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*The Open Nutrition Journal*, 2017, *11*, 27-38


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The Open Nutrition Journal

Content list available at: www.benthamopen.com/TONUTRJ/

DOI: 10.2174/1874288201711010027

REVIEW ARTICLE

# Review of Pro- and/or Prebiotics in Extensively Hydrolyzed or Amino Acid-Based Infant Formula for Food Allergy

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Received: January 27, 2017 Revised: March 30, 2017 Accepted: April 12, 2017

**Abstract:**

***Background:***

The incidence of food allergy is a growing health concern in the United States. Research suggests that there is a link between the gut microbiota and the development of allergy. As a result, researchers propose that gut microbial populations could affect the development and management of immunological disease.

***Objectives:***

The purpose of this review is to present current evidence of the advantages and disadvantages of probiotic and/or prebiotic addition to extensively hydrolyzed protein (EHF) and amino acid-based infant formulas (AAF) for the management of food allergy.

***Method:***

Only randomized controlled trials were included for review. The randomized controlled trials were limited to human subjects less than 12 years of age with a confirmed case of food allergy who were consuming EHF or AAF supplemented with probiotics and/or prebiotics.

***Results:***

Eleven studies were included for review. Probiotic and synbiotic addition was associated with an improvement in SCORAD index in EHF and AAF, and EHF significantly moderated immunologic and/or inflammatory responses. Probiotic addition to EHF benefited patients exhibiting hematochezia, and synbiotic addition resulted in softer stool, higher stool frequency, and decreased incidence of infection in some studies.

***Conclusion:***

Although few studies report statistically significant effects upon feeding prebiotics or probiotics with EHF and AAF on food allergy, this review sheds light on evidence that such inclusion may have positive impacts on SCORAD index, stool quality, immunologic and inflammatory factors, and incidence of infection.

**Keywords:** Food allergy, Infant formula, Microbiota, Probiotics, Prebiotics, Synbiotics.

## INTRODUCTION

The incidence of food allergy has increased worldwide and is a growing health concern in the United States, where an estimated 6% of children under 3 years of age and approximately 4% of adults are affected [1]. The risk of developing food allergies varies based on age, race, gender, and genetic factors. The National Health and Nutrition

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Examination (NHANES) survey found that non-Hispanic blacks, males, and children exhibit increased risk for the development of food allergies [2].

Food allergy is defined as adverse reactions to food involving specific Immunoglobulin E (IgE)-mediated, cell-mediated, or both IgE- and cell-mediated immunologic mechanisms [3]. IgE-mediated reactions include anaphylaxis, angiodemia, urticaria, wheezing, vomiting, rhinitis, and eczema. Non-IgE-mediated reactions include villous atrophy, eosinophilic proctocolitis, enterocolitis, and esophagitis and are associated with the consumption of cow's milk, soy, and other dietary proteins. In addition, affected infants may display symptoms of food allergy such as irritability and colic [4].

The clinical management of food allergy should involve both short-term interventions as well as long-term management to decrease the risk of future reactions. The primary strategy for long-term management is the adoption of an elimination diet. Specialized hypoallergenic formulas such as extensively hydrolyzed protein formulas (EHF) and amino acid-based formulas (AAF) are recommended for young patients including infants and children.

In infants diagnosed with food allergies, elimination diets (restriction in the offending protein) are generally followed for at least 9-12 months from diagnosis. Extensively hydrolyzed protein formula and AAF are utilized for infants who are unable to tolerate breastmilk, cow's milk or soy protein. Extensively hydrolyzed protein formulas have been safely consumed by infants with conditions such as severe inflammatory bowel disease and cow's milk allergy for more than 50 years, and is the first choice for allergic infants who cannot be breast fed. Amino acid-based formulas are recommended in the event when EHF is not tolerated. Both formulas in the U.S. are required to undergo extensive clinical testing in order to prove their compliance with established standards for hypoallergenicity [4].

Research suggests that there is a link between the gut microbiota and the development of allergy. The gut microbiota consists of more than 1000 bacterial species, and developments in sequencing technology have allowed researchers to better understand the diversity of all bacterial species within the gut microbiota [5]. As a result, researchers have proposed that the bacterial populations making up the gut microbiota impact systemic immunity and metabolism, and could therefore affect the development of immunological disease [6].

A recent study following 33 subjects diagnosed with food allergy from infancy to 5 years of age found that 84.8% of subjects experienced resolution of food allergy by 5 years of age. The research also indicated that high rates of breastfeeding in addition to early introduction of supplementary foods rich in prebiotics may impact the development of food allergy [7].

Mouse models have been utilized to understand the effects of gut microbiota on the development of food allergy. In a study focused on oral tolerance, gnotobiotic mice were transplanted with gut microbiota composed mainly of *Bifidobacterium* and *Bacteroides,* and as a result exhibited low sensitization to cow's milk [8]. These results suggest that the transplanted bacteria have a protective effect on food allergy development. Another study showed that the gut microbiota in a food allergy model displayed increased levels of *Lachnospiraceae*, *Lactobacillaceae*, *Rikenellacease*, and *Porphyromonadaceae,* indicating their possible role in the development of sensitization [9].

Studies focused on human models have indicated similar results. A recent study found that among infants displaying food allergen sensitization, *Enterobacteriaceae* were overrepresented and *Bacteroidaceae* were underrepresented at 3 months and 1 year. The risk of allergen development increased with higher *Enterobacteriaceae*/*Bacteroidaceae* ratio but decreased with microbiota diversity [10]. Two additional studies exist comparing the gut microbiota of the healthy infant versus the gut microbiota of infants exhibiting food allergy. Both studies found that infants with food allergy displayed different strains of microbiota as well as different levels of microbiota diversity than that of healthy infants [11, 12]. The development of atopic disease is also shown to be affected by composition of gut microbiota. A study by Nylund *et al.* found that the microbiota composition among infants with eczema did not differ from the controls at 6 months of age, but was more diverse and at 18 months of age, more similar to adult gut microbiota composition than control subjects [13].

Probiotics and prebiotics may alter the gut flora in a way that confers health benefits. Probiotics are live microorganisms that have demonstrated, in animal and *in vitro* studies, to have numerous beneficial impacts on overall health. Among these benefits are the improved protection from infection, development of innate immunity, improvement of gut barrier function, and the inhibition of gut colonization of pathogenic bacteria while allowing for the enhanced colonization of healthy bacteria [14 - 20]. One study found a link between the addition of prebiotics to infant formula and higher fecal levels of secretory immunoglobulin A, an antibody that plays a role in the defense of the gastrointestinal tract [21]. Prebiotics are non-digestible substances that provide a beneficial health impact by stimulating the growth or activity of advantageous bacteria in the gut. The term synbiotic refers to a combination of probiotics and prebiotics.

The evidence indicating the impact of gut microbiota composition on immune development and function presents an opportunity to re-evaluate the preventative and therapeutic strategies in response to food allergy. A recent study demonstrated that daily *Lactobacillus GG* supplementation resulted in a shift in intestinal microbial population to one with increased taxa associated with a decreased risk of allergy and atopy [22]. Altering the composition of an allergic patient's microbiota to one that has higher levels of bacteria associated with tolerance may be possible by the use of probiotics and/or prebiotics. However, reviews focused on the effect of probiotic and/or prebiotic supplementations as part of treatment for food allergy have been inconclusive. The purpose of this review is to present current evidence on the efficacy of probiotic, prebiotic, and synbiotic addition to EHF and AAF for the management of food allergy.

## MATERIALS AND METHODS

### Types of Studies

Randomized clinical trials are the only studies included in this review. Case reports, observational studies, systematic and narrative reviews, letters, editorials, and commentaries were excluded.

### Types of Participants

Studies included were limited to those with infant and child participants less than 12 years of age who were consuming EHF or AAF supplemented with probiotics and/or prebiotics and had a confirmed diagnosis of food allergy.

### Search Strategy

The databases PubMed (www.ncbi.nlm.nih.gov), Embase (Excerpta Medica database), FSTA (Food Science and Technology Abstracts), and MEDLINE were searched for articles published from January 1, 1946 to June 28, 2016. Google Scholar was searched to identify any literature not found in other databases

All databases were searched using the following terms: (prebiotic* OR probiotic* OR synbiotic*) AND (infants OR children OR child OR baby OR babies) AND ("amino acid" OR "extensively hydrolyzed").

## RESULTS

The Embase, FSTA, and MEDLINE searches yielded 139 results total. After application of inclusion and exclusion criteria Table (**1**), seven studies were included for review. The Pubmed search yielded 54 results. After application of inclusion and exclusion criteria, four additional studies were included for review. No additional studies were included from Google Scholar searches. Out of the 11 studies included, five measured the effects of probiotic addition, one measured the effects of prebiotic addition, and five measured the effects of synbiotic addition to EHF and AAF.

**Table 1. Inclusion and Exclusion Criteria.**

| | Inclusion Criteria | Exclusion Criteria |
|---|---|---|
| **Study Design** | Randomized Controlled Trials | Case reports, observational studies, systematic and narrative reviews, letters, editorials, and commentaries |
| **Population** | Human subjects, age <12 years, confirmed diagnosis of food allergy | Non-human subjects, age >12 years, not diagnosed or treated for food allergy |
| **Intervention** | Extensively hydrolyzed protein formula or amino acid-based formula supplemented with probiotics and/or prebiotics | Probiotic and/or prebiotic supplementation without being added to formula |
| **Outcomes** | Severity and extent of atopic eczema, fecal characteristics, tolerance, inflammatory factors, anthropometric factors, incidence of infection, allergic symptoms (respiratory, dermatological, gastrointestinal) | Symptoms unrelated to food allergy |

All five probiotic studies focused on the addition of probiotics to EHF for the management of cow's milk allergy. The outcomes for these studies included changes in fecal characteristics, development of tolerance, changes in SCORAD index (SCORing Atopic Dermatitis), changes in immunological and inflammatory factors, microbial cell counts in feces, and effects on wheezing and hospital admissions. *Lactobacillus* was included as part of the treatment in all five studies, and *Bifidobacterium* was part of the treatment in two of the studies.

Only one study involving prebiotic addition met inclusion and exclusion criteria. The primary outcome for the study was the effect on SCORAD index of infants with atopic dermatitis after prebiotic galacto-oligosaccharide (GOS) addition to EHF.

Among five of the studies focusing on synbiotic addition to EHF or AAF, three studies measured the effects of synbiotic addition to AAF in response to cow's milk allergy. The remaining two studies measured the effects of synbiotic addition to EHF in response to atopic dermatitis. The outcomes for these studies included anthropometric changes, formula tolerance, hypoallergenicity, changes in SCORAD index, fecal characteristics and bacterial composition of feces, changes in immunological factors, and incidence of infection. Out of the five studies, three studies used a synbiotic blend of *Bifidobacterium breve* M-16V + oligofructose, long-chain inulin, and acidic oligosaccharides. The remaining two studies used a synbiotic blend of *Bifidobacterium breve* M-16V + a mixture of short chain galacto-/long chain fructooligosaccharides.

**Table 2. Detailed Characteristics of Included Studies.**

| Study ID | Sample Size | Age Range (months) | Intervention | Dose | Duration (Days) | Outcome(s) | Findings |
|---|---|---|---|---|---|---|---|
| Probiotics | | | | | | | |
| Baldassarre 2010 | 62 | 0-12 | EHF + *Lactobacillus GG* *vs.* EHF alone | 2.5 x $10^7$ (CFU)/g | 28 | • Changes in fecal calprotectin levels<br>• Presence of occult blood in stool | • Fecal calprotectin decrease among EHF + LGG group was significantly larger than decrease among EHF - LGG group<br>• After 4 weeks, 0/12 patients in EHF + LGG group exhibited occult in blood stool, whereas 5/14 in the EHF - LGG group exhibited occult blood in stool |
| Hol 2008 | 119 | 0-6 | EHF + *Lactobacillus casei* CRL431 and *Bifidobacterium lactis* Bb-12 *vs.* EHF alone | $10^7$ (CFU)/g | 365 | • Development of clinical tolerance to cow's milk<br>• Analysis of T- and B-lymphocyte subsets<br>• Changes in SCORAD index<br>• Effects on wheezing and hospital admissions | • Tolerance development in probiotic group compared to placebo group after 6 months and 12 months was not significant.<br>• Treatment significantly decreased $CD3^+$ and $CD3^+CD4^+$ levels in the probiotic group<br>• SCORAD index significantly improved in probiotic group at 6 months and 12 months, and significantly improved in placebo group at 6 months only. After adjusting for baseline values, there was no significant change from baseline between probiotic and placebo groups.<br>• Treatment had no effect on hospital admissions and wheezing. |

*(Table 2) contd.....*

| Study ID | Sample Size | Age Range (months) | Intervention | Dose | Duration (Days) | Outcome(s) | Findings |
|---|---|---|---|---|---|---|---|
| Isolauri 2000 | 27 | 4.6 | EHF + *Bifidobacterium lactis* Bb-12 or *Lactobacillus GG* *vs.* EHF alone | LGG: 3 x $10^8$ (CFU)/g *Bifidobacterium lactis*: 1 x 109 (CFU)/g | 60 | • Extent and severity of atopic eczema<br>• Serum concentrations of soluble cell surface molecules, cytokines/chemokines, and urine concentrations of methyl-histamine and eosinophilic protein | • After 2 months, there was a significant decrease in SCORAD index for both probiotic groups<br>• After 6 months, the SCORAD index had decreased to 0 in all groups<br>• After 2 months, serum CD4 concentration decreased in both probiotic groups, but not the unsupplemented group<br>• Serum TGF-β1 concentrations decreased in those receiving *Bifidobacterium lactis* Bb-12, but increased in those receiving *Lactobacillus GG*<br>• Serum concentrations of IL-1ra, TNFα, GM-CSF, sICAM-1, RANTES and MCP-1α were unaffected by probiotics |
| Kirjavainen 2003 | 35 | 3.5-6.8 | EHF + viable *Lactobacillus GG* or heat-inactivated *Lactobacillus GG* *vs.* EHF alone | 1 x $10^9$ (CFU/g) | 52.5 | • Changes in SCORAD index<br>• Changes in microbial cell counts in feces | • Adverse gastrointestinal effects were noted among the heat-inactivated LGG group and this intervention was discontinued<br>• Mean decrease in SCORAD index was greater in the viable LGG group than in the placebo group<br>• Treatment groups did not show a significant change in bacterial numbers in fecal samples |
| Majamaa 1997 | 31 | 2.5-15.7 | EHF + *Lactobacillus GG* *vs.* EHF alone | 5 x $10^8$ (CFU)/g | 60 | • Changes in severity of atopic eczema<br>• Changes in intestinal inflammation *via* concentrations of fecal $\alpha_1$-antitrypsin, eosinophil cationic protein (ECP), and tumor necrosis factor-α (TNF-α)<br>• Changes in systemic immune response *via* concentrations of ECP and cytokine production of peripheral blood mononuclear cells | • The clinical score of atopic dermatitis (SCORAD) significantly improved within the EHF + LGG group only<br>• Concentrations of fecal α1-antitrypsin and TNF-α significantly decreased among EHF + LGG group only<br>• Fecal ECP concentrations were not affected by treatment |
| Prebiotics | | | | | | | |

*(Table 2) contd.....*

| Study ID | Sample Size | Age Range (months) | Intervention | Dose | Duration (Days) | Outcome(s) | Findings |
|---|---|---|---|---|---|---|---|
| Boženský 2015 | 120 | 1.5-2 | EHF + galacto-oligosaccharides *vs.* EHF alone | 0.5g / 100ml | 182.5 | • Severity of atopic eczema as measured by SCORAD index<br>• Changes in anthropometric factors, tolerance, and incidence of infection | • While both groups showed a decrease in SCORAD index, there was no significant difference in reduction between the two groups<br>• There was no significant difference in anthropometry, tolerance, or incidence of infection among the two groups |
| Synbiotics | | | | | | | |
| Burks 2015 | 110 | 0-8 | AAF + oligofructose, long-chain inulin, acidic oligosaccharides, *Bifidobacterium breve* M-16V *vs.* AAF alone | Prebiotic blend: 8 g/l (6.8 g/l oligofructose:inulin 9:1 and 1.2 g/l pAOS) *Bifidobacterium breve* M-16V: 1.47 x $10^9$ (CFU)/100 ml | 112 | • Changes in growth (weight, length, head circumference)<br>• Allergic symptoms consisting of dermatological (SCORAD index), respiratory, and gastrointestinal<br>• Changes in consistency, frequency, and color of stool<br>• Changes in formula intake<br>• Changes in fecal pH and fecal short-chain fatty acids | • An increase in weight, length, and head circumference was seen in both groups, but there was no significant difference between the groups<br>• 22% of subjects in the treatment group reported diarrhea, whereas 4% in the control group reported diarrhea<br>• 2% of subjects in the treatment group were treated for infection, whereas 18% in the control group were treated for infection<br>• SCORAD index and severity of other allergy symptoms decreased for both groups but there was no significant decrease between the groups<br>• Flatulence, stool frequency, and stool consistency was not significantly different between both groups<br>• Stool color showed significant improvement in the treatment group<br>• Fecal pH was significantly lower among the treatment group<br>• For the treatment group, acetic acid levels were significantly higher while propionic acid levels were lower |

*(Table 2) contd.....*

| Study ID | Sample Size | Age Range (months) | Intervention | Dose | Duration (Days) | Outcome(s) | Findings |
|---|---|---|---|---|---|---|---|
| Burks 2014 | 110 | 0-8 | AAF + oligofructose, long-chain inulin, acidic oligosaccharides, *Bifidobacterium breve* M-16V *vs.* AAF alone | Prebiotic blend: 8 g/l (6.8 g/l oligofructose:inulin 9:1 and 1.2 g/l pAOS) *Bifidobacterium breve* M-16V: 1.47 x $10^9$ (CFU)/100 ml | 112 | • Changes in growth (weight, length, head circumference)<br>• Formula tolerance<br>• Changes in SCORAD index<br>• Evidence of respiratory allergic symptoms<br>• Stool consistency, frequency, and color<br>• Incidence of adverse events and medication use | • Both formulas supported normal growth, there was no significant difference in growth between the two groups<br>• Both formulas were equally tolerated and reduced allergic symptoms with no significant differences between the two groups<br>• SCORAD index decreased in both groups with no significant differences between the two groups<br>• The treatment group reported softer and yellow/brown stools, whereas the control group reported dry and green/dark brown stools<br>• Incidence of infection and antibiotic use was lower in the treatment group |
| Harvey 2014 | 30 | 0-36 | AAF + *Bifidobacterium breve* M16-V and neutral fructo-oligosaccharides mixture *vs.* AAF alone | Prebiotic Mixture: 0.8 g/100 ml *Bifidobacterium breve* M16-V: 1.47 x $10^9$ CFU/100ml | 7 | •Acceptance of formula<br>• Hypoallergenicity (absence of clinical symptoms)<br>• Incidence of adverse events | • No serious adverse events were reported upon synbiotic addition<br>• Demonstrated at 95% confidence that at least 90% of infants would have no reaction to synbiotic addition, therefore establishing the formula as hypoallergenic |
| Van Der Aa 2010 | 90 | 0-7 | EHF + *Bifidobacterium breve* M16-V and short chain galacto-/long chain fructooligosaccharide mixture *vs.* EHF alone | Prebiotic Mixture: 0.8 g/100 ml *Bifidobacterium breve* M-16-V: 1.3 x $10^9$ CFU/100ml | 84 | • Severity of atopic dermatitis measured by SCORAD index<br>• Changes in total and specific IgE and eosinophilic granulocytes | • SCORAD index decreased for both groups, but there was no significant difference in SCORAD index between the groups at any time<br>• Total and specific serum IgE and eosinophilic granulocytes were unaffected by treatment<br>• In a subgroup of infants with elevated total or specific IgE, there was a significantly greater improvement in SCORAD index in the treatment group than in the control group |

*(Table 2) contd.....*

| Study ID | Sample Size | Age Range (months) | Intervention | Dose | Duration (Days) | Outcome(s) | Findings |
|---|---|---|---|---|---|---|---|
| Van Der Aa 2009 | 90 | 0-7 | EHF + *Bifidobacterium breve* M16-V and short chain galacto-/long chain fructooligosaccharide mixture *vs.* EHF | Prebiotic Mixture: 0.8 g/100 ml *Bifidobacterium breve* M-16V: 1.3 x $10^9$ CFU/100ml | 84 | • Bacterial composition, pH, lactate, and short-chain fatty acids in fecal samples<br>• Changes in fecal consistency and frequency | • The treatment group demonstrated a significantly higher detection rate *of B. breve* M16-V and concentrations of D-lactate and L-lactate than controls.<br>• The treatment group had a significantly lower fecal pH than controls<br>• The treatment group demonstrated higher proportions of acetic acid and lower proportions of butyric when compared to controls<br>• Fecal consistency was reported to be significantly softer and constipation was reported less often in the treatment group |

Abbreviations:
EHF: Extensively hydrolyzed protein formula
AAF: Amino acid-based formula

## SCORAD Index

Out of the 11 studies, eight listed change in SCORAD index as an outcome. SCORAD index reportedly improved in all eight studies in which probiotics and/or prebiotics were added to EHF or AAF. However, only three studies demonstrated statistically significant improvement in SCORAD index in the treatment group than in the control group [23, 24, 29]. One study found that SCORAD index was only significantly improved among patients who exhibited elevated total or specific IgE levels [25].

## Inflammatory and Immunological Factors

Inflammation is a significant factor in the manifestation of atopic disease, and certain strains of microflora have been shown *in vitro* to impact the immune system as well as the production of pro-inflammatory and anti-inflammatory cytokines [26 - 28]. Four of the included studies found that *Lactobacillus GG*, *Bifdobacterium lactis*, or a mix of *Bifidobacterium breve* with short chain galacto-/long chain fructooligosaccharides, when added to EHF, significantly led to moderate immunologic and/or inflammatory responses [23 - 25, 29]. These results provide further possible evidence of probiotic and synbiotic effectiveness in the reduction of atopic disease in food allergic individuals.

## Stool Characteristics

In patients exhibiting hematochezia, probiotic addition to EHF was beneficial. One study found that the consumption of EHF with the addition of *Lactobacillus GG* resulted in decreased fecal calprotectin levels, and it was also found that after 4 weeks, none of the patients in the treatment group had blood in their stool compared to 5 out of 14 in the control group that still had blood in their stool [30].

Results varied among studies with outcomes including fecal consistency and frequency. One study did not report any significant difference in stool consistency and frequency, whereas two other studies reported softer stools and higher stool frequency among treatment groups [31 - 33]. When outcomes included stool bacterial populations, the results varied. One study measuring the effects of *Lactobacillus GG* supplementation found that fecal bacterial counts were unaffected [32]; while another study measuring the effects of *Bifidobacterium breve* supplementation found that the treatment group demonstrated a significantly higher detection rate of *B. breve* in stools [33].

### Infection Incidence

Three of the included studies measured incidence of infection after probiotic and synbiotic supplementation to EHF and AAF. One study found that *Lactobacillus casei* and *Bifidobacterium lactis*, when added to EHF, did not have any effect on hospital admissions [29]. The remaining two studies found that *Bifidobacterium breve* and a mixture of prebiotics resulted in a lower incidence of infection and antibiotic use [31, 34].

### Growth

Changes in weight, length, and head circumference were outcomes included in two studies. Both studies found that synbiotic-supplemented AAF supported normal growth and both control and treatment groups experienced increases in weight, length, and head circumference, with no significant differences between the two groups [31, 34].

### Adverse Effects

There were very few adverse effects reported in the studies included. One study found that heat-inactivated *Lactobacillus* GG resulted in adverse gastrointestinal effects and was discontinued. However, the use of viable *Lactobacillus* GG in the same study did not have any adverse effects [32]. In a second study, diarrhea was reported in 4% of subjects in the control group and in 22% of subjects in the treatment group receiving AAF supplemented with *Bifidobacterium breve* and a prebiotic mixture of oligofructose, long-chain inulin, and acidic oligosaccharides [34].

## DISCUSSION

In summary, while the results of this review suggest that the advantages associated with probiotic and/or prebiotic addition to hypoallergenic formulas improved skin conditions and SCORAD index, decreased incidence of hematochezia and infection, improved inflammatory and immunological factors, improved fecal characteristics, and supported normal growth, very few studies reported a statistically significant effect of feeding prebiotics or probiotics.

The modulation of immunological and inflammatory factors as a result of probiotic addition to EHF is supported by a recent meta-analysis showing that probiotic supplementation was effective in reducing total IgE as well as the risk of atopic sensitization among patients [35]. Reduced IgE levels are one of the hallmarks in the development of allergen tolerance, and the results of this review as well as the meta-analysis suggests that probiotics may play a role in this process [36]. Probiotics affect IFN-γ secretion in patients with cow's milk allergy and IgE-associated atopic dermatitis. Decreased IFN-γ response is associated with the development of cow's milk allergy, and probiotic supplementation resulted in increased IFN-γ secretion and therefore likely contributes to the management of the condition [37].

This review found that in every instance that SCORAD index was an outcome for probiotic and/or prebiotic addition to hypoallergenic formula, SCORAD index improved. Despite the general improvements in SCORAD, only three out of eight studies with this outcome found the results to be significant. A Cochrane Review also reported mixed results of infant eczema outcomes in patients consuming probiotic supplements; however, the author noted that the varying outcomes may be attributed to heterogeneity among the studies [38]. This review shows that a possible driving factor for the effectiveness of probiotic and/or prebiotic supplementation in hypoallergenic formula on SCORAD index is the level of total or specific IgE in subjects in the treatment group. Not all studies reported the baseline IgE levels for their subjects, which may account for differing results.

In addition, research has shown that probiotic and/or prebiotic addition to hypoallergenic formula does not affect the formula's hypoallergenicity. One study measured the hypoallergenicity of an extensively hydrolyzed protein formula containing *Bifidobacterium lactis* and compared its hypoallergenicity to that of an EHF containing *Lactobacillus GG*. The study found that out of 66 participants, none had a reaction to EHF + *Bifidobacterium lactis* and only one had a reaction to EHF + *Lactobacillus GG*. Both formulas met hypoallergenicity criteria set by the American Academy of Pediatrics (AAP) [39]. Three studies included in this review found that AAF with synbiotics resulted in normal tolerance and either normal or increased growth in patients [31, 34, 40]. There were two cases of adverse gastrointestinal effects in this review; an increase in the incidence of diarrhea [34] and gastrointestinal intolerance due to heat-inactivated *Lactobacillus* GG [32]. The literature review did not report any serious contraindications to adding probiotic and/or prebiotic to hypoallergenic formula.

## CONCLUSION

The purpose of this paper was to review the use of probiotics and/or prebiotics in extensively hydrolyzed protein or amino acid-based formula and their impact on food allergy.

The strength of evidence in this review is limited by heterogeneity among the studies reviewed and the limited number of studies included. There are vast differences in factors such as sample size, age range, intervention, dose, and duration in the studies included in this review. In addition, the scientific community strongly lacks studies measuring the effects of probiotic and/or prebiotic addition to hypoallergenic formulas.

At present, there is not enough evidence to make any recommendation for inclusion of prebiotics or probiotics to EHF or AAF. Moving forward, future research should ensure adequate sample size and focus on using similar age groups, outcomes measures, and measuring techniques. The results of this review cannot be generalized and extrapolated to other prebiotic mixtures and probiotic strains. Different probiotic strains and prebiotic mixtures may yield different results.

Although there is insufficient evidence to draw a conclusion, the results of this review suggest that there may be benefits associated with hypoallergenic formulas supplemented with probiotics and prebiotics, and that further research in this area is warranted. It is also of note that both prebiotics and probiotics in the doses studied appear to be safe and well tolerated.

## CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

## ACKNOWLEDGEMENTS

Declared none.

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