Intestinal anti-inflammatory activity of goat milk and goat yoghurt in the acetic acid model of rat colitis

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Abstract

The intestinal anti-inflammatory effect of goat milk and goat yoghurt with addition of Lactobacillus acidophilus, with or without native bee honey, was evaluated in rats with 10% acetic acid-induced colitis. The pre-treatment with goat milk, goat yoghurt or sulfasalazine significantly improved the myeloperoxidase activity, levels of leukotriene B4 (LTB4), interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α). It also promoted a significant reduction in oxidative stress that could be seen by the reduction in malondialdehyde and the increase in glutathione. The benefit of the pre-treatments was also demonstrated in the preservation of colonic cytoarchitecture and the decreased expression of cyclooxygenase-2 and inducible nitric oxide synthase. These results suggest that goat milk and goat yoghurt exert protective effects similar to those of sulfasalazine on intestinal damage induced by acetic acid and that goat milk and goat yoghurt may act as functional foods in inflammatory bowel disease.

1. Introduction

Inflammatory bowel disease (IBD), which comprises Crohn's disease (CD) and ulcerative colitis (UC), is a debilitating and immunologically-mediated disease characterised by excessive inflammatory and effector mucosal responses; these responses lead to tissue destruction in the gastrointestinal tract (Viladomiu, Hontecillas, Yuan, Lu, & Bassaganya-Riera, 2013). The aetiology of IBD is unknown, but alterations in the intestinal immune system contribute to inflammation (Gálvez et al., 2001). This results in an increased synthesis and release of various pro-inflammatory mediators, including reactive oxygen species (ROS), nitrogen metabolites, eicosanoids, cytokines and chemokines, all of which contribute to the perpetuation of the inflammatory response in the intestine (Strober & Fuss, 2011). In addition, expression of inflammatory proteins such as cyclooxygenase (COX-2) and inducible nitric oxide synthase (iNOS), which are capable of producing pro-inflammatory mediators such as prostaglandin E2 (PGE2) and nitric oxide (NO), respectively, play an important role in IBD (Dong et al., 2003).

Milk and dairy products can provide nutritional support for patients with intestinal inflammation because their components confer benefits to gastrointestinal health and may be useful as part of the diet (Russ et al., 2010). Goat milk is a nutritional and therapeutic food with unique beneficial features that are superior to those of bovine milk (Slanacac et al., 2010). It can be consumed as an alternative to cows' milk because it is less allergenic and has better digestibility (García, Rovina, Boutoial, & López, 2014). Goat milk is an excellent matrix for development of functional foods (Silanikove, Leitner, Merin, & Prosser, 2010), and the nutritional benefits of goat dairy products can be improved by enriching them...
with strains of probiotics (Mukdhi, Haro, González, & Medina, 2013).

*Lactobacillus acidophilus*, *Bifidobacterium* spp., and *Lactobacillus casei* are lactic acid bacteria commonly used in fermented dairy products (Asharaf, Vasiljevic, Day, Smith, & Donkor, 2014). The desirable number of viable probiotic bacteria has not been firmly established; levels between 10⁶ to 10⁹ colony forming units (cfu) g⁻¹ have been suggested (Abadía-García et al., 2013). Probiotic foods provide health benefits because they help maintain a good balance and composition of intestinal flora and increase resistance against the invasion of pathogens (Tripathi & Giri, 2014). Yoghurt is a fermented dairy product that contains viable bacteria with health-promoting effects (Morelli, 2014). Studies in experimental models of IBD have demonstrated that yoghurt is effective at reducing inflammation (Gobbato, Rachid, & Perdigão, 2008; Leblanc, Chaves, & Perdigão, 2009). Although yoghurt from cows’ milk is largely consumed (Ranadheera, Evans, Adams, & Baines, 2012), there is a high demand for alternatives to cows’ milk due to problems associated with food allergy and gastrointestinal disorder and a desire for new dairy products (Haenlein, 2004; Ranadheera et al., 2012). Goat yoghurt constitutes an appropriate matrix for the inclusion of ingredients such as candied fruit, jam, honey, and nuts that are well liked by consumers (Garcia et al., 2014).

Research on the nutritional and therapeutic importance of goat milk, as well as the functional properties of probiotics, has led to the development of goat yoghurt with honey. Therefore, this study aimed to evaluate the intestinal anti-inflammatory effect of goat milk and goat yoghurt with addition of *L. acidophilus*, with or without native bee (*Melipona scutellaris*) honey, in rats with induced colitis.

### 2. Materials and methods

#### 2.1. Goat milk and goat yoghurt

Goat milk “in natura” was obtained from the Cooperativa dos Produtores Rurais de Monteiro Ltda – CAPRIBOM® – Brazil. Alpina Francesa goats were selected with 38.1 ± 2.6 kg of live weight, milk yield of 1.8 L day⁻¹ and maintained at an intensive system. The diets were prepared according to NRC (2007) guidelines to meet the milk production requirements of 2.0 kg day⁻¹, with 4% of fat.

For yoghurt preparation, goat milk “in natura” was pasteurised (65 °C, 30 min) and cooled, sugar was then added and a heat treatment (90 °C, 15 min) applied. After cooling to 45 °C the starter culture (YF-L903, Christian Hansen®, Valinhos, Minas Gerais, Brazil) comprising Streptococcus salivarius subsp. thermophilus and *Lactobacillus delbrueckii* subsp. bulgaricus (0.4 g L⁻¹) and probiotic culture (La-05, Christian Hansen®, Valinhos, Minas Gerais, Brazil) comprising *L. acidophilus* (0.1 g L⁻¹) were added. The yoghurt was fermented (4 h), cooled, and honey (10%) was added. The yoghurt was packed and stored under refrigeration. The final product had count of 107 cfu mL⁻¹.

#### 2.2. Reagents

All the reagents were obtained from Sigma Chemicals (São Paulo, SP, Brazil). Acetic acid and ethanol were obtained from Nova Chemicals. Nitric oxide synthase and cyclooxygenase antibodies were obtained from Santa Cruz Biotechnology, Inc. (Dallas, Texas, USA), and the kits for leukotriene B₄ (LTB₄) and cytokines tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) were obtained from R&D Systems (Minneapolis, MN, USA).

#### 2.3. Animals and experimental design

This study was approved by the Ethics Committee on Animal Use (CEUA/UFPB, protocol n° 0109/13). Sixty female Wistar rats ages 30 to 32d were obtained from the Biotechnology Center–Chiotec/UFPB. The animals remained in cages for growth with free access to water and food (Purina®) and maintained at a temperature of 22 °C ± 1 °C under a 12 h/12 h light/dark cycle. With. After growth, animals (190–240 g) were randomised into six groups (n = 10): non-colitic, colitic, goat milk, goat yoghurt (without honey), goat yoghurt with honey (10%), and sulfasalazine (250 mg kg⁻¹). All the groups were orally administered (by gavage) 1 mL of their respective product daily for fourteen days before the colitis induction and 24 h after the induction.

#### 2.4. Colitis induction

Colitis was induced by the method originally described by MacPherson and Pfeiffer (1978) and subsequently modified by Millar et al. (1996) with minor adjustments. The animals were fasted 24 h and then anaesthetised with ketamine/xylazine. They were rectally administered acetic acid (0.5 mL 10%, v/v, in 0.9% saline) by 2 mm diameter cannula 8 cm deep. After the administration, the animals were kept in a head-down position for 30 s and then returned to their cages to recover from anaesthesia. The rats in the non-colitic group received 0.5 mL saline intracolonically. Two days later, the animals were sacrificed, under ketamine (Venil, São Paulo, Brazil)/xylazine (Calmium, São Paulo, Brazil) anaesthesia, and their colons were removed to assess the macroscopic damage, histological and biochemical parameters.

#### 2.5. Macroscopic assessment of colonic damage

The colons of the animals were removed, placed on an ice-cold plate, cleaned of fat and mesentery, weighed, and the lengths were measured. The colon was opened longitudinally and evaluated for the extent and severity of macroscopic damage on a scale of 0–10 according to the model described by Bell, Gall, and Wallace (1995). Then, the colon was divided longitudinally into four sections and frozen at −80 °C until biochemical analysis. The fragment for the determination of glutathione was weighed and frozen at −80 °C with 1 mL of 5% (w/v) trichloroacetic acid.

#### 2.6. Biochemical evaluation of colonic damage

The determination of myeloperoxidase (MPO) in the colonic mucosa was performed by the method described by Krawisz, Sharon, and Stenson (1984). The results were expressed as U g⁻¹ of wet tissue, and one unit of myeloperoxidase activity was defined as that degrading 1 nmol min⁻¹ hydrogen peroxide at 25 °C. Colonic malondialdehyde (MDA) content was evaluated according to the method proposed by Esterbauer and Cheeseman (1990) and expressed as nmol g⁻¹ wet tissue. The total glutathione content determination was performed by the method described by Anderson (1985), and the results were expressed as nmol g⁻¹ tissue. Levels of LTB₄ and pro-inflammatory cytokines, TNF-α and IL-1β, were quantified by enzyme-linked immunosorbent assay (ELISA) using an ELISA kit (R&D Systems) according to the manufacturer’s protocol. The results were expressed as ng g⁻¹ wet tissue. Each colonic tissue was processed using tissue homogeniser (Lab 1000, LM-D160/1) for analysis of MPO, MDA, glutathione content, LTB₄ and cytokines.
2.7. Histological analysis

The material collected for the histopathologic evaluation was obtained from the most representative damage zone and was immediately fixed in 10% buffered formalin. Colon fragments were selected, embedded in paraffin, and 5 μm thick slices were obtained. Shortly after, the slices were stained with haematoxylin and eosin. Equivalent colonic segments were also obtained from the non-colitic group. The histological sections were evaluated by a pathologist for the degree of leukocyte infiltration, leukocyte distribution in the colonic tissue using the parameters (normal tissue, light infiltration, moderate infiltration and intense infiltration), and the presence/absence of the indicators of an inflammatory process such as oedema, loss of normal tissue and cytoarchitecture points of necrosis and destruction.

2.8. Immunohistochemical analysis of cyclooxygenase and inducible nitric oxide synthase

Three thin sections of colon (4 μm; n = 5) were obtained with a microtome and transferred to gelatine-coated slides. Each tissue section was then deparaffinized and rehydrated. The colon tissue slices were washed with 0.3% Triton X-100 in phosphate buffer, quenched with endogenous peroxidase (3% hydrogen peroxide), and incubated overnight at 4 °C with the following primary antibodies: COX-2, 1:600 and iNOS, 1:700 (Santa Cruz Biotechnology). After the slices were washed with phosphate buffer, they were incubated with a streptavidin-HRP-conjugated secondary antibody (Biocare Medical, Concord, CA, USA) for 30 min. Immunoreactivity to COX-2 and iNOS was visualised using a colorimetric-based detection kit following the protocol provided by the manufacturer (TreKAvdin-HRP Label + Kit from Biocare Medical). Known positive and negative controls were included in each batch. Using planimetry microscopy (Olympus BX50, Morphology Department/ UFRN) with a high-power objective (40×). The intensity of immunostaining was determined, and scores from 1 to 4 were given: 1, absence of positive cells; 2, small number of positive cells or isolated cells; 3, moderate number of positive cells; 4, large number of positive cells. The labelling intensity was evaluated by two previously trained examiners in a double-blind fashion. Three sections per animal were evaluated.

2.9. Analysis for immunofluorescence

Three tissue sections (n = 5) were deparaffinised with xylene and washed with various concentrations of ethanol and PBS. Antigen retrieval was performed with 10 mM sodium citrate, and 0.05% Tween 20 for 40 min at 95 °C. After the slices were washed with phosphate buffer and haematoxylin and eosin. Equivalent colonic segments were also obtained from the non-colitic group. The histological sections were evaluated by a pathologist for the degree of leukocyte infiltration, leukocyte distribution in the colonic tissue using the parameters (normal tissue, light infiltration, moderate infiltration and intense infiltration), and the presence/absence of the indicators of an inflammatory process such as oedema, loss of normal tissue and cytoarchitecture points of necrosis and destruction.

2.10. Statistical analysis

All results are expressed as the mean ± SEM. The differences between means were tested for statistical significance using a one-way analysis of variance (ANOVA) followed by the Tukey test. Nonparametric data (score) are expressed as the median (range) and were analysed using the Mann–Whitney test or Kruskal–Wallis test followed by Dunn’s test. Statistical significance was set at p < 0.05 using the statistical program GraphPad Prism 4.

3. Results

3.1. Effect of goat milk and goat yoghurt on macroscopic assessment of colonic damage

The damage induced by intrarectal instillation of 0.5 ml 10% (v/v) acetic acid in rats was characterised by severe inflammation and ulceration along the colonic tissue. The control rats given saline did not exhibit inflammation. The administration of goat milk, goat yoghurt or sulfasalazine (250 mg kg⁻¹) for fourteen days prior to the induction of colitis showed significant protection against damage (p < 0.05) with a consequent reduction in the extent and severity of colonic inflammation compared with the colitic group and no differences between the treatment groups (Table 1). In addition, the colonic segments showed bowel wall thickening and a significant increase in the colonic weight/length ratio in comparison with the non-colitic animals (p < 0.05) (Table 1). The colonic weight/length ratio, which is a reliable index of oedema tissue, was significantly reduced in colitic rats treated with goat milk, goat yoghurt or sulfasalazine (250 mg kg⁻¹; p < 0.05) (Table 1).

3.2. Effect of goat milk and goat yoghurt on myeloperoxidase activity, leukotriene B4 levels, malondialdehyde content and glutathione content

Colonic MPO activity (Fig. 1A) and LTB₄ levels (Fig. 1B) were high in the colitic group compared with the non-colitic group (p < 0.01). The administration of goat milk, goat yoghurt or sulfasalazine (250 mg kg⁻¹) before the colitis induction caused a significant reduction in MPO (p < 0.01) and LTB₄ (p < 0.01) compared with the colitic group. However, there was no change between the treated groups (Fig. 1A,B).

As a consequence of inflammation, a change in oxidative status in the colon was recorded (Fig. 1). The animals from the colitic group showed an increase in the MDA levels in comparison with the non-colitic group (p < 0.01) (Fig. 1C), as well as a significant

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Effects of goat milk, goat yoghurt and sulfasalazine on colonic macroscopic score and weight/length ratio in acetic acid rat colitis.³</th>
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<tr>
<td>Experimental groups</td>
<td>Damage score</td>
</tr>
<tr>
<td>Non-colitic</td>
<td>0 (/)</td>
</tr>
<tr>
<td>Colitic</td>
<td>8 (7–9)</td>
</tr>
<tr>
<td>Goat milk</td>
<td>5 (4–6)*</td>
</tr>
<tr>
<td>Goat yoghurt</td>
<td>4.5 (2–6)*</td>
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<tr>
<td>Goat yoghurt with honey</td>
<td>4 (4–5)*</td>
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<td>Sulfasalazine</td>
<td>4.5 (4–5)*</td>
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³ Damage scores are expressed as the median with the range in parentheses; weight/length ratio data are expressed as mean ± SEM (n = 10). An asterisk indicates p < 0.05 versus colitic group. All colitic groups statistically differ from non-colitic group (p < 0.05).
reduction in the glutathione levels (p < 0.01) (Fig. 1D). In addition, compared with the colitic group, the animals receiving either goat milk, goat yoghurt or sulfasalazine (250 mg kg\(^{-1}\)) was associated with an improvement in oxidative stress (p < 0.01) measured by a reduction in the MDA levels (Fig. 1C) and the prevention of glutathione depletion (Fig. 1D). The goat milk group differed from the sulfasalazine group (p < 0.05) in MDA (not shown), and the sulfasalazine group differed from the goat milk and goat yoghurt groups (p < 0.01) in glutathione (data not shown).

### 3.3. Effect of goat milk and goat yoghurt on pro-inflammatory cytokines levels

An increase in the levels of pro-inflammatory cytokines TNF-\(\alpha\) (Fig. 2A) and IL-1\(\beta\) (Fig. 2B) was observed in the animals in the colitic group compared with the animals in the non-colitic group (p < 0.01). There was a reduction in the cytokine levels in the animals receiving goat milk, goat yoghurt or sulfasalazine (250 mg kg\(^{-1}\)) compared with the colitic group (p < 0.01; Fig. 2) The sulfasalazine group differed from the non-colitic, goat milk and goat yoghurt with honey groups (p < 0.01) in TNF-\(\alpha\) levels (not shown).

### 3.4. Effect of goat milk and goat yoghurt on histopathological findings

The histological analysis of the colons of the animals in the colitic group revealed an intense leukocyte infiltration, loss of tissue architecture with the destruction of the epithelium and the consequent destruction of goblet cells, and the presence of haemorrhage (Fig. 3b). The goat milk group showed a mild infiltration, preservation of the epithelium and all the layers of the organ and absence of haemorrhage with vasodilation (Fig. 3c). A moderate infiltration with the presence of polymorphonuclear and slight haemorrhage was noted in the goat yoghurt group (Fig. 3d). In the goat yoghurt with honey group, a moderate infiltration, the presence of vasodilation and blood congestion in the crypts, followed by blood stasis in the submucosa were observed (Fig. 3e). Finally, the

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**Fig. 1.** Effects of goat milk (GM, \[\text{\textblacksquare}\]), goat yoghurt (GY, \[\text{\textblacksquare}\]), goat yoghurt with honey (GYH, \[\text{\textblacksquare}\]) and sulfasalazine (SA, \[\text{\textblacksquare}\]) on (panel A) myeloperoxidase (MPO) activity, (panel B) leukotriene B4 (LTB4) levels, (panel C) malondialdehyde (MDA) content and (panel D) glutathione content in colonic tissue in acetic acid rat colitis compared with control non-colitic (NC, \[\text{\textblacksquare}\]) and untreated colitic (C, \[\text{\textblacksquare}\]) rats. Data are expressed as means ± SEM (n = 10); **p < 0.01 versus colitic group.

**Fig. 2.** Effects of goat milk (GM, \[\text{\textblacksquare}\]), goat yoghurt (GY, \[\text{\textblacksquare}\]), goat yoghurt with honey (GYH, \[\text{\textblacksquare}\]) and sulfasalazine (SA, \[\text{\textblacksquare}\]) on (panel A) tumour necrosis factor-\(\alpha\) (TNF-\(\alpha\)) or (panel B) interleukin-1\(\beta\) (IL-1\(\beta\)) levels in colonic tissue in acetic acid rat colitis compared with control non colitic (NC, \[\text{\textblacksquare}\]) and untreated colitic (C, \[\text{\textblacksquare}\]) rats. Data are expressed as means ± SEM (n = 10); **p < 0.01 versus colitic group.
sulfasalazine group presented with preserved morphological structures and discrete inflammatory infiltrates of polymorphonuclear in the lamina propria and submucosa (Fig. 3f). The colons of the animals in the non-colitic group had normal tissue with full organ preservation and an absence of inflammatory processes (Fig. 3a).

3.5. Effect of goat milk and goat yoghurt on immunohistochemical analysis

The effects of goat milk and goat yoghurt on COX-2 and iNOS immunohistochemical analysis in acetic acid rat colitis are shown in Figs. 4 and 5, respectively. The non-colitic group exhibited a low expression of the mediators COX-2 and iNOS. The immunohistochemical analysis revealed that all treatments significantly reduced the expression of COX-2 and iNOS in the tissue ($p < 0.05$). The colitic group was strongly positive for the COX-2 and iNOS mediators evaluated ($p < 0.05$) compared with the non-colitic group.

3.6. Immunofluorescence analysis

Differences between the tissue location of the COX-2 and iNOS antibodies in the non-colitic, colitic, goat yoghurt, goat yoghurt with honey and sulfasalazine groups were observed. The COX-2 and iNOS signal was strongly diffuse (green) in the cells of the colitic group (Figs. 6A and 7A), moderately diffuse (green) in all mucosal layers in the goat milk, goat yoghurt, goat yoghurt with honey and sulfasalazine groups (Figs. 6A and 7A), and weak to absent (green) in the non-colitic (Figs. 6A and 7A). In the treated and non-colitic groups, an increased labelling of DAPI showing a decreased labelling of COX-2 and iNOS was observed. Densitometric analysis confirmed a significant reduction in COX-2 and iNOS immunoreactivity in goat milk, goat yoghurt, goat yoghurt with honey and sulfasalazine groups ($p < 0.001$; Figs. 6B and 7B).

4. Discussion

Inflammatory bowel disease (IBD) is a chronic condition characterised by an upregulation of pro-inflammatory mediators (mainly TNF-α and IL-1β, in the acute colitis) and uncontrolled immune responses. In this disease, the intestinal mucosa has permanent inflammation resulting from immune cell activation and infiltration from the circulation (Abraham & Cho, 2009; Podolsky, 2002). The inflammatory process of IBD is associated with the development of severe oxidative stress and production of ROS (Buffinton & Doe, 1995; Ioannidis, Varnalidis, Paraskevas, & Botsios, 2011; Opara, 2006) that can be demonstrated in experimental models of colitis (Guerra et al., 2015; Zorrilla et al., 2014).
The results of this study demonstrate the prophylactic effect of goat milk and goat yoghurt in an acute acetic acid colitis model. The administration of goat milk, goat yoghurt or sulfasalazine caused a marked reduction in the macroscopic colonic damage, decreased the inflammation along the colonic tissue of the animals, which was demonstrated in the histological study, reduced the leukocyte infiltration and preserved the tissue architecture of the organ, with consequent preservation of the epithelium. Cellular infiltration into the colonic mucosa was inhibited by the goat milk, goat yoghurt and sulfasalazine, which was confirmed by a reduction in MPO activity and LTB4 levels. LTB4 acts as an inflammation chemotactic mediator, and has an effect on the immigration and activation of leukocytes, suggesting its involvement in diseases such as inflammatory bowel disease (Wang et al., 2000).

Oxidative stress in the intestinal tissue was evaluated using the MDA levels from the tissue. Goat milk, goat yoghurt and sulfasalazine caused improvements in this marker. Additionally, glutathione, which acts as the first line of defence against oxidative damage, was used to evaluate the integrity of the antioxidant defence system and was significantly reduced in the rats with colitis induced by acetic acid. However, the administration of goat milk, goat yoghurt or sulfasalazine increased the glutathione levels to normal. These data are in agreement with other authors who have shown that the supplementation of glutathione in experimental models of colitis induced by TNBS is capable of contributing to the recovery from mucosal injury (Loguercio et al., 2003). Goat milk is reported to be a good source of antioxidants and is particularly rich in cysteine (Russ et al., 2010). Glutathione peroxidase, an important ingredient in goat milk, is part of a defence system against undesirable microorganisms (Slacanac et al. 2010). Moreover, goat milk proteins may be more easily digested, and for this reason, goat milk can be used as an alternative food in diets of...
patients with ulcerative colitis and ulcers (Park, 1994; Slaćanac et al., 2010).

The intestinal anti-inflammatory activity of goat milk and yoghurt could also be demonstrated by the decreased levels of pro-inflammatory cytokines. The groups that received goat milk, goat yoghurt or sulfasalazine showed a significant reduction in colonic TNF-α and IL-1β. The involvement of TNF-α in IBD is a very important factor that is shown by studies of treatments that decrease TNF-α in the intestinal mucosa, such as the study by Blandino et al. (2001), that proved the effectiveness of a synthetic organic compound that inhibited TNF-α and IL-1β by demonstrating the attenuation of colonic mucosa damage in an acetic acid colitis model.

The colonic inflammatory status was also characterised by increased colonic iNOS and COX-2 expression in comparison with non colitic animals. The expression of COX-2, enzyme present in the inductive sites of inflammation, stimulated by TNF-α, IL-1β and other mediators (Stenson, 2008), has been shown to play an important role in the intestinal inflammatory response, and the down regulation characterises improvement of colonic inflammation (Dong et al., 2003). As COX-2, pro-inflammatory cytokines such as TNF-α can induce iNOS activity and cause an increase in nitric oxide production in colonic epithelial cells, which is associated with the initiation and maintenance of inflammation in IBD (Kolios, Valatas, & Ward, 2004). Thus, the pre-treatment of colitic rats with goat milk, goat yoghurt or sulfasalazine downregulated iNOS and COX-2 expression in colonic tissue.

Goat milk is considered a natural source of oligosaccharides and can be applied in human nutrition because of its composition and concentration of oligosaccharides (Martinez-Ferez et al., 2006). Goat milk oligosaccharides have been shown to be effective in rats with experimental model of colitis induced by TNBS (Daddaoua et al., 2006) and dextran sulphate sodium (DSS) (Lara-Villoslada et al., 2006) by having anti-inflammatory effects, reducing the

Fig. 5. Panel A, effect of goat milk and goat yoghurt on iNOS immunohistochemical analysis in acetic acid rat colitis. For each antigen, three immunolabelled sections were analysed per animal (n = 5, 3 sections per animal): (a) non-colitic; (b) colitic; (c) goat milk; (d) goat yoghurt; (e) goat yoghurt with honey; (f) sulfasalazine. Magnification 40×, scale bar = 100 μm. Panel B, representative samples from treatment groups are shown with graphs summarising each group’s mean score and showing immunoreactivity to iNOS: non-colitic (NC, ■); untreated colitic (C, □); goat milk (GM, △); goat yoghurt (GY, □); goat yoghurt with honey (GYH, ▽); sulfasalazine (SA, ♦). Data are expressed as means ± SEM; *p < 0.05 versus colitic group.
intestinal damage and improving the expression of inflammatory markers and some genes, may be useful in IBD. Yoghurt is known for its nutritional, sensory and therapeutic properties (Gonzalez, Adhikari, & Sancho-Madriz, 2011) and is widely accepted by consumers. LeBlanc and Perdigón (2010) reported that yoghurt modulates the immune response by stimulating the production of cytokines and regulating this production to avoid exacerbating the inflammatory immune response. A study conducted by LeBlanc et al. (2009) showed that conventional yoghurt administration, before and after TNBS inoculation, exerted anti-inflammatory effects and reduced colonic damage by reducing pro-inflammatory cytokine levels. In the present study, the intestinal anti-inflammatory activity is primarily attributed to the goat milk, and this activity has been preserved with the preparation of goat yoghurt, since the preparation of this product, with the addition of probiotic and honey, did not change the parameters when compared with the goat milk.

Honey is reported to have anti-inflammatory properties (Alvarez-Suarez, Giampieri, & Battino, 2013; Bogdanov, Jurendic, Sieber, & Gallman, 2008). The phenolic compounds, vitamin C and enzymes present in honey have antioxidant activity (Khalil, Sulaiman, & Boukraa, 2010). In the present study, no differences

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Fig. 6. Panel A: representative confocal photomicrographs of COX-2 immunoreactivity (green) in colons of the animals from each group; the sections are nuclear counterstained with DAPI (blue); (a) non-colitic; (b) colitic; (c) goat milk; (d) goat yoghurt; (e) goat yoghurt with honey; (f) sulfasalazine. Negative control rat colon (a) had absent or weak COX-2 labelling in all mucosa layers; COX-2 labelling was strong in the colitic group (b); weak to moderate COX-2 labelling (red arrows) was seen in the group treated with goat milk (c), goat yoghurt (d), goat yoghurt with honey (e) and sulfasalazine (f). Scale bar 20 mm. Panel B, densitometric analysis confirmed a significant reduction in COX-2 immunoreactivity in goat milk (GM), goat yoghurt (GY), goat yoghurt with honey (GYH) and sulfasalazine groups (SA); *p < 0.05 versus colitic group (C); Kruskal–Wallis test followed by Dunn’s test. Non-colitic group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
in the inflammatory or oxidative stress parameters were observed between the goat yoghurt groups with or without honey. This discrepancy was perhaps due to the concentration used, because honey was added to improve the palatability of the product, or the view that goat milk has a peculiar flavour.

5. Conclusion

Goat milk and goat yoghurt have intestinal anti-inflammatory activity when given as a pre-treatment in the acetic acid-induced colitis model in rats. This result was shown in the reduction in colonic tissue damage, preservation of the cytoarchitecture of the tissue, and decrease in pro-inflammatory mediators accompanied by the improvement of oxidative stress. Therefore, these goat dairy products may be a valuable alternative to traditional medications and a potential functional food for the prevention of IBD.

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