

Comparison of Human Milk, Cow's Milk and Infant Formulas for Their Antifungal Effects against Environmental Fungi

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Aim: Breast-feeding is generally accepted as the optimal method of feeding infants. The purpose of the present study was to investigate and compare the antifungal effect of human milk with infant formula and cow's milk.

Materials and Methods: Eighty Sabouraud agar petri dishes were separated into eight groups. All petri dish lids were closed and incubated, after 15 minutes uncovered. The first group was used to detect the fungal flora of the environmental air. The second group was rubbed with human milk. The third group was rubbed with 0.9% NaCl solution. Fourth group was rubbed with cow's milk. Fifth, 6, 7 and 8. groups were rubbed with probiotic formula, prebiotic formula, pro-prebiotic formula and standard infant formula respectively. All statistical analyses were performed using the SPSS.

Results: The number of fungal colonies grown in human milk-rubbed dishes was significantly less than the other groups except group 7 (groups 1, 3, 4, 5, 6, 7 and 8) ($P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.05$, $P > 0.05$, $P < 0.0001$, respectively).

Conclusions: In vitro antifungal effect of human milk is significantly superior than cow's milk and formulas tested. Human milk is not only the best food for infants but also it protects infants from fungal infections and fungal allergies by means of antifungal effect. Prebiotic and pro-prebiotic formula also have antifungal effect.

Key Words: Milk, human, infant formula, cow's milk, probiotic, prebiotic, fungi, antifungal

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Anne Sütünün Antifungal Etkisinin İnek Sütü ve Formül Mamalarla Karşılaştırılması

Amaç: Annesütü ile beslenme genel olarak bebek beslenmesinin en uygun şekli olarak kabul edilir. Sunulan çalışmanın amacı anne sütünün antifungal etkisinin araştırılması ve inek sütü ile formül mamalarla karşılaştırılmasıdır.

Yöntem ve Gereç: Seksen adet Sabouraud agar besiyeri alınarak 8 gruba bölündü. Tüm petri kutuları kapakları açık durumda 15 dakika bekletildikten sonra kapatılarak inkubasyona bırakıldı. İlk grup çevre havasının fungal florasını tesbit etmek için kullanıldı. İkinci gruptaki besiyerlerine anne sütü uygulandı. Üçüncü gruba 0.9% NaCl solüsyonu uygulandı. Dördüncü gruba inek sütü uygulandı. Beş, 6, 7 ve 8. gruplara sırasıyla probiyotik formüle, prebiyotik formula, pro-prebiyotik formula ve standart formula uygulandı. İstatistiksel değerlendirmeler için SPSS programı uygulandı.

Bulgular: Anne sütü uygulanan besiyerlerinde üreyen fungal koloni sayıları diğer gruplardan 7.grup hariç anlamlı derecede düşüktü (grup 1, 3, 4, 5, 6, 7 ve 8) (sırasıyla; $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.05$, $P > 0.05$, $P < 0.0001$).

Sonuç: Anne sütünün in vitro antifungal etkisi inek sütü ve formül mamalardan anlamlı derecede üstündür. Anne sütü çocuklar için yalnızca en iyi besin maddesi olmakla kalmaz, aynı zamanda antifungal etkisi ile onları fungal infeksiyonlardan ve fungal allerjilerden korur. Prebiyotik ve pro-prebiyotik formülalar da antifungal etkiye sahiptir.

Anahtar Sözcükler: süt, insan, infant formula, inek sütü, probiyotik, prebiyotik, fungi, antifungal

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Introduction

Breast milk contains various antimicrobial substances, factors that promote immune development, constituents that promote tolerance/priming of the infant immune system, as well as anti-inflammatory components (1). These substances in human milk not only modulate and mature the immune system of premature or mature newborns but also protect them against infections. The incidence of gastrointestinal and respiratory infections in infants receiving human milk is lower than in those not breast-fed, because of the presence of the specific antibody and antiadhesion molecules in human milk (2). While many studies have focused on the anti-bacterial and anti-viral effects of milk or milk constituents, there are only few studies concerning the possible anti-fungal effects (3). Infant formula on the market today should be aimed at providing the best alternative to breast milk for infants of those women who are unable to continue breastfeeding. For these purposes, the following compounds have been added to formula: long-chain polyunsaturated fatty acids, probiotics, prebiotics and nucleotides (4). A number of studies involving the feeding of probiotics and prebiotics to infants have been published over the last decade (5). These studies have examined a wide range of health outcomes, including growth rates of infants, safety profile of formula, prophylaxis and alleviation of diarrheal disease, reduction in atopic diseases, necrotizing enterocolitis, and infections of the preterm infant. In addition, these studies have described microbiological alterations observed in response to probiotic and prebiotic feeding (6). As far as we know, there is few previous studies that investigate the antifungal effect of infant formula. The purpose of the present study was to investigate the antifungal effect of human milk, infant formula and cow's milk and compare their antifungal effects.

Material and Methods

Milk samples were obtained from lactating mothers of healthy term infants between the 3rd and 8th days of lactation after colostrum, with a breast pump, and the milk was placed into sterile containers. All samples were stored at 4°C and processed within 2 hours of collection. Eighty Sabouraud agar petri dishes were used. After 15 minutes uncovered in Ankara Emek district, in the same location, on an unrainy day, all petri dish lids were closed and incubated at room temperature. Then, the dishes

were separated into eight groups. The first group (group 1, $n=10$ dishes) was used to detect the fungal flora of the environmental air. The second group (group 2, $n=10$ dishes) was rubbed with a thin layer of human milk by a sterile pipette. The third group (group 3, $n=10$ dishes) was rubbed with 0.9% NaCl solution to determine whether the depletion of fungal growth in human milk-rubbed dishes was only caused by the prevention of contact of air to the agar or the antifungal effect of the human milk. Fourth group (group 4, $n=10$ dishes) was rubbed with a thin layer of fresh cow's milk. Fifth, sixth, seventh and eighth groups (group 5,6,7,8, $n=10$ dishes each one) were rubbed with a thin layer of probiotic formula, prebiotic formula, pro-prebiotic formula and infant formula (without pro-prebiotic) respectively. After 7 days of incubation, the colony-forming fungal growths of all dishes were evaluated, counted, and recorded by a microbiologist who did not know the groups of the dish numbers (7).

The study, carried out at Fatih University School of Medicine was approved by the local ethics committee and conducted in accordance with the ethical principles described by the Declaration of Helsinki.

Statistical Analyses

All statistical analyses were performed using the SPSS for Windows, version 13.0 (Chicago, IL, USA). Unless otherwise stated, results were expressed as means \pm standard deviation for numerical values and percentage for categorical values. Nonparametric and parametric tests were used as appropriate. $P < 0.05$ was considered statistically significant.

Results

The fungal growths in all groups are shown in Table 1 and Figure 1. There was no statistically significant difference between the number of fungal colonies grown in control group and in the 0.9% NaCl group. The number of fungal colonies grown in human milk-rubbed dishes in group 2 was less than all of the other groups (groups 1, 3, 4, 5, 6, 7 and 8), which was statistically significant except group 7 ($P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.05$, $P > 0.05$, $P < 0.0001$, respectively). The number of fungal colonies grown in prebiotic-rubbed dishes in group 6 and pro-prebiotic-rubbed dishes in group 7 was less than controls in group 1, and this difference was statistically significant

Table 1. Colony-forming fungal growth in all groups.

	Env. Fungal Flora (Group 1) (n = 10)	Env. Fungal Flora with Human Milk (Group 2) (n = 10)	Env. Fungal Flora with 0.9 NaCl (Group 3) (n = 10)	Env. Fungal Flora with cow's milk (group 4) (n = 10)	Env. Fungal Flora with probiotic formula (group 5) (n = 10)	Env. Fungal Flora with probiotic formula (group 6) (n = 10)	Env. Fungal Flora With pro+prebiotic formula (group 7) (n = 10)	Env. Fungal Flora With pro+prebiotic formula (group 8) (n = 10)
	Number of colonies	Number of colonies	Number of colonies	Number of colonies	Number of colonies	Number of colonies	Number of colonies	Number of colonies
	% (Ratio of colony to total)	% (Ratio of colony to total)	% (Ratio of colony to total)	% (Ratio of colony to total)	% (Ratio of colony to total)	% (Ratio of colony to total)	% (Ratio of colony to total)	% (Ratio of colony to total)
Rhizopus	75	28	78	79	68	43	40	79
Cladosporium	59	21	54	55	43	34	28	53
Penicillium	35	9	28	25	25	17	17	28
Alternaria	21	9	20	24	19	15	11	22
Mucor	17	7	19	16	15	13	8	18
Aspergillus	15	6	16	13	13	10	7	14
Hormodendrum	9	3	10	7	9	5	4	8
Lactaria	5	2	5	7	6	2	4	4
Monilia	4	1	4	3	3	2	2	4
Stemphylium	2	1	3	3	2	1	1	2
Myxomycele	2	1	2	3	2	1	1	3
Total	244	88	239	235	205	143	123	235
Mean number of colonies ± standard deviation	24.4 ± 11.0	28.8 ± 5.07	23.9 ± 9.08	23.5 ± 8.95	20.5 ± 2.91	14.3 ± 4.66	12.3 ± 5.22	23.5 ± 5.66

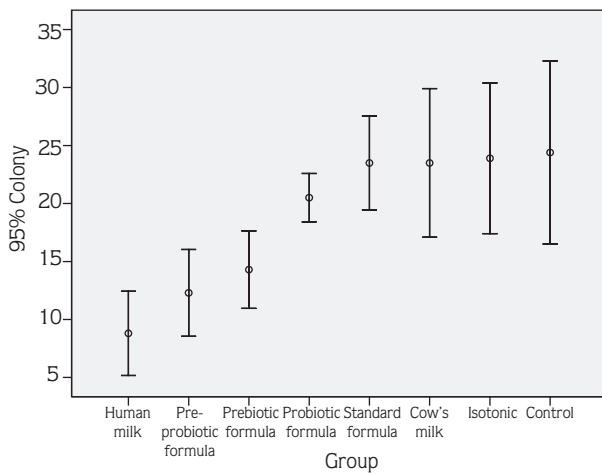


Figure 1. The comparison of fungal colonies between groups.

($P < 0.05$, $P < 0.01$, respectively). The number of fungal colonies grown in cow's milk-rubbed dishes in group 4, probiotic-rubbed dishes in group 5 and standard infant formula (without pro-prebiotic)-rubbed dishes in group 8 was less than controls in group 1, but this difference was not statistically significant. *Cladosporium*, *Alternaria*, *Aspergillus*, *Penicillium*, *Stemphyllium*, *Mucor*, *Hormodendrium*, *Lacaria*, *Monilia*, *Myxomycele* and *Rhizopus* sp. fungi were grown and fungus species were similar in all of the dishes. In the human milk group, the number of fungal colonies was significantly lower, but the percentages of fungal species were similar to the others.

Discussion

Studies in developed nations indicated a significantly lower frequency and/or severity of disease in breast-fed infants compared to those fed artificially (7). These differences suggest that human milk has protective and immunomodulatory activities that are lacking in even the best artificial formula (8). Recent reports associate artificial feeding of neonates with subsequent chronic diseases of later life, especially those with an autoimmune component (10). A systematic review concluded that breast-feeding appears to protect infants from the development of atopic diseases (eczema and food and respiratory allergies), particularly if there is a family history (11). While most studies have focused on anti-bacterial, anti-parasite and anti-viral activities, only few have focused on the anti-fungal effect of human milk (3).

Cummings *et al.* reported that phagocytosis and killing effect on *Candida albicans* by milk macrophages were identical to that of blood monocytes (12). Anderson *et al.* reported that human milk showed potent inhibitory effect on fungal growth and lactoferrin was responsible for the fungistatic effect (3).

In our previous study, we showed that human milk has antifungal effect *in vitro* (2). Fungal growth was significantly less in human milk-rubbed agars compared to simple Sabouraud agars and 0.9% NaCl solution-rubbed agars in that previous study suggesting an *in vitro* antifungal effect of human milk. In presented study, human milk showed same antifungal effect and we additionally found that there was significantly less fungal growth in human milk-rubbed agars than cow's milk-rubbed agars, probiotic formula-rubbed agars, prebiotic formula-rubbed agars and standard infant formula (without pro-prebiotic)-rubbed agars. This situation may suggest that an *in vitro* antifungal effect of human milk, which could be an important factor in protection from mycotic infections and allergic diseases in breast-fed infants, is superior than cow's milk and various kinds of infant formula. Fungi threaten human life not only by infections, but also by causing allergic reactions as aeroallergens (13). Before causing allergic reactions, mucosal blockage of fungi on entrance to the human body can prevent the onset of allergic bronchitis, asthma, and skin diseases such as urticaria. Providing the production of IgG-type blocking antibodies against the aeroallergens such as fungi is one of the mechanisms of vaccination in asthmatic patients (14). Because of its antifungal effect, human milk may be proposed to protect infants from allergic diseases caused by fungi *via* some mechanisms of induction of cytokines and secretory IgA. Anderson *et al.* found that human skim milk inhibits growth of *Candida albicans* and *Rhodotorula Rubra*, and this effect was caused by lactoferrin through its iron-binding capacity. In contrast, bovine milk had no inhibitory effects (3). The reason why cow's milk and kinds of infant formula's antifungal effect is lower than human milk may be the lactoferrin levels; that kind of infant formula does not contain lactoferrin and cow's milk contains lower levels of lactoferrin than human milk.

Fungal growth in human milk-rubbed agars was compared to pro-prebiotic formula-rubbed agars, but the difference did not reach statistically significant ranks. However, if we include more agars, it may reach

statistically significant levels; because number of fungal colonies grown in human milk–rubbed dishes was less than pro-prebiotic formula-rubbed agars and human milk have more antifungal effect than pre-probiotic formula.

In the present study, we found that prebiotic and pre-probiotic formula have also antifungal effects and the reason of this condition may be oligosaccharides in these formula. Prebiotics are nondigestible food components that benefit the host by selectively stimulating the growth or activity of non-pathogenic bacteria in the colon. Prebiotics (commonly oligosaccharides) added to infant feeds have the potential to prevent sensitization of infants to dietary allergens (15). Human milk also contains prebiotics (16). Oligosaccharides may be responsible in inhibition of fungal growth by stimulating the growth of non-pathogenic bacteria in the dishes in our study. More recent studies have been able to show clinical benefits with the use of a prebiotic mixture in infant formula. Firstly, it has been shown to decrease the risk of developing atopic dermatitis in high risk infants; secondly a reduced incidence of intestinal as well as upper airway infections in the first year of life. It can be hypothesized that prebiotics might play an important role as a new concept in allergy and infection prevention in infants (17). Antifungal effect of prebiotics and pre-probiotics may contribute to allergy and infection prevention in infants. Because of these effects prebiotic or pre-probiotic formula can be preferred rather than probiotic formula and standard formula in infants who can not be fed with human milk.

Probiotic formula did not have any antifungal effect in our presented study and the reason of this may be that

probiotic microorganisms can not proliferate in Saburoud dishes. If probiotic microorganisms had grown in Saburoud dishes, these microorganisms would have inhibited the fungal growth. Probiotics may play an important role in helping the body to protect itself from infections, especially along the colonized mucosal surfaces of the gastrointestinal tract. Probiotic products are available in many different forms worldwide, including pills, powders, foods, and infant formula (18). Human milk also contains probiotics (16). There is currently insufficient evidence to recommend the addition of probiotics for allergy prevention (19) Brouwer et al found that probiotic bacteria used in infants with Atopic Dermatitis did not have clinical or immunological effect. Their results indicated that oral supplementation with these probiotic bacterial strains did not have a significant impact on the symptoms of infantile atopic dermatitis (20). The fact that probiotic formula did not have antifungal effect may be contributed to this condition.

In conclusion, *in vitro* antifungal effect of human milk is significantly superior than cow's milk and kinds of infant formula. Human milk is not only the best food for infants but also it protects infants from fungal infections and fungal allergies by means of antifungal effect. To prevent infections and allergic diseases, human milk must be considered as the ideal food for newborns. Prebiotic and pre-probiotic formula have also antifungal effects. Prebiotic or pre-probiotic formula should be preferred rather than standard formula and probiotic formula in circumstances that human milk can not be given to infant, due to the antifungal effects and decreasing the risk of atopic diseases and infections.

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