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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 1×10^9 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 × 10^8cells/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

Table 1
Mutagens detected in goaťs milk.

Revertants (colonies/plate) of *Salmonella typhimurium* TA 98 in diluted milk (1/50)			
	La Perla Farm	Sunchales Farm	INTA Farm
Control	788 ± 33a	655 ± 51a	210 ± 28a
Treatment 25days	455 ± 47[b]	300 ± 33[b]	102 ± 36[b]
Treatment 50 days	289 ± 23[c]	126 ± 26[c]	60 ± 5[c]

Revertants of *S. typhimurium* TA 98 versus different goat's milk diluted 1/50. Revertants values are expressed as means ± SD. Different letters in column ([a,b,c]) indicate significant differences between the same farm with and without the administration of mixture of goat's probiotic. Spontaneous revertants of *S. typhimurium* TA 98: 13 ± 1 (colonies/plate) are considered Negative control.

ranging between (1.03–0.77 mg/mL).

The concentrations of histidine and biotin, required by Ames test to "start" his-strains, are: 0.124 mg/mL and 0.096 mg/mL respectively. For this reason, goat milk dilution 1:50 was performed. In this way final concentrations were: 0.018 (± 0.003) mg/mL (histidine) and we assumed that according previous works than the biotin concentration could be near to 0.030 mg/mL, value so minor than 1.50 mg/mL necessary to produce false positive.

3.2. Mutagens determination in milk

To evaluate the presence of mutagens, the milk samples were subjected to Ames test (Table 1). The three groups of milk exceeded twice the control value (13 ± 1 colonies/plate). However, INTA had the lowest values. There is a significant difference in the number of mutants found between to the farms. Fewer mutagens are found in the milk of goats that only eat the diet provided and where their circulation is restricted to controlled areas. Also in the area, there are practically no herbs can be consumed by the aridity of the soil.

Goats raised in semi-intensive areas that can circulate outside the pens for a few hours and consume forages native in a more humid climate have more mutagens in milk. However, further studies should be necessary in order to understand real values of this fact.

The results suggested the presence of mutagens in milk. For this reason, it is important to find antimutagenic agents. This group of agents include both natural and synthetic compounds. Based on their mechanism of action among antimutagens, several classes of compounds may be distinguished. These are compounds with antioxidant activity; compounds that inhibit the activation of mutagens; blocking agents; as well as compounds characterized by several modes of action. It was reported previously that several antitumor compounds act through the antimutagenic mechanism (Sloczynska et al., 2014). In recent years, several publications were focused on the screening of both natural and synthetic compounds for their beneficial muta/anti-mutagenicity profiles. The conjugated linoleic acid (CLA) present in goat milk is a known anticancer and antitumor agent (Parodi, 1997; Piperova et al., 2004,). The increased of CLA due probiotic consumption was previously demonstrated (Apás et al., 2015). Contrary, the casein of goats milk appear did not inhibit human breast cancer as the camel casein (Shariatikia et al., 2017)

The probiotics have antimutagenic and anticarcinogenic properties (Serban, 2014). The goat probiotic diminished the inflammation of intestine during the weaning period and dysplasia marker putrescine (Apás et al., 2015).

The evaluation of possible effects of administration of a probiotic diet to goats (Table 1), showed a notable reduction of bacterial mutants of 42, 54, and 51% after 25 days of ingesting MGP and 63, 81, and 71% after 50 days of ingesting MGP for La Perla, Sunchales and INTA farms, respectively.

In our work, the final values of INTA were lower than other farms, due to low initial values. The use of MGP decreased more than a third the number of bacterial mutants in respect to samples without probiotic treatment (Control), in all farms after 50 days of treatment. These results are in agreement with the ability of the MGP to remove mutagenic agents. In our laboratory the mutagen removal ability, *in vitro*, was tested, working with the same goat probiotic mixture (Apás et al., 2014). MGP was able to bind and detoxify potent mutagens, and this property can be useful in supplemented foods for goats. The mutagens remotion, improve small ruminant health and the food safety. The mutagens binding by probiotics were also reported by Hsieh and Chou (2006), but the authors studied soy milk.

The ability to retain aflatoxin by *L. casei* and bifidobacteria was previously reported (Hernández-Mendoza et al., 2009). The binding of lactic acid bacteria cell wall component to aflatoxin, reduces its negative health impacts (Haskard et al., 2001). Padma et al. (2011) postulated that the interaction between lactic acid bacteria and mutagen is due to the wall low molecular weight glycopeptides and that holding power could be due specific for each mutagen.

Green et al. (1980) have reported mutagenic activity in the chloroform extract of heat-sterilized milk. However, they did not inform that chemical substances were involved. In contrast, negative mutagenicity of heated milk was informed by other authors (Berg et al., 1990)

3.3. Physico- chemical analysis of samples with and without probiotic treatment

With the aim of knowing about the effects of probiotic feed mixture on the physicochemical parameters of the milk, density, acidity, fat, solid non fat and proteins concentration were determined (Table 2). The milk production increased after the 25 days of probiotic consumption, between 11 and 14% in the semi-extensive farms, and between 14 and 17% in the INTA farm. Future studies should be conducted to determine the significance and possible causes of this increased.

The chemical composition of milk determines its nutritional quality (Gallegos Sánchez et al., 2005), and its value as raw material for dairy industry. Our density values: 1.02–1.03 ± 0.1 (Table 2) showed no significant differences ($p \geq 0.05$) between the dairy of different farm and they were consistent with those found (1.029 ± 0.002) by Frau et al. (2012).

Acidity values were similar ($p \geq 0.05$) in all the conditions studied (Table 2) and also similar to the values reported (0.16 ± 0.02) by Frau

Table 2
Goat milk parameters.

Farm	Density (g/mL)	Acidity (g% lactic acid)	Fat Matter (g%)	Non Fat Solid (g%)	Proteins (g%)
La Perla control	1.02 ± 0.1[a]	0.18 ± 0.02[b]	3.56 ± 0.03[a]	8.68 ± 0.04[a]	3.55 ± 0.15[a]
La Perla treatment	1.02 ± 0.01[a]	0.19 ± 0.01[b]	4.00 ± 0.02[b]	8.88 ± 0.05[a]	3.67 ± 0.24[a]
Sunchales control	1.03 ± 0.01[a]	0.18 ± 0.02 [a]	3.79 ± 0.03 [a]	8.57 ± 0.19 [a]	3.67 ± 0.34[a]
Sunchales treatment	1.02 ± 0.01 [a]	0.18 ± 0.2 [a]	4.15 ± 0.02[b]	8.78 ± 0.23[a]	3.78 ± 0.034[a]
INTA control	1.03 ± 0.01[a]	0.17 ± 0.01[a]	4.33 ± 0.20[a]	8.99 ± 0.14[a]	3.95 ± 0.53[a]
INTA treatment	1.03 ± 0.01[a]	0.17 ± 0.01[a]	4.96 ± 0.21[b]	8.96 ± 0.18[a]	3.91 ± 0.56[a]

Analysis of quality parameters of goat milk in different establishments. Different letters ([a,b,c]) in the column of the each farm indicate significant differences (n = 10).

et al. (2012). The results indicate that the probiotic mixture added to goat diet did not influence the acidity values.

However, the milk fat concentration was statistically different into each farm. The percent of fat in each one was 12, 9, and 14, for La Perla, Sunchales, and INTA, respectively. Other authors studied the mixed breed Saanen goats and they have reported milk fat content of 3.4% ± 0.27 (Vega y León et al., 2004) and 4.21% ± 0.52 (Frau et al., 2007). The increase of fat, observed in our results, is correlated to previous researches carried out by our group, working with goats from INTA Catamarca (Apás et al., 2015). The MGP administration in lactating goats allowed obtaining milk, characterized by the improved concentration of beneficial compounds, mainly CLA.

Nadathur et al. (1998) determined the effect of fatty acids liberated of milk due to the action of a bacterial lipase. The antimutagenicity against N-methyl, N'-nitro, N-nitrosoguanidine of the milk increased proportionally with the enzymatic activity, suggesting that liberated fatty acids contributed to the increased antimutagenicity. In the same way, our results (Table 2) indicated that probiotic treatment had increased the fat concentration of goat milk. In previous work carried out in our laboratory, MGP was able to increase, in goat milk several non-saturated fatty acids (Apás et al., 2015).

The values of nonfat milk solids (Table 2) obtained from groups considered in this study, showed no significant differences ($p \geq 0.05$) between them. They are in accord with the results published by other authors: 8.27 ± 0.75 (Frau et al., 2012), but they are lower ($p \leq 0.05$) than those informed by Oliszewski et al. (2002) with 11.02 ± 0.12.

With respect to proteins, the probiotic consumption did not increase the total proteins of the milk (Table 2). These values were similar to those reported by Chacón (2005) (3.56 ± 0.24) and by Frau et al. (2012) (3.37 ± 0.31), but lower than that reported by Oliszewski et al. (2002) (5.13% ± 0.10).

Recently So et al. (2017) have published a review about the probiotics-mediated suppression of cancer. They have concluded that despite the encouraging laboratory studies, the benefits related to probiotic uses, should not be exaggerated before we get more results from human clinical trials.

4. Conclusion

These results reinforce the hypothesis that the addition of probiotics to goat diets could achieve food safety for consumers without compromising the organoleptic characteristics of goat milk product, as well as, provide multiple benefits to animal health. The probiotic mixture used in this work diminished the mutagens present in goat milk and this premise defines the first scientific results on this topic. The increased of antimutagenic ability could respond to observed modification of fat content. The present study supports the use of the mix probiotic to enhance the quality of goat's products.

Conflict of interest

None.

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