J. Dairy Sci. 99:1–12
http://dx.doi.org/10.3168/jds.2016-10930

Goat whey ameliorates intestinal inflammation on acetic acid-induced colitis in rats

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ABSTRACT

Complementary or alternative medicine is of great interest for the treatment of inflammatory bowel disease, with the aim of ameliorating the side effects of the drugs commonly used or improving their efficacy. In this study, we evaluated the ability of goat whey to prevent intestinal inflammation in the experimental model of acetic acid-induced rats and compared it to sulfasalazine. Pretreatment with goat whey (1, 2, and 4 g/kg) and sulfasalazine (250 mg/kg) on colitic rats improved colonic inflammatory markers, including myeloperoxidase activity, leukotriene B_4 levels, as well as the production of proinflammatory cytokines IL-1β and tumor necrosis factor-α. Furthermore, the administration of goat whey significantly reduced the colonic oxidative stress by reducing malondialdehyde levels and increased total glutathione content, a potent antioxidant peptide. The histological evaluation of the colonic specimens from colitic rats confirmed these beneficial effects, as goat whey preserved the colonic tissue, especially in those rats treated with the highest dose of goat whey or with sulfasalazine. The immunohistochemistry analysis of the colonic tissue evaluation also revealed a reduction in the expression of cyclooxygenase-2, inducible nitric oxide synthase, and matrix metalloproteinase-9, together with an increased expression of cytokine signaling-1 suppressor. These results suggest that goat whey exerted a preventive effect against the intestinal damage induced by acetic acid, showing a similar efficacy to that shown by sulfasalazine, therefore making it a potential treatment for human inflammatory bowel disease.

Key words: goat whey, intestinal inflammation, oxidative stress, cytokines, immunohistochemical

INTRODUCTION

Inflammatory bowel disease (**IBD**) is a chronic inflammatory condition, encompassing Crohn's disease and ulcerative colitis, with high incidence and prevalence in developed countries. The pathologies are characterized by pain, diarrhea, weight loss, frequent rectal bleeding, and alternating periods of relapse and remission. Both forms of IBD feature exacerbated uncontrolled intestinal inflammation that leads to poor quality of life and requires prolonged medical or surgical interventions (Molodecky et al., 2012; Nørgård et al., 2014). At present, the exact pathogenesis of IBD is not fully understood, as it is a complex and multifactorial chronic disease. Currently, there is general agreement that IBD is the result of the combined effects of 4 basic components: multiple genetic variations of the host, alterations in the composition of the intestinal microbiota, changes in the surrounding environment that are greatly affected by diet, and the abnormal reactivity of the intestinal mucosal immune response to an unknown antigen (Galvez et al., 2010; Sang et al., 2014; Magalhães et al., 2015).

Different studies have proposed that this inflammatory process is initially triggered by the increased permeability of the epithelial barrier to the luminal

Received January 21, 2016.
Accepted June 17, 2016.
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antigens, thus leading to an improper activation of the immune mucosal system. This results in an increased influx and activation of neutrophils and macrophages, with the concomitant production of several proinflammatory mediators, including reactive oxygen species, eicosanoids, cytokines, and chemokines (Dedon and Tannenbaum, 2004; Hyun and Mayer, 2006; Strober and Fuss, 2011). Moreover, colitis has been associated with the increased expression of inducible proteins such as cyclooxygenase (**COX**)-2 and inducible nitric oxide synthase (**iNOS**), which may play a key role in the modulation of inflammation (Ardizzone and Porro, 2005; Sakthivel and Guruvayoorappan, 2013). In fact, the synthesis of large amounts of nitric oxide by iNOS has been demonstrated to be pathogenic in human IBD (Kolios et al., 2004). Of note, it has been reported that the increased expression of iNOS is mediated through the activation of the transcription factor nuclear factor (**NF**)-κB and driven by different proinflammatory cytokines, such as tumor necrosis factor (**TNF**)-α, IL-1β and IFN-γ. Subsequently, NF-κB activation can also induce the expression of these cytokines, thus generating a vicious cycle, as well as that of many other genes involved in antiapoptotic and prometastatic processes such as matrix metalloproteinase (**MMP**) 2 and 9, respectively (Salas et al., 2002; Agarwal et al., 2004; Naugler and Karin, 2008; Rouzer and Marnett, 2009).

Treatment of IBD has 2 main purposes: to promote the remission of the symptoms of the acute period and then to maintain the remission by controlling the chronic inflammation, thus preventing the reactivation of the intestinal inflammatory process. When considering all of the above, the downregulation of the altered immune response is essential for the management of these patients. In fact, this is the main objective of the pharmacological therapy at present, which includes aminosalicylates (sulfasalazine or mesalazine), immunosuppressants (glucocorticoids or azathioprine), and biologicals (infliximab or adalimumab; Bernstein, 2015). However, although all these drugs have shown efficacy, an important proportion of side effects may limit their required long-term use (Siegel, 2011). In this context, new therapies that combine efficacy and safety in human IBD therapy are needed. Among these, dietary interventions with nutraceuticals or so-called functional foods seem to represent a safe alternative way to modulate the altered mucosal immune response that occurs in intestinal inflammation, which can be achieved mainly through their effect on intestinal microbiota, as has been reported for prebiotics (Roberfroid et al., 2010).

Whey is a by-product of cheese and contains nutritionally important and valuable components used in a functional food. In fact, whey is already considered as a functional milk fraction, with positive effects on health outcomes, as it contains proteins with a high nutritional value due to the presence of EAA, as well as considerable lactose content, oligosaccharides, and minerals (Hernández-Ledesma et al., 2011; Thum et al., 2015). The aim of our study was to evaluate the preventive effects of goat whey (**GW**) in an experimental model of intestinal inflammation induced by intracolonic instillation of acid acetic in rats, and compare its efficacy to sulfasalazine (**SAZ**), an aminosalicylate currently used in human IBD.

MATERIALS AND METHODS

Obtaining and Characterizing the GW

Goat cheese whey came from alpine brown goats confined at the experimental station of the Goat Division of the Federal University of Paraiba, in São João do Cariri, Paraiba, Brazil. The selected subjects weighed 40 ± 6 kg of BW and were 50 ± 10 d of lactation and had received full ration consisting of concentrate, Tifton hay, and forage cactus. The diets were formulated according to NRC (2007) to meet the required milk production of 1.5 kg per day with 4% fat.

Next, we proceeded to prepare the cheese curds and obtain whey (Oliveira et al., 2012). Initially, milk pasteurization (65 ± 1°C) was performed for 15 min followed by cooling to 45 ± 1°C. Then GW was produced by coagulation with additives in the following sequence: 0.5 mL/L of calcium chloride and 0.9 mL/L of commercial coagulant chloride (Ha-La, Chr. Hansen A/S, Hørsholm, Denmark). The mass was salted (NaCl 1% relative to the weight of the mass) and distributed in 250-g perforated form.

The whey obtained from the preparation of the cheese curd was immediately stored in polyethylene containers and subjected to spray-drying by a mini Buchi B290 Spray Dryer (Büchi Labortechnik AG, Flawil, Switzerland), using an inlet temperature of 130°C, feed rate of 15%, air flow of 40 L/min, and a compressor pressure of around 700 kPa for an approximate time of 2 h drying for 1 L of whey. Analyses were performed to characterize the GW to understand the moisture (DM basis), fat (Gerber butyrometer, Gerber Instruments, Effretikon, Zürich, Switzerland), protein (by the micro-Kjeldahl method), and lactose (by HPLC, Varian, Palo Alto, CA); all analyses were performed according to the methods described by the AOAC International (2005). The preparation of sialic acid whey solution and quantification were carried out following the methodology used by Sousa et al. (2015).