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# Effect of a Fermented Formula on Thymus Size and Stool pH in Healthy Term Infants

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**ABSTRACT:** To investigate the effects of fermented formula (FF) with *Bifidobacterium breve C50* and *Streptococcus thermophilus 065* on thymus size and stool pH of healthy term infants, ultrasound examinations and evaluations of thymus sizes and thymus indices (TI) and measurements of stool pH were performed in the same 90 term neonates on the 3rd d of life and on the 1st, 2nd, 3rd, and 4th mo of life. Thirty newborns were exclusively breast-fed while the remaining 60 were randomly assigned to receive either a FF or a standard formula (SF). The fecal pH of the breast-fed group was lower than the SF group ($p < 0.05$), although it was similar to that of the FF group on the third postnatal day, persisting for the entire 4 mo of the study. The difference in TI was statistically significant over repeated measurements among the groups. The FF infants showed a TI similar to the breast-fed newborns. Probiotic fermentation products have effects comparable to those of the bacteria composing the intestinal microflora supporting the idea that intestinal bacterial balance plays an important role in improving host immune responses. **(*Pediatr Res* 62: 98–100, 2007)**

The principal components of human milk modulating immune responses are immunoglobulin immune cells – macrophages, neutrophils, and lymphocytes – and humoral factors – lactoferrin, lysozyme , and hormones (1,2) that interact with the intestinal microflora during postnatal breast-feeding. The stool microflora of the breast-fed infant is predominantly composed of bifid-bacteria (3). The thymus provides the environment for T lymphocyte maturation. Sonographic studies show that the thymus grows until 8 mo of age decreasing to a stable size around 12 mo of age (4,5). Thymus size is dependent on whether the infant is breast-fed; the thymus size of breast-fed infants is twice the size of formula-fed infants at 4 mo of age (6). Now it is clearly known that infant feeding can modify the balance of intestinal flora and thus influence local and general immune response. One way to achieve this is using fermented formula, or formula containing probiotics or prebiotics (7,8). These products have been shown to have an effect on intestinal homeostasis and intestinal inflammation (9) and have been used to prevent and treat acute diarrhea. A new infant formula fermented with *Bifidobacterium breve C50* and *Streptococcus thermophilus 065* (FF) has been developed. These particular lactic-acid producing bacteria were selected because *in vitro* – in animal and human studies – they have been shown to have an effect on immune response and exert anti-inflammatory effects on gut-associated lymphoid tissue (GALT) (10,11). We hypothesized that fermented formula would result in a higher thymus index (TI) and a lower fecal pH over the first 4 months after birth mimicking the effects of breast milk.

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## MATERIALS AND METHODS

***Inclusion and exclusion criteria.*** Over a period of 6 mo, healthy newborns born at the Department of Neonatology of the University of Bari were considered eligible to be included in the study. Newborns were selected on the basis of their parents' willingness to participate in the study. The exclusion criteria were prematurity, delivery by caesarian section, and any prenatal condition that could influence the thymus volume, such as maternal infection and under nutrition. Thirty newborns were exclusively breast-fed. Unfortunately, our efforts to convince the mothers to breast-feed were often unsuccessful. Information regarding the study was given only when the need of formula was imminent. Sixty newborns, whose mothers decided at their births not to breast-feed, were randomly assigned to receive either FF or standard infant formula (SF). These children did not receive colostrum. Both formulas had the same basic nutritional composition per 100 ml of 71 kilocalories, 1.5 g protein, 8.3 g carbohydrates, and 3 g fat.

Written informed consent was obtained from all parents and the study protocol was approved by the Ethics Committee of the Ospedale Consorziale Policlinico, Università di Bari. All newborns underwent a clinical examination and evaluation of weight, length, and head circumference at scheduled visits on the 3rd d of life. They were re-evaluated at 1, 2, 3, and 4 mo of age. Stools were collected at each visit. All the enrolled neonates completed the follow-up schedule. Physicians examining the infants and performing ultrasonographic evaluations were blind to the feeding regimen of the individual subject.

Ultrasonography measurement of the thymus was performed during each visit. The organ measurements were done by the operator using a Hewlett Packard Point HX and a 7.5 Mz probe. The thymus appears as a well-defined, echo-poor structure in the anterior mediastinum. The trans-sternal plane was used to measure the largest transverse diameter of the thymus; perpendicular to the diameter this large sagittal area (longitudinal scan plane) was depicted by the monitor and measured by the computer. The two measurements were multiplied and recorded as the TI and results were expressed as the average of two determinations. The index is an estimation of the volume of the thymus and postmortem examinations have shown a high correlation among thymus index, weight, and volume (12).

Fecal samples were collected as fresh as possible and immediately frozen at −20°C until evaluation. pH was measured using a Handy-lab pH meter (Schott Glas, Mainz, Germany) equipped with an Inlab 423 pH electrode (Mettler –Toledo Columbo). Fecal pH was determined after 10% fecal suspension (wt/vol) in saline solution (0.15 M NaCl solution).

Statistical analysis was performed using SPSS v 11.5 for Windows. The longitudinal study was designed to evaluate differences in growth parameters, TI and fecal pH with a power of 0.80 and a $p$ value $<0.05$. ANOVA for repeated measures was used for TI and fecal pH among groups over time determination. Data were expressed as mean and SD.

The present investigation was conducted as a blind, randomized clinical trial to compare the effects of FF and breast milk on increasing TI and reducing fecal pH.

**Abbreviations: BF,** breast-fed; **FF,** fermented formula; **GALT,** gut associated lymphoid tissue; **SF,** standard formula; **TI,** thymus index



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**Table 1.** *Demographic characteristics of the newborns*

| Breast-feed infant (BF) | Formula feed infants (FF) | Standard formula feed infants (SF) |
|---|---|---|
| Mean gestational age 39.8 ± 1.26 | Mean gestational age 38.8 ± 1.06 | Mean gestational age 39.3 ± 1.13 |
| Birth weight (g) 3338 ± 491.2 | Birth weight (g) 3366 ± 342.2 | Birth weight (g) 3411 ± 432.1 |
| Apgar score 8.76 ± 0.78 | Apgar score 8.82 ± 0.78 | Apgar score 8.92 ± 0.29 |
| Male/Female 18/12 | Male/Female 17/13 | Male/Female 14/16 |

Values are given as mean and SD.

## RESULTS

Demographic data are shown in Table 1. No differences in growth parameters, Apgar scores, or gender were seen among the three groups at the beginning of the study. The fecal pH of the breast-fed group was lower than that of the SF group ($p < 0.05$) while it was similar to that of the FF group on the 3rd postnatal day; this value persisted during the 4 mo of the study (Table 2).

From the 1st postnatal evaluation and examination to the 3rd day of life, the TI of the breast-fed infants remained elevated compared with that of the FF and SF infants. The difference in TI was statistically significant over the repeated measurement among the BF and the other two groups (Fig. 1). The FF infants showed a TI similar to the breast-fed newborns (Fig. 1). None of the infants received antibiotics during the 4 mo of study.

## DISCUSSION

This randomized, double-blind study compares the effects of fermented formula with *Bifidobacterium breve C50* and *Streptococcus thermophilus 065* to breast milk and standard formula feeding on TI and fecal pH. The TI of breast-fed infants remained significantly higher than that measured in the formula-fed infants.

**Table 2.** *Value of stool pH*

| | 3rd day | 1st month | 2nd month | 3rd month | 4th month |
|---|---|---|---|---|---|
| BF | 5.03 ± 0.7 | 5.05 ± 0.2 | 5.04 ± 0.7 | 5.06 ± 0.3 | 5.04 ± 0.4 |
| FF | 5.12 ± 1.4 | 5.13 ± 0.3 | 5.11 ± 0.3 | 5.12 ± 0.7 | 5.15 ± 0.6 |
| SF | 5.86 ± 1.7* | 5.63 ± 0.5* | 5.93 ± 0.7* | 5.78 ± 0.6* | 5.83 ± 0.7* |

* vs breast-feed and FF feed $p < 0.001$.

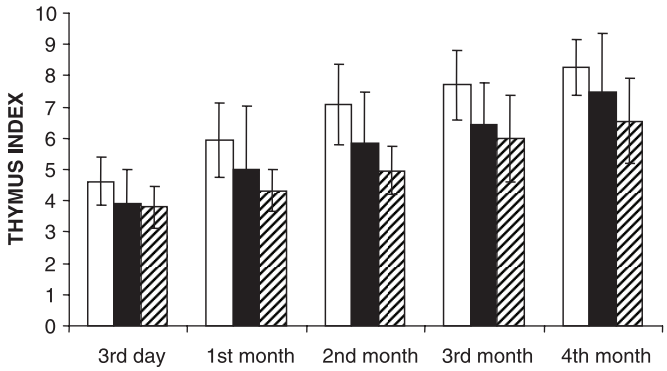


**Figure 1.** Thymus Index of the three groups in different evaluations. BF (□; $n = 30$ newborns) FF (■; $n = 30$ newborns) SF (□; $n = 30$ newborns).Values are expressed as mean and SD. TI increased over the time in a statistically different way in the three groups. BF *vs* SF ($p < 0.001$); BF *vs* FF $p < 0.036$; FF *vs* SF $p < 0.042$.

Infants receiving FF showed a TI and a fecal pH similar to that of breast-fed babies compared with infants receiving SF. Moreover, the TI in BF newborns was larger from the 3rd d of life. Other studies *in vivo* reported the relation between the intestinal flora and general immune system (13). The study by Tribould found that babies provided with FF had an increase in bifidbacteria associated with an increase in intestinal IgA antibody to poliovirus production after vaccination (14).

An increase in thymus size has already been reported in breast-fed infants (6). Breast milk contains a large number of immune components and some of these could be responsible for the modulation of the immune response (15). The role of the thymus in development and maturation of the newborn immune system plays a role in the differentiation and specialization of lymphoid cells populations. These cells will move toward other lymphoid districts and will contribute to tolerance against "self" antigens. Neuroendocrine regulation underlies adaptation to dynamic changes of the organism as environmental stimulations contribute to the full function of the thymus. It is widely known that the thymus plays a key role in the organic defense against infections and in the development of immune tolerance (16). Recently, it has been pointed out that the dimensions of the thymus at birth are associated with childhood mortality in developing countries. The determination of thymus dimensions at birth could be an important predictive factor of the immune competence level (17,18). Our data show that from the 3rd d of life breast-fed newborns had a TI significantly greater than those of the newborns in the other two groups. We speculated that this could be due to the effect of the components of the colostrums in breast milk that have stimulant effects on the immune system (19). We have no other explanation for this finding. The children belonged to the Caucasian race and the social factors in this group were homogeneous. Moreover, the breast-fed children persistently showed an increased TI. Jeppsen *et al.* (20) demonstrated an immune-modulating effect of breast milk with increases of CD4, CD8, and thymus index in breast-fed newborns relative to the formula-fed infants. This occurred from the 1st d of life until the 10th mo. The role of feeding in the development of the immune system, expressed incrementally by thymus volume, is clear. The same authors showed a decreased thymus size in uninfected infants of HIV-positive mothers fed on human donor milk compared with that of exclusively breast-fed newborns. The results are larger than that of the formula-fed infants group. This difference was clearly seen at 4 mo even though at birth those children had a small thymus size compared with infants of HIV-negative mothers (21). These data support the hypothesis that changes in intestinal microflora "related to different type of feeding" might influence the thymus size. Contrary to Hasselbach, Jeppesen, and our study data where the feeding clearly influences the dimensions of the thymus, independent of other clinical variables such as weight, length, and head circumference, Yekeler (22) demonstrated no correlation between thymus dimensions and type of feeding. A low power of the study (*i.e.*, a small number of formula-fed infants compared with the breast-fed controls) might explain these findings.

The relatively higher growth of the thymus in the breast-fed babies may also be explained by many variables. First, the increased production of CD4 and CD8 demonstrated from

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recent studies suggest that the larger size of the thymus is compatible with increased thymic activity and emission of T lymphocytes (23). Moreover even if the fetal immune system could be influenced during gestation, the composition of human milk plays a crucial role in the development of the infant immune system *via* several cytokines and growth factors (24). Bioactive IL-10 in human milk has immune-modulating, anti-inflammatory effects on the alimentary tract (25) and a protective role against necrotizing enterocolitis (26). IL-7, an essential factor for proliferation and survival of T cell precursors has been found in high concentrations in human milk from the first lactating week (27). Other studies underline the importance of IL-7 in conditioning T cell function outside the thymic environment as well (28,29).

The observation that TI is larger in the FF group compared with SF infants deserves further comment. Bacterial species similar to those used to ferment the study formula secrete moieties that retain anti-inflammatory properties after crossing an *in vitro* model of intestinal barrier (10). Therefore, unidentified soluble factors might explain the immune regulatory properties of the FF. As a complementary hypothesis, the fermentation process itself might influence the composition of intestinal microflora and, in turn, the infant immune system. Although we did not document a direct change in intestinal microflora composition, we demonstrate an acidic shift in fecal pH. This is indirect though reliable evidence of an increasing lactobacillar stool concentration (30,31). We are aware of the limitations of this method, which does not allow us to draw any conclusion about the specific composition of intestinal microflora. New techniques are emerging, such as those which use 16S ribosomal RNA (rRNA) gene sequences or strain specific Rep-PCR for evaluation of microbial populations (32,33). The modification of fecal pH associated with alteration of TI in our studies is a phenomenon that deserves further evaluation using these emerging technologies.

The active metabolites from this formula are of interest for newborns that are at higher risk of infections due to live bacteria, such as in premature infants with an underdeveloped immune system. Understanding the mechanism of the beneficial effect of the FF on endogenous microflora and immunogenic activities should provide new regimens for prevention and treatment of illness without the potential detrimental effects of giving live microorganisms. In conclusion, probiotic fermentation products may have, as in this case, beneficial effects comparable to those of the bacteria, which compose the optimal intestinal microflora. This strengthens the idea that intestinal bacterial balance plays an important role in improving host immune responses to different situations.

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