

Goat and Bovine Colostrum as a Basis for New Probiotic Functional Foods and Dietary Supplements

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Abstract

Because bovine and goat colostrum are a rich source of essential nutrients for new-borns, their use in functional foods has substantially increased in recent years, but not in combination with probiotic bacteria. Therefore, our aim was to assess the possibility of combining goat or bovine colostrum with probiotics. First, we compared the immunomodulatory effect of both goat and bovine colostrum with that of human milk by the 3-day *in vitro* stimulation of human peripheral blood mononuclear cells (hPBMCs). Luminex multiplex analysis was used to analyze cytokine production by the hPBMCs. Although goat colostrum had a more similar immunomodulatory effect to human milk than bovine colostrum, we, nevertheless, decided to compare the potential of all three as growth substrates. Using the agar plate method, the growth of bifidobacteria, lactobacilli and enterococci was determined. Growth of *Bifidobacterium* sp. in goat colostrum is significantly better ($P < 0.05$) than in bovine colostrum or human milk. Our results indicate that goat colostrum has significant potential for the development of new functional foods and dietary supplements with bifidobacteria.

Keywords: Colostrum; Cytokines; Probiotics; Bifidobacterium; Lactobacillus

Introduction

Colostrum is defined as first milk with specific composition produced by mammals immediately after parturition. It contains high levels of bioactive components, including growth factors, immunoglobulins, lactoperoxidase, lactoferrin, lysozyme, immunomodulatory peptides, oligosaccharides and others [1,2]. Bovine colostrum due to content of these antimicrobial components has been tested for the treatment and prevention of various infectious diseases caused by bacteria, viruses and protozoa [3]. Oligosaccharides with prebiotic effect are naturally present in colostrum and can improve the growth of probiotic microorganisms such as lactobacilli and bifidobacteria [4].

During the last twenty years an increase in studies investigating the positive effects of probiotics is observed. Some of the reported beneficial effects of probiotics are associated with immune response modulation and cholesterol lowering ability [5]. Other benefits include prevention and treatment of gastrointestinal diseases and allergies such as colitis, colon cancer and irritable bowel syndrome [6]. The combination of health benefits of probiotics and colostrum can help to supply a market of food supplements and functional food with new products. For this reason, aims of the presented study were to test bovine and goat colostrum as growth substrate for selected probiotic microorganisms and compared the immunomodulatory effect of tested colostrum with human milk on human mononuclear cells.

Methods

Colostrum

Bovine colostrum samples were collected from first milking within 2 hours postpartum from cows of Czech pied cattle (Kojcice, Czech Republic). Human milk samples were obtained from voluntary donors from Gynaecology and Obstetrics Clinic of General Faculty Hospital (Prague). Goat colostrum samples were collected from first milking within 2 hours postpartum (Betula Pendula, Czech Republic). After

milking of colostrum samples, the samples were immediately frozen and stored at -20°C . Colostrum samples were adjusted two different ways. For testing of growth ability of probiotic and potential probiotic microorganisms, milk substrates were pasteurized at 62.5°C for 30 min. Samples for immunomodulation were only defatted by centrifugation (6000 g, 15 min) [7].

Immunomodulation

Samples of human blood were obtained from healthy adult donors from Blood Transfusion Centre of General Faculty Hospital (Prague). Human peripheral blood mononuclear cells (hPBMCs) were isolated from blood by Ficoll-Hypaque gradient (Sigma-Aldrich, Switzerland). Following separation and purification hPBMCs were adjusted at the final concentration of 10^7 cells mL^{-1} . Mononuclear cells (0.1 ml) were stimulated in X-vivo medium (Cambrex, USA) with 0.1 ml of defatted human breast milk or bovine and goat colostrum for 3 days at 37°C . The total volume was 1 ml. Negative control was composed by unstimulated hPBMCs and *ex-vivo* medium. The microplates with samples were incubated for 3 days at 37°C . Levels of cytokine produced by stimulation of hPBMCs by different types of colostrum were determined using Fluorokine MAP Human Base Kit A (R&D Systems, USA) for IFN- γ , IL-4, IL8, IL-10, IL-12, IL-13 and IL-17 by multiplex analysis using Luminex 200 Analyzer (Luminex Corp., USA).

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Growth of selected microorganisms in animal colostrum and human breast milk

Tested strains were obtained from the Culture Collection of Dairy Microorganisms Laktoflora[†] (Czech Republic), from the Czech University of Life Sciences (Czech Republic) and biopsy samples, as shown in Table 1. After overnight cultivation, the tested bacterial cells were isolated from growth medium (Table 1) by centrifugation (6000 g, 7 minutes), washed with sterile saline solution and then finally adjusted in the same buffer at final concentration of $10^3 \sim 10^4$ CFU mL⁻¹. Before growth testing, colostrum samples were pasteurized at 62.5°C for 30 minutes, then cooled at 37°C and inoculated with 10% (v/v) of bacterial suspension. Inoculated samples of colostrum were cultivated in anaerobic jars at 37°C for 24 h. The counts of tested strains at 0 and 24 hours were determined using 10-fold serial dilution and cultivated onto MRS agar (MERCK, Germany) / M17 agar, as shown in Table 1.

Statistical analysis

The concentration of observed cytokines producing by PBMCs

was assessed by software Luminex IS 2.3 (Luminex Corp., USA). All statistical evaluations were evaluated using Kruskal-Wallis test. Differences were considered statistically significant at the level of $P < 0.05$.

Results

Immunomodulatory effect of human milk, bovine and goat colostrum on human peripheral blood mononuclear cells were compared based on the production of pro-inflammatory and regulatory cytokine. Selected interleukins (IL-1 α , IL-4, IL-8, IL-10, IL-17, IL-12 and IL-13) and interferon (INF)- γ were determined using multiplex analysis in contrast with other studies where levels of tested cytokines were evaluated by flow cytometry or ELISPOT assay [8,9]. Our results have shown that the production of IL-8 and IL-12 interleukins was not statistically different ($p < 0.05$) comparing all three samples. In all the remaining interleukins (ILN- γ , IL-10, IL-17, IL-1 α , IL-4 and IL-13) a significant difference between human and bovine colostrum was observed. Immunomodulatory effect of goat colostrum and human milk did not differ significantly (Figure 1).

Strain	Species	Origin	Growth condition
[†] CCDM 229	<i>B. animalis</i> ssp. <i>animalis</i>	original culture	MRS broth / agar 6.2 + L-cysteine hydrochloride; anaerobic, 37°C, 72 hours
[†] CCDM 562	<i>B. breve</i>	GIT of child	
[†] AVNB3-P1	<i>B. adolescentis</i>	GIT of child	
[†] JOV	<i>B. bifidum</i>	infant faeces	
[†] JKM	<i>B. bifidum</i>	infant faeces	
[†] CCDM 150	<i>L. rhamnosus</i>	curd	MRS broth / agar 5.7; anaerobic 37°C, 72 hours
[†] CCDM 66	<i>L. delbrueckii</i> ssp. <i>bulgaricus</i>	yogurt	
[†] CCDM 151	<i>L. acidophilus</i>	tabl. Biolacta	
[†] RL 25	<i>L. fermentum</i>	human faeces	
[†] DM1TA6-P	<i>L. casei</i> ssp. <i>paracasei</i>	GIT of child	M 17 broth / agar; aerobic 37°C, 72 hour
[†] CCDM 945	<i>Enterococcus faecium</i>	original culture	
[†] CCDM 922	<i>Enterococcus durans</i>	isolated	

[†]Culture Collection of Dairy Microorganisms Laktoflora, Czech Republic; [†]isolate obtained from the biopsy sample of child, [†]Czech University of Life Sciences, Prague Czech Republic

Table 1: Tested microorganisms.

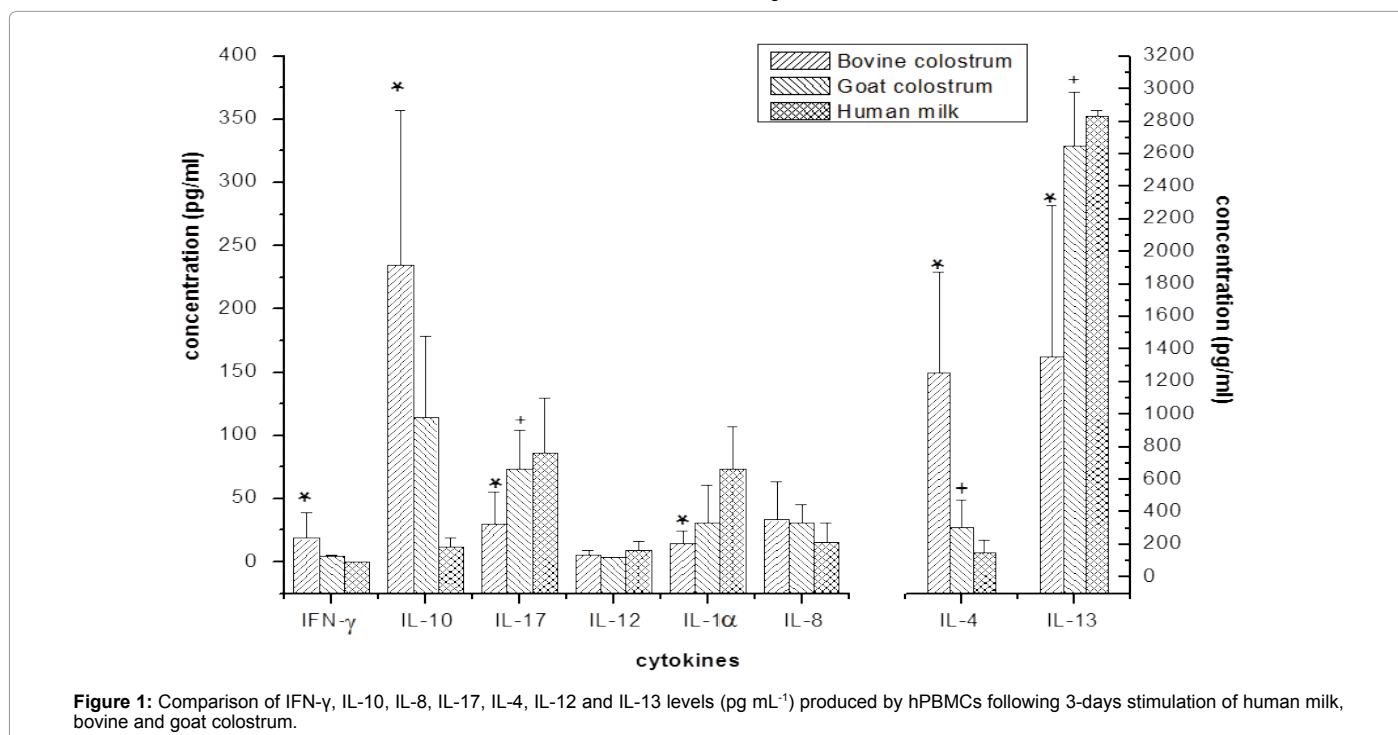


Figure 1: Comparison of IFN- γ , IL-10, IL-8, IL-17, IL-4, IL-12 and IL-13 levels (pg mL⁻¹) produced by hPBMCs following 3-days stimulation of human milk, bovine and goat colostrum.

Although goat colostrum has a more similar immunomodulatory effect to human milk than bovine colostrum, we compared the potential of all three as growth substrates for selected probiotic strains. As shown in Figure 2, we determined statistically significant difference between genera *Lactobacillus* sp., *Bifidobacterium* sp. and *Enterococcus* sp. The growth of *Bifidobacterium* sp. in goat colostrum was significantly better than in bovine colostrum or human milk (Figure 2). Growth of *Enterococcus* sp. was significantly lower in human milk than in other two used substrates. Lower counts of tested enterococci could cause their higher sensitivity to antimicrobial substances of human milk.

Discussion

Colostrum is an important source of protective and immunomodulatory components protecting newborns because their immune system is not fully developed after parturition [10]. Immunomodulatory components of colostrum generally include cytokines, chemokines, immunoglobulins and growth factors [1,11]. During the last years the development of methodological approaches for detection of various biological factors has increased rapidly. Immunoassays (enzymatic immunoassay, RIA) offer generally higher specificity, sensitivity, and reproducibility compared with bioassays [12]. Successfully used methods to detect multiple proteins are also proteomics and antibody-based protein arrays. Antibody-based protein arrays are primarily effective in characterizing the spectra of biologically active components in body fluids and cells [7,13]. The advantages of this determination are high numbers of proteins detected on an array and low volume of tested sample required. The protein array is also possible to use in basic and clinical research as well as in clinical practice due to a parallel identification of individual protein biomarkers.

Kverka et al. [12] investigated the cytokine profile in human colostrum and milk by multiplex protein array. They detected the previously described interleukin IL-8, epidermal growth factors (EGF), angiogenin and others, but they also found 32 cytokines which have

not been described before in these samples. The cytokine spectrum of individual human colostrum and milk was quite variable and the same proteins were not changed consistently over time [12]. High concentrations of cytokines (IL-1 β , IL-6, TNF- α , INF- γ), which are included in bovine or goat colostrum, can influence the immune response similarly as those in human colostrum [11,14]. Bovine colostrum also contains other immunomodulatory components (lactoferrin and insulin growth factors (IGF)-1) with the ability to regulate the secretion of cytokines [15]. The immunomodulatory effect of defatted bovine colostrum powder on human mononuclear cells isolated from peripheral blood of healthy donors was also tested in the study of Biswas et al. [2] mononuclear cells were stimulated by two different concentrations of bovine colostrum (0.1 and 10.1 $\mu\text{g mL}^{-1}$) and after 18-h or 24-h stimulation the production of IL-12 and IFN- γ was evaluated. The results have shown that bovine colostrum promoted the production of Th1 cytokine and it can be useful in prevention and treatment of several microbial infections. In present study, bovine colostrum stimulated a high production of IFN- γ , but also the production of Th2 cytokines IL-4 and IL-10. Increased production of IL-4 and IL-10 leads to inhibit the production of Th1 cytokines. In other studies of Wan et al. [14] and Yoshiaka et al. [16] tested the effect of oral administration of bovine colostrum on cytokine production *in vivo* and *in vitro* in mice. Their results indicate that immunomodulatory effect of bovine colostrum is pleiotropic on the production of cytokine and bovine colostrum can help in keeping the Th1 / Th2 balance.

The growth of tested strains may have been also caused by activity of bioactive components, such as lactoferrin, lysozyme and lactoperoxidase present in tested colostrum and human milk. The antimicrobial effect of bioactive components of milk is influenced by their synergistic manner and it does not depend on the level of their individual contribution [17]. Griffiths et al. [18] tested the ability of human and bovine lactoferrin to influence the growth of *B. bifidum*, *B. infantis*, *L. acidophilus* and enteric bacteria. The results has shown

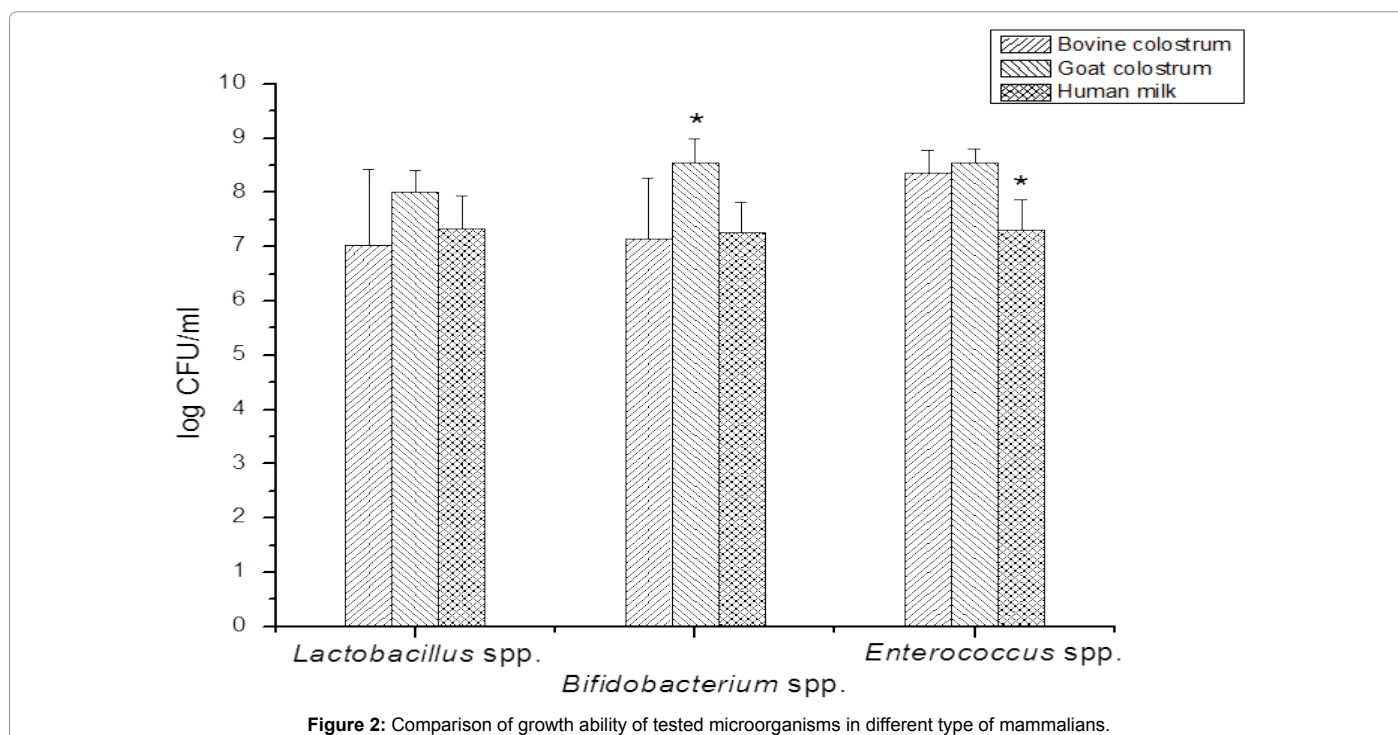


Figure 2: Comparison of growth ability of tested microorganisms in different type of mammals.

that lactoferrin in iron-limited forms and probiotics incorporated to biotherapeutic products can inhibit the overgrowth of enteric bacteria and so could help to balance of human gut microflora. In another study, Rada et al. [19] the growth of bifidobacteria in human milk was investigated. The results showed that both tested strains of *B. bifidum* grew in all human milk samples in contrast to strains of *B. animalis* spp. *lactis*, in which growth was decreased. *B. animalis* spp. *lactis* was the most sensitive to lysozyme in human milk (15-58 µg/l). The concentration of lysozyme in bovine colostrum (0.14-0.7 mg/l) and its lytic activity is less effective than lysozyme isolated from human milk [20]. Lower lytic activity of bovine lysozyme is caused by a different composition of amino acids than that found in human and albumen lysozyme [21].

In the summary, the results of the present study show that bovine and goat colostrum influences *in vitro* cytokine secretion of human mononuclear cells. The goat colostrum influenced the secretion of selected cytokines similarly to human milk than bovine colostrum. Nevertheless, its disadvantage is the poor availability and produced quantity. It is necessary to remark that this study has tested only a limited range of cytokines and that both colostrum may influence other cytokines and chemokines which play a role in immunomodulation. Further determination into the mechanism of cytokine production from human mononuclear cells and specific cell populations stimulated by mammalian colostrum or combination of colostrum and probiotics is warranted.

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