Considerations in Meeting Protein Needs of the Human Milk–Fed Preterm Infant

Julie Wagner, RD, LMNT, CNSC; Corrine Hanson, RD, PhD; Ann Anderson-Berry, MD

ABSTRACT
Preterm infants provided with sufficient nutrition to achieve intrauterine growth rates have the greatest potential for optimal neurodevelopment. Although human milk is the preferred feeding for preterm infants, unfortified human milk provides insufficient nutrition for the very low-birth-weight infant. Even after fortification with human milk fortifier, human milk often fails to meet the high protein needs of the smallest preterm infants, and additional protein supplementation must be provided. Although substantial evidence exists to support quantitative protein goals for human milk–fed preterm infants, the optimal type of protein for use in human milk fortification remains uncertain. This question was addressed through a PubMed literature search of prospective clinical trials conducted since 1990 in preterm or low-birth-weight infant populations. The following 3 different aspects of protein quality were evaluated: whey-to-casein ratio, hydrolyzed versus intact protein, and bovine milk protein versus human milk protein. Because of a scarcity of current studies conducted with fortified human milk, studies examining protein quality using preterm infant formulas were included to address certain components of the clinical question. Twenty-six studies were included in the review study. No definite advantage was found for any specific whey-to-casein ratio. Protein hydrolyzate products with appropriate formulations can support adequate growth and biochemical indicators of nutrition status and may reduce gastrointestinal transit time, gastroesophageal reflux events, and later incidence of atopic dermatitis in some infants. Plasma amino acid levels similar to those of infants fed exclusive human milk–based diets can be achieved with products composed of a mixture of bovine proteins, peptides, and amino acids formulated to replicate the amino acid composition of human milk. Growth and biochemical indicators of nutrition status are similar for infants fed human milk fortified with human milk protein and bovine milk protein.

Key Words: human milk fortifier, neonate, nutrition, preterm infant, protein quality, very low-birth-weight infants

The ideal nutrition regimen for the preterm infant could be defined as that which allows for growth that is similar in both quantity and quality to intrauterine fetal growth while providing for optimal functional development of body tissues without increasing the risk of adverse outcomes.1-5 Currently, the most practical method of assessing the nutrition status of the preterm infant is to...
compare extrauterine growth patterns with the intrauterine growth of a reference fetus of the same gestational age with a goal of achieving intrauterine growth rates.\textsuperscript{3,4,6} Fetal weight gain rates have been estimated at 16 to 18 g/kg per day for body weights between 500 and 1800 g.\textsuperscript{7}

Historically, providing preterm infants with nutrition sufficient to achieve intrauterine growth rates was often considered to be harmful and unattainable, particularly in the early days and weeks of life.\textsuperscript{4} Consequently, until recently, the vast majority of very low-birth-weight (VLBW; \(< 1500\) g at birth) and extremely low-birth-weight (ELBW; \(< 1000\) g at birth) infants exhibited growth failure, defined by weight less than the 10th percentile, by the time they reached 36 weeks' corrected gestational age.\textsuperscript{8,9} Inadequate nutrient provision, particularly protein provision, is recognized as the primary cause of extrauterine growth restriction.\textsuperscript{4,10} This is of particular concern because extensive evidence from large, well-designed studies of VLBW and ELBW infants has shown that growth restriction and inadequate energy and protein provision in the neonatal period result in adverse long-term neurodevelopmental outcomes.\textsuperscript{11-17} As a result, great efforts have been made to improve the nutrition status of preterm infants over the past decade, subsequently reducing the incidence of growth restriction.\textsuperscript{4} However, former preterm infants tend to have reduced linear growth, reduced fat-free mass, and increased fat mass compared with their term counterparts.\textsuperscript{2,10,18} This altered body composition can likely be attributed to high provision of fat and carbohydrate and inadequate provision of protein during the early neonatal period.\textsuperscript{10}

Current estimates of protein needs for preterm infants are summarized in Table 1. These expert recommendations range from 3.4 to 4.8 g/kg per day.\textsuperscript{1,6,7,19,20} Protein needs of the growing preterm infant are partially dependent on the degree of deficit acquired in the first days of life. Postnatally, approximately 3.5 g/kg per day of parenteral protein, with energy provision of 90 kcal/kg per day, is needed to achieve the same protein accretion that would be expected in utero.\textsuperscript{9} Preterm infants provided with inadequate protein early in life quickly accrue substantial deficits in total body protein.\textsuperscript{5,21} To achieve total body protein stores similar to intrauterine levels at the same gestational age, later protein provision must be increased beyond baseline needs to “catch-up” from this early deficit. To account for this, enteral protein provision should be slightly higher than the baseline amount expected to achieve intrauterine growth rates, as is accounted for at the higher ends of the recommended enteral intake ranges.

Several decades ago, data suggested that protein intakes greater than 5 g/kg per day were associated with hyperammonemia, elevated blood urea nitrogen, hyperaminoacidemias, metabolic acidosis, and other adverse outcomes for preterm infants.\textsuperscript{22} However, it is unlikely that these outcomes can be generalized to preterm infants with today’s level of clinical care who receive vastly improved nutritional product formulations and whose protein intake is unlikely to reach levels as high as 5 g/kg per day.\textsuperscript{2} Although total protein intake in the first week of life has been associated with small increases in blood urea nitrogen and decreases in serum bicarbonate levels,\textsuperscript{23} effects are not large enough to be clinically significant for the majority of VLBW

<table>
<thead>
<tr>
<th>Source</th>
<th>Recommendations</th>
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<tbody>
<tr>
<td>Life Sciences Research Office (2002)</td>
<td>3.4-4.3 g/kg/d with safe upper limit of 4.9 g/kg/d</td>
</tr>
<tr>
<td>Ziegler (2002)</td>
<td>Body weight 500-1200 g: 4.0 g/kg/d</td>
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<tr>
<td></td>
<td>Body weight 1200-1500g: 3.9 g/kg/d</td>
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<tr>
<td></td>
<td>Body weight 1500-1800g: 3.6 g/kg/d</td>
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<td></td>
<td>Body weight 1800-2200 g: 3.4 g/kg/d</td>
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<tr>
<td>Rigo (2005)\textsuperscript{19}</td>
<td>CGA 26-30 wk: 3.8-4.4 g/kg/d</td>
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<tr>
<td></td>
<td>CGA 30-36 wk: 3.4-4.2 g/kg/d</td>
</tr>
<tr>
<td>European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (2010)\textsuperscript{1}</td>
<td>Body weight (&lt; 1000) g: 4.0-4.5 g/kg/d</td>
</tr>
<tr>
<td></td>
<td>Body weight 1000-1800 g: 3.5-4.0 g/kg/d</td>
</tr>
<tr>
<td></td>
<td>Decreasing needs as discharge approaches if growth allows</td>
</tr>
<tr>
<td>Hay (2008)\textsuperscript{20}</td>
<td>CGA 24-30 wk: 3.6-4.8 g/kg/d</td>
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<tr>
<td></td>
<td>CGA 30-36 wk: 2.3 g/kg/d</td>
</tr>
<tr>
<td></td>
<td>CGA 36-wk term: 1.5-2 g/kg/d</td>
</tr>
</tbody>
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Abbreviation: CGA, corrected gestational age.
Protein intakes up to 4.9 g/kg per day are considered safe, and practitioners should err on the side of overestimating rather than underestimating protein needs.

The optimal feeding for all infants is mother’s own milk. Human milk contains bioactive components that provide numerous health benefits, including many protections specific to preterm infants. Preterm infants fed human milk have shorter neonatal intensive care unit (NICU) stays, decreased incidence of late-onset sepsis, retinopathy of prematurity, lower rates of metabolic syndrome and reduced blood pressure in adolescence, and improved cognitive and motor development as toddlers.

Perhaps the most significant effect of human milk feeding in the preterm infant population is the association with a significantly reduced incidence of necrotizing enterocolitis (NEC). Human milk intake, particularly early human milk intake, provides protection against NEC, and a dose response exists such that feeding a greater proportion of human milk provides increased protection against NEC.

Despite its many benefits, unfortified human milk does not provide adequate nutrition for the VLBW infant. Preterm infants fed unfortified human milk are at increased risk of poor growth, decreased bone mineralization, and late hyponatremia compared with those fed preterm infant formula. Multinutrient human milk fortifiers are designed to meet the macro- and micronutrient needs of VLBW infants when added to human milk to a final concentration of 24 kcal/oz. Fortification is typically initiated once enteral feedings reach approximately 80 to 100 mL/kg per day, although there are reports of successful initiation of fortification at total feeding volumes as low as 25 mL/day or even at the initiation of enteral feeding. There is currently no evidence that increasing the amount of fortifier incrementally over a period of multiple days provides any clinical benefit. If clinicians choose this practice, it should be done with the concurrent provision of adequate parenteral nutrition to meet full nutrient needs until goal levels of fortification are reached. The use of standardized feeding protocols that incorporate fortification guidelines is often an effective strategy for optimizing nutrient intake.

Despite the widespread use of human milk fortifiers, growth of preterm infants fed formula human milk still lags behind both expected intrauterine growth rates and growth rates of preterm infants fed preterm infant formula. Inadequate protein intake is believed to be the primary cause of reduced growth rates of preterm infants fed fortified human milk. Most currently available human milk fortifiers were designed to meet protein needs of preterm infants when added to early preterm milk.

Protein content of preterm milk decreases over the first 2 to 4 weeks after delivery, and protein provision, therefore, becomes inadequate as lactation progresses. Infants fed fortified donor human milk, which is provided primarily by mothers several months into lactation, are at particularly high risk for receiving lower than estimated protein intakes. Actual protein intakes have been found to be as much as 1.5 g/kg per day lower than assumed values when feeding human milk fortified with standard methods.

Additional protein can be added to fortified human milk by adding a fixed amount of a modular protein supplement to all fortified human milk at a level that ensures all infants receive protein delivery consistent with published guidelines even when receiving the minimum possible protein provision from human milk. This method acknowledges the low risk of providing a modest protein excess, which then allows for energy production from amino acids as occurs in utero. The quantity of additional protein needed to meet recommended protein intakes can be calculated using estimates of the protein content of term human milk and the protein content of the chosen multinutrient human milk fortifier. The quality or type of protein that should be used in human milk fortification to optimize clinical outcomes for preterm infants remains in question.

SEARCH STRATEGY

A PubMed literature search was conducted to examine 3 different aspects of protein quality in the context of preterm infant feeding. These included whey-to-casein ratios, hydrolyzed versus intact protein, and bovine milk protein versus human milk protein. The searches for whey/casein and hydrolyzed protein yielded minimal relevant results, so the search terms were liberalized to include studies of preterm infant formulas. Because as much as two-thirds of the total enteral protein must be provided with fortifiers and modular protein supplements to meet the high needs of the VLBW and ELBW infants when feeding human milk, it is reasonable to consider extrapolating findings from preterm formula to human milk fortification in the absence of adequate data in human milk–fed infants. Final specific PubMed search terms are listed in Table 2. Studies selected for inclusion in this review study were limited to prospective clinical trials conducted since 1990 in preterm or LBW infant populations comparing feeding types that addressed 1 or more of the aspects of protein quality in question. Twenty-six published studies were reviewed. Four examined whey-to-casein ratios, 13 examined hydrolyzed versus intact protein, and 9 examined bovine milk protein versus human milk protein.
The primary milk proteins casein and whey are naturally present in significantly different proportions in human milk compared with bovine milk, the protein source for the majority of human milk fortifiers currently available in the United States. Human milk contains a substantially higher percentage of more soluble whey protein, with a whey-to-casein ratio of approximately 60:40 for mature human milk compared with a ratio of 18:82 for bovine milk protein. All studies meeting the criteria for inclusion in the current review compared various whey-to-casein ratios of preterm formulas and showed no major clinical benefit to any particular ratio. No evidence was found for any clinically significant effect on acid-base status, growth outcomes, or gastric emptying. In 1 study, nitrogen and phosphorus absorption were higher with casein-predominant formulas, whereas fat absorption was higher with increasing whey content. Plasma amino acids differed with varying whey-to-casein ratios, reflecting the different amino acid profiles of the 2 proteins. Plasma threonine levels are generally higher with increasing whey-to-casein ratio, and phenylalanine and tyrosine levels are lower with increasing whey-to-casein ratio. The clinical significance of and optimal ranges for individual plasma amino acid levels in preterm infants are not entirely clear; an overall product formulation likely has a greater effect on clinical outcomes than whey-to-casein ratio alone. Bovine milk–based multini nutrient human milk fortifiers currently available in the United States contain either a mixture of whey and casein or exclusive whey protein.

Hydrolyzed Protein

Hydrolyzed protein refers to proteins that have been broken down into smaller peptides or free amino acids. Protein hydrolyzate products are increasingly being used in preterm infant populations, and their risks and benefits must be considered. Human milk fortifiers and preterm infant formulas consisting of hydrolyzed protein have been used in Europe for many years to reduce the risk of feeding intolerance and later allergic conditions. The nutritional adequacy of protein hydrolyzate products for preterm infants has been questioned. Larger peptides and proteins are better absorbed than shorter peptides and amino acids, and the processing necessary to reduce peptide size reduces the bioavailability of the amino acids and the organic calcium and phosphorus.

The studies included in this review study were conducted with preterm formula and yield mixed results. In a frequently cited study of 19 preterm infants fed 3 different hydrolyzed protein preterm formulas or a standard preterm formula, the infants fed the hydrolyzed formulas had reduced nitrogen absorption (83% vs 90%) and phosphorus absorption (78% vs 89%) compared with the infants fed a standard preterm formula. Additional studies have also found decreased nitrogen absorption and reduced growth rates in preterm infants fed hydrolyzate formulas when compared with those fed intact protein formulas with nitrogen content either equivalent to or greater than the hydrolyzed formula. Another study showed equivalent growth but reduced serum proteins and albumin with a partially hydrolyzed whey protein formula compared with an isonitrogenous intact protein formula.

Despite evidence of reduced nutrient bioavailability, data suggest that growth and biochemical outcomes equivalent to those of standard preterm formulas can be achieved when feeding preterm hydrolyzate formulas with approximately 10% higher total protein content and added calcium and phosphorus. Protein hydrolyzate preterm formulas have been found to reduce gastrointestinal transit time, accelerate feeding advancement, and reduce the number of gastroesophageal reflux events and esophageal acid reflux exposure in preterm infants. In another study, however, a hydrolyzed protein formula had no effect on gastric emptying. As with standard preterm formulas, extensive and partial hydrolyzate preterm formulas with adequate protein content have resulted in greater weight gain and plasma amino acid and urea levels compared with human milk fortified in a standard fashion without additional protein supplementation. Serum amino acid profiles reflect whey-to-casein ratios and formula amino acid composition and seem to be generally unaffected by protein hydrolysis.

Two studies examined the effect of hydrolyzed protein on later development of allergic disease in preterm infants. One study found reduced incidence of atopic dermatitis at 12 months but no other effect on allergic disease; the other found no...
biochemical or clinical evidence of allergic disease at 5 to 7 years. Additional studies are needed on the effect of protein hydrolyzate products on allergic conditions in preterm infants, particularly in the context of human milk fortification.

Although hydrolyzed protein may offer some benefits in preterm infant feeding, careful attention must be given to product formulation to ensure that protein and mineral content is adequate to compensate for reduced bioavailability.

**Bovine Milk Protein and Human Milk Protein**

Numerous studies suggest that human milk fortified with bovine milk protein results in equivalent growth and biochemical indicators of nutrition status to human milk fortified with human milk protein, particularly when the bovine protein source is formulated to replicate the amino acid composition of human milk. One study showed higher postprandial plasma amino acids and faster protein absorption with bovine milk–based preterm formula compared with human milk fortified with human milk protein.

One of the latest questions in regard to the optimal composition of human milk fortifier is whether the risk of NEC is affected by the fortifier protein source. In a recent study, infants with birth weights of 500 to 1250 g received mother’s own milk as available and were randomized to receive either human milk–based fortifier or bovine milk–based fortifier. In the absence of adequate volumes of maternal milk, the infants receiving the human milk–based fortifier received donor human milk fortified with human milk–based fortifier, and the infants receiving the bovine milk–based fortifier received standard preterm infant formula. The researchers found a 50% reduction in the incidence of all NEC cases and a 90% reduction in the incidence of surgical NEC cases in the group that received an exclusively human milk–based diet. Unfortunately, any differences in outcomes between bovine milk–based human milk fortifiers and standard preterm formula cannot be determined from this study because this group included infants who received both products. In addition, the baseline NEC rate in the group receiving bovine protein was 15.3%, considerably higher than baseline rates in many large NICUs, where bovine protein is regularly used for supplementation.

A related study examined infants fed exclusive diets of either donor human milk fortified with human milk–based fortifier or standard preterm formula. This study found fewer days on total parenteral nutrition in the human milk–based group compared with the preterm formula group (36 vs 27 days) in addition to reduced NEC rate in the human milk–based group. This study also had an unusually high baseline NEC rate (21%) in the group fed bovine protein and unusually long periods of total parenteral nutrition use in both groups.

A final study compared measures of oxidative stress in preterm infants fed standard preterm formula or human milk, varying percentages of which were fortified with bovine milk–based human milk fortifier. The group fed human milk with the highest

| TABLE 3. Comparison of Human Milk Fortifier and Protein Supplement Products |
|------------------|------------------|------------------|------------------|
| Product Name     | Product Type     | Protein Type     | Protein Provided When Added to Term Human Milk to 24 kcal/oz Fed at 150 mL/kg |
| Enfamil Human Milk Fortifier Acidified Liquid | Multinutrient liquid fortifier | Hydrolyzed bovine whey | 4.1 g/kg |
| Similac Human Milk Fortifier Concentrated Liquid | Multinutrient liquid fortifier | Intact bovine casein/whey | 3.1 g/kg |
| Prolact + HMF Human Milk Fortifier | Multinutrient liquid fortifier | Intact human milk protein | 3.1 g/kg |
| Similac Liquid Protein Fortifier | Protein modular liquid | Hydrolyzed bovine casein | NA |
| Nestlé Beneprotein | Protein modular powder | Intact bovine whey | NA |

Abbreviation: NA, not available.
percentage of total feeding volumes fortified with bovine human milk fortifier (≥50%) had the highest levels of F2-isoprostanes, a measure of oxidative stress. The clinical effect of this finding remains unclear.

Despite the shortcomings of these most recent studies, the effect of feeding bovine milk–based human milk fortifier on incidence of NEC in preterm infants deserves additional attention, and any potential effect of protein hydrolysis should also be examined.

**RECOMMENDATIONS FOR PRACTICE**

The reviewed studies show no definite advantage of any specific whey-to-casein ratio. There may be potential benefits of hydrolyzate products when carefully formulated to compensate for their nutritional shortcomings, although data specific to human milk fortifiers are not currently available. It is still unclear whether there is a true effect of human milk–based human milk fortifier on the incidence of NEC. Better study design and a more representative baseline patient population are needed in future studies.

In clinical practice, choices in fortification of human milk are limited by product availability. Until recently, many of the most nutritionally appropriate products available in the United States were powders. Because of the increased risk of bacterial contamination with powder products, the use of sterile liquid alternatives

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**TABLE 4. Summary of Recommendations for Research and Practice**

<table>
<thead>
<tr>
<th>What we know</th>
<th>Adequate neonatal growth results in improved neurodevelopmental outcomes</th>
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<tbody>
<tr>
<td></td>
<td>Protein needs of preterm infants are estimated at 3.4-4.8 g/kg/d and are affected by catch up needs</td>
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<tr>
<td></td>
<td>Human milk is the optimal feeding for preterm infants but requires fortification with macro- and micronutrients</td>
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<tr>
<td></td>
<td>Human milk fortified in a standard fashion fails to meet protein needs of the smallest preterm infants, and additional modular protein supplements are needed</td>
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<td></td>
<td>Whey-to-casein ratio in studies of preterm infant formula has minimal effect on clinical outcomes but has not been well studied in human milk fortifiers</td>
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<tr>
<td></td>
<td>Hydrolyzed protein sources may provide benefits for some preterm infants but must be carefully formulated to compensate for decreased nutrient bioavailability</td>
</tr>
<tr>
<td></td>
<td>Similar growth and biochemical outcomes are achieved with bovine milk–based and human milk–based fortifiers</td>
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<table>
<thead>
<tr>
<th>What needs to be studied</th>
<th>Growth and biochemical outcomes in preterm infants fed human milk fortified with protein hydrolyzate human milk fortifier</th>
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<tr>
<td></td>
<td>Comparison of rates of later allergic disease between human milk fortifier with intact and hydrolyzed protein</td>
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<tr>
<td></td>
<td>Additional studies on the effect of bovine milk–based human milk fortifier on incidence of NEC and other clinical outcomes</td>
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<tr>
<td></td>
<td>Comparison of NEC rates between human milk fortifier with intact and hydrolyzed bovine protein</td>
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<table>
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<tr>
<th>What we can do today</th>
<th>Support feeding with human milk</th>
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<tbody>
<tr>
<td></td>
<td>Aim to achieve intrauterine fetal growth rates</td>
</tr>
<tr>
<td></td>
<td>Provide early parenteral protein and initiate early enteral feeding to help minimize early protein deficits</td>
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<tr>
<td></td>
<td>Take early deficits into consideration when estimating enteral protein needs</td>
</tr>
<tr>
<td></td>
<td>Provide modular protein supplements in addition to human milk fortifier as needed for adequate protein provision with human milk from later stages of lactation</td>
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<tr>
<td></td>
<td>Choose fortifier products whose protein composition is formulated to replicate the amino acid composition of human milk</td>
</tr>
<tr>
<td></td>
<td>When selecting fortifier products, consider the potential benefits of hydrolyzed protein as well as the need for greater total protein content and addition of calcium and phosphorus to compensate for reduced bioavailability</td>
</tr>
<tr>
<td></td>
<td>Support development of fortifier products that meet protein needs of our high-risk patients</td>
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Abbreviation: NEC, necrotizing enterocolitis.
is recommended whenever possible and the use of powder products in NICUs is discouraged.78,80

Currently, there are 3 primary liquid multinutrient products intended exclusively for use in human milk fortification available in the United States,52,53,81 and these products vary in all aspects of protein quality. One of these is a human milk–based product81 that may be prohibitively expensive for many institutions and requires an additional modular protein supplement to meet the protein needs of the smallest premature infants. Another liquid product was designed to meet full protein needs for most preterm infants without the need for an additional modular protein supplement.83 This product contains partially hydrolyzed whey protein and is acidified, bringing the pH of the fortified human milk down from 6.8 to 4.7.82 Acidification has been found to significantly reduce milk white blood cell count, lipase activity, and total protein content compared with unaltered human milk.82 A retrospective chart review conducted by these authors revealed a higher incidence of metabolic acidosis, and slower growth despite increased delivery of protein and calories in infants fed this fortifier compared with those fed powder human milk fortifier.83 Another center has reported similar findings of poor growth, acidosis, and intolerance to this product.84 The third liquid multinutrient product85 contains intact mixed bovine protein and requires the addition of a modular protein supplement to meet the needs of most VLBW infants. Commercially available modular protein products at this time include an intact bovine whey powder85 and a liquid hydrolyzed casein product designed specifically for use in pediatric populations.86 Currently available fortifier and supplement products are summarized in Table 3.

RECOMMENDATIONS FOR RESEARCH

Prospective clinical trials examining protein quality of human milk fortifiers in preterm infant populations are limited. Clinical practice and research recommendations are summarized in Table 4.

Clinical trials are needed to examine the nutritional quality of protein hydrolyze human milk fortifiers and their associated clinical outcomes, including incidence of NEC and later development of allergic disease, when compared with intact protein human milk fortifiers. Future research should also include additional studies on the effect of bovine milk–based human milk fortifiers on growth, the incidence of NEC, and other clinical outcomes without out the confounding variable of supplemental standard preterm formula. Finally, fortifier product development should be expanded to include additional high-protein nonacidified liquid fortifier products that allow clinicians to nourish optimally the human milk–fed preterm infant.

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58. Maggo L, Zappa AA, Savazzini G, Valsasina R, Schubert W. Higher urinary excretion of essential amino acids in preterm infants fed pro-
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