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IN VITRO DIGESTIBILITY OF DIFFERENT SPECIES OF DUCKWEED

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ABSTRACT

High feed cost of ingredients for compounding concentrates and shortage of quality pasture for ruminant all year round is a major constraints for ruminant production in Nigeria. This result in starvation, malnutrition and growth retardation of animals during the dry season. This challenge called for a research into alternative feed or feedstuff that could be utilized for ruminants. In the light of the above, some aquatic weeds especially duckweed (*Lemna spp*) that has not been fully explored as feed stuffs for ruminant in Nigeria were considered. In this context, evaluating the proximate composition and in vitro digestibility of different species of duckweed is essential. Different species of lemna which include *Lemna paucicosta*, *Spirodela polyrhiza* and *Lemna aequinoctialis* were collected, weighed and ovendried at 60^{o}C,rumen liqour was collected from 3 WAD rams for invitro gas fermentation for degradability, organic matter digestibility (OMD) short chain fatty acid (SCFA) and metabolizable energy (ME). Data were analysed using descriptive statistics and ANOVA at α0.05. In vitro fermentation results showed significant differences in metabolizable energy ranges from 3.60 to 4.26MJ/kg DM and organic matter digestibility which ranges from 73.91 to 310.24%. There was no significant difference in short chain fatty acids and Methane production, reinforcing the relationship between duckweed digestibility and methane emissions. Moreso, the result also revealed significant difference in Dry matter degradability which ranges from (82.70-88.60). In conclusion, the study highlighted the potential of duckweed as a high-nutrient feed additive for livestock, offering an eco-friendly alternative to conventional feed ingredients.

Key word: *Lemna*, proximate analysis, ruminant

DESCRIPTION OF PROBLEM

High feed cost of ingredients for compounding concentrates and shortage of quality pasture for ruminant all year round is a major constraints for ruminant production in Nigeria. This result in starvation, malnutrition and growth retardation of animals during the dry season. This challenge called for a research into alternative feed or feedstuff that could be utilized for ruminants. In the light of the above, some aquatic weeds especially duckweed (Lemna spp) that has not been fully explored as feed stuffs for ruminant in Nigeria were considered. Duckweed has garnered attention as a promising alternative feed resource for livestock due to its rapid growth, high protein content, and efficient nutrient uptake (Kadlec *et al.*, 2016). Thriving in shallow water environments, duckweed benefits from optimal conditions for rapid growth, including abundant sunlight, which it uses for photosynthesis to produce essential nutrients (Appenroth *et al.*, 2017). Its ability to grow quickly and efficiently makes it an attractive option for various applications.

Duckweed found in pond, fresh water, dam and stream is a major problem for aquaculture management in which considerable effort are being made for its consistent evacuation. Therefore, using duckweed as a forage for ruminants will reduce the menace it causes to fish farming, irrigation and drainage canal and water quality. Duckweed is rich in vitamins, minerals, and antioxidants, making it a valuable dietary supplement for livestock (Wang *et al.*, 2020). Duckweed, a small floating aquatic plant from the Lemnaceae family, is increasingly recognized for its ecological and economic potential (Hauwa and Gasim, 2023). Additionally, duckweed

is rich in essential amino acids, vitamins, and minerals, making it a potentially valuable component of livestock and aquaculture feeds. However, to fully understand its applicability and optimize its use in animal nutrition, it is essential to determine the in vitro analysis of different species of duckweed.

MATERIALS AND METHODS

The experiment was carried out at the Ruminant Laboratory Department of Animal Science University of Ibadan, Nigeria. Duckweed samples (*Lemna pauciocostata, Spirodela polyrhiza and Lemna aequinoctialis*) were collected from different ponds around (Olodo) Egbeda Local Government, Ibadan and Oyo town and identified at the Department of Botany University of Ibadan, Nigeria. The samples collected were weighed and oven dried at 60^{o}C until a constant weight was obtained, milled and kept in Ziploc bag for further analysis.

In vitro gas production

Rumen liquor was obtained from four West African dwarf rams. The method of collection was described by (Babayemi and Bamikole, 2006a) using suction tube from rams fed with 60% concentrates (Soya bean meal 20%, Wheat offal 15%, corn bran 10%, PKC 10%, and salt 1%) and 40% *Panicum maximum* at 5% of their body weight for 3 days. The rumen liquor was collected into a thermoflask to a temperature of 39°C from the rams before they were offered the morning feed.

Incubation period was as reported by (Menke and Steingass, 1988) using 50ml calibrated transparent plastic syringe with tilted silicon tube. The sample weighing 200mg with three replicates was carefully dropped into the syringe and thereafter, 30ml inoculums containing cheese cloth strained rumen liquor and buffer, under continuous flush with carbon-dioxide (CO_2) was dispensed using another 30ml calibrated syringe. The syringe was tapped and pushed upwards by the plunger in other to completely eliminate air in the inoculums. The silicon tube in the syringe was then tightened by a clip so as to prevent escape of gas.

Incubation was carried out 3, 6, 9, 12, 15, 18, 21 and 24hours.

Determination of Gas Production Characteristics

The volume of gas produced at intervals was plotted against the incubation time and from the graph, the gas production characteristics were estimated using the equation. Y = a + b (1 - et) to estimate gas production characteristics as described by Ørskov and McDonald (1979)

Where Y = Volume of gas produced t = time of incubation, a = intercept (gas produced from the soluble fraction), b = gas produced from insoluble fraction, c= gas production rate for the insoluble fraction b.

From the gas production parameters other variables such as Organic matter digestibility (OMD);

Metabolizable energy (ME), and Short chain fatty acids (SCFA) were estimated according to the equations of (Menke and Steingass 1988). They were obtained through the following procedures:

ME (cal/kg DM) = 2.20 + 0.136GV + 0.057CP + 0.0029CF. (Menke and Steingass, 1988)

OMD% = 14.88+ 0.889GV + 0.45CP + 0.651XA. (Menke and Steingass, 1988)

SCFA = 0.0239GV - 0.0601 (Getachew *et al*., 2000)

Where GV, CP, CF and XA are Total gas production (ml/200mgDm) crude protein, crude fibre and ash of the incubated samples respectively

Methane Determination

To estimate methane generation from the substrate, 4 ml of 10M NaOH was added immediately after evacuation from the incubator using a 5 ml capacity syringe. The substance was introduced into the silicon tube attached to the 60ml syringe. The clip was then opened when NaOH was released gradually. The content was agitated vigorously while the plunger

shifted to fill the vacuum generated by CO2 absorption. The methane volume was recorded during calibration. To ascertain the real gas generated, the mean volume of gas created from the blanks was subtracted from the volume of gas produced for each sample (Fievez and Babayemi, 2007).

Statistical analysis

All data obtained were subjected to descriptive statistics and analysis of variance (ANOVA) using (SAS, 1999) package and means were separated at P>0.05 using Duncan Multiple Range Test.

RESULTS AND DISCUSSION

Shown in Table 1 is the in-vitro fermentation characteristics of different species of duckweed. It was revealed that no soluble fraction 'a' (0) was produced for T2 but T1 had (0.33ml) and T3 (0.67ml) has the highest and there is no significant different across the treatments. The results align with findings from (Zhao *et al*., 2018), who reported a values in the range of 0.1 to 0.6. Their study highlighted that higher initial gas production correlates with higher soluble carbohydrate content, which facilitates rapid microbial digestion. There is no significant different in the gas production from soluble fraction (y) value with T2 and T3 (2.67ml) having the lowest values and T1 (3.00ml) having the highest value, these align with (Wang *et al*., 2018) who reported values ranging from 2.60 to 3.20. The slightly higher maximum value in their study might be due to optimized pre-treatment methods or nutrient supplementation during cultivation, which can enhance the degradability of the substrate.

T3 recorded the highest initial gas production among the duckweed species tested. The gas production is an indication of microbial degradation of the samples (Babayemi *et al.*, 2004, Fievez *et al.*, 2005).

(Table 2) revealed the in vitro parameters of different species of duckweed. It was noticed that the metabolizable energy (ME) in (MJ/kg) of T1 (4.09) was significantly different from T3 (3.60) having the lowest value but significantly the same with T2 (4.26) having the highest value. The values were in line with (Leng *et al*., 1995) who reported (2.8 to 4.20 MJ/kgDM) depending on the species and cultivation conditions. Metabolizable energy **(**ME**)** values are influenced by species, harvesting stage, and water nutrient content (Iqbal, 1999). The organic matter digestibility (OMD) of T1 (243.48) and T2 (310.24) were significantly the same but significantly different from T3 (73.91) having the lowest value which alligns to (Wang *et al*., 2016) on Lemna minor reported OMD values ranges from 75.01% to 250.05% which noted that environmental factors such as nutrient availability and light intensity could significantly affect the OMD of duckweed. There is no significantly different across all treatment between SCFA values with T1 and T2 (0.98) having the highest values and T3 (0.90) having the lowest value, these results align closely with (Wang *et al*., 2017) values ranging from 0.85 to 0.95, the research suggested that duckweed has a relatively high capacity for SCFA production, especially under optimized conditions of anaerobic digestion, such as a stable pH and sufficient microbial inoculation.

Methane production of different species of duckweed is presented in figure 1. It was observed that lemna species produces relatively small methane production. T2 produces highest methane value of (2.00mg/200mg) while the lowest value was observed in T1 and T3 (1.67mg/200mg). However, there was no significant different observed among the treatment which is almost similar to (Zhao *et al*., 2018) who reported methane production values ranging from 1.50 to 2.10 for duckweed under anaerobic digestion. Their findings indicated that the methane yield depends significantly on the protein content of the duckweed, as protein degradation produces ammonia, which enhances methane production (Zhao *et al*., 2018).

The dry matter degradability result is illustrated in figure 2. The dry matter degradability of Lemna varied significantly across the treatments with T3 (88.60%) having the highest value and T2 (82.70) having the lowest value. This parameter is

an essential indicator of the feedstock's digestibility and its potential efficiency in biogas production systems. The DMD values observed in this study align with findings from (Zhao *et al*., 2019), who reported DMD values of 80.50% to 87.90% for Lemna minor. However, the study attributed the high degradability of duckweed to relative low lignin content and high protein concentration, which facilitates microbial digestion. Also, the similarity in values between the studies and the current work highlights the consistent digestibility of Lemna species across different conditions

Table 1. In vitro fermentation characteristics of different species of duckweed

PARAMETERS	T1	T2	T3	SEM
a	0.33	0.00	0.67	0.08
a+b	4.67	4.33	4.67	0.18
b	4.33	4.33	4.00	0.14
t	15.00^{b}	15.00^{b}	21.00^{a}	0.51
y	3.00	2.67	2.67	0.17
y^{1}	0.40	0.40	0.50	0.02
c	0.06	0.07	0.04	0.00

$^{a, b, c}$ Means across the rows with different superscripts differ significantly at P< 0.05

T1=*Lemna paucicostata* **T2**= *Spirodela polyrhiza* **T3**=*Lemna aequinoctialis* y= gas volume produced at time "t'', a= gas produced from soluble fraction, b= potential gas production from insoluble fraction, c= gas production rate constant (h^{-1}) for insoluble fraction (b), a+b= total degradable fraction, t= time of incubation, SEM= Standard error of the mean

Table 2. In-vitro Parameters of Duckweed Species

PARAMETERS	T1	T2	T3	SEM
OMD	243.48^{a}	310.24^{a}	73.91^{b}	6.11
SCFA	0.98	0.98	0.90	0.03
ME	4.09^{a}	4.26^{a}	3.60^{b}	0.02

$^{a, b, c}$ Means across the rows with different superscripts differ significantly at P< 0.05; OMD: Organic matter digestibility (%), ME: Metabolizable energy (MJ/KgDM), SCFA: Short Chain Fatty Acids (µmol).**T1**= *Lemna paucicostata* **T2**= *Spirodela polyrhiza* **T3**= *Lemna aequinoctialis*

CONCLUSION AND APPLICATION

Results of in vitro fermentation studies indicate that metabolizable energy (ME) and organic matter digestibility (OMD) are species specific, but short-chain fatty acid (SCFA) production is less variable, implying similar microbial fermentation rate across species. These results emphasize the need to use high-quality species of duckweed, which is beneficial for energy availability, digestion, and overall feed value, and, consequently, for the health and productivity of the animal.

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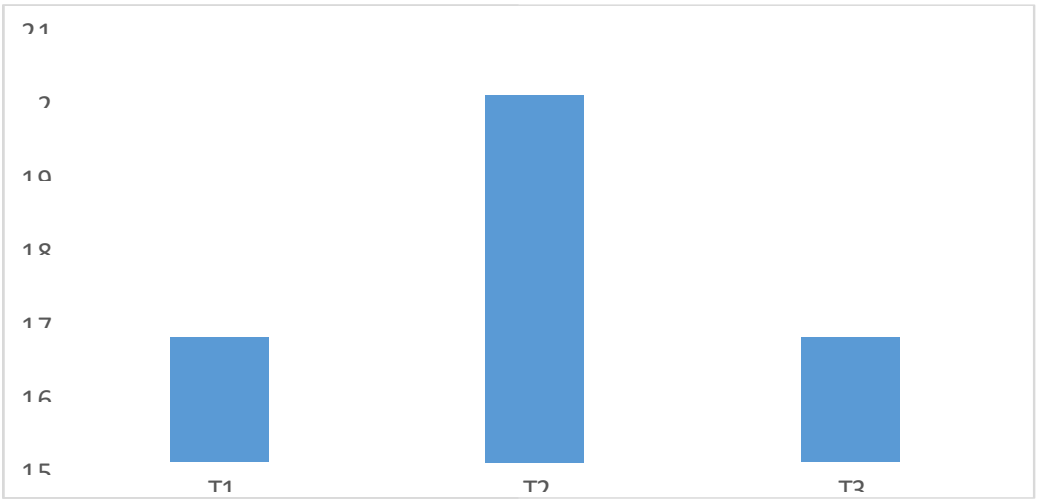

Figure 1. Methane production of different species of duckweed.

T1=*Lemna paucicostata,* **T2**= *Spirodela polyrhiza,* **T3**=*Lemna aequinoctialis*

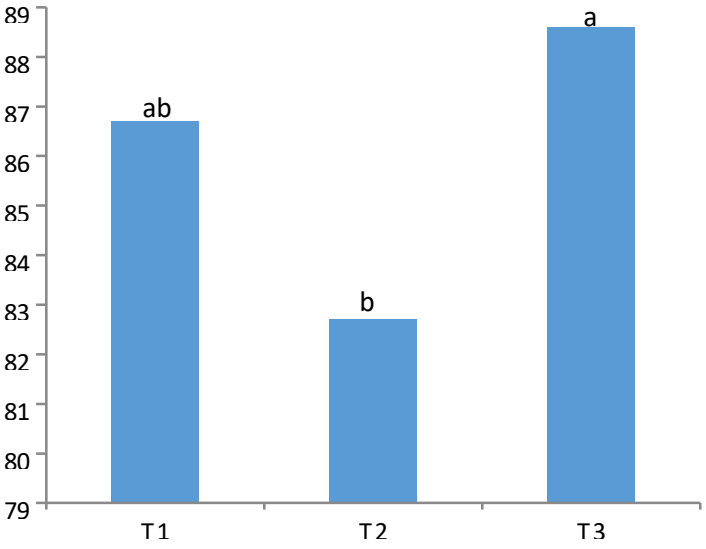

Figure 2. Dry Matter Degradability of different species of duckweed

T1= *Lemna* paucicostata **T2**= Spirodela polyrhiza**T3**= Lemna aequinoctialis