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Probiotic yogurt offers higher immune-protection than probiotic whey beverage

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ABSTRACT

The probiotics can be useful to improve the immune response in experimental challenges, like the exhausting exercise, that cause immunosuppression. We have evaluated a probiotic yogurt and a probiotic whey beverage using an exhausting physical-exercise protocol with rats. Wistar rats were given a daily 4-mL supplement of each type of conventional or probiotic yogurt and whey beverage, manufactured with lactic culture *Streptococcus thermophilus* TA040 and *Lactobacillus bulgaricus* LB340, and probiotic culture *Lactobacillus acidophilus* LA 14 and *Bifidobacterium longum* BL 05. The effects on the immune system were compared to those of pair-treated cohorts receiving for 14 days. Results demonstrated that the probiotic yogurt outperformed the probiotic whey beverage in blood-cell indicators (neutrophils and lymphocytes), cytokines (TNF-α and IL-1β) and various standard health parameters. In conclusion, in this study, the treadmill exercise assay successfully produced immunosuppression in the rat and the combination of the nutrients and probiotic bacteria of the yogurt reduces more effectively the adverse effects developed over the prolonged strenuous exercise than did a similar probiotic whey beverage.

1. Introduction

Dairy foods are the main types of food matrices supplemented with probiotic bacteria and they have a positive reputation among consumers (Granato, Branco, Cruz, Faria, & Shah, 2010). Among the dairy products, yogurt/fermented milks have been the subject of several studies all over the world and different benefits for human health have been reported after their ingestion (Wang et al., 2012). The regular consumption of probiotic foods can provide health benefits (Cruz, Buriti, Souza, Faria, & Saad, 2009), among which improvements in the immune system increasing resistance to infection in the upper respiratory tract of athletic subjects (de Vrese et al., 2006), common infections and gastrointestinal illnesses (West et al., 2011).

The probiotics are described as useful for athletes in combat against oxidative stress (Martarelli et al., 2011), improvement in mucosal immunity (Cox, Pyne, Saunders, & Fricker, 2010) and general immunity (Gleeson et al., 2012). Stressful situations such as those derived from intense physical exercise can increase the incidence of gastrointestinal disease episodes, particularly of diarrhea during heavy training (Mackinnon, 2000), and are likely to cause increased susceptibility to infections of the upper respiratory tract (Mackinnon, 2000) due in part to suppression of the functions of the immune system succeeding intense exercise sessions prolonged to exhaustion (Pedersen & Hoffman-Goetz, 2000). Probiotics can prevent illnesses during heavy training and competition, which is one of the priorities for athletes, technicians and exercise scientists, who are interested in minimizing gastrointestinal disorders, particularly diarrhea, during travel for international competitions, since these adversely affect adaptation periods and physical performance (Pyne & Gleeson, 1998).

It has been demonstrated that consumption of probiotic can enhance the immune system and health of sedentary and exercised subjects (Corthesy, Gaskins, & Mercenier, 2007; Fang, Elina, Heikki, & Seppo, 2000; Gleeson, Nieman, & Pedersen, 2004). Therefore probiotics could be used to indirectly maximize athletic performance by preventing the immunosuppression caused by prolonged sessions of intense physical exercise, thus reducing the athlete's susceptibility to disease (Nichols, 2007) and the incidence of acute infections, diarrheas and their associated symptoms (Guarino, Lo Vecchio, & Canani, 2009).

Considering the benefits that food supplementation with probiotics could bring to athletes, directly or indirectly, reducing gastrointestinal disorders and improving the immune system, preventing infections of the upper respiratory tract, the objective of this study was to evaluate the efficiency of two different probiotic matrices, yogurt and whey

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; HDL, high-density lipoprotein; IL-1β, interleukin 1 beta; Protal, total protein; TG, triacylglycerols; TNF-α, tumor necrosis factor alpha.

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beverage, in favoring maintenance of the immune system in Wistar rats exercised to the point of exhaustion after receiving the probiotic supplements for 14 days. The probiotic strains used in this study were *Lactobacillus acidophilus* LA 14 and *Bifidobacterium longum* BL 05 (Danisco, São Paulo, Brazil). These probiotic bacteria have been used in several commercial dairy products available on the Brazilian market, as well as in previous studies covering dairy foods from a technological point of view (Cruz, Castro, Faria, Bogusz, et al., 2012; Cruz, Castro, Faria, Lollo, et al., 2012; Cruz et al., 2010). Recently the ingestion of fresh Minas cheese supplemented with these probiotic strains attenuated exercise-induced immune suppression in Wistar rats (Lollo, Cruz, et al., 2012).

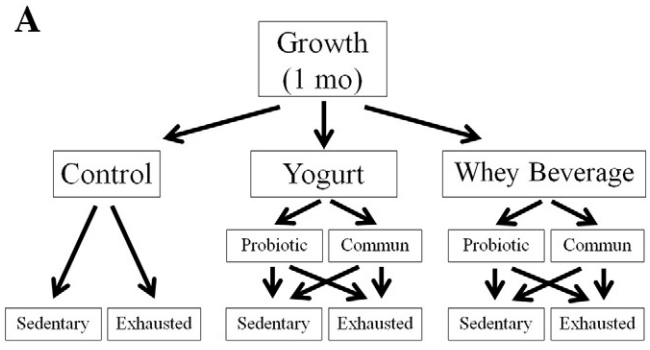
2. Material and methods

2.1. Animals

Male (21-day-old, specific-pathogen-free) Wistar rats, bred at the Multidisciplinary Center for Biological Research (University of Campinas, SP, Brazil), were housed (~22 °C, 55% relative humidity, inverted 12-h light/12-h dark cycles) in individual growth cages, with free access to commercial chow (Labina, Purina, Brazil) and water at all times, until they reached a body weight of 150.3 ± 8.3 g. The research methodology was approved by the Ethics Committee on Animal Experimentation (CEEA-UNICAMP, protocol 2345-1). The animals were randomly assigned to 1 of 10 groups (n = 6 per group), depending on whether the diet was probiotic yogurt, conventional yogurt, whey probiotic beverage, whey beverage or control and whether the rats were exhausted or remained sedentary. Every day the animals were gavaged with 4 mL of the appropriate beverage under test, the control group being fed 4 mL water (Fig. 1). This study adhered to the American College of Sports Medicine animal care standards.

2.2. Yogurt and whey beverage processing

The yogurt and whey beverage were processed according to methods published elsewhere (Castro, Cruz, Bisinotto, et al., 2013; Castro, Cruz, Rodrigues, et al., 2013; Cruz et al., 2010), the only difference being the exclusion of glucose oxidase from the former. The whey probiotic beverage was manufactured considering a formulation with 49% cheese whey and 51% milk (% v/v). No addition of pulp fruit and sweetened agent was performed. The lactic (*Streptococcus thermophilus* TA040 and *Lactobacillus bulgaricus* LB340, Danisco, São Paulo, Brazil) and probiotic cultures were added to obtain concentrations of approximately 6 and 8 log cfu/g in both the yogurt and the whey beverage. The cultures were commercial freeze-dried cultures for direct vat inoculation, and adequate distribution was ensured by manual homogenization for about 2 min.



B

Protocol to exhaustion

Time (min)	Speed
1 to 90	15 meters/minute
91 to 100	20 meters/minute
150 to exhaustion	22 meters/minute

Fig. 1. Whey bev.: whey beverage; Probiotic whey bev.: Probiotic whey beverage.

2.3. Lactic and probiotic bacteria count

The lactic and probiotic count was performed using standardized microbiological methods published elsewhere (Cruz et al., 2013). The count of *S. thermophilus* TA040 was quantified using M17 agar (Oxoid, São Paulo, Brasil) following incubation under aerobic conditions at 37 °C for 48 h while for *L. bulgaricus* it used Man, Rogosa and Sharpe (MRS, Oxoid, São Paulo, Brasil) and was previously acidified to pH 5.4 with acetic acid, and the plates were incubated at 37 °C for 48 h, under aerobic conditions. Towards the probiotic strains, *L. acidophilus* count procedure was performed in Man, Rogosa and Sharpe (MRS) agar supplemented by 0.15% (wt/vol) bile salts (Oxoid, São Paulo, Brasil), incubated at 37 °C for 72 h under aerobic conditions while *B. longum* the count was carried out in duplicate by deep plating in sodium lithium-propionate chloride agar with 0.5 g/L of LiCl and 0.75 g/L of sodium propionate and incubating under anaerobic conditions at 37 °C for 3 days. The viable counts of lactic and probiotic bacteria were determined after 1 and 14 days of refrigerated storage in both products and ranged from 9.1 to 9, 8.7 to 8.6, 8.5 to 7.5 and 7.2 to 6.9 log cfu/g of *S. thermophilus*, *L. bulgaricus*, *L. acidophilus* and *B. longum*, respectively.

2.4. AA composition and proximate composition of the yogurt, whey beverage and diets

The amino acids (AA) were extracted with methanol and derivatized with phenylisothiocyanate (White, Hart, & Fry, 1986), and the phenylthiohydantoin derivatives were submitted to chromatography using a Luna C-18, 100 , 5 μm, 250 × 4.6 mm column (00G-4252-EQ, Phenomenex, Torrance, CA), at 50 °C. Quantification was by comparison with a standard mixture with dl-2-aminobutyric acid as the internal standard (Sigma-Aldrich Corp., St Louis, MO). The free AA were determined by extracting 1.25 g samples in 80% ethanol, and adding 0.1 M HCl plus 500 μL of α-aminobutyric acid as the internal standard, in a 5 mL volumetric flask. The mixture was sonicated for 10 min and further homogenized for 1 h, followed by centrifugation at 8500 ×*g* for 15 min. The supernatant was filtered through a 0.22 μm membrane and a 40 μL aliquot was derivatized as described above for the injection of 20 μL into the liquid chromatograph.

Moisture, total ash, protein and lipids were determined according to AOAC (2002) methods. The total carbohydrate content was inferred by difference. The experimental diets were isonitrogenous (approximately 16% protein, dry basis), isolipidic, and isocaloric (approximately 360 kcal/100 g). Table 1 shows the proximate composition and AA contents of the yogurt, whey beverage and diets. The AA composition and proximate composition were performed with three samples of each product/diet.

2.5. Exhaustion protocol

The rats were introduced to the treadmill by running for 10 min at 10 m/min on the day before being brought to exhaustion. The exhaustion test was applied following the time–speed schedule shown in Fig. 1B (Lollo, Cruz, et al., 2012).

2.6. Biochemical parameters

Two hours after the exhaustion session, blood samples were collected in *BD-Vacutainers* tubes (Becton Dickinson, Franklin Lakes, NJ), kept at 4 °C, and centrifuged at 3000 ×*g* (4 °C, 12 min) to obtain serum for the