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Acetogenic and Acid Utiliizng Bacterial Content Analysis on Ruminal Fluid of Water Buffalo *(Bubalus bubalis*) Calves

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Microbial digesta of water buffaloes was investigated. The study aimed to isolate, characterize and differentiate the morphological and cultural analysis of acetogenic and acid utilizing bacteria present from the digestive tract of the calves. The alteration of the feeds diet (raw milk and supplemented feeds diet) from the birth of the calves until the 30th was observed. Samples were isolated in anaerobic condition and incubated at 37^{0}C for 24-48 hours for visible growth of microorganisms. Bacteria were subjected to gram staining for further characterizing and differentiating them according to its morphological and cultural properties Different substrates and inhibitors were applied to selective media to assure the growth of specific acetogens and acid utilizers. There were six isolates isolated based on morphological and cultural characteristics identified until genus level, namely; *Oscillospira sp., Megasphera, sp., Selemonas sp., Streptococcus sp., Lactobacillus sp. and Eubacterium sp.*. There were more acetogenic and acid utilizing bacteria isolated from the digestive tract fluid collected on 30th day compared to the first day.

Keywords: acetogenic bacteria, acid-utilizing bacteria, acetogenesis, digestive tract fluid

Introduction

Water buffalos are commonly found in lower preferment such as around the tropical and subtropical forest. These animals are considered as terrene mammals that prefer to be dependent among the muddy, plain and riverside kind of habitat. Water buffalos have a life span of twenty (20) years of age according to Dairy Production and Products of Food Agriculture Organization of United Nations (2015). Nowadays livestock holders, particularly the farmers

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living in tropic areas chose ruminants such as buffalos, carabaos, and goats for rearing of animals. Nagpal *et al*. (2010) studied buffaloes fed with foliages, forages, raw milk and the nutritive value of supplemented feeds diets and obtaining different bacterial microorganisms that has been altered by external factors such as diet, feeding frequency and ruminant-host interactions.

They stated that the ruminant's performance was established according to the microbial activities inside the rumen as affected by dietary feeds intake. Thus, rumen has been considered as one of the most powerful fibrolytic fermentation systems known (Pan wang *et al.,* 2012).

Rumen microbiology has been on the forefront of modern livestock productivity related researches (Singh *et al.,* 2015). The ruminal microorganisms contribute to the different activities such as metabolism and cell-wall degradation (Lee *et al*., 2000). Ranging from 50 to 82% of the rumen are microorganisms that efficiently utilize the substrates. They serve as fiber digesters and gratify the responsibility inside that environmental digestibility and bacteria have been reported to be primarily responsible for the degradation of plant proteins. Puniya (2015) elaborated that bacteria, protozoa, fungi and viruses exist in close proximity in the rumen, where bacteria predominate, nearly 95% of the total microbial community.

The competence of the rumen microbiota has been considered in improving the animal performance which is acknowledged by its desirable traits in terms of production. Singh *et al.* (2012) elaborated that the diversity in the rumen digesta is dominated by the population of the predominant microbial attachment that provide the host with nutrients. Interaction has been processed through the involuted chemical substance that requires the degradation to be done accordingly in micro-systematic view.

These kind of microorganisms occur through the acetyl-CoA pathway which inhabit the the rumen (Stewart *et al.,* 1997) have fibrolytic and acetogenic activities according to their biochemistry. Through acetogenesis, bacteria enhance the efficiency of increasing microbial proteins. Their capability of reducing CO_2 to acetate is conditional to the growth and substrate utilization. Presence of microorganisms also indicates modification of acid-utilizers that enhance their productivity due to the production of vitamins and acids in rumen that serve as energy source for the ruminants (Katra *et al.,* 2009).

This investigation on the microbial biota of ruminal fluid of buffalo calves was conducted in order to isolate and characterize acetogenic and acid utilizing bacteria based on the morphological analysis on 1^{st} and 30^{th} day associated with feeds diet.

Materials and methods

Experimental Calves. There were ten (10) buffalo calves used in this study. They were placed in a dams right after they were born to record their birthday, sex, dam, sire. Specifically, a floor area of 2.6 m^2 served as the shelter of the experimental newly born buffalo calves. Inside was a set up of rice straw beddings together with two pails. A total of 10 experimental calves were first fed with colostrum before they were engaged in different diets. Then after, they were randomly assigned in each treatment and pre-weaned for 30days. Five (calves) were fed with raw milk, calf starter pellets and forages t and other five calves were fed with raw milk with forages with the absence of pellet concentrates.

Collection of Digestive Tract Fluid and Isolation of Acetogenic and Acid Utilizing Bacteria. Liquors were collected from the rumen of calves fed with raw milk, forages, starter pellets and calves fed with forages and milk only. This was done through esophageal tubing by large syringe. The collection started during day 1 and day 30^{th} with a range of 100ml-200 ml of fluid has been obtained. The samples were kept immediately in the test tubes with rubber stopper under freezing conditions of -28˚C.

Selective media were used to obtain acetogens and acid utilizing bacteria. Basal medium for acid utilizing bacteria was used and Simmon's Citrate Agar for acetogens with inhibitors was used for specific growth of the bacteria. The samples were centrifuged for 5-10minutes and 1 ml was obtained for dilution up to -12 vortex. 1ml diluted fluid ws transferred to the liquid media with an input of carbon dioxide in an anaerobic condition. Solidification of the media was done after 15-20 minutes, then incubated for 24-48 hours under 37^{O}C. Visible colonies were isolated again for the production of the lawn culture and incubated for 48 hours. Then after, cultures in the lawn were isolated to obtain the pure culture of different isolates.

Morphological Identification. Isolates were visible after 48 hours of incubation. They were subjected to gram staining for the identification. After obtaining a loop of isolated bacteria from the culture, smear it in glass slide, heat fixed flooded with crystal violet and rinsed. The smear was then covered with gram iodine. After 20 seconds, the slide was rinsed with dH2O. Then after, the stain was washed with 95% ethanol it was rinsed and washed with water. The bacteria were flooded with safranin-O for 30 seconds and was rinse with dH2O and blot-dried the slide.

Morphological characteristics of isolates were studied under the microscope and recdorded. Dehority (2003) Laboratory Manual was used in classification and morphology of rumen microorganisms. Sizes, shapes, gram

stain and appearance of microorganisms was the basis in identifying the obtained microscope under the compound microscope. The bacteria found were described according to the gram stain and morphologically described according to width, diameter and length. Bacteria diversity was determined up to the genus level according to the two sampling dates specifically during the 1.0 µm and day 30.

Results

Through morphological analysis, six pure cultures were isolated. These were *Oscillospira sp.*, *Megasphera sp.*, *Selemonas sp.*, *Streptococcus sp.*, *Lactobacillus sp.*, and *Eubacterium sp.*

Oscillospira sp. (Fig. A) is gram negative with large rods or filaments, motile with a diameter of 3.0-6.0 µm. It is divided by closely spaced cross walls into numerous disc-shaped cells and its endospores are normally center located. *Megasphera sp.* (Fig. B) is gram positive and large coccus that is arranged singly or in chains. It is considered a non-motile through the absence of flagella with a diameter of 2.5-3.0 µm. *Selemonas sp.* (Fig. C) is gram negative and characterized as large crescent-shaped rod cells with a tuft of flagella on one side. They have a diameter of 0.8-1.0 by 2.0-7.0 µm. *Streptococcus sp.* (Fig. D) are gram positive and spherical to elongated cocci. It usually occurs in single, pairs and chain with a diameter of 0.8-0.9 µm. *Lactobacillus sp.* (Fig. E) is gram positive and straight and curved rods having a diameter of 0.6-1.5 µm. It occurs singly, pairs and in short chains. *Eubacterium sp.* (Fig. F) is a gram positive short rod to coccoid and is arranged singly, pairs and short chains having a diameter of 0.5-.9 µm.

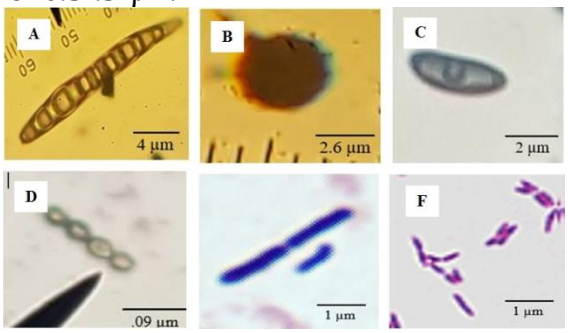

Figure 1. isolated from digestive fluid of calves. *A. Oscillospira sp. B. Megasphera sp. C. Selemonas sp. D. Streptococcus sp. E. Lactobacillus sp. F. Eubacterium sp.*

Different diets influenced ruminant's established production of microorganisms for further digestion in *B.bubalis* calves fed with milk, forages and feeds produced more diversified bacteria inside the rumen. The diversity

was observed to be greater in Day 30 and found in both treatments. Acetogenic and acid-utilizing bacteria gradually increased diversity as the calves were exposed to feeds in their diets. This result imply that using replacement diets instead of milk, can be used as pre-weaning diets and would not influence the distribution of acetogenic and acid utilizing bacteria.

Table 1. The Presence and Absence of Identified Isolates

	Control (Forages, Milkand Feeds)		Treatment (Forages)	
	D1	D30	D1	D30
Oscillospira sp.	present	present	negative	present
Megasphera sp.	negative	present	negative	present
Selemonas sp.	present	present	negative	present
Streptococcus sp.	present	present	present	present
Lactobacillus sp.	present	present	present	present
Eubacterium	present	present	negative	present

Discussions

Presence of microorganisms was abundant during the 30th day of weaning of the calves while it was less abundant during their 1st day. Control treatment that involved the feeding of milk, forages and feeds alters the rumen microbial ecosystem in which according to Mackie *et al*. (2003) broad presence of *Oscillospira* spp. in various rumen are most likely highest in abundance especially in associated diets. Fernando *et al*. (2010) emphasized that detection of *Lactobacillus* sp, and *Selemonas* sp. has significant fold increase as the animals adapt in a high concentration diet. This yields the great abundance of the two species most likely in the control group. *Streptococcus* and *Eubacterium sp*. also dominated the rumen as reported also by Cai *et al* (2002). Ouwerker *et al.* (2003) proved that *Megasphera sp*was found in rumen microbial community throughout the trial of altering diets upon the ruminants. High-grain or high-forages adaptation programs were widely used with feedlot cattle and buffalo to balance enhanced growth performance (Roe *et al*., 2010).

The acetogenic and acid utilizing bacteria isolated from digestive tract fluid of calves of *B. Bubalis* are *Oscillospira sp., Megashera sp., Selemonas sp.,. Streptococcus sp., Lactobacillus sp.* and *Eubacterium sp.* The diversity of the acetogenic and acid utilizing microbiota is not influenced by the replacement diet.

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