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RUMINAL VOLATILE FATTY ACIDS, TOTAL SUGARS, MILK YIELD AND QUALITY OF DAIRY COWS FED WATER HYACINTH [*Eichhornia crassipes* (Mart.) Solms] and BANANA (*Musa sp.*) PSEUDOSTEM

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ABSTRACT

Ruminally cannulated Holstein Friesian x Sahiwal dairy cows were fed diets containing Napier grass (NG), 40% water hyacinth (WH) and 40% banana pseudostem (BP) following a 3x3 Latin Square Design. Rumen fluid samples collected after feeding were analyzed for volatile fatty acids and total sugars using gas chromatography and phenol-sulfuric acid methods, respectively. Average daily milk yield and quality (crude fat, crude protein, total solids) were determined. Results showed that propionic acid (19.62-25.74 mmol/l) was significantly different (*P*<0.05) while acetic acid (43.58-60.26 mmol/l) and butyric acid (10.74-15.91 mmol/l), total sugars (1.39-1.79 mg/ml), average daily milk yield (9.09-9.33 l/d), milk fat (3.21-4.39%), solids not fat (8.22-8.82%), total solids (11.83-12.98%) and crude protein (3.12-3.33%) were not significantly different (*P*>0.05) for cows fed with diets containing NG, WH and BP. These results suggest that WH and BP can be fed at 40% without any effect on rumen metabolism and milk yield and quality.

Key words: crude fat, crude protein, gas chromatography, milk yield, volatile fatty acids

INTRODUCTION

Considered as the world's worst aquatic plant and the most reproductive plant, water hyacinth covers about 20% of Laguna de Bay's surface area. Due to its rapid growth rate and propagation, water hyacinth causes various problems such as clogging of drains, interference with water transport, water flow, irrigation, and hydropower and water supply systems. Also, there is depletion in the water's biodiversity (Tellez *et al.*, 2008) and an increase in the occurrence of diseases such as malaria and bilharzia (Gunnarsson and Petersen, 2007).

On the other hand, one of the main issues confronting the banana industry is poor by-product utilization. A huge percentage of banana plants are discarded resulting in environmental hazards; thus, causing an imbalance in the ecosystem. Although it rots easily under natural conditions and the effects are short-lived, it is a temporary contaminant and

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has a foul odor. Currently, million tons of banana pseudostems are produced and growers are facing various problems in disposing of the collected banana pseudostems.

Therefore, an effective economic means of reducing environmental problems caused by water hyacinth and banana is proposed. The efficiency of both plants as an alternative ruminant feed can contribute to sustainable weed control and by-product utilization.

Ruminal microorganisms produce enzymes necessary for the fermentation process, thereby allowing ruminants to digest forages (Burns, 2008). Cows utilize the energy absorbed for milk production and other activities.

The main products of ruminal fermentation are volatile fatty acids (VFAs) and microbial biomass. Volatile fatty acids produced are the primary source of energy for the animal. Propionate is used as a source of glucose for gluconeogenesis while acetate and butyrate are used for fattening animals and as a source of long-chain fatty acid synthesis (Russell *et al.*, 1992). Also, carbohydrate digestion is reflected by the amount of glucose produced by the ruminant (Avila *et al.*, 2015). On the other hand, microbial protein is absorbed in the intestine and used a protein source.

The determination of ruminal fermentation and microbial ecosystem in pursuit of improving the feeding process for ruminants to achieve better production have been the focus of numerous studies (Castillo-Gonzalez *et al.*, 2014). Although there are numerous studies on the use of water hyacinth and banana pseudostem as feed material in other countries (Rahman and Huqueet, 2002; Abdel-Sabour 2010; Alvarez *et al.*, 2015; Su *et al.*, 2018), there are no studies about the use of such plants in the Philippines. It is important to examine the potential of these plants as feeding material so that proper management and technologies can be developed. The main objective of this study is to determine the milk yield and quality, ruminal volatile fatty acids and total sugars of dairy cows fed diets containing water hyacinth and banana pseudostem.

MATERIALS AND METHODS

Three ruminally fistulated Holstein Friesian x Sahiwal dairy cows were used in a 3x3 Latin square design fed with diets containing Napier grass (NG; *Pennisetum purpureum*), 40% water hyacinth (WH; *Eichhornia crassipes*) and 40% banana pseudostem (BP; *Musa sp.*). Each period is composed of 10 days adaptation and 4 days incubation. Rumen fluid was collected 2 hours after feeding every 13th day of each period. The samples collected were filtered through four layers of cheesecloth, then 2-3 drops of toluene to every 10 ml of rumen fluid were added. The pH of the solutions was measured using a calibrated pH pen. Preserved samples were stored in a polyethylene bottle at -20 ^{o}C until further analysis.

Milk samples were collected during the incubation period for each trial. Samples were analyzed immediately for fat, solids-not-fat and protein content using EKOMILK Ultra Milk Analyzer. Total solids (%) was calculated using Equation 1. The protein content of the milk samples was also determined.

$$\text{Total Solids (\%)} = \text{Fat(\%)} + \text{SNF(\%)} \quad (1)$$

The method of Luo *et al.* (2015) for the analysis of volatile fatty acids with modification was used in the present study. Two hundred fifty microliters of 25% ortho-phosphoric acid was added to 1 ml rumen fluid. Samples were centrifuged for 30 minutes at 3000 rpm to separate the precipitated protein. The supernatant was diluted with methanol at 1:3 (v/v) as adapted from Mandake *et al.* (2013) prior to gas chromatography.

Volatile fatty acids were analyzed using Shimadzu GC-2010 Plus equipped with SPL-2010 Plus split/splitless injection unit, with injection volume of 1 µl, operated in split mode (split ratio 50:1) fitted to a flame ionization detector, using a capillary column (SH-Stabilwax-DA, 30m x 0.32 mmID x 0.25 µm, Shimadzu) with N_2 as the carrier gas. As adapted from Luo *et al.* (2015), the flow rate was set at 1.0 ml/min., column temperature at 100 °C for 1 min, increasing at a rate of 20 °C/min to 190 then maintained for 3 minutes. The temperatures for injector and detector were maintained at 220 and 250 °C, respectively. Analysis was done for 7.5 minutes.

For total sugars, the method was adapted from Dubois *et al.* (1956). One milliliter sample was pipetted into a pre-acid washed test tube. One milliliter of 5% phenol was added afterward. Concentrated sulfuric acid (H_2SO_4) was added immediately. The resulting solution was mixed using a vortex mixer, left to stand for 10 minutes then cooled in a water bath at room temperature for 20 minutes. The glucose standards were prepared at 0, 0.02, 0.04, 0.06, 0.08, and 0.10 mg/ml.

The absorbance of the solutions from the phenol-sulfuric method was read at 490 nm using a UV-Vis Spectrophotometer. Total sugar content was calculated using equation 2.

$$\text{Total Sugars, } \frac{mg}{mL} = \left(C_{glc}, \frac{mg}{mL}\right)(DF) \quad (2)$$

The collected data were analyzed using one-way Analysis of Variance (ANOVA).

RESULTS AND DISCUSSION

The average milk yield for cows fed diets containing NG, 40% WH and 40% BP is 9.50±1.87, 9.17±2.14 and 9.08±1.53 liters, respectively. There was no significant difference (P>0.05) observed in the milk produced by cows; therefore, WH and BP can be a good substitute for NG up to 40% for the ruminant diet, giving a comparable yield for milk.

Milk produced by a cow can be affected by its breed. Friesian and Sahiwal cross gives higher milk yield than the purebred Sahiwal, without eating as much as the Friesian. The data obtained in the study is comparable to the observations of Meyn and Wilkins (1974) with approximately 8 kg/day average milk yield.

Table 1 shows the fat, solids-not-fat, total solids and protein content of the milk obtained from cows fed NG, 40% WH, and 40% BP diets. The fat content of milk produced by Holstein Friesian x Sahiwal cows used in this study agrees with the values reported by Talukder *et al.* (2013) wherein the milk fat of crossbred cows range from 3.96 to 4.50%. Fat content of crossbred cows are much higher than the purebred with 3.5% rate.

According to Hidanah *et al.* (2016), acetic and butyric acid are precursors of milk and body fat while propionic acid for body fat production. Also, acetate and butyrate are utilized by the ruminant in milk fat production while propionic acid reduces it. However, a ratio of 3:1 or below of acetic to propionic acid indicates production of low-fat content in milk (McDonald *et al.*, 2002). Moreover, the observed acetic acid to propionic acid ratio for cows fed NG, 40% WH, and 40% BP diets agrees with the suggested ratio, whereas milk fat was observed to exceed 1% or low-fat milk.

Rumen microorganisms hydrolyze plants' structural and nonstructural carbohydrates into absorbable energy in the form of volatile fatty acids. Nonstructural carbohydrates such as starch, pectin and sugars are fermented by NSC bacteria including *Butyrivibrio fibrisolvens* (Bryant, 1973; Russell *et al.*, 1992). Structural carbohydrate bacteria, on the other hand, ferments structural carbohydrates such as cellulose, hemicellulose, and lignin (Brooks, 2010).

In the rumen, the major portion of the carbohydrate is broken down into acetic, propionic and butyric acids. Hence, this study focused on the presence and concentration of these three major metabolites in the rumen fluid of Holstein Friesian x Sahiwal cows fed with NG, 40% WH, and 40% BP diets. The VFA concentrations in the rumen fluid of cows fed with NG, 40% WH, and 40% BP diets were not significantly different ($P>0.05$) for acetic acid and butyric acid (Table 2).

As shown in Table 3, the rumen fluid obtained from dairy cows fed with NG, 40% WH and 40% BP diets contain total sugar concentrations of 1.79±0.67, 1.69±0.54 and 1.39±0.14 mg/ml, respectively. It can be observed that there is no significant difference ($P>0.05$) in total sugar production between the two diets. Therefore, in terms of total sugar production, WH and BP diets are comparable to NG.

The phenol-sulfuric acid method was used to determine the total sugar content of the rumen fluid obtained from cows fed diets with NG, 40% WH and 40% BP. Sulfuric acid breaks down disaccharides, polysaccharides and oligosaccharides to monosaccharides. Pentoses are dehydrated to furfural while hexoses to hydroxymethyl furfural (Nielsen, 2010). Dehydration of a D-glucose is shown in Figure 1.

Table 1. Fat, solids-not-fat, total solids and protein (%) content of the milk.

Diet	Napier Grass	Water Hyacinth	Banana Pseudostem	*P-va*lue
Fat^{ns}	4.39±1.37	4.16±0.80	3.21±2.26	0.7477
Solids-Not-Fat^{ns}	8.22±0.63	8.82±0.27	8.62±0.92	0.6752
Total Solids^{ns}	12.61±0.89	12.98±0.97	11.83±1.35	0.5891
Protein^{ns}	3.12±0.22	3.33±0.11	3.25±0.31	0.6542

Table 2. Volatile fatty acid content (mmol/l) of the rumen fluid obtained from cows fed NG, 40% WH and 40% BP diets.

Diet	Napier Grass	Water Hyacinth	Banana Pseudostem	*P-value*
Acetate^{ns}	47.86±10.79	60.26±9.67	43.58±12.58	0.1643
Propionate	19.62±4.01^{a}	25.74±4.30^{b}	17.03±2.81^{a}	0.0237
Butyrate^{ns}	12.03±4.18	15.91±4.57	10.74±3.34	0.3055

Table 3. Total sugar content (mg/ml) of the rumen fluid from cows fed NG, 40% WH and 40% BP diets.

Diet	Napier Grass	Water Hyacinth	Banana Pseudostem	*P*-value
Mean^{ns}	1.79	1.69	1.39	0.9207
SD	0.67	0.54	0.14	

Hydroxymethyl furfural formed from dehydration of glucose reacts with phenol to form a yellow gold-colored solution as shown in Figure 2. The absorbance of the solutions was then measured using UV-Vis Spectrophotometer set at 490 nm.

At 40% inclusion, WH and BP are comparable to NG as a feed ingredient for dairy cows. The results for total sugars, milk yield, and milk quality were not significantly different ($P>0.05$). For VFA, the results were not significantly different for acetic acid and butyric acid but significantly different for propionic acid ($P<0.05$). In general, WH and BP are a sustainable source of low-cost feed materials.

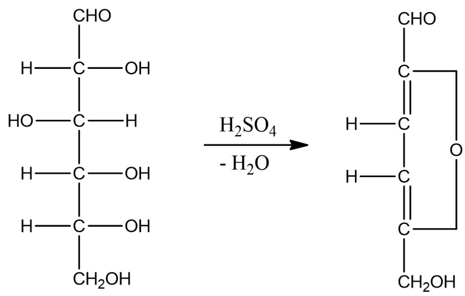

Figure 1. Dehydration of glucose to hydroxymethylfurfural.

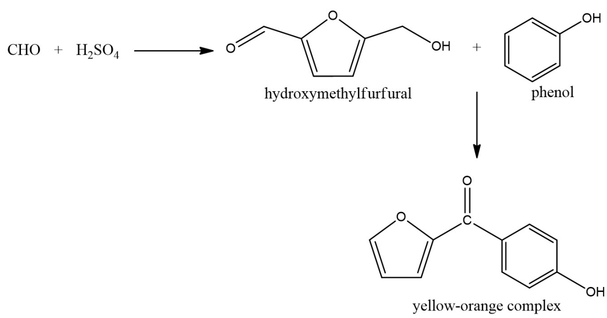

Figure 2. Reaction of carbohydrates in phenol-sulfuric acid method.

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