

## Feeding *Lactobacilli* as probiotic and proportion of *Escherichia coli* in the intestine of calves

S. M. Amanullah\*, M. S. Alam, R. N. Subarna, R. Bateen<sup>1</sup>, K. S. Huque and A. Sultana  
Bangladesh Livestock Research Institution, Savar, Dhaka-1341, Bangladesh

### Abstract

A study was conducted to determine the ability of oral *Lactobacillus* bacteria as probiotic to increase the *Lactobacilli* and decrease the *Escherichia coli* (*E. coli*) population in the intestine of calves. Bacteria were isolated from yoghurt (*Dahi*) with selective de Man, Rogosa and Sharpe (MRS) agar media and identified as *Lactobacillus*, followed by mass production of bacterial cells and freeze drying. Four one-day-old calves were divided into two groups. One group (n=2) was fed freeze-dried bacterial cells and remaining group (n=2) was a control. After 60 days, one calf from each group was slaughtered to enumerate *Lactobacillus* and *E. coli* bacteria on intestinal wall. The number of colony-forming units (cfu) of *Lactobacillus* was significantly ( $p>0.01$ ) higher in the intestinal wall of *Lactobacillus*-fed calf than in the control. On the other hand the number of *E. coli* was significantly ( $p>0.01$ ) lower in *Lactobacillus*-fed calf. (*Bangl. vet.* 2009. Vol. 26, No. 1, 17-22)

### Introduction

Newborn, milk-fed calves are often severely affected by diarrhoea commonly called “scours” (Davis and Drackley, 1998). Strategies used to face this challenge are improvements in sanitation, individual hutches, oral antibiotics, and fortified colostrum supplements (Otterby and Linn, 1981). However, antibiotics have adverse side-effects with implications for human health. Probiotics are alternative products. According to Fuller (1989), probiotics are “live microbial feed supplements, which beneficially affect the host animal by improving its intestinal microbial balance” and they are expected to prevent digestive disorders and/or increase performance (Wren, 1987; Fox, 1988). Beneficial effects on the growth of rats (Hargrove and Alford, 1978, 1980; Wong *et al.*, 1983) and piglets (Rodriguez, 1994; Anonymous, 1999) have been shown for some strains of lactic acid bacteria (LAB) administered orally during the first few days of life. LAB is the most commonly used organisms in probiotic preparations, especially *Lactobacilli* (Fuller, 1989). These are found in large numbers in the gut of healthy animals. They are generally regarded as safe by the Food and Drug Administration (FDA) in United States of America. Adhesion or colonization to the gut surface is considered an important property of indigenous probiotic strains, such as some *Lactobacillus* and *Bifidobacteria* strains, which are able to perform colonization and immune stimulation (Havenaar *et al.*, 1992). The colonisation of epithelial surfaces

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<sup>1</sup> Department of Animal Science, Faculty of Animal Husbandry, Bangladesh Agricultural University, Mymensingh, Bangladesh

\* Correspondence: E-mail:- sm\_aman03@yahoo.com

by probiotic bacteria tends to exclude pathogenic species. This study was undertaken to investigate the ability of *Lactobacilli* isolated from yoghurt (*Dahi*) in removing *E. coli* from intestine in newborn calves.

## Materials and Methods

This study was conducted in three steps.

### *a) Isolation and identification of bacteria*

*Isolation of Lactobacillus bacteria:* Selective MRS agar media was used. The samples were inoculated through a pour-plate method. After inoculation, plates were incubated at 37°C for 24-48 hours.

### *Phenotypic characterization*

*Gram's stain:* Gram's stain was used as described by Collins and Lyne (1980).

*Catalase test:* To check the production of enzyme catalase, a drop of 3% hydrogen peroxide was placed on a clean microscope slide. A visible amount of bacterial growth was added aseptically with the help of an inoculating loop. Both were mixed and observed for gas bubble production.

*Identification tests:* The strains showing Gram (+) and Catalase (-) were identified by the following tests:

*Sugar fermentation:* One percent solutions of lactose, sucrose, glucose, maltose and mannitol were prepared by dissolving one gram of sugar in 10 ml of distilled water and sterilized by passing through 0.45 µm filter. Nutrient broth was prepared by dissolving 0.8g of Nutrient broth powder in 100ml distilled water, one ml of phenol red was added, and it was autoclaved at 121°C for 15 minutes and cooled at room temperature. Five ml of broth and 100 µl of sugars were taken into sterilized test tubes, labelled and placed at room temperature for 24 hours to check for contamination. After 24 hours, the purified colonies were inoculated into test tubes and incubated at 37°C for 48 hours. If the colour changed from red to yellow, the test was positive.

*Sodium chloride (NaCl) utilization test:* NaCl solution was prepared at 4% and 6.5%. Colonies were inoculated in MRS broth containing NaCl in test tubes. Test tubes were incubated at 37°C for 48-72 hours.

### *b) Mass production of bacterial cells and probiotic powder*

The isolated bacteria were transferred to MRS broth for multiplication. After 48 hours of incubation culture cells were harvested by centrifugation at 6000 rpm for 30 minutes. The cell suspension was washed once with sterile saline, and then inoculated in sterile 10% skim milk solution. Finally, it was freeze-dried to get probiotic *Lactobacilli* powder.

### *c) Intestinal colonization of Lactobacilli*

*Animals and their management:* Four male calves aged one-day were distributed in two groups of two calves in each group and kept in an open shed with other calves in the farm. All calves were allowed to feed twice a day through suckling.

*Feeding of probiotic:* Prepared probiotic powder was given orally with water @ 0.5g/day/calf before evening suckling. All drenching bottles were sterilized with boiling water for half an hour before and after drinking.

*Slaughter of calves and Sample collection:* At 60 days, one calf from each group was selected at random for slaughtering. After removal of skin, the abdomen was washed with sterile water; intestine was removed and placed onto a sterile surgical cloth. A 10 to 15-cm segment from three different portions of each of small and large intestines was cut and placed into a sterile stainless steel tray.

*Enumeration of Lactobacillus from the mucus of small intestine (modified from Fuller et al., 1981) :*

Each segment of the intestine was cut longitudinally, the contents removed and washed gently with sterile water. The mucus was collected by scraping gently with a glass slide from previously marked one square cm area of the wall. Mucus was weighed and kept in test tube. Distilled water was added to this mucus at a ratio of nine ml of water to one ml of mucus. It was then vortexed, and centrifuged for 15 minutes. The supernatant was serially diluted ten-fold and plated on MRS and Eosin Methylene Blue (EMB) agar plates and incubated at 37° C for 72 hours to identify the number of *Lactobacillus* and *E. coli* cfu.

## **Results and Discussion**

### *Isolation and identification*

The bacteria were characterized on their morphological, cultural, physiological and biochemical characteristics as described by Collins and Lyne (1980). The results are shown in Table 1. The colonies were round and white on MRS agar. Colonies in the form of mosaic were observed on MRS plates. More than one colony was observed in most cases (Plate 1). Cultural and morphological characteristics were examined with the help of microscope. The bacteria on MRS agar were Gram-positive, small rod-shaped and single or in pairs (Plate 2).

The isolated bacteria used lactose, glucose, sucrose and maltose but not mannitol. Growth of bacteria was observed in MRS broth containing 4% and 6.5% NaCl and 0.3% methylene blue solution. Negative response was observed with catalase test.

### *Probiotic powder preparation*

The powder containing probiotic was tested for its cfu count of *Lactobacillus*, immediately after freeze drying and each week up to one month. On average the

number of *Lactobacillus* was  $53.3 \times 10^8$  cfu/g. The variation in different weeks was negligible. The shelf-life of probiotic was not determined after one month.

Table 1. Identification of bacteria

Tests	Results
Phenotypic characterization	
Gram's stain	Gram positive, small rod, in single or pair form
Catalase test	-
Sugar fermentation test	
Lactose, glucose, sucrose, maltose	Acid production
Mannitol	-
Growth in NaCl solution	
Growth in 4% NaCl	+
Growth in 6.5% NaCl	+
Growth in 0.3% Methylene blue	+

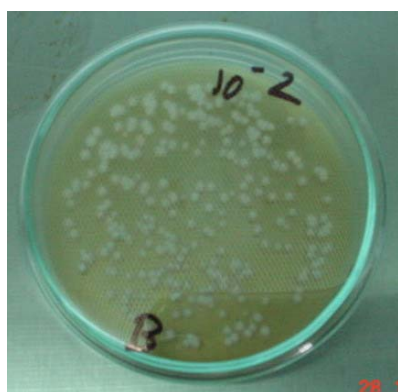


Plate 1

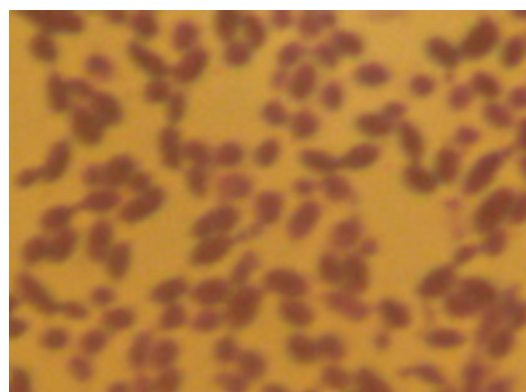


Plate 2

Table 2. Colony forming units (CFU) of *Lactobacillus* and *E. coli* in the intestine of calves as affected by feeding probiotic *Lactobacillus*

Bacteria	Control	Probiotic	SEM	Significant
<i>Lactobacillus</i> ( $\times 10^7$ cfu/inch <sup>2</sup> )	7.7	13.0	3.5	**
<i>E. coli</i> ( $\times 10^5$ cfu/inch <sup>2</sup> )	9.1	5.4	0.41	**

#### Colonization in calf intestine

At the end of the trial, there was a significant difference ( $p < 0.01$ ) between control and treated calves in terms of *Lactobacillus* and *E. coli* population in the intestine (Table 2). In calves of control group, the number of *Lactobacillus* was  $7.7 \times 10^7$  cfu/inch<sup>2</sup> of intestinal wall as against  $13.0 \times 10^7$  cfu/inch<sup>2</sup> in the probiotic-treated calves. On the other hand, substantial reduction in *E. coli* was observed in probiotic-fed calf. On average the number of cfu of *E. coli* in the intestinal wall of probiotic-fed calf was  $5.4 \times 10^5$  cfu/inch<sup>2</sup>, as compared to  $9.1 \times 10^5$  cfu/inch<sup>2</sup> in control.

Increases in numbers of *Lactobacilli* in the intestinal flora accompanied by reductions in numbers of coliform bacteria are apparently normal in development of intestinal flora of calves. It does suggest that the *Lactobacilli* in the developing intestinal flora exert a controlling effect on coliform bacteria. Several investigators observed increases in numbers of *Lactobacilli* accompanied by a decrease in coliforms after oral administration of *L. acidophilus* (Buchanan and Gibbons 1974; Fuller, 1973; Gilliland and Speck, 1977 and Morishita *et al.*, 1971). Several reports indicated greater declines in numbers of coliforms after feeding *L. acidophilus* to humans (Muralidhara *et al.* 1977; Speck, 1976). Muralidhara *et al.* (1977) also observed that feeding massive numbers of *Lactobacilli* to young piglets resulted in a decrease in numbers of coliforms in the intestinal tract.

## Conclusions

It can be concluded that the probiotic *Lactobacilli* isolated from yoghurt (*Dahi*) are able to increase the proportion of *Lactobacillus* and decrease the proportion of *E. coli* in the intestine of newborn calves, which may open an opportunity for the dairy farmers to use an alternative to antibiotics to improve calf health.

## References

- Anonymous 1999: Probiotiques: une procédure d'homologation stricte, gage de crédibilité. *La revue de l'alimentation animale hors-série* 6 – 8.
- Buchanan MP, Gibbons NE 1974: *Bergey's manual of determinative bacteriology*. 8<sup>th</sup> ed. Williams and Wilkins Co., Baltimore, MD.
- Collins CH, Lyne PM 1980: *Microbiological Methods*. Vol. 4. Butterworths, London, pp. 215-217.
- Davis CL, Drackley JK 1998: *The Development, Nutrition and Management of the Young Calf*. Iowa State University Press, Ames, IA, USA.
- Fox SM 1988: Probiotics: Intestinal inoculants for production animals. *Veterinary Medicine* **83** 806– 830
- Fuller R 1973: Ecological studies of the *Lactobacillus* flora associated with the crop epithelium of the fowl. *Journal of Applied Bacteriology* **36** 131.
- Fuller R 1989: Probiotics in man and animal. *Journal of Applied Bacteriology* **66** 365– 378.
- Fuller R, Houghton SB, Brooker BE 1981: Attachment of *Streptococcus faecium* to the duodenal epithelium of the chicken and its importance in colonization of the small intestine. *Applied and Environmental Microbiology* **41** 1433-1441.
- Gilliland SE, Speck ML 1977: Antagonistic action of *Lactobacillus acidophilus* toward intestinal and food borne pathogens in associative cultures. *Journal of Food Protection* **40** 820-823.
- Hargrove RE Alford JA 1978: Growth rate and feed efficiency of rats fed yogurt and other fermented milks. *Journal of Dairy Science* **61** 11-19.
- Hargrove RE Alford JA 1980: Growth response of weanling rats to heated, aged, fractionated and chemically treated yogurts. *Journal of Dairy Science* **63** 1065– 1072.

- Havenaar R, Huisintveld JHJ 1992: *Probiotics: a general view. In: Lactic acid bacteria in health and disease*. Vol 1. Amsterdam: Elsevier Applied Science Publishers.
- Morishita Y, Mitsuoka T, Kaneuchi CC, Yamamoto S, Ogata M 1971: Specific establishment of Lactobacilli in the digestive tract of germ-free chickens. *Japan Journal of Microbiology* **15** 531-534.
- Muralidhara KS, Sheggeby GG, Elliker PR, England DC, Sandine WE 1977: Effect of feeding Lactobacilli on the coliform and Lactobacillus flora of intestinal tissue and feces from piglets. *Journal of Food Protection* **40** 288-292.
- Otterby DE, Linn JG 1981: Advances in nutrition and management of calves and heifers. *Journal of Dairy Science* **64** 1365-1377.
- Rodriguez ML 1994: *Bacterias productoras de acido lactico: Efectos sobre el crecimiento y la flora intestinal de pollos, gazapos y lechones*. Doctoral thesis, Facultad de Veterinaria, Universidad Complutense, Madrid.
- Speck ML 1976: Interactions among Lactobacilli and man. *Journal of Dairy Science* **59** 338-342.
- Wong NP, McDonough FE, Hitchins AD 1983: Contribution of *Streptococcus thermophilus* to growth-stimulating effect of yogurt on rats. *Journal of Dairy Science* **66** 444- 449.
- Wren WB 1987: Probiotics—Fact or Fiction, *Animal Health and Nutrition, Large Animal Veterinary* Nov/Dec, 28- 30.