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Beneficial Effects of Fermented Sugarcane Residue with Goat Probiotic On Gut Health

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Abstract **– Sugarcane is rich in dietary fibers that have beneficial properties in gastrointestinal tract. In addition the sugarcane is well known for its protective role in mammalian health. However, the sugarcane industry produces vast quantities of residues. Sugarcane blunting (waste produced by the sugarcane industry) was fermented under different conditions: using native microbiota (control), using specific goat probiotics, including *Lactobacillus reuteri* DDL19 (Treatment N°1), *Lactobacillus alimentarius* DDL 48 (Treatment N°2), *Enterococcus faecium* DDE 39 (Treatment N°3), *Bifidobacterium bifidum* DDBA (Treatment N°4) and a probiotic mixture containing equal amounts of the four probiotics (Treatment N°5). The fermented sugarcane is a synbiotic supplement for goats because it contains goat probiotic as well as dietary fibers considered as prebiotic. Firstly, the ensilages were microbiologically examined and then administered simultaneously to different batches of six goats each immediately after weaning. We evaluated the five different treatments during three consecutive periods of seven days each (ingest-repose), starting immediately after weaning. The best results were obtained with the probiotic mixtures of strains belonging to different genera and species. The ingestion of the Treatment 5 was able to diminish coliforms and enhance the beneficial *Bifidobacterium* and lactic acid bacteria by at least one logarithmic unit with respect to the control. Furthermore, the incidence of diarrhea in this experimental group was lower than the control group. The intestinal flora was improved in all cases in which sugar cane fermented with the probiotic strains was consumed. The residue is a good vehicle for probiotic strains, providing sugars and dietary fibers in a period of drought and in geographical areas of poor pastures. In this work, we use an industrial solid waste (highly polluting) as synbiotic supplement food for goats.**

Keywords **– Goats, Probiotic, Silage, Sugarcane.**

I. Introduction

Breeding ruminant livestock is one of the major industries in the northwest region of Argentina, where sugarcane is a major agricultural product. Sugarcane blunting refers to the upper part of the cane that usually contains lower sugar concentrations than the rest and is normally discarded, producing abundant waste. Therefore, finding a use for this residue would be more beneficial for the environment. Fermentation of sugarcane blunting by inoculation of a probiotic ruminant lactic acid bacteria (LAB) plus two LAB isolated from sugarcane was developed and found to prolong shelf life of silage and increase microbiological safety of feed for goats [1]

Weaning is always a delicate step in the livestock industry in general because it coincides with profound physiological changes of the animal's life [2]. The diarrheic process, typical during the early months of life, is a condition that is difficult to prevent, treat and control [3]. Consequently, probiotic administration one week after weaning would allow animals to adapt to their new feeding practices.

An increasingly common trend is the application of potentially beneficial microorganisms to supplement host defenses in order to achieve good sanitary conditions. Most Lactobacilli have the ability to survive gastric passage and can colonize the intestinal tract of humans and other mammals [4]. LAB are present in nearly all silages.

II. Materials and Method

Strains

The goats probiotic *Lactobacillus alimentarius* DDL48, *Lactobacillus reuteri* DDL 19, *Bifidobacterium bifidum* DDBA, and *Enterococcus faecium* DDE39 were isolated from fresh faecal samples of healthy goats in north-west Argentina [5]. Fresh cultures of these strains were obtained from MRS broth (pH 5.5), with the exception of *Bifidobacterium bifidum* DDBA, which was grown in MRS broth containing 1% lactose.

Plants and silages

For evaluation of fermentation conditions, blunting of *Saccharum officinarum* (sugarcane) of the variety PR980 from Tucumán city (Argentina) were used. Sugarcane (n=15) from the third cut was harvested manually after 6 months of growth. Blunting from the harvested sugarcane was produced using a cutter forest at a 5-10 cm cut size

and blunting samples (4.0 kg) were placed into plastic bags.

Fermentation of blunting sugarcane

Six different fermentations were carried out as described by Apás et al [1]. The control sample (C) was not inoculated, whereas Treatment N°1 (T1) was inoculated with *Lactobacillus alimentarius* DDL 48 at ≈ 1-3 $\times 10^8$ colony forming units (CFU)/g fresh forage weight, Treatment N°2 (T2) was inoculated with *Lactobacillus reuteri* DDL 19 at ≈ 1-3 $\times 10^8$ CFU/g fresh forage weight, Treatment N°3 (T3) was inoculated with *Enterococcus faecium* DDE 39 at ≈ 1-3 $\times 10^8$ CFU/g fresh forage weight, Treatment N°4 (T4) was inoculated with *Bifidobacterium bifidum* DDBA at ≈ 1-3 × 10^8 CFU/g fresh forage weight, and Treatment N°5 (T5) was the probiotic mix containing *L. alimentariu*s DDL 48, *L. reuteri* DDL 19, *E. faecium* DDE 39 and *B. bifidum* DDBA (in a 1:1:1:1 ratio) at a final total concentration of ≈ 1-3 × 10^8 CFU/g fresh forage weight.

Bacteria in suspension were applied to forage (10 mL/4.00 kg forage) and thoroughly mixed with the forage in a 114 L capacity plastic bag (pore diameter 200 µ). Forage (4.00 kg) was ensiled in triplicate in polyethylene bag silos that were vacuum packed and heat-sealed. Bag silos were incubated at 21±2°C for 30 days. The pH was determined in silages homogenized diluted 1/10.

Analytical methods

Dry matter was defined as the total sample weight, i.e., the water in the sample expressed in g/kg of silage or as a percentage (100 less than ambient relative percent humidity). For all samples taken at different times of silage fermentation (0 and 30 days), 10-12 g of the crushed blunting was placed in previously dried and weighed petri plates. The prepared plates were heated to 105°C in a furnace (Tecno-Dalvo, Buenos Aires, Argentina) until a constant weight was obtained. The silages were analyzed in triplicate for dry matter (DM; AOAC method 934.01, 2003), N (AOAC method 976.05, 2003), ash (AOAC method 942.05, 2003), and crude fibres (AOAC method 930.10, 1995).

The pH was determined with a pH-meter equipped with a glass electrode, which was calibrated against standard buffer solutions (Sigma, USA) at pH 4.0 and 7.0.

Microbiological assays of fermented blunting

Culture incubation was carried out in triplicate. Dilutions were homogenized in a Stomacher 400 blender (Seward-Tekmar, Norfolk, UK) to determine the number of viable cells. Samples from each incubated culture were collected at different times after the ensilage started (0, 15 and 30 days).

For quantification of microbes, 100 g of crushed blunting was diluted into 900 mL of sterile peptone water. The mixture was homogenized and the homogenate diluted 1/10. From this homogenate, serial dilutions were prepared. Cell numbers (CFU/mL) were counted by plating 0.1 mL of homogenate in the appropriate media.

MRS plus cycloheximide 0.0035g/ml (Merck-Germany) pH 5.5 was use for LAB, Mac-Conkey agar media (Britania L495, Argentina) for Enterobacteriaceae, KF (Difco, 212226) for enumeration of *Enterococcus* (Brodsky and Schliemann, 1976) plus 1% TTC (2,3,5-Triphenyl tetrazolium chloride) the bacteria were incubated at 37ºC for 48 h. The medium HHD (dehydrated: Lactic Acid Bacteria Differential Broth. Cod MI 086 from HI Media Laboratories Pvt Limited, Bombay - 400 086, India) plus AnaeroGen (Oxoid) was used for *Bifidobacterium*, this bacteria was incubated at 37ºC for 72 h. Saboraud plus penicillin: chloramphenicol 1:1 at 1.5% was used for both fungi and yeast and was incubated at 30°C for 96 h.

Animals and feeding

The work was carried out simultaneously with six batches of six Creole goats per batch. The groups of goats were kept isolated to minimize contact with animals of a different group and thus prevent cross-contamination. Immediately after weaning, 45-day-old goats selected by body weight (initial average weight: 8.40 ± 0.95 kg) were used to evaluate the effect of probiotics on intestinal fermentative activities and weight enhancement for a period of 50 days. The animals were kept in individual pens and supplied with water *ad libitum*; body weight was recorded weekly. A visual inspection, a subjective individual health status and the occurrence of diarrhea were recorded daily. All procedures involving animals, their handling and treatment were approved by the Universidad Nacional de Tucumán Ethical Committee for Animal Use. The research was conducted at a private farm located in Termas de Río Hondo, a province of Santiago del Estero, Argentina. This region is 80 km from our laboratory in Tucumán. Termas has a dry subtropical climate with little rainfall and very salty soil. During the study period (50 days), the temperature reached a maximum of 40°C during the day and a minimum of 20°C at night and early morning. The daily diet composition administrated was (Ingredients of diet g fresh matter/group/day): forage blunting sugarcane, 1200; alfalfa, 1200; Corn grain, 800; NaCl 6.0; urea 9.6 and Vitamins and protein by meat and bone meal (HCH, SA: 2309.90.10 Chenha International Co,. Ltd; Korea), 100.

The sugarcane forage supplied in the feed was inoculated with 1-3×10^8 CFU/g of *Lactobacillus reuteri* DDL 19 (T1), *Lactobacillus alimentarius* DDL 48 (T2), *Bifidobacterium bifidum* DDBA (T3), *Enterococcus faecium* DDE 39 (T4), and the last treatment group (T5) was supplemented with a mixture of all the probiotics in a 1:1:1:1 ratio at a final concentration of 1-3×10^8 CFU/g forage. The goats were divided into one Control and five Treatment groups. In each group, goats received two feeds per day. For the control group(C), the sugarcane was not inoculated with a probiotic.

A previously described protocol for the administration of probiotics was followed to prevent any probiotic adaptation of goat gut that could translate into a lack of beneficial response [6]. The protocol was as follows: 7 days of supplemented diet (first probiotic ingestion), 7 days without supplement, 7 days of supplemented diet (second probiotic ingestion), 7 days without supplement and 7 days of supplemented diet (third probiotic ingestion).

Microbiological assays of fecal sample

Fresh, individual faeces samples were collected weekly by rectal swab to perform microbial assessments throughout the assay period. The samples were stored in ice and processed within 6 h of collection. Fecal bacteria were investigated by diluting 1 g wet faeces in 10 mL (total volume) of physiological sterile solution. After homogenization of the mixture by vortexing, 1 mL of the suspension was added to 9 mL of physiological sterile solution. The samples were serially diluted, and 0.1 mL of each dilution was plated in triplicate on the appropriate agar medium to examine microbial populations. Dilutions were inoculated into different culture media according to the organisms to be investigated. Trypticase soy agar (TSA) (Merck-Germany) was used for total aerobic counts, MRS plus cycloheximide 0.0035g/ml (Merck-Germany) pH 5.5 for LAB, Mac-Conkey agar media (Britania L495, Argentina) for Enterobacteriaceae, KF (Difco, 212226) for enumeration of *Enterococcus* and HHD (dehydrated: Lactic Acid Bacteria Differential Broth. Cod MI 086 from HI Media Laboratories Pvt Limited, Bombay -400 086, India) plus AnaeroGen (Oxoid) for *Bifidobacterium*. Average results, obtained from each triplicate determination, were expressed as CFU/g fresh faeces.

Diarrhea occurrence

Faeces consistency and the number of fecal deposits were visually recorded daily.

Feed conversion

Feed conversion efficiency was determined as total feed consumed per kg of live goats during the entire study period.

Statistical analyses

The data were analyzed with non parametric variance analysis of Kruskal-Wallis and Connover´s test was applied. The significance level used was 5%. The position measures studied were medians (Me) and as a measured of variability was the medians deviation (DMe), whose mathematical expression was:

$$DMe = \frac{1}{n}.\sum_{i=1}^{n}\left|x_i - Me\right|$$

Quadratic error was defined from DMe:

$$SEM_e = \frac{DMe}{\sqrt{n}}$$

To know the differences between the medians, in percentage, Relatives Changes Rates (RCR) were calculated:

$$RCR_{Me} = \frac{(Mei - Mej)}{Mej}.100$$

$$\text{Whit}\begin{cases} Mei := Median.of.group.i \\ Mej := Median.of.group.j \end{cases}$$

III. RESULTS

Chemical and microbiological analysis of the silage fermentation process

Chemical analysis of the silage of fresh *Saccharum officinarum* (sugarcane) was carried out in order to evaluate its properties. The dry matter was 33.78 %. Chemical composition (g/kg DM) of the ensile of fresh *Saccharum officinarum* (sugarcane): Ash 36 ± 1; Crude protein 14 ± 1; Nitrogen 2.3 ± 1.3; Total fiber 115.7 ± 1.9; Carbohydrates 142.2 ± 2.3.

Prior to testing, the cane was dried in the sun for three hours, and the initial moisture was measured to be approximately 30-31%. After thirty days, the moisture content of the silo bag with the probiotic mixture (T5) showed a higher content of dry matter compared to the other treatments and the control. The DM in control (C) silage was 28.06% (± 1.18) and the DM in T1, T2, T3, T4 and T5 were 29.91(± 1.63); 30.22(± 1.39); 31.65 (± 1.85); 32.59 (± 1.03) and 33.78 % (± 1.23), respectively. The microbiota of the fermentation process was determined at 0, 15 and 30 days fermentation for the five treatments and the control sample (Table1). In all fermented silages, a decrease in the count of fungi and yeasts was observed after 30 days with respect to initial values. This decrease was 1.52 (Log CFU/g) in spontaneous fermentation (control). In all inoculated fermentations, large decreases were observed compared to C. The decreases were 1.77, 2.32, 1.81, 1.90, and 2.49 (Log CFU/g) for T1, T2, T3, T4, and T5, respectively. However, after 15 days T2 and T5 demonstrated significant differences with respect to the control fermentation (Table 1).

On average the supplementation of *Lactobacillus* DDL 19 (T1) was associated with higher presence of LAB after 15 days of experiment. Nevertheless, after 30 days incubation T1, T2, T4, and T5 had 0.79, 0.85, 0.19, and 2.03 Log CFU/g higher than the control, respectively.

Significant differences in the Log CFU/g of Enterococci and *Bifidobacterium* with respect to the control was observed after 30 days incubation only in T4 and T5.

Significant reductions in the population of coliforms were observed. The decreases were 2.40, 2.66, 2.91, 2.80, 2.91, and 3.55 Log CFU/g for control, T1, T2, T_3, T4, and T5, respectively, after 15 days fermentation. However, after 30 days only treatments T3, T4, and T5, showed a significantly higher decrease with respect to the control.

Lactobacillus, *Bifidobacterium* and *Enterococcus* demonstrated significant increases in T5 compared to spontaneous fermentation (control). The results indicate that fermentation of sugarcane blunting is an adequate medium for introduction of ruminant probiotic bacteria, as showed in our previous work [1].

A greater and faster pH decrease contributes to a higher yield of safety products. After 15 days incubation, pH analysis during silage fermentation indicated that the initial pH of 5.55 (average) lowered to 3.45 (± 0.06), 3.38 (± 0.04), 3.17 (±0.07), 3.78 (± 0.04), 3.40 (±0.03), and 3.00 (±0.01) for control, T1, T2, T3, T4, and T5, respectively. The lowest pH (3.00) found after 15 days incubation in T5 suggested that this condition appears to be a more effective way of avoiding detrimental contamination. It is necessary to reduce pH and stabilize it in the shortest time possible. Thus, the faster the drop in pH, the greater number of nutrients is retained in the silo;

for this reason it is paramount to promote the growth of LAB as quickly as possible after sealing the silo bags [7].

Fecal flora

At the beginning of the assay period the goats were 45±6 days old (initial body weights were included in Table 3), and the consumption of probiotic was initiated from the very first day of the weaning process. The minimal bacterial fecal populations of Enterobacteria, LAB, *Bifidobacterium* and Enterococci were observed at this time. In this instance some differences were observed between the groups: C and T5 has lower concentration of total aerobic microorganism and coliforms than the other groups; T2 has higher level of Lactobacilli; and T1 and T2 have lower *Bifidobacterium* count than the other groups. However, these initial differences were not observed or were not maintained after probiotic administration.

After 7 days of a probiotic-supplemented diet, the fecal microbiological populations showed significant differences between C and T1, T2, T3, and T4 groups for Lactobacilli; T2, T3 and T4 for Bifidobacteria; and T1, T4, and T5 for coliforms.

The consumption of probiotic was reinitiated after 7 days of no probiotic treatment. The age of the goats at this period was 59±6 days and the body weight (median of each group) was 8.42, 8.57, 8.34, 8.32, 8.58, and 8.59 kg for C, T1, T2, T3, T4, and T5, respectively.

Results obtained after resuming the diet are summarized in Table 2. In this period, a significant increase with respect to the control was observed in the number of *Enterococcus* cells in goats receiving fermented sugarcane inoculated with *Enterococcus faecium* DDE39 (T4) and, unexpectedly, in the T1 and T3 groups, which were 0.78, 0.37, and 0.74 Log CFU/g respectively. In addition, increased numbers of viable cells of *Bifidobacterium* of 1.26, 1.23, 0.95, and 1.21 Log CFU/g were observed for T1, T3, T4, and T5, respectively. The treatments 2, 3, and 5 displayed a significant increase, 0.56, 0.64, and 0.86 Log CFU/g, in LAB with respect to C. Conversely, lower levels of coliforms (P: 0001) were observed in the faeces of T5 animals that were supplemented with mixed probiotic (6.93 Log CFU/g) and faeces of T4 animals (7.25 Log CFU/g) compared to C (7.52 Log CFU/g).

At the beginning of the third probiotic ingestion, the age of the goats at this period was 73±6 days, and the body weight was 9.08, 9.32, 8.98, 8.99, 9.38 and 9.58 kg for C, T1, T2, T3, T4, and T5, respectively. After the third probiotic ingestion, the total aerobic counts in fecal samples obtained from goats were higher with respect to control only for T1, T2, and T5. However, during this period the *Enterococcus* levels increased with all the samples, showing a significant increase with respect to C only by T2 and T5 (Table 2). Cell numbers of *Lactobacillus* were (0.96 Log CFU/g) higher in faeces in T5 with respect to Control. Regarding C concentration of *Bifidobacterium* present in faeces, the increase grew only by 0.41, 0.59, and 0.93 Log CFU/g for groups T2, T3, and T5, respectively. Following this period, a significant reduction in quantity of Enterobacteria with respect to the C was observed in T1, T2, T3, and T5 (Table 2).

A comparison between the three ingestion periods of each cell numbers within a treatment indicated that the Log CFU/g of total aerobes increased 1.22, 1.26, 1.26, 1.20, 0.79 and 1.37 for C, T1, T2, T3, T4 and T5, respectively. The amounts of *Lactobacillus* diminished in all the groups with the exception of T5 in which case there was an increase of 0.54 Log CFU/g. For *Enterococcus* the increase was: 0.83, 0.67, 0.79, 0.74, 0.81, and 1.13; Bifidobacteria increased 0.33, 0.71, 0.81, 0.84, 0.39 and 1.28 Log CFU/g for C, T1, T2, T3, T4, and T5, respectively. The amount of Enterobacteria increased 2.11, 1.59, 1.38, 1.49, 1.82, and 0.68 for C, T1, T2, T3, T4, and T5, respectively.

In summary, T5 have a significantly higher amount of *Lactobacillus*, Bifidobacteria and *Enterococcus* as well as lower Enterobacteria with respect to the control.

Diarrhea occurrence

During the first period of probiotic ingestion, two goats in the control group and two goats among all treatment groups experienced diarrhea. Diarrhea was observed for two days in a goat that received only *Enterococcus* (T3) and for three days in a goat in the group receiving *Lactobacillus reuteri* (T2) and in two goats in the control group (C). Following this period, only four goats in the control group experienced diarrhea, with watery faeces of soft consistency but not simultaneously. This behavior was observed between the second and the third exposure to fermented sugarcane without probiotic. Furthermore, an occurrence of diarrhea after the first period was only observed in the control group.

Production parameters

No significant changes were observed in terms of body weight in any treatments with respect to the control. Differences in feed consumption and feed conversion ratio among the different feeding treatments (T1-T4) were insignificant. The highest feed conversion was observed in goats fed with T5-fermented silage (Table 3).

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Table 1: Evolution of microbial profile during sugarcane probiotic fermentation

Results are expressed as median. Treatment: T1 inoculated with *Lactobacillus alimentarius* DDL 48; T2 inoculated with *Lactobacillus reuteri* DDL 19; T3 was inoculated¸ with *Enterococcus faecium* DDE 39; T_4 inoculated with *Bifidobacterium bifidum* DDBA; and T_5 was the probiotic mix containing *L. alimentarius* DDL 48, *L. reuteri* DDL 19, *E. faecium* DDE 39 and *B. bifidum* DDBA (in a 1:1:1:1 ratio). [A] Indicate that these values differ significantly with respect to the respective control presented in the same column ($P \leq 0.05$)

Treatments	Yeast and fungus(CFU/g)			Lactobacilli (CFU/g)			Enterococci (CFU/g)		
	0 days	**15 days**	**30 days**	**0 days**	**15 days**	**30 days**	**0 days**	**15 days**	**30 days**
C	7.40	7.02	5.88	7.05	7.51	7.30	6.61	7.60	7.00
T1	7.45	6.33	5.68	7.50	8.50[A]	8.09[A]	6.63[A]	6.15[A]	6.90
T2	7.50	6.05[A]	5.18[A]	7.65[A]	8.10	8.15[A]	6.55	7.02[A]	7.06
T3	7.51[A]	7.05	5.70	7.05	7.20	7.61[A]	6.60	7.45	7.05
T4	7.58[A]	6.27	5.68	7.05	7.28	7.49	6.70	7.60	7.60[A]
T5	7.50	6.05A	5.01A	7.70A	8.29	9.33[A]	6.69	7.50	7.58[A]
Kruskall-Wallis (H,p)	H=11.38 p=0.0433	H=15.35 p=0.0088	H=15.13 p=0.0093	H=14.63 p=0.0116	H=16.39 p=0.0058	H=16.58 p=0.0054	H=11.59 p=0.0397	H=13.99 p=0.0152	H=14.39 p=0.0129
SEM	0.0115	0.0849	0.0570	0.0659	0.1144	0.1249	0.0106	0.0881	0.0525

Treatments	Bifidobacteria (CFU/g)			Enterobacteria (CFU/g)		
	0 days	**15 days**	**30 days**	**0 days**	**15 days**	**30 days**
C	6.36	6.25	6.59	6.45	6.03	4.05
T1	6.32	6.42[A]	6.50	6.38	5.03	3.72
T2	6.70	6.95	6.60	6.62[A]	5.08	3.71
T3	6.75	6.35	6.80	6.45	5.02	3.65[A]
T4	6.18	6.41	6.15[A]	6.53	5.09	3.62[A]
T5	6.29	7.00[A]	7.10[A]	6.55	4.59[A]	3.00[A]
Kruskall-Wallis (H,p)	H=15.37 p=0.0084	H=13.74 p=0.0172	H=16.11 p=0.0065	H=14.11 p=0.0144	H=12.06 p=0.0335	H=14.91 p=0.0100
SEM	0.0427	0.0564	0.0524	0.0175	0.0651	0.0478

Table 2. Evolution of microbial profiles in fecal goat´s during sugarcane probiotic administration

Results are expressed as median. Different superscripts in the same row, within each time studied, indicate significant differences with respect to control. C: Control. Treatment: T1 inoculated with *Lactobacillus alimentarius* DDL 48; T2 inoculated with *Lactobacillus reuteri* DDL 19; T3 was inoculated with *Enterococcus faecium* DDE 39; T4 inoculated with *Bifidobacterium bifidum* DDBA; and T5 was the probiotic mix containing *L. alimentarius* DDL 48, *L. reuteri* DDL 19, *E. faecium* DDE 39 and *B. bifidum* DDBA (in a 1:1:1:1 ratio).

	Microorganism	**Control**	**T1**	**T2**	**T3**	**T4**	**T5**		**SEM**
Intestinal microflora of Creole goats (Log CFU/g faeces) before probiotic ingestion.	Total aerobic	6.47	6.64[B]	6.58[B]	6.60[B]	6.56	6.64[B]	H: 24.87 p: 0.0001	0.0092
	Lactobacilli	6.50	6.53	6.64[B]	6.49	6.58	6.53	H: 18.08 p:0.0028	0.0112
	Enterococci	5.86	5.89	5.73	5.79	5.99	5.97[B]	H: 25.29 p: 0.0001	0.0166
	Bifidobacteria	6.10	6.02[B]	6.00[B]	6.18	6.11	6.08[B]	H: 27.22 P 0.0001	0.0105
	Enterobacteria	5.31	5.54[B]	5.49[B]	5.61[B]	5.58[B]	5.39	H: 24.48 p:0.0002	0.0207
Intestinal microflora of Creole goats (Log CFU/g faeces) after probiotic ingestion.	Total aerobic	7.73	6.64[B]	7.94	7.53	7.88	7.83	H: 17.28 p: 0.0039	0.0502
	Lactobacilli	5.26	6.42[B]	6.19[B]	6.14[B]	6.00[B]	5.85	H: 22.07 p:0.0005	0.0572
	Enterococci	5.38	6.00[B]	5.49	5.53	5.20	5.51	H: 23.21 p: 0.0003	0.0391
	Bifidobacteria	5.72	5.95	6.00[B]	6.11[B]	5.99[B]	5.87	H: 16.12 p: 0.0064	0.0293
	Enterobacteria	6.42	5.79[B]	6.32	5.37	6.29[B]	5.56[B]	H: 29.45 p≤0.0001	0.0756

Intestinal microflora of Creole goats (Log CFU/g faeces) after second probiotic ingestion.	Total aerobic	7.26	7.14	6.90[B]	7.39	7.47	7.53	H: 22.51 p: 0.0004	0.0438
	Lactobacilli	6.12	6.85	7.20[B]	7.13[B]	6.50	7.39[B]	H: 28.57 p≤0.0001	0.0703
	Enterococci	5.85	6.22[B]	6.07	6.63[B]	6.59[B]	5.94	H: 25.89 P: 0.0001	0.0596
	Bifidobacteria	6.41	7.28[B]	6.87	7.41[B]	7.06[B]	7.29[B]	H: 30.66 p≤0.0001	0.0518
	Enterobacteria	7.52	7.71	7.37	7.26	7.25[B]	6.93[B]	H: 25.53 p:0.0001	0.0471
Intestinal microflora of Creole goats (Log CFU/g wet faeces) after third probiotic ingestion.	Total aerobic	7.69	7.90[B]	7.84[B]	7.80	7.35	8.01[B]	H: 30.99 p≤0.0001	0.0263
	Lactobacilli	6.11	6.11	6.23	5.95	6.04	7.07[B]	H: 28.54 p≤0.0001	0.0395
	Enterococci	6.69	6.56	6.52B	6.53	6.80	7.10[B]	H: 28.55 p≤0.0001	0.0355
	Bifidobacteria	6.43	6.73	6.81[B]	7.02[B]	6.49	7.36[B]	H: 32.80 p≤0.0001	0.0544
	Enterobacteria	7.42	7.13[B]	6.87[B]	7.10[B]	7.40	6.07[B]	H: 32.35 p≤0.0001	0.0547

[B] Indicate that these values differ significantly with respect to the respective control presented in the same row (P≤0.05)

Table 3: Production parameters

C (Control) goat in the control group (C).T5 goat in the T5 group feeding with the probiotic mix containing *L. alimentarius* DDL 48. *L. reuteri* DDL 19. *E. faecium* DDE 39 and *B. bifidum* DDBA (in a 1:1:1:1 ratio). [C] Indicate significant differences (P<0.05).

Days	Body Weight (kg) (BW)		Food Consumed (FC).		Efficient (BW / FC)	
	C	T5	C	T5	C	T5
0	8.12	8.08	-	-	-	-
10	8.05	8.30	1.59	1.73	27.9	31.3
20	8.60	8.70	1.80	1.86	27.7	29.0
30	8.85	9.10	1.99	2.03	26.1	27.4
40	9.08	9.25	2.30	2.29	23.8	24.1
50	9.55	9.58	2.48	2.41	23.8	23.2
K-W (H.p)	H:30.12	p:0.0015	H:48.53	p<0.0001	H :0.27	0.6905
SEM		0.1297		0.1338		

IV. Discussion

In silage, the forage must be at least 30% of dry matter (DM). Wet silage facilitates the development of clostridia, stimulates the production of butyric acid, induces formation of alcohol by yeast and consequently reduces quality. In contrast to the control group that had a low DM, the samples fermented with goat probiotics have appropriate values of DM, indicating that probiotics led to a bioconversion of silage that renders it more stable microbiologically. Moreover, the fermented silage T5 probiotic mix increases the concentration of *Lactobacillus*, *Bifidobacterium* and *Enterococcus*, compared with uninoculated silage after 30 days of fermentation. These results indicate a longer survival of inoculated bacterial genera, suggesting that the fermentation of sugarcane blunting is an adequate medium for introduction of ruminant probiotic bacteria.

The administration of a mixture of goat probiotic enhances the maximum number of viable LAB cells. Furthermore, the mixed probiotic produced a decrease in Enterobacteria levels, which is advantageous from a veterinary medical perspective because these bacteria are frequently associated with infections [8]. The protective effect of LAB against coliforms in other mammals has also been reported. Moreover, it has been reported that some mixed probiotics may protect the livestock from bacterial infections [9], [10]. The Enterobacteria reduction in pigs, due to mixed probiotic administration, was reported after the second week of the trial [10], we obtained the same results for goats but in week three of the trial.

On the other hand, after the third probiotic ingestion, we found a significant increase in *Bifidobacterium* population. This situation can be considered beneficial because these bacteria compete with anaerobes, including clostridia, in bowels.

Neonatal-calf diarrhea is an important cause of morbidity and mortality in young ruminants. Two different probiotic preparations, containing six *Lactobacillus* spp. of bovine and human origin, were successful in reducing the overall mortality, incidence of diarrhea and fecal coliform

counts in calves [11]. Reduction in the incidence of diarrhea was observed in calves fed milk fermented with a mix of LAB [12]. Together with our results, this suggests that probiotic use correlates with improved health of ruminants. Dose, timing and duration of the administration of probiotics may be a factor affecting efficacy; a higher dose of probiotic given for a short period of time seems to be more effective than lower doses [13]. In concordance with this observation, the protocol used appears to be effective after 30 days of application. Another determinant may be the age of the animals; during early life, colonization patterns are unstable and newborn animals are then susceptible to environmental pathogens. Studying the dynamics of microbial fecal microbiota in Creole goats found that the minimum bacterial concentration was observed between 90 to 150 days [9]. This observation is in concordance with a reduction observed in the bacterial fecal populations of Enterobacteria, LAB, Bifidobacteria, and Enterococci in the same time frame. Initial colonization is of great importance to the host because the bacteria can modulate expression of genes in epithelial cells thus creating a favorable habitat for themselves [14]. In our case, the administration during the post-weaning period produced a protective and beneficial effect with respect to the control group.

One approach in probiotic application could be to use mixtures of strains belonging to different genera or species. Timmerman *et al*. in 2004 [15] reviewed studies that provided evidence for multi-strain probiotics being more effective than mono-strain probiotics. Apás *et al*. (2010) [6] studied the same probiotic mixture used in T5, administered orally using milk as a vehicle. The probiotic mixture dispensed was able to modify microflora balance by reducing Enterobacteria like *Salmonella*/*Shigella* and increasing LAB and Bifidobacteria. Probiotic supplementation was associated with a tenfold reduction in fecal putrescine, a cancer and bacterial disease marker. The Ames test indicated a 60% decrease in mutagen concentration in faeces. For this reason, the probiotic mixture appears to have a protective effect in goats. Although this paper investigated the comparative effects of probiotic administered individually or in mixtures in a different substrate, our results indicate that the probiotic mixture is the best alternative to increase and ensure ruminant livestock health.

On the other hand, *Lactobacillus alimentarius* DDL48 isolated from healthy goats was reported to inhibit the development of fungi and parasites [9]. Such microbiota contribute to the digestion of nutrients and form a protective layer on mucosal surfaces that inhibits growth of pathogens [16]; for example, Lactobacilli possess antagonistic activity against potential pathogens such as *Salmonella* and *Escherichia coli* [7]. LAB, including *Lactobacillus* and *Bifidobacterium* are normal microbiota of the complex ecosystem of the gastrointestinal tract. Competitive exclusion of pathogens is an important benefit derived from probiotic bacteria. The effect of probiotic LAB on competitive exclusion of pathogens has been demonstrated in human mucosal material *in vitro* [17], *in vivo* in chickens [18] and pigs [10].

Sugarcane blunting could be considered as a good alternative as a vehicle of goat probiotic. Sugarcane bagasse could be utilized as a roughage source for goats [19] since it is rich in dietary fiber. Dietary fiber has important beneficial properties in the upper and lower gastrointestinal tract. For this reason they are considered as prebiotics. They contribute to the establishment of a *healthier* microbiota where Bifidobacteria and/or Lactobacilli become predominant and exert possible health-promoting effects at the expense of more harmful bacteria species [3]. Besides, numerous investigations on the protective roles of sugarcane (*Saccharum officinarum* L.) derivatives showed that the extracts of sugarcane exhibit antiproliferative, antioxidative, and hepatoprotective abilities [20]. In addition, sugarcane showed multiple biological activities including antimutation and antioxidation [21]. Furthermore, the addition of sugarcane adds carbohydrates to the diet and it was previously informed that the presence of glucose in supplemented food enhances the survival of LAB inoculants in rumen fluid [22]. These factors, *a priori*, promote the advantage of fermented sugarcane blunting silage. On the other hand, it is well known that when ruminant diets are supplemented, there is often a concomitant reduction in pasturing time because the animals' energy demand is reduced [23].

Synbiotics may be defined as a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract [24]. The concept of synbiotics in ruminants, to our knowledge, has not been widely applied [3]. The administration of goat probiotic into a prebiotic vehicle (blunting of sugarcane) could be considered as symbiotic treatments. Previous reports suggest a synergistic effect coupling probiotics and prebiotics in the reduction of food-borne pathogenic bacterial populations [25].

The effects of probiotics and prebiotics on the growth performance of livestock are contradictory with the improvements significant only in some feeding trials. These results applied in livestock production should be analyzed with care because the administration of prebiotics can also cause a satiety effect in animals and so jeopardize the attempt to achieve weight gain [3]. In cattle, the overall results derived from probiotic administration are favorable; however, effects on performance have not been consistent [26], [27]. According to our results the highest feed conversion was observed in the T5 group. However, insignificant changes were observed regarding body weight with respect to the control.

We conclude that the residues of sugarcane fermented probiotic acting as a vehicle were easily ingested by goats. Synbiotic administration (probiotics and fermented sugar cane residue) increases the presence of LAB beneficial for the intestines and dramatically lowers the potential for harmful microorganisms. Therefore, this paper presents the use of agricultural residues as a vehicle for probiotics for goats with beneficial effects for animal health and

economic benefits as well as a reduction in ecologically-damaging solid waste.

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