

Effects of using Water Hyacinth (*Eichhornia crassipes* L.) in the Diet of Swamp Buffaloes on Nutrient Digestibility, Rumen Environment, Purine Derivatives, and Nitrogen Retention

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Abstract: The present experiment aims to evaluate the effects of incremental levels of water hyacinth (WH) in Para grass (*Brachiaria mutica*) based on the diet on nutrient intake, digestibility, and nitrogen retention of growing swamp buffaloes. Four male buffaloes of 305 ± 8.40 kg were allocated in a 4x4 Latin square design. The treatments were 25, 50, 75, and 100% WH (DM basis), replacing the Para grass (PG) corresponding to WH25, WH50, WH75, and WH100 treatment. Urea-molasses cake was used to supply the dietary crude protein intake so that this was 210 g/100 kg live weight per day for all the treatments (DM basis). The results showed that although the dry matter (DM), neutral detergent fiber (NDF), and metabolizable energy (ME) intake were not significantly different ($P>0.05$) among the treatments, these were numerically higher for the WH50 treatment. Especially in the daily ME intake (MJ/kg LW) was 39.1, 43.4, 41.7, and 39.9 for the WH25, WH50, WH75, and WH100 treatments, respectively. With 50% WH replacing PG in the diet, it gave potential for better nutrient digestibility, nitrogen retention, and daily weight gain in the present study. In conclusion, WH could be used to replace PG in buffalo diet up to 100%. At a level of 50% replacement of WH to PG in the buffalo, the diet was optimum based on the utilization of nutrients and energy of WH, lower feed cost, and environmental improvement.

Keywords: Swamp buffalo, water plants, nutrition, microbial protein synthesis.

1. INTRODUCTION

The water buffaloes raised in Vietnam are mainly swamp types, closely associated with mud, water, and rice production. In the Mekong delta (MD), a rice basket of Vietnam, during the flooding season, it is found that they can thrive well in acidic water by diving into grazing water plants underwater in the daytime and consuming local supplements such as rice bran, broken rice, etc. in the night time. In recent years the buffaloes in Vietnam have been raised essentially for meat, while less for work due to an increase of mechanization in agricultural production. The buffalo beef demand has been enhanced in the whole country. Interestingly, the buffalo population decreased from 2.88 million heads (2010) to 2.35 million heads (2019), with the annual reduction being 2.25 %, while the buffalo beef produced in domestic markets increased from 83,600 tons (2010) to 95,100 tons (2019) with the annual increase of 1.28 % [1, 2]. Buffalo beef has become a special meat preferred by Vietnamese to make many delicious and nutritious dishes such as buffalo meat hot pot, Stir-fried fresh buffalo meat, Stewed buffalo ribs with lemongrass, etc., in well-

hyacinth (WH, *Eichhornia crassipes*) has been known and expensive restaurants in many cities. Water natural water plants with large bio-mass, low acid detergent fiber, and good crude protein content (similar to the tropical grasses) but high moisture content under alluvial or acidic water conditions. It well develops in the MD, with a tremendous amount available in most canals and rivers, and has sometimes caused the problems of environment and waterway transportation [3]. The water hyacinth was also studied as the feed source in fresh or silage forms for cattle, sheep, and chicken [4-6] with the prospective application. However, the studies for utilizing WH as the main coarse feed in swamp buffalo diets have still been limited. Therefore, this study aimed to determine the optimum levels of freshwater hyacinth in the buffalo diet based on the nutritional effects of intake, digestibility, rumen environment, and nitrogen retention.

2. MATERIALS AND METHODS

Location

This experiment was carried out at the experimental farm Nam Can Tho, Phong Dien district of Can Tho city, and Department of Animal Science, College of Agriculture, Can Tho University, Vietnam.

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Experimental Design

Para grass (*Brachiaria mutica*) is a common natural grass that popularly develops in the MD and is widely used to feed buffaloes and cattle. The experiment was arranged as a 4x4 Latin square design with four male swamp buffaloes of 305 ± 8.40 kg. The treatments were 25, 50, 75, and 100% WH (DM basis), replacing the Para grass (PG) corresponding to WH25, WH50, WH75, and WH100 treatment.

Feeding Procedure

Before entering the experiment, all the buffaloes were individually offered Para grass and urea-molasses cake supplementation for 7 days to calculate the daily dry matter of PG intake, and then WH replacement to PG was applied for the experiment. PG was fed ad libitum, while the urea-molasses cake (UMC), including urea, soybean meal, rice bran, molasses, and minerals [7], was used to supply the dietary crude protein intake so that this was 210 g/100 kg live weight per day [8] for all the treatments (DM basis).

The buffaloes were fed twice a day at 7.30 AM and 3.30 PM and each time 50% of the ration was offered. Fresh water was available all the time. The prior feeding of urea molasses cake was done, and then next for the WH and PG. PG was chopped from 10-20 cm, while fresh WH was made from 20 to 30 cm in length.

The experiment followed the ethical management process on experimental animals of the Can Tho University of Vietnam, No. 3965/QĐ ĐHCT. Published on October 15-2021.

Measurements

Feed and nutrient intakes, nutrient digestibility, rumen parameters, purine derivatives, nitrogen retention, and daily weight gain were measured.

The experimental period was 14 days, with 7 days for adaptation and 7 days for taking rumen fluid samples and recording. During the 7-day collection period, feeds were offered and refused, and feces and urine were collected daily, weighed, and pooled weekly for chemical analysis.

Dry matter (DM), organic matter (OM), crude protein (CP), and total ash (Ash) of feed samples were determined according to [9]. Neutral detergent fiber

(NDF) was analyzed [10], and metabolizable energy (ME) was calculated [11]. Urine nitrogen analysis was done by the Kjeldahl method. The apparent nutrient digestibility of DM, OM, CP, NDF, and nitrogen balance was determined [12].

Rumen fluid was collected using an esophagus tube at 3 hours post-feeding; samples were weighed and pooled over the day collection period. Rumen ammonia was measured by the Kjeldahl method and VFAs analysis was done by Gas-liquid chromatography [13].

Allantoin was measured using HPLC [14], while Uric acid was measured on an autoanalyzer [15]. Then purine derivative (PD) was calculated by the equation Y (PD) = Allantoin (mmol/d) + Uric acid (mmol/d). The estimation of microbial nitrogen synthesis (MN) of the swamp buffalo was calculated following the equations [16]:

$$Y = 0.12 * X + 0.2 * W^{0.75}$$

The estimation of microbial nitrogen synthesis (MN).

$$MN \text{ (gN/buffalo/d)} = 0.727 * X$$

Where:

Y: Total purine derivatives in urine

X: Purine of microbes absorbed

$W^{0.75}$: Metabolic live weight

MN: Microbial nitrogen synthesis

Live weight was determined at the initial and end of the experimental period using the electrical scale (Tru-Test, Limited Auckland, New Zealand) to calculate the daily weight gain.

Statistical Analysis

The recorded data were analyzed using the software of Minitab Reference Manual Release16.2 [17], ANOVA procedure, following the 4x4 Latin square model, and when a comparison between 2 treatments was made, the Tukey test of the Minitab was used.

3. RESULTS AND DISCUSSION

Chemical Composition of Feeds

The chemical composition of the feeds used in the experiment is presented in Table 1.



Photo 1: Water hyacinth (WH) and collection for the Exp.

Table 1: Chemical Composition (%) of Feeds Used in the Trial

Item	DM	OM	CP	PDF	ADF	Lig	Ash	ME*, MJ/kgDM
Water hyacinth	8.45	85.36	12.34	56.59	35.56	3.86	14.64	9.52
Para grass	17.9	87.6	12.8	64.4	40.6	6.88	12.4	9.77
Urea-molasses cake (UMC)	76.5	84.4	32.4	14.9	33.9	6.16	15.6	10.2

DM: dry matter, OM: organic matter, CP: crude protein, ADF: Acid detergent fiber, NDF: neutral detergent fiber, Lig: Lignin, and ME*: Metabolizable energy [11].

Table 1 showed that DM content of WH (8.45 %) was lower than PG (17.94%). OM and CP content was similar between WH and PG, while NDF, ADF, and Lignin content of WH (56.6, 35.6, and 3.86%, respectively) were lower than PG (64.4, 40.6, and 6.88 %, respectively). These criteria also indicated that WH could be a promising forage to replace PG in common diets of buffalo diets. In addition, it can provide a significant amount of water in the diet. The urea-molasses cake could be a good supplement for buffaloes in the experiment with 32.4 %CP and 10.2

MJ/kgDM. The CP (12.3 %) and Lignin (3.86 %) in the experiment were similar to those reported by Nigam [18], being 13.3% and 3.50 %, respectively. At the same time, the WH ash of 14.6 % in the present study was also similar to that stated by Dada [19], being 14.1 %.

Daily Feeds, Nutrients, and ME Intakes

The feeds, nutrients, and ME intakes of buffaloes in different treatments are shown in Table 2.

Table 2: Daily Feeds, Nutrients, and ME Intakes of Buffaloes in Different Treatments

Item	Treatment				± SEM	P
	WH25	WH50	WH75	WH100		
Daily intakes, kg DM/head						
WH	1.01 ^a	2.31 ^b	3.18 ^b	4.33 ^c	0.276	0.001
PG	2.98 ^a	2.26 ^a	1.05 ^b	0.00 ^c	0.224	0.001
UMC	0.435	0.212	0.360	0.338	0.135	0.477
WH/PG, %	25.4	50.4	75.1	100.0	0.131	0.001
DM	4.43	4.78	4.59	4.67	0.135	0.162
OM	3.84	4.14	3.94	3.99	0.126	0.235
CP	0.646	0.642	0.643	0.644	0.003	0.696
NDF	2.56	2.79	2.53	2.50	0.129	0.167
ADF	1.72	1.82	1.69	1.66	0.064	0.157
*ME, MJ/ani.	43.2	46.7	44.2	44.7	2.546	0.402

WH25, WH50, WH75, and WH100: WH replacing PG at levels 25, 50, 75, and 100 %. This means different letters within the same row significantly differ at the 5% level. DM: dry matter, OM: organic matter, CP: crude protein, ADF: Acid detergent fiber, NDF: neutral detergent fiber, Lig: Lignin, and *ME: Metabolizable energy (Bruinenberg *et al.*, 2002).

Table 2 showed that daily WH, PG, and UMC intakes confirm the experimental design. The daily DM intake was not significantly different among the treatments. However, it was numerically higher for the WH50, WH75, and WH100 treatments than the 25WH treatment. Similarly, the daily ME intake (MJ/ani.) was 46.7, 44.2, and 44.7 for the WH50, WH75, and WH100 treatments, respectively, compared to that of the WH25 treatment (43.2). The results showed that DM and ME intakes of the WH50 treatment were relatively higher than the others. It also seemed to indicate that this level of 50WH:50PG was appropriate for the buffalo intakes with a better association in both two plants based on the nutrients and digestibility (Table 3). In a previous experiment on swamp buffaloes (233±25.0 kg) with a range of dietary CP (% DM) being 5.4, 6.6, 8.5, and 10.5 as the treatments, the daily DM intake (kgDM) was from 4.17 to 5.60, and the CP intake (kgDM) was from 0.21 to 0.60 [20].

Apparent Nutrient Digestibility

In Table 3, the apparent nutrient digestibility is shown.

In general, the digestibilities of nutrients of buffaloes in treatments were not significantly different ($P>0.05$).

There was a slightly higher value of digestibility DM, OM, CP, NDF, and ADF for the WH50 treatment as compared to the others. The digestibility (%) of DM and CP ranged respectively from 68.1÷72.4 and 66.5÷71.6 among the treatments. These digestibility coefficients were higher than those from other reports on swamp buffaloes, being 53.1 and 63.3, respectively, with the diets including Para grass, rice straw, and sesbania [8]; and being 56.0-60.8 for DM with diets including rice straw, cassava pulp, ground corn, soybean meal and urea [20]. Thus, WH replacing other common forages in the buffalo diets could improve nutrient digestibility.

Rumen Function

The rumen parameters measured for evaluating the rumen function are shown in Table 4.

The rumen parameters of the buffaloes in the experiment showed that pH was not affected by the dietary treatments. In contrast, N-NH₃ concentration in the treatments at 3 h was higher, and it was gradually reduced when increasing the WH percent in the diets ($P<0.05$). Similarly, VFA concentration (μmol/ml) at 3 h after feeding was significantly different ($P<0.05$) among the treatments with the higher value (87.2) for the WH50 treatment, while there was a reduction of VFA

Table 3: Apparent Nutrient Digestibility (%) of Buffaloes in Different Treatments

Item	Treatment				± SEM	P
	LB25	LB50	LB75	LB100		
DM	70.2	72.4	71.0	68.1	2.430	0.414
OM	71.2	73.4	71.8	68.7	2.329	0.339
CP	67.4	71.6	67.6	66.5	3.062	0.424
NDF	70.2	72.7	71.7	69.4	3.685	0.810
ADF	63.5	66.3	65.2	64.8	2.015	0.592

WH25, WH50, WH75, and WH100: WH replacing PG at levels 25, 50, 75, and 100 %.

Table 4: Rumen pH Values, N-NH₃ (mg/100ml), and Total Volatile Fatty Acid (VFAs) Concentration (μmol/ml) at 3 h after the Feeding of Buffaloes in the Treatments

Item	Treatment				P	± SE
	WH25	WH50	WH75	WH100		
pH	6.98	7.02	6.95	7.05	0.372	0.058
N-NH ₃	25.2 ^a	25.0 ^a	20.0 ^{ab}	17.5 ^b	0.010	1.73
VFAs	86.6 ^a	87.2 ^a	75.5 ^{ab}	74.2 ^b	0.018	3.56
<i>Acetic acid</i>	61.1 ^a	60.6 ^a	52.4 ^b	52.7 ^b	0.015	2.67
<i>Propionic acid</i>	18.5	19.5	17.3	16.1	0.082	2.82
<i>Butyric acid</i>	4.98	4.62	4.34	4.12	0.287	0.672

WH25, WH50, WH75, and WH100: WH replacing PG at levels 25, 50, 75, and 100 %. This means different letters within the same row significantly differ at the 5% level.

concentration for the WH75 and WH100 (Table 4). The reduction of N-NH₃ and VFA concentration could be caused by the higher water consumption when increasing the WH from WH75 to WH100 (lower DM content compared to PG). However, interestingly, these did not negatively affect nutrient digestibility in the present experiment. Thus the optimum WH percent in diets should be considered for improving the rumen environment, and WH could be utilized for feeding the water buffaloes. It was observed that the total VFAs of swamp buffaloes fed rice straw and urea-treated rice straw at 4 h after feeding was from 96.7 – 115 µmol/ml [21], and similarly, this was 90.4 µmol/ml at 3 h after feeding in swamp buffaloes in the Mekong delta [22].

Purine Derivatives and Microbial Nitrogen Synthesis

Purine derivatives excretion in urine and microbial nitrogen synthesis of the buffaloes in the experiment are presented in Table 6.

Table 5 showed that Allantoin, uric acid, purine derivatives, and estimated microbial nitrogen in

buffaloes were not significantly different ($P < 0.05$) among the treatments. However, these values were slightly higher for the WH50 treatment. The urinary allantoin and uric acid (mmol/ani./d) of swamp buffaloes were found to be from 22.4 to 37.4 and from 4.90 to 9.10 by Wanapat and Rowlinson [21]. In the present study with the 210gCP/100 kgLW intake, urinary PD excretion (µmol/kgW^{0.75}) was from 226 to 365 compared to the results presented by Paengkoum *et al.* [20] being from 287 to 403 in the experiment mentioned above. This experiment's estimated MN (gN/ani./d) was from 45.8 to 80.0, with gradual reductions of the PD and MN from WH75 to WH100 treatment.

Nitrogen Balance and Daily Weight Gain

Nitrogen balance and daily weight gain of the buffaloes are presented in Table 6.

Nitrogen intake, excretion, and retention were not significantly different ($P > 0.05$). However, there was a higher value of nitrogen retention for the WH50 treatment compared to others. Although a short time of

Table 5: Allantoin, Acid Uric and Purine Derivatives (PD) in Urine, and Microbial Nitrogen (MN) Synthesis in the Treatments

Item	Treatment				±SEM	P
	WH25	WH50	WH75	WH100		
Allantoin, mmol/head/d	19.9	24.3	18.8	18.6	2.52	0.183
Uric acid, mmol/head/d	3.43	4.81	4.11	3.65	0.602	0.208
PD, mmol/head/d	23.3	29.1	22.9	22.2	3.15	0.351
PD, µmol/kgW ^{0.75}	299	365	276	266	31.9	0.075
MN, gN/head/d	54.2	80.0	49.9	45.8	18.9	0.352
MN, gN/kgW ^{0.75}	0.728	1.092	0.626	0.549	0.234	0.201

WH25, WH50, WH75, and WH100: WH replacing PG at levels 25, 50, 75, and 100 %. This means different letters within the same row significantly differ at the 5% level.

Table 6: Nitrogen Retention and Daily Weight Gain of the Buffaloes in the Treatments

Item	Treatment				±SEM	P
	WH25	WH50	WH75	WH100		
Nitrogen balance, g/head/d						
<i>Intake</i>	103.5	103.8	103.5	103.6	0.271	0.669
<i>Excretion</i>	72.8	67.2	70.0	71.3	7.13	0.873
<i>Retention</i>	30.7	36.6	33.5	32.3	7.10	0.858
Nitrogen retention, g/kgW ^{0.75}	0.448	0.565	0.435	0.490	0.082	0.449
Live weight at initial, kg	307	305	306	307	0.707	0.416
Daily weight gain, g	277 ^b	598 ^a	321 ^b	330 ^b	48.8	0.002

WH25, WH50, WH75, and WH100: WH replacing PG at levels 25, 50, 75, and 100%. This means different letters within the same row significantly differ at the 5% level.

2 weeks for recording the change of buffalo live weight, it was observed that the reference values of daily weight gain (g) were significantly different ($P < 0.05$) among the treatments, with the highest value for the WH50 treatment (598). In one experiment of swamp buffaloes (231 kg) in Vietnam with an intake of 150-200 gCP/100 kgLW, Nha *et al.* [8] found that the nitrogen retention (g/d) and the weight gain (g) being 214 and 335, respectively, which was lower than these of the WH50 treatment but similar to the others of the present experiment.

In general, using WH replacing PG (%) from 25 to 100 in the diets of swamp buffaloes indicated that DM and ME intakes, nutrient digestibility, rumen parameters, urinary PD excretion, and nitrogen retention did not have any negative effects. However, there was a slight, although not significant, improvement in those at the WH50 treatment. The better MN synthesis, nitrogen retention, and daily weight gain in this treatment could be explained by the appropriate CP intake in all the treatments and improved ME intake. Thus the improvements could support the daily weight gain of the WH50 treatment as compared to others. Especially the microbial nitrogen synthesis in ruminants was improved by the synchronization of ME sources and CP in the rumen, which was confirmed by other authors [23-25].

4. CONCLUSIONS AND RECOMMENDATIONS

In conclusion, water hyacinths could be used to replace grass in swamp buffalo diets. The better results are with 50% water hyacinth (DM basis) in the basal grass diet to better utilize this water plant.

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