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Identification of *Lactobacillus* sp. On The Rumen of Young Calves (*Bubalus bubalis)* Given Different Diets

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Lactobacilli are important integral of microbial flora ofthe rumen and intestines of ruminants. In this study, young water buffalos were adapted to varied feeding rations treatment with the hypothesis that alteration of different diets will have an effect to the microbial diversity on host physiology. Ten young water buffalos were subjected to gradually adapt to the weaning diets. Digestive fluids were collected from the calves on two sampling periods (1st and 30th days) in accordance to different feeding rations. Lactobacilli were isolated from the digestive fluids collected and assessed based on their morphological and cultural characteristics. Eight isolates were observed (*Prevotella ruminicola, Butyrivibrio fibrisolvens, Bifidobacterium* sp. *Lactobacillus plantarum, Weissella paramesenteroides, L. salivarius, L. ruminis* and *L. mucosae).* It was also observed that there were more lactose fermenting bacterial isolates on the 30th day.

Keywords: lactose fermenting, water buffalo, digestive fluid, digestive microbiota

Introduction

The domestic water buffalo *Bubalus bubalis*, contributes a significant share of global milk production and it is widely known as the major milk producing animal in several countries like in the Philippines. Buffaloes are usually kept by small scale producers in developing countries, who raise one or two animals in mixed crop-livestock systems (FAO, 2015). Lactobacilli are an important integral of microbial flora of the rumen and intestines in ruminants. They are among the incumbent of the rumen of young calves and participate in

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its functions. The number of lactobacilli in the digestive tract fluid content of calves increases after birth reaching 10^8 -10^9/g, similar numbers of lactobacilli are present in the intestines of calves (Karney *et al.*, 1986). Profiling the lactobacilli presence in the digestive tract fluid of calves may give animal nutritionists better understanding of microbial dynamics they formulated more appropriate diets for weaning calves. Moreover, the identification and culture of some lactobacilli from the digestive tract fluid may lead to potential production of fermentative microbes with enhanced digestive capabilities that may help to develop efficient diet for different ages of the calves. Thus, it is possible to replace the milk diet at early stage with another to increase milk availability to human consumption. Also, the generated baseline information will be useful in improving calf rearing specially on early weaning, production and management.

This study cultured anaerobically and identified using morpho-cultural approach lactose-fermenting bacteria in the digestive tract fluids of buffalo calves fed with different diets at different periods of weaning.

Materials and methods

Experimental Calves and Sample Collection

Ten (10) newly born BM buffalo calves at the Philippine Carabao Center-Gene Pool Farm were used in the study. The calves were separated from their dams immediately after calving and were kept in nursery pens. The birthday, sex, dam, sire and birth weight of each calf were determined and recorded. The calves were randomly assigned into two dietary treatments. Treatment 1 had five calves fed raw milk, calf starter and forage and Treatment 2 had five (5) calves fed with forage up to thirty days.The digestive tract fluid samples were collected using esophageal (rubber) tubing by means of suction using a large syringe. The first source of digestive tract fluid came from the calves at birth.The collection of digestive tract fluid was done at their birth one (1) day old and followed by thirty (30) days old calves. The collected digestive tract fluids (100-200 ml) were kept immediately in sterile vials with rubber stopper and transported using the thermo flask and placed in a refrigerator under a freezing conditions (-27 ˚C) until time of use. The samples were centrifuged for 5-10minutes and 1 ml was obtained for dilution up to -12 vortex.

Microbial Analysis.

For isolation of bacteria, one (1) ml diluted fluid was transferred to the liquid media with an input of carbon dioxide in an anaerobic condition incubated for 24-48 hours under 37◦C. Solidification of selective Lactobacillus

Anaerobic MRS for 15 minutes was used to isolate lactose-fermenting bacteria. Visible colonies were isolated again for the production of the lawn culture and incubated for 48 hours. Then, pure culture of different isolates were obtained. Visible isolates after 48 hours of incubation were subjected to gram staining for the characterization of isolates.

For further characterization of isolates, microscopic morphological properties such as sizes, shapes, and appearance of microorganisms were determined. The identification of isolates were based on standard rumen microbial book and manuals of Hobson *et al.* (1997); Stewart *et al.* (1997) and Staley *et al.* (1990) and validated by a rumen microbiologist. The diversity of genera of microorganisms were compared between the two treatements at day 1 and day 30 of feeding.

Results

There were eight (8) pure cultured and was identified using morphological and cultural analyses. The following bacteria were seen to be more abundant on the 30th day. The bacteria cultured were *Prevotella ruminicola*, *Butyrivibrio fibrisolvens, Bifidobacterium* sp. *Lactobacillus plantarum*, *Weissella paramesenteroides, L. salivarius, L. ruminis* and *L. mucosae*, *Prevotella ruminicola*, cells were oval to rod-shaped, in irregular granules, singly or in pairs, non-motile, non-spore. About 0.8-1.0 by 0.8-3.0 µm in size. Cells were ocassionally encapsulated and were gram negative. *Butyrivibrio fibrioslvens* were straight or curved rods, singly or in chains. 0.3-0.8 by 1.0-5.0 µm, with tapered and rounded ends and were classified as gram negative. *Lactobacillus ruminis* were rods, 0.5-0.7 by 1.0-2.0 µm, occurring singly, and were gram positive. *Bifidobacterium sp.* gram-positive, anaerobic, branched rod-shaped bacteria are about 10 µm in length. *L. Plantarum* were small (0.5 – 1.2 x 1.0 – 10µm), elongated rod-like bacilli assembled in pairs or in chains of variable length. *Lactobacillus mucosae* were an obligate rod shaped usually 1.0-5.0 µm. Genus Weissella were Gram-positive, catalase-negative, non-endospore forming cells with coccoid or rod-shaped morphology.

Table 1 shows the different bacteria that dominated the rumen of a buffalo calves fed with varying diets on Day 1 and Day 30. Results showed that the same bacteria were found in calves regardless of diets given.

Table 1. The Presence of Identified Isolates in Calves fed varying diets on Day 1 and Day 30

	Control		Treatment	
	D1	D30	D1	D30
Prevotella ruminicola	+	+	-	+
Butyrivibrio fibrisolvens	-	+	-	+
L. plantarum,	+	+	-	+
Weissella paramesenteroides	-	+	+	+
L. salivarius.	+	+	+	+
L. ruminis	+	+	+	+
L. mucosae	+	+	-	+
Bifidobacterium sp.	+	+	-	+

(-) Indicate the absence of bacteria; (+) Indicate the presence of bacteria

Discussions

Pita *et al.* (2014) states that *Prevotellaruminicola* and *Butyrivibriofibrioslvens* dominates in the rumen and showed greater diversity profiles of the microbiome. Abundance of *Bifidobacterium sp.* was mostly isolated from ruminants especially during the milk feeding period of life (Bunesova, *et al*., 2014). Another dominating bacteria are the *Lactobacillus sp. (L. salivarus, L. mucosae, L. ruminis* and *L. plantarum)* as stated by Sakamoto *et al.* (2016). They also indicate that the forestomach does not function like the rumen of ruminants, but that lactic acid fermentation does take place in this compartment. Yin (2014) emphasized that *W. paramesenteroides* was diverse especially in an altered feeds diet wherein they are present usually in milk and forages. Distribution of lactose-fermenting bacteria increased as the calves were introduced to diets which were composed of forages, milk and feeds as supported based on the presence and absence of lactose-fermenting bacteria on the two sampling dates. Animal production is influenced mainly by the characteristics of the diet, as with, the nutritional value of forage and concentrate, the fiber content as well the time of ingestion and rumination on calves.

The profile of lactose-fermenting bacteria in digestive tract fluid of young water buffalos *(Bubalus bubalis)* given different diets is composed of *Prevotella ruminicola, Butyrivibrio fibrisolvens, Lactobacillus ruminis, Bifidobacterium sp., L. plantarum, L. mucosae, Weissella paramesenteroides,* and *L. salivarius.* The weaning diet of the calves did not influence the diversity of lactose-fermenting bacteria on the 30th day.

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