponin (24.59%), alkaloid (14.96%), tannin (4.32%) and phenol (4.87%) were highest in *N. maculata.* The level of tannin ranged from 0.48% in *E. crassipes* to 4.32% in *N. maculata,* saponin ranged between 16.45% in *E. crassipes* and 24.59% in *N. maculata*. However, the presence of tolerable tannin and saponin contents in plants had been reported to be beneficial in ruminants nutrition. Feedstuffs containing saponin had been shown to be defaunating agents (Teferedegne, 2000) and capable of reducing methane production

(Babayemi *et al.,* 2004). The tannin level in the three weeds is below the 6.00% toxic level for small ruminants and it could be an added advantage as a natural additive in the diet of ruminants, since forages or feeds containing tannin have potential of forming complexes with protein and the tannin-protein used as by-pass protein in the rumen (Barry and McNabb, 1999) by diminishing rumen protein fermentation, thus improving on the availability of protein to ruminants at the lower gut.

Table 5 presents the metabolizable energy (ME), organic matter digestibility (OMD), short chain fatty acids (SCFA), methane (CH_4) and total gas production. The ME, SCFA and OMD values of the aquatic weeds were similar ($P>0.05$), while CH_4 and total gass production were significantly ($P<0.05$) influenced by species difference. Although, 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. In this study, the higher production of gas and the eventual preponderance of SCFA in *N. maculata* probably showed an increased proportion of acetate and butyrate but may mean a decrease in propionate production (Babayemi *et al.,* 2006). However, the ME values that averaged 4.74 MJ/Kg DM in the aquatic weeds suggests their potential to make energy available to the ruminants. Thus, the aquatic weeds have an advantage of being used as feed for small ruminants especially during the dry season.

Table 4: Anti-nutrients composition of *Eichhornia crassipes,Nymphaea lotus and Nymphaea maculata* (n = 3)

Components %	*Eichhornia crassipes*	*Nymphaea lotus*	*Nymphaea maculata*	SEM
Phytate	8.50^{a}	7.20^{b}	2.50^{c}	0.71
Alkaloid	8.15^{b}	8.84^{b}	14.96^{a}	1.09
Saponin	16.45^{c}	21.88^{b}	24.59^{a}	1.22
Oxalate	1.98^{b}	3.29^{a}	2.30^{b}	0.21
Tannin	0.48^{b}	0.57^{b}	4.32^{a}	0.65
Phenol	0.57^{b}	0.32^{b}	4.87^{a}	0.76

abc = Means on the same row with different superscripts are significantly varied ($P<0.05$

Table 5: Metabolizable energy (MJ/Kg DM), organic matter digestibility (%), short chain fatty acids (µmol), methane (ml) and total gas production of *Eichhornia crassipes, Nymphaea lotus and Nymphaea maculata* (n = 3)

Parameters	*Eichhornia crassipes*	*Nymphaea lotus*	*Nymphaea maculata*	SEM
ME (MJ/Kg DM)	4.75	4.74	4.75	0.02
OMD (%)	44.89	44.89	44.88	0.51
SCFA (µm	0.17	0.17	0.18	0.01
CH_4 (ml)	6.00^{c}	9.00^{a}	8.00^{b}	0.78
Total gas (ml/200mgDM)	11.00^{c}	15.00^{b}	20.00^{a}	2.31

abc = Means on the same row with different superscripts are significantly varied ($P<0.05$)
ME = Metabolizable energy; OMD = Organic matter digestibility; SCFA = Short chain fatty acid; CH_4 = Methane

CONCLUSION

Nutrient composition of the three aquatic weeds showed that the plants might be useful sources of protein and energy in ruminant feeding. The presence of tannin and saponin below toxic level in the plants exhibited the tendency to suppress methanogenesis and improve the availability of protein to ruminant animals at the lower gut. The observed values of crude protein, gross energy, metabolizable energy and short chain fatty acids in *E. crassipes, N. lotus* and *N. maculata* connote the plants' ability to meet nutrient requirements of small ruminant in the tropical regions particularly during the dry season.

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