4 Feeding Intolerance and Faecal Calprotectin in Preterm Neonates on 3 Different Feeding Protocols: A Comparative Study

By

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ABSTRACT

Background: Feeding intolerance and Necrotizing Enterocolitis (NEC) remain significant challenges in the care of preterm neonates, contributing substantially to morbidity and mortality in these vulnerable preterms.

Objective: This study aimed to compare the effects of breast milk, preterm formula, and hydrolyzed formula on feeding intolerance, fecal calprotectin levels and NEC development in preterm neonates.
Patients and Methods: This prospective study was conducted over six months in the Neonatal Intensive Care Unit (NICU) at Children's Hospital, Ain Shams University, involving 45 preterm infants. Patients were divided into three groups (n=15 each): Group I (mainly breastfed), Group II (preterm formula with ≤30%breast milk), and Group III (hydrolyzed formula with ≤30% breast milk). Demographic, natal, anthropometric data and abdominal X-ray findings were collected and fecal calprotectin levels were measuredat two points: before initiating enteral feeding and after achieving full enteral intake or upon NEC diagnosis. Faecal Calprotectin was quantified using the **fCAL turbo test, a particle-enhanced turbidimetric immunoassay done on cobas c 503 analyzer.**
Results: Baseline characteristics were comparable across all groups. All groups showed significant increases in abdominal circumference and feeding volume over time, but only Group I (breastfed) demonstrated significant weight gain. Group III (hydrolyzed formula) showed a significant decrease in gastric residuals. Crucially, fecal calprotectin levels were significantly lower in Group I ($p=0.016$), with a highly significant greater percentage reduction ($p=0.007$) compared to both formula groups. Calprotectin levels correlated negatively with gestational age and anthropometrics and positively with delayed feeding initiation and meconium passage across groups.
Conclusion: Preterm babies who are mainly breastfed are less likely to develop feeding intolerance and NEC. This highlights the crucial anti-inflammatory benefits of breast milk, advocating for its prioritization in preterm infant nutrition.
Keywords: Preterm Neonates, Feeding Intolerance, Fecal Calprotectin, Breast Milk, Necrotizing Enterocolitis, Formula Feeding.

Introduction

Feeding intolerance (FI) is defined as difficulty to digest enteral feedings and is accompanied by an increase in gastric residuals, abdominal distension, and/or reflux***(Cresi and Maggiora, 2018)***.The common causes of FI in preterm infants include low intestinal motility, enzymatic digestion, bacterial colonization, hormonal response, and local immunity ***(Albraik et al., 2022)***. It is very common among preterm infants and is the main obstacle against progression and early achievement of full enteral intake ***(Embleton et al., 2023)***.Feeding intolerance occurs in the early stage of NEC formerly known as grade 1 according to BELL's score ***(Bell et al., 1978)***.

Trophic feeding and early advance of enteral feeding by maternal own milk (MOM) protect preterm infants from development of feeding intolerance ***(Alshaikh et al., 2021).***Breast feeding is the single best feeding option especially in preterms as it plays an important role in development as well as reducing the risks of NEC, sepsis and mortality ***(Zukova et al., 2021).***Insufficient amount of breast milk and its improper storage are the main obstacles hindering its use ***(Wilde, 2021)***.

Preterm formulas were developed to meet the relatively high protein, energy, and mineral requirements which are necessary to support the rate of growth in the preterm infant.Preterm formula has many disadvantages as it is associated with high incidence of severe gut disorders, NEC and other infections(***Hay and Hendrickson, 2017)***.

When human milk is unavailable, hydrolyzed formulas may be used empirically (starterformula) or therapeutically to improve feeding tolerance or reduce gastro-esophageal reflux. The possible reasons for these effects includethe accelerated gastric emptying and intestinal transit, more efficient enteric peptide digestion and stimulation of small intestinal enzymatic and motile activity ***(Ng et al., 2017).***Yet, it has lower calories intake (70 kcal/100ml) compared to preterm formula ***(D'Auria et al.,2021)***and might result in lower gain.

Fecal calprotectinis a biomarker used for diagnosis of NEC and has many advantages as it is fast, accessible, not invasive, cheap and has high sensitivity for NEC ***(Pathirana et al., 2018).*** Studies showed that the sensitivity and specificity of faecal calprotectin as a diagnostic marker were 76-100% and 39-96.4%, respectively ***(Wang et al., 2019).*** Yet, it has many disadvantages as increased levels are also seen in gastrointestinal malignancies, infections, polyps and with the use of non-steroidal anti-inflammatory drugs ***(Suryani et al., 2018)***.

Patients and methods:

Ethical Consideration:

1- Approval was obtained from the Scientific Research Ethics Committee at the Faculty of Medicine, Ain Shams University, before starting work on the study.
2- Written consent was taken from the legal guardians of the patient.
3- Confidentially of the data was done by using a code number for every participant.
4- The authors received no financial support for the research, authorship, and/or publication of this article.
5- No conflict of interest regarding study or publications.
6- All parents of participating neonates had the rights to accept or refuse to participate in this study.

Sample Size Calculation:

The sample size was calculated using the G*Power software, with an effect size of 0.40, power of 80%, and alpha error of 0.05. This yielded a required sample of 45 patients and 15 neonates per group to detect a significant difference in faecal calprotectin level using One Way ANOVA test ***(Arisanti and Wibowo, 2019).***

Inclusion Criteria :

Preterm neonates with gestational age ranging from 27 week to less than 37week admitted from first day of life before initiation of feeding.

Exclusion Criteria:

- Intra uterine growth retardation
- Full term babies
- All babies with congenital gastrointestinal anomalies, bowel surgery, inborn error of metabolism and Hypoxic-ischemic encephalopathy (HIE).
- Contraindications of enteral intake.

Study Procedure:

This Prospective study was carried out through the period from December 2024 to june 2025in the neonatal intensive care unit (NICU), Children's Hospital, Ain Shams University

Preterm babies who were admitted to NICU, Children's Hospital, AinShams University.

They were classified into 3 groups by simple random method

First group: were mainly breast fed.

Second group: were preterm formula fed and breast milk doesn't exceed 30% from daily fed.

Third group: Were hydrolyzed formula fed and breast milk doesn't exceed 30% from daily fed.

All studied cases were subjected to:

1. **Full history taking:**

Antenatal, Natal, Postnatal history: Including: maternal disease during pregnancy, parity, mode of delivery, causes of preterm labor and resuscitation data including: APGAR score at 10 min.

2. **Examination:**

- General assessment (vital signs, anthropometric measurement: Weight, length, head circumference)

- Systemic examination: Cardiovascular, Chest, Abdominal examination and Complete neurological examination**.**

All groups were daily followed up by the following parameters:

1. Weight measurement.
2. Abdominal examination (abdominal girth measurement, intestinal sound auscultation, abdominal palpation for any organomegaly).
3. Mode of feeding and average increase of feeding.
4. Total parenteral nutrition (TPN) if given.
5. Recording of average bowel motion /day.
6. Recording of feeding intolerance as (abdominal distension, residual, vomiting, melena and no intestinal sound).

3. Laboratory investigations:

- CBC analysis was performed using an automated hematology analyzer which utilizes Coulter Principle for cell counting and flow cytometry for leukocyte differentiation.
- CRP concentrations were determined using ELISA kits.
- Quantitative measurement of fecal calprotectin in small stool sample at two time points: one before initial enteral feeding and the other one after reaching full enteral intake or diagnosis of NEC(at day 6).

Fresh stool samples were collected from diapers of studied neonates in sterile containers. Faecal samples were extracted and diluted to a final concentration of 1:500 using the CALEX® cap. The prepared extracts were stored at the ASU laboratory till the analysis at 2-8 °C up to 3 days or frozen (-20°C) for long-term storage**.** Faecal calprotectin assay was determined by particle-enhanced turbidimetric immunoassay on Cobas c503 analyzer (Roche Diagnostics) using the fCAL turbo kit (Bühlmann Laboratories, Switzerland). The assay is based on antigen-antibody reactions forming insoluble complexes, with turbidity directly proportional to calprotectin concentration. Calibration was performed with kit-provided calibrators. The measuring range for the fCAL turbo assay on the cobas c 503 analyser was 30-2000 µg/g.

4. Radiological Evaluation: Abdominal X-ray when NEC is suspected.

Statistical analysis:

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp). The quantitative data were presented as mean, standard deviations and ranges when parametric and median, inter-quartile range (IQR) when data found non-parametric. Also, qualitative variables were presented as number and percentages.

The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following:

P-value > 0.05: Non-significant (NS)

P-value < 0.05: Significant (S)

P-value < 0.01: Highly significant (HS).

Results: Table (1): demographic data and clinical data of all the studied patients

		No. = 45
Gender	Female Male	22 (48.9%) 23 (51.1%)
PROM	No Yes	27 (60.0%) 18 (40.0%)
Preeclampsia	No Yes	28 (62.2%) 17 (37.8%)
Diabetes	No Yes	35 (77.8%) 10 (22.2%)
Vaginal bleeding	No Yes	25 (55.6%) 20 (44.4%)
Gestational age	Mean±SD Range	32.67 ± 2.17 29 – 36
Mode of delivery	CS NVD	29 (64.4%) 16 (35.6%)
APGAR at 10 min	Median (IQR) Range	9 (8 - 9) 7 – 9
Respiratory support	NC HFNC NCPAP MV	3 (6.7%) 8(24.4%) 23 (51.1%) 11 (24.4%)
Start of feeding (hours)	Median (IQR) Range	48 (36 - 48) 12 – 60
First pass meconium (hours)	Median (IQR) Range	24 (12 - 36) 1 – 48
Weight (kg)	Mean±SD Range	1.87 ± 0.5 0.8 - 2.5
Length (cm)	Mean±SD Range	43.13 ± 4.47 35 – 49
Abdominal girth (cm)	Mean±SD Range	26.09 ± 1.4 24 – 29

Table 1: this table show the demographic and clinical data of all the studied patients**.**

Table (2): Follow up of the studied parameters in group I (breast fed)

Group I		Day 1 No. = 15	Day 2 No. = 15	Day 3 No. = 15	Day 4 No. = 13	Day 5 No. = 11	Day 6 No. = 5	Test value	P-value	Sig.
Weight (kg)	Mean ± SD Range	1.87 ± 0.52 0.7 – 2.46	1.84 ± 0.51 0.7 – 2.43	1.80 ± 0.51 0.72 – 2.38	1.83 ± 0.42 1 – 2.35	1.79 ± 0.43 1 – 2.37	1.97 ± 0.44 1.32 – 2.39	8.249	0.036	S
Abdominal gith (cm)	Mean ± SD Range	25.73 ± 1.10 24 – 27	25.80 ± 1.21 24 – 28	26.13 ± 0.92 25 – 27	26.08 ± 1.12 25 – 28	25.73 ± 1.19 24 – 28	26.60 ± 0.89 26 – 28	2261.37•6	0.000	HS
Intestinal sound	Absent Audible	0 (0.0%) 15 (100.0%)	0 (0.0%) 15 (100.0%)	1 (6.7%) 14 (93.3%)	0 (0.0%) 13 (100.0%)	1 (9.1%) 10 (90.9%)	1 (20.0%) 4 (80.0%)	6.066*	0.532	NS
Pass stool	No 1 times 2 times 3 times	4 (26.7%) 3 (20.0%) 8 (53.3%) 0 (0.0%)	3 (20.0%) 7 (46.7%) 4 (26.7%) 1 (6.7%)	2 (13.3%) 4 (26.7%) 8 (53.3%) 1 (6.7%)	0 (0.0%) 3 (23.1%) 8 (61.5%) 2 (15.4%)	1 (9.1%) 3 (27.3%) 4 (36.4%) 3 (27.3%)	1 (20.0%) 1 (20.0%) 3 (60.0%) 0 (0.0%)	20.056*	0.517	NS
Mode of feeding	Ryle Oral	15 (100.0%) 0 (0.0%)	15 (100.0%) 0 (0.0%)	14 (93.3%) 1 (6.7%)	10 (76.9%) 3 (23.1%)	8 (72.7%) 3 (27.3%)	4 (80.0%) 1 (20.0%)	9.462*	0.221	NS
Increasing of feeding (ml/kg)	Mean ± SD Range	16.67 ± 4.88 10 – 20	18.67 ± 5.16 10 – 30	20.00 ± 5.55 10 – 30	23.08 ± 6.30 10 – 30	21.82 ± 6.03 10 – 30	20.00 ± 0.00 20 – 20	176.333•	0.001	HS
Abdominal Distension	No Yes	15 (100.0%) 0 (0.0%)	14 (93.3%) 1 (6.7%)	14 (93.3%) 1 (6.7%)	13 (100.0%) 0 (0.0%)	10 (100.0%) 0 (0.0%)	4 (80.0%) 1 (20.0%)	5.330*	0.619	NS
Residual	No Yes	15 (100.0%) 0 (0.0%)	14 (93.3%) 1 (6.7%)	13 (92.9%) 1 (7.1%)	12 (100.0%) 0 (0.0%)	7 (100.0%) 0 (0.0%)	3 (75.0%) 1 (25.0%)	5.933*	0.547	NS

Table (2): demonstrates that in **Group I**, the **mean weight (kg)** decreased from day 1 [1.87 ± 0.52] to day 5 [1.79 ± 0.43] but then significantly increased by day 6 [1.97 ± 0.44] ($p = 0.036$). The **abdominal girth** also showed a highly significant increase by day 6 [26.60 ± 0.89] compared to day 1 [25.73 ± 1.10] ($p < 0.001$). Similarly, the **mean feeding increase (ml/kg)** rose progressively, peaking at day 4 [23.08 ± 6.30] and remaining significantly higher by day 6 [20.00 ± 0.00] than at baseline [16.67 ± 4.88] ($p = 0.001$).In contrast, **intestinal sound presence**($p = 0.532$),**pass stool frequency** ($p = 0.517$), **mode of feeding** ($p=0.221$),**abdominal distension**($p = 0.619$),and **residual**($p = 0.547$) showed no statistically significant differences across follow-up days.

Table (3): Follow up of the studied parameters in group II (premature formula fed)

Group II		**Day 1 No. = 15**	**Day 2 No. = 15**	**Day 3 No. = 15**	**Day 4 No. = 13**	**Day 5 No. = 9**	**Day 6 No. =8**	**Day 7 No. = 2**	**Day 8 No. = 1**	**Test value**	**P-value**	**Sig.**
Weight	Mean ± SD	1.75 ± 0.49	1.72 ± 0.49	1.68 ± 0.48	1.77 ± 0.46	1.81 ± 0.39	1.81 ± 0.44	1.91 ± 0.01	1.93 ± 0.00	0.574	0.479	NS
	Range	0.85 – 2.49	0.83 – 2.47	0.8 – 2.39	0.8 – 2.35	1.11 – 2.25	1.05 – 2.27	1.9 – 1.92	1.93 – 1.93			
Abdominal girth (cm)	Mean ± SD	25.80 ± 1.42	25.80 ± 1.37	26.13 ± 1.46	26.58 ± 1.16	26.11 ± 0.93	26.25 ± 0.89	25.00 ± 0.00	25.00 ± 0.00	5143.744•	0.000	HS
	Range	23 – 28	23 – 28	24 – 29	25 – 29	25 – 27	25 – 27	25 – 25	25 – 25			
Intestinal sound	Absent	0 (0.0%)	0 (0.0%)	1 (6.7%)	1 (8.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3.800*	0.802	NS
	Audible	15 (100.0%)	15 (100.0%)	14 (93.3%)	11 (91.7%)	9 (100.0%)	8 (100.0%)	1 (100.0%)	1 (100.0%)			
Pass Stool	No	0 (0.0%)	3 (20.0%)	7 (46.7%)	4 (33.3%)	0 (0.0%)	1 (12.5%)	0 (0.0%)	0 (0.0%)	31.107*	0.071	NS
	1 times	9 (60.0%)	6 (40.0%)	3 (20.0%)	4 (33.3%)	4 (44.4%)	0 (0.0%)	1 (100.0%)	0 (0.0%)			
	2 times	6 (40.0%)	6 (40.0%)	4 (26.7%)	4 (33.3%)	5 (55.6%)	7 (87.5%)	0 (0.0%)	1 (100.0%)			

	3 times	0 (0.0%)	0 (0.0%)	1 (6.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			
Mode of feeding	Ryle	15 (100.0%)	14 (93.3%)	14 (93.3%)	11 (91.7%)	8 (88.9%)	6 (75.0%)	1 (100.0%)	1 (100.0%)	4.870*	0.675	NS
	Oral	0 (0.0%)	1 (6.7%)	1 (6.7%)	1 (8.3%)	1 (11.1%)	2 (25.0%)	0 (0.0%)	0 (0.0%)			
Increasing of feeding (ml/kg)	Mean ± SD	14.67 ± 5.16	16.67 ± 4.88	18.33 ± 5.77	22.00 ± 6.32	23.33 ± 5.00	25.00 ± 5.35	20.00 ± 0.00	20.00 ± 0.00	255.769•	0.000	HS
	Range	10 – 20	10 – 20	10 – 30	10 – 30	20 – 30	20 – 30	20 – 20	20 – 20			
Abdominal Distension	No	15 (100.0%)	13 (86.7%)	11 (73.3%)	8 (66.7%)	8 (100.0%)	8 (100.0%)	1 (100.0%)	1 (100.0%)	11.538*	0.116	NS
	Yes	0 (0.0%)	2 (13.3%)	4 (26.7%)	4 (33.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			
Residual	No	15 (100.0%)	14 (100.0%)	12 (85.7%)	9 (81.8%)	6 (100.0%)	6 (100.0%)	1 (100.0%)	1 (100.0%)	7.479*	0.380	NS
	Yes	0 (0.0%)	0 (0.0%)	2 (14.3%)	2 (18.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			

Table (3) demonstrates that in **group II**, the mean **abdominal girth** high significantly increased by day 6 [26.25 ± 0.89] compared to day 1 [25.80 ± 1.42], with p-value <0.001. Similarly, the **mean feeding increase (ml/kg)** rose progressively from day 1 [14.67 ± 5.16] to day 6 [25.00 ± 5.35], showing a significant upward trend (p <0.001). No significant changes were observed for **weight** (p = 0.479), **intestinal sound presence** (p = 0.802), **pass stool frequency** (p = 0.071), **mode of feeding** (p = 0.675), **abdominal distension** (p = 0.116), or **residual** (p = 0.380) across follow-up days.

Table (4): Follow up of the studied parameters in group III (hydrolysed formula fed)

Group III		Day 1 No. = 15	Day 2 No. = 15	Day 3 No. = 15	Day 4 No. = 13	Day 5 No. = 13	Day 6 No. = 9	Day 7 No. = 2	Day 8 No. = 2	Test value	P-value	Sig.
WT	Mean ± SD Range	1.78 ± 0.53 0.86 – 2.47	1.74 ± 0.53 0.84 – 2.43	1.71 ± 0.52 0.82 – 2.38	1.80 ± 0.48 0.82 – 2.38	1.80 ± 0.47 0.8 – 2.35	1.69 ± 0.70 0 – 2.38	1.08 ± 0.36 0.82 – 1.33	1.09 ± 0.37 0.83 – 1.35	1.519	0.253	NS
Abdominal girth (cm)	Mean ± SD Range	25.87 ± 1.85 23 – 29	26.00 ± 1.73 23 – 29	26.33 ± 1.72 24 – 29	26.54 ± 1.27 24 – 29	26.62 ± 1.33 24 – 29	26.00 ± 1.00 24 – 27	25.00 ± 1.41 24 – 26	25.00 ± 1.41 24 – 26	1276.438•	0.018	S
Intestinal sound	Absent Audible	0 (0.0%) 15 (100.0%)	0 (0.0%) 15 (100.0%)	1 (6.7%) 14 (93.3%)	0 (0.0%) 13 (100.0%)	1 (7.7%) 12 (92.3%)	0 (0.0%) 9 (100.0%)	0 (0.0%) 2 (100.0%)	0 (0.0%) 2 (100.0%)	4.129*	0.764	NS
Pass stool	No 1 time 2 times 3 times	0 (0.0%) 6 (40.0%) 7 (46.7%) 2 (13.3%)	3 (20.0%) 5 (33.3%) 6 (40.0%) 1 (6.7%)	4 (26.7%) 5 (33.3%) 5 (33.3%) 1 (6.7%)	3 (23.1%) 1 (7.7%) 7 (53.8%) 2 (15.4%)	2 (15.4%) 4 (30.8%) 5 (38.5%) 2 (15.4%)	0 (0.0%) 1 (11.1%) 8 (88.9%) 0 (0.0%)	0 (0.0%) 1 (50.0%) 1 (50.0%) 0 (0.0%)	0 (0.0%) 1 (50.0%) 1 (50.0%) 0 (0.0%)	18.248*	0.633	NS
Mode of feeding	Ryle Oral	15 (100.0%) 0 (0.0%)	15 (100.0%) 0 (0.0%)	14 (93.3%) 1 (6.7%)	11 (84.6%) 2 (15.4%)	10 (76.9%) 3 (23.1%)	6 (66.7%) 3 (33.3%)	2 (100.0%) 0 (0.0%)	2 (100.0%) 0 (0.0%)	11.524*	0.117	NS
Increasing of feeding (ml/kg)	Mean ± SD Range	14.67 ± 5.16 10 – 20	15.00 ± 5.19 10 – 20	18.33 ± 5.77 10 – 30	20.00 ± 4.71 10 – 30	20.83 ± 6.69 10 – 30	24.44 ± 7.26 10 – 30	15.00 ± 7.07 10 – 20	20.00 ± 0.00 20 – 20	396.487•	0.000	HS
Abdominal Distension	No Yes	15 (100.0%) 0 (0.0%)	14 (93.3%) 1 (6.7%)	11 (73.3%) 4 (26.7%)	10 (76.9%) 3 (23.1%)	11 (84.6%) 2 (15.4%)	9 (100.0%) 0 (0.0%)	2 (100.0%) 0 (0.0%)	2 (100.0%) 0 (0.0%)	8.990*	0.253	NS
Residual	No Yes	15 (100.0%) 0 (0.0%)	15 (100.0%) 0 (0.0%)	10 (71.4%) 4 (28.6%)	11 (100.0%) 0 (0.0%)	10 (90.9%) 1 (9.1%)	6 (100.0%) 0 (0.0%)	2 (100.0%) 0 (0.0%)	2 (100.0%) 0 (0.0%)	14.722*	0.039	S

Table (4) illustrates that in **group III**; the **abdominal girth** increased significantly by day 5 [26.62 ± 1.33] compared to day 1 [25.87 ± 1.85] (p = 0.018). The **mean feeding increase (ml/kg)** also demonstrated a highly significant upward trend, rising from day 1 [14.67 ± 5.16] to day 6 [24.44 ± 7.26] (p < 0.001). Additionally, presence of **residual** decreased significantly from [4 (28.6%)] at day 3 and [1 (9.1%)] at day 5 and with no residual reported by day 6 in all remaining patients(p = 0.039).No statistically significant differences were observed for**weight** (p = 0.253) **intestinal sound presence** (p = 0.764), **pass stool frequency** (p = 0.633), **mode of feeding** (p = 0.117), or **abdominal distension** (p = 0.253) across the study period.

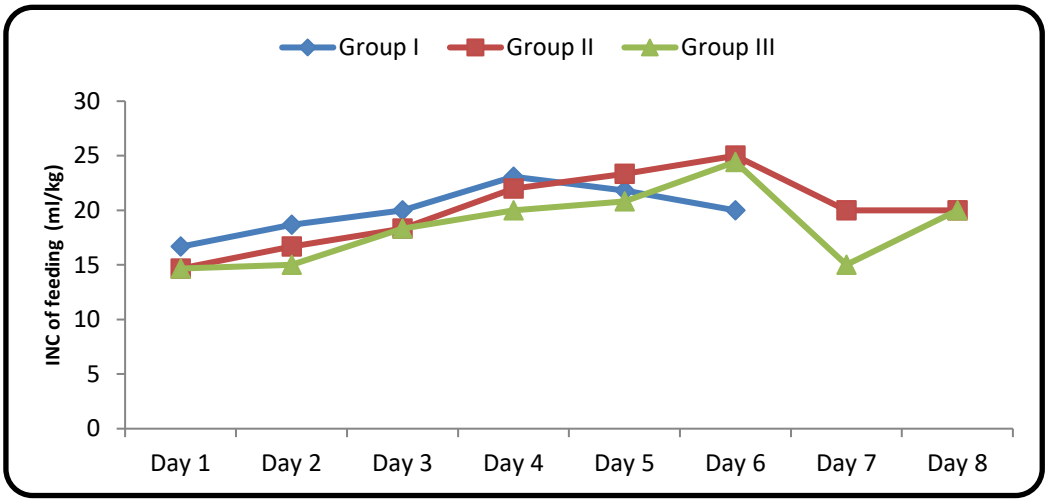

Figure (1): Comparison between the three studied groups regarding Increasing of feeding (ml/kg) at different times of measurements

Table (5): Comparison between the three studied groups regarding faecal calprotectin level at two time points: one before initial enteral feeding and the other one after reaching full enteral intake or diagnosis of NEC.

Calprotectin µg/g		**Group I**	**Group II**	**Group III**	**Test value**	**P-value**	**Sig.**
		No. = 15	**No. = 15**	**No. = 15**			
1stmeasurement	Median (IQR)	2050 (2000 – 2200)	2100 (2050 – 2150)	2100 (2050 – 2100)	0.603≠	0.740	NS
	Range	2000 – 2200	2000 – 2200	2000 – 2200			
2nd measurement	Median (IQR)	340 (250 – 500)	480 (420 – 1780)	500 (430 – 1760)	8.237≠	0.016	S
	Range	100 – 1820	250 – 1980	300 – 1950			
Range	Normal ≤ 600	13 (86.7%)	10 (66.7%)	11 (73.3%)	1.684	0.431	NS
	Abnormal > 600	2 (13.3%)	5 (33.3%)	4 (26.7%)			
Post hoc analysis							

	Group I VS Group II	Group I VS Group III	Group II VS Group III
Second	0.026	0.007	0.868

Table (5) reveals that **faecal calprotectin level before** enteral feeding, was similarly elevated across all groups, **but it** differed significantly between groups **after** reaching full enteral intake or NEC diagnosis (p = 0.016). **Group I** showed markedly lower post-intervention levels [median (IQR): 340 (250–500) compared to **group II** [480 (420–1780)] and **group III** [500 (430–1760**)**.Post hoc analysis confirmed significant differences between **group I vs. group II** (p = 0.026) and **group I vs. group III** (p = 0.007), but not between **group II vs. group III** (p = 0.868).

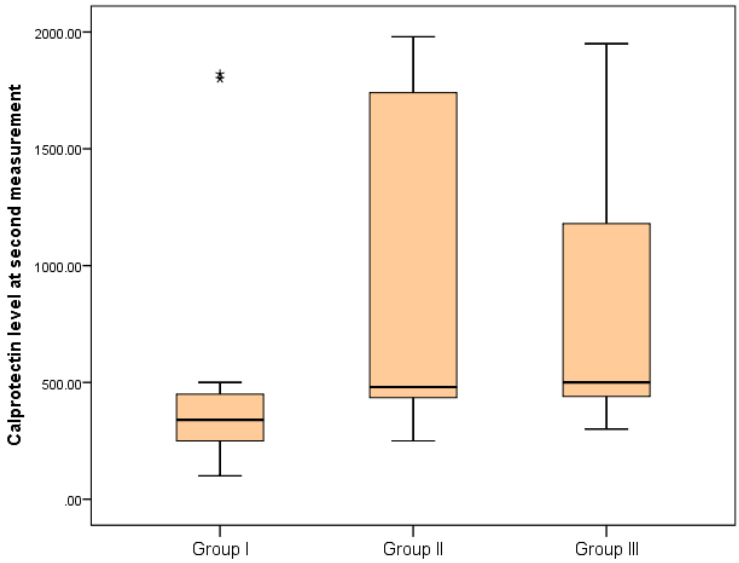

Figure (2): Comparison between the three studied groups regarding calprotectin level at second measurement

Table (6): Comparison between faecal calprotectin level at first and second measurements in each group

		First measurement	Second measurement	Test value	P-value	Sig.
Group I	Median (IQR)	2050 (2000 – 2200)	340 (250 – 500)	3.411	0.001	HS
	Range	2000 – 2200	100 – 1820			
Group II	Median (IQR)	2100 (2050 – 2150)	480 (420 – 1780)	3.408	0.001	HS
	Range	2000 – 2200	250 – 1980			
Group III	Median (IQR)	2100 (2050 – 2100)	500 (430 – 1760)	3.412	0.001	HS
	Range	2000 – 2200	300 – 1950			

Table (6) indicates a **significant decrease** in faecal calprotectin levels between the first and second measurements across **all groups** (p = 0.001) for each group.

Discussion

Feeding intolerance and its complication of necrotizing enterocolitis (NEC) contribute substantially to morbidity and mortality in these vulnerable preterms ***(Cuna et al;2018).***The immature gastrointestinal tract of preterm infants is particularly susceptible to inflammation and injury, making optimal enteral nutrition a critical factor in their development and overall health***(Indrio et al., 2022)***.For preterm neonates, the analysis of faecal calprotectin is important due to its ability to anticipate intestinal inflammation early. Hence, early diagnosis and timely intervention to avoid severe conditions like NEC***(YOON, 2014).***

This cross-section study included 45 preterms admitted in the Neonatal Intensive Care Unit (NICU) at Children's Hospital, Ain Shams University, following approval from the Research Ethics Committee of Ain Shams University Hospitals. Our patients were divided into three groups. The first group received mainly breast milk. The second group was primarily fed preterm formula while, the third group received hydrolyzed formulawith breast milk not exceeding 30% of their daily intake in the second and third group.

Our study revealed that the most common cause of preterm labor was premature contraction (46.7%), followed by PROM (40%).

Spontaneous preterm labor due to uterine contractions is a common cause globally, but its incidence and management differ widely. In a large multicenter study across **Europe**, spontaneous preterm labor accounted for over 40% of all preterm deliveries ***(Khodadadi et al., 2025)***.also, In South Africa, ***(Ramokolo et al., 2019)*** reported spontaneous contractions as the leading cause of preterm delivery, particularly in women with low socioeconomic status and high stress levels.

In a study conducted in **Nigeria** by **(*Oloyede and Akinlusi., 2020)***PROM accounted for 30% of preterm births, highlighting infections and inadequate antenatal care as major risk factors.Similarly, in contrast, in **high-income countries**, PROM still features prominently but is often better managed due to earlier detection and prophylactic interventions. A study from the **U.S.*(Panneflek et al., 2024)*** found PROM to be responsible for around 20–25% of preterm births.

Our study revealed that the median age of starting feeding was 48 hours postnatally ranging from 12 hours to 60 hours.

Similarly, a multicenter study in 2022 involving 210 preterm neonates admitted to neonatal intensive care units (NICUs) in Northwest Ethiopia found that the median time to initiate trophic feeding (TF) was 42 hours, Factors such as gestational age, APGAR score, and presence of respiratory distress syndrome significantly influenced the timing of feeding initiation***(Kebede et al., 2022).***

In contrast, A study in the **United States*(Monzon et al., 2023)***found that **early enteral feeding (<24 hours)** was standard practice in over 80% of NICUs, especially for infants >28 weeks' gestation, due to its benefits in **gut** maturation, shorter duration of parenteral nutrition, and reduced risk of **late-onset sepsis**.In the **UK**, the **National Neonatal Audit Programme (NNAP)** reported that **80–90% of preterm infants** received trophic feeds within 24 hours of

admission unless contraindicated ***(Ismail et al; 2022*)**.

Our study revealed that the median age of passing meconium was 24 hours ranging from 1 hours to 48 hours.

In a study by ***(Masi et al., 2019)*** in Italy, preterm neonates <34 weeks gestation had a mean time to meconium passage of 36–48 hours, with delays more common in infants with co-morbidities like respiratory distress syndrome (RDS) or sepsis.***(Patel et al., 2017)*** in the United States found that in preterm infants <32 weeks, meconium passage often occurred beyond 48 hours, especially in cases requiring mechanical ventilation or delayed feeding.Therefore, the median of 24 hours in our study reflects relatively early gastrointestinal activity, possibly due to prompt initiation of feeding or fewer early-onset complications in the sample group.

Our study found a statistically significant increase in the percentage of infants passing stool three times in Group III (hydrolysed formula) compared to other groups. This suggests that hydrolysed formula feeding may be associated with increased stool frequency in preterm infants. Increased stool frequency is often considered an indicator of enhanced gastrointestinal motility and digestion.

Similar findings have been reported by ***(Vivatvakin et al., 2020)***, who noted that hydrolysed protein formulas could improve digestion and stooling patterns, especially in infants with immature or sensitive gastrointestinal systems. However, stool frequency alone does not necessarily indicate optimal feeding tolerance, ***(Mysonhimer et al., 2022).***

Prior to enteral feeding, first fecal calprotectin levels were similarly elevated across all three groups (p=0.740). After reaching full enteral intake or NEC diagnosis, there was a statistically significant difference in calprotectin levels between the groups (p=0.016). A highly significant difference was observed in the percentage reduction of faecal calprotectin levels across the three groups (p=0.007), with Group I showing the largest median decrease.

faecal calprotectin levels in preterm infants have been reported to range widely, with values >2000 μg/g documented in the first 48–72 hours of life ***(Mihatsch et al., 2021)***. Such high early levels may reflect physiological inflammation as the immature gut mucosa adapts to extrauterine life. Additionally, these levels could indicate neutrophil activity associated with prenatal stress, hypoxia, or initial microbial exposure. Importantly, this initial elevation does not necessarily predict pathology, and longitudinal trends are likely more informative than single early measurements.

Some studies have suggested that persistently high or rising levels of calprotectin may correlate with the risk of developing NEC or sepsis ***(Thymann et al., 2023)***.

Thymann et al (2023)found no difference between maternal breast milk and hydrolyzed formula.**Yoon et al (2014)** found no effect of feeding type or method on FCP, while **Li et al (2014)** found FCP was higher in breastfed infants than formula-fed infants.

Moussa et al. (2016) noted a significant increase in FCP in the study group with feeding intolerance compared to the control group. agreeing with **Aydemir et al (2012),Yoon et al (2014)**, and **Frargy and Hassan (2014)** They also noted thatFCP levels rose around the time of initial signs of GI illness and highly increasing in NEC.

In our study findings; in 3 Groups, calprotectin levels were **negatively correlated with the increasing volume of feeding (ml/kg)**

Moussa et al. (2016) found no significant correlation between FCP level and volume of enteral feeding, which agreed with **Thymann et al. (2023)**. This, however, differed from **Costa et al. (2020)**, who reported that FCP levels decreased with increasing enteral feeding volume, and from **Xuet al. (2022)**, who found that FCP increased with increasing feeding volume.

<u>This study had some limitations as:</u>

- Relatively small sample size.
- The study was conducted at a single center, which may limit generalizability**.**

Conclusion

Preterm babies who are mainly breastfed are less likely to develop feeding intolerance and NEC. This highlights the crucial anti-inflammatory benefits of breast milk, advocating for its prioritization in preterm infant nutrition.

The study also reinforces the known associations between prematurity, feeding parameters, and intestinal health, as reflected by calprotectin levels.

Additionally, the study confirms the usefulness of fecal calprotectin as a non-invasive biomarker for monitoring intestinal inflammation and feeding tolerance in preterm neonates.

Recommendations

Based on the study's findings, here are the recommendations:

1. **Prioritize Breast Milk Feeding for Preterm Neonates:** The study strongly suggests that breast milk significantly reduces feeding intolerance, as indicated by the greater decrease in faecal calprotectin levels in the breastfed group compared to formula-fed groups.
2. **Advocate for Donor Human Milk when Mother's Own Milk is Unavailable:** Given the clear benefits of breast milk in modulating gut inflammation, if a mother's own milk is insufficient or unavailable, the use of donor human milk should be strongly considered as the preferred alternative.
3. **Monitor Faecal Calprotectin as a Marker of Gut Health:** Faecal calprotectin proved to be a valuable biomarker for assessing feeding intolerance and NEC
4. **Consider Individualized Feeding Protocols:** highlight the need for individualized feeding protocols for preterm neonates, adjusting based on clinical signs, growth patterns, and potentially calprotectin levels.

Authors contribution

Study conception and design (**Marwa El-Deeb**, **Basma Shehata**, **Sara Salem**)

Marwa: conceptualized the research question and designed the methodology; **Basma**: co-designed the study protocol and statistical analysis plan; **Sara**: contributed to refining the study objectives and methodology.

Data acquisition (**Yasmin El-gazar**, **Sara Salem**)

Yasmin: led patient recruitment and data collection; **Sara**: supported clinical assessments and data entry.

Data analysis and interpretation (**Marwa El-Deeb**, **Basma Shehata**)

Marwa: performed statistical analysis and generated key findings; **Basma:** contributed to interpretation and critical evaluation of results.

Writing – review & editing(Marwa El-Deeb,BasmaShehata)

Marwa, Basma: critically reviewed and revised the manuscript for intellectual content.

All authors reviewed and approved the final manuscript.

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