The science behind our products

Althéra, Alfaré & Alfamino

Clinical studies

Mothers should be encouraged to continue breastfeeding while avoiding all milk and milk products from their own diet. This usually requires qualified dietary counseling to completely exclude hidden sources of cow's milk protein. If a decision to use a special formula intended for infants is taken, it is important to give instructions on correct preparation methods, emphasising that unboiled water, unsterilised bottles or incorrect dilution can all lead to illness. Formula for special medical purposes intended for infants must be used under medical supervision.

Rapp M et *al.* 2013: Characterization of an extensively hydrolysed whey infant formula with a low bitterness (abstract)

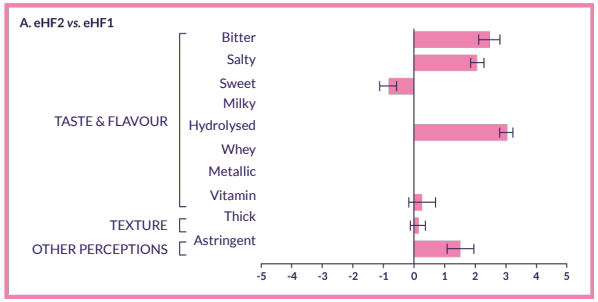
Study objective: To compare the peptide size distribution and allergenicity of several leading commercial whey-based and casein-based extensively hydrolysed infant formulas (eHF), and to define how the level of hydrolysis and recipe impacts sensory profile.

Methods: The eHF1 (Althéra) and eHF3 (Pepti-Junior) are whey-based eHFs, the eHF2 (Nutramigen) is a casein-based eHF. Size exclusion chromatography was used to characterise the peptide size distribution, beta-lactoglobulin (βLG) was quantified by ELISA and rat basophil cell leukemia cell line (RBL) assay was used to assess cross linking activity of the residual βLG. The sensory profile (comparative profile, one sample *versus* a reference) was carried out with a trained professional panel of 11 adult subjects.

Results:

- eHF1 and eHF2 were the most hydrolysed with respectively 99% and 98% of their peptides below 1000 Da.
- Whey-based hydrolysed formula, eHF3, was much less hydrolysed with only 74% of its peptides below 1000 Da.
- Residual βLG specific allergenicity of whey-based extensively hydrolysed infant formulas was similar to that of the casein-based eHF2.
- eHF1 was significantly less bitter, less salty, and slightly sweeter than casein-based eHF2. Comparison of eHF1 to eHF3 showed that eHF1 was slightly bitter and saltier than eHF3.

eHF1 is less bitter than eHF2 and only slightly bitter than the less hydrolysed eHF3



Conclusion: All tested eHFs showed very similar low allergenicity in vitro, despite significant differences in residual βLG and peptide size profiles. The sensory data underlines that comparable degree of hydrolysis can deliver different sensory basic taste characteristics, important for mother and infant's acceptance.

Althéra has low residual allergenicity and is more palatable than a casein-based formula making it more suited for long term compliance.

Note: Althéra and Alfaré have the same protein hydrolysate thus the studies that confirm hypoallergenicity apply to both products. Other attributes are not comparable.

Rapp M, Martin-Paschoud C, Nutten S et al. Characterization of an extensively hydrolyzed whey infant formula with a low bitterness. Clinical and Translational Allergy. 2013; 3(Suppl 3):P132. Presented at the Food Allergy and Anaphylaxis meeting in February 2014.

Niggemann B et *al.* 2008: Safety and efficacy of a new extensively hydrolysed formula for infants with cow's milk protein allergy

Study objectives: To compare the tolerance and growth of infants with CMPA who were fed an extensively hydrolysed formula (eHF) containing lactose (Althéra) with those who were fed with an amino acid formula (AAF; Neocate).

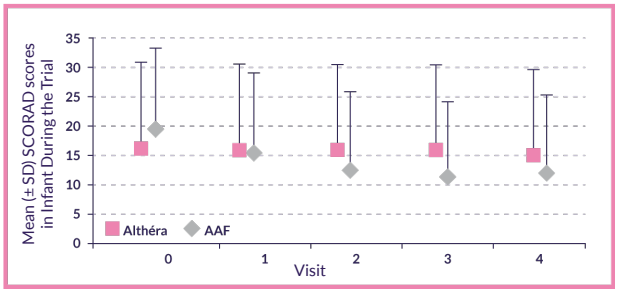
Subjects and methods: A prospective, multi-centre, randomised, reference-controlled study of 77 infants <12 months old with suspected CMPA were enrolled. In 66 of these, CMPA was confirmed by oral challenge in a double-blind, placebo-controlled food challenge (DBPCFC) or by a medical history of severe allergic reaction to cow's milk and a positive skin prick test. These infants were then tested for their reaction to eHF and AAF in a DBPCFC.

End points: Allergenicity of Althéra vs. AAF prior to randomisation, and evaluation after 30, 60, 90 and 180 days of feeding of: growth, allergy symptoms, blood analysis, tolerance and stool characterisitcs

Results:

- No significant difference in growth measurements between the 2 groups. Length and head circumference were similar to Euro-growth standards, but weight was slightly lower.
- Gastro-intestinal and respiratory tract symptoms of allergy similar in the 2 groups.
- SCORAD scores for atopic dermatitis remained constant throughout the study in infants-fed eHF, there was a slight decrease in those fed AAF.

SCORAD scores remained constant throughout the trial period in the eHF group and tended to decrease in the AAF group



Conclusion: **Infants-fed eHF had significantly fewer incidents of vomiting than infants-fed AAF and a significantly higher frequency of soft stools. The new eHF is safe and well tolerated in infants diagnosed with CMPA.**

Althéra can be safely prescribed in infants with CMPA, since it is well tolerated and ensures adequate growth.

Note: Althéra and Alfaré have the same protein hydrolysate thus the studies that confirm hypoallergenicity apply to both products. Other attributes are not comparable.

Niggemann B, von Berg A, Bollrath C et al. Safety and efficacy of a new extensively hydrolysed formula for infants with cow's milk protein allergy. Pediatr Allergy Immunol. 2008;19(4):348-54.

Vandenplas Y et *al.* 2013: Treating cow's milk protein allergy: a double- blind randomised trial comparing two extensively hydrolysed formulas with probiotics

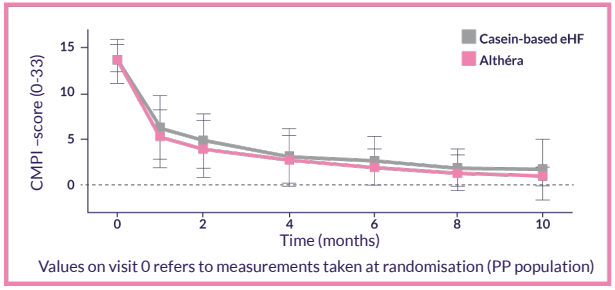
Study objective: To determine whether a diet with extensively hydrolysedwhey (eWH) or extensively hydrolysed casein (eCH) protein is the best option in the treatment of cow's milk protein allergy.

Methods: Infants with suspected CMPA were treated with an eWH or eCH, and efficacy was assessed with a symptom-based score developed by the authors. Diagnosis of CMPA was based on a positive challenge. If positive, the same eWF/eCH was continued. If negative, a standard starter and follow-up formula were given up to the age of 12 months.

Results:

- An open challenge was performed on 85/116 (73%) infants suspected of CMPA on clinical grounds and was positive in 59/85 (69%).
- Significant statistical and clinical reductions in symptom-based scores in both groups were reported after 1 month.
- Total and specific IgE and skin prick test results were similar in both groups.
- Both hydrolysates were enriched with probiotics, which were recovered in the gastrointestinal flora.
- eWH-standard formula sequence led to better growth at the age of 1 year than the other three feeding regimens tested.

Mean (± SD) CMPI Scores in Infants During the Trial



Values on visit 0 refers to measurements taken at randomisation (PP population)

Conclusion: **The eWH and eCH are equally effective. The symptom-based score is a useful tool to evaluate the efficacy of dietary treatment in infants with CMPA.**

Althéra is safe, effective and supports normal growth similar to casein-based formulas. The CMPI is an effective tool to evaluate therapeutic formula efficacy.

Note: Althéra and Alfaré have the same protein hydrolysate thus the studies that confirm hypoallergenicity apply to both products. Other attributes are not comparable.

Vandenplas Y, Steenhout P, Planoudis Y et al. Treating cow's milk protein allergy: a double-blind randomised trial comparing two extensively hydrolysed formulas with probiotics. Acta Paediatr.2013;102(10):990-8.

Sorensen RU et *al.* 2007: Weaning to a Partially Hydrolyzed Whey Formula at Three Months of Age Protects Against the Later Development of Anti-Cow Milk IgE Antibodies in Comparison to Whole Cow's Milk or Extensively Hydrolyzed Whey Formulas (abstract)

Study objective: To test the hypothesis that the introduction of a partially hydrolysed whey (pHW) formula at the time of weaning would protect against the development of anti-cow milk IgE antibodies in infants at risk for developing allergy, while the use of routine cow's milk formula (intact protein, RCMF) or extensively hydrolysed cow milk proteins (eHW) would not have this effect.

Subjects and methods: Infants (n=242) with a family history of allergy were enrolled at birth and assigned to one of 4 groups: a group that continued breast feeding, and three randomised groups weaned to either RCMF, pHW or eHW formula. All infants were breast fed for 3 months, then study formulas was were given for 1 month. After 4 months of age, all formula groups received the same CMRF given to one of the groups during the intervention period.

Results:

- None of the study participants developed anti-cow milk IgE antibodies during intervention.
- Total IgE was lower in the breast fed group than in the 3 groups that received cow's milk formulas initially at 1 year of age.
- Infants in both the RCMF and the eHW groups developed specific anti-cow's milk IgE antibodies.
- Only one child in the group that received pHW developed a low concentration of specific anti-cow's milk IgE antibodies.

Conclusions: Results support the observation in experimental animals that pHW may induce tolerance while an eHW, although not allergenic during exposure, does not prevent later sensitisation to cow milk proteins.

Althéra has low allergenicity and is therefore a good option in the management of CMPA.

Note: Althéra and Alfaré have the same protein hydrolysate thus the studies that confirm hypoallergenicity apply to both products. Other attributes are not comparable.

Sorensen RU, Inostroza J, Hebel E et al. Weaning to a Partially Hydrolyzed Whey Formula at Three Months of Age Protects Against the Later Development of Anti-Cow Milk IgE Antibodies in Comparison to Whole Cow's Milk or Extensively Hydrolyzed Whey Formulas. Abstract 473 presented at the AAAAI congress 2007.

Dupont C *et al.* 2009: Breast-milk, partially hydrolysed formula, and extensively hydrolysed formula: immediate and long term effects in infants at risk of allergy (abstract)

Study objective: To evaluate the efficacy of partially hydrolysed formula (pHF; NAN HA) *versus* extensively hydrolysed formula (eHF; Althéra) to prevent cow's milk allergy by inducing specific oral tolerance to cow's milk proteins in high-risk infants.

Subjects and methods: Prospective, parallel, controlled, multicenter and randomised birth cohort of at risk infants (Kjellman score of ≥2), receiving blindly during the 1st year of life, (pHF) and (eHF), in comparison with a breastfed reference group (BF), weaned with either pHF (BF-pHF) or eHF (BF-eHF). Population: 141 (ITT) and 104 (PP) children in the pHF group; 138 (ITT) and 90 (PP) in the eHF group; of which 40 (ITT) and 32 (PP) and 38 (ITT) and 31 (PP) respectively were partially breastfed.

Results:

- Comparable growth parameters in all groups except for higher length and weight gains during the second trimester in BF-eHF *vs.* BF-pHF and in formula BF *vs.* formula.
- eHF and pHF infants had higher haematocrite, haemoglobin, leucocytes, lymphocytes and RBC than BF infants at 4 months.
- Haemoglobin was higher in formula infants than in BF infants at 1 year.
- eHF infants exhibited less bronchitis, P=0.040, coughing, P=0.025, gastroenteritis, P=0.022, gastroesophageal reflux, P= 0.046 than pHF ones.
- Total IgE increased less from 4 months to 1 year of age in the pHF group vs. eHF group, respectively from 6.55 ± 8.62 to 22.47 ± 49.35 KU/mL vs 8.96 ± 11.01 to 31.84 ± 49.30 KU/mL, P=0.048.

Conclusions: Feeding children at risk for allergy with partially hydrolysed formula leads to normal growth parameters during the first year of life. Interestingly, the development of total IgE is restrained with pHF as compared with eHF, indicating a potential effect long term tolerogenic effect.

Althéra supports normal growth parameters comparable to a pHF and breast fed babies weaned with pHF or Althéra.

Dupont C, Basuyau JP, Soulaines P et al. Breast-milk, partially hydrolysed formula, and extensively hydrolysed formula: immediate and long term effects in infants at risk of allergy. Abstract 134 presented at the EAACI congress 2009.

Francavilla R *et al*. 2012: Effect of lactose on gut microbiota and metabolome of infants with cow's milk protein allergy

Study objective: To investigate the influence of lactose on the composition of the gut microbiota and metabolome of infants with cow's milk protein allergy.

Methods: Infants prospectively enrolled received an extensively hydrolysed formula with no lactose for 2 months followed by an identical lactose containingformula for an additional 2 months. Healthy, age-gender-matched infants were used as controls. Before and after the introduction of lactose in the diet the following determinations were performed: enumeration of cells present in the feces using FISH, counts of viable bacterial cells and gas-chromatography mass spectrometry/solid-phase microextraction analysis.

Results:
The addition of lactose:
- Significantly increased the counts of Bifidobacteria and lactic acid bacteria ($P < 0.01$).
- Decreased Bacteroides/clostridia ($P < 0.05$) reaching counts found in healthy controls.
- Significantly increased the concentration of total short-chain fatty acids ($P < 0.05$).

Conclusions: The addition of lactose to an eHF is able to positively modulate the composition of gut microbiota by increasing the total fecal counts of Lactobacillus/Bifidobacteria and decreasing that of Bacteroides/Clostridia. The positive effect is completed by the increase of median concentration of short chain fatty acids, especially for acetic and butyric acids demonstrated by the metabolomic analysis.

Althéra contains lactose which helps promote healthy gut microbiota.

Francavilla R, Calasso M, Calace L et al. Effect of lactose on gut microbiota and metabolome of infants with cow's milk protein allergy. Pediatr Allergy Immunol. 2012;23(5):420-7.

Vandenplas Y *et al*. 2010: Safety and adequacy of anoptimized formula for pediatric patients with cow's milk-sensitive enteropathy

Study objective: To evaluate thesafetyand nutritional adequacyof new Alfaréfor paediatric patients with clinical indications for the enteral use of semi-elemental diet.

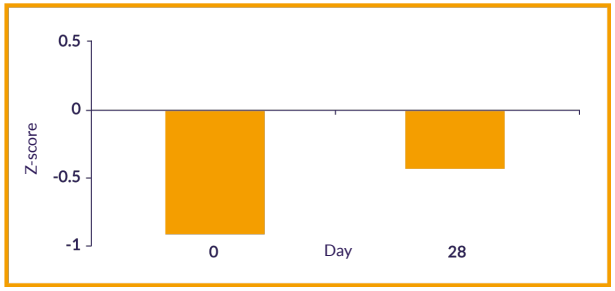
Methods: A prospective, open trial.Safetywas measured as normal growth based on Euro-growth standards for body mass index (BMI)-for-age z-scores, and nutritionaladequacywas evaluated based on measurements of blood parameters. 47 patients <32 months old, having a gestational age of ≥ 26 weeks, and weighing ≥ 1,500 g were enrolled, and fed with New Alfaré for 4 weeks. Weight, length and blood parameters were measured at the beginning and end of the study. Signs of tolerance to theformula(amount offormula intake, gastrointestinal symptoms and stool characteristics) were recorded daily by the parents. 25 patients completed the study with all measurements.

Results:

- Significant increase in the mean BMI-for-age z-score (P<0.05) and albumin concentration (P<0.01) after 4 weeks.
- Significant decrease in mean plasma threonine concentration (P=0.01).
- Mean tryptophan concentration tended to increase by the end of the study (P=0.06).
- Significant increase in the concentration of some long chain fatty acids DGLA and EPA.
- No adverse events reported.

Conclusions: These results show that "New Alfaré" is safe and nutritionally adequate for paediatric patients with cow's milk-sensitive enteropathy.

Mean BMI-for-age z-scores of patients in the per protocol population relative to Euro-growth reference at beginning and end of the study



Using a new hydrolysis technique, Alfaré is well tolerated in paediatric patients with cow's milk-sensitive enteropathy.

Note: Vandenplas Y et *al*. Minerva Pediatr.2010 Aug;62(4):339-45 and Vandenplas Y et *al*. Amino Acids. 2010 Mar;38(3):909-14 use data from the same study.

Vandenplas Y, Plaskie K. Safetyandadequacyof anoptimizedformulafor pediatric patients with cow's milk-sensitive enteropathy. Minerva Pediatr.2010;62(4):339-45.

Vandenplas Y *et al*. 2010: Safety and adequacy of a semi-elemental formula for children with gastro-intestinal disease

Study objective: To evaluate the nutritional adequacy of a new Alfaré diet in 47 children with functional gastro-intestinal disorders

Methods: A prospective, open trial.Safetywas measured as normal growth based on Euro-growth standards for body mass index (BMI)-for-age z-scores, and nutritionaladequacywas evaluated based on measurements of blood parameters. 47 patients <32 months old, having a gestational age of ≥ 26 weeks, and weighing ≥ 1,500 g were enrolled, and fed with New Alfaré for 4 weeks. Weight, length and blood parameters were measured at the beginning and end of the study. Signs of tolerance to theformula(amount offormula intake, gastrointestinal symptoms and stool characteristics) were recorded daily by the parents. 25 patients completed the study with all measurements.

Results:

- In total, 533 litres of "New-Alfaré" was consumed during 775 trial-days.
- Mean intake per infant was 85.8 ± 26.8 kcal/kg/day or 122.5 ± 38.3 ml/kg/day.
- Weight and length evolution during the 4 weeks trial were within normal range.
- Significant increase in mean BMI-for-age z-score ($P<0.05$) and albumin concentration ($P<0.01$) after 4 weeks.
- Significant decrease in plasma threonine concentration ($P = 0.01$) and tryptophan concentration increased ($P = 0.06$).
- No adverse events reported.

Conclusions: These results show that "New Alfaré" is safe and nutritionally adequate for paediatric patients with gastrointestinal disease.

Using a new hydrolysis technique, Alfaré is well tolerated in GI compromised infant and nutritionally complete.

Note: Vandenplas Y et *al*. Minerva Pediatr.2010 Aug;62(4):339-45 and Vandenplas Y et *al*. Amino Acids. 2010 Mar;38(3):909-14 use data from the same study.

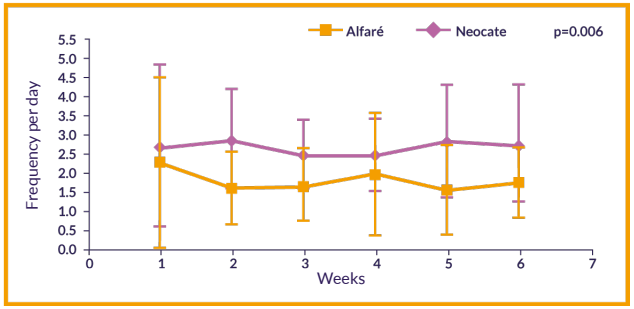
Vandenplas Y, Plaskie K, Hauser B. Safety and adequacy of a semi-elemental formula for children with gastro-intestinal disease. Amino Acids. 2010;38(3):909-14.

Schappi M *et al*. 2006: Omega 3PUFA enriched semi-elemental diet for protracted diarrhoea (abstract)

Study objective: To treat children with non-crohn's disease small intestinal inflammatory disorders with a whey protein hydrosylate enriched with Ω3PUFA formula A (Alfaré).

Methods: 20 children (aged 1/12 to 2 years) with enteropathy and inflammation were randomised into 2 groups in parallel. 10 received formula A and 10 formula B (Neocate) over a 6 week period. Growth, stool output, routine biochemistry, plasma amino acids, erythrocyte lipid profile and small intestinal biopsies were assessed before, and after treatment period.

Stool frequency



Results:

- Enteropathy improved in A and B but the inflammatory cell density was less in A than B (mean score 0.91 vs 2.02).
- δ weight and length Z scores were 0.66 ± 0.54 and 0.51 ± 0.86 in A, 0.65 ± 0.92 and 0.18 ± 0.28 in B (p= NS).
- Stool output was significantly reduced in A vs B (P= 0.006).
- Serum albumin rose from 36 ± 5 to 38 ± 4 in A, B from 32 ± 7 to 35 ± 5g/L (P = NS).
- Red cell lipids C20-5n3 and C22-6n3 were significantly higher and C14, C20-1n9, C22-2N6, C24-1n9 lower in A vs B (P < 0.01).
- Taurine status improved significantly in A (δ + 14 ± 3.5 µmol/l) vs B (δ -27 7.8 µmol/l) P = 0.001 other biochemical parameters and plasma amino acids did not change significantly.

Conclusions: Improved Ω3PUFA status results in down regulation of intestinal inflammation and secretion with better control of the protracted diarrhoea and small intestinal mucosal inflammation.

Alfaré promotes growth and is effective in inducing remission in infants with GI disorders.

Note: Schappi M et *al*. Abstract PG3-14 presented at ESPGHAN 2006 and Milla P et *al*. Abstract P0583 presented at ESPGHAN 2004 use data from the same study.

Schappi M, Fläck S, Haschke F et al. Omega 3PUFA enriched semi-elemental diet for protracted diarrhoea. Abstract PG3-14 presented at ESPGHAN 2006.

Milla P *et al.* 2004: A new semi-elemental diet for small intestinal inflammatory disease (abstract)

Study objective: To treat children with non-crohn's disease small intestinal inflammatory disorders with a whey protein hydrosylate enriched with Ω3PUFA formula A (Alfaré).

Methods: 20 children (aged 1/12 to 2 years) with enteropathy and inflammation were randomised into two groups in parallel. 10 received formula A and 10 formula B (Neocate) over a 6 week period. Growth, stool output, routine biochemistry, plasma amino acids, erythrocyte lipid profile and small intestinal biopsies were assessed before, and after treatment period.

Results:

- Enteropathy and inflammation went into remission in 9/10 with A and B.
- Weight increase ranged from 0.4- 1 .53 kg in A and 0.5- 1 .32 kg in B.
- Serum albumin rose from a mean of 32 ± 2 to 36 ± 3 in A in B from 30 ± 2 to 35 ± 4g/L.
- No significant difference between formulas A and B in the parameters measured, however A was more palatable than B and tube feeding was used significantly less in A 2/1 0 than B 6/1 0 ($p<0.5$).

Conclusions: Alfaré was at least as good as Neocate in inducing a remission in this small group of children with protracted diarrhoea and small intestinal mucosal inflammatory disease. It may have some practical advantages over currently used formulas as it appears to be taken more readily by mouth.

Alfaré promotes growth and is effective in inducing remission in infants with GI disorders; it also is more palatable than an amino acid based formula.

Note: Schappi M et *al.* Abstract PG3-14 presented at ESPGHAN 2006 and Milla P et *al.* Abstract P0583 presented at ESPGHAN 2004 use data from the same study.

Milla P, Schappi M, Flack S et al. A new semi-elemental diet for small intestinal inflammatory disease. Abstract P0583 presented at ESPGHAN 2004.

Nowak-Wegrzyn A *et al*. 2014: Evaluation of hypoallergenicity of a new, amino acid-based formula

Study objective: To determine whether a new AAF with a different carbohydrate and lipid composition meets the AAP hypoallergenicity criteria.

Methods: Children (2 months to ≤12 years of age) with recently confirmed CMPA participated in double-blind placebo controlled food challenges (DBPCFC) with a new AAF (Test) and a commercially available AAF (Control) in a randomised, crossover fashion over a 2-7 day period. CMPA was confirmed within 6 months prior to enrollment by elevated serum cow's milk (CM)-IgE levels, positive skin prick test to CM extract, or positive CM oral challenge. Subjects were monitored during the DBPCFCs for immediate allergic reactions via a rigorous and comprehensive scoring system and observed for 1 hour prior to discharge. If both blinded challenges were tolerated, subjects participated in a week-long open challenge of a minimum 8 ounces daily intake of the Test formula at home. Formula intake, stool characteristics, incidence of flatulence, emesis and spit-up were documented.

Results:

- 37 children (mean age of 4.57 ± 3.68 years, 49% male) with recently confirmed CMPA were enrolled;
  4 subjects did not complete the first DBPCFC due to refusal to ingest a full serving of formula (2 Control, 2 Test).
- 33 subjects completed DBPCFCs with both the Test and Control formulas.
- No subjects had an acute allergic reaction to either formula.
- The lower bound 95% confidence interval for meeting the definition of hypoallergenicity was 91.3%.
- Average intake over the 7-day open challenge period of Test formula was 9.06 ounces daily.
- No unusual stool patterns, allergic symptoms, or signs of intolerance were reported.

Conclusions: The new Test AAF meets the AAP (American Academy of Pediatrics) criteria for hypoallergenicity and can be recommended for the management of CMPA.

Alfamino is hypoallergenic and effective in children with confirmed CMPA.

Nowak-Wegrzyn A, Czerkies L, Collins B et al. Evaluation of hypoallergenicity of a new, amino acid-based formula. Clin Pediatr (Phila). 2014. pii: 0009922814557785. [Epub ahead of print].