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# Goat milk mutagenesis is influenced by probiotic administration

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ABSTRACT

In recent years, goat's milk has shown a number of advantages over the milk from other ruminant species. Some milk substances can alter the milk quality and also present potential carcinogenic activity. In this work, chemical composition and mutagenic compounds were determined using Ames test on goat milk from different geographical locations of northern Argentina. In Tucumán, two extensive farms were analyzed, one of them near to soybean culture, La Perla Farm, where the goaťs milk showed 788 ± 33 colonies/plate and the Sunchales Farm, which milk contained 655 ± 51 colonies/plate. The third is a public organization with semi-extensive INTA Farm, where Ames test indicated 210 ± 28 colonies/plate. A goat probiotic mixture (GPM): *Lactobacillus reuteri* DDL19, *Lactobacillus alimentarius* DDL48, *Bifidobacterium bifidum* DDBA and *Enterococcus faecium* DDE39 was orally administered as a treatment to diminish toxic substances. Ames test after a 25-days treatment with probiotics bacteria, showed 455 ± 47; 300 ± 33, and 102 ± 36 colonies/plate from goat milk obtained from La Perla, Sunchales and INTA farms, respectively. After a 50-days treatment, the Ames test detected 289 ± 23, 126 ± 26, and 60 ± 5 colonies/plate, in goat milk from La Perla, Sunchales and INTA farms, respectively. Moreover, the probiotic administration did not modify the milk physical-chemical composition; with the exception of fatty acid. The diminution of the mutagen capacity of milk could respond to observed modification on fat content. These results reinforce previous results about adsorption of mutagens by the strains contained in the GPM and define the first scientific results on this topic. The GPM, added to goat diet, did not influence protein and nonfat solids contents, acidity and density values, but allowed the obtaining of milk characterized by improving the concentration of beneficial compounds. The study supports the use of probiotics to enhance the quality of goat products.

## 1. Introduction

Goat breeding (*Capra hircus*) worldwide has been associated with marginal sectors and poor countries. The farming systems of goats are primarily extensive and limited technology is used, which has resulted in lower cost to producers, but at the same time has added factors that threaten the meat and milk quality. Grazing often occurs in areas which are affected by biological factors such as mycotoxins, toxic natural components of plants, as well as soil and/or water contamination from agricultural practices (seed treatment, fungicides, pesticides, etc.). Commonly, the drinking sources for animals are affected and some of the fore mentioned components can appear in animal products, especially dairy foods (Ruiz et al., 2008). In addition, several antibiotics are used for livestock in order to prevent microbial infections and promote the animal growth. However, about 80% of the antibiotics are excreted into the environment in animal manure which facilitates the development of antibiotic resistant strains (Salcedo and Kim, 2017). Moreover, bacteria resistant to antibiotics were found in animal guts without antibiotic administration (Zutic et al., 2013) as well as in bovine and goat milk (Chung et al., 2009)

The use of probiotic could avoid or diminish the inadequate use of antibiotics and produces health benefices and antimutagenicity (Apás et al., 2010, 2014,2015).

The addition of probiotics to animal diet provides a good alternative for improving their health (Draksler et al., 2004; s et al., 2008, 2010;). This situation is being translated into benefits for the society, such as the removal of mutagenic and carcinogenic compounds, which can be present in fecal samples (Apás et al., 2014). However, it is still necessary; to be determined whether probiotic bacteria are efficient to diminish the mutagens which may be presented in milk. To our

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knowledge, the mutagenic properties of goaťs milk according to its components, remains unknown.

In synthesis, the administration of the probiotic mixture decreases the concentration of mutagens in feces. On the other hand, the same probiotics increase the concentration of polyunsaturated organic acids with antimutagenic properties in milk. Therefore, we hypothesize that the administration of probiotics may also decrease the amount of mutagens in milk.

The objective of this study was to determine the effects of the probiotic consumption on the possible mutagenic properties of goat milk.

## 2. Material and methods

### 2.1. Bacteria strains

In this study, we used the mixture of goat probiotics (MGP) integrated by *Lactobacillus reuteri* DDL 19, *Lactobacillus alimentarius* DDL 48, *Bifidobacterium bifidum* DDBA and *Enterococcus faecium* DDE 39; each strain was cultured in an appropriate broth (Apás et al., 2010)

The mix in a relation 1:1:1:1 at a final total concentration of $1 \times 10^9$ colony-forming unit (CFU)/mL suspended in sterile milk. *Salmonella typhimurium* TA 98 was grown in nutrient Broth I (Oxoid Australia, West Heidelberg, Australia) in the presence of 25 mg/mL of ampicillin. Tests of histidine requirement, rfa mutation, uvrB mutation and R-factor were carried out to confirm the genotypes of S. *typhimurium* TA 98. Before the mutagenicity test, *S. typhimurium* cells were grown at 37 °C for 16–18 h until reaching 1–2.$10^8$ CFU/mL.

### 2.2. Lactating goats

The work was carried out with batches of 10 adult lactating goats in each farm during the whole trial. The selection criteria of the goats that participated in the trial (Saanen-Creole of three years old) implied that they are healthy, without previous administration of antibiotics in the last six months, which are in their second calving. The udders were checked and the goats showed an abnormal number of somatic cells in the initial intake were discarded. During the test, they did not raise and kept in a separate pen from other cattle. The administration of probiotics began in all animals between day 20 and 22 of the calving.

The geographical location of the farms was different but within 150 km. Two farms raised animals extensively and applied traditional methods. One of them is adjoining to a soybean cultivated field (La Perla), locality of Taco Ralo, Tucumán, Argentina; and the other from a distance of 23 km (Sunchales) locality of Lamadrid, Tucumán, Argentina. Farms located in a semi-humid climate with 320 m above sea level.

The third farm was controlled by a governmental organization; the National institute of agricultural technology (INTA) that is located in Sumalao, Catamarca, Argentina. Farm with a semi-arid climate at 505 m above sea level.

In all the farms the diet consisted in alfalfa, crushed maize grain, salt and a complex of vitamins and minerals according Apás et al. (2015). In the semi-extensive farms, the goats graze and browse during the morning and in the evening and at night they remain in the corral, where the same feed is supplied to the goats of the intensive breeding establishment.

The probiotic was orally administered at a dosage of 10 mL/day/goat. The management protocol was similar to that described by Apás et al. (2015).

All procedures involving the animals, their handling and treatments were approved by the Ethics Committee for the use of animals. The udders of the goats were cleaned and the total milk collected from the milking was collected in sterile vials, mixed and placed at 4 °C. The assays were carried out the immediately before and after 25 and 50 days of probiotic administration. The milk samples were stored at −20 °C during 2 days until processing

### 2.3. Determination of mutagenic compounds in goat milk

The antimutagenic activity of the mixture of goat probiotics (MGP) was determined by measuring the inhibition of *S. typhimurium* TA 98 mutation, in goat milk samples (Maron and Ames, 1983). A sample was considered mutagenic when the number of revertants colonies was at least twice the negative control yield (MUI ≥ 2) and showed a significant response in the variance analysis. The mutagens Positive control was sodium azide Sigma-Aldrich (0.5 μg/plate). Negative control: *S. typhimurium* TA 98 in sterile distilled water. Negative and positive control cultures gave the number of revertants per plate that were within the normal limits, previously found in our laboratory (Apás et al., 2014).

### 2.4. Antimutagenic activity of probiotic bacteria

The milk obtained was diluted (1/50) in phosphate buffer. On hundred microliter of this dilution was mixing with equal volume of a culture of 16–18 h of *S. typhimurium* TA 98 strain (approximate cell density 2.0 × $10^8$cells/mL). The mix was incubated with agitation in a shaker (150 rpm, 120 min, 37 °C). Then 200 μL were mixed with 2 mL top agar.

The top (overlay) agar for the Ames assay was prepared with 0.6% (w/v) agar, 0.5% (w/v) NaCl, supplemented with 0.5 mM L-histidine (Sigma-Aldrich) and 0.5 mM d-biotin (Merck, Germany). The mixture was then gently mixed and finally poured onto a plate containing glucose agar (glucose 2% w/v and agar 1.5% w/v). When the top agar had solidified, the plates were incubated in the inverted position at 37 °C for 48 h and *his*$^+$ revertant colonies were counted. Antimutagenic activity of probiotic bacteria was measured as reduction of number colonies from samples treated with probiotic bacteria, in comparison to the control (without probiotic bacteria), according to Apás et al. (2014).

### 2.5. Physicochemical analysis of samples of goat milk with and without probiotic treatment

It was conducted using an ultrasonic milk analyzer EKOMILK. The following parameters were measured: fat, nonfat solids, protein, density, acidity.

Histidine was determined according to the HPLC method for the determination of biogenic amino acids and amines using gradient chromatography and pre-column derivatization with o-phthaldialdehyde (OPA) (Alberto et al., 2002)

### 2.6. Statistical analysis

Data were represented as a mean ± standard deviation and were submitted to multivariate ANOVA using Info-Stat statistical software (InfoStat, 2012); P-values of $< 0.05$ were considered statistically significant.

## 3. Results and discussion

### 3.1. Development of the determination of mutagens in milk

According to our knowledge, this is the first time Ames test is used in goat milk. In order to carry out the Ames test, the experimental conditions were determined. One of the drawbacks is that the amount of histidine and biotin contained in milk could allow the growth of auxotrophic and hence could be associated with false positive results. The technique requires only a basal concentration of histidine and biotin. According to previous studies (Park and Haenlein, 2006; Bedoya Mejía et al., 2012), goat milk has histidine (0.89 mg/mL) and biotin (1.50 mg/mL). In the goats milk studied the amount of histidine was