In vitro Gas Production, Proximate Composition, Heavy Metal Composition and Phytochemical profile of Kelp (*Laminaria pallida*) and Water Hyacinth (*Eichhornia crassipes*)

[1]Yusuf, A. O. and [1]Owolabi, A. J.
Department of Animal Production and Health, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.
Corresponding author: john.ayobami969@gmail.com, +2347030544028

Abstract

Alternative feed sources have become imperative to sustain ruminant production, especially in tropical regions. Aquatic plants offer a suitable potential to achieve sustainable ruminant production. Thus, this study was conducted to determine the in vitro gas production, proximate composition, heavy metal and phytochemical profile of water hyacinth (WH) (Eichhornia crassipes) and kelp (seaweed), which are both considered as potential ruminant feed resources. Grounded water hyacinth plants and seaweed were analysed for in vitro gas production, proximate, phytochemical and heavy metal concentrations. In vitro gas production peaked at 29.7 in kelp and 22.7 in WH at 48 hours of incubation. Phytochemical screening revealed presence of phenol, flavonoids, tannins and saponin with WH having 0.59, 0.68, 0.23 and 1.10%, respectively while kelp was 0.88, 1.33, 0.35 and 2.17%, respectively. The composition of lead, cadmium, arsenic, mercury, and chromium in WH were 0.0016, 0.48, 2.275, 0,200 and 0.016mg/kg respectively, while kelp had 0.006, 0.016, 1.400, 0.016 and 0.005mg/kg respectively. Percentage composition of dry matter, ash, moisture content, crude protein, crude fibre, ether extract, NDF, ADF, and ADL in WH were 85.34, 49.06, 14.66, 10.51, 22.66, 11.48, 66.04, 43.44, and 20.71% respectively, while kelp had 83.12, 26.21, 16.88, 13.62, 14.95, 13.53, 56.00, 31.49 and 15.33%, respectively. The results showed that both WH and kelp could be potential feed resources in ruminant production without causing any harmful effects to the animals. However, in vivo studies of both plants are recommended to better ascertain their effects on ruminants.

Keywords: water hyacinth, seaweed, in vitro, ruminants.

Production de Gaz *In Vitro*, Composition Proximale, Composition en Métaux Lourds et Profil Phytopharmaceutique de la Laminaire (*Laminaria pallida*) et de la Jacinthe d'Eau (*Eichhornia crassipes*)

Résumé

Les sources de nourriture alternatives sont devenues impératives pour soutenir la production des ruminants, notamment dans les régions tropicales. Les plantes aquatiques offrent un potentiel approprié pour atteindre une production durable de ruminants. Ainsi, cette étude a été réalisée pour déterminer la production de gaz in vitro, la composition proximale, les concentrations en métaux lourds et le profil phytopharmaceutique de la jacinthe d'eau (JE) (Eichhornia crassipes) et de la laminaire (algue), toutes deux considérées comme des ressources alimentaires potentielles pour les ruminants. Les plantes de jacinthe d'eau et les algues séchées ont été analysées pour la production de gaz in vitro, la composition proximale, les concentrations phytopharmaceutiques et les métaux lourds. La production de gaz in vitro a atteint un maximum de 29,7 dans la laminaire et de 22,7 dans la JE après 48 heures d'incubation. Le dépistage phytopharmaceutique a révélé la présence de phénols, de flavonoïdes, de tanins et de saponines, la JE ayant respectivement 0,59, 0,68, 0,23 et 1,10 %, tandis que la laminaire avait 0,88, 1,33, 0,35 et 2,17 %, respectivement. La composition en plomb, cadmium, arsenic, mercure et chrome dans la JE était respectivement de 0,0016, 0,48, 2,275, 0,200 et 0,016 mg/kg, tandis que la laminaire avait respectivement 0,006, 0,016, 1,400, 0,016 et 0,005 *mg/kg. La composition en matière sèche, cendres, teneur en humidité, protéines brutes, fibres brutes, extrait éther, NDF, ADF et ADL dans la JE était respectivement de 85,34, 49,06, 14,66, 10,51, 22,66, 11,48, 66,04, 43,44 et 20,71 %, tandis que la laminaire avait respectivement 83,12, 26,21, 16,88, 13,62, 14,95, 13,53, 56,00, 31,49 et 15,33 %. Les résultats montrent que la JE et la laminaire pourraient être des ressources alimentaires potentielles dans la production de ruminants sans causer d'effets nocifs pour les animaux. Cependant, des études in vivo des deux plantes sont recommandées pour mieux déterminer leurs effets sur les ruminants.*

Mots-clés : jacinthe d'eau, algue, *in vitro*, ruminants

Introduction

Animal production in Sub-Saharan Africa is basically a smallholder farmers' affair attracting minimum investment in housing, feed and healthcare (Staatz and Dembele, 2008, Salami *et al*., 2010). Herding, tethering and confinement of animals are the three distinct management systems. Among the many constraints to production beneath these systems, those associated with shortages in quantity, quality and deficiency of feed throughout the long dry season are of primary concern (Osakwe *et al.,* 2000).

Ruminants in Nigeria depend largely on natural fields for their feed (Abubakar *et al.,* 1998). Although, natural pastures are adequate for maintenance and small weight gain during the wet season, the reverse is the case during the dry season (Oladotun *et al*., 2003; Odeyinka and Okunade, 2005). Hence, an animal could gain weight during the raining season and just to lose it during subsequent drying season arising from starvation during prolonged dry season (Abubakar *et al.,* 1998). Consequently, the use of unconventional feed resources has been advocated as a way out of this problem. Some of the unconventional feed resources that have been used included *Leucaena leucocephala, Enterolobium cyclocarpum* and aquatic plant like *Eichhornia crassipes* (water hyacinth) (Tham, 2012; Mahmoud, 2013) and seaweed (kelp) (Makkar *et al*., 2016; Min *et al*., 2021).

Major limiting factor to livestock production in tropical Africa is the shortage and fluctuating nature of forage quality and quantity all year round (Ajayi *et al.,* 2005). Most accessible ruminant feeds during the dry season have been described as fibrous and low in digestibility leading to poor livestock productivity (Richard *et al.,* 1994). According to Benninson and Paterson (2003) the potency of using browse plants for ruminant nutrition in the tropics has been affirmed to improve intake of poor-quality roughages, improve development rates and increase reproduction efficiency in ruminants. Water hyacinth and Kelp are known for their high growth rate (Dada, 2002; Thomas, 2002) and past research have indicated its potential as a ruminant feed resource. However, research has reported that these aquatic plants can accumulate metal contaminants like arsenic, lead, mercury and cadmium (Zheng *et al*., 2016; Banach *et al*., 2020) despite the numerous nutritional benefits that they offer (Adelodun *et al*., 2020; Morais *et al*., 2020)

In vitro gas production is used to measure fermentation properties of ruminant feed stuffs (Rymer *et al*., 2005; Posada *et al.,* 2016) wherein the gas produced is primarily the result of carbohydrate fermentation, including starch, cellulose, and hemicelluloses, to volatile fatty acids (VFA). The volatile fatty acids produced are mainly acetate, propionate, and butyrate and, to a lesser extent, succinate, format, lactate, and ethanol. The gases include carbon dioxide, methane, and traces of hydrogen (Makkar 2004; Posada and Noguera 2005). This study was therefore aimed at estimating the *in vitro* gas production, proximate, phytochemical and heavy metal composition of both water hyacinth and kelp.

Materials and Methods

In vitro gas production**:** This was determined following the procedure of Menke and Steingass (1988). A sensitive scale was used to measure 200mg of the samples in triplicates and then placed into 100mL graduated glass syringes. The rumen fluid (inoculum) was collected inside a pre-warmed flask (39^0C) early in the morning (6.00am) from culled cattle at an abattoir located in Odo eran, Abeokuta. The inoculum was strained through two layers of cheese cloth and mixed with sodium and ammonium bicarbonate buffer (35g $NaHCO_3$ plus 4g NH_4HCO_3 per litre) at a ratio of 1:2(v/v) to prevent lowering of the pH of the rumen fluid which could result in decreased microbial activities.

Thirty millimetres of the buffered inoculum were then added to each syringe containing the milled samples and the gas released was read directly on the graduated syringe. The syringes were positioned vertically in a water bath kept at 39˚C. A blank syringe containing 30mL of the buffered inoculums only was included as control. All the syringes were gently shaken 30 minutes after commencement of incubation and four times daily at regular intervals thereafter. Gas production was recorded at 0, 3, 6, 12, 24, 36, and 48 hours of incubation.

The data from *in vitro* gas production was fitted to the non-linear equation (Larbi *et al*., 1996): V (ml/0.2 g DM) = $GV(I-e^{-ct})$

Where V is the potential gas production, GV is the volume of gas and *ct* is the fractional rate of gas production.

Chemical Analysis

Samples were analysed for dry matter (DM) by drying in an oven 105 °C for 12 h. After drying and subjection to particle size reduction, chemical composition was carried out on each feed sample and the whole water hyacinth according to the methods of AOAC (2000). Dried samples were milled through a 1mm screen. Ash was determined after ignition in a muffle furnace at 500 + 15°C for 4 hours. The nitrogen (N) content was used to estimate crude protein (CP) after which samples were digested in concentrated sulphuric acid. Ether extract (EE) was determined using petroleum ether (60-80ºC) extraction in a Soxhlet extracting system.

The neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to the method of Van Soest *et al.* (1991).

Determination of Heavy Metals

Ten grams each of kelp and water hyacinth sample contained in conical flask was digested with 5mL of phosphoric acid, heated on a heating mantle for about an hour, until dryness; 100mL of distilled water were added, thoroughly shaken and filtered into a 100mL standard flask and the filtrate was made up to mark with distilled water. Aliquots were analysed for lead, cadmium, mercury, chromium and arsenic using atomic absorption spectrophotometer, model Shimadzu AA-6680 (Nwude et al., 2010).

Phytochemical Screening

The phytochemical constituents (phenol, flavonoid, tannin and saponin) were determined using standard methods as described by Harborne (1973).

Results and Discussion

Table 1: Phytochemical composition (%) of water hyacinth and kelp

Treatment	Phenol	Flavonoid	Tannin	Saponin
Water Hyacinth	0.59	0.68	0.23	1.10
Kelp (Sea Weed)	0.88	1.33	0.35	2.17

Table 1 shows the phytochemical constituents of whole water hyacinth and kelp. The water hyacinth used in this study contained phenol, flavonoid, tannin and saponin containing 0.59, 0.68, 0.23 and 1.10% respectively, while that of kelp were 0.88, 1.33. 0.35 and 2.17%. respectively. The values obtained for flavonoid (0.68%) and tannin (0.23%) were lower than those reported by Rorong *et al.,* 2012 who used distilled water and three levels of methanol for his analysis. Considering the flavonoid content level of the two plant extracts, kelp is of higher nutritional potential in diet of ruminants as it helps fight off free radicals. A threshold concentration of 5% for tannin has been reported beyond which there may be rejection of browse by goat and wild browsers (Cooper and Own-Smith, 1985). In sheep and cattle, dietary levels of 2 and 5% respectively have also been reported to have adverse effects on digestibility (Mclead, 1994). The levels reported in this study shows that both water hyacinth and kelp will be acceptable by ruminants.

Table 2: Heavy metal concentration of water hyacinth and kelp

Treatment	Lead (mg/kg)	Cadmium (mg/kg)	Arsenic (mg/kg)	Mercury (mg/kg)	Chromium (mg/kg)
Water Hyacinth	0.016	0.480	2.275	0.200	0.016
Kelp (Sea Weed)	0.006	0.016	1.400	0.160	0.005

Table 2 shows the heavy metal concentration of water hyacinth (WH) and kelp. Research (Akobundu, 1987; Ogunlade, 1992) have confirmed the use of WH as having the capability of absorbing heavy metals from industrial and domestic effluents. Laboratory analysis has also shown that water hyacinth is of a high absorptive capacity (Soerjani, 1984). Concerns have also been raised about possible heavy metal contamination in sea weeds from mining, petrochemical industries, municipal waste etc. as sea weeds are directly consumed by humans. Seaweed can rapidly accumulate elevated concentrations of metals such as cadmium and copper (Guadtry *et* al., 2007; Jarvis and Bielmyer-Fraser, 2015). Some heavy metals have bio-importance as trace elements but, the bio-toxic effects of many of them in human and animal biochemistry are of great concern. The levels reported in this study were within certified measurements by WHO, 1995; WHO, 1997; USEPA, 2007. Also, an unpublished research paper by Owolabi on *in vivo* feeding trial of sun-dried water hyacinth with West African dwarf goats showed no significant increase in the blood serum heavy metal of the goats at the end of the feeding trial.

Table 3: Proximate composition of water hyacinth and kelp

Treatment	Water Hyacinth	Kelp (Seaweed)
Dry Matter	85.34	83.12
Ash	49.06	26.21
Moisture Content	14.66	16.88
Crude Protein	10.51	13.62
Crude Fibre	22.66	14.95
Ether Extract	11.48	13.53
Neutral Detergent Fibre (NDF)	66.04	56.00
Acid Detergent Fibre (ADF)	43.44	31.49
Acid Detergent Lignin (ADL)	20.71	15.33

Table 3 shows the proximate composition of water hyacinth and kelp. The whole water hyacinth in this study contained 14.66% moisture, 10.51% crude protein, 11.48% ether extract, 22.66% crude fibre, 49.06% ash, 66.04% NDF, 43.44% ADF and 20.71% ADL. The results on the proximate composition of water hyacinth in this study contradicts the finding of Abdelhamid and Gadar, 1991 and Dung, 2001 who both reported higher dry matter (95% and 87%), lower ash (13.1% and 25.7%), higher crude protein (20% and 12.8%), lower neutral detergent fibre (62.3% and 63.5%), higher ether extract (35% and 38%) and acid detergent fibre (29% and

33.7%) relative to that obtained in this study. This could be as a result of the parts of the plant used in this study as the previous studies used only the shoot, while this study used both the shoot and root.

The proximate composition of kelp in this study contained 83.12% dry matter, 13.62% crude protein, 13.53% ether extract, 14.95% crude fibre, 26.21% ash, 56% NDF, 31.49% ADF and 15.33% ADL. The results obtained in this study are comparable to the results obtained in a similar study on various species of seaweed (Banerjee *et al*., 2009). The slight variations in this study could be as a result of the specie of seaweed, the location in which the plants were harvested and stages of maturity e.g. flowering, fruiting or early stage (all these could be responsible for the variations in crude protein, crude fibre as well as fibre fraction components)

Figure 1: *In vitro* gas production of water hyacinth and kelp

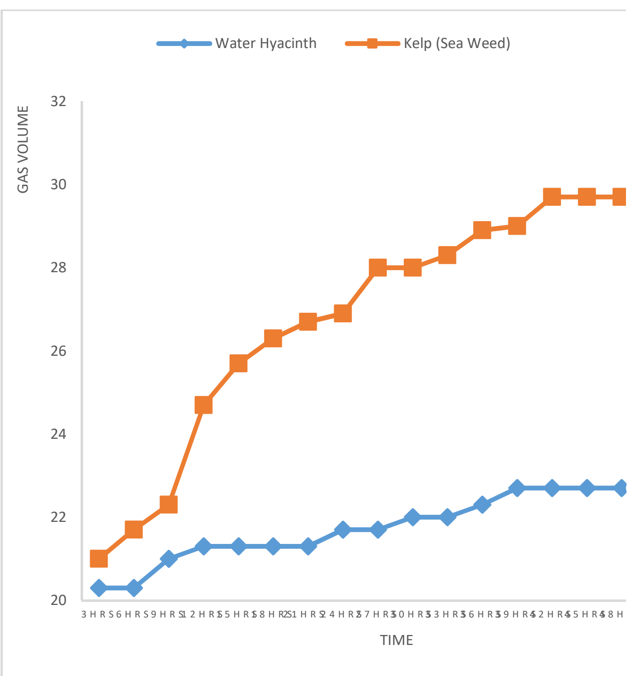

Figure 1 shows the graphical representation of the *in vitro* gas production of water hyacinth and kelp. This result shows kelp to have a higher gas production than water hyacinth. This could be due to kelp having a higher crude protein and lower crude fibre content compared to water hyacinth which had a lower crude protein and higher crude fibre.

Figure 2: Graphical analysis of phytochemical screening between water hyacinth and kelp

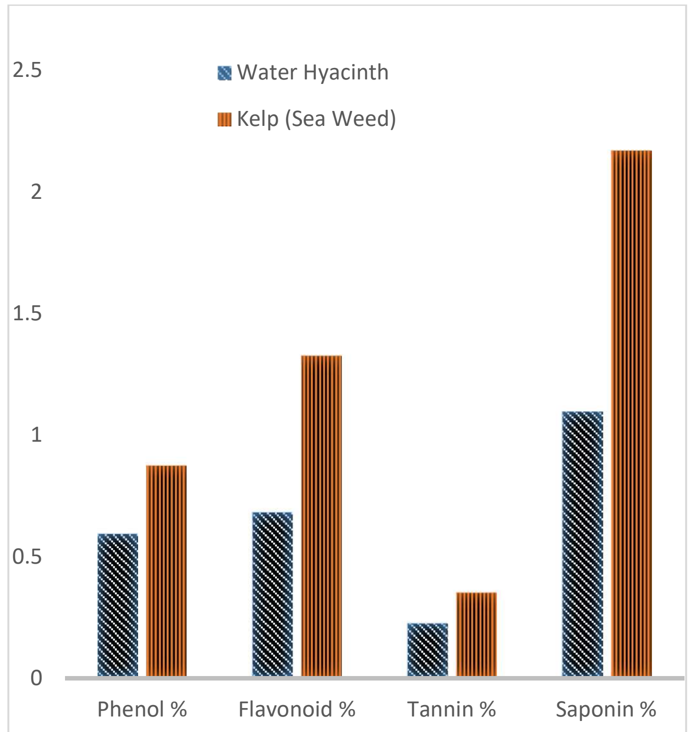

Figure 2 shows the phytochemical constituents of water hyacinth and kelp. It can be deduced from the graph that kelp has a higher content of phenol, flavonoid, tannin and saponin Flavonoid has been known for decades to promote anti-inflammatory activity in animals, according to the findings of Pizoorno *et al*., 2010. According to Kim Evans 2011 flavonoids and proanthocyanidins are often found in fruits and vegetables, and they are powerful cancer prevention agents. Especially, flavonoids are potent antioxidants, and the body uses them to lower inflammation, reduce blood pressure and fight off radicals, which cause disease and aging, sometimes from the inside out. The concentration of flavonoid to the total antioxidant activity of components in food can be very high because daily intake can vary between 50-500 mg according to research review.

Figure 3: Graphical analysis of heavy metal concentration between water hyacinth and kelp

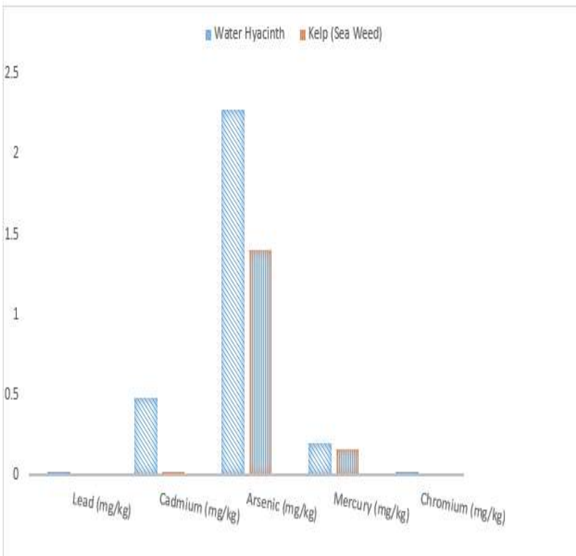

Figure 3 shows the heavy metal concentration between water hyacinth and kelp. From the graph, water hyacinth had a higher level of concentration compared to kelp with the most significant in arsenic where water hyacinth had 2.275% compared to kelp which had 1.4% and cadmium where water hyacinth had 0.48% and kelp had 0.016%. The differences in this value could be as a result of the absorptive capabilities of both sample and/or the concentration of heavy metals in the waters where they were harvested.

Conclusion

It is apparent that both plants have nutritive value which animals can effectively benefit from when included in their diets. Though the results of this study revealed presence of anti-nutritional factors (ANFs) and heavy metals, their concentrations were found to be below the critical levels and therefore are not harmful to ruminant animals; with kelp showing a higher potential than water hyacinth as a beneficial feed resource for ruminants. However, further research *in vivo* could succinctly define the production potentials of these plants and effects from long term exposure of ruminants to them.

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