Goat Milk Yoghurt by Using Lacto-B Culture Modulates the Production of Tumor Necrosis Factor–Alpha and Interleukin–10 in Malnourished Rats

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Abstract

Total spleen lymphocytes, lymphocyte proliferation, tumor necrosis factor–α (TNF-α), and interleukin–10 (IL-10) in spleen lymphocyte culture were studied in malnourished Wistar rats fed with goat milk yoghurt. Malnourished rats were created by using standard feed restriction as much as 50% of normal rats for 21 d. Goat milk yoghurt containing three types of microorganism e.g., Lactobacillus acidophilus, Sterptococcus thermophilus and Bifidobacterium longum derived from Lacto-B culture in powder form. After 21 d, the rats continued to receive restricted feeding and supplemented with goat milk yoghurt for 7 d. Total splenocytes were counted by hemocytometer. Splenocytes proliferation was expressed as stimulation index, whereas the TNF-α and IL-10 of spleen lymphocyte culture were measured by ELISA technique. The total number of splenocytes and stimulation index of splenocytes in moderate malnourished and normal rats supplemented with goat milk yoghurt was not significantly different. The level of TNF-α in the rat supplemented with goat milk yoghurt was lower (p<0.05) than the control group, whereas the level of IL-10 in the rat supplemented with goat milk yoghurt was higher (p<0.05) than the control group. In conclusion, goat milk yoghurt supplementation in malnourished rats could decrease TNF-α as a representation of the pro-inflammatory cytokine, while it increases IL-10 as a representation of the anti-inflammatory cytokine.

Key words: goat milk yoghurt, IL-10, lymphocyte proliferation, malnutrition, TNF-α

Introduction

Goat milk has many advantages in terms of percentage of an over-sized fat droplets smaller than cow’s milk (Sil’anikove et al., 2010). Because the goat milk fat globule has a greater surface area, and lipases in the gut are supposedly able to attack the lipids more rapidly. Almost 20% of the fatty acids of goat milk fall into the short-chain fatty acids category (C4:O to C12:O) compared with 10-20% for cow milk. Lipases attack the ester linkages of the shorter-chain fatty acids more readily, so these differences may contribute to more rapid digestion and absorption of goat milk fat (Arora et al., 2013). Some physicochemical properties of goat milk such as smaller fat globules, higher percentage of short and medium chain fatty acids, and softer curd formation of its proteins are advantageous for higher digestibility and healthier lipid metabolism relative to cow milk (Park, 1994). Thus goat milk has been recommended for either infants, old, convalescent people (Kompan and Komprej, 2012), and goat milk-based diet (animal fat) has a beneficial effect and thus its consumption may be recommended especially in cases of malabsorption syndromes (Alferez et al., 2001). Goat milk has a nutritional value similar to that of cow milk and could be used as an alternative to cow milk for rehabilitating undernourished children (Razafindrakoto et al., 1993).

The prevalence of malnutrition in Indonesia is still relatively high despite a declining trend from 2007 to 2010. The proportion of malnourished children under five in Indonesia decreased from 18.4% in 2007 to 17.9% in 2010 (Riskesdas, 2010). Protein malnutrition disrupts the normal ecology of the microflora affecting strictly anaerobes, impairs host immune response and antibacterial defenses, enhances the susceptibility to infection, and leads to mucosal atrophy (Dock et al., 2004). The direct relationship between malnutrition and death is mainly due to the immunodeficiency status and consequently, greater
susceptibility to infectious agents (Franca et al., 2009). The severity of the malnutrition depends on the degree of nutrient imbalance and the interaction between nutrients and the age of the host (Nunez et al., 2013). Severe protein malnutrition, mainly in newborns and small children, also provokes thymus atrophy that, in turn, reduces thymus cell number and also severely affects the development of peripheral lymphoid organs. The immediate consequence of this atrophy is leucopenia, decreased CD4/CD8 ratio and increased number of immature T cells in the periphery (Savino, 2002).

The spleen has a very high blood flow, that it filters off cell-free fluid from the blood into the lymphatic system and that the rate of filtration is modulated by neuronal and humoral factors (Semaeva et al., 2010). The spleen was chosen as a representative of systemic lymphoid organ to evaluate the systemic immune system status (Ramiro-Puig et al., 2007).

The induction of proinflammatory and anti-inflammatory cytokines is important in determining whether the immune system is successful in providing protection against specific pathogenic organisms (Al-Bana et al., 2008). Production TNF-α by bone marrow cells is also significantly lower in malnourished animals (França et al., 2009).

Probiotic yoghurt intake was associated with significant anti-inflammatory effects that paralleled the expansion of peripheral pool of putative T regulatory (Treg) cells in inflammatory bowel disease (IBD) patients (Baroja et al., 2007). The concept that probiotics act via the induction of regulatory cells is inherently attractive, because it seems unlikely that they could act by adequately replacing all bacteria in the intestinal microflora that are capable of causing inflammation in experimental mice/individuals susceptible to IBD-like inflammation or IBD (Giacinto et al., 2005). Several different mechanism of probiotics are: 1) involves altering the composition of the intestinal microbiota by producing bacteriocins, which are products that eliminate certain bacteria, or by altering pH, which will alter the growth characteristics of certain bacteria, 2) involves altering the epithelial barrier function of the intestine, and 3) have important immunoregulatory activity, so certain probiotics and their products can activate regulatory T cells and regulatory pathways, leading to downregulation of inflammation (Sartor, 2011). The previous study by Giacinto et al. (2005) showed that probiotic administration was associated with an early increase in the production of IL-10 and an increased number of regulatory CD4+ T cells. The increase in CD4+ CD25high T cells correlated with the decrease in the percentage of TNF-α or IL-12-producing monocytes and dendritic cell (DC) (Baroja et al., 2007).

Recently, yoghurt are often added by probiotics to improve health effects, and originally yoghurt is made using a starter Lactobacillus bulgaricus and Streptococcus thermophilus cultures that can be derived from the powder or liquid culture. Probiotic such as Bifidobacterium longum reported by the previous study (Pavlovic et al., 2006) grew better in goat milk than in cow milk. According to Dock et al. (2004), S. thermophilus and L. helveticus enhance the recovery of gut atrophy induced by malnutrition.

In this study, yoghurt was made directly using Lacto-B powder which inoculated into the milk. Lacto-B is a non dairy powder-containing probiotic consists of B. longum, L. acidophilus, and S. thermophilus known as treatment As Safe (GRAS). Multi-strain and multi-species probiotics have improved functionality as compared to single strain (Dash, 2009). This study aimed to determine the effect of goat milk yoghurt which is made with culture from Lacto-B powder on total of rat splenocytes, splenocyte proliferation, levels of TNF-α and IL-10 in splenocyte culture supernatant of malnourished rats.

Materials and Methods

Goat milk yoghurt preparation
Goat milk yoghurt was prepared from goat milk of Etawah Crossbred from Indonesia and culture of Lacto-B powder. Goat milk was pasteurized at 85°C for 30 min and cooled to 43-45°C. After cooled, pasteurized goat milk was inoculated with 3% Lacto-B powder (Novell Pharmaceutical Laboratories) containing S. thermophilus, L. acidophilus and B. longum and incubated at 45°C for 6 h (Dave, 1998; Sunarlim, 2009 with slight modification).

Experimental animals
Three week old male Wistar rats weighing 25.5-41.0 g, were individually caged and housed. During 7 d the rats fed unrestricted amounts of a standard laboratory diet AIN-93G (Reeves et al., 1993) and then randomly assigned to two groups: one maintained in the same conditions (control) and another group fed 50% of the intake of the control’s (restricted or malnourished) (Rosso et al., 1981) for 21 d. Both groups were allowed to drink water ad libitum. After 21 d, the rat were assigned to four groups: 1) Malnourished rats (MC), 2) Malnourished rats with goat milk
yoghurt (MY), 3) Normal rats (NC), and 4) Normal rats with goat milk yoghurt (NY). MC and NC groups (control without yoghurt) were given aquadest with a volume equal to the volume of treated yoghurt. Aquadest as control or yoghurt were given orally with force feeding. The dose of goat milk yoghurt was 2 mL/100 g body weight/day, whose consumption is equivalent to the children consumption of 100 mL yoghurt/day (WHO, 2006). Each group of rat using 7 replications (n=7 rats). Rat body weight was measured every 3 d. All of groups were treated for 7 d, and then were killed. Lymphocytes are isolated from rat spleen for cultured. All procedures related to animal experiment were conducted following the recommendation of Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine Universitas Gadjah Mada, Indonesia (KE/FK/753/EC).

Lymphocyte proliferation assay

Lymphocyte proliferation was assayed according to Jain et al. (2008) with slight modifications. Spleen was removed and placed in 10 mL of RPMI 1640 (Sigma) media containing 10% fetal bovine serum (FBS) (Gibco) and 2% penicillin-streptomycin (Gibco). The spleen was washed with RPMI media. Aseptically collected spleen tissues were gently teased with sterile needles and forceps to release splenocytes into the RPMI 1640 (Sigma) media containing 10% FBS and 2% penicillin-streptomycin. Tissue suspensions were allowed to stand for 2 min to sediment large tissue clumps. The upper portion containing splenocytes was collected and centrifuged at 1000×g for 5 min at 40°C. Suspension was incubated with erythrocyte lysis buffer (0.17 M Tris HCl and 0.16 M NH₄Cl, pH 7.2) for 1 min and washed twice by centrifuging as above with RPMI 1640 medium. Cell viability was checked by trypan blue (0.4% solution) and counted by haemacytometer. Viable splenocytes (1.5×10⁶ cells/mL) were finally cultured in heat inactivated 10% FBS enriched RPMI 1640 medium supplemented with or without mitogen 5 µg/mL phytohaemagglutinine (PHA) (Murex) and incubated at 37°C in a humidified atmosphere of 5% CO₂ incubator for 72 h. Then, 10 µL of methyithiazol tetrazolium (MTT) (5 mg/mL) was added in each well and incubated for 4 h at 37°C. Acidified isopropanol (100 µL of 0.1 N HCl in anhydrous isopropanol) was added and mixed thoroughly to dissolve the dark blue crystals of formazan. Formazan quantification was performed using an Enzyme-linked immunosorbent assay (ELISA) plate reader with 550 nm. The lymphocyte proliferation was expressed as stimulation index (SI) which was calculated as the corrected absorbance of mitogen-stimulated cells divided by the corrected absorbance of unstimulated cells (Keller et al., 2005).

Measurement of cytokine levels (TNF-α and IL-10)

Splenocytes were cultured as described above, and supernatant was collected after 72 h to analyze cytokine levels. Rat specific ELISA kits for measurement of TNF-α and IL-10 (eBioscience, Bender MedSystem, Vienna, Austria) were used. Assays were performed according to instruction provided by the manufacturers. Briefly, microwell plate was washed twice with Wash Buffer. The Standard was reconstituted with 250 µL aquabidest for Standard of TNF-α, and 380 µL of aquabidest for IL-10, and then prepared standard with 1:2 dilution in small tube. For TNF-α: 225 µL of reconstituted standard TNF-α (concentration = 5 ng/mL) was pipetted into S1 tube (standard 1) containing 225 µL Sample Diluent (concentration = 2500 pg/mL). This procedure was done for the next tubes until the concentration of the final tube (S7) was 39.1 pg/mL. The procedure for analysis of IL-10 was the same as TNF-α analysis. For IL-10: 225 µL of reconstituted standard (concentration = 2000 pg/mL) was pipetted into S1 tube (standard 1) containing 225 µL Sample Diluent (concentration = 1000 pg/mL). The S1 standard (225 µL) was transferred into S2 standard tube (concentration = 500 pg/mL), and continue this procedure was done for the next tubes until the concentration of the final standard (S7) was 15.6 pg/mL. Each standard (100 µL) was pipetted into well for standard, and 100 µL of Sample Diluent was pipetted into well for blank. Each well for sample was filled with 50 µL of Sample Diluent and added 50 µL sample. Biotin conjugate solution was added into microwell plate, and covered with adheive film. The plate was incubated at room temperature (18-25°C) for 2 h. Adhesive film was removed, and washed microwell plate 4 times with 400 µL Wash Buffer for each well. Streptavidin-HRP solution (100 µL) was added into all well, and covered with adhesive film. The plate was incubated at room temperature for 1 h. Micro plate was washed 4 times, whereas washing for plate in IL-10 assay as much as 3 times, and added 100 µL tetramethyl-benzidine (TMB) substrate into wells. The plate was incubated at room temperature for 10 min, and avoided from direct exposure to light. The enzyme reaction was stopped by pipetting 100 µL of Stop Solution into each well. The absorbance of each microwell was read on ELISA plate reader 405 nm. Washing for plate in IL-10 assay as much as 3 times.
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Statistical analysis
The data of total splenocytes, lymphocyte proliferation, TNF-α and IL-10 levels of supernatant lymphocyte culture in malnourished and normal rats supplemented with goat milk yoghurt, were analyzed by ANOVA using SPSS 12.0 (2003).

Results
To study the effects of goat milk yoghurt containing *S. thermophilus*, *L. acidophilus* and *B. longum* on improvement the immunity of malnourished rats, the goat milk yoghurt has been supplemented for 7 d after 21 d received restricted feeding and continued restricted feeding together with goat milk yoghurt supplemented.

Effects on body weight
The rat body weight that received 50% restricted feeding and normal rats for 21 d and after supplemented with goat milk yoghurt is showed on Fig. 1.

A 50% restricted feeding resulted in moderate malnutrition, because the weight gain of malnourished rats in this study after malnutrition period for 21 d only 30.56% of normal rats. After restriction feeding period, the malnourished rats have lower (*p*<0.05) body weight than the normal rats (Table 1).

Body weight of rats after 7 d received goat milk yoghurt and continue restricted feeding given in Table 2.

Based on Table 1 and 2, increasing of body weight of malnourished rat supplemented with goat milk yoghurt was higher (near twice: 89.40–73.93=15.47 g) than the control (88.16–78.77=9.39 g) rats. However, the body weight of malnourished rats significantly lower (*p*<0.05) than the normal rats (Table 2).

Effect on total splenocytes
To study the immunity of malnourished rats, splenocytes counts were quantified. The results showed that the average of splenocytes counts of malnourished and normal rats treated with goat milk yoghurt was not significantly different with the control rats (Table 3).

Effects on lymphocyte proliferation
Measurement of the proliferative response of lympho-

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![Graph](https://via.placeholder.com/150)

**Fig. 1. Body weight (g) of malnourished and normal rats.** (day 0-6: adaptation; day 6-27: restriction feeding; day 27-33: yoghurt supplementation and continued restricted feeding. MC: Malnourished-control; MY: Malnourished-yoghurt; NC: Normal-control; NY: Normal-yoghurt)

**Table 1. Body weight (g) of rats after 21 d of restriction feeding and normal rats before supplemented with goat milk yoghurt**

<table>
<thead>
<tr>
<th>Rats</th>
<th>Before supplemented goat milk yoghurt</th>
<th>Control</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malnourished (Restricted feeding)</td>
<td>73.93±3.48a</td>
<td>78.77±5.03a</td>
<td>76.35±4.86a</td>
</tr>
<tr>
<td>Normal</td>
<td>119.79±9.28b</td>
<td>117.61±10.15b</td>
<td>118.70±9.41b</td>
</tr>
</tbody>
</table>

Superscript with the different letters indicates significantly different (*p*<0.05).

**Table 2. Body weight (g) of malnourished and normal rats treated with goat milk yoghurt**

<table>
<thead>
<tr>
<th>Rats</th>
<th>After supplemented Goat milk yoghurt</th>
<th>Control</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malnourished</td>
<td>89.40±4.56a</td>
<td>88.16±5.97a</td>
<td>88.78±5.14a</td>
</tr>
<tr>
<td>Normal</td>
<td>148.92±9.27b</td>
<td>144.67±11.86b</td>
<td>146.80±10.46b</td>
</tr>
</tbody>
</table>

Superscript with the different letters indicates significantly different (*p*<0.05).

**Table 3. Splenocytes count (×10⁶) of malnourished and normal rats treated with goat milk yoghurt**

<table>
<thead>
<tr>
<th>Rats</th>
<th>Goat milk yoghurt</th>
<th>Control</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malnourished</td>
<td>43.00±18.04a</td>
<td>32.93±11.25a</td>
<td>37.96±15.36</td>
</tr>
<tr>
<td>Normal</td>
<td>38.71±8.90a</td>
<td>47.07±17.32a</td>
<td>42.89±13.92</td>
</tr>
<tr>
<td>Average</td>
<td>40.85±13.85</td>
<td>40.00±15.83</td>
<td>40.42±14.60</td>
</tr>
</tbody>
</table>

Superscript with the same letter indicates not significantly different.
cytes is the most commonly used technique for evaluating cell-mediated immune response. Quantitative analysis of proliferative response involves measuring the number of cells in culture in the presence and absence of a stimulatory agent such as an antigen or a mitogen. Concanavalin A (Con A) and PHA stimulate T cells (Meydani and Ha, 2000).

Lymphocyte proliferation that was expressed as an stimulation index of lymphocytes in malnourished and normal rat was not significantly different. Furthermore, no significant changes were also observed in stimulation index of lymphocytes of rat treated with goat milk yoghurt for 7 d compared to the control in normal and malnourished rat (Fig. 2).

The average of lymphocyte stimulation index in the malnourished and normal rats supplemented with goat milk yoghurt was 1.62±0.68 and 1.60±0.72, respectively.

**Effect on TNF-α level in splenocyte culture supernatant**

Levels of TNF-α was significantly decreased (p<0.05) in supernatant of cutured splenocytes from rat received goat milk yoghurt for 7 d compared to the control rat (Fig. 3), whereas TNF-α levels in malnourished and normal rat were not significantly different. The average of TNF-α in rats supplemented with goat milk yoghurt was 19.90±5.87 pg/mL, whereas in the control rat was 25.01±4.08 pg/mL.

**Effect on IL-10 level in splenocyte culture supernatant**

Goat milk yoghurt that supplemented in malnourished and normal rats increased level of IL-10 in spleen lymphocyte culture significantly (p<0.05) (Fig. 4). The average of IL-10 rats supplemented with goat milk yoghurt was 78.05±23.72 pg/mL, whereas in the control rat was 53.80±18.79 pg/mL.

**Discussion**

Malnutrition causes decreasing the weight depending on degree of malnutrition. According to Cortés-Barberena et al. (2008), the mild malnutrition or first degree when the weight deficit reached 10-25%; moderate or second degree when the weight deficit reached 25-40%; and severe or third degree when the weight deficit was greater than 40% of that of age-matched control rats. Increasing of rat body weight in this study after receiving goat milk yoghurt was nearly twice when compared to the control rat 7 d before. This is accordance with the previous study (Prada et al., 2007), in the rats treated with low protein diets for 60 d, 30 d of recovery feeding showed that body weight was twice when compared to that observed in the group one month before and no significant difference in proteinemia, albuminemia, circulating free fatty acid (FFA) levels and liver lipid content between the groups of low protein diets and normal protein. The low
of body weight in malnourished rats in this study also similar to the other previous study using rats 5 wk old with 55% dietary restricted (Hubert et al., 2000) and protein restricted (Prada et al., 2007). Decreasing this body weight gain through by reducing the intake of calories or other nutrients per unit body mass (Hubert et al., 2000). The children on goat milk outgained the cow milk children in bodyweight over the 2-wk trial period and fat absorption tended to be better in the goat milk children (Razafindrakoto et al., 1993).

Splenocytes counts of moderate malnourished and normal Wistar rats in this study similar to the previous study (Cortés-Barberena et al., 2008), total splenic lymphocytes from normal Wistar rats was not significantly different with the moderate malnourished rats, but was significantly different between normal or moderate and severe malnourished rats. Maybe moderate malnutrition are not causing spleen damage that produces lymphocytes, but in the case of severe malnutrition occurs spleen damage. This possibility supported by the study effect of severe malnutrition in Wistar rats showed that malnutrition plays an important role in thymus and spleen atrophy by increasing the rate of spontaneous apoptosis, and indicate that severe malnutrition is associated with a significant increase of spontaneously apoptotic cells in the thymus (9.8-fold) and spleen (2.4-fold). These alterations may contribute to the immune suppression found in malnourished organisms (Ortiz et al., 2008).

The total number of lymphocyte from normal rats was higher than the diabetic rat (260±23 vs. 123±25) × 10⁶/spleen, because that lymphocytes are more prone to injury in the diabetic state (Chi et al., 1982). In this study, the number of lymphocytes in normal or malnourished rat was lower (Table 3) than the lymphocytes in the previous study by Chi et al. (1982). This differences may be due to differences of age and species of rats, because lymphocytes number may also decrease with age, whereas splenic morphology is affected by species, age and genetic (Cesta, 2006), that also effects on lymphocytes number. According to Cheung and Nadakavukaren (1983), that age related to cellularity changes (cells/g of tissue) of the spleen of rats and the spleen increase in weight with age. In this study, source of lymphocytes using Wistar rats and previous study by Chi et al. (1982) using mixed of Wistar and Sprague Dawley rat lymphocytes.

Lactic acid bacteria (LAB) of goat milk yoghurt in this study was 9.1×10⁸ CFU/mL and Bifidobacteria was 2.0×10⁶ CFU/mL. Supplementation this goat milk yoghurt for 7 d did not effect on lymphocyte number in spleen of normal and malnourished rats. This results similar to the previous study by Galdeano and Perdigon (2006), L. casei with dose of 10⁸ CFU/mouse/day, that fed for 7 d did not influence the T-cell population of lamina propria in the mice small intestine.

There was no different between splenocyte proliferation response in moderate malnourished and normal rats fed goat milk yoghurt, because of the possibility of moderate malnutrition in rats no significant damage of the spleen as occurs in severe malnutrition. Giving yoghurt with a dose of 2 mL/day that containing LAB 9.1×10⁸ CFU/mL and bifidobacteria 2.0×10⁶ CFU/mL have no effect on proliferative response in normal and malnourished rats. First, this is because in normal (healthy) rats, yogurt supplementation is only used for the maintenance, and there is a homeostasis in the immune system and also there was no significant damage to the spleen in moderate malnutrition. Tolerance and homeostasis are maintained by specialized subsets of lymphocytes. Subsets of CD4⁺ T cells have drawn most of the attention so far, and there are several phenotypes, depending on the type of cytokines or surface molecules they express (Corthesy et al., 2007). Second, this possibility to occur spleen lymphocyte proliferation response is required a certain dose and duration of administration of probiotics and optimal mitogen concentration as well. According to Kirjavainen et al. (1999), L. rhamnosus GG (LGG) and Propionibacterium freudenreichii subsp. shermanii JS (PJS) have specific dose and duration-dependent immunomodulatory effects on the proliferative activity of B and T lymphocytes and may also reduce lymphocyte sensitivity to the cytotoxic effects of lectin mitogens. A significant decrease in basal lymphoproliferation (by 32 to 42%) was observed with PJS treatment after the 3- and 7-d periods; however, this activity returned to control levels after 14 d of treatment, which also resulted in significantly enhanced T-cell proliferation at optimal and supraoptimal ConA concentrations (by 24 to 80%). The 14 d LGG treatment also enhanced the latter activity (by 119%). In this study using 5 μg/mL of PHA mitogen-specific T cell proliferation alone, so it is not stimulated B cells, and the PHA dose may also not optimal to induce lymphocyte proliferation response. Study by Palacios et al. (2007) using 60 μg/mL T-cell mitogen PHA, showed that in vitro lymphocyte proliferation stimulated by T-cell mitogens decreased with age in free-living vertebrate.

After supplemented with goat milk yoghurt, the average of IL-10 was higher (Fig. 4) than TNF level (Fig. 3). The results showed that goat milk yoghurt containing L. aci-
dophilus, S. thermophilus and B. longum tend to induce anti-inflammatory cytokine in malnourished and normal rat. This results similar to the previous study using probiotic E. coli, that the probiotic strain was able to downregulate the increased levels of TNF-α both in the intestine from colitis rats, and in plasma and lungs in mice treated with LPS (Arribas et al., 2009). In addition, the ability of this probiotic bacterium E. coli to reduce TNF-α production in inflammatory conditions was also demonstrated in the trinitrobenzene sulfonic acid (TNBS) model of rat colitis or when it was evaluated in the dextran sodium sulfate (DSS)-induced colitis in mice (Grabig et al., 2006). TNF-α producing cells in the thymus increased significantly in the malnourished control group compared with the wellnourished control group, and no significant differences were observed among the test groups, milk, probiotic fermented milk, and bacterial-free supernatant, where the values obtained were similar to the wellnourished control group (Nunez et al., 2013). According to study by Galdeano et al. (2011), the administration of probiotic fermented milk as a dietary supplement during the re-nutrition process in a murine immunodeficiency model by malnutrition showed could be a good adjuvant diet to improve the gut and systemic immune response for the protection against Salmonella infection (Galdeano et al., 2011). This probiotics interact with immunocompetent cells using the mucosal interface and modulate locally the production of proinflammatory cytokines (Borruei et al., 2002). These proinflammatory cytokines are responsible for the presence of a chronic systemic inflammatory state, which shares numerous characteristics with the “acute-phase response” found in critically ill patients (Mantovani et al., 2004). Practically, measurement of circulating levels of TNF-α would enable not only to identify malnourished patients in a more rapid and cost-effective way, but would also help to select those who might benefit from some type of nutritional intervention. Usually, malnourished patients had higher values of IL-1 and TNF-α (16.7 and 28.0 pg/mL) (Correia et al., 2007).

Goat milk yoghurt containing L. acidophilus, S. thermophilus and B. longum that supplemented in malnourished rats could increase the level of IL-10 in splenocytes culture supernatant and also in normal rats. This result similar to the previous study (Nunez et al., 2013) using mice that received probiotics fermented milk containing L. delbrueckii subsp. bulgaricus (10^8 CFU/mL) and S. thermophilus (10^8 CFU/mL) and the probiotic bacterium L. casei DN-114-001 (10^8 CFU/mL) during re-nutrition period showed significantly increased number of IL-10 producing cells in the thymus. The increases of IL-10 can contribute to the decrease in cellular apoptosis observed after re-nutrition.

Probiotics in fermented milk was the most effective re-nutrition supplement to improve the histology of the thymus, decreasing cellular apoptosis in this organ (Nunez et al., 2013). Other previous study reported the effects of E. coli Nissle 1917 on the secretion of the cytokine IL-10 in Con A-activated splenocytes obtained from mice after LPS-induced septic shock (Arribas et al., 2009). That effects are dose dependent, which was shown after performing additional experiments with different doses of the probiotic E. coli coli strain Nissle 1917 (10^6-10^9 CFU per mice) (Hockertz, 1997).

IL-10 is secreted by T lymphocytes, and monocytes play a central role in downregulating inflammatory cascades by suppressing the secretion of proinflammatory cytokines such as TNF-α (Kuhn et al., 1993; Van Furth et al., 1996). IL-10 also inhibits the production of chemokines by leukocytes and down regulates the expression of intercellular adhesion molecule 1 and chemoattractant proteins by endothelial cells, thus inhibiting leukocyte migration to the site of infection. In turn, TNF-α can induce the production of IL-10 upon stimulation with endotoxin (Van Furth et al., 1996).

In this study, goat milk yoghurt using Lacto-B powder directly, which consists of mixture three types of bacteria as much as 1×10^9 CFU/mL. The total of LAB in the goat milk yoghurt was 9.1×10^9 CFU/mL and bifidobacteria was 2.0×10^6 CFU/mL. This number of lactic acid bacteria still in the range according to Nunez et al. (2013). Anti-inflammatory effects such as stimulation of IL-10-producing cells, are strain-dependent traits, and their effectiveness also depends on the concentrations used and the method of administration. Oral administration of a probiotic mixture that consisted of B. longum Bar 33 and L. acidophilus Bar 13 prevented inflammation and mucosal ulcerations in a trinitrobenzene sulfonic acid- (TNBS-) induced colitis mouse model (Roselli et al., 2009). This protection was associated with an upregulation of IL-10 that caused an inhibition of the TNBS-induced increase of the CD4+ population, downregulation of IL-12, and a different pattern of Foxp3+ CD4+ CD25+ Treg cells in the intraepithelial and lamina propria lymphocytes (Mengheri, 2008). In other probiotic mixture containing L. acidophilus, L. casei, L. reuteri, B. bifidium, and S. thermophilus induced both Tcell and B-cell hyporesponsiveness and down-regulated T helper (Th) 1, Th2, and Th17 cytokines without apoptosis induction in mice. It also induced gener-
ation of CD4⁺ Foxp3⁺ Tregs from the CD4⁺CD25 population and increased the suppressor activity of naturally occurring. Conversion of T cells into Foxp3⁺ Tregs is directly mediated by regulatory dendritic cells (rDCs) that express high levels of IL-10, TGF-β, COX-2, and indoleamine 2,3-dioxygenase. The therapeutical effect of the probiotics is associated with enrichment of CD4⁺ Foxp3⁺ Tregs in the inflamed regions (Kwon et al., 2010). The forkhead family protein Foxp3 (forkhead box P3) is a transcription factor highly expressed in CD4⁺ Tregs. It is a regulator of T-cell tolerance and is necessary for the development and function of Tregs (Ziegler, 2006).

The other previous study showed that a yoghurt produced with a pool of potentially probiotic LAB strains was effective in inhibiting the propagation of a dimethylhydrazine- (DMH-) induced colon cancer in mice by increasing the number of IL-10-secreting cells, increasing cellular apoptosis and decreasing procarcinogenic enzymes (de LeBlanc and Perdigon, 2010). By increasing IL-10 levels and in consequently decreasing inflammatory cytokines such as TNF-α and IFN-γ, some LAB can prevent the appearance of local inflammatory diseases and could be used as an adjunct therapy with conventional treatments (de LeBlanc et al., 2011).

Components of food that is consumed, naturally have the potential to interact with a variety of lymphoid cells throughout the gastrointestinal tract. Protein administered orally, has been known to interact with specific secondary lymphoid organs such as tonsils and Peyer’s patches. Complex interactions that occur on the side (lymphoid organs) is crucial for the development of the specific immune response. Several factors can influence the interactions, including the form of solids or liquids from food component and oral exposure methods (Sfeir et al., 2004). Immunomodulating requires intact or active peptides that are able to achieve immunocompetent cells. Most of the proteins are absorbed through the apical membrane of epithelial cells, which do not bind to the receptor will undergo lysosomal degradation (Perdue, 1999).

For the occurrence of the immune responses depends on the interaction with the active component with the immunocompetent cells, and immunogenicity of LAB in intestinal depends on degree of interaction with lymphoid tissue (Meydani and Ha, 2000). According to Sfeir et al. (2004), complex interactions between protein and immunocompetent cells is crucial for occurrence the specific immune response. Active component in yoghurt consists of bacterial and non-bacterial components that can role as immunomodulators (Meydani and Ha, 2000). Cell surface components in lactic acid bacteria, such as peptidoglycan, lipoproteins, and diacylated lipopeptide, act as ligands for immune cells that bind to Toll-like receptor (TLR) 2 and TLR1 or 6 heterodimers on the macrophage cell surface and then stimulate nitrite oxide (NO) production (Itoh et al., 2012).

The LAB are Gram-positive bacteria and their cell walls comprise a complex mixture of glycolipids, lipoproteins and phosphorylated polysaccharides embedded in a thick layer of peptidoglycan (PGN), a polymer of β-(1,4)-linked N-acetylgalactosamine and N-acetylmuramic acid, cross-linked by short peptides. TLR2 has been shown to recognize lipoteichoic acid (LTA), lipoarabinomannan, lipoprotein (LP) and PGN, and is likely to be involved in the recognition of LAB. Lactic acid bacteria act as immunoregulators through interaction of lipoprotein with TLR2 and as immunostimulators through interaction of peptidoglycan with nucleotide-binding oligomerization domain-2 (NOD2). As dendritic cell (DC) are potent stimulators of naïve T cells and express several pattern recognition receptors (PRRs), they serve as an important link between the microbiota and polarization towards T helper type 1 (Th1), Th2- or regulatory T-cell-dominated environments. Cell surface-expressed and intracellularly expressed PRRs collectively recognize lipid, carbohydrate, protein and nucleic acid structures that are broadly expressed by different groups of microorganisms (Zeuthen et al., 2008).

Recognition of microbial components by TLRs initiates signal transduction pathways, which triggers expression of genes. These gene products control innate immune responses and further instruct development of antigen-specific acquired immunity. Individual TLRs play important roles in recognizing specific microbial components (Takeda and Akira, 2005).

The presence of LAB is thought to be essential for yoghurt to exert immunostimulatory effects but components of nonbacterial yogurt, such as whey protein, short peptides, and conjugated linoleic acid (CLA), are believed to contribute to yoghurt’s beneficial effects as well. It is proposed that the LAB that survive through the gastrointestinal (GI) tract, whether intact or modified, can bind to the luminal surface of M cells. LAB-bound M cells reaching to the dome region of Peyer’s patch (PP) cells stimulate local immune response, which can further activate the local immune system, resulting in stimulation of the local and the systemic immune response (Meydani and Ha, 2000). Whey protein had activity as an anti-allergy, anti-inflammatory, immunomodulatory and antioxidant. One of the proteins in the milk whey fraction which has the broadest
range of biological activities related to the body's defense system, is lactoferrin, which among other acts as immunomodulator (Shimazaki et al., 1998; Zimecki and Kruzel, 2000). Study by Ward et al. (2002), showed that lactoferrin had been known inhibit allergen-induced skin inflammation in both mice and humans, most likely secondary to TNF-α production. The potency of probiotic bacteria to regulate immune responses is a complex interaction between the host immune system and different bacterial compounds, including chromosomal DNA and cell wall components as well as soluble metabolites (Corthesy et al., 2007).

The other non-bacterial component in goat milk yoghurt was prebiotics oligosaccharides. According to Wu et al. (2006), goat milk showed rich in sources of prebiotic oligosaccharides that resemble milk oligosaccharides (breast milk) (Bode, 2006), and in contrast to other prebiotics such as inulin or frukto short-chain oligosaccharides (Lara-Villoslada et al., 2006). The similarities found between human and goats’ milk oligosaccharides in terms of the presence of neutral (galactosyl-lactose and lacto-N-hexaose) and sialylated (3-, 6-sialyl-lactose and disialyl-lactose) structures suggest that the latter may, to some extend, carry out or resemble physiological activities described for the former (Martinez-Ferez et al., 2006). Goat milk oligosaccharides also have anti-inflammatory effects in rats with experimental colitis and may be useful in the management of inflammatory bowel disease (Daddaoua et al., 2006).

In conclusions, supplementation of goat milk yoghurt in malnourished rats could decrease the pro-inflammatory cytokine, while it increases the anti-inflammatory cytokine, represented by TNF-α and IL-10 levels in the splenocyte culture supernatant, respectively. However, that goat milk yoghurt feeding does not give any effect on total splenocyte numbers and splenocyte proliferation.

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References

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