

# Effect of a Probiotic Compound in Rumen Development, Diarrhea Incidence and Weight Gain in Young Holstein Calves

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**Abstract**—It has been proven that early establishment of microbial flora in digestive tract of ruminants, has a beneficial effect on their health condition and productivity. A probiotic compound, made from five bacteria isolated from adult bovine cattle, was dosed to 15 Holstein newborn calves in order to measure its capacity of improving body weight gain and reduce diarrhea incidence. The test was performed in the municipality of Cajicá (Colombia), at 2580 m.a.s.l., throughout rainy season, with environmental temperature that oscillated between 4 to 25 °C. Five calves were allotted to control (no addition of probiotic). Treatments 1, and 2 (5 calves per group) received 10 ml Probiotic mix 1 and 2, respectively. Probiotic mixes 1 and 2 were similar in microbial composition but different in production process. Probiotics were added to the morning milk and dosed on a daily basis by a month and then on a weekly basis for three additional months. Diarrhea incidence was measured by observance of number of animals affected in each group; each animal was weighed up on a daily basis for obtaining weight gain and rumen fluid samples were extracted with oro-esophageal catheter for determining level of fiber and grain consumption.

**Keywords**—Calve, diarrhea, probiotic, rumen microorganisms.

## I. INTRODUCTION

THE direct provision of microorganisms has demonstrated benefits in dairy animal's health like increase in the gain of weight, milk production and total digestibility of the diet components [1]. With the early inoculation of microorganisms it is tried to quickly activate ruminal flora and to promote a beneficial effect in the animal health. Similarly, the faster degradation of vegetal fibers will reduce the high cost that feeding represents in the first stage of the development [2].

In bovine's production systems, is critical stage of the growth the transition that animals must do from their monogastric condition, fed with milk, to the herbivore condition, in which their pre-gastric fermentative cameras must be completely active to make the digestion of the fibrous resources [3]. It is well known that the activation of microflora in charge of this fermentation must be made before the weaning, so that it is a gradual and not a sudden event [4].

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In dairy cattle farms located at the high tropic areas of the Andean region, it has been observed a high incidence of diarrheas and mortality in calves, mainly in the first weeks of life [5]. With the present work we look for demonstrating that with early inoculation of ruminal microorganisms in calves, the transition to the herbivore condition occurs in shorter time interval and thus, microbial flora begins to degrade vegetal resources with more efficiency. This condition will generate a favorable repercussion in the health state of calves and, in addition, less days of provision of milk would represent an economic benefit for the dairy farmers.

## II. MATERIALS AND METHODS

### A. Animal Groups

Fifteen animals were selected and divided in 3 groups of 5 calves each one, first denominated control (without treatment provision), the second probiotic mixture 1 and third one probiotic mixture 2. Microbial composition of probiotic mixtures 1 and 2 were similar, but their production processes are substantially different, thus a different biological action was expected for each one. The experiment was carried out during the months of June to November of 2008 at *Fagua*, a dairy farm property of Alqueria S.A. This farm is located in the municipality of Cajicá (Colombia), 2580 m.a.s.l.

The animals were distributed in paddocks in groups of 5 individuals, a different treatment each. In order to facilitate their identification and handling, animals were identified with a color cord around the neck, with different color for each treatment, white for the control group, blue for probiotic mixture 1 and red for probiotic mixture 2.

### B. Viability

Animals that were part of the experiment were put under viability evaluation using the Apgar's methodology [6], which gives a numerical valuation to the physical conditions of the animal, considering vitality and reflections. This valuation was made for having a notion of the homogeneity of the group and also as an indicator of future performance during the experiment.

### C. Probiotic Administration

Milk administration was made using a 5 positions deposit (milkbar). Probiotics were added in the morning milk and dosed on a daily basis by a month and then on a weekly basis for three additional months.

#### D. Diarrhoea Presence and Weight Gain

Presence of diarrheas in the animals was determined by direct observation of the number of animals affected in each group. The growth of the animals was measured making a periodic determination of the increase in their corporal mass. Each animal was weighed on a weekly basis the first four weeks of post birth and then every fifteen days until completing 16 weeks age.

#### E. Blood Analysis

Blood samples were taken to in each calf at the birth time and on days 7, 21, 42 and 63 by jugular puncture using vacutainer needle. Samples were taken to clinical laboratory and sanguineous chemistry was evaluated. The evaluated variables were hematocrite, total plasmatic proteins, albumen, fibrinogen and immunoglobulin. Sanguineous total solids were directly quantified in a random sample of hematologic content. Result is given as percentage of cells in volume of blood. This procedure is also known as complete sanguineous count. Total plasmatic proteins were measured by Biuret method, whose principle is the formation of protein-copper ion compounds in alkaline solution, which are measured by colorimetric methods.

#### F. Rumen Fluid

Samples of ruminal fluid of each calf were extracted using oro-esophageal catheter, with the purpose of determining the level of fiber and grain consumption. These samples were taken on days 28, 50, 71 and 92 after birth.

#### G. Statistics

A statistical design of blocks completely random was used. With this model it was tried to verify the effectiveness of the probiotic in field, being used few animals, validating the methodology for applying it in a later study with greater number of animals. In the same way, it was considered average repeated statistical test in the time, to verify the effectiveness of the product in time.

$$Y_{ij} = \mu + B_i + T_j + E_{ij}$$

$\mu$  = Average population

$B_i$  = Block, period (3), time for the moment of the block.

$T_j$  = Treatments. (The three described above)

$E_{ij}$  = experimental Error

### III. RESULTS AND DISCUSSION

The conformation of the experimental group was made between the months of June and July of 2008 and the data of their viability condition, according to Apgar's methodology, indicated that most of the animals were in optimal condition at the time of his birth (Fig. 1). As observed in the three groups, average condition is superior to normal, evident indicative of the good genetics available in the farm and of the good general condition of the mothers.

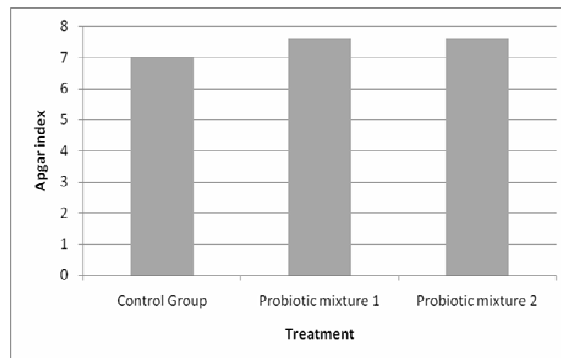


Fig. 1 Calves viability measured as Apgar's index

In calves treated with probiotic mix 1 or 2, diarrhea incidence was 20 % whereas in animals or the control group increased in scours in 60 %. The calves' growth showed a tendency ( $P > 0.05$ ) in favor of the effect of treatment with probiotic mixture 2 on daily gain of weight. In Fig. 2, the results indicate near 90 grams average of daily weight gain on individuals of this group. In all cases, an optimal corporal condition was shown.

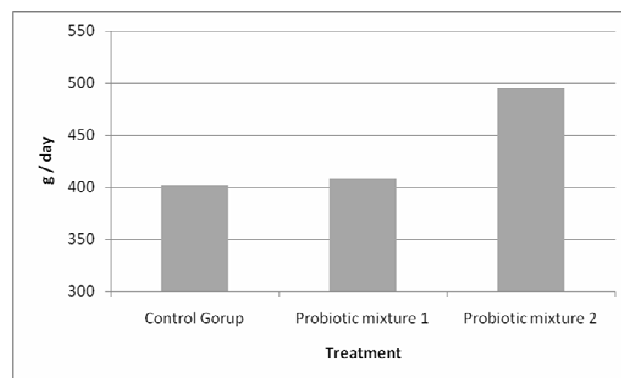


Fig. 2 Daily weight gain on calves

The results for sanguineous variables are shown in Figs. 3, 4 and 5. Hematocrit increased in the animals dealt with both probiotic compounds. This condition was not expected and was not observed in the group control, although never reached levels that indicated dehydration. This greater amount of erythrocytes and proteins circulating in blood brings beneficial effects in the development of calves. A high amount of red globules is wished in the organism because it helps to transport with more agility and efficiency nutritional molecules for the cells as well as oxygenates the tissues [7]. Fig. 3 shows that while hematocrit falls with time in control group, for the probiotic mixtures 1 and 2 groups, red globules amount increases ( $P > 0.05$ ), without exceeding in any case the values considered risky for the health (dotted lines).

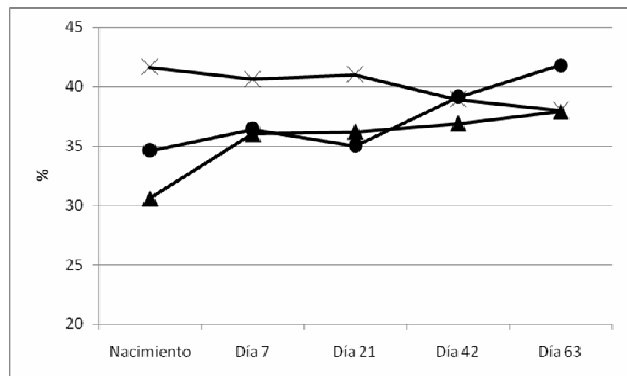


Fig. 3 Hematocrit variation on Control Group (X), Probiotic mix 1 (▲), and Probiotic mix 2 (●)

Iron is a component of all the cells and is indispensable for the synthesis of erythrocytes. Its transport until the bony marrow occurs by means of transport proteins. The greater amount of proteins in blood, greater is going to be the transport towards the bony marrow, which explains the behavior of the hematocrit in the group probiotic mixture 2 in correlation with the behavior of plasmatic proteins. Iron also promotes greater voluntary food consumption and consequently, an increase of weight in these animals [8].

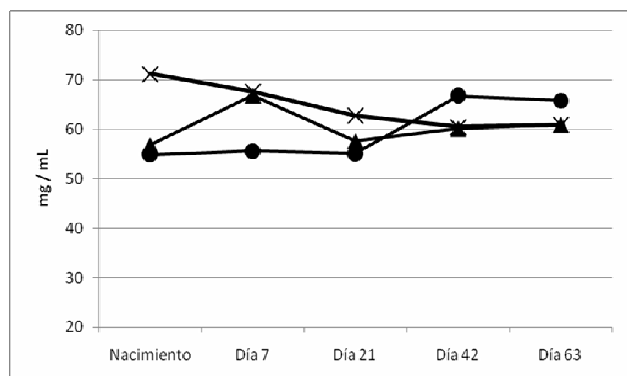


Fig. 4 Total plasmatic proteins on Control Group (▲), Probiotic mix 1 (X), and Probiotic mix 2 (●)

Albumen and immunoglobulin levels of the 3 groups did not show significant differences; nevertheless all had levels over the optimal one, which indicates that they had good nutrition and good immunity transference throughout the experiment.

Although the animals had a development adapted to the conditions of the dairy cattle productive system in the savannah of Bogota, their conditions of handling are commonly associated to environmental stress (low temperatures and high humidity) that triggers respiratory impairments. At several moments of their development, calves demonstrated inflammatory processes, presumably related to respiratory problems. This was observable in fibrinogen data which are over the optimal one recommended (2 to 4 mg/mL). Fibrinogen is an acute phase reactant that increases in tissue

damage processes or inflammation events. It is one of the most important determinants of erythrocyte sedimentation [9]. In the experiment, values between 5 and 10 mg/mL were associated to permanent calves' events of cough and congestion in the respiratory routes.

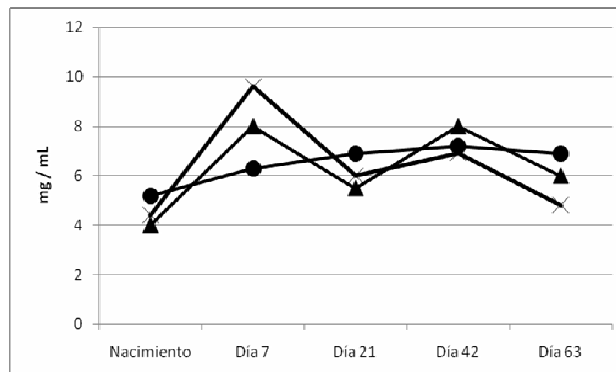


Fig. 5 Fibrinogen variation on Control Group (X), Probiotic mix 1 (●), and Probiotic mix 2 (▲)

The volume of ruminal content recovered from the control group (4 mL) was smaller than rumen content recovered from probiotic (mix 1 and 2) treated animals that peaked to 8 and 8.7 mL, respectively. Appearance of these both ruminal contents showed an intense green color with presence of small fragments of fiber (not larger than 1 cm) as well as presence of grains. On the contrary, rumen content recovered from the control group showed great abundant presence of saliva, a milky color appearance and unprocessed larger fragments of fiber.

#### IV. CONCLUSION

Administration of probiotic mixtures 1 and 2 to newborn calves showed an important beneficial effect by reducing the incidence of diarrhea (40 % less).

Treatment of Probiotic mixture 2 showed an average daily weight gain (495 g), clearly superior to the other groups where differences were not observed.

Significant differences for plasmatic variables of hematocrit and total proteins were observed. Treatment with probiotic mixture 2 demonstrated an increase in these indicators, whereas for variables IgG, albumen and fibrinogen, the statistical analysis did not show a conclusive effect of the treatments.

Rumen content extracted from calves indicated that animals administered with probiotic mixtures 1 and 2 had a more developed rumen than control group capable of degradation of fiber and grains.

In no case the inclusion of the microbial inducer produced some problem to the health for the calves including in this test.

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