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Protein hydrolysate versus standard formula for preterm infants (Review)

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[Intervention Review]

Protein hydrolysate versus standard formula for preterm infants

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ABSTRACT

Background

When human milk is not available for feeding preterm infants, protein hydrolysate rather than standard cow's milk formulas (with intact proteins) are often used because they are perceived as being tolerated better and less likely to lead to complications. However, protein hydrolysate formulas are more expensive than standard formulas, and concern exists that their use in practice is not supported by high-quality evidence.

Objectives

To assess the effect of feeding preterm infants with hydrolysed formula (versus standard cow's milk formulas) on the risk of feed intolerance, necrotising enterocolitis, and other morbidity and mortality in preterm infants.

Search methods

We used the standard Cochrane Neonatal search strategy including electronic searches of the Cochrane Central Register of Controlled Trials (CENTRAL; 2017, Issue 4), Ovid MEDLINE, Ovid Embase, and the Cumulative Index to Nursing and Allied Health Literature (CINAHL) (to April 2017), as well as conference proceedings and previous reviews.

Selection criteria

Randomised and quasi-randomised controlled trials that compared feeding preterm infants with protein hydrolysate versus standard (non-hydrolysed) cow's milk formula.

Data collection and analysis

Two review authors assessed trial eligibility and risk of bias and extracted data independently. We analysed treatment effects as described in the individual trials and reported risk ratios and risk differences for dichotomous data, and mean differences for continuous data, with respective 95% confidence intervals (CI). We used a fixed-effect model in meta-analyses and explored potential causes of heterogeneity in sensitivity analyses. We assessed quality of evidence at the outcome level using the GRADE approach.

Main results

We identified 11 trials for inclusion in the review. All trials were small (total participants 665) and had various methodological limitations including uncertainty about methods to ensure allocation concealment and blinding. Most participants were clinically stable preterm infants of gestational age less than about 34 weeks or birth weight less than about 1750 g. Fewer participants were extremely preterm, extremely low birth weight, or growth-restricted. Most trials found no effects on feed intolerance assessed variously as mean prefeed gastric residual volume, incidence of abdominal distention or other concerning gastrointestinal signs, or time taken to achieve full enteral feeds (meta-analysis was limited because studies used different measures). Meta-analysis found no effect on the risk of necrotising enterocolitis (typical risk ratio 1.10, 95% CI 0.36 to 3.34; risk difference 0.00, 95% CI -0.03 to 0.04; 5 trials, 385 infants) (low quality evidence; downgraded for imprecision and design weaknesses).

Authors' conclusions

The identified trials provide only low quality evidence about the effects of feeding preterm infants with protein hydrolysate versus standard formula. The existing data did not support conclusions that feeding with protein hydrolysate affects the risk of feed intolerance or necrotising enterocolitis. Further large, pragmatic trials are needed to provide more reliable and precise estimates of effectiveness and cost-effectiveness.

PLAIN LANGUAGE SUMMARY

Hydrolysed formula for preterm infants

Review question: does feeding preterm infants with cow's milk formula containing predigested (hydrolysed) proteins rather than whole proteins improve digestion and reduce the risk of severe bowel problems?

Background: preterm infants often find cow's milk formula more difficult to digest than human milk, and cow's milk formula may increase the risk of severe bowel problems for preterm (born before their due date) infants. If preterm infants are fed with cow's milk formula (when human milk is unavailable), then using a formula in which the protein is already partially digested (called hydrolysed) rather than a standard formula (with intact proteins) might reduce the risk of these problems. However, hydrolysed formulas are more expensive than standard formulas, and may have specific side effects not seen with standard formulas. Given these concerns, we have reviewed all the available evidence from clinical trials that compared these types of formula for feeding preterm infants.

Study characteristics: in searches of medical databases up to April 2017, we found 11 trials; most were small (involving 665 infants in total) and had methodological weaknesses.

Key results: the data from these trials provided no strong or consistent evidence that feeding preterm infants with hydrolysed formula rather than standard formula improved digestion or reduced the risk of severe bowel problems.

Conclusions: the currently available evidence suggested that feeding preterm infants with hydrolysed formula (rather than standard formula) during their initial hospital admission has no important benefits or harms. However, this finding is not yet conclusive, and larger and better quality trials are needed to provide evidence to help clinicians and families make informed choices about this issue.

SUMMARY OF FINDINGS FOR THE MAIN COMPARISON [Explanation]

Hydrolysed compared to non-hydrolysed formula for feeding preterm infants

Patient or population: feeding preterm infants
Setting: neonatal unit
Intervention: hydrolysed formula (protein hydrolysate)
Comparison: non-hydrolysed formula

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	No of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with non-hydrolysed formula	Risk with hydrolysed formula				
Feed intolerance	Study population		RR 2.71 (0.29 to 25.00)	161 (3 RCTs)	⊕⊕◯◯ **Low**	Limited data from 3 small RCTs with imprecise estimate of effect size
	13 per 1000	34 per 1000 (4 to 316)				
Necrotising enterocolitis	Study population		RR 1.10 (0.36 to 3.34)	385 (5 RCTs)	⊕⊕◯◯ **Low**	Methodological limitations in included trials, and imprecise effect size estimate
	32 per 1000	35 per 1000 (12 to 107)				

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: confidence interval; **RCT:** randomised controlled trial; **RR:** risk ratio.

BACKGROUND

Hydrolysed cow's milk formulas, originally developed for infants with cow's milk protein allergy or intolerance, are used as enteral feeding alternatives for preterm infants for whom human milk is unavailable. These formulas contain hydrolysed rather than intact proteins, and may also differ from standard cow's milk formulas in carbohydrate, lipid, and micronutrient type and content (Oldaeus 1997). Their use as a sole or supplemental enteral feed source for preterm infants has increased since the late-1990s, particularly in high-income countries, because they are perceived as being tolerated better, and less likely to lead to complications, than standard cow's milk formulas (Zuppa 2005). However, hydrolysed formulas are more expensive than standard formulas, and concern exists that their use in practice is not supported by high quality evidence (Foucard 2005).

Description of the condition

Human breast milk is recommended as the best form of enteral nutrition for preterm infants (AAP 2012). Breast milk proteins, carbohydrates, fats, and micronutrients have been optimised by evolution for neonatal digestion and absorption. Breast milk contains many non-nutrient factors including immunoglobulins and lactoferrin that promote intestinal adaptation and maturation, improve enteral feed tolerance, and protect against infection and inflammatory disorders (Agostoni 2010; Arslanoglu 2013).

When sufficient human breast milk is unavailable, cow's milk-based formulas are used for feeding preterm infants, either as the sole enteral diet or as a supplement to human breast milk (Klingenberg 2012). Feeding preterm infants with standard cow's milk formulas rather than human breast milk is, however, associated with higher rates of feed intolerance and necrotising enterocolitis (Quigley 2014). Feed intolerance and interruption of enteral feeds is a major contributor to cumulative nutrient deficits and postnatal growth restriction in very preterm infants (Embleton 2001; Cooke 2016). Slow postnatal growth is associated with neurodevelopmental impairment in later childhood and with poorer cognitive and educational outcomes (Brandt 2003; Embleton 2013a; Leppanen 2014). Necrotising enterocolitis affects about 5% of very preterm infants. Infants who develop necrotising enterocolitis experience more infections, have lower levels of nutrient intake, grow more slowly, have longer durations of intensive care and hospital stay, and are more likely to die or be disabled than gestation-comparable infants who do not develop necrotising enterocolitis (Morgan 2011; Pike 2012; Yee 2012).

Description of the intervention

Standard cow's milk formulas can be grouped broadly as 'term' formulas (designed for term infants; nutrient content based on the composition of mature breast milk) and nutrient-enriched 'preterm' formulas (designed for preterm or low birth weight infants; energy-enriched and variably protein- and mineral-enriched) (Fewtrell 1999). Concern exists that standard cow's milk formulas (either 'term' or 'preterm') are poorly tolerated, especially by very preterm infants, because the immature infant's gastrointestinal tract is less efficient than that of term infants at digesting intact cow's milk proteins and fats (Ewer 1994; Lindberg 1998).

Hydrolysed formulas

'Hydrolysed' protein formulas, containing protein digested chemically (acid/alkali) or enzymatically (protease) to oligopeptides, are often used for feeding preterm infants, especially infants with feed intolerance or clinical features (such as episodic apnoea, oxygen desaturation or bradycardia) that are attributed to gastro-oesophageal reflux, or following gastrointestinal surgery or necrotising enterocolitis (Zuppa 2005).

Several brands of hydrolysed formulas (both 'term' and 'preterm') are available commercially and these are grouped broadly depending on degree of hydrolysis:

- extensively hydrolysed: residual free amino acids and peptides with molecular weights less than 1.5 kDa to 3.0 kDa;
- partially hydrolysed: residual peptides with molecular weights of 3.0 kDa to 10.0 kDa.

This distinction is mainly relevant to the putative hypo-allergenic properties of hydrolysed formulas and there are limited data regarding its functional relevance to preterm infants. Formulas also vary by the predominant protein source (casein versus whey-casein) as well as by carbohydrate (lactose, maltodextrin) and fat (cow, vegetable) type and content (BNFC 2016).

How the intervention might work

Although developed as hypo-allergenic alternatives to standard cow's milk formulas for infants at risk of cow's milk protein intolerance or allergy, the evidence for this effect in term infants is very weak (Boyle 2016; Osborn 2017). In preterm infants, hydrolysed formulas are mostly used for their perceived benefits in reducing the risk of feed intolerance and necrotising enterocolitis. When human milk is unavailable, hydrolysed formulas may be used empirically (starter formula) or therapeutically to improve feeding tolerance or reduce gastro-oesophageal reflux. The possible mechanisms for these effects include accelerated gastric emptying and intestinal transit, more efficient enteric peptide digestion, and stimulation of small intestinal enzymatic and motilin activity (Mihatsch 2001b; Zuppa 2005). If better feed tolerance reduces the time taken to establish full enteral feeding in very preterm infants, this may reduce the adverse infectious or metabolic consequences of prolonged exposure to parenteral nutrition.

Several potential adverse effects of hydrolysed formulas are recognised. Osmolality increases when protein is hydrolysed into smaller peptides, and these higher osmolarity fluids delivered to the small

intestine may increase the risk of necrotising enterocolitis. Furthermore, if bioactive proteins such as immunoglobulin or lactoferrin are hydrolysed, this may reduce their putative benefits in reducing the risk of infection or necrotising enterocolitis. It is possible that some peptides created by artificial hydrolysis have diminished or harmful functional activities (Embleton 2013b). Concern about micronutrient bioavailability in hydrolysed formulas also exists, particularly whether bone minerals are less well absorbed in the absence of intact casein proteins (Zuppa 2005).

Why it is important to do this review

Given the potential for protein hydrolysate formulas (rather than standard cow's milk formulas) to improve enteral feed tolerance and prevent adverse outcomes in preterm infants, we undertook a systematic review of the randomised trial data to help to inform practice and research.

OBJECTIVES

To assess the effect of feeding preterm infants with hydrolysed formula (versus standard cow's milk formulas) on the risk of feed intolerance, necrotising enterocolitis, and other morbidity and mortality in preterm infants.

METHODS

Criteria for considering studies for this review

Types of studies

Randomised or quasi-randomised controlled trials, including cluster-randomised controlled trials.

Types of participants

Preterm (less than 37 weeks' gestation) newborn infants who received cow's milk formula as their sole or supplemental enteral diet.

Types of interventions

Hydrolysed cow's milk formula versus standard (non-hydrolysed) cow's milk formula or another type of hydrolysed cow's milk formula. Formula was to be allocated as at least 20% of intended enteral diet for at least two weeks to allow measurable effects on growth rates and episodes of feed intolerance. Trials should have compared formulas with similar energy and protein levels (i.e. hydrolysed 'preterm' formula versus non-hydrolysed 'preterm' formula, or hydrolysed 'term' formula versus non-hydrolysed 'term' formula).

We planned separate comparisons of trials that assessed:

- *empirical* use of hydrolysed formulas;
- *indicated* (therapeutic) use of hydrolysed formulas to treat infants with feed intolerance, gastro-oesophageal reflux (and associated apnoea, desaturation or bradycardia), or following gastrointestinal surgery or necrotising enterocolitis (as defined by the primary investigators).

Types of outcome measures

Primary outcomes

- Number of infants with at least one episode of feed intolerance that resulted in cessation or reduction in enteral feeding (enteral feeds reduced or ceased for more than four hours), or mean number of episodes of feed intolerance during trial period, or both.
- Infants with at least one episode of necrotising enterocolitis (modified Bell stage 2/3) (Walsh 1986) (unless indicated use following necrotising enterocolitis).

Secondary outcomes

- Time to full enteral feeding independent of parenteral fluids (days).
- Growth: time to regain birth weight, and subsequent rates of weight (grams/kilogram/day), length (millimetre/week), and head growth (millimetre/week) during hospital admission.
- Duration of hospital admission (days).
- Measures of bone mineralisation:
    - serum alkaline phosphatase level at 36 to 40 weeks' postmenstrual age or
    - bone mineral content assessed post-term by dual energy x-ray absorptiometry (DEXA) or
    - clinical or radiological evidence of rickets on long-term follow-up.
- Late-onset invasive infection diagnosed more than 72 hours after birth as determined by culture from a normally sterile site: cerebrospinal fluid, blood, bone or joint, peritoneum, pleural space or central venous line tip; or findings on autopsy examination consistent with invasive microbial infection.
- Mortality: all-cause until 28 days and during hospital admission.
- Neurodevelopmental outcomes assessed by a validated test after 12 months' post-term: neurological evaluations, developmental scores and classifications of disability, including auditory and visual disability.
- Allergy or atopy diagnosed after 12 months' post-term: asthma, eczema, allergic rhinitis or conjunctivitis, food allergy,

allergic sensitisation (skin prick, or specific or total immunoglobulin E level) (Boyle 2016).

Search methods for identification of studies

Electronic searches

We searched the Cochrane Central Register of Controlled Trials (CENTRAL, 2017, issue 4), Ovid MEDLINE (1946 to April 2017), Ovid Embase (1974 to April 2017), Ovid Maternity & Infant Care Database (1971 to April 2017), and CINAHL (1982 to April 2017) using a combination of the following text words and MeSH terms described in Appendix 1. We limited the search outputs with the relevant search filters for clinical trials as recommended in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011). We did not apply any language restrictions.

We searched ClinicalTrials.gov and the World Health Organization's International Trials Registry and Platform (www.who.int/ictrp/en/) for completed or ongoing trials.

Searching other resources

We examined reference lists in previous reviews and included studies. We searched the proceedings of the annual meetings of the Pediatric Academic Societies (1993 to 2016), the European Society for Paediatric Research (1995 to 2016), the Royal College of Paediatrics and Child Health (2000 to 2017) and the Perinatal Society of Australia and New Zealand (2000 to 2016). Trials reported only as abstracts were eligible if sufficient information was available from the report, or from contact with the authors, to fulfil the inclusion criteria.

Data collection and analysis

We used the standard methods of Cochrane Neonatal.

Selection of studies

We screened the title and abstract of all studies identified by the search strategy and two review authors independently assessed the full articles for all potentially relevant trials. We excluded those studies that did not meet all the inclusion criteria and we stated the reason for exclusion. We discussed any disagreements until consensus was achieved.

Data extraction and management

Two review authors (DN and WM) extracted data independently using a data collection form to aid extraction of information on design, methodology, participants, interventions, outcomes and treatment effects from each included study. We discussed any disagreements until we reached a consensus. If data from the trial reports were insufficient, we contacted the trialists for further information.

Assessment of risk of bias in included studies

We used the criteria and standard methods of Cochrane Neonatal to assess the methodological quality of any included trials. Two review authors (DN and JKA) assessed risk of bias across key domains (Appendix 2) and resolved disagreements in consultation with a third review author (WM). We requested additional information from the trial authors to clarify methodology and results when necessary. We did not exclude trials on the basis of risk of bias, but we did plan to conduct sensitivity analyses if applicable to explore the consequences of synthesising evidence of variable quality (Higgins 2011).

Measures of treatment effect

We analysed the treatment effects in the individual trials using Review Manager 5 (RevMan 2014) and reported risk ratio (RR) and risk difference (RD) for dichotomous data and mean difference (MD) for continuous data, with respective 95% confidence intervals (CI). We determined the number needed to treat for an additional beneficial outcome (NNTB) or an additional harmful outcome (NNTH) for analyses with a statistically significant difference in the RD.

Unit of analysis issues

The unit of analysis was the participating infant in individually randomised trials and the neonatal unit (or subunit) for cluster-randomised trials. For cluster-randomised trials, we planned to undertake analyses at the level of the participant while accounting for the clustering in the data using the methods recommended in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011).

Dealing with missing data

Where data were missing, and could not be derived as described, we approached the analysis of missing data as follows.

- We contacted the original study investigators to request the missing data.
- Where possible, we imputed missing standard deviations (SDs) using the coefficient of variation (CV) or calculated from other available statistics including standard errors, CIs, t values and P values.
- If the data were assumed to be missing at random, we analysed the data without imputing any missing values.
- If this could not be assumed, then we planned to impute the missing outcomes with replacement values, assuming all to have a poor outcome. We planned sensitivity analyses to assess

any changes in the direction or magnitude of effect resulting from data imputation.

Assessment of heterogeneity

Two review authors assessed clinical heterogeneity, with a meta-analysis conducted only when both authors agreed that study participants, interventions and outcomes were sufficiently similar.
We examined the treatment effects of individual trials and heterogeneity between trial results by inspecting the forest plots. We calculated the I^2 statistic for each analysis to quantify inconsistency across studies and described the percentage of variability in effect estimates that may be due to heterogeneity rather than to sampling error. If we detected moderate or high heterogeneity (I^2 greater than 50%), we would explore the possible causes (e.g. differences in study design, participants, interventions or completeness of outcome assessments).

Assessment of reporting biases

If more than 10 trials were included in a meta-analysis, we planned to examine a funnel plot for asymmetry.

Data synthesis

We used the fixed-effect model in Review Manager 5 for meta-analyses (as per Cochrane Neonatal recommendations) (RevMan 2014). Where moderate or high heterogeneity existed, we planned to examine the potential causes in subgroup and sensitivity analyses.

Quality of evidence

We assessed the quality of evidence for the main comparisons at the primary outcomes level using the GRADE approach, as outlined in the GRADE handbook (Schünemann 2013; see Appendix 3). Two review authors independently assessed the quality of the evidence for outcomes identified as critical or important for clinical decision-making (feed tolerance and incidence of necrotising enterocolitis). We considered evidence from randomised controlled trials as high quality but downgraded the evidence one level for serious (or two levels for very serious) limitations based upon the following: design (risk of bias), consistency across studies, directness of the evidence, precision of estimates and presence of publication bias. We used the GRADEpro GDT Guideline Development Tool to create a 'Summary of findings' table to report the quality of the evidence.

Subgroup analysis and investigation of heterogeneity

We planned subgroup analyses by:

- gestational age at birth: very preterm (less than 32 weeks) infants versus infants born at 32 weeks or later;
- indication (for therapeutic use): postsurgery versus postnecrotising enterocolitis versus feeding intolerance or gastro-oesophageal reflux;
- extent of protein hydrolysis (as defined by manufacturers): extensively versus partially hydrolysed formula.

Sensitivity analysis

We planned sensitivity analyses to determine if the findings were affected by including only studies of adequate methodology (low risk of bias), defined as adequate randomisation and allocation concealment, blinding of intervention and measurement, and less than 10% loss to follow-up.

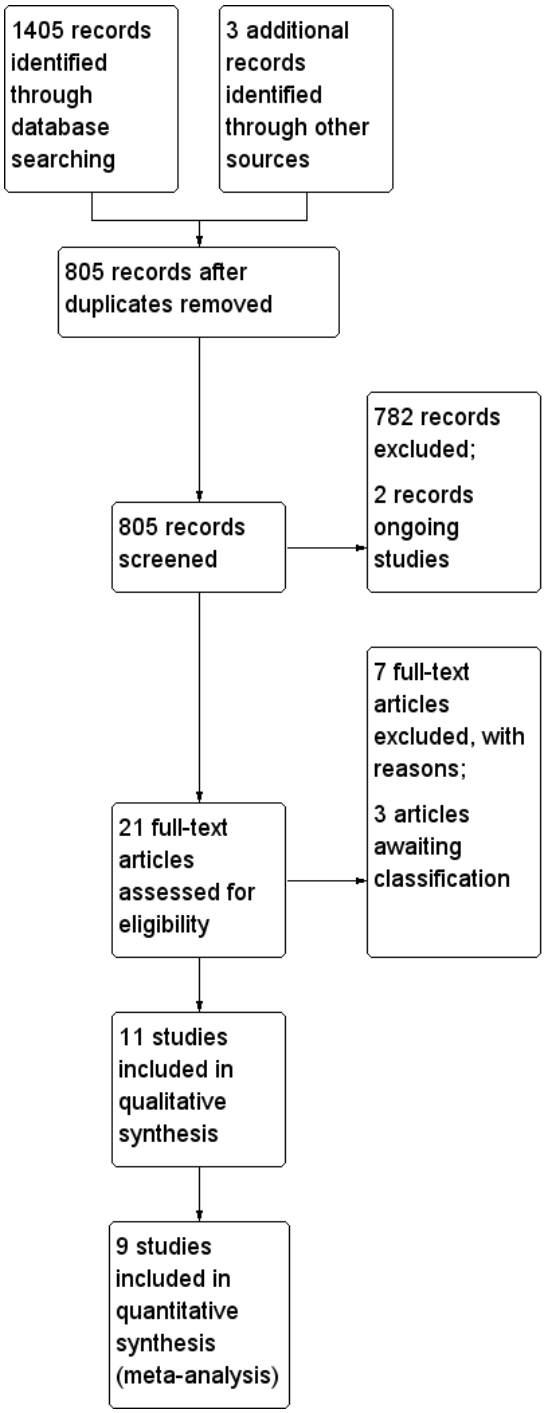
RESULTS

Description of studies

Results of the search

From the preliminary search, we identified 1405 records, including three records from other sources. After removing duplicates, we screened 805 separate records against titles and abstracts for relevance. Among these, we excluded 782 articles outright. We shortlisted 21 articles for full-text assessment, with two articles identified as on-going trials (ACTRN12613000481774; Yin 2015).
We included 11 trials (Characteristics of included studies table) and excluded seven studies (Characteristics of excluded studies table). See Figure 1.

Figure 1. Study flow diagram.



One trial is awaiting further data (Del Moral 2017), and two trials await English language translation to allow assessment of eligibility for inclusion (Dobryanskyy 2015; Luo 2016).

Included studies

We included 11 trials (Huston 1992; Schweizer 1993; Raupp 1995; Pauls 1996; Picaud 2001; Riezzo 2001; Mihatsch 2002; Szajewska 2004; Maggio 2005; Florendo 2009; Baldassarre 2017). Most of the included trials were undertaken during the 1990s and 2000s by investigators in neonatal units in Europe (mainly Germany and Italy) and North America. For further details, see Characteristics of included studies table.

Participants

In total, 665 infants participated in the included trials. Most participants were clinically stable preterm infants of gestational age less than about 34 weeks or birth weight less than about 1750 g. Few participants were extremely preterm, extremely low birth weight or growth-restricted. Most of the trials specifically excluded infants with congenital anomalies, or gastrointestinal or neurological problems.

Interventions

All the trials assessed the *empirical* use of protein hydrolysate formulas; none assessed *indicated* use.

Trials varied according to brand of formula studied. All trials except one assessed a "preterm" (nutrient-enriched) hydrolysed formula; Schweizer 1993 assessed a "term" hydrolysed formula. Most trials used a whey-casein-based hydrolysate. Two trials used a predominantly casein-based hydrolysate (Huston 1992; Riezzo 2001). Most studies assessed a partially hydrolysed formula. Three trials use an extensively hydrolysed formula (Schweizer 1993; Mihatsch 2002; Baldassarre 2017). One (three-arm) trial randomly allocated infants to receive a partially hydrolysed formula, an extensively hydrolysed formula, or a standard preterm formula (Szajewska 2004). Control diets were preterm non-hydrolysed formulas in all except Riezzo 2001 where the control diet was a standard term formula.

No trials compared hydrolysed cow's milk formula versus another type of hydrolysed cow's milk formula.

Trial participants received the intervention or control formulas on commencing enteral feeds either as a sole diet or a supplement when mother's own milk was not available or insufficient. One trial specifically excluded participants post hoc if mother's own milk formed more than 10% of enteral intake (Mihatsch 2002). In general, trial feeds were allocated for several weeks (at least two weeks), or until participating infants reached a specified weight (typically about 1.8 kg).

Outcomes

The outcomes reported most commonly were feed intolerance (reported in various ways but often without accompanying numerical data), growth parameters during the study period or until hospital discharge, and adverse events (including mortality and necrotising enterocolitis). None of the trials reported long-term growth and neurodevelopmental outcomes.

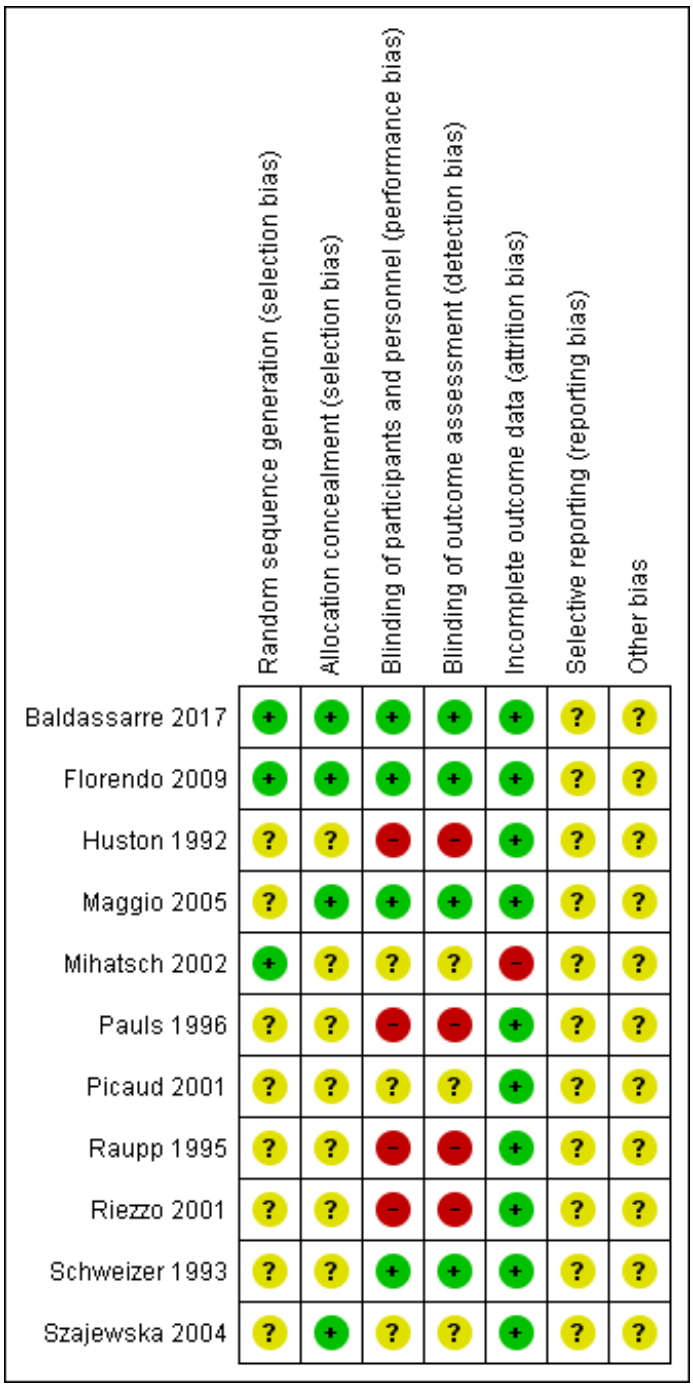
Excluded studies

We excluded seven studies (Rigo 1994; Rigo 1995; Mihatsch 1999; Mihatsch 2001a; Agosti 2003; Corvaglia 2013; Logarajaha 2015). The reasons for exclusion are described in the Characteristics of excluded studies table.

Risk of bias in included studies

Quality assessments are detailed in the Characteristics of included studies table and summarised in Figure 2.

Figure 2. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.



Allocation

Three trials reported adequate allocation concealment methods (sealed, numbered envelopes; central randomisation in blocks) and were at low risk of bias (Szajewska 2004; Maggio 2005; Florendo 2009). None of the remaining trials reported sufficient details to assess if or how allocation concealment was achieved.

Blinding

Four trials reported blinding of investigators, and carers or parents (Schweizer 1993; Maggio 2005; Florendo 2009; Baldassarre 2017). It is probable that the other trials were not blinded as the reports did not describe any methods that might achieve this.

Incomplete outcome data

Most trials were likely to be at low risk of bias because of incomplete assessment of the trial cohort. In one trial, the investigators recruited 129 infants initially then excluded 42 participants post hoc because they had received more than 10% of their enteral intake as human milk (Mihatsch 2002).

Selective reporting

We were unable to assess reliably whether selective reporting occurred as we did not have protocols or other indicators of prespecified outcomes for any of the trials.

Other potential sources of bias

We did not identify any other potential sources of bias in the reports.

Effects of interventions

See: **Summary of findings for the main comparison** Hydrolysed compared to non-hydrolysed formula for feeding preterm infants

Empirical use of protein hydrolysate versus standard formula (Comparison 1)

1. Feed intolerance (Outcome 1.1)

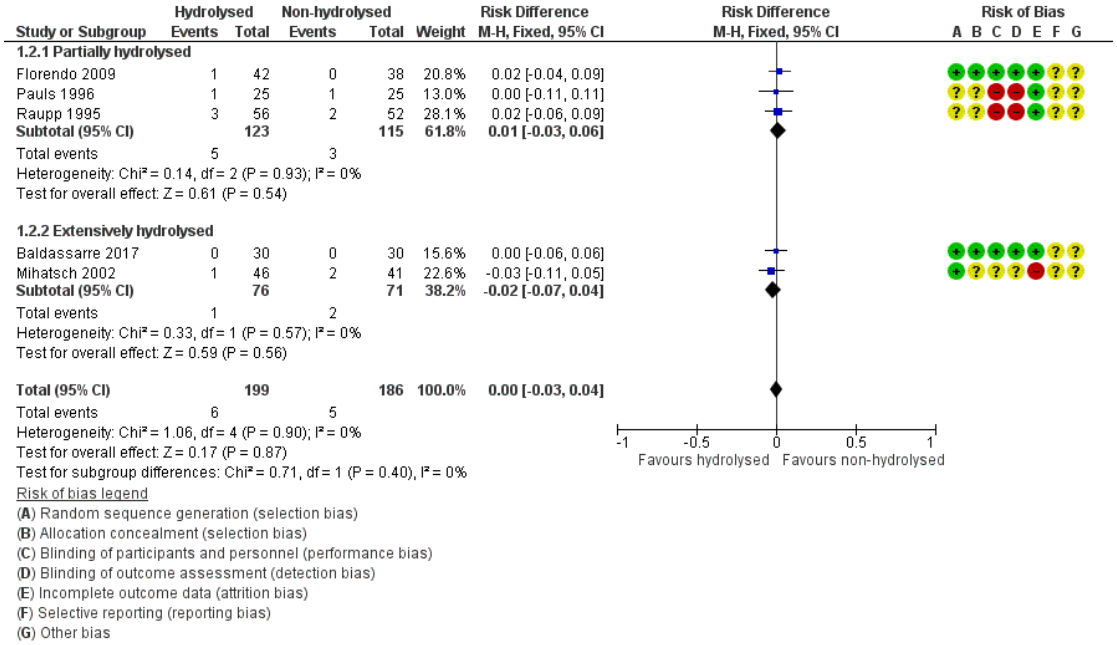
Two trials reported numerical data on the incidence of feed intolerance (Maggio 2005; Florendo 2009; Baldassarre 2017). Meta-analysis found no statistically significant effect (typical RR 2.71, 95% CI 0.29 to 25.00; typical RD 0.02, 95% CI -0.03 to 0.08) (I^2 not applicable) (Analysis 1.1).

The other trials did not report any numerical data but described their findings narratively. These found no differences in measures of gastric residual volumes (Pauls 1996; Mihatsch 2002), frequency of regurgitation (Riezzo 2001), or vomiting or diarrhoea (Szajewska 2004). Raupp 1995 reported that "both formulas were well tolerated." The remaining trials did not report any measures of feed intolerance (Huston 1992; Schweizer 1993; Picaud 2001).

2. Incidence of necrotising enterocolitis (Outcome 1.2)

Meta-analysis of data from five trials (385 infants) found no difference (typical RR 1.10, 95% CI 0.36 to 3.34; typical RD 0.00, 95% CI -0.03 to 0.04) (I^2 = 0%) (Analysis 1.2; Figure 3).

Figure 3. Forest plot of comparison: 1 Hydrolysed versus non-hydrolysed formula, outcome: 1.2 Necrotising enterocolitis.



The other trials did not report this outcome, although in most it seems likely that none of the participants developed necrotising enterocolitis.

The quality of evidence for the primary outcomes was low because of methodological limitations in the included trials (including uncertainty about allocation concealment and blinding), and imprecision of effect size estimates (Summary of findings for the main comparison).

3. Time to full enteral feeding (Outcome 1.3)

Most trials did not report time to full enteral feeds (Huston 1992; Raupp 1995; Riezzo 2001; Szajewska 2004; Maggio 2005; Florendo 2009).

Mihatsch 2002 reported that the median time to full enteral feeding was shorter in the intervention group (10 days versus 12 days in the control group).

Four trials reported no difference:

- Schweizer 1993: 24 days versus 25 days (SD not reported);
- Pauls 1996; no data reported;
- Picaud 2001: 16 (SD 8) days versus 17 (SD 8) days (MD -1.00 days, 95% CI -8.36 to 6.36).
- Baldassarre 2017: 11 days versus 10 days (SD not reported)

4. Growth: time to regain birth weight, and subsequent rates of growth during hospital admission (Outcomes 1.4 to 1.6)

Four trials did not report any growth data (Pauls 1996; Riezzo 2001; Szajewska 2004; Baldassarre 2017). The other trials reported some data on growth parameters during the study period or until hospital discharge, but most did not provide sufficient data for inclusion in the meta-analysis (Huston 1992; Schweizer 1993; Raupp 1995; Mihatsch 2002).

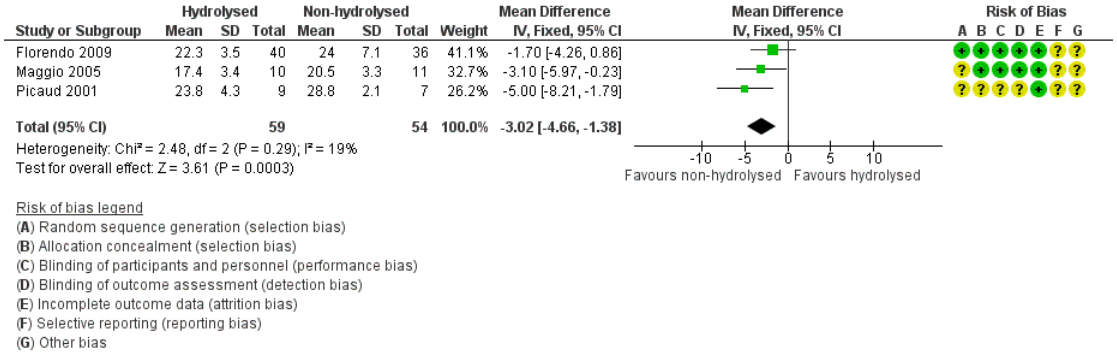
Time to regain birth weight

One trial reported days to regain birth weight (Schweizer 1993). This trial found no difference (10 days in the intervention group versus 9 days in the control group; SD not reported).

Weight gain

Three trials reported rates of weight gain over the study period or until hospital discharge (Picaud 2001; Maggio 2005; Florendo 2009). Meta-analysis showed that weight gain was slower in the infants fed with hydrolysed formula (MD -3.02 g/kg/day, 95% CI -4.66 to -1.38) (Analysis 1.4; Figure 4).

Figure 4. Forest plot of comparison: 1 Hydrolysed versus non-hydrolysed formula, outcome: 1.4 Weight gain (g/kg/day).



Length change

Meta-analysis of data from two trials (97 infants) found no difference in length change (MD -0.04 mm/week, 95% CI -1.24 to 1.15) (Analysis 1.5).

Head circumference growth

Meta-analysis of data from two trials (97 infants) found no difference in head circumference growth (MD 0.27 mm/week, 95% CI -0.39 to 0.94) (Analysis 1.6).

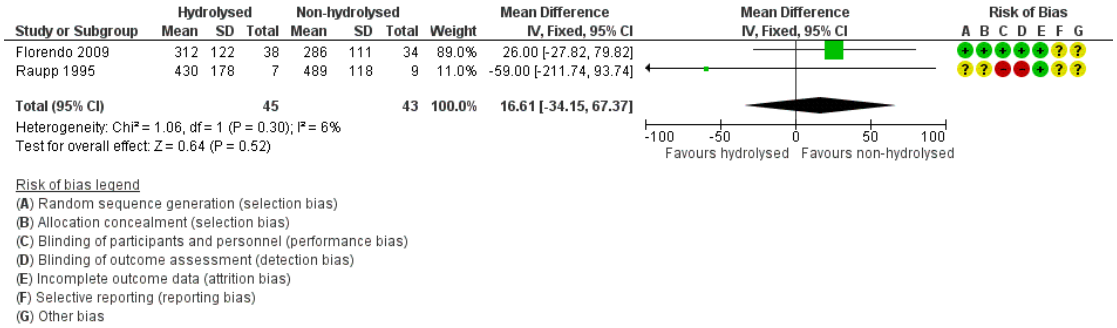
5. Duration of hospital admission

None of the trials reported the duration of hospital admission.

6. Measures of bone mineralisation (Outcome 1.7)

Two trials reported measures of bone mineralisation (Raupp 1995; Florendo 2009). Neither trial, nor a meta-analysis of data from both trials, showed a difference in serum alkaline phosphatase level at 36 to 40 weeks' postmenstrual age (MD 16.6 IU/L, 95% CI -34.1 to 67.4) (Analysis 1.7; Figure 5). None of the trials reported bone mineral content assessed post-term or clinical or radiological evidence of rickets on long-term follow-up.

Figure 5. Forest plot of comparison: 1 Hydrolysed versus non-hydrolysed formula, outcome: 1.7 Serum alkaline phosphatase (IU/L).



7. Late-onset invasive infection (Outcome 1.8)

Only one trial reported the incidence of late-onset invasive infection (Baldassarre 2017). There was no difference in the incidence of microbiologically confirmed bacteraemia (typical RR 1.50, 95% CI 0.27 to 8.34; typical RD -0.03, 95% CI -0.11 to

0.17) (Analysis 1.8).

8. Mortality

None of the trials reported the incidence of mortality.

9. Neurodevelopmental outcomes

None of the trials reported neurodevelopmental outcomes.

10. Allergy or atopy diagnosed after 12 months' post-term (Outcome 1.8)

One trial assessed allergy or atopy (Szajewska 2004). The trial found no difference in the incidence of "any allergic disease" (atopic dermatitis, gastrointestinal symptoms, wheezing) at 12 months (RR 0.62, 95% CI 0.27 to 1.42; RD -0.13, 95% CI -0.36 to 0.10) (Analysis 1.9).

Subgroup analyses

- Gestational age at birth: very preterm (less than 32 weeks) infants versus infants born at 32 weeks or later: subgroup data not available.
- Indication (for therapeutic use): postsurgery versus post-necrotising enterocolitis versus feeding intolerance or gastro-oesophageal reflux: not applicable as all trials assessed empirical use.
- Extent of protein hydrolysis (as defined by manufacturers): data for subgroup analysis sufficient for necrotising enterocolitis (outcome 1.2) only. Three trials used a partially hydrolysed preterm formula (Raupp 1995; Pauls 1996; Florendo 2009). Two trials used an extensively hydrolysed formula (Mihatsch 1999; Baldassarre 2017). Meta-analysis found no evidence of a subgroup effect (test for subgroup differences: $Chi^2 = 0.75$, df = 1 (P = 0.39), $I^2 = 0\%$) (Figure 3).

Indicated use of protein hydrolysate versus standard formula (Comparison 2)

We found no trials comparing protein hydrolysate versus standard formula.

DISCUSSION

Summary of main results

These data from 11 small randomised controlled trials provided only low quality evidence about how feeding preterm infants (typically stable infants of gestational age less than 34 weeks at birth) with protein hydrolysate rather than standard cow's milk formula affects the risk of feed tolerance, necrotising enterocolitis or other adverse outcomes. Limited data did not indicate any important effects on growth, although a meta-analysis of data from three trials suggested that weight gain was slower in infants fed with protein hydrolysate compared with isocaloric preterm formula. There are currently no data available to assess the effects on growth and neurodevelopmental outcomes beyond the initial hospital admission.

Overall completeness and applicability of evidence

These findings should be interpreted and applied cautiously. The primary outcome, feed intolerance, was reported in various ways, and together with the paucity of numerical data, this precluded meta-analysis. Trials generally reported that feeding with protein hydrolysate did not affect measures such as the prefeed gastric residual volume or the need to cease enteral feeding. Similarly, few trials reported the impact of the intervention on the time to achieve full enteral feeding, and the trials that did report this outcome found no statistically significant or clinically important effects.

Although a meta-analysis of five trials (385 participants) found no effect on the risk of necrotising enterocolitis, there were insufficient data to exclude a more modest but still important effect size. The lower bound of the 95% CI was consistent with a 3% absolute risk reduction (i.e. one fewer infant developing necrotising enterocolitis for every 33 infants who received protein hydrolysate formula). Because necrotising enterocolitis is a relatively rare outcome, affecting about 5% of very preterm infants, much larger trials would be needed to provide a more precise estimate of the effect of feeding with protein hydrolysate versus standard formula (Yee 2012).

Data on growth parameters are limited, as are data on other adverse outcomes. Furthermore, uncertainty remains about longer-term impact on growth or development. As concerns exist that hydrolysed proteins may be utilised less efficiently than intact proteins by preterm infants, and that concomitant mineral uptake may be lower, trials that assess the effects on both short- and long-term growth and body composition (including bone health) may help to inform policy and practice (Senterre 2016).

Another major applicability limitation of this review is that all the included trials were undertaken at healthcare facilities in high-income countries, and none in low-income countries. Therefore, this evidence may be of limited applicability to practices in resource-limited settings where, globally, most preterm and low birth weight infants are cared for (Imdad 2013).

All the included trials assessed the effect of empirical (primary) use of protein hydrolysate for feeding preterm infants. We found no trials that assessed the indicated use of protein hydrolysate versus standard formula for preterm infants with feed intolerance, gastro-oesophageal reflux (and associated apnoea, desaturation or bradycardia), or following gastrointestinal surgery or necrotising enterocolitis. Although indicated use of protein hydrolysate is common,

based on perceptions that formulas with intact proteins may be tolerated poorly by infants with intestinal trauma or compromise, there is no evidence from trials to inform this practice (Lapillonne 2016).

Quality of the evidence

The GRADE assessments indicated that the quality of evidence for the primary outcomes was 'low' because of methodological limitations in the included trials (including uncertainty about allocation concealment and blinding), and imprecision of effect size estimates (Summary of findings for the main comparison).
Most of the included trials were funded or supported by the manufacturers of the formulas being assessed but the funders were not involved in trial design or analysis. However, there remains some concern that formula manufacturers may promote study findings of trials of specialist formulas selectively as part of a marketing strategy that subverts UNICEF Baby Friendly Initiative regulations (Cleminson 2015).

Potential biases in the review process

It is possible that our findings were subject to publication and other reporting biases. We attempted to minimise this by screening the reference lists of included trials and related reviews and searching the proceedings of major international perinatal conferences to identify trial reports that were not (or were not yet) published in full form in academic journals. The meta-analyses that we performed did not contain sufficient trials to explore symmetry of funnel plots as a means of identifying possible publication or reporting bias.

AUTHORS' CONCLUSIONS

Implications for practice

This review provides only low quality evidence regarding any benefits or harms of feeding preterm infants with protein hydrolysate versus standard formula. Although there are no trial data to suggest an effect on the risk of feed intolerance or necrotising enterocolitis, the total number of infants studied was small (665 infants) and the data that could be abstracted from published studies for inclusion in meta-analyses were limited.

Implications for research

Further, high-quality randomised controlled trials are needed to assess the benefits and safety of protein hydrolysate versus standard cow's milk formulas for feeding very preterm infants when maternal breast milk is insufficient or not available. Trials could assess primary (empirical) use and secondary (indicated) use in infants with feed intolerance or gastro-oesophageal reflux, or following gastrointestinal surgery or necrotising enterocolitis. Trials should aim to ensure the participation of extremely preterm, extremely low birth weight or growth-restricted infants so that subgroup analyses can be planned for these infants at higher risk of necrotising enterocolitis. Given that protein hydrolysate preterm formulas is more expensive than standard preterm formula, trials could justifiably include a cost-benefit analysis.

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* *Indicates the major publication for the study*

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Baldassarre 2017

Methods	RCT.
Participants	Preterm infants (28-33 weeks' gestational age; birth weight 700-1750 g and appropriate to gestational age), within 24 hours of first enteral feeding (and whose mother did not plan to exclusively breastfeed)
Interventions	Extensively hydrolysed casein infant formula (n = 33). Standard cow's milk-based preterm infant formula (n = 35).
Outcomes	Enteral intake (ml/kg/day) during first 14 days after birth. Feeds intolerance measures (abdominal distention, regurgitation/emesis, feedings withheld ≥ 4 hours or bloody stools) Necrotising enterocolitis. Invasive infection.
Notes	University of Bari-Policlinico Hospital, Neonatology and Neonatal Intensive Care Unit, Department of Biomedical Science and Human Oncology, Bari, Italy Trial date: 2014-2016. Trial registration: clinicaltrials.gov/ct2/show/NCT01987154. Further information provided by investigators (August 2017).

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer generated.
Allocation concealment (selection bias)	Low risk	Sealed opaque envelopes.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	"Double-blind."
Blinding of outcome assessment (detection bias) All outcomes	Low risk	"Double-blind."
Incomplete outcome data (attrition bias) All outcomes	Low risk	60/68 enrolled infants completed trial and contributed to outcome analysis
Selective reporting (reporting bias)	Unclear risk	Protocol not available.

Baldassarre 2017 (*Continued*)

Other bias	Unclear risk	Funded by Mead Johnson Nutrition.

Florendo 2009

Methods	RCT.
Participants	Preterm infants (≤ 32 weeks' gestational age, ≤ 1750 g at birth) receiving ≤ 25% breast milk as total enteral intake
Interventions	Empirical use of partially hydrolysed whey-casein preterm formula (n = 42) Intact preterm formula (n = 38).
Outcomes	Feed intolerance (interruption of enteral feeds). Necrotising enterocolitis.
Notes	Division of Neonatology, University of Tennessee Center for Health Sciences, Memphis, TN, USA Trial date: 2004-2005.

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Computer-generated.
Allocation concealment (selection bias)	Low risk	Sequentially labelled, sealed opaque envelopes.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	"Double-blind," ready-to-feed colour coded cartons.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	"Double-blind."
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete outcome data for 74/80 participants. 1 infant in the control group developed sepsis and 1 infant from the hydrolysed formula group developed necrotising enterocolitis and was withdrawn
Selective reporting (reporting bias)	Unclear risk	Protocol not available.
Other bias	Unclear risk	Funded by Nestle (manufacturer of the trial formula).

Huston 1992

Methods	RCT.
Participants	Preterm very low birth weight infants.
Interventions	Empirical use of partially hydrolysed casein hydrolysate formula (with either 40% or 60% medium chain triglyceride) Non-hydrolysed preterm formula. Total n = 60.
Outcomes	Food tolerance. Growth rates.
Notes	Department of Pediatrics, Emanual Children's Health Care Centre, Portland, OR, USA Trial date: early 1990s. Reported as abstract only.

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Insufficient information - only abstract available.
Allocation concealment (selection bias)	Unclear risk	Insufficient information - only abstract available.
Blinding of participants and personnel (performance bias) All outcomes	High risk	Unlikely to be blinded.
Blinding of outcome assessment (detection bias) All outcomes	High risk	Unlikely to be blinded.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported for all participants.
Selective reporting (reporting bias)	Unclear risk	Protocol not available.
Other bias	Unclear risk	Funder: Mead Johnson Nutritional Group.

Maggio 2005

Methods	RCT.
Participants	Preterm infants (≤ 34 weeks' gestational age, ≤ 1750 g at birth)
Interventions	Empirical use of partially hydrolysed whey-based formula* (n = 10) Conventional preterm formula* (n = 11).

Maggio 2005 (*Continued*)

Outcomes	Growth rates from inclusion until hospital discharge. Feed intolerance (no infants had enteral feeds interrupted).
Notes	Division of Neonatology, Department of Paediatrics, Catholic University of the Sacred Heart, Rome, Italy Trial date: 1998-2000. * Energy content of both formulas: 75 kCal/100 mL.

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Randomised schedule generated - not specified how.
Allocation concealment (selection bias)	Low risk	Sealed opaque envelopes.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Study and control formulas identical in colour and smell.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Study and control formulas identical in colour and smell.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported for all participants.
Selective reporting (reporting bias)	Unclear risk	Protocol not available.
Other bias	Unclear risk	Funded by Humana (manufacturer of the trial formula).

Mihatsch 2002

Methods	RCT.
Participants	Very low birth weight (< 1500 g) infants.
Interventions	Empirical use of extensively hydrolysed (whey-casein) preterm formula* (n = 41) Standard preterm formula* (n = 46).
Outcomes	Necrotising enterocolitis. Proportion of enteral feeds with gastric residual volumes > 5 mL/kg birth weight
Notes	Division of Neonatology and Pediatric Critical Care, Department of Pediatrics, Ulm University, 89070 Ulm, Germany Trial date: 1999-2001. * Energy content of both formulas: 80 kCal/100 mL.

Mihatsch 2002 (*Continued*)

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Computer-generated.
Allocation concealment (selection bias)	Unclear risk	Sealed envelopes - unclear if opaque.
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	"Double-blind"- same appearance, but investigators acknowledged taste different
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	"Double-blind."
Incomplete outcome data (attrition bias) All outcomes	High risk	129 infants recruited initially, then 42 excluded post hoc because they received > 10% of enteral intake as human milk
Selective reporting (reporting bias)	Unclear risk	Protocol not available.
Other bias	Unclear risk	Funder: Milupa GmbH, Germany (manufacturer of the trial formula)

Pauls 1996

Methods	RCT.
Participants	Very low birth weight (< 1500 g) infants.
Interventions	Empirical use of partially hydrolysed whey-casein formula* (n = 25) Non-hydrolysed protein formula* (n = 25).
Outcomes	Mean gastric residual volume (% of intake). Time to full enteral feeds. Necrotising enterocolitis.
Notes	Kinderklinik, Freie Universitat Berlin, Germany. Trial date: early 1990s. Reported as an abstract only. * Energy content of both formulas: 80 kCal/100 mL; protein content: hydrolysed formula 2.9 g/100 mL vs non-hydrolysed formula 2.7 g/100 mL
Risk of bias	

Pauls 1996 (*Continued*)

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Insufficient information - only abstract available.
Allocation concealment (selection bias)	Unclear risk	Insufficient information - only abstract available.
Blinding of participants and personnel (performance bias) All outcomes	High risk	Unlikely to be blinded.
Blinding of outcome assessment (detection bias) All outcomes	High risk	Unlikely to be blinded.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported for all participants.
Selective reporting (reporting bias)	Unclear risk	Protocol not available.
Other bias	Unclear risk	Funder: not stated.

Picaud 2001

Methods	RCT.
Participants	Preterm newborns with birth weight < 1500 g and aged < 15 days old when commencing enteral feeds
Interventions	Empirical use of partially hydrolysed formula* (n = 9). Standard preterm formula* (n = 7). Until 40 weeks' postmenstrual age.
Outcomes	Rate of weight gain during initial hospital admission. Nitrogen balance studies.
Notes	Edouard Herriot Hospital, Claude Bernard University, Lyon, France Trial date: late 1990s. * Energy content of both formulas: 80 kCal/100 mL, but nitrogen content 10% higher in standard preterm formula

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Not stated.

Picaud 2001 (*Continued*)

Allocation concealment (selection bias)	Unclear risk	Sealed envelopes - unclear if opaque.
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Investigators unaware of formula, unclear if carers or parents aware
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Investigators unaware of formula, unclear if carers or parents aware
Incomplete outcome data (attrition bias) All outcomes	Low risk	All infants assessed for primary outcomes.
Selective reporting (reporting bias)	Unclear risk	Protocol not available.
Other bias	Unclear risk	Funder: Nestle (manufacturer of the trial formula).

Raupp 1995

Methods	RCT.
Participants	Neonates, bodyweight 1000-1799 g.
Interventions	Empirical use of partially hydrolysed whey-casein formula* (n = 56) Non-hydrolysed preterm formula* (n = 52).
Outcomes	Biochemistry. Bone mineralisation. Blood/serum. Necrotising enterocolitis.
Notes	University Children's Hospital of Düsseldorf. *Energy content of both formulas: 80 kCal/100 mL.

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Information not available.
Allocation concealment (selection bias)	Unclear risk	Information not available.
Blinding of participants and personnel (performance bias) All outcomes	High risk	Unblinded.

Raupp 1995 (*Continued*)

Blinding of outcome assessment (detection bias) All outcomes	High risk	Unblinded
Incomplete outcome data (attrition bias) All outcomes	Low risk	All infants assessed for primary outcomes.
Selective reporting (reporting bias)	Unclear risk	Protocol not available.
Other bias	Unclear risk	Funder: Nestle (manufacturer of the trial formula).

Riezzo 2001

Methods	RCT.
Participants	Preterm infants (n = 36).
Interventions	Partially hydrolysed casein preterm formula* (n = 18). Standard (whey-casein) formula* (n = 18).
Outcomes	Proportion of infants who had > 1 episode of regurgitation or vomiting per day
Notes	Department of Pediatrics, Neonatology Section, University of Bari, Bari, Italy Trial date: 2000. Energy content of hydrolysed formula (80 kCal/100 mL) higher than control standard term formula (68 kCal/100 mL). Because this did not report growth rates (the reason for specifying similar energy levels in comparison formulas), we made a consensus decision to include the trial

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Unclear evidence provided - only stated that infants were randomly assigned
Allocation concealment (selection bias)	Unclear risk	Unclear evidence provided - only stated that infants were randomly assigned
Blinding of participants and personnel (performance bias) All outcomes	High risk	Unblinded.
Blinding of outcome assessment (detection bias) All outcomes	High risk	Unblinded.

Riezzo 2001 (*Continued*)

Incomplete outcome data (attrition bias) All outcomes	Low risk	All infants assessed for primary outcomes.
Selective reporting (reporting bias)	Unclear risk	Protocol not available.
Other bias	Unclear risk	Funder: not stated.

Schweizer 1993

Methods	RCT.
Participants	Preterm infants (formula fed).
Interventions	Extensively hydrolysed whey-casein term formula (Alfare)* (n = 26) Non-hydrolysed preterm formula (Prematil)* (n = 26).
Outcomes	Time to regain birth weight. Time to full enteral feeding. Mean number of high gastric residual volumes per day.
Notes	Kinderklinik der Stadt, Klinlken, Dortmund. Trial date: 1991-1993. * Energy content of hydrolysed formula (70 kCal/100 mL) lower than control standard preterm formula (80 kCal/100 mL). Because this did not report growth rates (the reason for specifying similar energy levels in comparison formulas), we made a consensus decision to include the trial

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Insufficient information - only abstract available.
Allocation concealment (selection bias)	Unclear risk	Insufficient information - only abstract available.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	"Double-blinded."
Blinding of outcome assessment (detection bias) All outcomes	Low risk	"Double-blinded."
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported for all participants.
Selective reporting (reporting bias)	Unclear risk	Protocol not available.

Schweizer 1993 (*Continued*)

Other bias	Unclear risk	Funder: not stated.

Szajewska 2004

Methods	RCT.
Participants	Preterm infants, bodyweight < 2500 g with ≥ 1 first-degree relative with atopy
Interventions	Extensively (n = 26) or partially hydrolysed whey-casein preterm formula* (n = 32) Standard preterm formula* (n = 32).
Outcomes	Allergic disease in infancy. Feed intolerance.
Notes	Primary aim to assess effects on allergy and atopic disease. In hospital feed tolerance, growth or adverse outcomes not reported. We contacted corresponding author to seek these data in December 2016 *Energy content of both formulas: 80 kCal/100 mL. 33% "dropout" prior to assessment at 4-5 months' post-term.

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Randomised schedule generated - unspecified how.
Allocation concealment (selection bias)	Low risk	Sealed numbered envelopes - not stated if opaque, but codes concealed from investigators until trial completed
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	"Double-blind" but study and control formulas not identical in texture and smell
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	"Double-blind" but study and control formulas not identical in texture and smell
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported for all participants.
Selective reporting (reporting bias)	Unclear risk	Protocol not available.
Other bias	Unclear risk	Funded by Ovita Nutricia Research Foundation.

n: number of infants; RCT: randomised controlled trial.

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Agosti 2003	Not an RCT.
Corvaglia 2013	Cross-over RCT with cross-over at each enteral feed.
Logarajaha 2015	Cross-over RCT with cross-over at 24 hours.
Mihatsch 1999	Cross-over RCT with initial formula allocation for 5 days only
Mihatsch 2001a	Cross-over RCT with initial formula allocation for 5 days only
Rigo 1994	5-arm RCT with term infants receiving different types of hydrolysed formula (3 different whey hydrolysate formulas, a soy-collagen hydrolysate formula, or a whey-casein hydrolysate formula)
Rigo 1995	Not an RCT.

RCT: randomised controlled trial.

Characteristics of studies awaiting assessment *[ordered by study ID]*

Del Moral 2017

Methods	RCT (double blind). Randomisation sequence was generated by computer, allocation by sealed envelopes
Participants	Very low birth weight or very preterm infants (stratified by 2 birth weight categories (500-1000 g and 1001-1500 g)) who survived > 3 days after birth and for whom breast milk was not available or insufficient for requirements
Interventions	Empirical use of 100% whey protein partially hydrolysed preterm formula (n = 62) vs intact preterm formula (n = 73) Breast milk allowed if available and the different formulas were given to supplement when no breast milk available (postrandomisation exclusion if breast milk > 25% of total enteral intake)
Outcomes	Time to achieve full feeds. Number of days from initiating oral feeds to achieve full feeds Mortality. Necrotising enterocolitis.
Notes	Principal investigator: Teresa del Moral, Department of Pediatrics, Miller School of Medicine, University of Miami, FL, USA Contacted tdelmoral@miami.edu in July 2017 seeking data. Trial date: 2004-2005.

Del Moral 2017 (*Continued*)

	Funded by Nestle. Study discontinued because the increasingly common use of breast milk meant recruitment was much slower than planned

Dobryanskyy 2015

Methods	RCT.
Participants	Very low birth weight (< 1500 g) infants.
Interventions	Hydrolysed formula (n = 35) vs standard preterm formula (n = 25)
Outcomes	Feed intolerance. Time to full enteral feeding. Necrotising enterocolitis. 9 additional infants (originally randomised) who died were excluded
Notes	Article in Ukrainian. Awaiting translation.

Luo 2016

Methods	RCT.
Participants	"Very/extremely" low birth weight infants.
Interventions	Hydrolysed protein formula vs preterm formula.
Outcomes	Feed intolerance. Growth rates.
Notes	Article in Chinese. Awaiting translation.

RCT: randomised controlled trial.

Characteristics of ongoing studies *[ordered by study ID]*

ACTRN12613000481774

Trial name or title	Effects of a New Hydrolyzed Powdered Formula on Feeding Tolerance in Preterm Neonates: a Randomised Placebo-Controlled Study
Methods	RCT.
Participants	60 newborns with birth weight <1500 g.

ACTRN12613000481774 (*Continued*)

Interventions	Powdered hydrolysed formula vs standard preterm formula.
Outcomes	Time to reach full enteral feeding (120 kCal/kg/day).
Starting date	2013.
Contact information	Prof Gianluca Terrin, University of Rome "La Sapienza", Italy
Notes	Trial has not proceeded due to lack of funding (personal communication from Prof Terrin)

Yin 2015

Trial name or title	Extensively Hydrolyzed Milk Protein Formula in Preterm Children
Methods	RCT.
Participants	370 preterm infants < 34 weeks' gestational age who could not be breastfed
Interventions	Extensively hydrolysed (100% whey protein) formula (66 kCal/100 mL) vs preterm formula (80 kCal/100 mL) fed until discharge from the neonatal intensive care unit
Outcomes	Incidence of feed intolerance. Time to achieve full enteral nutrition.
Starting date	2016.
Contact information	Zhongda Hospital Southeast University, Nanjing, China. Contacted lipingyin_zd@163.com in November 2016 seeking data
Notes	Registered with the Chinese Clinical Trial Registry (ChiCTR-IOR-14005696) in 2014

RCT: randomised controlled trial.

DATA AND ANALYSES

Comparison 1. Hydrolysed versus non-hydrolysed formula

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Feed intolerance	3	161	Risk Ratio (M-H, Fixed, 95% CI)	2.71 [0.29, 25.00]
2 Necrotising enterocolitis	5	385	Risk Difference (M-H, Fixed, 95% CI)	0.00 [-0.03, 0.04]
2.1 Partially hydrolysed	3	238	Risk Difference (M-H, Fixed, 95% CI)	0.01 [-0.03, 0.06]
2.2 Extensively hydrolysed	2	147	Risk Difference (M-H, Fixed, 95% CI)	-0.02 [-0.07, 0.04]
3 Time to full enteral feeding	1	16	Mean Difference (IV, Fixed, 95% CI)	-1.0 [-8.36, 6.36]
4 Weight gain (g/kg/day)	3	113	Mean Difference (IV, Fixed, 95% CI)	-3.02 [-4.66, -1.38]
5 Length gain (mm/week)	2	97	Mean Difference (IV, Fixed, 95% CI)	-0.04 [-1.24, 1.15]
6 Head circumference growth (mm/week)	2	97	Mean Difference (IV, Fixed, 95% CI)	0.27 [-0.39, 0.94]
7 Serum alkaline phosphatase (IU/L)	2	88	Mean Difference (IV, Fixed, 95% CI)	16.61 [-34.15, 67.37]
8 Late-onset invasive infection	1	60	Risk Ratio (M-H, Fixed, 95% CI)	1.5 [0.27, 8.34]
9 Any allergic disease	1	63	Risk Ratio (M-H, Fixed, 95% CI)	0.62 [0.27, 1.42]

Analysis 1.1. Comparison 1 Hydrolysed versus non-hydrolysed formula, Outcome 1 Feed intolerance.

Review: Protein hydrolysate versus standard formula for preterm infants

Comparison: 1 Hydrolysed versus non-hydrolysed formula

Outcome: 1 Feed intolerance

Study or subgroup	Hydrolysed n/N	Non-hydrolysed n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Baldassarre 2017	0/30	0/30			Not estimable
Florendo 2009	3/42	1/38		100.0 %	2.71 [0.29, 25.00]
Maggio 2005	0/10	0/11			Not estimable
Total (95% CI)	**82**	**79**		**100.0 %**	**2.71 [0.29, 25.00]**

Total events: 3 (Hydrolysed), 1 (Non-hydrolysed)

Heterogeneity: not applicable

Test for overall effect: Z = 0.88 (P = 0.38)

Test for subgroup differences: Not applicable

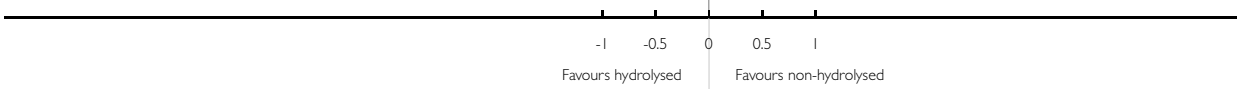
Analysis 1.2. Comparison 1 Hydrolysed versus non-hydrolysed formula, Outcome 2 Necrotising enterocolitis.

Review: Protein hydrolysate versus standard formula for preterm infants

Comparison: 1 Hydrolysed versus non-hydrolysed formula

Outcome: 2 Necrotising enterocolitis

Study or subgroup	Hydrolysed n/N	Non-hydrolysed n/N	Risk Difference M-H,Fixed,95% CI	Weight	Risk Difference M-H,Fixed,95% CI
1 Partially hydrolysed					
Florendo 2009	1/42	0/38		20.8 %	0.02 [-0.04, 0.09]
Pauls 1996	1/25	1/25		13.0 %	0.0 [-0.11, 0.11]
Raupp 1995	3/56	2/52		28.1 %	0.02 [-0.06, 0.09]
Subtotal (95% CI)	**123**	**115**		**61.8 %**	**0.01 [-0.03, 0.06]**
Total events: 5 (Hydrolysed), 3 (Non-hydrolysed)					
Heterogeneity: Chi^2 = 0.14, df = 2 (P = 0.93); I^2 =0.0%					
Test for overall effect: Z = 0.61 (P = 0.54)					
2 Extensively hydrolysed					
Baldassarre 2017	0/30	0/30		15.6 %	0.0 [-0.06, 0.06]
Mihatsch 2002	1/46	2/41		22.6 %	-0.03 [-0.11, 0.05]
Subtotal (95% CI)	**76**	**71**		**38.2 %**	**-0.02 [-0.07, 0.04]**
Total events: 1 (Hydrolysed), 2 (Non-hydrolysed)					
Heterogeneity: Chi^2 = 0.33, df = 1 (P = 0.57); I^2 =0.0%					
Test for overall effect: Z = 0.59 (P = 0.56)					
Total (95% CI)	**199**	**186**		**100.0 %**	**0.00 [-0.03, 0.04]**
Total events: 6 (Hydrolysed), 5 (Non-hydrolysed)					
Heterogeneity: Chi^2 = 1.06, df = 4 (P = 0.90); I^2 =0.0%					
Test for overall effect: Z = 0.17 (P = 0.87)					
Test for subgroup differences: Chi^2 = 0.71, df = 1 (P = 0.40), I^2 =0.0%					

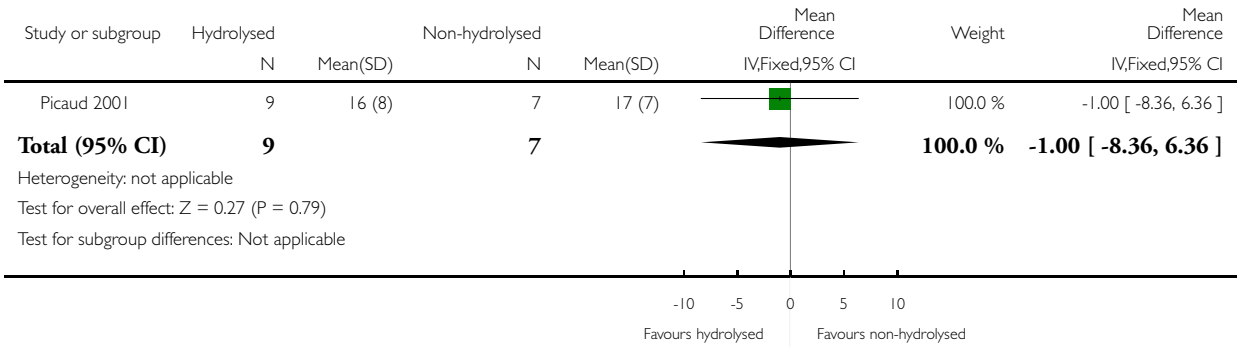


Analysis 1.3. Comparison 1 Hydrolysed versus non-hydrolysed formula, Outcome 3 Time to full enteral feeding.

Review: Protein hydrolysate versus standard formula for preterm infants

Comparison: 1 Hydrolysed versus non-hydrolysed formula

Outcome: 3 Time to full enteral feeding

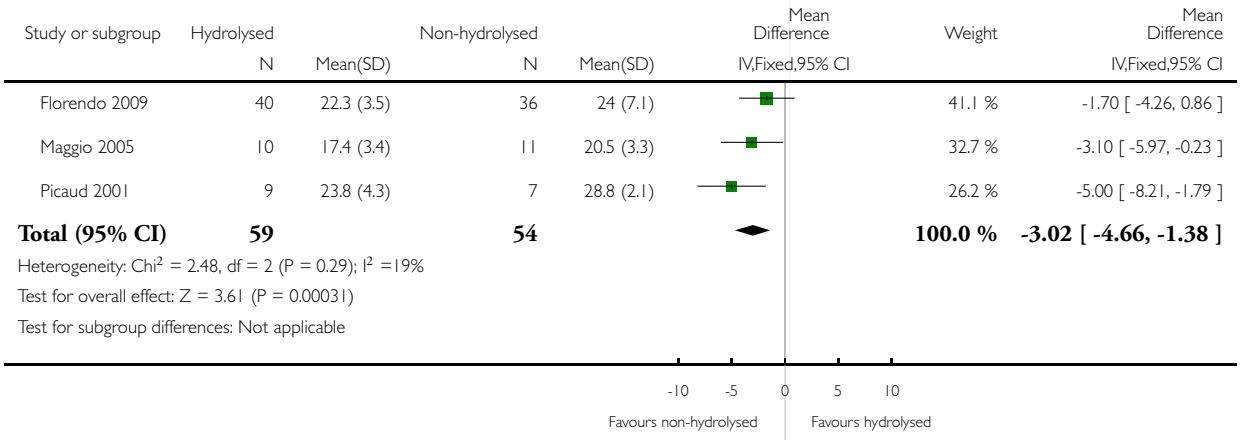


Analysis 1.4. Comparison 1 Hydrolysed versus non-hydrolysed formula, Outcome 4 Weight gain (g/kg/day).

Review: Protein hydrolysate versus standard formula for preterm infants

Comparison: 1 Hydrolysed versus non-hydrolysed formula

Outcome: 4 Weight gain (g/kg/day)



Analysis 1.5. Comparison 1 Hydrolysed versus non-hydrolysed formula, Outcome 5 Length gain (mm/week).

Review: Protein hydrolysate versus standard formula for preterm infants

Comparison: 1 Hydrolysed versus non-hydrolysed formula

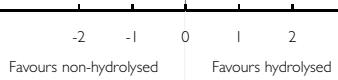
Outcome: 5 Length gain (mm/week)

Study or subgroup	Hydrolysed N	Mean(SD)	Non-hydrolysed N	Mean(SD)	Mean Difference IV,Fixed,95% CI	Weight	Mean Difference IV,Fixed,95% CI
Florendo 2009	40	10 (3)	36	10 (3)		77.8 %	0.0 [-1.35, 1.35]
Maggio 2005	10	12.7 (2.7)	11	12.9 (3.2)		22.2 %	-0.20 [-2.73, 2.33]
Total (95% CI)	**50**		**47**			**100.0 %**	**-0.04 [-1.24, 1.15]**

Heterogeneity: Chi^2 = 0.02, df = 1 (P = 0.89); I^2 =0.0%

Test for overall effect: Z = 0.07 (P = 0.94)

Test for subgroup differences: Not applicable

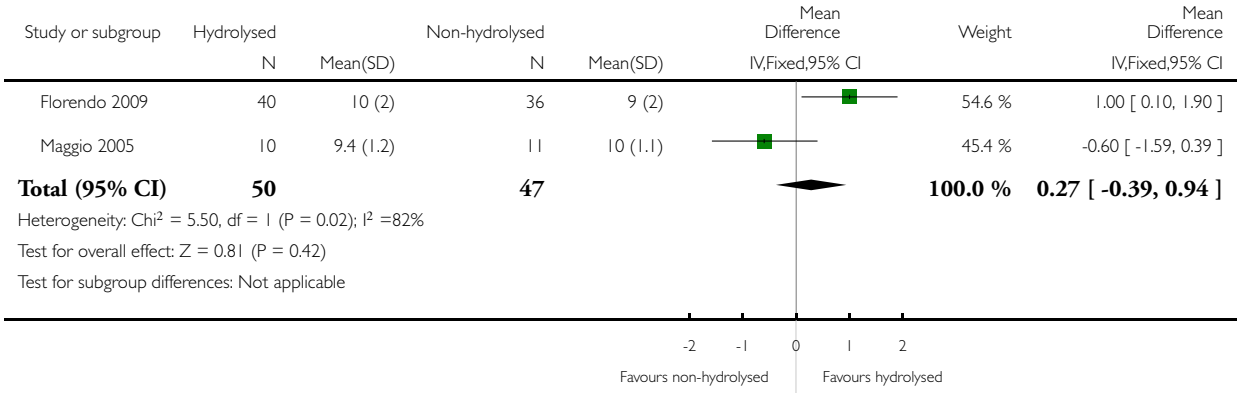


Analysis 1.6. Comparison 1 Hydrolysed versus non-hydrolysed formula, Outcome 6 Head circumference growth (mm/week).

Review: Protein hydrolysate versus standard formula for preterm infants

Comparison: 1 Hydrolysed versus non-hydrolysed formula

Outcome: 6 Head circumference growth (mm/week)

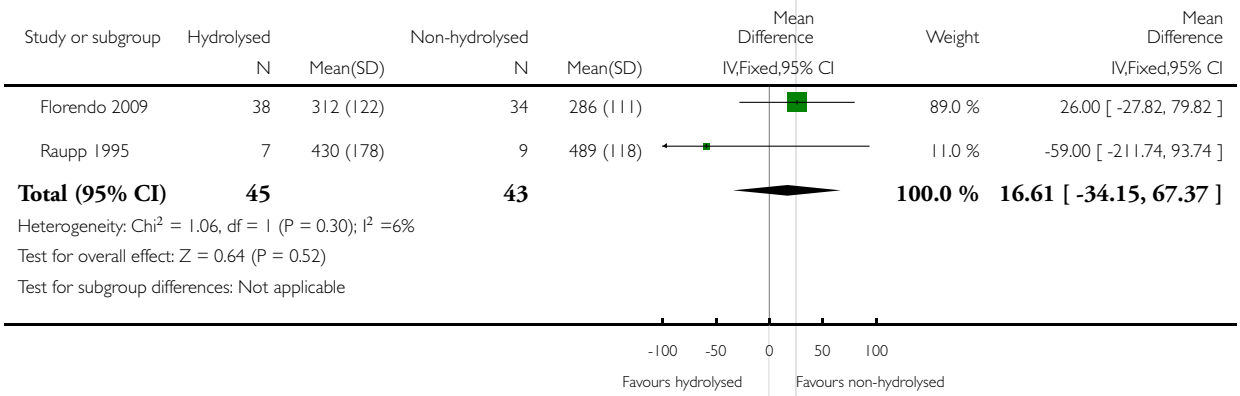


Analysis 1.7. Comparison 1 Hydrolysed versus non-hydrolysed formula, Outcome 7 Serum alkaline phosphatase (IU/L).

Review: Protein hydrolysate versus standard formula for preterm infants

Comparison: 1 Hydrolysed versus non-hydrolysed formula

Outcome: 7 Serum alkaline phosphatase (IU/L)



Analysis 1.8. Comparison 1 Hydrolysed versus non-hydrolysed formula, Outcome 8 Late-onset invasive infection.

Review: Protein hydrolysate versus standard formula for preterm infants

Comparison: 1 Hydrolysed versus non-hydrolysed formula

Outcome: 8 Late-onset invasive infection

Study or subgroup	Hydrolysed n/N	Non-hydrolysed n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Baldassarre 2017	3/30	2/30		100.0 %	1.50 [0.27, 8.34]
Total (95% CI)	**30**	**30**		**100.0 %**	**1.50 [0.27, 8.34]**

Total events: 3 (Hydrolysed), 2 (Non-hydrolysed)

Heterogeneity: not applicable

Test for overall effect: Z = 0.46 (P = 0.64)

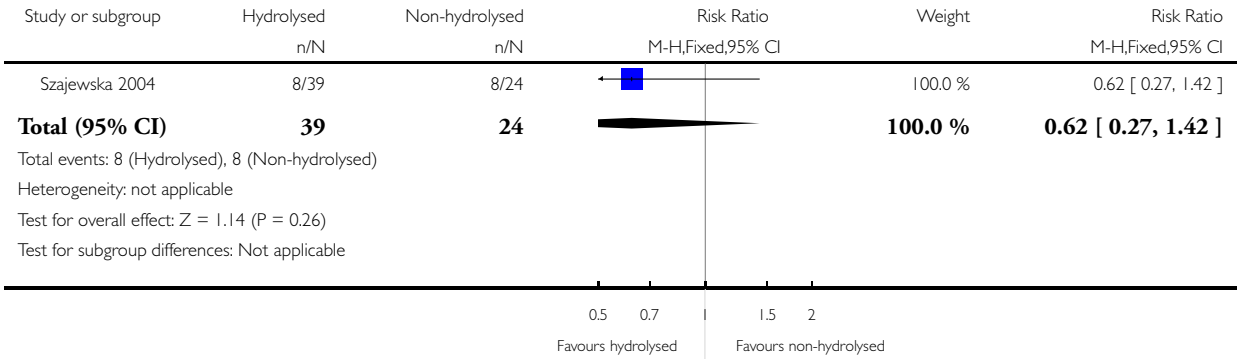
Test for subgroup differences: Not applicable

Analysis 1.9. Comparison 1 Hydrolysed versus non-hydrolysed formula, Outcome 9 Any allergic disease.

Review: Protein hydrolysate versus standard formula for preterm infants

Comparison: 1 Hydrolysed versus non-hydrolysed formula

Outcome: 9 Any allergic disease



APPENDICES

Appendix 1. Electronic search strategy

Database: Ovid MEDLINE(R) Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R)
<1946 to present>
MEDLINE searched 5 May 2017; 453 records identified
---
1 exp Infant, Newborn/ (561559)
2 Premature Birth/ (10039)
3 (neonat$ or neo nat$).ti,ab. (231791)
4 (newborn$ or new born$ or newly born$).ti,ab. (150586)
5 (preterm or preterms or pre term or pre terms).ti,ab. (60963)
6 (preemie$ or premie or premies).ti,ab. (142)
7 (prematur$ adj3 (birth$ or born or deliver$)).ti,ab. (13989)
8 (low adj3 (birthweight$ or birth weight$)).ti,ab. (30615)
9 (lbw or vlbw or elbw).ti,ab. (7084)
10 infan$.ti,ab. (388569)
11 (baby or babies).ti,ab. (62128)
12 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 (954384)
13 (hydroly$ adj3 (formula$ or milk or protein$ or whey)).ti,ab. (7903)
14 (hypoallergen$ adj3 (formula$ or milk or protein$ or whey)).ti,ab. (273)
15 (Nutramigen or Nutriprem or Pregestamil or Profylac or Nan or Aptamil Pepti or Pepti-Junior or Pepdite or Infatrini or Similac or Gold Prem Pro or Alimentum).ti,ab. (1787)

16 13 or 14 or 15 (9804)
17 randomized controlled trial.pt. (462115)
18 controlled clinical trial.pt. (94040)
19 randomized.ab. (403274)
20 placebo.ab. (188761)
21 drug therapy.fs. (1991821)
22 randomly.ab. (280150)
23 trial.ab. (422286)
24 groups.ab. (1725818)
25 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 (4099198)
26 exp animals/ not humans.sh. (4396754)
27 25 not 26 (3544583)
28 12 and 16 and 27 (453)

Database: Ovid Embase <1974 to 2017 week 18>
Searched via Ovid 5 May 2017 391 records identified
---
1 exp Infant, Newborn/ (515450)
2 Premature Birth/ (48076)
3 (neonat$ or neo nat$).ti,ab. (286253)
4 (newborn$ or new born$ or newly born$).ti,ab. (176250)
5 (preterm or preterms or pre term or pre terms).ti,ab. (79839)
6 (preemie$ or premie or premies).ti,ab. (200)
7 (prematur$ adj3 (birth$ or born or deliver$)).ti,ab. (17865)
8 (low adj3 (birthweight$ or birth weight$)).ti,ab. (36327)
9 (lbw or vlbw or elbw).ti,ab. (9053)
10 infan$.ti,ab. (436861)
11 (baby or babies).ti,ab. (80736)
12 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 (1027154)
13 (hydroly$ adj3 (formula$ or milk or protein$ or whey)).ti,ab. (8679)
14 (hypoallergen$ adj3 (formula$ or milk or protein$ or whey)).ti,ab. (417)
15 (Nutramigen or Nutriprem or Pregestamil or Profylac or Nan or Aptamil Pepti or Pepti-Junior or Pepdite or Infatrini or Similac or Gold Prem Pro or Alimentum).ti,ab. (1685)
16 13 or 14 or 15 (10503)
17 clinical trial/ (918094)
18 randomized controlled trial/ (442254)
19 randomization/ (73053)
20 single blind procedure/ (26265)
21 double blind procedure/ (136826)
22 crossover procedure/ (50503)
23 placebo/ (303176)
24 randomi?ed controlled trial$.tw. (153302)
25 rct.tw. (23420)
26 random allocation.tw. (1658)
27 randomly allocated.tw. (26937)
28 allocated randomly.tw. (2222)
29 (allocated adj2 random).tw. (858)
30 single blind$.tw. (19050)
31 double blind$.tw. (176511)
32 ((treble or triple) adj blind$).tw. (674)
33 placebo$.tw. (251481)
34 prospective study/ (366226)

35 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 (1721674)
36 case study/ (45994)
37 case report.tw. (332856)
38 abstract report/ or letter/ (1009473)
39 36 or 37 or 38 (1380654)
40 35 not 39 (1677110)
41 12 and 16 and 40 (391)

Database: Maternity & Infant Care Database (MIDIRS) <1971 to March 2017>
Searched via Ovid 5 May 2017 20 records identified
1 (neonat$ or neo nat$).ti,ab. (37541)
2 (newborn$ or new born$ or newly born$).ti,ab. (17281)
3 (preterm or preterms or pre term or pre terms).ti,ab. (21945)
4 (preemie$ or premie or premies).ti,ab. (48)
5 (prematur$ adj3 (birth$ or born or deliver$)).ti,ab. (3526)
6 (low adj3 (birthweight$ or birth weight$)).ti,ab. (9603)
7 (lbw or vlbw or elbw).ti,ab. (2624)
8 infan$.ti,ab. (55675)
9 (baby or babies).ti,ab. (26173)
10 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 (103259)
11 (hydroly$ adj3 (formula$ or milk or protein$ or whey)).ti,ab. (144)
12 (hypoallergen$ adj3 (formula$ or milk or protein$ or whey)).ti,ab. (28)
13 (Nutramigen or Nutriprem or Pregestamil or Profylac or Nan or Aptamil Pepti or Pepti-Junior or Pepdite or Infatrini or Similac or Gold Prem Pro or Alimentum).ti,ab. (33)
14 11 or 12 or 13 (188)
15 10 and 14 (178)
16 limit 15 to randomised controlled trial (20)

Database: CINAHL
Search date: 9 June 2017

Search ID#	**Search terms**	**Search options**
S1	(MH "Infant, Newborn+")	**Search modes** - Boolean/Phrase
S2	TX ((neonat* or neo nat*)) OR TX ((newborn* or new born* or newly born*)) OR TX ((preterm or preterms or pre term or pre terms)) OR TX ((preemie$ or premie or premies)) OR TX ((prematur* N3 (birth* or born or deliver*))) OR TX ((low N3 (birthweight* or birth weight*))) OR TX ((lbw or vlbw or elbw)) OR TX infan* OR TX ((baby or babies))	**Search modes** - Boolean/Phrase
S3	S1 OR S2	**Search modes** - Boolean/Phrase
S4	TX ((hydroly* N3 (formula* or milk or protein* or whey))) OR TX ((hypoallergen* N3 (formula* or milk or protein* or whey))) OR TX ((Nutramigen or Nutriprem or Pregestamil or Profylac or Nan or Aptamil Pepti or Pepti-	**Search modes** - Boolean/Phrase

(*Continued*)

	Junior or Pepdite or Infatrini or Similac or Gold Prem Pro or Alimentum))	
S5	S3 AND S4	**Search modes** - Boolean/Phrase
S6	S3 AND S4	**Limiters** - Clinical Queries: Therapy - High Sensitivity **Search modes** - Boolean/Phrase (210 records)

Appendix 2. Risk of bias

- Random sequence generation: we categorised the method used to generate the allocation sequence as:

  ○ low risk of bias: any random process (e.g. random number table; computer random number generator; coin tossing; shuffling of cards or envelopes; throwing of dice; drawing of lots; minimisation (may be implemented without a random element; this is considered equivalent to being random));

  ○ high risk of bias: any non-random process (e.g. sequence generated by odd or even date of birth; date (or day) of admission; sequence generated by hospital or clinic record number; allocation by judgement of the clinician; allocation by preference of the participant; allocation based on results of a laboratory test or series of tests; allocation based on availability of the intervention);

  ○ unclear risk of bias: insufficient information about the sequence generation process to permit judgement.

- Allocation concealment: we categorised the method used to conceal the allocation sequence as:

  ○ low risk of bias: randomisation method described that would not allow investigator/participant to know or influence the intervention group before eligible participants entered the study (i.e. central allocation, including telephone, web-based, and pharmacy-controlled randomisation; sequentially numbered drug containers of identical appearance; sequentially numbered, opaque, sealed envelopes);

  ○ high risk of bias: open random allocation schedule (i.e. list of random numbers); assignment envelopes used without appropriate safeguards (e.g. if envelopes were unsealed or non-opaque or were not sequentially numbered); alternation or rotation; date of birth; case record number; any other explicitly unconcealed procedure;

  ○ unclear risk of bias: randomisation stated but no information provided on method used.

- Blinding of participants and personnel: we assessed blinding of participants, clinicians and carers, and outcome assessors separately for different outcomes and categorised the methods as:

  ○ low risk of bias: no blinding or incomplete blinding, but review authors judged that the outcome was not likely to be influenced by lack of blinding; blinding of participants and key study personnel ensured, and unlikely that blinding could have been broken;

  ○ high risk of bias: no blinding or incomplete blinding, and the outcome was likely to be influenced by lack of blinding; blinding of key study participants and personnel attempted, but likely that blinding could have been broken, and the outcome was likely to be influenced by lack of blinding;

  ○ unclear risk of bias: insufficient information to permit judgement.

- Incomplete outcome data: we described the completeness of data including attrition and exclusions from the analysis for each outcome and any reasons for attrition or exclusion where reported. We assessed whether missing data were balanced across groups or were related to outcomes. We categorised completeness as:

  ○ low risk of bias: no missing outcome data; reasons for missing outcome data unlikely to be related to true outcome (for survival data, censoring unlikely to introduce bias); missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups; for dichotomous outcome data, the proportion of missing outcomes compared with observed event risk not enough to have a clinically relevant impact on the intervention effect estimate; for continuous outcome data, plausible effect size (difference in means or standardised difference in means) among missing outcomes not sufficient to have a clinically relevant impact on observed effect size; missing data imputed by appropriate methods;

○ high risk of bias: reason for missing outcome data likely to be related to true outcome, with imbalance in numbers or reasons for missing data across intervention groups; for dichotomous outcome data, the proportion of missing outcomes compared with observed event risk enough to induce clinically relevant bias in intervention effect estimate; for continuous outcome data, plausible effect size (difference in means or standardised difference in means) among missing outcomes enough to induce clinically relevant bias in observed effect; 'as-treated' analysis done with substantial departure of the intervention received from that assigned at randomisation; potentially inappropriate application of simple imputation;

○ unclear risk of bias: insufficient information to permit judgement.

- Selective reporting: we assessed reporting bias due to selective outcome reporting as:

○ low risk of bias: study protocol was available, and all the study's prespecified (primary and secondary) outcomes that were of interest in the review were reported in the prespecified way; study protocol was not available, but it was clear that published reports included all expected outcomes, including those that were prespecified;

○ high risk of bias: not all the study's prespecified primary outcomes were reported; one or more primary outcomes were reported by measurements, analysis methods or subsets of data (i.e. subscales) that had not been prespecified; one or more reported primary outcomes were not prespecified (unless clear justification for their reporting was provided, such as an unexpected adverse effect); one or more outcomes of interest in the review had been reported incompletely, so that they could not be entered into a meta-analysis; the study report failed to include results for a key outcome that would be expected to have been reported for such a study;

○ unclear risk of bias: insufficient information to permit judgement.

- Other bias: we analysed bias due to problems not covered elsewhere in the table:

○ low risk of bias: study appeared free of other sources of bias;

○ high risk of bias: study had a potential source of bias related to the specific study design used; stopped early because a data-dependent study design was used; stopped early as the result of a data-dependent process (including a formal stopping rule); had extreme baseline imbalance; was claimed to be fraudulent; had some other problem;

○ unclear risk of bias: insufficient information to assess whether an important risk of bias existed; insufficient rationale or evidence to suggest that an identified problem would introduce bias.

Appendix 3. GRADE

GRADE considers that evidence from randomised controlled trials is 'high' quality but that assessment may be downgraded based on consideration of any of five areas:

- design (risk of bias);
- consistency across studies;
- directness of the evidence;
- precision of estimates; and
- presence of publication bias.

This results in an assessment of the quality of a body of evidence in one of four grades:

- High: we are very confident that the true effect lies close to that of the estimate of the effect.
- Moderate: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
- Low: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
- Very low: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

CONTRIBUTIONS OF AUTHORS

All authors developed the protocol.

DN and WMG: screened the search outputs, assessed study eligibility, extracted and synthesised data.

DN and JK assessed risk of bias across key domains and undertook the GRADE assessment with WMG.

All authors revised the final review.

DECLARATIONS OF INTEREST

Nicholas Embleton has conducted research with support from manufacturers of infant formula including Nestec SA (Switzerland), Wyeth UK and Nutricia UK but did not receive any payment, support or benefit in kind for contribution to this review.

The other authors do not have any declarations of interest.

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DIFFERENCES BETWEEN PROTOCOL AND REVIEW

Two trials compared formulas with different energy densities (Schweizer 1993; Riezzo 2001). These did not report growth rates (the reason for prespecifying similar energy levels) so we made consensus decision to include them in the review.