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Serum Metabolites and Urine Oxalates Concentration of Growing Dairy Bull Calves Fed Water Hyacinth [*Eichhornia Crassipes* (Mart.) Solms]

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ABSTRACT
The study was conducted to evaluate the serum metabolites and urine oxalate concentration of dairy bull calves fed different inclusion rates of water hyacinth at the Dairy Training and Research Institute (DTRI), Dairy Farm, College of Agriculture and Food Science and at the College of Veterinary Medicine, University of the Philippines Los Baños, College, Laguna from August 2017 to December 2017.Sixteen Holstein Friesian-Sahiwal crossbred growing dairy bull calves with average weight of 87.30 kg were used as experimental animals. WH at 0, 10, 20 and 40% dry matter (DM) basis substitution of Napier grass in the total mixed ration served as treatments in four (4) randomized complete blocks, each block consisting of calves at the same weight range. Feeding of WH did not affect serum calcium (Ca) concentration but showed differences in serum magnesium (Mg) concentration. Oxalate crystals were not found in the urine despite the presence of oxalic acid in the chemical analysis made in the WH. It could be concluded that WH can be fed to growing dairy bull calves at 10-20% inclusion rate to ensure its optimal utilization.

Keywords: *serum metabolites, calcium, magnesium, water hyacinth*

INTRODUCTION
One of the most common animals raised in rural areas is cattle. These mammals are of great importance to humans because aside from producing meat and milk, they can also be used as draft animals in small farmlands. Some people grow cattle because of tradition, while some as a source of extra income. Water hyacinth (WH) is considered as an invasive and a nuisance aquatic plant. Considering WH as an alternative feed source for ruminants is vital and timely considering that the ruminant industry is being challenged by the decreasing pasture areas due to land use conversion and that feeds are becoming more expensive.

Alternative feed sources are important during dry season when forage for ruminants are scarce. Availability of forage limits production performance. The high protein content in the leaves of WH (19.5%) makes it a potential fodder source for cows, goats, pigs, ducks and tilapia fingerlings in other countries (Sophal *et al.*, 2010).

Several authors reported the presence of oxalate crystal in WH. These crystals may cause mouth irritation and may cause lesions in the stomach, intestines, liver and kidneys. These salts bind with Calcium (Ca) or Magnesium (Mg) and precipitates in the urine which may lead to renal lithiasis (Nampoothiri, 2017; Rahman et al., 2012; Franceschi and Nakata, 2005).

The study aimed to evaluate the effects of feeding different inclusion rates of WH on serum metabolites and urine oxalates of growing dairy bull calves.

MATERIALS AND METHODS
Ethical Approval
The experiment was conducted in accordance with the protocol set by the Institutional Animal Care and Use Committee (IACUC) of the University of the Philippines Los Baños (UPLB), College, Laguna.

Site and Duration of the Study
The experiment was carried out at the Dairy Training and Research Institute (DTRI) Dairy Farm, College of Agriculture and Food Science, and at the College of Veterinary Medicine, UPLB, College, Laguna from August 2017 to December 2017.

Experimental Design
Sixteen apparently healthy growing dairy bull calves (Holstein Friesian-Sahiwal crossbreeds) approximately four (4) months of age with an average weight of 8.30 kg were divided into four (4) groups and assigned to one of the four (4) treatments in a randomized complete block design

(RCBD) with the weight of calves as the blocking factor. Each treatment was composed of four (4) calves with each replicate calf housed in individual stalls. The treatments were composed of the TMR with 0, 10, 20, and 40% substitution of Napier grass by WH on dry matter basis.

Experimental Animals and their Management

Sixteen apparently healthy growing dairy bull calves, approximately four (4) months of age with average weight 87.30 kg were used as experimental animals. Each calf was housed in individual stalls using nylon rope inserted in plastic hose tied around their neck. The length of the rope was enough for the animals to move comfortably. The health status of the calves such as external and internal parasites, respiratory problems, calf scours and other health problems that occurred was monitored during the experimental period. The physiological values were also checked. The animals were quarantined for one month.

Feeding Management

WH was fed as a part of the total mixed ration (TMR) composed of Napier grass, silage, concentrate, molasses and salt. Chopped fresh aerial part of WH with inclusion rates of 10, 20 and 40% substitution rate of DM from Napier grass was incorporated in the TMR for the whole duration of the experiment.

Silage was either corn, sorghum or mombasa (*Panicum maximum*) grass added with molasses while the concentrate was a mixture of yellow corn, copra meal expeller, soybean meal, vegetable oil, molasses, salt, limestone, mineral premix, antioxidant and mold inhibitor. Fresh, clean water and *ad libitum* feeding were offered in the morning and provided throughout the duration of the experiment.

Serum Calcium and Magnesium and Urine Oxalates Concentration of Growing Dairy Bull Calves Fed Different Inclusion Rates of Water Hyacinth

Five milliliters (ml) of blood was collected from the jugular vein of the 16 calves using a vacutainer needle attached to a yellow top vacutainer tube. The serum was collected by centrifugation of blood samples at 3000 rpm for 10 minutes and then stored at −20°C until further analysis.

To identify oxalate crystals in the urine, two to five ml of urine samples were collected and placed in sterile vials. Urine samples were analyzed within 24 hours by using urine strips and through microscopic examination.

Statistical Analysis

Data gathered were analyzed using the MIXED procedure of SAS (SAS Institue,2014). The model considered treatments as fixed effects and blocks as random effects. Least significant means were used to compare treatment means. Statistical significance and tendencies were set at P value ≤ 0.05.

RESULTS AND DISCUSSION

Serum Calcium and Magnesium and Urine Oxalates Concentration

Serum calcium and magnesium, and urine oxalates of 16 Holstein Friesian-Sahiwal crossbreed dairy bull calves representative of treatment groups fed 0, 10, 20, and 40% WH with four (4) calves per treatment were determined and compared (Table 1 and 2).

No significant differences were observed among the different levels of WH inclusion on the serum calcium concentration samples taken from the experimental animals. In contrast, magnesium concentrations at 0% and 10% WH inclusion differed from 20% and 40% inclusion levels. In the present study, two (2) of the calves in 20% WH and three (3) calves in the 40% WH inclusion suffered from diarrhea for 2-4 days during the 10th week of the feeding trial, hence lower magnesium levels were observed.

Table1. Calcium and magnesium concentration of growing dairy bull calves fed TMRs with different inclusion rates of WH, mg/dl.

Parameter	Treatments				Mse	*P*-Value
	0% WH[1]	10% WH[2]	20% WH[3]	40% WH[4]		
Calcium	8.52	8.26	7.84	8.76	1.18	0.8592
Magnesium	2.24[a]	2.14[a]	1.76[b]	1.77[b]	0.08	0.0016

Means in the same row with different superscript are significantly different at *P*-value< 0.05.

Legend: [1] 0%WH – Napier grass as basal diet; [2]10% WH- 10 % WH in the diet; [3]20% WH -20%WH in the diet; [4]40% WH - 40% WH in the di

Radostits (2000) as cited by Naik *et al*. (2010) reported that the efficiency of the absorption of magnesium during intestinal transit is reduced in cases of diarrhea.

In this study, results of the colorimetric method reveal that serum calcium and magnesium collected from the experimental calves fed different inclusion rates of WH are within acceptable limits. Furthermore, in the present study, WH and Napier grass have a Ca concentration of 1.3 and 0.36 while Mg is 0.5 and 0.30, respectively. The values of Ca and Mg for WH and Napier grass do not conform with the study of Franceschi and Nakata (2005) that the ratio of Ca and Mg is 2:1. Moreover, Mg acts as antagonist to calcium. According to Hadzimusici and Krnici (2012), the value of magnesium in the blood is a reflection of the nutritious value of animal feed and that the level of magnesium in blood is a reflection of its feed intake rather than its reserves in the organism.

Oxalates were not found in the serum because calcium and magnesium are still within normal range. Rahman *et al*. (2012) reported that oxalic acid is one of the anti-nutritional factors found in forage which can bind with dietary Ca or Mg to form insoluble Ca or Mg oxalate. These salts cause a decrease in serum Ca or Mg levels or may lead to renal failure due to their precipitation of these salts in the kidneys. In addition, Rahman *et al*. (2012) established that a high dietary intake of Ca may decrease oxalate absorption and its subsequent urinary excretion. The present study is not in agreement with Heuze *et al*. (2015) in a report in Feedipedia that some authors also reported the presence of sharp calcium oxalate crystals which cause mouth irritation and may also harm the digestive tract and may cause renal lesions. Heuze (2015) reported that fresh aerial part of the WH was offered to ruminants at 25% inclusion for a period of 28 days. Needle like structures which were oxalate crystals were observed in WH tissues. Franceschi and Nakata (2005) stated that Calcium oxalate is found in animals and is usually associated with a pathological condition known as renal lithiases.

In the same manner, oxalate crystals in the urine was determined from two to five ml of urine samples. All parameters which underwent clinical microscopy gave results which fell within acceptable limits. Oxalates were not also found in the urine samples (Table 2). Ca in the TMR offered to experimental calves in the present study ranged from 0.63-1.12%. The amount of Ca in the TMR could possibly be the reason why oxalates crystals were not formed in the urine because according to Rahman *et al*. (2012), a high dietary intake of Ca may decrease oxalate absorption. A 100 kg calf needs only 0.43% Ca. Moreover, Tham (2012) reported that WH in India was composed of 5.8% oxalates which was higher than the oxalates observed in the present study.

Although WH analysis show the presence of oxalates amounting to 4.57%, this did not manifest in the serum and urine analysis made. This could be attributed to the nature of digestive system of ruminants such that oxalates present in the feed were not absorbed, hence no oxalates were found in the serum and urine.

Table2. Urinalysis results of dairy bull calves fed with TMRs with different inclusion rates of water hyacinth.

PARAMETER	TREATMENTS			
	0% WH[1]	10% WH[2]	20% WH[3]	40% WH[4]
Color	Light yellow	Light yellow	Light yellow	Light yellow
Transparency	Clear to hazy	Clear	Clear to hazy	Clear to hazy
Ph	8.0	7.0-8.0	8.0	7.0-8.0
Specific gravity	1.010-1.005	1.010-1.015	1.01-1.005	1.008-1.015
Urobilinogen	Negative	Negative	Negative	Negative
Nitrite	Negative	Negative	Negative	Negative
Bilirubin	Negative	Negative	Negative	Negative
Albumin	Trace to +1	Trace	Trace	Trace
Sugar	Negative	Negative	Negative	Negative
Ketones	Negative	Negative	Negative	Negative
Epithelial cells	Rare to few	Rare	Rare to few	Rare
*RBC	0-2 cells/HPF	0-2	0-2	0-2
*Pus Cells	0-2 cells/HPF	0-2	0-2	0-2
Amorphous urates	-	-	-	-
Amorphous phosphates	Rare to few	Rare to none	Rare to few	Rare to few
Bacteria	Moderate to few	Rare	Moderate to few	Moderate to few
Triple phosphate	Many to none	Few to none	Many to none	-

Legend: [1] 0%WH – Napier grass as basal diet; [2]10% WH- 10 % WH in the diet; [3]20% WH -20%WH in the diet; [4]40% WH - 40% WH in the diet. *RBC and Pus cells –unit of measurement is cells/HPF (High Power Field)

Moreover, these findings are consistent with the study of Wu *et al*. (2012) in an evaluation of the toxicity of WH leaves for animal feeding. In the study, after feeding for 7 and 28 days, the body weight of all the mice increased. The results of hematological analysis, clinical biochemical analysis, histopathological evaluation, general dissection or investigations of internal organs, appearance and behavior observations did not indicate any adverse effects from the diet containing Water hyacinth leaf protein (WHLP). It is therefore concluded that water hyacinth leaves are not acutely toxic.

Furthermore, the present study does not conform with the findings of several authors (Heuze, 2015; Nampoothiri, 2017) that WH causes irritations in the stomach and intestines due to the presence of oxalate crystals though oxalates were found in WH from the analysis conducted in the present study, it did not cause lesions in the stomach and intestines.

CONCLUSION

This study revealed that inclusion of water hyacinth on the feeds of the experimental animals did not affect the serum calcium concentration. However, magnesium concentration with greater levels in treatments with 0 and 10% WH differed with 20 and 40% WH. Oxalates were not found in urine samples of animal fed different inclusion rates of WH.

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