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*Article*

# Adjusted Versus Targeted Fortification in Extremely Low Birth Weight Preterm Infants: FORTIN STUDY. A Randomized Clinical Trial

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**Abstract:** Fortified human milk is the first choice for preterm infants. Although individualized fortification is recommended, the optimal method for this population remains uncertain. We conducted a comparative study assessing the growth effects of adjusted (AF) and targeted fortification (TF) in extremely low birth weight (ELBW) infants. This single-center, randomized, controlled clinical trial was conducted at a tertiary neonatal unit in Spain. Eligible participants were premature infants with a birthweight <1000g exclusively fed with human milk. A total of 38 patients were enrolled, 15 of them randomised to AF group and 23 to TF group. AF was based on BUN concentration and TF on human milk analysis. The primary outcome was weight gain velocity (g/kg/day).No significant differences were found in weight gain velocity at 28 days, at 36 weeks of postmenstrual age, at discharge nor during intervention. Protein intake was significantly higher in AF group (5.02 g/kg/day vs 4.48 g/kg/day, p= 0.001). No differences were found in lipids, carbohydrates and energy intake; neither in weight z score change between the different time points; nor in length and head circumference growth. Both AF and TF are comparable methods of fortification and provide appropriate growth rate in ELBW infants.

**Keywords:** preterm infants; neonatal nutrition; growth; human milk; human milk fortification; blood urea nitrogen; individualized fortification

## 1. Introduction

Human milk is the first choice for preterm infants and it is associated with multiple short and long-term benefits [1–7]. When mother's own milk (MOM) is not available, donor human milk (DHM) is the best alternative [8,9].

Despite its advantages, MOM or DHM do not meet the nutritional requirements of preterm infants, especially in terms of protein, calcium and phosphorus. This is particularly true for extremely low birth weight (ELBW) infants due to their accelerated growth [1,10–13]. The goal of fortification is to increase the nutrient concentration of human milk (HM) to the level required to optimize growth [8,14–19].

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There are three approaches for fortifying HM for ELBW infants. Standard fortification (SF), the most widely used strategy, consists of adding a fixed amount of multicomponent fortifier per 100 ml of HM during the entire fortification period [1,16]. This method assumes a fixed estimated HM composition with a protein content of 1.5 g/dL, without considering temporal or inter-individual variations [13]. However, this assumption may not be accurate [20] and this strategy has been shown to lead to suboptimal growth in ELBW infants [15,21–24].

Individualized fortification is emerging as a potential solution to mitigate the inherent variability in HM composition. However, the optimal fortification strategy remains uncertain [24]. It involves two different methods: adjusted fortification (AF) based on blood urea nitrogen (BUN) concentration as a marker of protein nutrition [26–29] and targeted fortification (TF) based on regular analysis of HM and fortification adjustment according to the actual macronutrient composition [30,31]. It allows tailor-made fortification to meet the required nutritional recommendations. Despite the theoretical advantages of this nutritional strategy, its implementation involves significant challenges [32–38].

The aim of this study is to compare the effects of AF and TF on growth and neonatal morbidity in ELBW preterm infants.

## 2. Materials and Methods

### *2.1. Study Design*

Prospective, single center, randomized, controlled, interventional study performed in a tertiary referral neonatal unit at La Paz University Hospital (Madrid, Spain). The study protocol was approved by the local ethics committee. Written informed parenteral consent was obtained. The trial was registered at ClinicalTrials.gov with identification number NCT04982133.

A Priori Research Hypothesis

Targeted fortification will improve postnatal weight gain and growth over adjusted fortification.

### *2.2. Participants*

Preterm infants with birthweight <1000g fed with MOM or DHM were eligible for the study when they reached an enteral feeding volume ≥100 mL/kg/day. Exclusion criteria: major congenital malformation, chromosomopathies, metabolic disease or gastrointestinal surgery.

Randomization

Patients whose parents signed the informed consent were randomized to a fortification method. A stratified randomization method based on intrauterine growth restriction (IUGR) defined as birth percentile <3 or <10 with altered prenatal doppler was initially proposed. However, given the low incidence of IUGR in our population, it was changed to a simple randomization method. A list of unique randomization codes was followed and assigned consecutively to each study participant. Siblings of multiple births were randomized individually. It was not possible to blind the medical team to the group allocation but the investigators only did fortification changes. The results of the HM analysis in AF group were blinded until the end of the study.

### *2.3. Nutritional Intervention*

Infants were randomly assigned to one of the two parallel treatment groups on top of standard fortification with a multicomponent fortifier (PreNAN Human milk fortifier™, Nestlé, Vevey, Switzerland) powder with a recommended dosage of 1g per 25 mL providing an additional 0.7g of fat, 1.42g of protein, and 1.3g of carbohydrates per 100 mL of HM. Study arms were the following: (1) AF modified fortification based on BUN concentration; (2) TF based on HM macronutrient content.

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Nutritional intake was assessed twice a week. The intervention was continued until 36 weeks of postmenstrual age (PMA) or until discharge, whichever came first. The study was also discontinued when DHM was replaced by formula due to hospital criteria (weight >1500g or age >6 weeks). Parenteral and enteral feeding regimens were standardized by internal feeding guidelines, which remained unchanged during the study period. The target volume of enteral nutrition was 150-180ml/kg/day. MOM feeding was supplemented with DHM when necessary to achieve adequate enteral intake.

2.3.1. Adjusted "Modified" Fortification

Adjustments were made by BUN concentration weekly [26,27]:

- BUN <10mg/dl: protein fortification was increased one level by adding a protein fortification module [oligopeptides (Clinical Nutrition, SA, Barcelona, Spain) 0.5g/100ml, 1g/100ml or 1.5g/100ml].
- BUN 10-16mg/dl: no nutritional changes were performed if growth was adequate, defined as no decrease in weight z-score on Fenton curves compared to the previous weekly weight, after the postnatal weight loss phase [39,40].
- BUN >16mg/dl: fortification was decreased one level if growth was adequate. If BUN >10mg/dl but growth was inadequate defined as decrease in weight z-score on Fenton curves, an insufficient intake of other macronutrients was inferred, and non-protein kilocalories were supplemented with lipids in the form of medium-chain triglycerides [MCT 1-2ml/kg (Nutricion Medica, Madrid, Spain)] and glucose polymer powder [0.97g carbohydrates/g (Fantomalt, Nutricia, Netherlands) 2g/100ml].

Multicomponent fortification was never withdrawn regardless of BUN value. The adjusted "modified" fortification scheme is illustrated in Figure 1.

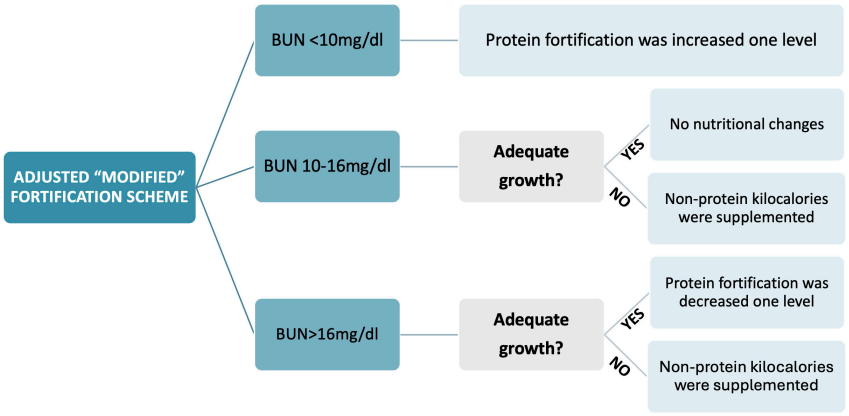


**Figure 1.** Adjusted "modified" fortification scheme.Adequate growth was defined as no decrease in weight z-score on Fenton curves compared to the weight measurement obtained during the previous weekly nutritional assessment.

2.3.2. Targeted Fortification

Adjustments were made according to the twice-weekly macronutrient analysis of HM. Monocomponent modules (oligopeptides, MCT or glucose polymer powder) were added to reach the nutritional targets recommended by the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) in relation to enteral intake in preterm infants [41]: protein 3.5-4.5g/kg/day, carbohydrate 11.6-13.2g/kg/day, lipids 4.8-6.6g/kg/day and energy 110-135 kcal/kg/day. Initial fortification with the multicomponent fortifier was never withdrawn even though some macronutrients exceeded the nutritional recommendations.

 doi:10.20944/preprints202407.2219.v1



*2.4. Analysis of Human Milk*

Macronutrient composition analysis of MOM or DHM was performed in both groups twice a week. The analysis focused on the predominant type of human milk, defined as the HM type that represented more than 50% of the intake during the previous 3 days. The analysis was performed on an 8ml aliquot obtained from a 24-hour pool of fresh HM. If fresh milk was not available, frozen milk was used, thawed 24 hours in a refrigerator at 4°C, according to recommendations for thawing HM [42]. All samples were analyzed using a milk analyzer based on near infrared (NIR) spectroscopy (MilkoScanTM Mars, FOSS Analytical A/S, Hillerød, Denmark). HM composition of protein (true protein, g/100ml), carbohydrate (lactose, g/100ml), and fat (g/100 ml) were obtained, as well as a calculated value of Kcal/100ml. Regarding energy calculation, we utilized the following standard equation to determine the energy content of human milk: we multiplied grams of protein by 4 kcal/g, grams of carbohydrates by 4 kcal/g, and grams of fats by 9 kcal/g, and summed these values to obtain the total kcal/dL.

Analysis of HM composition is integrated into routine laboratory analysis. Laboratory professionals perform equipment verification and control and they own a quality accreditation (UNE-EN-ISO-15189 standard).

*2.5. Biochemical Analyses*

In AF group serum urea, calcium, phosphorus and alkaline phosphatase in venous blood was performed weekly. In TF group the same serum measurements were performed, but only 28 days after the beginning of fortification, at 36 weeks PMA and at discharge. Serum urea was determined by the urease method. An automated clinical analyzer (Siemens Atellica Healthineers, Siemens Healthineers, Erlangen, Germany) was used.

*2.6. Outcomes*

The primary outcome was weight gain velocity (g/kg/day) in the following periods: birth to 28 days after the initiation of fortification, birth to 36 weeks PMA, birth to discharge and during the intervention. Weight was recorded daily to the nearest 5g using an electronic scale (Kern MBC 15K2DM, Kern and Sohn, Balingen, Germany) or an incubator with adequate calibration (Babyleo TN500, Draeger, Lübeck, Germany).

Weight gain velocity was calculated according to Patel et al formula [43]: [1000 x ln(Wn/W1)]/(Dn-D1), where W1 is the weight at the start and Wn is the weight at the final day of the observation. D1 is the starting day and Dn is the final day of the observation period.

Secondary outcomes were:

1. Length and head circumference (HC) growth. Cranial-heel length was measured on a length board in supine position (Añó sayol, Barcelona, Spain), and HC was measured with a non-stretching tape to the nearest 0.5 cm.
2. Standard deviation score (SDS) differences, based on Fenton graphs [41], for weight, length and HC between the periods studied (SDS different time points – SDS birth). The research team performed all anthropometric measurements.
3. Actual enteral macronutrient intake (g/kg/day and Kcal/kg/day).
4. Nutritional achievements: initiation of enteral nutrition (hours), exclusive enteral nutrition (150 ml/kg/day) (days), parenteral nutrition cessation (days), start of HM fortification (days) and volume of enteral feeding at the beginning of fortification (ml/kg/day).
5. Predominant HM type (>50% of intake during the study period): MOM or DHM.
6. Perinatal characteristics and neonatal morbidities: early-onset sepsis [44], intraventricular hemorrhage ≥ grade II [45], white matter injury [46], significant ductus arteriosus [47,48], necrotizing enterocolitis defined as Bell's stage >2 [49], retinopathy [50], moderate-severe bronchopulmonary dysplasia [51,52], cholestasis [53], late-onset sepsis [44] and death.
7. Neonatal intensive care unit (NICU) and hospital stay (days).

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*2.7. Sample Size and Statistical Analysis*

Sample size was calculated based on the results of previous studies, where the reported weight gain for TF was 19.9 ± 2.7 g/kg/day [30], and for AF was 17.5 ± 3.2 g/kg/day [26]. The calculation was performed for a test power of 80%, resulting in a sample size of 38 participants. Losses of 15% were assumed.

Qualitative data is described in absolute frequencies and percentages and quantitative data by mean and standard deviation or median and interquartile range, based on its distribution. The normality of the continuous variables was studied using Kolmogorov-Smirnov test.

The association between variables were studied using chi-square test for categorical variable and Student's t-test, as a parametric test, or Mann-Whitney U test, as a non-parametric test, for continuous variables. To study the relationship between quantitative variables, Pearson's correlation, or its non-parametric equivalent Spearman's correlation, were used. Generalized linear repeated measures models were used to assess the relationship between protein intake and urea values.

All statistical tests were considered bilateral and p-values less than 0.05 were considered significant. Data was analyzed with SAS 9.4 statistical software (SAS Institute, Cary, NC).

## 3. Results

38 patients were included from April 2021 to April 2023 (104 preterm infants <1000g were admitted to the NICU in this period; **Figure 2**). 15 patients were randomized to AF group and 23 to TF group.

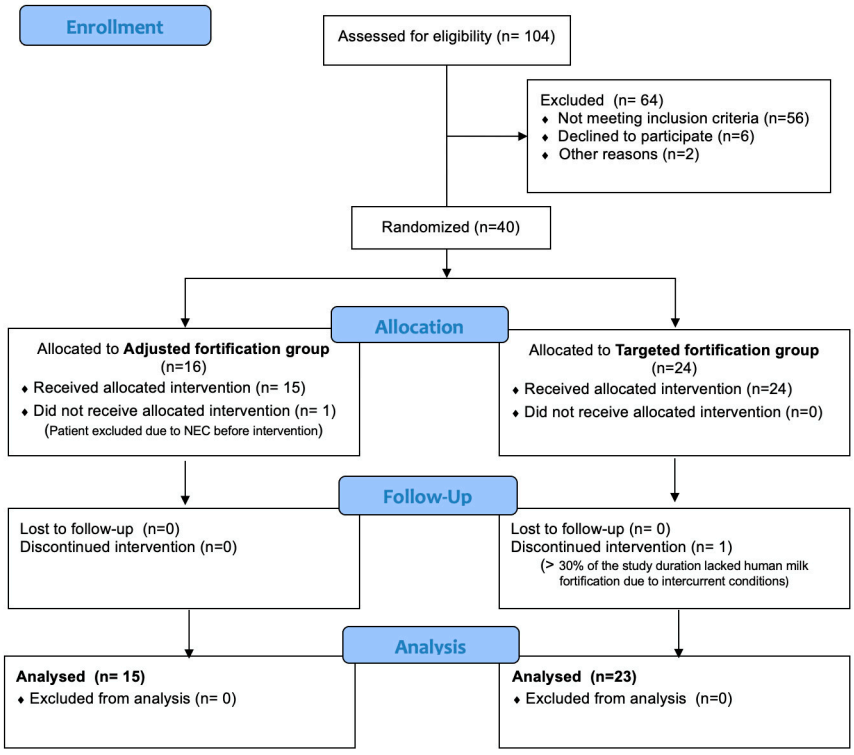


**Figure 2.** CONSORT flow chart.

Mean (SD) gestational age was 27 weeks (2) and birth weight (interquartile range-IQR) was 835g (710-894). Baseline general characteristics were comparable between both arms **(Table 1).** Other characteristics were as follows in the AF and TF group, respectively: complete lung maturation 87% vs 74%; incidence of chorioamnionitis 13% vs 35%; born by cesarean section 87% vs 74%.

The mean (SD) duration of the intervention was 50 (14) days in the AF group and 46 (16) days in the TF group (p=0.40).

 doi:10.20944/preprints202407.2219.v1



**Table 1.** General characteristics of study population.

| General characteristics of study population | Adjusted fortification (n =15) | Targeted fortification (n=23) | p-value |
|---|---|---|---|
| **Gestational age (weeks)** Mean (CI 95%) | 27 (26.27, 28.01) | 27 (25.88, 27.79) | 0.64 |
| **Birth weight (g)** Mean (CI 95%) | 765 (669.79, 860.74) | 819 (767.77, 870.58) | 0.26 |
| **Birth weight z-score** Mean (CI 95%) | -0.8 (-1.42, -0.38) | -0.9 (-0.79, 0.18) | 0.09 |
| **Length birth (cm)** Mean (CI 95%) | 32.5 (31.18, 33.82) | 33.5 (32.51, 34.51) | 0.19 |
| **Head circumference at birth (cm)** Mean (CI 95%) | 23.3 (22.42, 24.17) | 23.6 (22.96, 24.16) | 0.58 |
| **Male No (%)** | 9/15 (60) | 11/23 (47.8) | 0.46 |
| **Multiple gestation No (%)** | 4/15 (26.7) | 11/23 (47.8) | 0.19 |
| **Umbilical cord pH** Median (CI 95%) | 7.29 (7.17, 7.38) | 7.33 (7.23, 7.36) | 0.12 |
| **Initial weight loss (%)** Mean (CI 95%) | 6.7 (4.30, 9.43) | 6.18 (4.23, 8.12) | 0.73 |
| **Birth weight regain (day)** Median (CI 95%) | 6 (4.86, 7.81) | 7 (5.34, 7.96) | 0.47 |
| **Weight at hospital discharge (g)** Mean (CI 95%) | 2691 (2364.26, 3017.07) | 2816 (2543.68, 3088.49) | 0.54 |
| **Length at hospital discharge (cm)** Median (CI 95%) | 45 (43.57, 46.50) | 45 (43.96, 46.29) | 0.66 |
| **Head circumference at discharge (cm)** Mean (CI 95%) | 33.7 (32.85, 34.45) | 33.2 (32.52, 33.96) | 0.43 |

CI: confidence interval.

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*3.1. Nutritional Strategy*

Enteral nutrition was started at 24 hours in both groups (IQR AF 20-48 hours and TF 20-30 hours, p=0.5), exclusive enteral nutrition was reached at 17 (IQR 13-26) days in AF group and at 15 (IQR 12-25) days in TF group (p=0.48). No differences were found between groups in age (SD) at parenteral nutrition discontinuation [AF 17 (7) days vs TF 16 (9) days, p=0.91].

Fortification was initiated in AF group at 15 (IQR 11-22) days and in TF group at 14 (IQR 10-21) days (p=0.67). There was no significant difference in the volume of enteral nutrition at which fortification was initiated [AF 146ml/kg/day (IQR 121-152) vs TF 145ml/kg/day (IQR 123-155), p=0.89]. Predominant type of feeding was MOM in both groups with a frequency of 73% in AF group and 52% in TF group (p=0.192).

No differences were found in HM composition between groups in terms of protein, fat, lactose and energy. Protein intake was significantly higher in AF group [5.02 (0.51) g/kg/day vs 4.48 (0.17) g/kg/day, p= 0.001]. No significant differences were found in lipids, carbohydrates and energy intakes between the two arms **(Table 2).**

The same number of nutritional assessments were performed in both groups (mean 14, SD 4). Nutritional modifications (IQR) per patient were also similar (AF: 3 (2-4), TF: 4 (2-7); p=0.165). In AF group non-protein kilocalories were added in 40% of the patients.

**Table 2.** Macronutrient analysis of human milk (HM) and actual macronutrient intake in adjusted fortification group and targeted fortification group.

| HM analyses | Adjusted fortification (n=15) | Targeted fortification (n=23) | p-value | Intake | Adjusted fortification (n=15) | Targeted fortification (n=23) | p-value |
|---|---|---|---|---|---|---|---|
| **Protein HM (g/100ml)** Mean (CI 95%) | 1.34 (1.25, 1.45) | 1.27 (1.21, 1.35) | 0.20 | **Protein g/kg/day** Mean (CI 95%) | **5.02(4.73, 5.30)** | **4.48 (4.41, 4.56)** | **0.001** |
| **Lipid HM (g/100ml)** Median (CI 95%) | 7.32 (7.23, 7.77) | 7.61 (7.35, 7.79) | 0.10 | **Lipid g/kg/day** Mean (CI 95%) | 7.15 (6.45, 7.85) | 6.56 (6.39, 6.74) | 0.10 |
| **Carbohydrates HM (g/100ml)** Median (CI 95%) | 3.30 (3.16, 3.90) | 3.20 (3.05, 3.34) | 0.80 | **Carbohydrates g/kg/day** Mean (CI 95%) | 15.15 (14.09, 16.22) | 15.15 (14.54, 15.76) | 0.99 |
| **Energy HM (kcal/100ml)** Median (CI 95%) | 65.66 (63.67, 69.96) | 64.08 (62.78, 65.57) | 0.156 | **Energy kcal/kg/day** Mean (CI 95%) | 144.72 (135.62, 153.83) | 137.63 (134.94, 140.32) | 0.13 |

CI: confidence interval.

*3.2. Growth Outcomes*

No significant differences were found in weight gain at 28 days after initiation of fortification, at 36 weeks of PMA, at discharge nor during the intervention **(Table 3)**. No differences were observed in the difference in weight z score (SD) between the different studied time points: neither at 28 days after initiation of fortification [AF -0.82 (0.62) vs TF -1.18 (0.60), p=0.09] nor at 36 weeks PMA [AF -1 (0.67) vs TF -1.23 (0.75), p=0.36] nor at discharge [AF -0.9 (1.03) vs TF -1.13 (0.57), p=0.38].

**Table 3.** Weight gain (g/kg/day) in adjusted fortification group and targeted fortification group.

| Weight gain g/kg/day | Adjusted fortification (n =15) | Targeted fortification (n=23) | p-value |
|---|---|---|---|
| **From birth to 28 days from the start of HM fortification** Mean (CI 95%) | 14.06 (12.31, 15.81) | 13.24 (12.13, 14.36) | 0.38 |

 doi:10.20944/preprints202407.2219.v1



| | | | |
|---|---|---|---|
| **From birth to 36 weeks PMA** Median (CI 95%) | 15.31 (13.37, 16.42) | 14.02 (13.16, 16.41) | 0.53 |
| **From birth to discharge** Median (CI 95%) | 14.53 (13.01, 15.46) | 14.04 (13.35, 15.12) | 0.68 |
| **During the intervention** Mean (CI 95%) | 16.33 (15.26, 17.40) | 16.89 (15.44, 18.34) | 0.56 |

PMA: Postmenstrual age. CI: confidence interval.

There was no effect of fortification type on length and HC growth, neither assessed in cm/week nor on changes in z score **(Table 4).**

**Table 4.** Length and head circumference growth in adjusted fortification group and targeted fortification group.

| **Length growth cm/week** | **Adjusted fortification (n =15)** | **Targeted fortification (n=23)** | **p-value** | **Head circumference growth cm/week** | **Adjusted fortification (n =15)** | **Targeted fortification (n=23)** | **p-value** |
|---|---|---|---|---|---|---|---|
| **From birth to 28 days from the start of HM fortification** Mean (CI 95%) | 0.91 (0.78, 1.05) | 0.86 (0.74, 0.98) | 0.536 | **From birth to 28 days from the start of HM fortification** Mean (CI 95%) | 0.78 (0.65, 0.93) | 0.73(0.64, 0.83) | 0.44 |
| **From birth to 36 weeks PMA** Median (CI 95%) | 0.95 (0.89, 1.08) | 0.88 (0.70, 1.49) | 0.38 | **From birth to 36 weeks PMA** Median (CI 95%) | 0.85 (0.76, 0.94) | 0.86 (0.70, 1.18) | 0.86 |
| **From birth to discharge** Median (CI 95%) | 0.91 (0.89, 1.07) | 0.94 (0.79, 1.33) | 0.47 | **From birth to discharge** Median (CI 95%) | 0.83 (0.75, 0.91) | 0.79 (0.67, 1.09) | 0.41 |

PMA: Postmenstrual age. CI: confidence interval.

*3.3. Biochemical Parameters*

Statistically significant differences were observed in urea at 36 weeks of PMA [AF 23mg/dl (IQR 21-29mg/dl) vs TF 15mg/dl (IQR 11.7-23mg/dl); p= 0.026] and at the end of the intervention [AF 24mg/dl (IQR 21-32mg/dl) vs TF 20mg/dl (IQR 16-22mg/dl); p= 0.018]. No differences were found in phosphorus, calcium or alkaline phosphatase values at any point.

A positive correlation was found between protein intake and serum urea concentration (p= 0.002, **Figure 3**).

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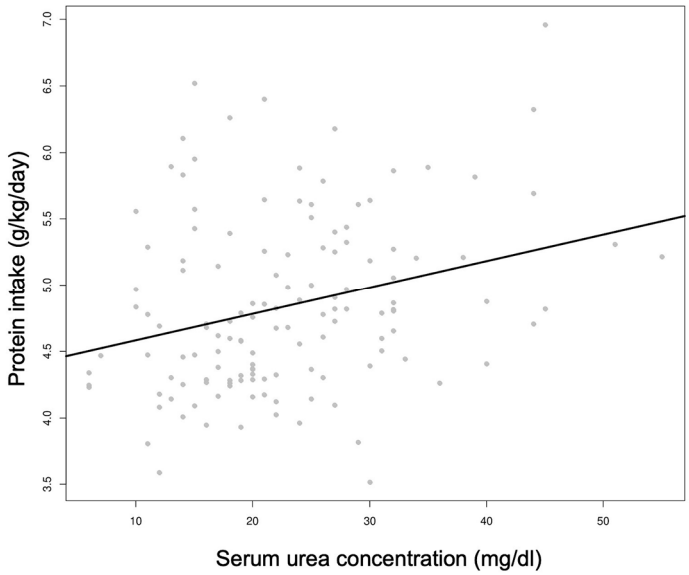


**Figure 3.** Correlation between protein intake and serum urea concentration.

*3.4. Neonatal Morbidity and Hospital Stay*

No differences were found in the main neonatal outcomes except for the presence of significant ductus arteriosus (AF group 47% vs TF group 78%, p= 0.045). Late onset sepsis was highly prevalent in both groups, with at least one episode of sepsis in 53% of patients in AF group and 65% in TF group (p=0.46).

No differences were found in NICU stay (SD) [59 (34) days in AF group vs 58 (26) days in TF group, p=0.915], hospital stay [84 (IQR 74-102) days in AF vs 81 (IQR 74-102) days in TF, p=0.79] and PMA at discharge [39.1 (IQR 38,4-40,6) weeks vs 38.4 (IQR 37.9-40.3) weeks p=0.40]. There was no difference in weight (SD) at the end of the intervention [2127 (481) g vs 2120 (437) g, p=0.96] nor at hospital discharge [2690.7 (589.4) g vs 2816.1 (630) g, p=0.542].

## 4. Discussion

The present study demonstrates no differences on postnatal weight gain and growth between AF and TF at different times of analysis. To our knowledge, this study is unique with respect to increased energy intakes and measurement of actual macronutrient intake in both groups. Our study took a rigorous approach to investigate the impact of individual fortification on growth of ELBW preterm infants. Previous studies have demonstrated the benefit in growth of TF vs SF [30,54–56]. A randomized clinical trial (RCT) comparing the three fortification methods found similar weight gain in AF and TF group but higher than SF. In this study protein intake was significantly higher in the individualized versus SF group (protein intake was 4.3g/kg/day in AF group, 4.5g/kg/day in TF group and 3.6g/kg/day in SF group) [57].

Rochow et al [58] compared SF with TF. Similar to our study, fortification was initiated with a standard fortifier and fortification modules were added to adjust all HM macronutrients. The modules used for fat and protein fortification were different from ours. They found greater weight at 36 weeks and greater growth velocity in TF group.

However, few studies have compared AF method with TF. Bulut et al [59] compared the effect on growth of AF versus TF finding a positive effect on growth in TF group. Only protein was supplemented in both fortification groups and BUN limit used to increase protein intake was lower than in our study (5mg/dl vs 10 mg/dl).

Different from Arslanoglu et al [26,27], that only increased protein intake, we increased caloric intake in the form of non-protein kilocalories for those patients with an inadequate growth response

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despite acceptable BUN concentration. This "modified" adjusted method, with a more complete adjustment, might justify the absence of differences in growth between the two groups.

Protein intake is the main growth factor when energy intake is appropriate [25,60]. In our study, protein intake in TF group was within the recommended range [25], meanwhile AF group exceeded the nutritional recommendations with a protein intake of 5.02 g/kg/day. This “superfortification” was not linked to a corresponding rise in non-protein kilocalories, as no differences were found in energy, carbohydrate, and lipid intake. Therefore, as energy intake becomes a limiting factor when protein intake is high, it is likely that protein intakes >4.5g/kg/day were not associated with a parallel increase in growth due to an inability to metabolize these proteins. Other explanation maybe a potential ceiling effect for enteral protein supply, at least for the population studied, indicating that an enteral protein intake exceeding 4.5 g/kg/d might not further improve weight gain in this population. Our results are in line with data recently reported by Miller et al [61], who also found no influence of increased enteral protein intake on weight gain in infants of similar gestational age at birth.

Plasma urea, the product of amino acid oxidation, is considered an estimator of protein intake. A target urea level of 21-34mg/dl or BUN 10-16mg/dl has been proposed with limited evidence [1]. We found significantly higher urea levels in the high protein intake group and a positive correlation between protein intake and plasma urea levels. This finding is consistent with other authors supporting the use of serum urea as an estimator of protein intake but appropriate levels should be defined [62].

Average growth rates during intervention were 16-17g/kg/day, lower than previously reported with similar protein intakes but in recommended ranges [25,58]. In our study, late-onset sepsis was not an exclusion criterion, possibly explaining lower weight gain. As growth is similar between both groups, it is not surprising that no differences are found in the main neonatal morbidities nor hospital stay.

On the other hand, we used modular products for enteral feeding available in our NICU but evidence is scarce on their optimal composition for individual fortification.

Each method of fortification has several advantages and disadvantages. AF does not require a HM analyzer and urea determinations are part of routine blood tests in newborns. However, it requires frequent blood samples involving pain, discomfort and risk of anemia. AF may also involve a delay in nutritional adjustment as a metabolic response is needed [26,63].

TF has the advantage of adapting to changes in HM composition and adjusting different macronutrients to meet nutritional recommendations. The number of times per week that HM should be tested to achieve an accurate intake has not been defined. Several studies suggest that twice-weekly HM testing may provide adequate macronutrient intakes [33,64]. In addition, it requires the availability of trained staff in sample collection and maintenance and calibration of the HM analyzer. Therefore, it implies the need to consider material and human resources for its implementation. In any case, both fortification methods in our study required comparable nutritional modifications.

The strengths of the present study are the determination of actual macronutrient intakes with twice-weekly milk analyses in all infants and the length of intervention larger than previous studies. The macronutrients intake analysis in both groups make possible to evaluate the effect of both types of fortification on macronutrient intake. The study had limitations due to the inability to stratify by IUGR. However, a multivariate analysis was conducted to assess the impact of IUGR on the response variables, with no significant effect observed. In addition, a minor error in the estimation of macronutrient intake was accepted, as we based it on the composition of the predominant HM and on two days measurement per week of estimated intakes. These approaches allowed the study applicability in case results would be positive. Furthermore, the attending physician was not blinded having the possibility of visualizing serum urea in TF group. This did not cause any bias since the research team always established the nutritional strategy.

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## 5. Conclusions

Both AF and TF provide adequate growth rate in ELBW infants. Therefore, the most plausible individualized fortification method should be chosen according to the resources available. To verify effects on neurocognitive outcome larger follow up is needed.

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**Institutional Review Board Statement:** The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of La Paz University Hospital (ethical approval code HULP: 5704, date 30 November 2020)

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request. Researchers submitting a methodologically sound proposal should contact miguel.saenz@salud.madrid.org. In order to access, requesters will need to sign a data access agreement.

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