



ORIGINAL ARTICLE

Effects of human milk fortifier with iron on the bacteriostatic properties of breast milk[☆]

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Abstract

Objective: To compare bacterial growth in pure colostrum versus colostrum with human milk fortifier (HMF) containing iron.

Methods: The growth of *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* in 78 samples of pure colostrum or colostrum with added iron-containing HMF was compared. For qualitative analysis, filter paper discs were immersed in samples from each group and incubated for 48 hours with 10^1 colony forming units (CFUs)/mL of each strain. For quantitative assessment, 1 mL of each strain containing 10^7 CFUs/mL was homogenized with 1 mL of either colostrum or colostrum with human milk fortifier, seeded into a Petri dish, and incubated at 37 °C. Twenty-four hours later, the number of CFUs was counted.

Results: The qualitative analysis showed no difference in bacterial growth. In the quantitative evaluation, *E. coli* growth in the control group was $29.4 \pm 9.7 \times 10^6$ CFU/mL, while in the HMF group it was $31.2 \pm 10.8 \times 10^6$ CFU/mL. The difference between the average growth was $1.9 \pm 4.9 \times 10^6$ CFU/mL ($p=0.001$). There were no differences in *S. aureus* and *P. aeruginosa* growth.

Conclusion: Addition of iron at this concentration reduces breast milk bacteriostatic action against *E. coli*.

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Efeitos do aditivo do leite materno com ferro sobre as propriedades bacteriostáticas do leite materno

Resumo

Objetivo: Comparar o crescimento bacteriano em colostro puro e colostro com aditivo do leite materno contendo ferro.

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Métodos: Foram comparadas 78 amostras de colostro puro ou colostro com adição de aditivo do leite materno contendo ferro para avaliar o crescimento de *Escherichia coli*, *Staphylococcus aureus* e *Pseudomonas aeruginosa*. Para a análise qualitativa, discos de papel-filtro foram imersos em amostras de cada grupo e incubados por 48 horas com 10^1 Unidades Formadoras de Colônias/mL de cada cepa. Para a avaliação quantitativa, 1 mL de cada cepa contendo 10^7 Unidades Formadoras de Colônias/mL foi homogeneizado com 1 mL, tanto de colostro puro quanto de colostro com aditivo do leite materno, espalhado em placa de Petri e incubado a 37 °C. O número de Unidades Formadoras de Colônias foi contado 24 horas depois.

Resultados: A análise qualitativa não mostrou nenhuma diferença no crescimento bacteriano. Na avaliação quantitativa, o crescimento de *Escherichia coli* (EC) no grupo C foi de $29,4 \pm 9,7 \times 10^6$ CFU/mL, enquanto no grupo FM85 foi de $31,2 \pm 10,8 \times 10^6$ CFU/mL. A diferença entre o crescimento médio foi de $1,9 \pm 4,9 \times 10^6$ CFU/mL ($p = 0,001$). Não houve diferenças no crescimento de *Staphylococcus aureus* e *Pseudomonas aeruginosa*.

Conclusão: A adição de ferro a essa concentração reduz a ação bacteriostática do leite materno contra *Escherichia coli*.

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Introduction

It is well known that breast milk is the optimal food to be offered to the newborn due to its unique growth and immunologic factors.^{1,2} It has been shown that premature newborns also have better outcomes when fed with breast milk.²⁻⁴ However, due to the high-energy needs of premature newborns, breast milk alone is often insufficient to meet their nutritional requirements, especially in premature infants who weigh less than 1,500 g.⁴⁻⁶ In such small infants, energy, protein, calcium, phosphorus, iron, and sodium intakes should be increased, and the advantages of breast milk maintained by adding human milk fortifier (HMF) to breast milk.^{4,6-10} The only HMF available at the moment of this study in Brazil had been modified to contain an increased amount of iron (0.28 mg of Fe per 1 gram of product).

Among all immunologic benefits of breast milk, the bacteriostatic capacity of lactoferrin is remarkable.¹¹ Lactoferrin is an iron-binding protein that has been shown to have activity against bacteria, viruses, and fungi;^{11,12} to stimulate the immune system and the mucosa immune function;¹¹ and to have antioxidant and anti-carcinogenic effects.¹¹⁻¹³ Bovine lactoferrin supplementation has been shown to prevent sepsis in very-low-birth-weight neonates, and has been shown to reduce respiratory tract illness and increase hematocrits in healthy bottle-fed infants.^{14,15} In human breast milk, lactoferrin acts at the newborns' mucosa and protects them from infection by binding to iron and depriving it from pathologic bacteria that need iron to proliferate.⁷⁻¹¹ In order to maintain this bacteriostatic capacity, lactoferrin needs to be in an environment with a low iron concentration. If exogenous iron is added to breast milk, the benefits of lactoferrin might be impaired, which in turn might increase the risk of infection in newborns.⁷⁻¹⁰

The aim of this study was to compare pathogenic bacterial growth in colostrum *versus* colostrum supplemented with iron-enriched HMF.

Materials and methods

Colostrum samples were collected from lactating mothers who delivered at term during the period of 2010 and 2011. 10 mL of breast milk was collected from each mother. The inclusion criteria were lactating white healthy mothers who delivered in term. The exclusion criteria were mothers who had cesarean deliveries, receiving antibiotic treatment, on suspicion of infection, or with history of smoking.

The mothers were approached by the researcher after delivery, always accompanied by obstetrics and gynecology resident physicians.

The participants were asked about their pre-pregnancy body weight and age. They were instructed on how to collect the breast milk in an aseptic fashion. Sample collection was performed manually or with a manual suction pump, according to the mother's preference. Mothers who chose to use the manual suction pump received an ethylene oxide-sterilized pump containing a flask, a polypropylene tube, and a latex plunger, and were verbally oriented on how to use the pump (according to manufacturer's instructions).

The samples were collected in sterilized tubes and were closed with sterilized rubber stoppers. Mothers who chose to use a pump collected their samples in a coupled tube, closed with a polypropylene stopper. In the laboratory, each sample was transferred to another sterile tube. Each of these tubes was identified with a label containing the mother's name and the sample number, as well as the day and hour of collection.

The samples were kept in a refrigerator at a temperature of 4 °C to 6 °C, and analyzed within 72 hours. Each sample was divided into two samples of 5 mL, one to be analyzed as control (pure human milk), and the other with added HMF. HMF was added immediately before the analyses, in a proportion of 5%, which resulted in 0.25 g of fortifier for each 5 mL of breast milk (manufacturer's instructions). The fortifier was weighed with an analytical balance.

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