

## DUCKWEED AS A NON-CONVENTIONAL FORAGE FOR RUMINANTS IN THE TROPICS

Deborah Erinola AJAYI<sup>1</sup>, Sunday Oloruntoba OMOTOSO<sup>2\*</sup>, Rashida Bukola FAGBENRO<sup>1</sup>, Evelyn Oluwatoyin OYOGHO<sup>1</sup>, Yusuf Aramide Adelaja OSO<sup>1</sup>, Olaniyi Jacob BABAYEMI<sup>1</sup>

<sup>1</sup>Department of Animal Science, University of Ibadan, Nigeria

<sup>2</sup>Institute of Agricultural Research and Training, Obafemi Awolowo University, Ibadan, Nigeria

### ABSTRACT

The high cost of grain-based concentrates and crop residues are increasingly affecting profitable ruminant farming in Nigeria because of inadequate green forage all year round. Duckweeds are aquatic plants which constitute a nuisance in an earthen fish pond. However, information about their forage value is limited. Hence, this study was conducted to evaluate the nutritive value, secondary metabolites and *in vitro* fermentation characteristics of duckweed from earthen fish ponds. Duckweed represented as T1, T2, T3, T4 and T5 from different locations were collected, dried and analysed for chemical composition and *in vitro* gas production using buffered rumen fluid from goats. Cumulative gas production was measured at 3 to 48 hours of incubation periods. Results indicated that crude protein content was similar, while NDF, ADF and ADL were significantly different, with mean values of 54.11 %, 29.53 % and 11.57 %, respectively. Saponin content (0.407–0.468 %) was higher than alkaloids (0.312–0.433 %) and total phenols (0.158–0.175 %). Calcium and phosphorus varied from 0.09 to 0.54 % and 0.01 to 1.05 %, respectively. Lead and cadmium ranged from 0.01 to 0.17 mg.kg<sup>-1</sup> and 0.01 to 0.03 mg.kg<sup>-1</sup>, respectively. Gas from the insoluble fraction (b), potential gas (a+b), the rate (c) and volume (Y) of gas produced were comparable. Cumulative gas produced increased as hours of incubation progressed, with gas volumes higher (3.93 mL/200 g DM) at 48 hr and least (1.07 mL/200 g DM) at 12 hr post-incubations. Metabolizable energy was similar and ranged from 3.13 to 3.68 MJ/Kg DM. Organic matter digestibility was higher (40.58 %) for T1 and comparable with T5 (36.76 %). Short-chain fatty acids (ranged = 0.02–0.05 µmol/200 mg DM) were comparable. *In vitro* dry matter degradability (ranged = 24.00 to 39.67 %) was significantly different. In conclusion, duckweed from earthen fish ponds is fairly degradable *in vitro*, and the nutrient contents elucidate its forage value for ruminants.

**Key words:** duckweed; gas production; non-conventional forage; secondary metabolites

### INTRODUCTION

Forages are feed resources for ruminants in humid and Sub-Saharan African countries, where the high cost of grain-based rations could no longer guarantee profitable ruminant production systems. Meanwhile, the availability of quality forage all year round has always been affected by unpredictable variations of seasons and recently, by climate change. The consequences of climate change often result in feed

crises and the performance of ruminants below the optimal level of productivity. The poor nutritive value of tropical forages limits the efficiency of animal production (Halmemies-Beauchet-Filleau *et al.*, 2018). Based on the premises above, an unwanted aquatic plant species with a potential forage value though largely unexplored that can be added to ruminant's feed resource base in Nigeria is duckweed. Duckweed is a free-floating aquatic plant species that grow on calm surface waters such as ponds and lakes (Ziegler *et al.*,

**Copyright:** © 2023 Ajayi et al.

\*Correspondence: E-mail: tobsum2006@gmail.com  
Sunday Oloruntoba Omotoso, Institute of Agricultural Research and Training, Obafemi Awolowo University, Ibadan, Nigeria

Received: July 24, 2023  
Accepted: December 1, 2023



2015). Most of the fish farmers in Nigeria consider its growth on surfaces of fish ponds as a nuisance. However, Luhana (2022) reported that duckweeds are valuable plant species for resolving crucial environmental issues. Duckweed can be consumed in fresh or dried forms by ruminants (Heuze and Tran, 2015). The fresh form of duckweed improved nitrogen use efficiency in West African dwarf goats (Babayemi *et al.*, 2006). A 20 and 30 % inclusion levels of duckweed in Taiwan grass-based diets improved nutrient digestibility, nitrogen retention and  $\text{NH}_3\text{-N}$  levels in Pelibuey lambs though with a decreased dry matter intake (Zetina-Cordoba *et al.*, 2013). However, Gule *et al.* (2023) reported that up to 50 % supplementary proportion of duckweed in commercial feed offered Horo ram increased growth performance, yield and quality of carcass.

Considerable dry matter yields (68.8 and 73.0 tons  $\text{ha}^{-1}$  per annum) of duckweed have been reported under outdoor pond fertilised (Mohedano *et al.*, 2012) and field cultivation (Hassan and Chakrabarthi, 2009) conditions, respectively. Moreover, duckweed is potentially rich in protein (Appenroth *et al.*, 2017), which is highly degradable in the rumen (Heuze and Tran, 2015). The considerable amounts of lipids (Yan *et al.*, 2013), minerals (van der Spiegel *et al.*, 2013) and lower fibre fraction, especially lignin (Heuze and Tran, 2015), in duckweed species make it valuable forage for ruminants. In contrast, the high oxalic and phytic acids, condensed tannins and phenolic concentrations (Negesse *et al.*, 2009; van der Spiegel *et al.*, 2013), ditto harmful heavy metals, often accumulated in duckweed species (Showqi *et al.*, 2018), might limit its use either as a sole diet or in combination with other feedstuffs for livestock. Nevertheless, the nutritionally desirable components of duckweed species demand its evaluation either by *in vivo* or *in vitro* techniques to validate the digestibility and subsequent impact on the performance of ruminants when offered as feed.

Duckweeds, though an unwanted aquatic plant species in aquaculture/fish pond management systems, as explained by the high cost of removal from surfaces of ponds (Yahaya *et al.*, 2022), has not been adequately accepted for feeding ruminants in Nigeria, despite their use as feed resources in aquacultures and monogastric elsewhere (Gwaze and Mwale, 2015). *In vitro* gas production reveals the extent of digestibility of feed and fermentation in ruminants (Sallam, 2007) and assesses the feed's nutritive value, especially pasture forage resources (Rittner and Reed, 1992). To justify the use of duckweed as an alternative and

novel aquatic-derived fodder in ruminants' feeding strategies, preliminary investigation via *in vitro* gas production technique is paramount. This study, therefore, assessed the nutrient and mineral potential, secondary metabolites and *in vitro* gas production characteristics of duckweed to estimate the short-chain volatile fatty acids, metabolizable energy, organic matter digestibility and apparent *in vitro* dry matter degradability.

## MATERIAL AND METHODS

### Location of study and collection of duckweed

The study was carried out at the Animal Science Department, University of Ibadan. Duckweed was harvested in July during the rainy season, from the surfaces of earthen fish ponds at Olodo, Igangan, Igboora, Eruwa, Lanlate located in Ibadan/Ibarapa, Oyo State, Nigeria, Southwest geo-political zone (Latitude, 70.15°N and 70.55°N and Longitude, 30°E and 30.30°E). The area has a humid climate with an annual rainfall of 1,250 mm. Harvesting of duckweed was carried out using a sieve (4 mm diameter mesh size). Other plant species harvested with the duckweed were removed and samples from each location were pooled, kept in sterile polythene bags and taken to the laboratory for authentication of the species. The sample collected was wilted under shade for 12 hours and weighed. The samples were oven-dried at 60 °C until constant weight was attained to determine the dry matter, ground to pass through a 1 mm screen size and kept in sterile containers until chemical analysis.

### Chemical and mineral analyses of duckweed

Dried and ground samples of duckweed (Lemna species) were analysed for crude protein, ether extract and ash contents according to AOAC (2005). Fibre fractions (neutral detergent fibre, acid detergent fibre and lignin) were determined as reported by Van Soest *et al.* (1991). After wet digestion of ash in nitric and perchloric acid mixture (ratio = 4.1 v/v), the mineral concentrations (calcium, potassium and sodium) were determined according to the method of AOAC (2005) using a flame photometer (Jenway Digital Flame Photometer, Model: PFP7). Other minerals (phosphorus, cadmium, lead and chromium) were analysed using Spectronic 20 spectrophotometer and Atomic Absorption Spectrophotometer (Buck 200, VGP Model, Buck Scientific), accordingly.

### Quantitative determination of alkaloids, saponin and total phenols

The samples of duckweeds were analysed quantitatively for alkaloids and saponin concentrations using standard procedures of Tambe and Bhambar (2014) and Obdoni and Ochuko (2002), respectively. Total phenols were analysed by the Folin-Ciocalteu spectrophotometric method using tannic acid as a standard (Porter *et al.*, 1986). All determinations were carried out in triplicates.

### *In vitro* gas production procedure

The *in vitro* fermentation of duckweed was carried out, as described by Menke and Steingass (1988). Rumen liquor from West African dwarf goats ( $n = 6$ , average live weight of  $26 \pm 0.12$  kg) offered *Megathyrus maximus* and concentrate *ad libitum* was used for the study. Rumen liquor was collected into a pre-warmed thermoflask (at  $39 \pm 1$  °C) before morning feeding using a suction tube (Babayemi and Bamikole, 2006). The rumen fluid was diluted using the reduced buffer medium in the proportion 1:2 (v/v) under flushing with CO<sub>2</sub>. Dried duckweed samples (200 mg) from each location (T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub>, and T<sub>5</sub>) were incubated separately in buffered rumen fluid using 120 mL calibrated transparent plastic syringes with plungers and tilted silicon tube under anaerobic conditions for 48 hours at 39 °C. Gas production was measured at 3-hour intervals from 3 to 48 hours. All the incubated samples, including blanks (inoculum), were replicated four times.

The average volume of gas produced from the blanks was deducted from the volume of gas produced for each sample. The volume of gas produced at different intervals was plotted against the incubation time, and the gas production characteristics were estimated from the graph using the equation  $Y = a + b(1 - e^{-ct})$  of Orskov and McDonald (1979) to estimate gas production characteristics, where:

$Y$  = volume of gas produced at time,  $t$  = time of incubation,  $a$  = intercept (initial gas produced from the soluble fraction),  $b$  = gas produced from insoluble fraction,  $c$  = gas production rate for the insoluble fraction/rate of degradability ( $b$ ),  $a + b$  = final gas produced. At 48 h post-incubation, the *in vitro* dry matter degradability (1VDMD) was determined following the method described by Aderinboye *et al.* (2016). The residual material was oven-dried at 48°C for at least 48 hours, weighed, and the percentage of *in vitro* apparent dry matter (DM)

degradability was calculated as follows:

$$\% \text{ 1VDMD} = \frac{\text{Substrate DM incubated} - (\text{Residual DM} - \text{Blank DM}) \times 100}{\text{Substrate DM incubated}}$$

where: DM = Dry matter.

Metabolizable energy (ME, MJ/Kg DM) and Organic matter digestibility (OMD %) were calculated according to Menke and Steingass (1988) equations:  $ME = 2.20 + 0.136 \text{ GV} + 0.057 \text{ CP} + 0.0029 \text{ CF}$ ;  $OMD = 14.88 + 0.889 \text{ GV} + 0.45 \text{ CP} + 0.651 \text{ XA}$ .

Short-chain fatty acids (SCFA,  $\mu\text{mol}$ ) were calculated as described by Getachew *et al.* (2002) after 48 hours *in vitro* gas production as  $SCFA = 0.0239 \text{ GV} - 0.0601$ , where:  $GV = 48 \text{ h total gas volume (mL.200 mg}^{-1})$ ,  $CP = \text{crude protein (\%)}$ ,  $CF = \text{crude fibre (\%)}$  and  $XA = \text{ash (\%)}$ .

### Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the General Linear model procedures of SAS (Statistical Analysis System, version 9.4) (2002) for a completely randomised block design. Means were separated using the Duncan Multiple Range Test and significance was declared at  $p < 0.05$ .

## RESULTS AND DISCUSSION

### Chemical composition and secondary metabolites of duckweed

The chemical composition of duckweed (*Lemna* species) across the locations of collection (Table 1) was significantly different ( $p < 0.05$ ) except for the crude protein content. Ether extract content of duckweed though comparable across the locations, the range (3.55 to 8.95 % EE) obtained was higher than the 3.45–4.68 % reported by Chatterjee *et al.* (2019) for *Lemna minor*. The range of ash content (2.25–24.02 %) for the duckweed despite a relatively lower values in T<sub>2</sub> and T<sub>3</sub>, revealed the mineral potential of duckweed, as reported by Chatterjee *et al.* (2019). The fibre fractions of a ration are vital determinant of dry matter intake and digestibility. The mean value of NDF (54.11 %) content for duckweed across the locations was lower compared to 57.4 g/100 g DM reported for duckweed of *Lemna* species (Huque *et al.*, 1996). The mean ADF (29.53 %) content was higher than values (20.3, 21.5 and 22.7 g/100 g DM) documented by Huque *et al.* (1996) for *Lemna* and

other duckweeds (*Spirodela* and *Wolffia*), respectively, and similarly the ADL content reported by Chatterjee *et al.* (2019) for *Lemna minor*. Higher content of structural cell wall fraction in duckweed (*Lemna* spp.), according to Huque *et al.* (1996), was associated with long hairy roots. Meanwhile, NDF (51.67–57.03 %), ADF (24.66–33.45 %) and ADL (10.10–12.96 %) contents of duckweed obtained aligned with the fibre fractions that could increase feed intake and promote ruminal digestibility (McDonald *et al.*, 1995). Observed significant variations in the chemical composition of duckweed across locations are indicative of the nutrient content of the growth media, as established by Appenroth *et al.* (2017). Remarkably, the mean values for the contents of CP (10.50 %), EE (6.22 %), ash (13.09 %), NDF (54.11 %), ADF (29.53 %) and ADL (11.57 %) in duckweed, which was in the range of nutrients required by ruminants for optimal performance, elucidate its potential as a valuable alternative or non-conventional forage.

Secondary metabolites from plants are critical to the bioavailability of nutrients and the degradability of feed in ruminants. The duckweed across the locations had a relatively comparable content of saponin, phenols and alkaloids (Table 1). Saponin content in duckweed (0.407–0.468 %) across the locations was higher than 0.0032 % observed in *Lemna minor*

(Negesse *et al.*, 2009). This range of values for secondary metabolites (Saponin = 0.407–0.468 %, Total phenol = 0.158–0.175 % and Alkaloids = 0.312–0.433 %) revealed a higher concentration of saponin in duckweed, which confirmed the findings of Ife *et al.* (2021), that duckweed species contain saponin. Saponins suppress fat accumulation (Zhao *et al.*, 2018) and the proliferation of ruminal protozoa counts (Wang *et al.*, 2000). Alkaloids play a significant role in modulating rumen microbial functions of animals under thermal or pH stress (Estrada-Angulo *et al.*, 2016), specifically in the activity of methanogenic archaea to mitigate methane production (Khiaosa-ard *et al.*, 2020). The range of alkaloids (0.312–0.433 %), observed in the duckweed, was higher than the recommended limits of 0.25–0.5 ppm for calf and beef cattle (Coufal-Majewski *et al.*, 2016). Nevertheless, the negative impact of higher alkaloid concentration on ruminants could be counteracted by high saponin or tannin content (Jensen, 2012) in forages. Moreover, duckweed can manipulate the ruminal fermentation pathway for propionate production with a lesser acetate, reduce enteric methane and ammonia emissions, when included in ruminant diets. On the other hand, the mean total phenol content (0.168 %), observed in duckweed, was lower than values of 1.3 % to 2.9 % reported by

**Table 1. Chemical composition and secondary metabolite of duckweed (*Lemna* species) from earthen fish ponds (n = 4)**

| Nutrients (%)            | Duckweed            |                    |                      |                     |                     | Mean  | SEM  | P-value |
|--------------------------|---------------------|--------------------|----------------------|---------------------|---------------------|-------|------|---------|
|                          | T <sub>1</sub>      | T <sub>2</sub>     | T <sub>3</sub>       | T <sub>4</sub>      | T <sub>5</sub>      |       |      |         |
| Dry matter               | 6.51 <sup>c</sup>   | 8.23 <sup>b</sup>  | 12.14 <sup>a</sup>   | 4.67 <sup>e</sup>   | 5.28 <sup>d</sup>   | 7.37  | 1.24 | <.0001  |
| Crude protein            | 17.50               | 11.67              | 10.50                | 8.17                | 4.67                | 10.50 | 2.02 | 0.3898  |
| Ether extract            | 8.95 <sup>a</sup>   | 5.43 <sup>ab</sup> | 6.02 <sup>ab</sup>   | 3.55 <sup>b</sup>   | 7.15 <sup>ab</sup>  | 6.22  | 0.72 | 0.1637  |
| Ash                      | 22.83 <sup>b</sup>  | 2.25 <sup>d</sup>  | 2.28 <sup>d</sup>    | 14.07 <sup>c</sup>  | 24.02 <sup>a</sup>  | 13.09 | 2.54 | <.0001  |
| NDF                      | 55.49 <sup>b</sup>  | 57.03 <sup>a</sup> | 51.67 <sup>e</sup>   | 52.91 <sup>d</sup>  | 53.45 <sup>c</sup>  | 54.11 | 0.54 | <.0001  |
| ADF                      | 29.81 <sup>c</sup>  | 32.20 <sup>b</sup> | 33.45 <sup>a</sup>   | 27.50 <sup>d</sup>  | 24.66 <sup>e</sup>  | 29.53 | 0.87 | <.0001  |
| ADL                      | 11.51 <sup>c</sup>  | 12.80 <sup>b</sup> | 12.96 <sup>a</sup>   | 10.10 <sup>e</sup>  | 10.48 <sup>d</sup>  | 11.57 | 0.38 | <.0001  |
| Secondary metabolite (%) |                     |                    |                      |                     |                     |       |      |         |
| Saponin                  | 0.468 <sup>a</sup>  | 0.453 <sup>b</sup> | 0.464 <sup>a</sup>   | 0.407 <sup>c</sup>  | 0.411 <sup>c</sup>  | 0.441 | 0.01 | <.0001  |
| Phenols                  | 0.167 <sup>bc</sup> | 0.158 <sup>d</sup> | 0.166 <sup>c</sup>   | 0.175 <sup>a</sup>  | 0.173 <sup>ab</sup> | 0.168 | 0.01 | 0.0010  |
| Alkaloids                | 0.345 <sup>bc</sup> | 0.312 <sup>c</sup> | 0.371 <sup>abc</sup> | 0.428 <sup>ab</sup> | 0.433 <sup>a</sup>  | 0.378 | 0.01 | 0.0283  |

<sup>abcde</sup>Means in the same row with different superscripts differ significantly (p < 0.05); SEM = Standard error of mean; T<sub>1</sub> = Duckweed from Eruwa; T<sub>2</sub> = Duckweed from Lanlate; T<sub>3</sub> = Duckweed from Igboora; T<sub>4</sub> = Duckweed from Iggangan; T<sub>5</sub> = Duckweed from Olodo; NDF = Neutral detergent fibre; ADF = Acid detergent fibre; ADL = Acid detergent lignin.

Dewanji and Matai (1996) in *Lemna* spp. The presence of phenols in the duckweed suggests its potential to prevent bloat, reduce gastrointestinal nematode counts (Waghorn and McNabb, 2003) and exhibit antimicrobial capacities (Gonzalez-Renteria *et al.*, 2020).

### Mineral concentration in duckweed

The macro- and trace minerals in duckweed were significantly different ( $p < 0.05$ ; Table 2). It is apparent that duckweed is high in trace minerals. The mean value of phosphorus (0.69 %) was higher than 0.59 % reported for aquatic plants (Chatterjee *et al.*, 2019), while the calcium content in duckweed was lower than 1.11 %, documented for aquatic plants by the same authors. The trace minerals (Zn, Cu, Fe, Pb and Cd) observed in duckweed indicated accumulation of heavy metals, which could negatively impact the health of livestock and humans. Meanwhile, the mean values of Zn, Cu, Fe, Pb and Cd (0.46 mg.kg<sup>-1</sup>, 0.26 mg.kg<sup>-1</sup>, 79.31 mg.kg<sup>-1</sup>, 0.07 mg.kg<sup>-1</sup> and 0.02 mg.kg<sup>-1</sup>, respectively) in duckweed, except Fe, were below the findings of Daud *et al.* (2018) for *Lemna minor*. More importantly, the trace minerals or heavy metals observed in duckweed from this study were below the levels that could affect the health and performance of ruminants. Variations in the mineral contents of duckweed from this study might not be unconnected with the nutrient concentration of the growth media, periods of pond fallow and the uptake of minerals.

### *In vitro* fermentation characteristics of duckweed

The mean values of *in vitro* fermentation characteristics of duckweed incubated for 48 hr are presented in Table 3. *In vitro* fermentation characteristics of duckweed from across the locations differed significantly ( $p < 0.05$ ), though the values of gas produced from the insoluble fraction (*b*), potential gas produced (*a + b*), the rate (*c*) and volume (*Y*) of gas produced were similar and comparable. Overall, volumes of gas produced by duckweed were generally lower. However, gas volumes (*Y*) were higher in T<sub>5</sub> (3.33 mL/200 mg DM) and lower in T<sub>3</sub> (1.33 mL/200 mg DM) groups. Gas production is essentially the aftermath of carbohydrate fermentation to acetate, propionate and butyrate, though a higher gas production from highly fermentable nutrients contributes to SCFAs production (Remesy *et al.*, 1995). The range of short-chain fatty acids produced in duckweed was lower (0.02 to 0.05) compared to values (0.62–0.77) reported for ensiled cassava foliage with Guinea grass (Binuomote and Babayemi, 2012). The lower SCFA rate of degradation recorded possibly suggests lower carbohydrate content, hence, lower potential gas produced, which is also likely due to presence of secondary metabolites in duckweed. Metabolizable energy (ME) was similar and comparable with values ranging from 3.13 to 3.68 MJ/Kg DM across treatments. Organic matter digestibility (OMD) was more (40.58 %) for duckweed collected at Eruwa (T<sub>1</sub>) and comparable with the 36.76 % in duckweed from Olodo (T<sub>5</sub>).

**Table 2. Mineral concentration in duckweed from earthen fish ponds (n = 4)**

| Minerals           | Duckweed            |                    |                    |                    |                    | Mean  | SEM  | P-value |
|--------------------|---------------------|--------------------|--------------------|--------------------|--------------------|-------|------|---------|
|                    | T <sub>1</sub>      | T <sub>2</sub>     | T <sub>3</sub>     | T <sub>4</sub>     | T <sub>5</sub>     |       |      |         |
| Phosphorus (%)     | 0.65 <sup>d</sup>   | 0.76 <sup>c</sup>  | 1.05 <sup>a</sup>  | 0.99 <sup>b</sup>  | 0.01 <sup>e</sup>  | 0.69  | 0.09 | <.0001  |
| Calcium (%)        | 0.25 <sup>c</sup>   | 0.09 <sup>e</sup>  | 0.23 <sup>d</sup>  | 0.54 <sup>a</sup>  | 0.40 <sup>b</sup>  | 0.30  | 0.04 | <.0001  |
| Zinc (mg/kg DM)    | 0.48 <sup>b</sup>   | 0.39 <sup>c</sup>  | 0.20 <sup>e</sup>  | 0.97 <sup>a</sup>  | 0.27 <sup>d</sup>  | 0.46  | 0.07 | <.0001  |
| Copper (mg/kg DM)  | 0.24 <sup>b</sup>   | 0.19 <sup>c</sup>  | 0.24 <sup>b</sup>  | 0.37 <sup>a</sup>  | 0.25 <sup>b</sup>  | 0.26  | 0.02 | <.0001  |
| Iron (mg/kg DM)    | 112.00 <sup>a</sup> | 81.50 <sup>b</sup> | 83.50 <sup>b</sup> | 51.20 <sup>d</sup> | 68.00 <sup>c</sup> | 79.31 | 5.36 | <.0001  |
| Lead (mg/kg DM)    | 0.15 <sup>b</sup>   | 0.02 <sup>c</sup>  | 0.01 <sup>c</sup>  | 0.01 <sup>c</sup>  | 0.17 <sup>a</sup>  | 0.07  | 0.02 | <.0001  |
| Cadmium (mg/kg DM) | 0.02 <sup>b</sup>   | 0.03 <sup>a</sup>  | 0.01 <sup>c</sup>  | 0.02 <sup>b</sup>  | 0.02 <sup>b</sup>  | 0.02  | 0.00 | 0.0046  |

<sup>abcde</sup>Means in the same row with different superscripts differ significantly ( $p < 0.05$ ); SEM = Standard error of mean; T<sub>1</sub> = Duckweed from Eruwa; T<sub>2</sub> = Duckweed from Lanlate; T<sub>3</sub> = Duckweed from Igboora; T<sub>4</sub> = Duckweed from Iangan; T<sub>5</sub> = Duckweed from Olodo.

**Table 3. *In vitro* fermentation characteristics of duckweed incubated for 48 (n = 4)**

| Duckweed       | <i>b</i>           | <i>a + b</i>       | <i>c</i>           | <i>Y</i>            | ME                 | OMD                 | SCFA               | IVDMD               |
|----------------|--------------------|--------------------|--------------------|---------------------|--------------------|---------------------|--------------------|---------------------|
| T <sub>1</sub> | 3.33 <sup>ab</sup> | 3.33 <sup>ab</sup> | 0.02 <sup>ab</sup> | 2.33 <sup>abc</sup> | 3.68 <sup>a</sup>  | 40.58 <sup>a</sup>  | 0.02 <sup>ab</sup> | 29.33 <sup>c</sup>  |
| T <sub>2</sub> | 4.67 <sup>a</sup>  | 4.67 <sup>a</sup>  | 0.03 <sup>a</sup>  | 3.00 <sup>ab</sup>  | 3.53 <sup>a</sup>  | 25.75 <sup>d</sup>  | 0.05 <sup>a</sup>  | 24.00 <sup>d</sup>  |
| T <sub>3</sub> | 2.67 <sup>b</sup>  | 2.67 <sup>b</sup>  | 0.04 <sup>a</sup>  | 1.33 <sup>bc</sup>  | 3.20 <sup>ab</sup> | 23.46 <sup>d</sup>  | 0.00 <sup>b</sup>  | 26.33 <sup>cd</sup> |
| T <sub>4</sub> | 4.33 <sup>a</sup>  | 4.33 <sup>a</sup>  | 0.02 <sup>a</sup>  | 2.33 <sup>abc</sup> | 3.29 <sup>ab</sup> | 31.57 <sup>bc</sup> | 0.04 <sup>a</sup>  | 39.67 <sup>a</sup>  |
| T <sub>5</sub> | 4.67 <sup>a</sup>  | 4.67 <sup>a</sup>  | 0.03 <sup>a</sup>  | 3.33 <sup>a</sup>   | 3.13 <sup>ab</sup> | 36.76 <sup>ab</sup> | 0.05 <sup>a</sup>  | 33.00 <sup>b</sup>  |
| Mean           | 3.93               | 3.93               | 0.03               | 2.46                | 3.37               | 31.62               | 0.03               | 30.47               |
| SEM            | 0.30               | 0.30               | 0.00               | 0.29                | 0.11               | 1.86                | 0.01               | 1.48                |
| P-value        | 0.0016             | 0.0016             | 0.0508             | 0.0668              | 0.1395             | <.0001              | 0.0016             | <.0001              |

<sup>abcd</sup>Means along the same column with different superscripts are significantly different ( $p < 0.05$ ); SEM = Standard error of mean; T<sub>1</sub> = Duckweed from Eruwa; T<sub>2</sub> = Duckweed from Lanlate; T<sub>3</sub> = Duckweed from Igboora; T<sub>4</sub> = Duckweed from Igangan; T<sub>5</sub> = Duckweed from Olodo; *Y* = volume of gas produced at time 't'; *a* = gas produced from the soluble fraction; *b* = gas produced from insoluble fraction, *c* = rate of gas production for the insoluble fraction; *a + b* = final gas produced; ME = Metabolizable Energy (MJ/Kg DM); SCFA = Short Chain Fatty Acid ( $\mu\text{mol}$ ); OMD = Organic Matter Digestibility (%); IVDMD = *In vitro* dry matter degradability (%).

The reduced rate of degradation for duckweed in this study is also evident in the lower values (24.00–39.67 %) of *in vitro* dry matter degradability, which is below 50 %. Obtained IVDMD values in duckweed differed from the range of values (610–875 g.kg<sup>-1</sup>), reported by Huque *et al.* (1996) for *in sacco* dry matter degradability of duckweed species (*Spirodela*, *Lemna* and *Wolffia*) at 48 hours incubation using cattle. Reasons for the wide variation in values of dry matter degradability in this study, compared to the findings of Huque *et al.* (1996), might be ascribed, amongst other factors, to differences in the rumen liquor/inocula of animals and techniques of evaluation used, diets fed to animals as well as growth media of the feed samples (duckweed). Generally, the slow release of essential nutrient media for the proliferation of

cellulolytic microbes needed for adhesion to substrate and rapid degradation, perhaps due to lignin content and phenolic compounds in duckweed (Table 1), might explain the significantly lower fermentation characteristics, metabolizable energy and organic matter digestibility, compared to most conventional feed (e.g. cereal grains, tubers, etc.) estimated *in vitro*.

#### ***In vitro* cumulative gas production by duckweed**

The trend of cumulative gas production in duckweed across the location of collections varied ( $p < 0.05$ ) with the hours of incubation (Table 4). The mean gas volume from duckweed was more (3.93 mL/200 g DM) at 48 hours post-incubation than cumulative gas (1.07, 2.53 and 2.81 mL/200 g DM) produced at other periods (12, 24 and 36 hrs) of incubation. The significant

**Table 4. *In vitro* cumulative gas production (mL/200 g DM) by duckweed post-incubation (n = 4)**

| Hours (hr) of incubation | Gas production volume/duckweed |                   |                   |                   |                   | Mean | SEM  | P-value |
|--------------------------|--------------------------------|-------------------|-------------------|-------------------|-------------------|------|------|---------|
|                          | T <sub>1</sub>                 | T <sub>2</sub>    | T <sub>3</sub>    | T <sub>4</sub>    | T <sub>5</sub>    |      |      |         |
| 12                       | 1.00 <sup>c</sup>              | 1.33 <sup>b</sup> | 0.33 <sup>d</sup> | 1.00 <sup>c</sup> | 1.67 <sup>a</sup> | 1.07 | 0.12 | <.0001  |
| 24                       | 2.00 <sup>d</sup>              | 2.33 <sup>c</sup> | 2.67 <sup>b</sup> | 3.00 <sup>a</sup> | 2.67 <sup>b</sup> | 2.53 | 0.09 | <.0001  |
| 36                       | 2.33                           | 3.00              | 2.67              | 3.00              | 3.00              | 2.81 | 0.11 | 0.1536  |
| 48                       | 3.33 <sup>c</sup>              | 4.67 <sup>a</sup> | 2.67 <sup>d</sup> | 4.33 <sup>b</sup> | 4.67 <sup>a</sup> | 3.93 | 0.21 | <.0001  |

<sup>abcd</sup>Means in the same row with different superscripts differ significantly ( $p < 0.05$ ); SEM = Standard error of mean; T<sub>1</sub> = Duckweed from Eruwa; T<sub>2</sub> = Duckweed from Lanlate; T<sub>3</sub> = Duckweed from Igboora; T<sub>4</sub> = Duckweed from Igangan; T<sub>5</sub> = Duckweed from Olodo.

increase in the volumes of gas produced by duckweed as hours of incubation advanced corroborates the reports of Khan *et al.* (2002) for aquatic plants. This observation suggests a direct relationship between fermentation patterns with substrate retention time in the rumen. Gasmi-Boubaker *et al.* (2005) affirmed a positive correlation between crude protein and gas production of browse species.

Although, gas production volumes of duckweed for this study revealed a negative correlation with crude protein content, it was observed that the relatively higher crude protein (10.50 %) and lower NDF (51.67 %) components for T<sub>3</sub> (Table 1) did not result in a higher gas production volume (Table 4). According to Khan *et al.* (2002), aquatic plants are reportedly high in undegradable protein. In other words, the relatively lower cumulative gas production volumes observed (Table 4) imply, that the protein fraction in the duckweed was not readily available for rumen microbial breakdown, possibly attributed to the protein-binding effect of phenolic compounds besides the possibility of lower fermentable carbohydrate. Plant secondary metabolites inhibit microbial growth (Forbey *et al.* 2009), degradation of feed and consequently, a reduction in metabolizable energy. Nevertheless, differences in chemical composition (fibre fractions, CP and EE) and the presence of secondary metabolites identified in the duckweed (Table 1) could be responsible for the variations in volumes of cumulative gas produced.

## CONCLUSION

The crude protein and fibre fraction, an appreciable amount of calcium, phosphorus and lower levels of heavy metals/trace minerals in duckweed (*Lemna* spp.) justify its use as non-conventional forage. Saponin was of a higher concentration and *in vitro* fermentation of duckweed revealed a lower rate of degradation and gas production, which explains the protein-binding effect of phenolic compounds. Therefore, the nutrient content of duckweed elucidates its value as non-conventional forage for ruminants. Further *in vivo* studies to evaluate the responses of ruminants to duckweed-based rations and their effects on quality of ruminant products are required.

## AUTHOR'S CONTRIBUTIONS

Conceptualization: Babayemi, O. J.

Methodology: Babayemi, O. J., Fagbenro, R. B., Omotoso, S. O., OSO, Y. A. A.

Investigation: Ajayi, D. E., Omotoso, S. O., Fagbenro, R. B., Oyogho, E. O.

Data curation: Ajayi, D. E., Oyogho, E. O., OSO, Y. A. A., Omotoso, S. O.

Writing-original draft preparation: Omotoso, S. O., Fagbenro, R. B.

Writing-review and editing: Omotoso, S. O., Fagbenro, R. B., Babayemi, O. J.

Project administration: Ajayi, D. E., Oyogho, E. O., Fagbenro, R. B., Babayemi, O. J.

All authors have read and agreed to the published version of the manuscript.

## INFORMED CONSENT STATEMENT

Not applicable.

## DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author.

## CONFLICT OF INTEREST

There is no conflict of interest with any individual or organization regarding the materials discussed in this manuscript.

## REFERENCES

- Aderinboye, R. Y., Akinlolu, A. O., Adeleke, M. A., Najeem, G. O., Ojo, V. O. A., Isah, O. A. & Babayemi, O. J. (2016). *In vitro* gas production and dry matter degradation of four browse leaves using cattle, sheep and goats inocula. *Slovak Journal of Animal Science*, 49(1), 32–43.
- Appenroth, K. J., Sree, K. S., Bohm, V., Hammann, S., Vetter, W., Leiterer, M. & Jahreis, G. (2017). Nutritional value of duckweeds (*Lemnaceae*) as human food. *Food Chemistry*, 217, 266–273.

- Association of Analytical Chemists. (2005). *Official methods of analysis* (15<sup>th</sup> edn.). Arlington, VA: Association of Official Analytical Chemists: AOAC International.
- Babayemi, O. J. & Bamikole, M. A. (2006). Effects of *Tephrosia candida* DC leaf and its mixtures with guinea grass on *in vitro* fermentation changes as feed for ruminants in Nigeria. *Pakistan Journal of Nutrition*, 5(1), 14–18.
- Binuomote, R. T. & Babayemi, O. J. (2012). Chemical composition and *in vitro* fermentation characteristics of ensiled cassava (*Manihot esculentus*, Crantz) tops and guinea grass mixture. *Continental Journal of Agricultural Science*, 6(3), 16–20.
- Chatterjee, A., Mohammad, A., Bhakat, C., Mandal, D. K., Rai, S. & Dutta, T. K. (2019). Comparative nutritional evaluation of wetland plants available in old alluvial zone of West Bengal. *Journal of Agricultural Engineering and Food Technology*, 6(3), 245–249.
- Coufal-Majewski, S., Stanford, K., McAllister, T. A., Blakley, B., McKinnon, J., Chaves, A. V. & Wang, Y. (2016). Impacts of cereal ergot in food animal production. *Frontiers in Veterinary Science*, 3(1), DOI:10.3389/fvets.2016.00015
- Daud, M. K., Ali, S., Abass, Z., Zaheer, I. A., Riaz, M. A., Malik, A., Hussain, A., Rizwan, M., Zia-ur-Rehman, M. & Zhu, S. J. (2018). A potential of duckweed (*Lemna minor*) for the phytoremediation of landfill leachate. *Journal of Chemistry*, 1–9. <https://doi.org/10.1155/2018/3951540>.
- Dewanji, A. & Matai, S. (1996). Nutritional evaluation of leaf protein extracted from three aquatic plants. *Journal of Agricultural Food and Chemistry*, 44(8), 2162–2166.
- Estrada-Angulo, A., Aguilar-Hernandez, A., Osuna-Perez, M., Nunez-Benitez, V. H., Castro-Perez, B. I., Silva-Hidalgo, G., Contreras-Perez, G., Barreras, A., Plascencia, A. & Zinn, R. A. (2016). Influence of quaternary benzo phenanthridine and protopine alkaloids on growth performance, dietary energy, carcass traits, visceral mass, and rumen health in finishing ewes under conditions of severe temperature-humidity index. *Asian-Australasian Journal of Animal Science*, 29(5), 652–658.
- Forbey, J. S., Harvey, A. L., Huffman, M. A., Provenza, F. D., Sullivan, R. & Tasdemir, D. (2009). Exploitation of secondary metabolites by animals: a response to homeostatic challenges. *Integrative and Comparative Biology*, 49(3), 314–328.
- Gasmi-Boubaker, A., Kayouli, C. & Buldgen, A. (2005). *In vitro* gas production and its relationship to *in situ* disappearance and chemical composition of some Mediterranean browse species. *Animal Feed Science and Technology*, 123, 303–311.
- Getachew, G., Makkar, H. P. S. & Becker, K. (2002). Tropical browses: content of phenolic compounds, *in vitro* gas production and stoichiometric relationship between short chain fatty acids and *in vitro* gas production. *Journal of Agricultural Science*, 139, 341–352.
- Gonzalez-Renteria, M., del Carmen Monroy-Dosta, M., Guzman-Garcia, X., Hernandez-Calderas, I. & Ramos-Lopez, M. A. (2020). Antibacterial activity of *Lemna minor* extracts against *Pseudomonas fluorescens* and safety evaluation in a zebrafish model. *Saudi Journal of Biological Sciences*, 27(12), 3465–3473.
- Gule, A. H., Derese, D. B., Erge, C. H., Girgo, U. G. & Ejeta, H. K. (2023). Effect of dietary supplementation of duckweed on growth performance, carcass and non-carcass traits of horro rams fed on a commercial-based diet. *Heliyon*, 9(7), e17820 <https://doi.org/10.1016/j.heliyon.2023.e17820>
- Gwaze, F. R. & Mwale, M. (2015). The prospect of duckweed in pig nutrition: A Review. *Journal of Agricultural Science*, 7(11), 189–199.
- Halmemies-Beauchet-Filleau, A., Rinne, M., Lamminen, M., Mapato, C., Ampapon, T., Wanapat, M. & Vanhatalo, A. (2018). Alternative and novel feeds for ruminants: nutritive value, product quality and environmental aspects. *Animal*, 12(s2), s295–s309.
- Hassan, M. R. & Chakrabarthi, R. (2009). Use of algae and aquatic macrophytes as feed in small-scale aquaculture: A Review. *FAO Feed and Aqua Technical Paper*, 531. FAO, Rome, Italy.
- Heuze, V. & Tran, G. (2015). Duckweed. Retrieved on 26 July 2017 from <https://www.feedipedia.org/node/15306>.
- Huque, K. S., Chowdhury, S. A. & Kibria, S. S. (1996). Study on the potentiality of duckweeds as a feed for cattle. *Asian-Australasian Journal of Animal Science*, 9, 133–137.
- Ife, I., Olatunde, S., Ogbon, O. & Umukoro, J. E. (2021). Processing techniques on phytochemical content, proximate composition, and toxic components in duckweed. *International Journal of Vegetable Science*, 27(3), 294–302.
- Jensen, T. L. (2012). Livestock foraging behaviour in response to sequence and interactions among alkaloids, tannins, and saponins. *All Graduate Theses and Dissertations*, 1217. <https://digitalcommons.usu.edu/etd/1217>
- Khan, M. J., Steingass, H. & Drochner, W. (2002). Evaluation of some aquatic plants from Bangladesh through



- mineral composition, *in vitro* gas production and *in situ* degradation measurements. *Asian-Australasian Journal of Animal Science*, 15(4), 537–542.
- Khiaosa-ard, R., Mahmood, M., Lerch, F., Trautinger, F. P., Petri, R. M., Münnich, M. & Zebeli, Q. (2020). Physicochemical stressors and mixed alkaloid supplementation modulate ruminal microbiota and fermentation *in vitro*. *Anaerobe*, 65, 102263.
- Luhana, K. (2022). Duckweeds: The tiny creatures for resolving the major environmental issues. In: Arora, S., Kumar, A., Ogita, S., Yau, Y. Y. (eds.) *Innovations in Environmental Biotechnology*, Springer, Singapore. [https://doi.org/10.1007/978-981-16-4445-0\\_38](https://doi.org/10.1007/978-981-16-4445-0_38)
- McDonald, P., Edwards, R. A. & Greenhalgh, J. F. D. (1995). *Animal Nutrition 5<sup>th</sup>*, Longman, London, UK.
- Menke, K. H. & Steingass, H. (1988). Estimation of the energetic feed value obtained from chemical analysis and gas production using rumen fluid. *Animal Research and Development*, 28, 7–55.
- Mohedano, R. A., Costa, R. H. R., Tavares, F. A. & Filho, B. P. (2012). High nutrient removal rate from swine wastes and protein biomass production by full-scale duckweed ponds. *Bioresource Technology*, 112, 98–104.
- Negesse, T., Makkar, H. P. S. & Becker, K. (2009). Nutritive value of some non-conventional feed resources of Ethiopia determined by chemical analyses and an *in vitro* gas method. *Animal Feed Science Technology*, 154(3–4), 204–217.
- Obdoni, B. O. & Ochuko, P. O. (2002). Phytochemical studies and comparative efficacy of the crude extracts of some homostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Science*, 8(2), 203–208.
- Orskov, E. R & McDonald, I. (1979). The estimation of protein degradability in the rumen from incubation measurements weighed according to rate of passage. *Journal of Agricultural Science*, 92, 499–503.
- Porter, L. J., Hrstich, L. N. & Chan, B. G. (1986). The conversion of procyanidins and prodelphinidins to cyaniding and delphinidin. *Phytochemistry*, 25, 223–230.
- Remesy, C., Demigne, C. & Morand, C. (1995). Metabolism of short-chain fatty acids in the liver. In: Cummings, J. H., Rombeau, J. L., Sakata, T. (eds.) *Physiological and Clinical Aspects of Short-chain Fatty Acids*. (Cambridge University Press, Cambridge, pp. 171–190.
- Rittner, U. & Reed, J. D. (1992). Phenolics and *In vitro* degradability of protein and fiber in West African browse. *Journal of Science of Food and Agriculture*, 58, 21–28.
- Sallam, S. M. A., Nasser, M. E. A., El-Waziry, A. M., Bueno, I. C. S. & Abdalla, A. L. (2007). Use of an *in vitro* rumen gas production technique to evaluate some ruminant feedstuffs. *Journal of Applied Sciences Research*, 3(1), 34–41.
- SAS, 2002. User's Guide: Statistics, Version 9.1. SAS Institute, Inc., Cary, NC, USA
- Showqi, I., Lone, F. A. and Naikoo, M. (2018). Preliminary assessment of heavy metals in water, sediment and macrophyte (*Lemna minor*) collected from Anchar Lake, Kashmir, India. *Applied Water Science*, 8(80), 1–11.
- Tambe, V. D. & Bhambar, R. S. (2014). Estimation of total phenol, tannin, alkaloid and flavonoid in *Hibiscus tiliaceus* Linn. wood extracts. *Journal of Pharmacognosy and Phytochemistry*, 2, 41–44.
- Van der Spiegel, M., Noordam, M. Y. & Fels-Klerx, H. J. (2013). Safety of novel protein sources (insects, microalgae, seaweed, duckweed, and rapeseed) and legislative aspects for their application in food and feed production. *Comprehensive Reviews in Food Science and Food Safety*, 12, 662–678.
- Van Soest, P. J., Robertson, J. B. & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, 74, 3583–3597.
- Waghorn, G. & McNabb, W. (2003). Consequences of plant phenolic compounds for productivity and health of ruminants. *Proceedings of the Nutrition Society*, 62(2), 383–392.
- Wang, Y., McAllister, T. A., Yanke, L. J. & Cheeke, P. R. (2000). Effects of steroidal saponins from *Yucca schidigera* extract on ruminal microbes. *Journal of Applied Microbiology*, 88(5), 887–896.
- Yahaya, I. O., Anurudu, N. F., Fagbenro, R. B., Omotoso, S. O. & Babayemi, O. J. (2022). Awareness and nutrient composition of duckweed (*Lemna* spp.) as a potential feed for ruminants. *Tropical Journal of Engineering, Science and Technology*, 1 (1), 122–134.
- Yan, Y., Candreva, J., Shi, H., Ernst, E., Martienssen, R., Schwender, J. & Shanklin, J. (2013). Survey of the total fatty acid and triacylglycerol composition and content of 30 duckweed species and cloning of a  $\Delta 6$ -desaturase responsible for the production of  $\gamma$ -linolenic and stearidonic acids in *Lemna gibba*. *BMC Plant Biology*, 13(1), 1–14.
- Zetina-Cordoba, P., Ortega-Cerilla, M. E., Ortega-Jimenez, E., Herrera-Haro, J. G., Sanchez Torres-Esqueda, M. T., Reta-Mendiola, J. L., Vilaboa-Arroniz, J. & Munguia-Ameca, G. (2013). Effect of cutting interval of Taiwan grass (*Pennisetum purpureum*) and partial substitution with duckweed (*Lemna* sp. and *Spirodela* sp.) on intake,

- digestibility and ruminal fermentation of Pelibuey lambs. *Livestock Science*, 157, 471–477.
- Zhao, Y. C., Xue, C. H., Zhang, T. T. & Wang, Y.M. (2018). Saponins from sea cucumber and their biological activities. *Journal of Agriculture, Food and Chemistry*, 66, 7222–7237.
- Ziegler, P., Adelman, K., Zimmer, S., Schmidt, C. & Appenroth, K. J. (2015). Relative *in vitro* growth rates of duckweeds (*Lemnaceae*) – the most rapidly growing higher plants. *Plant Biology*, 17(1), 33–41.