
Management of bovine protein allergy: new perspectives and nutritional aspects

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Objectives: Awareness of the considerable incidence of bovine protein allergy in infancy makes it necessary to eliminate cow's milk antigens from the diet in special cases. The primary objective of this review is to discuss the nutritional aspects and appropriate use of hypoallergenic formulas as a substitute for cow's milk formulas and to present new alternative feeding modalities in this field.

Data Sources: Related articles in PubMed (National Library of Medicine) were reviewed. This review is a synthesis of these sources along with the discussions with experts in this field and the expert opinion of the authors.

Results: Soy protein-based and hydrolyzed milk protein formulas are the most commonly used alternative protein sources in the case of bovine protein allergy. Despite the adequacy of their nutritional values, there are still some problems to be solved regarding these formulas. In addition, a considerable percentage of the infants with bovine protein allergy may also present allergenic reactions to soy proteins as well as to hydrolyzed milk proteins. Thus, there still exists chaos in the selection of the most appropriate formula for infants sensitized to cow's milk protein. Rice protein-based formulas, probiotics, and prebiotics are expected to be new effective alternatives.

Conclusions: Bovine protein allergy constitutes an important place in childhood food allergies. Soy protein-based and hydrolyzed protein formulas have some disadvantages and risk of allergenic activity that is not to be underestimated. So, substitution of cow's milk protein with an alternative protein source in sensitized infants has become an art rather than a science. Rice protein, prebiotics, and probiotics appear to be valuable alternatives giving hope for the future.

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INTRODUCTION

Although human milk is the optimal primary nutritional source for infants in the first year of life, there are conditions where its substitution by an infant formula is inevitable. Formulas containing cow's milk protein are commonly used to fulfill the nutritional needs of these infants. Because of the high incidence of bovine protein allergy, which affects from 2.2 to 7.5% of children mainly in their first year of life,^{1,2} hypoallergenic and hypoimmunogenic formulas from alternative protein sources have been introduced.³⁻⁷

In this article the nutritional aspects of the two most commonly used formulas (soy protein-based formulas and hydrolyzed protein formulas) will be discussed, and new perspectives related to alternative sources in bovine protein allergy will be presented.

NUTRITIONAL ASPECTS RELATED TO HYPOALLERGENIC FORMULAS

Soy Protein-Based Formulas

Characteristics of the formulas. For more than 60 years, soy-based infant formulas have been fed to millions of infants

worldwide. Soy-based formula is fed to 18% of infants in the United States, 13% in New Zealand, 7% in Britain, and nearly 5% in Italy at some time during the first year of life.⁸

Protein content

The protein content is a soy isolate supplemented with L-methionine, L-carnitine, and taurine to provide protein at 2.45 to 3.1 g/100 kcal or 1.65 to 2.1 g/dL.³ It has been demonstrated that at a protein intake of 1.8/100 kcal, methionine is required to improve nitrogen balance, whereas at intakes of 2.2 and 2.6 g/100 kcal methionine supplementation improves weight gain, urea nitrogen excretion, and albumin synthesis.⁴

Carnitine is added to soy formula to the level in breast milk because it is deficient in foods of plant origin and is required for the optimal mitochondrial oxidation of long-chain fatty acids.⁵

Taurine, an amino acid functioning as an antioxidant and constituting the major conjugate of bile acids in early infancy, is also added to soy formulas.⁵

Mineral absorption

Until 1980, mineral absorption from soy formulas was erratic because of poor stability of the suspensions and presence of excessive soy phytates capable of binding strongly with divalent cations in the formula. But today, after the advent of low phytate soy formulas, bone mineralization, serum levels of calcium, phosphorus and alkaline phosphatase are equivalent to those found in infants fed with cow's milk-based formulas.^{6,9-11} The presence of two different carbohydrates in

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the formula, sucrose and corn syrup hydrolysates, also offer an additional advantage for optimizing calcium absorption.⁶

The soy phytates and fiber oligosaccharides also bind iron and zinc, thus soy-based formulas are iron- and zinc-fortified. Table 1 demonstrates comparatively in vitro availability of calcium, iron, and zinc from first-age formulas and human milk. These results have been obtained from an availability study conducted by Bosscher et al.¹²

Problems to be solved. Despite the clinical studies^{3,5,6,13} demonstrating that full-term infants fed on soy-based protein formulas have shown normal growth with an adequate nutritional status of protein and with normal bone mineralization, there are still some problems to be solved: 1) aluminum toxicity; 2) manganese toxicity; and 3) exposure to phytoestrogens.

1) Aluminum toxicity

The aluminum content of soy-based formulas is high when compared with that of human milk (600 to 1,300 ng/mL vs 4 to 65 ng/mL) because of the mineral salts used in formula production.⁵ Increased deposition in bone and in the central nervous system, and reduced skeletal mineralization (osteopenia) resulting from the competition of aluminum with calcium for absorption are the potential findings of aluminum toxicity, particularly in the presence of reduced renal function in preterm infants.^{5,6,14} Term infants with normal renal function do not seem to be at risk for aluminum toxicity arising from soy-based formulas.¹²

2) Manganese toxicity

The manganese content of soy-based formulas is also higher than that of cow's and human milk (200 to 300 µg, 50 to 100 µg, and 3 to 8 µg, respectively).⁶ Little is known about the potential toxicity of these high manganese levels. Long-term effects on central nervous system still remain an important subject to be investigated.

3) Exposure to phytoestrogens

Phytoestrogen is a general term used to define classes of nonsteroidal compounds that are either of plant origin or derived from the in vivo metabolism of their precursors, present in several plants eaten by human beings.¹⁵ Based on their chemical structure, phytoestrogens are divided into three main groups: isoflavonoids, lignans, and coumestans.¹⁶ Flavonoids are naturally occurring phytochemicals in plants that can not be synthesized by humans and are thought to have positive effects on human health ("functional food"). More than 4,000 flavonoids have been identified and included in

six classes. Isoflavonoids occur predominantly in legumes and soy protein-containing food.¹⁷

When ingested, isoflavones are metabolized extensively in the intestinal tract, absorbed, transported to the liver, and then undergo enterohepatic recycling. Intestinal bacterial glucosidases cleave the sugar moieties and release the biologically active isoflavones, daidzein and genistein, and in the adult these can be further biotransformed by bacteria to the specific metabolites, equol, desmethylangolensin, and *p*-ethylphenol. All of these phytoestrogens are then eliminated, mainly by the kidney, and therefore share the physiologic features and behavior of endogenous estrogens.¹⁷ The absence of equol in the urine of 4-month-old infants fed soy-based formulas has been attributed to the lack of a fully developed gut microflora (at least during the first months of life) and highlights their key role in the formation of endocrine active compounds.¹⁸

There is a paucity of data on physiologic, developmental, and endocrine effects of soy phytochemicals during infancy. The daily exposure of infants to isoflavones in soy-based infant formulas is six- to 11-fold higher on a body weight basis than the dose that has hormonal effects in adults consuming soy foods.^{17,19} These high levels led to questions about the potential long-term effects of these hormone-like molecules.²⁰ However, the results of a recent study conducted by Strom et al²¹ demonstrate that exposure to soy formula in infancy does not appear to lead to different general health or endocrinologic and reproductive outcomes in adulthood than exposure to cow's milk formula. Several explanatory mechanisms have been suggested for these reassuring findings about the safety of soy formulas despite reported high levels of phytoestrogens (Table 2).^{18-20,22,23}

Conclusions and recommendations on the appropriate use of the soy-based formulas.

Term infants

For term infants, when mother's milk is not available, isolated soy protein-based formulas are safe and effective alternatives to provide appropriate nutrition for normal growth and development (Table 3). However, it should be kept in

Table 1. In Vitro Availability of Calcium, Iron, and Zinc from First-Age Infant Formulas and Human Milk: Results of an Availability Study

Source	Iron (%)	Calcium (%)	Zinc (%)
Human milk	8.12 ± 0.27	13.1 ± 0.8	13.1 ± 0.7
Whey-based formula	1.28 ± 0.28	13.3 ± 1.2	6.7 ± 0.6
Casein-based formulas	0.48 ± 0.22	21.2 ± 0.6	8.5 ± 1.6
Soy-based formulas	1.48 ± 0.28	13.0 ± 1.2	2.3 ± 0.4

Table 2. Possible Explanations of the Absence of Long-Term Biologic Effects in Infants with High Levels of Phytoestrogens

Dietary estrogens are nonsteroidal in structure and vastly less potent than endogenous estrogens. Metabolically active aglycones are extensively metabolized by infants into glucuronide and sulfate conjugates with a weaker biologic activity.
Equol, a mammalian-derived compound with a high estrogenic potency, was not detected in the plasma and urine of these infants possibly because of immaturity in the bacterial enzymes in early life or underdevelopment of specific gut bacteria required for metabolic biotransformation.
Genistein and diadzein may be lightly bound to many of the proteins that bind estrogens.
The infant's reduced gastrointestinal transit time may result in less absorption of soy isoflavones.

Table 3. Soy Protein-Based Formulas in Term Infants: Results from Clinical Studies

Normal growth and development in term neonates fed methionine-supplemented isolated soy protein-based formulas. ^{3,5,6}
Serum albumin concentration is normal. ⁵
Bone mineralization is equivalent to that documented with cow's milk-based formulas. ^{5,9-11}
Use of these formulas do not interfere with the normal immune responses to oral immunization with polio vaccine. ⁵

mind that infants with reduced renal function or with renal failure might be at potential risk for aluminum toxicity.

Preterm infants

Preterm infants demonstrate significantly less weight and length gain, lower serum albumin and phosphorus levels, higher alkaline phosphatase levels, decreased bone mineralization, and higher incidence of osteopenia of prematurity when they are fed soy protein-based formulas.⁵

These findings combined with the concerns about the substantial risk of aluminum toxicity lead to the conclusion that soy protein-based formulas should not be fed to low birth weight preterm infants.

Hydrolyzed Protein Formulas

Characteristics of the formulas. These formulas are obtained by the breakdown of proteins by heat and enzymatic hydrolysis. There are two types of these formulas distinguishable from one another with respect to the degree of hydrolysis: 1) partially hydrolyzed formulas and 2) highly hydrolyzed formulas.

Partially hydrolyzed formulas contain a definite proportion of proteins that are intact or only slightly degraded with peptides having a molecular weight between 8,000 and 20,000 Da.

Highly hydrolyzed formulas contain peptides that usually have a molecular weight <5,000 Da (normally available products are below 1,200 Da).²⁴

The enzymatic or thermal treatment of proteins does not seem to have an impact on the nutritional value of the protein source. Available data about the nutritional value of hydrolysates indicate that they are nutritionally acceptable and that they produce adequate weight and size gains.²⁴

More recently, the use of hydrolyzed protein formulas has been suggested in preterm infants to improve gastric emptying by accelerating the gastrointestinal transit time.²⁵

Problems to be solved.

1) Nitrogen absorption

Nitrogen absorption with these formulas is still questionable, as the transport of small peptides or individual amino acids may not be as efficient as it is when they have been initially hydrolyzed by microvillus membrane peptidases.²⁶ In preterm infants, the technology necessary to perform partial protein hydrolysis seems to reduce significantly nitrogen absorption and whey protein bioavailability.²⁷ Healthy term infants fed with a highly hydrolyzed formula had lower body

weight gain and higher levels of blood ureic nitrogen when compared with breast-fed infants.²⁸

2) Plasma amino acids

Isolauro et al²⁹ demonstrated that plasma concentrations of essential amino acids were lower in infants fed with highly hydrolyzed formulas when compared with breast-fed babies. In a more recent study,³⁰ infants fed with partially hydrolyzed formulas have been documented to have lower concentrations of plasma albumin and some essential amino acids (threonine, lysine) in comparison to standard formula-fed infants.

3) Other problems

Other disadvantages of these formulas include their high cost, and high degree of osmolality, which may provoke osmotic diarrhea and directly damage the enterocyte.³¹

Conclusions and recommendations on the appropriate use of the hydrolyzed protein formulas.

It becomes evident from the above mentioned concerns that it is not yet possible to make a final evaluation about the nutritional adequacy of hydrolyzed protein formulas. The results of the clinical studies are contradictory and long-term prospective studies are lacking. At the moment it is advisable to use these formulas very carefully and only in selected cases.

NEW PERSPECTIVES

It is certain that the management of children sensitized to cow's milk protein requires the exclusion of cow's milk antigens from the diet. However, the ongoing concerns regarding the commonly used hypoallergenic formulas (soy-based and hydrolyzed milk protein formulas) lead to the confusion of which formula should be used. Despite soy protein-based formulas are widely used as an alternative source, reactions to soy protein may occur in 17 to 47% of children sensitized to cow's milk proteins.³² Also, hydrolyzed milk protein formulas may still present residual allergenic activity, including anaphylaxis.³³ Thus, the potential risks of allergy and the presented evidence about some disadvantages of these formulas emphasize the necessity to look for alternative plans of nutrition in sensitized infants. Rice protein formulas, probiotics, and prebiotics seem to be noteworthy and to give hope for the future.

Rice Protein-Based Formulas

Characteristics of the formulas. Rice is considered a hypoallergenic food, as indicated by being chosen as one of the first solid food for infants and by its use in most of the elimination diets for food allergy diagnostic programs.³⁴

A rice protein-based formula has been recently marketed (Risolac Plasmon, PLADA srl, Milan, Italy). Isolated rice proteins are treated by enzymatic hydrolysis to obtain an extensively hydrolyzed protein formula. This process further reduces the allergenicity of the rice protein. After the hydrolysis process, this formula contains peptides with a molecular weight <4,000 Da (86.8% of peptides <2,000 Da, and 13.2% between 2,000 and 4,000 Da). A study in animals³⁵ suggests that this formula based on hydrolyzed rice proteins has a very

low sensitizing capability. Guinea pigs receiving an intravenous injection of hydrolyzed rice protein did not show any fatal reaction, and very low levels of specific IgG to rice proteins were observed.

The digestibility of the carbohydrate and fat components of rice protein-based formulas are as complete as those of casein diets.³⁶

Additional positive aspects of these formulas include their being gluten-free, highly digestible, and very close to our "nutritional culture" (more so than soy).

Problems to be solved.

1) Protein content and nitrogen absorption

The low protein content of all common rice varieties has made them unlikely candidates for infant formulas, despite their generally high biologic value.

Digestibility of protein, as with all rice products, is moderately low with a nitrogen absorption lower than that from cow's milk protein. It would seem that the inferior nitrogen absorption is the result of the unique nature of rice protein, being primarily present as protein bodies, some of which remain resistant to enzymatic attack.³⁷ This aspect can be compensated for by the high biologic value of the rice protein, making nitrogen retention equal to that from casein.³⁸

2) Plasma amino acids

Because of the low protein content of rice, lysine and arginine have to be added to rice protein-based formulas to increase the level of essential amino acids.

Plasma-free amino acids are consistent with the low essential/total amino acids ratio found in cereals, with slower absorption rate, and with the possibility that leucine acts as a limiting amino acid.³⁸

Conclusions and recommendations on the appropriate use of the rice protein-based formulas. Even if available data seem to confirm the acceptability, tolerance, and nutritional value of rice protein-based formulas, clinical studies in human infants should be performed to evaluate the allergenicity of these formulas, and the growth of infants fed with rice protein for a long period of time. Also the effects of these formulas on stool consistency should be considered; in fact, excessive hardening of the stool pattern could be a problem.

Probiotics and Prebiotics

Evidence of an inverse link between infections early in life and allergy has led to the suggestion that the rapid rise in atopy might be a result of improved hygiene leading to a deprivation of the immune system.³⁹⁻⁴¹ Food allergy is frequently the first manifestation of these disorders.⁴² The essential role of the gut microflora in the development of the immune system indicates that a close relationship between allergic sensitization and the development of the intestinal microflora may occur in infancy.⁴³ Confirming this hypothesis, studies^{39,44-46} have recently underlined the involvement of the intestinal microflora in the development of allergic diseases. Active microbial stimulation in the intestinal tract early in life triggers off a protective effect against allergic diseases by various immunologic mechanisms (Table 4).^{39,41,45,47-49}

Table 4. Mechanisms of the Promotion of Clinical Tolerance to Allergens by Certain Microbial Exposures

Type of the immunologic response	Mechanisms
Conversion of T-helper-2 type immunity towards T-helper-1 type immunity	Key role in the promotion of clinical tolerance by avoidance of the cytokines IL-4, IL-5, and IL-13 released as a result of Th-2 activation leading to IgE production and allergy
Generation of transforming growth factor β	Inhibition of the production of proinflammatory cytokines from macrophages, B and T cells Effective downregulation of Th2-driven allergenic activity Enhancement of the repair of the injured epithelium
IgA production	Mucosal immune defense

Thus, probiotic and/or prebiotic effect could have a role in the prevention of these disorders.

Probiotics are cultures of potentially beneficial bacteria of healthy gut microflora, and one strain, *Lactobacillus rhamnosus* (Lactobacillus GG), has been found safe and effective in treatment of allergic inflammation and food allergy.^{39,44} The recent results of two published placebo-controlled randomized clinical trials^{39,50} demonstrate that primary prevention of allergy in infants is possible with Lactobacillus GG supplementation when it is administered to their mothers prenatally and during breast-feeding. In both studies probiotic administrations to the mothers have offered a safe and effective mode to promote protection against atopic diseases in babies during infancy and the first 2 years of life, respectively.

Prebiotics are defined as a substrate of food ingredient that is nondigestible for the host, but is fermented selectively by some of the intestinal flora.⁵¹ In breast-fed infants, the intestinal microflora is dominated by bifidobacteria and lactobacilli, and this microbial pattern produces beneficial effects on the intestinal function and on the development of the immune system.⁵² The effect of human milk on the intestinal flora is caused by its content of selective agents which can stimulate the growth of bifidobacteria and lactobacilli. Oligosaccharides, a major component of human milk,⁵³ have been identified as a "bifidogenic" factor of human milk.^{54,55} Recently, it has been shown that human milk oligosaccharides are resistant to enzymatic digestion in the upper gastrointestinal tract.⁵⁶ Now it is possible to have commercially available nondigestible oligosaccharides and supplement the formulas with these ingredients known as prebiotics. A recent study⁵⁷ clearly demonstrated that supplementation of a term infant formula with a mixture of galacto- and fructo-oligosaccharides has a dose-dependent stimulating effect on the growth of bifidobacteria and lactobacilli in the intestine of healthy term infants.

CONCLUSION: A GLIMMER OF HOPE

New formulas containing rice protein are under investigation to feed infants with bovine protein allergy. The low solubility and low organoleptic value of the rice proteins brings the necessity of protein enrichment with other vegetable protein derived from leguminosa other than soy.

The addition of *Bifidobacteria* or *Lactobacillus* cultures to infant formulas to modify the fecal flora of the bottle-fed infants is used today, and it is becoming more and more popular. Some viable probiotics have certainly specific effects on the intestine. Nonviable probiotic cultures are also used in infant formulas. Controversies still exist about probiotic strains to be used because the mechanisms of action are not yet well understood and the long-term effects are totally unknown. Prebiotics are able to modify the intestinal microflora stimulating the growth of supposed health-promoting endogenous microorganisms, essentially bifidobacteria and lactobacilli. Prebiotics have also been found to stimulate intestinal absorption of calcium, magnesium, iron, and to interfere in the immunomodulation processes, probably through microflora changes.

When using probiotics or prebiotics, we try to modify an extremely complex microbial ecosystem. Better understanding of the short- and long-term risks on the balance of the healthy intestinal infant microflora, on long-term control of autochthonous flora, on inappropriate activation of the mucosal immune system (with probiotics or prebiotics), or of introducing a foreign bacteria strain (with probiotics), is certainly an important duty for the future, but today any adverse microbial or immunologic consequences have not been observed.

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