

Original Article

## COMPARATIVE EFFECTS OF BREAST MILK, COW MILK, AND NESTLÉ NAN PRO FORMULA ON ANTHROPOMETRIC CHARACTERISTICS, SERUM BIOCHEMICAL PARAMETERS, AND URINARY MINERAL EXCRETION IN INFANTS

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### ABSTRACT

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Infant feeding practices significantly influence growth and mineral metabolism during early life. This study compared the effects of breast milk, cow milk, and Nestlé NAN Pro formula on anthropometric measurements, serum biochemical parameters, and urinary mineral excretion among infants aged 6–12 months. A total of 90 healthy infants were equally divided into three feeding groups. Breastfed infants showed significantly higher body weight ( $8.6 \pm 0.17$  kg), body length ( $72.3 \pm 0.64$  cm), and head circumference ( $45.1 \pm 0.21$  cm) compared with infants receiving cow milk ( $p < 0.05$ ). Serum calcium ( $9.7 \pm 0.12$  mg/dL), magnesium ( $2.31 \pm 0.06$  mg/dL), and vitamin D levels ( $32.6 \pm 1.33$  ng/mL) were significantly higher in the breast milk group, whereas serum phosphorus ( $6.3 \pm 0.12$  mg/dL) and alkaline phosphatase ( $292.6 \pm 7.3$  IU/L) were elevated in the cow milk group ( $p < 0.05$ ). Similarly, urinary calcium ( $5.9 \pm 0.32$  mg/dL), urinary phosphorus ( $16.7 \pm 0.51$  mg/dL), and calcium/creatinine ratio ( $0.31 \pm 0.04$ ) were significantly greater among infants fed cow milk. In contrast, breastfed infants demonstrated comparatively lower urinary mineral excretion and a more favorable urinary pH. This study concluded that breastfeeding was associated with better growth and balanced mineral metabolism, while Nestlé NAN Pro formula showed outcomes closer to breast milk. Cow milk feeding during infancy was linked with altered biochemical and urinary mineral profiles. These findings support breastfeeding as the preferred feeding practice during the first year of life.

**Keywords:** Nutrition, vitamin D, calcium, Cow milk, Breast milk.

### 1. INTRODUCTION

Nutrition plays an important role in skeletal development, growth, and health during infancy (Pinto *et al.*, 2023). The first year of life is regarded as the critical period for rapid physical growth in infants, while the demand for nutrition also increases, particularly for minerals like calcium and phosphorus, which are involved in bone metabolism (Cunha *et al.*, 2015). During infancy, the mineral imbalance has a poor effect on growth and influences children to metabolic and skeletal problems in life (Norris *et al.*, 2008). Therefore, the intake and maintenance of minerals are critical for neuromuscular function, metabolic homeostasis, and normal bone mineralization (Yaşar *et al.*, 2018). Consequently, infant feeding is regarded as one of the major elements of nourishing and biological status throughout early childhood (Draper *et al.*, 2024).

Breast milk is an optimal source of nutrition for infants because of its high bioavailability of nutrients and immunological properties (Lokossou *et al.*, 2022). In addition, breast milk also contains sufficient macronutrients and ideal concentrations of phosphorus, calcium, magnesium, vitamin D, and bioactive factors that are involved in supporting bone formation and physiological growth (Szyller *et al.*, 2024). The calcium-to-phosphorus ratio in human milk is also involved in skeletal development and in greater mineral absorption (Radlović *et al.*, 2025). Moreover, breastfed infants exhibit enhanced

nutrient utilization and gastrointestinal tolerance in infants who received an alternate source of feeding in place of the mother's feeding (Jiang *et al.*, 2022). Breastfeeding has many benefits for infants as compared to other feeding sources due to the maternal, social, economic, and cultural factors (Rollins *et al.*, 2016). Cow milk is also commonly used in some populations, but its composition is different from human milk (Lajnaf *et al.*, 2023). It imposes a higher renal solute weight on juvenile kidneys due to the higher concentrations of protein, phosphorus, and certain minerals in the milk (Qasir *et al.*, 2025). The higher phosphorus intake in cow milk inhibits calcium homeostasis and contributes to variations in bone-related biochemical markers (Bergman *et al.*, 2009). The unmodified cow milk is associated with intestinal intolerance, micronutrient inequality, and hostile metabolic characteristics in infants (Manyi-Loh *et al.*, 2026).

The commercial infant milk, such as Nestlé NAN Pro also used as an alternative feeding option for the infants, which is manufactured by following the nutritional composition of human milk (Baker *et al.*, 2021). It is given to the infants when breastfeeding may not be feasible, and it is involved in the normal infant growth and development (Wallenborn *et al.*, 2021). It involved essential vitamins and minerals such as calcium, phosphorus, magnesium, and vitamin D, which improve the nutrient bioavailability and digestive tolerance while maintaining a suitable mineral balance (Hoque *et al.*, 2023).

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The biochemical responses observed differ from those observed with the commercial milk as compared to breastfeeding because of alterations in the nutrient composition and absorption effectiveness (Adhikari *et al.*, 2022). The biochemical parameters extensively used to assess the mineral absorption and bone health in infants it includes the serum calcium, serum phosphorus, alkaline phosphatase, magnesium, and vitamin D (Matejek *et al.*, 2025). Serum calcium and phosphorus are regarded as vital indicators of skeletal mineralization, while alkaline phosphatase activity, which indicates the bone turnover, and osteoblastic activity (Sardiwal *et al.*, 2013). Similarly, vitamin D is vital for maintenance of mineral homeostasis and calcium absorption (Baker *et al.*, 2021).

Urinary parameters provide important information regarding the mineral maintenance and excretion patterns, which include the urinary phosphorus, urinary calcium, and calcium-to-creatinine ratio (Nennich *et al.*, 2006). Any change in the urinary indicators can disrupt the calcium metabolism and increase renal mineral damage linked with dietary intake (Lokossou *et al.*, 2022). Several studies have examined the nutritional adequacy of breastfeeding and other formula feeding, but limited study available on the properties of breast milk, cow milk, and commercial formulas on both serum biochemical markers and urinary mineral excretion in infants (Sánchez *et al.*, 2021). It is necessary to determine the feeding practices that support the healthy growth and mineral maintenance during infancy. Therefore, the present study was designed to determine the effects of breast milk, cow milk, and Nestlé NAN Pro formula on anthropometric measurements, serum biochemical parameters, and urinary calcium/creatinine ratio between the infants aged 6–12 months.

## 2. METHODS AND MATERIALS

### Study Design and Participants:

This study was designed to compare the effects of different feeding practices on anthropometric measurements, serum biochemical parameters, and urinary mineral excretion between infants aged 6 to 12 months. Three groups were designed, and 90 healthy infants were selected between infants of 6 to 12 months from the pediatric outpatient clinics and community health centers in the Maternal and Pediatric Teaching Hospital, Soran, Erbil, Kurdistan Region, Iraq. 90 healthy infants were selected and divided into three groups: one group was based on breast milk feeding source, the second group was on cow milk, and the third group contained infants fed with Nestlé NAN Pro milk. Each group contained thirty infants, and the study ended after ninety days. Infants were divided based on their major milk feeding practice, started on the parental survey, and only infants receiving one primary milk source (breast milk, cow milk, or Nestlé NAN Pro formula) were included in the study group.

### Data Collection and Anthropometric Assessment:

The questionnaire was prepared and contained age, sex, feeding duration, primary milk source, complementary feeding practices, and history of vitamin D or calcium supplementation. The questionnaire was then completed by the parents of the selected infants. Infants with congenital anomalies, premature birth, low birth weight, chronic systemic or renal diseases, metabolic bone disorders, acute infections, current calcium or vitamin D supplementation, or mixed feeding practices were excluded from the study. All infants had received age-appropriate matching foods according to pediatric feeding recommendations, and the information on food collected from the parents to ensure similar feeding practices in the groups. The anthropometric measurements were taken by using the established method of Toschke *et al.* (2007). The body weight of infants was measured by using the digital infant weighing scale (SECA 354, Germany) while the length was recorded by using the infantometer (SECA 417, Germany). The head circumference was measured with the non-stretchable measuring tape. It was positioned around the broadest occipital-frontal circumference. Body mass index (BMI) was calculated by using the following equation:

$$\text{BMI} = \frac{\text{Weight (Kg)}}{\text{Height (m)}^2} \quad (1)$$

### Blood Sample Collection and Biochemical Analysis:

Disposable syringes were used, and 5 mL of venous blood was collected from each infant by a trained phlebotomist. The blood was then transferred into plain gel tubes and clotted at room temperature for 30 minutes. After that, the samples were centrifuged in a centrifuge (Hettich EBA 20, Germany) at 3000 rpm for 10 minutes to isolate the serum. The serum was then aliquoted into sterile Eppendorf tubes and kept at  $-20^{\circ}\text{C}$  until the biochemical analysis. The colorimetric Arsenazo III method was applied for the assessment of serum calcium concentration by using the diagnostic kits (Randox Laboratories Ltd., UK), while the ammonium molybdate UV colorimetric method was used for the determination of serum phosphorus level. An enzymatic kinetic assay was used for recording the alkaline phosphatase, which was based on the hydrolysis of p-nitrophenyl phosphate under alkaline conditions, while the xylydyl blue colorimetric process was used for the estimation of serum magnesium concentration. Serum vitamin D levels were measured using ELISA kits (Human Vitamin D ELISA Kit, Abcam, Cambridge, UK; Cat. No. ab213966), with a detection range of 3–120 ng/mL, a sensitivity of 2.5 ng/mL, an intra-assay coefficient of variation (CV) of <10%, and an inter-assay CV of <12% and absorbance was recorded by UV-Visible spectrophotometer (UV-1800, Shimadzu, Japan) (Hashim *et al.*, 2023; Hawiyes *et al.*, 2025).

### Urine Sample Collection and Analysis:

Before the collection of the sample, the perineal area of each infant was cleaned with sterile distilled water. The sterile pediatric urine collection bags were used for the sample collection of random spot urine samples. It was then transported into the sterile polypropylene bottles and transported to the laboratory for examination. The colorimetric Arsenazo III method with diagnostic kits (Randox Laboratories Ltd., UK) was used for the assessment of urinary calcium concentration, while the ammonium molybdate UV colorimetric method was applied for the determination of urinary phosphorus level. The kinetic Jaffe reaction method was used for measuring the urinary creatinine level in the infants, which was based on the synthesis of a colored complex between the creatinine and alkaline picrate (Goyal *et al.*, 2025). The urinary calcium-to-creatinine ratio was calculated by using the following formula.

$$\text{Ca/Creatinine Ratio} = \frac{\text{Urinary Calcium(mg/dL)}}{\text{Urinary Creatinine(mg/dL)}} \quad (2)$$

Urinary pH was recorded directly after the sample collection by using the digital pH meter (HI2211, Hanna Instruments, Romania).

### Statistical Analysis:

All the data were analysed by SPSS software (version 25.0), and a one-way analysis of variance was performed to determine the differences in the feeding groups. Post hoc comparisons were performed using the Tukey test to find significant differences, and superscript lettering was also used to designate significant differences between the groups.

## 3. RESULTS

Table 1 demonstrates the demographic and anthropometric features of feeding type. There was no significant difference ( $p = 0.821$ ) in the age of the children with the breast milk, cow milk, and NAN Pro, which demonstrates a similar age in the groups. Body weight was significantly different ( $p = 0.031$ ) between the groups. The body weight ( $8.6 \pm 0.17$  kg) was found in infants receiving breast milk, while the  $8.2 \pm 0.13$  kg body weight was found in infants receiving cow milk. The NAN Pro group recorded a weight of  $8.4 \pm 0.22$  kg in breastfed infants. Similarly, the significant changes ( $p = 0.048$ ) in body length were also recorded in the feedings. Breastfed infants showed the highest length ( $72.3 \pm 0.64$  cm) while infants fed cow milk recorded lower length values ( $69.3 \pm 0.54$  cm). Infants receiving NAN Pro formula were observed  $70.3 \pm 0.63$  cm length. Head circumference was also significantly different ( $p = 0.037$ ) between the

groups; the maximum head circumference was recorded as  $45.1 \pm 0.21$  cm in the breast milk group, and the NAN Pro group recorded  $44.6 \pm 0.29$  cm, while the cow milk group documented the lowest values ( $43.6 \pm 0.19$  cm). No significant ( $p = 0.059$ ) changes were recorded in BMI values in the groups. These results demonstrated that both Breast milk and NAN Pro groups showed significantly higher serum calcium, magnesium and vitamin D levels and lower phosphorus and ALP than the cow milk group as compared with the cow milk group.

Urinary biochemical markers observed noticeable changes in the groups. The elimination of urinary calcium was  $5.9 \pm 0.32$  mg/dL was significantly ( $p = 0.001$ ) higher in the cow milk group while the  $3.7 \pm 0.79$  mg/dL was recorded in the breast milk group and  $4.9 \pm 0.26$  mg/dL was recorded in the NAN Pro groups. In contrast, no significant changes ( $p = 0.721$ ) were recorded in the urinary creatinine concentrations in the groups. The significant ( $p = 0.005$ ) changes

were observed in the urinary calcium-to-creatinine ratio in the feeding groups. The cow milk infants group recorded ( $0.31 \pm 0.04$ ), while lower ratios ( $0.19 \pm 0.06$ ) were observed in the breastfed and  $0.22 \pm 0.06$  in NAN Pro infants' groups. Similarly, urinary phosphorus levels were increased in infants in the cow milk group ( $16.7 \pm 0.51$  mg/dL) compared to the breast milk ( $12.6 \pm 0.42$  mg/dL) and NAN Pro group ( $14.7 \pm 0.61$  mg/dL), which represents the significant differences ( $p = 0.007$ ) in the groups.

Urinary pH varied significantly ( $p = 0.031$ ) in the groups. The nearly similar values were recorded in breast milk and NAN Pro groups,  $6.3 \pm 0.08$  and  $6.3 \pm 0.06$ , respectively. While the cow milk group exhibited lower ( $5.8 \pm 0.07$ ) urinary pH. The findings of this study indicate that cow milk feeding is associated with higher mineral elimination and changed urinary balance as compared to breast milk and NAN Pro groups (Table 3).

**Table 1:** Comparison of Demographic and Anthropometric Parameters among Infants Fed with Breast Milk, Cow Milk, and NAN Pro Formula.

| Parameter                | Breast Milk        | Cow Milk           | NAN Pro            | p-value |
|--------------------------|--------------------|--------------------|--------------------|---------|
| Age (months)             | $8.3 \pm 0.11^a$   | $8.4 \pm 0.12^a$   | $8.6 \pm 0.22^a$   | 0.821   |
| Weight (kg)              | $8.6 \pm 0.17^a$   | $8.2 \pm 0.13^b$   | $8.4 \pm 0.22^a$   | 0.031   |
| Length (cm)              | $72.3 \pm 0.64^a$  | $69.3 \pm 0.54^b$  | $70.3 \pm 0.63^a$  | 0.048   |
| Head Circumference (cm)  | $45.1 \pm 0.21^a$  | $43.6 \pm 0.19^b$  | $44.6 \pm 0.29^a$  | 0.037   |
| BMI (kg/m <sup>2</sup> ) | $16.47 \pm 0.15^a$ | $17.08 \pm 0.32^b$ | $17.04 \pm 0.64^a$ | 0.059   |

**Note:** Values are expressed as Mean  $\pm$  SE. Total sample size = 90 ( $n = 30$  per group). Means with the same superscript letter are not significantly different, whereas means with different superscript letters differ significantly ( $p < 0.05$ ).

**Table 2:** Comparison of Serum Biochemical Parameters among Infants Fed with Breast Milk, Cow Milk, and NAN Pro Formula.

| Parameter                   | Breast Milk       | Cow Milk          | NAN Pro            | p-value |
|-----------------------------|-------------------|-------------------|--------------------|---------|
| Serum Calcium (mg/dL)       | $9.7 \pm 0.12^a$  | $8.6 \pm 0.14^c$  | $9.4 \pm 0.22^b$   | 0.006   |
| Serum Phosphorus (mg/dL)    | $5.3 \pm 0.06^b$  | $6.3 \pm 0.12^a$  | $5.5 \pm 0.07^b$   | 0.005   |
| Alkaline Phosphatase (IU/L) | $231.3 \pm 6.7^b$ | $292.6 \pm 7.3^a$ | $244.7 \pm 6.7^b$  | 0.004   |
| Serum Magnesium (mg/dL)     | $2.31 \pm 0.06^a$ | $1.9 \pm 0.06^b$  | $2.1 \pm 0.07^a$   | 0.005   |
| Vitamin D (ng/mL)           | $32.6 \pm 1.33^a$ | $25.7 \pm 1.21^b$ | $30.31 \pm 1.31^a$ | 0.004   |

**Note:** Values are expressed as Mean  $\pm$  SE. Total sample size = 90 ( $n = 30$  per group). Means with the same superscript letter are not significantly different, whereas means with different superscript letters differ significantly ( $p < 0.05$ ).

**Table 3:** Comparison of Urinary Biochemical Parameters among Infants Fed with Breast Milk, Cow Milk, and NAN Pro Formula.

| Parameter                  | Breast Milk        | Cow Milk          | NAN Pro           | p-value |
|----------------------------|--------------------|-------------------|-------------------|---------|
| Urinary Calcium (mg/dL)    | $3.7 \pm 0.79^b$   | $5.9 \pm 0.32^a$  | $4.9 \pm 0.26^b$  | 0.001   |
| Urinary Creatinine (mg/dL) | $21.62 \pm 0.63^a$ | $20.8 \pm 0.68^a$ | $21.4 \pm 0.61^a$ | 0.721   |
| Ca/Creatinine Ratio        | $0.19 \pm 0.06^b$  | $0.31 \pm 0.04^a$ | $0.22 \pm 0.06^b$ | 0.005   |
| Urinary Phosphorus (mg/dL) | $12.6 \pm 0.42^b$  | $16.7 \pm 0.51^a$ | $14.7 \pm 0.61^b$ | 0.007   |
| Urine pH                   | $6.3 \pm 0.08^a$   | $5.8 \pm 0.07^b$  | $6.3 \pm 0.06^a$  | 0.031   |

**Note:** Values are expressed as Mean  $\pm$  SE. Total sample size = 90 ( $n = 30$  per group). Means with the same superscript letter are not significantly different, whereas means with different superscript letters differ significantly ( $p < 0.05$ ).

#### 4. DISCUSSION

This study examined different feeding types and found a significant impact on the infant anthropometric, biochemical, and urinary biochemical parameters. This study's findings showed better weight, length, and head circumference in breast milk-fed infants, and NAN Pro formula also documented nearly similar results to the breastfed infants, while the cow milk group demonstrated lower anthropometric measurements. These findings are similar to the outcomes of Patro-Golab *et al.* (2009), who documented the optimal growth of infants with breastfeeding and also observed better development during the early phase of life. Almeida *et al.* (2021) demonstrated that breastfeeding enhances infant development and growth due to the balanced nutrient, mineral, and bioactive compound profiles. Moreover, Hair *et al.* (2013) reported that human milk is regarded as the best indicator of infant growth and observed the positive effect on weight gain, body length, and head circumference in the first two years of life. This study observed no significant difference of BMI in the groups. Almeida *et*

*al.* (2021) demonstrated that increased body weight proportionally with body length during early infancy.

This study recorded the lower anthropometric measurements with the cow milk group, which indicated the poor mineral and protein composition in the cow milk of infants. Kalbermatter *et al.* (2021) documented that cow milk hinders growth during the early life of infants. Similarly, Scholz-Ahrens *et al.* (2020) demonstrated a poor effect on growth with cow milk feeding and noted that it contains higher protein and mineral levels, which contribute to metabolic stress. Rapa *et al.* (2019) examined that the metabolic stress due to higher mineral and protein concentration can affect the nutrient utilization and immature kidneys. In contrast, this study reported that NAN Pro also described a similar nutritional profile to breast milk and supports the growth. Similar findings were also reported by Lamana *et al.* (2021) that formula-fed infants described nearly similar growth outcomes to the breastfed infants. However, Wallenborn *et al.* (2021) reported no significant differences in growth parameters between lactated and formula-fed infants, which indicated that growth is influenced by ge-

netic, ecological, and balancing feeding factors.

This study described the better effect on biochemical indicators with breast milk and then observed the best result with NAN Pro over cow milk feeding. This result demonstrated the well-adjusted mineral absorption in breastfed and NAN Pro-fed infants. Basile *et al.* (2006) reported higher levels of serum calcium, magnesium, and vitamin D concentrations with breastfeeding in infants. Infants with cow milk feeding showed higher levels of serum phosphorus and alkaline phosphatase (Morais *et al.*, 2021). Bergman *et al.* (2009) described that human milk increased mineral absorption and bone metabolism due to higher bioavailability of calcium and proper calcium-to-phosphorus ratios, which changed the metabolism. Similar studies were also reported by Christmann *et al.* (2016), who recorded higher phosphorus intake from cow milk, which affects the calcium homeostasis and increases the activity of alkaline phosphatase in infants. This study recorded the higher Vitamin D concentrations in the breastfed infants followed by NAN Pro and cow milk groups. Similar findings were also documented by Berti *et al.* (2017), who recorded a positive effect on vitamin D and bone health during early life with the breast milk and fortified infant formulas. Kvammen *et al.* (2018) examined a lower level of vitamin D in infants with cow milk feeding as compared to breastmilk feeding, which was associated with decreased mineral metabolism and decreased growth outcomes. The breastfed infants attributed this to better availability of calcium in human milk, while high phosphorus and protein contents of cow milk contribute to changes in mineral metabolism and improved alkaline phosphatase activity (Bergman *et al.*, 2009).

This study revealed the differences in the urine biochemical parameters among the different feeding groups. This study observed a higher level of urinary calcium, calcium-to-creatinine ratio, and urinary phosphorus levels with the cow milk feeding in the infants. Similarly, Fidan *et al.* (2016) also reported higher levels of biochemical parameters, which demonstrated the higher renal mineral load and changed urinary mineral balance. In contrast, breastfed and NAN Pro-fed infants represented the lesser urinary mineral losses and more promising urinary pH values. These findings are similar to the outcomes of Reid *et al.* (2015) and Constable *et al.* (2019), who described the lower urinary pH with the cow milk feeding, which was associated with the high protein and phosphorus intake from cow milk. Shaw *et al.* (2023) also reported a higher level of renal solute load and urinary mineral elimination in infants with cow milk as compared to other feeding sources. However, some previous studies have reported no significant changes in urinary mineral elimination in different feeding groups, suggesting that complementary feeding practices influence urinary biochemical parameters.

The study was limited by its single-center design and the absence of detailed dietary intake assessment. However, the findings support breastfeeding as the preferred infant feeding practice, with fortified infant formula as a suitable alternative.

## 5. CONCLUSION

This concluded the study of alterations in growth, biochemical, and urinary mineral excretion parameters in the groups. Breastfed infants revealed the most promising anthropometric and biochemical consequences, which indicated optimal nutritional composition and high bioavailability of nutrients in human milk. This demonstrated that breastfeeding can be used as the gold standard for infant nutrition. However, when breastfeeding is not feasible, the NAN Pro formula can be used as a suitable alternative, but the unmodified cow milk in infants should not be used during early infancy due to its negative effects on growth and mineral absorption.

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## Ethical Statment:

The research was permitted by the Ethics Committee of the Nursing Department - Soran Technical College Council/ Erbil Polytechnic University. Issue Number (11132), Date: 11/10/2025. Ethical consent statements were obtained for all participants.

## Conflict of Interests:

The authors declare that they have no conflicts of interest related to the publication or funding of this manuscript.

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## Author Contributions:

A.H.A., Data Collection, Laboratory Analysis, and Writing. A.U., Literature Review. J.H.R., Design of the Project, Data Analysis and Review & Editing. H.A.M. reviewed the final manuscript.

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