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REGULAR ARTICLES

Effect of water hyacinth (*Eichhornia crassipes*) hay inclusion in the diets of sheep

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Abstract The aim of this study was to evaluate the effects of replacing Tifton-85 hay (0, 20, 40, 60 and 80 % on a dry matter basis) with water hyacinth hay (*Eichhornia crassipes*) on intake and digestibility of nutrients, feeding behaviour, rumen and blood parameters of sheep. Five uncastrated male sheep, cannulated in the rumen, with an average body weight of 40 kg were assigned in a 5 × 5 Latin square design. The water hyacinth hay contained 870 g/kg dry matter (DM), 159 g/kg crude protein (CP), 547 g/kg neutral detergent fibre (NDF) and 461 g/kg total digestible nutrients (TDN). The DM intake and digestibility of NDF and non-fibre carbohydrates (NFC) were linearly reduced by replacing the Tifton-85 hay with water hyacinth hay. Similarly, there was a linear reduction of rumination time and efficiencies of feeding and rumination of DM and NDF. The concentrations of urea, glucose, AST and GGT in blood plasma were not changed by replacing the Tifton-85 hay with water hyacinth hay. Although water hyacinth hay reduced the intake and digestibility of some nutrients, the Tifton-85 hay replacement could be economically advantageous for sheep feeding.

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Introduction

The floating aquatic plant *Eichhornia crassipes*, popularly known as water hyacinth, is native to tropical and subtropical regions of the world and found in lakes, rivers and reservoirs (Martins et al. 2016). According to Patel (2012), this free-floating, perennial plant is indigenous to Brazil, the Amazon basin and the Ecuador region and was introduced as an ornamental species to adorn water bodies in many countries. Due to the high vegetative multiplication capacity, water hyacinth is considered to be an invasive plant and can reach up 17.5 tons per hectare per day (Rezania et al. 2015). Because this floating macrophyte grows in polluted water with organic contaminants and due to its capacity to accumulate heavy metals in the roots it is often used in wastewater treatment plants, absorbing nutrients from the water (Vitória et al. 2015; El-Zawahry et al. 2016).

The high growth rates and large capacity of dispersal of water hyacinth reflect the ability to compete successfully with other aquatic plants. In addition, it decreases the dissolved oxygen levels in water bodies, leading to a reduction of aquatic fish production (Shu et al. 2015), preventing the growth and abundance of phytoplankton under large mats (Gichuki et al. 2012). The alarming proliferation of water hyacinth affects human activities (ship and boat navigation, recreation, fisheries and tourism) and causes economic losses (Patel 2012). It also presents risks to public health causing the proliferation of diseases, since snails serving as a vector for the parasite schistosomiasis (bilharzia) reside in the tangled weed mat (Borokoni and Babalola 2012).

The control of water hyacinth propagation includes mechanical, chemical and biological control methods, which have often

been insufficient (Gichuki et al. 2012). Thus, to reduce environmental impact and promote sustainable use of the water hyacinth, some studies have been conducted to assess its effects in animal feeding (Peixoto et al. 2012; Tham and Udén 2013; Mako 2013). Hossain et al. (2015) reported that *E. crassipes* can be utilised as feed for animals, especially ruminants, as basal feed resources or supplements, as it contains moderate crude protein content (10.5 % of dry matter (DM)). It can also assist farmers by ensuring sustainable production with the lowest cost diets for cattle. Due to the higher availability and adequate nutritive value, the water hyacinth can be studied and could become an alternative for farmers as an unconventional feed for their livestock during a shortage of forage, to minimise feed cost with concentrated feed and maximised production.

Since water hyacinth (WH) grows in water polluted with organic contaminants, Valk (2015) suggested that WH needs pre-treatment before it can be used as livestock feed, avoiding detrimental effects to the animal. Therefore, the aim of this study was to evaluate the chemical composition of WH (*E. crassipes*) hay and the effects of replacing Tifton-85 hay with WH hay on nutrient intake and digestibility, ingestive behaviour and ruminal and blood parameters of sheep.

Material and methods

The experiment was conducted in the Animal Science Department at the Universidade Federal Rural de Pernambuco (UFRPE), located in Recife, Pernambuco state, Brazil. Five sheep (non-defined racial standard) cannulated in the rumen, with an average body weight of 40 kg, were assigned in a 5 × 5 Latin square design. The animals were kept in metabolic cages provided with individual feeders and drinkers. The experiment lasted 85 days divided into five periods of 17 days, with 10 days for diet adaptation and 7 days for sample and data collection.

WHs (*E. crassipes*) were collected on Apipucos Weir, in the city of Recife, Pernambuco state, Brazil. After collection, WHs were sun-dried for 6 days and kept over black plastic sheeting. Subsequently, WHs were turned several times a day, to maintain uniformity of the material until it contained less than 15 % humidity. The WH hay was processed in a forage chopper. Experimental treatments consisted of different replacement levels (0, 20, 40, 60 and 80 % on a DM basis) of Tifton-85 hay with WH hay (Tables 1 and 2). Diets were offered twice a day, at 8:00 and 16:00 hours, as total mixed ration. Feed leftovers were weighed daily to adjust voluntary intake, and 10 % of leftovers was allowed.

Faecal DM production was determined by total faeces collection twice a day (7:00 and 16:00 hours), using faecal collection bags that were maintained for 24 h in the animals, within 5 days of each collection period. The samples were stored in a freezer at −10 °C, which were later thawed, dried partially in a forced oven at 55 °C for 72 h and ground in a Willey mill with a 1-mm screen. The DM, mineral matter (MM), crude protein (CP) and ether extract (EE) were analysed as described by AOAC (2000). Neutral detergent fibre (NDF) was analysed as described by Mertens (2002). Neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) (Licitra et al. 1996) were measured using the Kjeldahl method. To estimate the non-fibre carbohydrates (NFC), the following equation was utilised: NFC (g/kg) = 1000 − [(CP − urea-derived CP + urea) + NDFap + EE + ash], as proposed by Hall (2000). Total digestible nutrients (TDN) were calculated by the following equation proposed by NRC (2001): $TDN\ (\%) = CP_d + 2.25\ EE_d + NFC_d + NDF_d - 7$ (subscript means digestible).

On the first day of the collection period, ingestive behaviour assessments (feeding, rumination and idle) were conducted by instantaneous scanning (Johnson and Combs 1991), in 10-min intervals, adapted to a 24-h period (Martin and Bateson 1993). DM and NDF feeding and rumination efficiencies (g/h) were calculated by dividing the intake of each nutrient by the total feeding time (feed efficiency) and rumination time (rumination efficiency).

On the last day of each collection period, rumen fluid samples (100 mL) were collected at 0, 2, 4 and 6 h after morning feed. To determine the ruminal pH, an automatic potentiometer was used. Blood samples were collected by jugular venipuncture in Vacutainer® tubes with and without anticoagulants on the last

Table 1 Chemical composition of feed used in the diet (g/kg DM)

Feed	DM (g/kg)	CP	EE	NDFap	TDN	Calcium	Phosphorus	Sodium
Tifton-85 hay	890	63.9	16.3	681	556	4.20	1.70	0
Water hyacinth hay	870	159	9.00	547	461	11.3	3.60	0
Ground corn	882	95.0	35.0	108	872	0.30	2.50	0
Soybean meal	901	513	49.0	219	815	3.00	6.00	0
Urea	990	0	0	0	0	0	0	0
Salt	990	0	0	0	0	174	3.00	396
Soybean oil[a]	996	0	990	–	207	0	0	0

DM dry matter, *OM* organic matter, *CP* crude protein, *EE* ether extract, *NDFap* neutral detergent fibre corrected for ash and protein, *TDN* total digestible nutrients

[a] Valadares Filho et al. (2015)

Table 2 Proportion of ingredients and chemical composition of the diet

	Replacement levels (%)				
	0	20	40	60	80
Ingredients (g/kg)					
Tifton-85 hay	600	480	360	240	120
Water hyacinth hay	0	120	240	360	480
Ground corn	264	296	325	322	350
Soybean meal	122	90	54	40	10
Urea	2.0	2.0	2.0	4.0	3.0
Salt	2.0	2.0	2.0	2.0	2.0
Soybean oil	0	0	7.0	22	25
Mineral supplement[a]	10	10	10	10	10
Chemical composition (g/kg DM)					
Dry matter (g/kg)	890	887	885	885	882
Crude protein	141	137	131	139	133
Ether extract	25.0	23.7	29.0	42.2	43.8
FDNap	464	444	424	404	385
Total digestible nutrients	716	710	691	691	674

NDFap neutral detergent fibre corrected for ash and protein

[a] Nutrients/kg of product: calcium = 173.7 g, phosphorus = 70 g, sodium = 148 g, magnesium = 1320 mg, iron = 2200 mg, cobalt = 140 mg, manganese = 3690 mg, zinc = 4700 mg, iodine = 61 mg, selenium = 45 mg, sulphur = 12 g and fluorine = 700 mg

day of data collection in each period, 4 h after the first feed. The aliquots of serum from blood samples were obtained by centrifugation at 2500 rpm and were stored in Eppendorfs at -20ºC until performing the analyses for the biochemical doses of metabolites: creatinine, urea, glucose, cholesterol, total protein, albumin, globulin, triglycerides, aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), alanine aminotransferase (ALT), calcium and phosphorus. Blood metabolite analysis was conducted with Doles® commercial kits with the colorimetric system, in a Doles®.D-250 semi-automatic biochemistry analyser.

Data were subjected to analysis of variance and regression, using the GML procedure of the Statistical Analysis System (SAS) program, considering the 5 % level of probability for a type I error. Replacement levels of Tifton-85 hay with WH hay (Y), collection time (T) and the interaction between these two factors (Y × T) were used as fixed effects to assess rumen pH in the 5 × 5 Latin square design. Repeated measures design over time was used, with (0, 2, 4 and 6 h after feeding) sampling times, which were repeated once within each experimental unit (animal × period).

Results

WH hay showed contents of 870 g/kg DM, 159 g/kg CP, 547 g/kg NDF, 246 g/kg NDF and 461 g/kg TDN (Table 1). DM, OM, CP, NDF, NFC and TDN intakes were linearly reduced ($P<0.05$) with the replacement of Tifton-85 hay with WH hay (Table 3). Except for DM, OM and CP ($P>0.05$), there was a linear reduction ($P<0.05$) in the apparent digestibility of NDF and NFC (Table 3). Linear reductions ($P<0.05$) were observed for rumination time and feeding and rumination efficiencies. However, feeding time was not changed ($P>0.05$) (Table 4).

The pH decreased ($P<0.05$) due to collection times, but there was a linear increase ($P<0.05$) with WH hay levels (Table 5). Concentrations of urea, total protein, glucose, triglycerides, AST, GGT, ALT and calcium in blood plasma were unchanged ($P>0.05$) with the substitution of Tifton-85 hay with WH hay (Table 6). However, linear reduction ($P<0.05$) was observed in albumin, ALP and phosphorus concentrations, and linear

Table 3 Intake and digestibility of nutrients in sheep fed diets containing different replacement levels of Tifton-85 hay with water hyacinth hay

Item	Replacement levels (%)					SEM	*P* value
	0	20	40	60	80		
Intake (g/day)							
Dry matter	1180	1130	950	820	680	26.4	<0.05
Organic matter	1100	1040	850	730	600	23.9	<0.05
Crude protein	90	90	80	80	70	2.3	<0.05
Neutral detergent fibre	480	450	360	290	230	11.7	<0.05
Non-fibre carbohydrates	500	490	400	340	290	10.7	<0.05
Total digestible nutrients	820	800	650	550	440	23.4	<0.05
Digestibility (g/kg)							
Dry matter	730	728	724	722	716	11.6	>0.05
Organic matter	762	758	748	728	720	12.2	>0.05
Crude protein	652	644	643	642	640	13.9	>0.05
Neutral detergent fibre	679	670	628	565	557	14.8	<0.05
Non-fibre carbohydrates	908	891	886	882	843	8.2	<0.05

SEM standard error of the mean

Table 4 Ingestive behaviour of sheep fed diets containing different replacement levels of Tifton-85 hay with water hyacinth hay

Item	Replacement levels (%)					SEM	*P* value
	0	20	40	60	80		
Feeding time (h/day)	3.98	3.18	3.13	3.45	3.17	0.15	>0.05
Rumination time (h/day)	8.92	8.38	8.97	7.82	7.08	0.86	<0.05
Idle time (h/day)	11.18	12.52	11.98	12.82	13.83	0.33	<0.05
Feeding efficiency of DM (kg/h)	0.31	0.36	0.33	0.26	0.23	0.02	<0.05
Feeding efficiency of NDF (kg/h)	0.16	0.17	0.15	0.11	0.09	0.01	<0.05
Rumination efficiency of DM (kg/h)	0.14	0.13	0.11	0.11	0.10	0.003	<0.05
Rumination efficiency of NDF (kg/h)	0.07	0.06	0.05	0.05	0.04	0.002	<0.05

SEM standard error of the mean

increases were observed in concentrations of creatinine, globulin and cholesterol (Table 6).

Discussion

The chemical composition of WH differs according to the region where it is collected. As WH is an aquatic plant, it depends on the nutrients available in the environment. The protein content of WH hay of 159 g CP/kg DM was higher than the 63.9 g CP/kg DM content of Tifton-85 hay (Table 1). Peixoto et al. (2012) reported a CP content of 252 g/kg DM in WH originating from Billings Reservoir (São Paulo, Brazil); Tham and Udén (2013) reported a CP of 174 g/kg DM in ensiled WH in Vietnam.

Reduction of NDF and TDN contents of diets (Table 2) could explain the decrease in DM and intake of other nutrients. According to Allen (2000), forage NDF content is highly correlated with DM intake. Lower energy input in diets with increasing WH hay levels explains TDN intake reduction in sheep. The absence of intake limitation by physical factors (rumen fill) explains lower time and rumination efficiency in sheep fed with higher WH hay levels.

Similar to this study, Mako (2013) observed DM intake reduction in goats fed with dehydrated WH replacing guinea grass, implying that it could be used as sole forage or at a high proportion in the diet of ruminants. However, by providing a fibre source (rice straw), Khan et al. (2002) found 67 % increase in DM intake of steers.

NDF digestibility reduction explains the decrease in fibre intake and can also explain reductions in the intake of other nutrients. As the fibre was not a limiting factor for intake, the higher content of rapidly degraded carbohydrates probably provided higher rumen outflow rate and lower fibre digestion time. Tham and Udén (2013) observed NDF digestibility increase in cattle fed with increasing levels of ensiled WH combined with a fibre source.

The average ruminal pH in sheep fed WH hay showed a normal range (5.5 to 7.0) for maximum microbial growth and maximum fibre ruminal digestion (Hoover and Stokes 1991), with the ideal fibre digestion range between 6.7 and 7.1.

Sheep fed with increasing WH hay levels showed increased concentrations of creatinine, globulin and cholesterol. Creatinine concentration is not associated with animal feeding (Gonçalves et al. 2014), but has been directly related to muscle mass, as it is a product of muscle metabolism and, as a result, is significantly correlated to live weight (Damptey et al. 2014). Regarding globulin, Damptey et al. (2014) reported that high globulin concentrations are indicative of an animal's immune state, in response to diseases and infections. WH hay did not cause animal intoxication in this study, as globulin levels were kept within the normal range for sheep (Table 6). Lower DM intake, as well as lower energy intake in diets with higher WH hay levels, could explain the increase in cholesterol levels, which were likely caused by mobilisation of body lipid reserves. According to Homem Junior et al. (2010), blood cholesterol may increase with the mobilisation of body reserves due to reduced intake.

Reduction in albumin concentrations may be related to a reduction in protein intake with increased WH hay levels. According to Kaneko et al. (1997), this metabolism is related to the protein content in the diet of ruminants. Due to the lack of effect of WH hay increasing levels on AST, ALT and GGT concentrations, which are related to hepatic metabolism, it could be inferred that there was no animal intoxication. According to González and Silva (2003), these enzymes act as blood biomarkers to assess metabolic disorders and liver function. The average total protein (75.5 g/L) was considered normal,

Table 5 Average pH of the rumen fluid as a function of collection time and inclusion level of water hyacinth hay

Item	Replacement levels (%)					SEM	*P* value
	0	20	40	60	80		
Hour 1 (0 h)	6.63	6.80	6.95	7.04	7.19	0.04	<0.05
Hour 2 (2 h)	6.30	6.51	6.59	6.69	6.85	0.04	<0.05
Hour 3 (4 h)	6.19	6.15	6.42	6.57	6.62	0.04	<0.05
Hour 4 (6 h)	6.27	6.29	6.43	6.54	6.63	0.03	<0.05

SEM standard error of the mean

Table 6 Metabolic profile of sheep fed diets containing different replacement levels of Tifton-85 hay with water hyacinth hay

Item	Replacement levels (%)					Reference value[a]	SEM	*P* value
	0	20	40	60	80			
Creatinine (μmol/L)	76.6	78.7	83.6	82.2	86.5	106–168	0.76	<0.05
Urea (mmol/L)	8.60	8.38	7.77	8.17	6.76	2.0–8.0	0.21	>0.05
Total proteins (g/L)	73.6	75.1	77.6	73.3	78.0	60.0–79.0	1.0	>0.05
Albumin (g/L)	35.7	33.4	33.9	32.9	31.7	24.0–39.0	0.54	<0.05
Globulin (g/L)	37.8	41.7	43.6	40.4	46.3	30.4–57.0	1.3	<0.05
Glucose (mmol/L)	4.38	4.57	4.32	4.47	4.74	5.0–8.0	0.16	>0.05
Cholesterol (mmol/L)	1.74	1.73	2.03	2.16	2.60	4.9–7.6	0.05	<0.05
Triglycerides (mmol/L)	0.42	0.32	0.40	0.41	0.35	NI	0.021	>0.05
AST (U/L)	74.4	61.8	66.0	70.2	69.1	0.0–90.0	2.0	>0.05
GGT (U/L)	38.2	39.6	39.1	36.7	35.1	20.0–52.0	1.1	>0.05
ALP (U/L)	526	522	489	440	448	68.0–387	13.4	<0.05
ALT (U/L)	15.7	13.6	11.5	12.6	12.6	30.0–40.0	0.63	>0.05
Calcium (mmol/L)	3.00	2.63	3.00	2.15	2.68	11.5–12.8	0.076	>0.05
Phosphorus (mmol/L)	2.32	2.29	2.14	2.10	1.66	5.0–7.3	0.066	<0.05

AST aspartate aminotransferase, *GGT* gamma glutamyl transferase, *ALP* alkaline phosphatase, *ALT* alanine aminotransferase, *NI* no information in the literature, *SEM* standard error of the mean

[a] Kaneko et al. (1997)

indicating that the WH hay provided adequate protein supply. The lack of WH hay effect on serum glucose was expected since this metabolite is not very sensitive to dietary energy intake variations (Contreras 2000).

The results of this study demonstrate the need for further studies to assess WH effects on sheep performance. Although WH hay reduced the intake and digestibility of some nutrients, the Tifton-85 hay replacement could be economically advantageous for sheep feeding in areas with great availability of this aquatic plant.

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Compliance with ethical standards

Statement of animal rights All procedures performed in studies involving animals were in accordance with the ethical standards of the Institutional Ethics Committee on Animal Use—CEUA—at the Universidade Federal Rural de Pernambuco where the studies were conducted.

Conflict of interest The authors declare that they have no competing interests.

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