Comparison between Fecal Calprotectin, Bifidobacteria and Lactobacillus Levels in Exclusively Breastfed Infants Versus Formula Fed Infants

Thesis

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List of Abbreviations

AMP	**:**	Antimicrobial proteins
APRIL	**:**	A proliferation-inducing ligand
BFHI	**:**	Baby Friendly Hospital Initiative
BMI	**:**	Body mass index
BSSL	**:**	Bile salt stimulating lipase
CD	**:**	Crohn's disease
CRP	**:**	C-Reactive protein
C-section	**:**	Cesarean section
CTR-ir	**:**	Calcitonin receptor-immunoreactivity
DCs	**:**	Dendritic cells
DHA	**:**	Docosahexanoic acid
EGF	**:**	Epidermal growth factor
ELISA	**:**	Enzyme-linked immunosorbent assay
Epo	**:**	Erythropoietin
FC	**:**	Fecal Calprotectin
FXR	**:**	Farnesoid X receptor
GALT	**:**	Gut associated lymphoid tissues
GI	**:**	Gastrointestinal
Git	**:**	Gastrointestinal tract
GPCRs	**:**	G protein-coupled receptors
HB-EGF	**:**	Heparin-binding growth factor
HIV	**:**	Human immunodeficiency virus

HMOs	**:**	Human milk oligosaccharides
HRP	**:**	Horseradish peroxidase
HSV-1	**:**	Herpes simplex virus type 1
HTLV-1	**:**	Human T- lymphotropic virus 1
IBD	**:**	Inflammatory bowel diesease
IBS	**:**	Inflammatory bowel syndrome
IECs	**:**	Intestinal epithelial cells
IgA	**:**	Immunoglobulin A
IGF	**:**	Insulin-like growth factor
IQR	**:**	Interquartile range
LAZ	**:**	Length-for-age
LCPUFAs	**:**	Long chain polyunsaturated fatty acids
LDL	**:**	Low density lipoprotein
LPS	**:**	**Lipopolysaccharide**
MFG	**:**	Milk fat globule
MUAC	**:**	**Mid-upper arm circumference**
NEC	**:**	Neonatal necrotizing enterocolitis
Neu5AC	**:**	N-Acetylneuraminic acid
NF	**:**	Nuclear factor
NS	**:**	Non-significant
PRR	**:**	Pattern recognition receptor
RBC	**:**	Red blood cells
rRNA		Ribosomal ribonucleic acid
RSV	**:**	Respiratory syncytial virus

SCFAs	**:**	Shortchain fatty acids
SIDS	**:**	Sudden infant death syndrome
SIgA	**:**	Secretory Immunoglobulin A
SVD	**:**	Spontaneous vaginal delivery
T2D	**:**	Type 2 diabetes
TLR4	**:**	Toll-like receptor 4
TNF-α	**:**	Tumor necrosis factor alpha
UC	**:**	ulcerative colitis
VEGF	**:**	Vascular endothelial growth factor
WAZ	**:**	Weight-for-age
WHO	**:**	World Health Organization
WLZ	**:**	Weight-for-length

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Introduction

The human intestinal microbiota is a complex and dynamic ecosystem with 300-500 different species of bacteria and plays an important role in maintaining host health, since it is involved in nutrition, pathogenesis, and immunology .The gut microbiota of an individual is influenced by different dietary habits during life. The gut microbiota of children fed a modern western diet compared to a rural diet has been characterized, and the effect of dietary polyphenols on the human gut microbiota has been reported ***(Lee et al., 2015).***

The development of the intestinal microbiota occurs primarily during infancy. Mutualistic interactions between the colonizing intestinal bacteria and the host are essential for healthy intestinal and immunological development ***(Wang et al., 2015).***

Human milk satisfies the nutritional requirements of the infant and confers protection against pathogens through the transmission of maternal antibodies (IgA) and other antimicrobial factors. The World Health Organization (WHO) recommends exclusive breastfeeding of children up to 6 months of age in order to ensure that the growing

infant receives the full nutritional benefits of breast-milk ***(Arrieta et al., 2014).***

Feeding type has been demonstrated to influence microbiota composition directly, by providing the substrates for bacterial proliferation and function and sources of bacterial contamination (originating from the nipple and surrounding skin, and milk ducts for breast milk from the dried powder, the equipment used for preparation and the water used for suspension for formula milk and indirectly, by modulating the morphology, cell composition and physiology of the intestinal mucosa, and the pancreatic function***(Guaraldi et al., 2012).***

Importantly, breast milk also contains prebiotic human milk oligosaccharides (HMOs) – sugar polymers that promote the growth of specific microbial communities, including Bifidobacterium spp. Bifidobacteria in the infant gut are important for inhibiting the growth of pathogenic organisms, modulating mucosal barrier function, and promoting immunological and inflammatory responses ***(Mueller et al.,2015).***

The synergy of the probiotic and prebiotic components of human breast milk provides breastfed infants with a stable and relatively uniform gut microbiome compared

with formula-fed babies .While consumption of infant formula containing probiotics has also been shown to promote the development of a neonatal gut microbiome similar to that of breastfed infants, maternal breast milk remains the ideal source of nutrition for infants ***(Mueller et al., 2015).***

The presence of calprotectin in feces is proportionate to neutrophil migration to the gastrointestinal mucosa. Calprotectin has immunomodulating and anti-proliferative effects aswell as an important role in neutrophil defense against bacterial infections ***(Chitkara et al., 2009).***

When bound to calcium, calprotectin has a high heat resistance and is stable in stool samples for up to one week at room temperature ***(Roseth et al., 1992; Roseth et al., 1997).*** These properties allow calprotectin to be eliminated intact in the feces and give it an advantage as a non-invasive biochemical marker for the screening of intestinal inflammation, compared with other markers that are currently used (lactoferrin, neutrophil elastase, and leukocyte esterase) ***(Bonnin Tomas et al., 2007)***.

Healthy infants in the first months of life have high calprotectin concentrations compared with children. The

kind of feeding influences the fecal calprotectin concentration ***(Savino et al., 2010).***

Golden et al, found faecal calprotectin concentrations to be significantly lower in breast-fed than in formula-fed infants during the ‘‘pre-weaning’’ period ***(Golden et al., 2002).***

Lactobacillus supplementation reduced the level of calprotectin, a marker of cow’s milk allergic colitis, and allowed a better recovery of the intestinal mucosa in infants suffering from hematochezia ***(Baldassarre et al., 2010).***

Arvola and colleagues reported a group of infants suffering rectal bleeding who was largely breast-fed,the cow’s milk elimination diet in a subset of infants did not affect the duration of rectal bleeding. Compared to the control group, infants suffering from rectal bleeding had lower bacteria counts and their populations of Bifi-dobacterium and Lactobacillus were around ten times lower than in healthy Infants. The authors thus suggested the possibility of probiotic intervention aimed at normalizing the level of bifidobacteria and lactobacilli***(Arvola et al., 2006).***