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Comparative Effect of Breast Milk and Infant Formulae on Neonatal Gut Microbiome within Katsina Metropolis

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Abstract

Numerous studies conducted in recent years have highlighted the intricate nature of the neonatal gut microbiome, influenced by various intrinsic and extrinsic factors. One significant factor in this regard is the type of feeding, which has a substantial impact on the development of intestinal microbiota in early infancy. This study aimed to compare the effects of breast milk and infant formulae on the gut microbiota of newborns in Katsina metropolis. Faecal samples were obtained from 46 neonates (33 exclusively breastfed, 10 formula-fed, and 3 mix-fed) and analyzed using a culture-dependent method. Colony enumerations and pH measurements were conducted for comparison between the groups. The mean weight of the participants was 2.88±0.1 kg, with exclusively breastfed infants (BFI) weighing significantly more (p = 0.03) than formula-fed infants (FFI). The bacteria selected for analysis (Bifidobacterium spp., Staphylococcus spp., Escherichia spp., and Lactobacillus spp.) were present in all feeding groups. Among BFI, similar levels of Escherichia spp. and Bifidobacterium spp. (61.17 CFU/g and 61.38 CFU/g respectively) were observed. Staphylococcus spp. constituted the majority of the bacterial load (32%) in both BFI and FFI groups. Apart from Escherichia spp. (p = 0.01), no significant differences were noted in the levels of all cultured bacteria across the feeding groups. The disparity in Escherichia spp. load was evident between BFI and MFI (p = 0.01), as well as FFI and MFI (p = 0.02) only. There was no overall significant correlation between bacterial load and mode of delivery within the feeding groups (p = 0.6). The average faecal pH of breastfed infants (5.09±01) was significantly lower (p = <0.001) compared to the formula-fed group (5.9±0.1). Despite advancements in enriching infant formulae with probiotics and other bifidogenic substances, subtle differences in fecal bacterial load compared to breast milk persist, highlighting the significant influence of both feeding methods on the composition and functionality of the neonatal gut microbiome.

Keywords: Breastfeeding, Formula-feeding, Fecal sample, Bacterial load

INTRODUCTION

The intestinal microbiota is a dynamic and complex ecosystem consisting of hundreds of variable microbes, mainly bacteria (10^11-12 bacteria/g of colonic content, forming 60% of the total faecal mass) (Ervin *et al.*, 2013). Directly involved in nutrition and immunology, the gut microbiome plays a pivotal role in maintaining host health (Sánchez *et al.*, 2017), influencing the proliferation and differentiation of epithelial cells. Deregulation of the gut microbiota is implicated in the pathogenesis of various immunological, metabolic, and cardiovascular diseases in the human body (Guaraldi & Salvatori, 2012). The type of feeding introduced during the neonatal period influences the shaping of the gut microbiota in early infancy and lifelong health (Tanaka & Nakayama, 2017).

The choice of feeding method is highly personal and often influenced by many factors (Kozhimannil *et al.*, 2014). Globally, only about 38% of infants are exclusively breastfed. In the United States, 75% of newborns initiate breastfeeding from birth, but by the age of three months, 67% of them rely on infant formula for some of their nutrition (U.S Food and Drug Administration, USFDA, 2014). In Nigeria, only 23% of mothers practice exclusive breastfeeding (UNICEF, 2017). A further study on breastfeeding

mothers in Zaria metropolis, Northern Nigeria, reported that only 2% of mothers (n=106) practice exclusive breastfeeding (Olayemi *et al.*, 2015).

Breast milk substitutes are marketed through complex networks that are beyond the control of individual regional governments (Kent, 2015). The national Agency for Food and Drug Administration and Control (NAFDAC) in Nigeria, for instance, organizes annual awareness intensification workshops to ensure compliance with the International Code of Marketing of Breast Milk Substitutes (BMS); however, continued violation of such regulations remains a challenge. Therefore, as the nutrition industry globalizes, there is a growing need for the study and analysis of different infant feeding methods to provide appropriate guidance and recommendations by health experts.

This study is intended to compare the effect of breastmilk and infant formulae on gut microbiota of newborns in Katsina metropolis. This will help not only in providing evidence-based emphasis on the importance of breastfeeding in early life among Katsina community, but also in understanding the uses and limitations of infant formulae. Therefore, the aim of this study was to compare the effects of breastmilk and infant formulae on neonatal gut microbiome in terms of fecal pH, fecal bacterial load, mode of delivery, as well as weight outcomes.

MATERIALS AND METHODS

Study Population

Healthy term neonates who presented for routine immunization, birth certification, and medical follow-up were targeted for this randomized culture-dependent study. Inclusion criteria involved neonates younger than two weeks of age, without prematurity, antibiotic exposure, or perinatal morbidities like sepsis, asphyxia, and congenital abnormalities. A total of 46 neonates, consisting of 33 exclusively breastfed, 10 formula-fed, and 3 mix-fed neonates, were recruited from the vicinity of Turai Umaru 'Yar'adua Maternity and Children Hospital (TUYMCH) and Federal Teaching Hospital (FTH) Katsina, with prior acquisition of signed consent from informed parents. No external influence was exerted on parents' choice of feeding method.

Collection of Fecal Samples

Faecal samples were collected from retrieved soiled diapers as soon as spontaneous defecation was noticed. Pre-collection evaluation for exclusion of contaminated samples included the assessment of topical application of diaper rash remedies such as medicated powders, sheer butter, antimicrobials, or heavy skin treatments that could potentially alter faecal sample pH or its microbial integrity.

Determination of Fecal pH

Potable pH meter (LPPCOLTD®, model number GLH-LK006, with a readability range of 0.01-14.00) equipped with Hamilton's glass electrode was utilized to determine fecal pH following the creation of a 10% fecal suspension (wt./v) in distilled water.

Culture and Enumeration

Following logarithmic dilution of fecal samples with distilled water from 10^{-1} to 10^{-6}, we inoculated 0.2 ml from dilutions 10^{-5} and 10^{-6} onto De Man-Rogosa-Sharpe (MRS) agar, Eosin Methylene Blue (EMB) agar, Mannitol Salt Agar (MSA), and MRS (+ L-Cystine supplement) agar in duplicates. These selective media were chosen to isolate *Lactobacillus spp.*, *Escherichia spp.*, *Staphylococcus spp.*, and *Bifidobacterium spp.* from the cultured samples. Colonies were then enumerated and quantified as Colony Forming Units (CFU) per 1g of wet fecal content. Bacterial isolates were confirmed using the VITEK 2® bacterial identifier with Gram-positive cocci and Gram-negative bacilli cartridges (ID-GPC and ID-GNB) at an accuracy level of 85-90%.

Statistical Analysis

SPSS v25 for Windows was utilized for intergroup analysis of findings. One-way ANOVA was employed to analyze differences among all three feeding groups. Further post-hoc analysis of significant *p*-values was carried out using the tukey HSD tool. Additionally, the chi-square (x^2) test was used to analyze the relationship between mode of delivery and feeding groups.

RESULTS

A total of 46 newborns were enrolled in this study, comprising 21 males and 25 females, with a mean age of 2.51 ± 0.62 weeks (range; 2–4 weeks). There were no significant differences in age and gender distribution among the feeding groups (p = 0.5 and 0.19, respectively). The average weight of the study participants was 2.88 ± 0.52 kg (range; 1.9–4.0 kg), with a significant correlation found between weight and the feeding method (p = 0.03). All values are presented as mean ± standard deviation (SD) in Table 1.

A total of 26 participants were recruited from the premises of TUYMCH, while the remaining 20 were recruited at FMC Katsina. NAN® (n=7) was observed to be the preferred formula brand of choice (61%) among mothers of newborns on

formulae and combined feeding (n=13) (Table 2).

The average fecal pH of exclusively breastfed infants was 5.09 ± 0.06, which was significantly lower (p < **0.001)** than the formula-fed group (5.9 ± 0.09). **Significant** differences (p **< 0.001)** in fecal pH were observed among all feeding groups **in** multiple inter-group comparisons. The pH values measured for BFI and FFI groups clustered around the mean, leading to a low standard deviation (Table 3**).**

Table 1: Basic characteristics of study participants (n=46)

Group	(n)	Gender		Delivery mode		Age (weeks)	Weight (kg)
		Male	Female	VD	CS		
BFI	33	15	18	31	2	2.55 ± 0.67	2.99 ± 0.50
FFI	10	6	4	7	3	2.30 ± 0.48	2.70 ± 0.50
MFI	3	0	3	3	0	2.67 ± 0.58	2.30 ± 0.40
Total	46	21	25	41	5	2.51 ± 0.62	2.88 ± 0.52
p-values		[a]0.19				[b]0.5	[c]0.03

Keys:BFI: Breastfed Infants VD: Vaginal Delivery
FFI: Formula-fed Infants CS: Caesarean Section
MI: Mix-fed Infants

[a]Inter-group difference in gender was evaluated by the chi-square (x2) test.
b difference in age among feeding groups was tested by One-way ANOVA.
c difference in weight among feeding groups was tested by One-way ANOVA, followed by Tukey's post-hoc analysis

Table 2: Distribution of participants based on recruitment facility and formula choice

Feeding groups	TUYMCH Katsina	FTH Katsina		
BFI	20	13		
FFI	5	5	Formulae brands	n
			NAN1®	7
			Peak®	1
			My Boy®	1
			Friso®	1
MFI	1	2	Friso®	2
			NAN2®	1
Total	26	20		

Table 3: pH of Faecal Samples of The Study Participants

Feeding group	N	pH (mean ± SD)
BFI	33	5.09 ± 0.06
FFI	10	5.88 ± 0.09
MFI	3	5.48 ± 0.12

pH differences between the feeding groups were tested using One-way ANOVA, followed by Tukey Post-hoc analysis.
Differences in pH between and among all groups were statistically significant ($p < 0.001$).

The selectively-cultivated bacteria (*Bifidobacterium spp.*, *Staphylococcus spp.*, *Escherichia spp.*, and *Lactobacillus spp.)* were present in all feeding groups. *Bifidobacterium spp.* showed a higher count in breastfed neonates (61.38 CFU/g) compared to formula-fed neonates (40.90 CFU/g), *although the difference was not* statistically significant ($p = 0.07$). Among BFI, nearly equal counts of *Escherichia spp.* (61.17 CFU/g) *and Bifidobacterium spp.* (61.38 CFU/g) were observed, with *Staphylococcus spp.* being the dominant species (81.67 CFU/g) in the group. A similar dominance of Staphylococcus spp. (69.80 CFU/g) was seen in the FFI group (Table 4). Apart from the respective counts of *Escherichia spp. among the feeding groups* ($p = 0.01$), no significant differences were found in the counts of other cultivated bacteria across the groups. Further post-hoc analysis (Tukey HSD) revealed significant differences in *Escherichia spp.* counts between BFI and MFI ($p = 0.01$), as well as FFI and MFI ($p = 0.02$) *(Fig 1)*.

Table 4: Composition of selected microbiota among feeding groups

Bacterial genera	Unit	BFI (n=33)	FFI (n=10)	MFI (n=3)	#p-value
Staphylococcus spp.	(CFU/g)	81.67 ± 83.30	69.80 ± 14.74	47.33 ± 20.04	0.69
	(%)	32.5	34.4	13.8	
Escherichia spp.	(CFU/g)	61.17 ± 33.19	62.15 ± 19.69	120.8 ± 43.40	0.01
	(%)	24.3	30.6	35.1	
Lactobacillus spp.	(CFU/g)	47.17 ± 44.01	30.30 ± 7.74	45.33 ± 16.24	0.48
	(%)	18.8	14.9	13.1	
Bifidobacterium spp.	(CFU/g)	61.38 ± 63.52	40.90 ± 15.81	130.7 ± 70.00	0.69
	(%)	24.4	20.1	38.0	
	Total				
	(CFU/g)	251.39	203.15	344.16	
	(%)	100	100	100	

#Differences between all feeding groups were tested using One-way ANOVA, followed by Tukey's Post-hoc analysis.

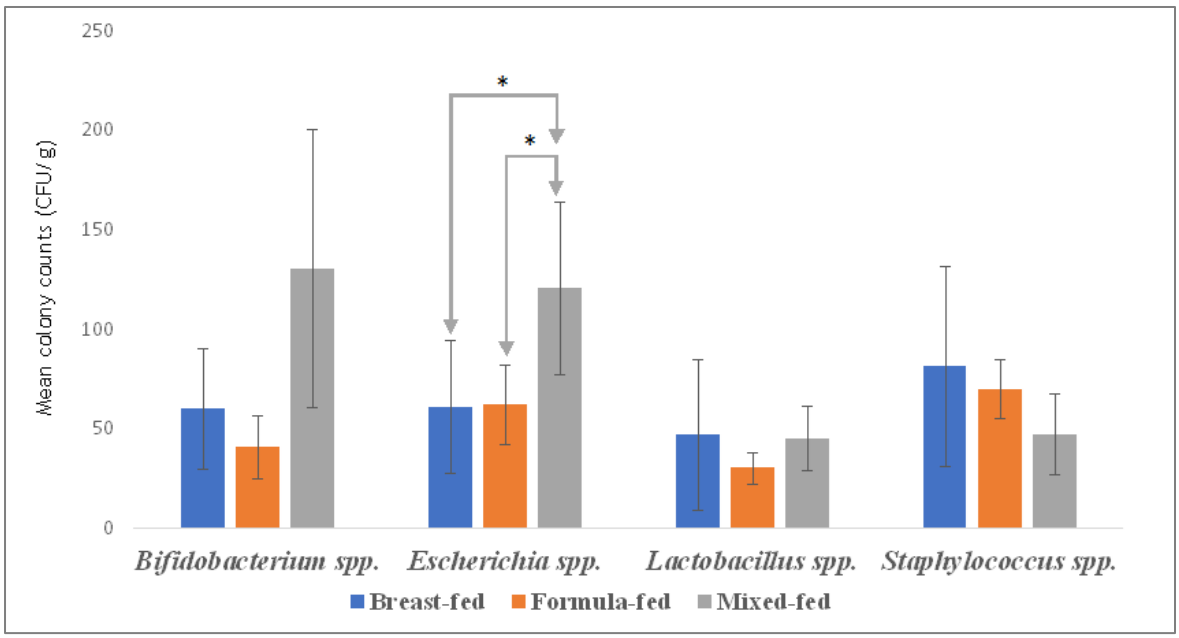

Figure 1: Bacterial Colony Counts (CFU/g) for Each Feeding Group

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Figure 1: Clustered column distribution showing mean colony counts (CFU/g) against cultivated species for each feeding group. ***** Denotes statistically significant difference between feeding groups ($p < 0.05$). Differences between the remaining microbial loads by feeding groups were statistically insignificant ($p > 0.05$)

There was no significant correlation ($p > 0.05$) in the cultivated bacterial load between and among the feeding groups (breastfed and formula-fed) and modes of delivery (vaginal and caesarean) in this study.

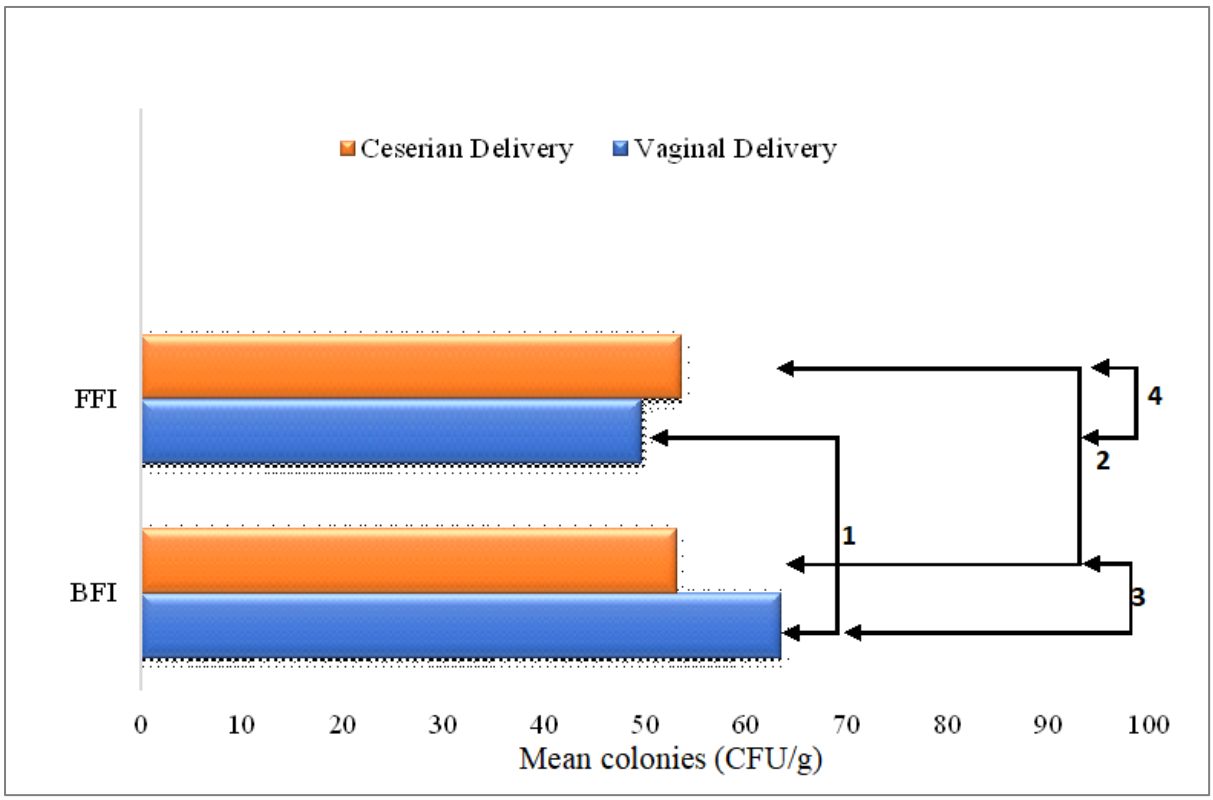

Figure 2: Relationship Between Infant Feeding Methods and Modes of Delivery
Figure 2: Clustered column chart showing relationship between feeding types, mode of delivery, and average bacterial load. Numbers **1-4** indicate *p*-values for inter-group comparison (**1** = 0.37; **2** = 0.96; **3** = 0.72; **4** = 0.56) tested by independent samples *t*-Test.

DISCUSSION

The importance of infant gut microbiota has been highlighted, not only for intestinal health but also for long-term health into adulthood (Fjalstad *et al.*, 2018). This study, which relied on culture-dependent methods, aimed to assess the impact of infant feeding practices on specific bacterial loads through fecal analysis. Additionally, fecal pH and weight, as anthropometric outcomes, were also examined and discussed given their consistent relevance.

Newborns recruited in this study were between the ages of 2-3 weeks at the time of sample collection, with no significant difference in age ($p = 0.5$) among feeding groups (Table 1). This finding aligns with a previous study of 91 infants ($p = 0.342$) by Ma *et al.*, (2020). Consistent with the results of Indrio *et al.*, (2007) in a study of 90 breastfed and formula-fed neonates, there was no significant difference ($p = 0.19$) in gender among feeding groups in our study. This specific age group was chosen to minimize dissimilarities between exclusively breastfed infants by ensuring uniform exposure to transitional breastmilk.

During infancy, weight is one of the most important anthropometric parameters for assessing nutritional status, with centile charts being the best monitoring tool (Marques *et al.*, 2004). The mean weight of the study participants was 2.88 ± 0.52 kg (range; 1.9-4.0 kg), showing a significant correlation between weight and feeding method ($p = 0.03$) (Table 2). This differs from the findings of Otaigbe *et al.* (2008), who reported an average weight of 3.76±0.57kg in their study of 309 newborns

delivered at the University of port harcourt Teaching Hospital. The discrepancy can be attributed to not only geographical variations between the study locations but also ethno-cultural differences among the study populations. The north-south weight dichotomy has been well-established by Fayehun and Asa (2020) in a retrospective study of 9,244 live births between 2008 and 2018. In our study, exclusively breastfed infants weighed significantly more (p = 0.03) than formula-fed and mixed-fed infants. Similarly, a UK millennium cohort study of 10,533 3-year-olds from infancy revealed a significant association between feeding type and weight, with formula-fed children showing higher average weight gain over time compared to their breastfed counterparts (Griffiths *et al.*, 2009). This suggests a potential long-term impact of formula feeding in contrast to the limited scope of our study.

Faecal pH has long been established as a key predictor of infant gut health, as well as pathogenic derangements in gut microbiome balance (Duar *et al.*, 2020). The neonatal gut acidity corresponding to specific feeding method also plays a significant role in gut microbiome shaping (Wang *et al.*, 2020). The findings from this study show the average faecal pH of exclusively breastfed infants to be 5.09 ± 0.06, significantly lower (p = <0.001) than the formula-fed group (5.9 ± 0.09). A similar degree of pH variation was previously reported by Indrio *et al.* (2007)after faecal analysis of 90 three-day-old newborns (30 of which were exclusively breastfed). It is known that lower faecal pH is driven by the fermentative bioactivity of *Bifidobacterium* spp. and has a significant role in protection against intestinal inflammation, as well as defense against pathogenic bacteria (Duar *et al.*, 2020).

There was a significant relationship (p <0.001) between exclusively breastfed and formula-fed newborns concerning CS delivery (Figure 2). This differs from the results of Bäckhed *et al.*,(2015) in a cohort study of Swedish infants during the first year of life; the microbes that newborns are exposed to during delivery are notably influenced by the mode of birth, where vaginal delivery exposes newborns to the commensals of the mother's birth canal, leading to variations in microbial composition. This discrepancy in findings may be attributed to the smaller number of participants examined in this study within a specific timeframe. Additionally, a recent study of 36 four-day-old newborns using 16S rRNA sequencing demonstrated a significantly higher diversity of bacteria in vaginally-delivered newborns compared to their caesarean-delivered counterparts (Akagawa *et al.*, 2019).

CONCLUSION

The findings in this study suggest that despite the recent supplementation of infant formulae with bifidogenic (pre and probiotic) substances, differences with breastmilk, although narrowed, still exist. Therefore, breast milk, whose beneficial health effects are undoubtedly unique, remains the recommended food of choice for infants in the first six months of life in the Katsina metropolis. Furthermore, findings from this study revealed no definitive advantages conferred by formula-feeding over breastfeeding in terms of infant weight and fecal acidity.

LIMITATION

In this study, only a relatively small number of newborns were identified as formula-fed within the research timeframe due to the COVID pandemic, thereby limiting the presentation of newborns at hospital facilities. Moreover, the grouping of participants was based on self-report, which is a potential source of measurement bias where mothers may incorrectly recall details of every feed used. Additionally, certain confounding factors such as the mother's socio-economic status, BMI, and parity, which are known to affect infant weight gain and nutritional outcomes, were not analyzed in this study. Finally, formula-fed newborns were allowed to receive their parents' chosen brands of infant formulae without any direct or indirect influence on their choices, therefore, cause-effect relationships could not be established in this study.

RECOMMENDATION

This study serves as a foundation for a more in-depth exploration of the diversity of the gut microbiota and lays the groundwork for future local investigations into the impacts of various infant feeding practices on neonatal health and well-being. Thus, additional extensive research is essential to more precisely delineate the

effects and health consequences of distinct feeding methodologies. Such efforts could aid in the development of local and national policies aimed at endorsing optimal infant feeding practices. Furthermore, systematic growth monitoring by healthcare professionals utilizing growth charts and WHO reference standards will be instrumental in overseeing and assessing the effectiveness of these policies.

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