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Closer to the Gold Standard: an Appraisal of Formulae Available in Italy for Use in Formula-fed Infants

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Infant formulae are the only alternatives to breast milk for infants who are unable to continue breastfeeding through the first year of life. They aim to provide formula-fed infants with the same structural and functional benefits observed in breastfed infants. To achieve this, bioactive nutrients have been added to infant formulae in recent years: long-chain polyunsaturated fatty acids for neurodevelopment; probiotics and prebiotics for local gastrointestinal defence; and nucleotides for promoting the immune response. Changes in protein quantity and quality allow infant formulae to achieve a balance between providing the correct plasma amino acid profile and reducing the protein intake, which could prevent obesity in later life. Hydrolysed proteins may help prevent atopic disorders. Many short-term trials have been published but long-term follow-up data are needed in infants who have been fed the newer infant formulae, to fully understand the role of bioactive nutrients.

KEY WORDS: Bioactive nutrients; Probiotics; Prebiotics; Long-chain polyunsaturated fatty acids; Infant formula

Introduction

FROM NUTRIENT COMPOSITION TO NUTRIENT FUNCTION

Breast milk is required for optimum short- and long-term growth in infancy and the importance of this has been recognized by the World Health Organization (WHO). The lower incidence of infectious diseases in breastfed babies has been shown in both developing and industrialized countries,[1] and recent studies have also demonstrated that breastfeeding is associated with a higher intelligence quotient (IQ) and a lower prevalence of obesity, during both infancy and adolescence.[2,3] The effect of breastfeeding on the prevention of atopy and allergic symptoms in the paediatric population has not been well defined.[4] The WHO suggests that exclusive breastfeeding should be carried out for the first 6 months of life in all infants, in both developing and industrialized countries. In situations where a mother is unable to breastfeed, infant formula should provide a safe and nutritionally adequate human milk substitute. The incidence of breastfeeding is increasing considerably in the developed world countries:[5] for example, recent epidemiological research has demonstrated the rising

rate in Italy.[6] Nevertheless, the number of European mothers who breastfeed beyond 4 – 6 months is well below optimum levels.[5] In addition, Italian mothers often switch very rapidly during the first year of life to either modified or unmodified whole cows' milk.[7] Within this context, the first educational goal should be to explain the functional advantages of prolonged breast-feeding to mothers. If, for whatever reason, human milk becomes unavailable to an infant, then during the first 6 months of life a new-generation cows' milk formula that is closer to the gold standard of human milk should be used, followed by an appropriate follow-on formula.

Cows' milk formulae that are currently available should be evaluated to see whether they:

(i) Provide the best alternative to human milk for infants who cannot be breastfed during the first 6 months of life;
(ii) Can substitute for human milk with the aim of approaching the structural and functional effects observed with breast-feeding.

At present, the nutrient composition and daily variability of human milk cannot be reproduced in formulae feeds. The origins of the main nutrients (e.g. proteins, lipids and carbohydrates), which are derived from non-human milk sources and biosynthetic processes, dictate that the quantities and the proportions of nutrients found in cows' milk formulae do not exactly reflect those present in human milk.[8] As a consequence, the protein and lipid intakes differ between breastfeeding and formula feeding,[8,9] and bacterial intestinal flora – which is considered the best biomarker for dietetic carbohydrate concentrations – is very different in breastfed and formula-fed infants.[10] The absorption and bioavailability of the main micronutrients (e.g. calcium and iron) seem to depend on the macronutrient composition of infant formulae.[11] Recent developments in infant formulae have therefore been targeted towards reproducing the functional effects, rather than the quantitative composition, of human milk. The term 'functional nutrient' has become part of the scientific language and several new nutrients have been added to (or are being studied with a view to being added to) infant formulae. These include:

(i) Long-chain polyunsaturated fatty acids (LCPUFA), for cerebral tissue composition and for neurological development;
(ii) Probiotics and prebiotics, for intestinal bacterial flora and local immune defence at a gastrointestinal level;
(iii) Nucleotides, to promote the immune response;
(iv) Possible quantitative and qualitative protein composition modifications, to balance the plasma amino acid profile (this is particularly significant for neuro-transmitter function in the early stages of cerebral development). Reducing the protein intake in the early years of life is recognized as an important factor for the prevention of excessive weight gain and obesity;
(v) Hydrolysed protein introduction for the prevention of atopy.[12]

These nutritional developments have enabled dietetic product manufacturers to conduct research, through which they have established the optimum proportions of different functional nutrients in infant formulae. Consequently, new-generation cows' milk formulae can provide functionally positive dynamic effects when selected ingredients are added to different types of milk. This is in contrast to earlier-generation infant formulae in which the balance of nutrients was undertaken in a purely 'quantitative' manner. In practice, the

concept that infant formula is a substitute for cows' milk has been replaced by a new concept which suggests that infant formula is a real alternative to human milk, when this is not available to the infant.

Points to be considered when evaluating infant formulae

Nutrients added to infant formulae may have purely nutritional functions or they may have defence, immune, pro-anabolic and neurotrophic roles, so serving as functional compounds. Therefore, during the evaluation of the nutritional identity of different cows' milk formulae our attention will focus on these components:

(i) Protein, glucid and lipid quantity and quality;
(ii) Immunoregulatory factors including indigestible carbohydrates (prebiotics), acidifying molecules, lactic bacteria (probiotics) and nucleotides.

PROTEIN QUANTITY AND QUALITY

Compared with breastfed infants, formula-fed infants consume 65 – 70% more protein.[13] A different plasma amino acid profile has been associated with formula-fed infants[13,14] together with a different glucose-insulin axis.[15,16] A lower rate of body mass growth in the 6 – 12-month period seems to be associated with breastfeeding compared with formula feeding, although the differences disappear in the second year of life.[9,17,18] Breastfed babies have a lower prevalence of excess weight and obesity in infancy, which varies in a manner that is dependent upon the duration of breastfeeding.[19] The 'protein hypothesis' represents an interesting biological explanation for the development of excessive weight gain and obesity, and an ongoing European trial aims to verify this point.[20] As a consequence of these findings, the ideal composition of any infant formula that aims to be a breast-milk substitute should result in a similar plasma amino acid profile. Many studies have shown that formula-fed infants have significantly higher plasma levels of numerous amino acids and urea than breastfed infants.[13] Casein-predominant formula (whey:casein ratio of 18:82) produces higher plasma levels of tyrosine and phenylalanine and lower plasma levels of tryptophan compared with whey-predominant formula (whey:casein ratio of 60:40);[14] while hyperthreoninaemia is a well-known phenomenon in infants receiving a whey-predominant formula.[13] Picone *et al.*[21] have demonstrated that a milk formula with a whey:casein ratio of 50:50 produces an amino acid profile that is closer to that of human milk compared with the more commonly used formulae containing whey: casein ratios of 60:40 or 20:80.[13] Despite feeding infants with a cows' milk formula containing a reduced total quantitative protein level and a whey:casein ratio of 50:50, it has still not been possible to produce a plasma amino acid profile that can be superimposed on to that of the breastfed infant.[21] The proteins in infant formulae are not species-specific; consequently, they cannot be reduced below certain limits as the levels of some essential amino acids, in particular tryptophan, could fall below essential levels.

At present, two approaches can be used to reduce the protein concentration of infant formula without altering the plasma amino acid profile:

(i) Removal of the whey protein glycomacropeptide, with a consequent reduction in the plasma threonine concentration (which is usually increased in infants fed with a whey-predominant formula);[22]
(ii) Formula enrichment with α-lactoalbumin, a tryptophan-rich protein which limits the amount of other amino acids

in a low-protein infant formula.[23] In the neonate, tryptophan and its metabolites are essential for optimal cerebral development, including the correct development of the hunger, satiety and sleep-wake rhythm regulation systems.[23] Human milk contains elevated concentrations of tryptophan, the precursor of serotonin and melatonin, compared with other neutral amino acids, and tryptophan transport across the blood–brain barrier is optimal.[23] In contrast, cows' milk formula provides lower tryptophan levels with higher concentrations of neutral amino acids. This seems to be associated with the lower concentrations of α-lactoalbumin in cows' milk compared with human milk. Infants fed with a low-protein infant formulae enriched with α-lactoalbumin had serum amino acid profiles that were more similar to those of breastfed infants.[24] New methods of fractionating whey protein, which remove glycomacropeptide that is rich in threonine and poor in tryptophan, and increase the tryptophan-rich α-lactoalbumin proportion, may allow formula-fed infants to achieve a plasma amino acid profile similar to that of breastfed infants.

PROBIOTICS AND PREBIOTICS

Probiotics (micro-organisms that have beneficial effects on the host) and prebiotics (indigestible probiotic substrates) have become familiar terms in the clinical and scientific communities. Probiotics and prebiotics are mainly recognized for their ability to modulate the intestinal flora. Whether probiotics – either ingested directly or stimulated by the ingestion of prebiotics – act independently on the immune system by local or systemic means, and the mechanisms involved in such actions, are not fully understood. Bifidobacteria and *Lactobacillus* represent the main beneficial strains of organisms that are used in probiotic supplementation, but the optimum quality and strains of probiotics are not absolutely certain. Documented evidence of the positive effects on the host organism exists for *L. rhamnosus GG*, *L. reuteri*, *L. acidophilus*, *Bifidobacterium* species and *Saccharomyces boulardi*.[25] Their probiotic biological activities might be explained by their adhesion to enterocytes, which could potentially inhibit the attachment of enteropathogenic strains of bacteria. The production of bacteriocines and short-chain fatty acids (mainly butyric acid) could also inhibit replication of pathogenic bacteria.[25]

Probiotics have been employed with success in the therapy and prevention of some forms of gastroenteritis.[25,26] A medium-term study has demonstrated that some probiotic strains of bacteria may be able to modify allergic inflammatory processes, and their effects continue after ingestion of the probiotic has stopped.[27]

Prebiotics, which mainly consist of indigestible oligosaccharides, are ingredients added to foods that can positively influence the host organism by stimulating the growth and/or activity of bacterial strains already present in the host organism, particularly in the colon. Prebiotics are used to modify microflora composition by stimulating the colonization of the gut by commensals that provide recognized benefits to the host organism. There is evidence that prebiotics increase the quantities of bifidobacteria and *Lactobacillus* species and decrease *Escherichia coli* and *Clostridia* species quantities in the gut.[28] On an industrial level, the main substances that can selectively influence and be advantageous to the intestinal microflora are lactulose, vegetable-derived

fructo-oligosaccharides (FOS) and synthetic galacto-oligosaccharides (GOS). Recent studies have evaluated the addition of prebiotic oligosaccharides consisting of 90% GOS and 10% FOS (up to 0.8 g/dl) to infant formula. This type of infant formula supplementation was associated with a dose-dependent elimination of bifidobacteria and *Lactobacillus* in the stools.[29,30] Prebiotics have also been associated with regulating frequency of evacuation and softer stool consistency.[29,30] All of the prebiotic-supplemented infant formulae were well tolerated and no side-effects have been linked to their use.[28] The ESPGHAN Committee on Nutrition has published two reports on the use of probiotics and prebiotics in dietetic products for infancy (Table 1).[28,31]

The use of a separate fermentation process for proteins is a third possible mechanism of infant formula supplementation. This produces active metabolites during milk fermentation that stimulate the host organism's defences in a way analogous to those observed with probiotic and prebiotic infant formula supplementation.[32,33]

LONG-CHAIN POLYUNSATURATED FATTY ACIDS FOR NEUROLOGICAL DEVELOPMENT

The observation that breastfed babies have better neurological development compared with formula-fed infants[34] has led to hypotheses suggesting a possible role for LCPUFA, particularly arachidonic acid (AA, 20:4n-6) and docosahexaenoic acid (DHA, 22:6n-3), which are derived from linoleic acid (LA, 18:2n-6) and α-linolenic acid (ALA, 18:3n-3), respectively (Fig. 1).

TABLE 1:
The ESPGHAN Committee on Nutrition: conclusions and recommendations on the inclusion of probiotics and prebiotics in infant dietetic products[28,31]

Data are still limited on the safety and long-term effects of probiotic preparations added to infant and follow-on formulae and dietetic products for infancy. Some data suggest a short-term benefit of some probiotic strains in neonates and small infants with infectious diarrhoea
Only use bacterial strains for which the identity and genetic stability have been demonstrated by culture and molecular methods
Probiotic-containing infant formulae should only be marketed after a careful evaluation of the benefits and safety. Fewer controls are needed for follow-on formulae because the infant has already been exposed to environmental organisms and has developed a mature immune response
The evidence that some probiotic-based preparations show relative benefits should be recognized on the basis of diarrhoea severity, effective prevention of diarrhoeal episodes, and short to medium-term preventive effects on atopic eczema
Available data on the use of prebiotics in infant dietetic products are still limited. General recommendations on the use of prebiotic molecules with preventive or therapeutic effects during infancy cannot therefore be given
Some prebiotics can increase the total number of bifidobacteria in the stools and they can make the stools 'soft'
Negative effects of the mixture of oligosaccharides used in dietetic products for infancy have not been documented
Future studies should define the type and dosage of oligosaccharides with a presumed prebiotic activity, optimal dosages and length of the administration, relative safety aspects of administration, and the potential short- and long-term effects

The hypothesis that LCPUFA play a role in the performance of the nervous system is supported by experimental, anatomo-pathological and clinical data.[35] In the 1980s, animal studies demonstrated a possible role for dietary LCPUFA in improving the neuro-physiological response, in particular the visual processes.[36] Several studies have demonstrated beneficial effects associated with ALA administration, which increases the availability of substrate for DHA synthesis.[37] A study of sudden infant death syndrome demonstrated that breastfed infants had a significantly greater amount of DHA in their cerebral cortex grey matter compared with formula-fed infants.[38] These findings have focused attention on the role of DHA in the early stages of neurodevelopment, and clinical studies are now evaluating the long-term effects of DHA on IQ.[39]

Even very low birth-weight infants can synthesize LCPUFA from their precursor molecules, but the fundamental problem is whether they can produce an adequate quantity of LCPUFA to allow optimum neurological development.[40] How the roles of AA and DHA in infancy are defined in terms of whether they are essential, conditionally essential or semi-essential fatty acids is not yet known, although this issue has been thoroughly investigated using meta-analyses.[41,42] Numerous organizations and international committees have considered the definition of LCPUFA function in infancy.[43] While the dietary supply of LCPUFA is always associated with a biochemical response (i.e. higher plasma levels of LCPUFA), doubts have been raised about the functional meaning of these increased levels in terms of their impact on neurological development. While a systematic literature analysis confirms the benefits of using dietary LCPUFA for premature infants, the situation in term

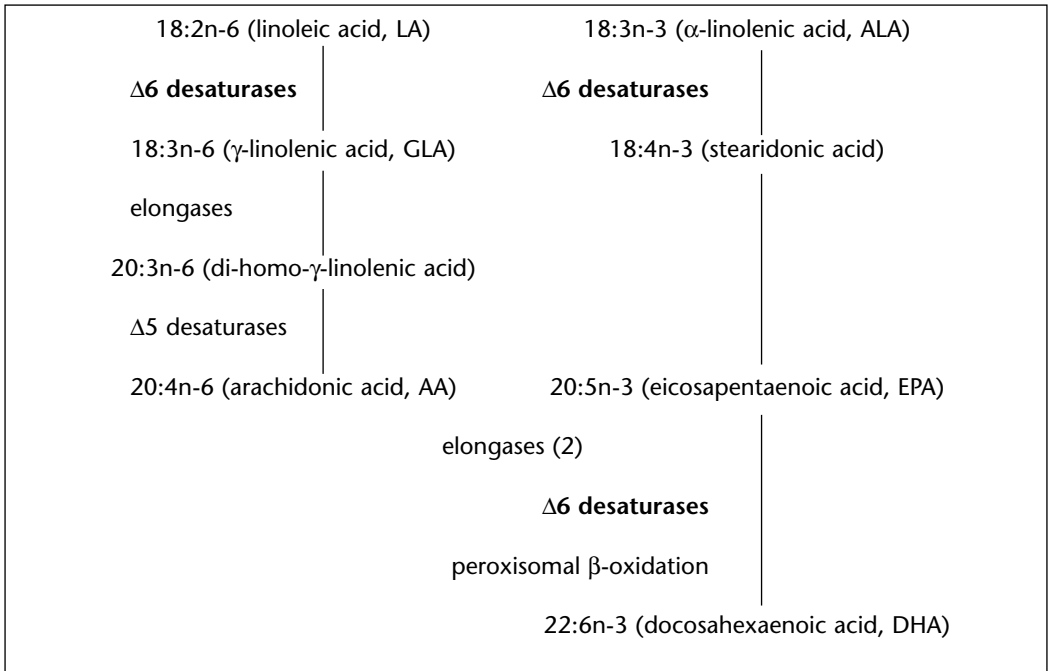

FIGURE 1: Synthesis of long-chain polyunsaturated fatty acids and their essential fatty acid precursors

infants remains inconclusive as there are several diverse interpretations of the available data, in addition to a lack of medium- and long-term data.[44] According to leading researchers, cows' milk formulae for term infants should contain at least 0.2% of the total fatty acids as DHA and 0.35% as AA, while cows' milk formulae for pre-term infants should contain at least 0.35% DHA and 0.40% AA.[43]

Systematic reviews and current recommendations confirm the lack of adverse effects associated with dietetic LCPUFA supplementation.[41 – 43] A meta-analysis of data from several clinical trials demonstrated that LCPUFA administration, either alone or in combination with AA, was not associated with reduced development of stature or weight parameters in term infants.[45]

NUCLEOTIDES

Human milk contains a much higher concentration of nucleotides than cows' milk.[46] Nucleotides can be extracted and added to infant formula, and their potential for stimulating the immune response has resulted in an ever-greater number of nucleotide-supplemented infant formulae becoming available.[46]

Nucleotides and their associated metabolites play key roles in numerous biological processes.[47] They can be synthesized by the organism and are therefore not considered essential nutrients, but some studies have demonstrated that nucleotides ingested as part of the diet can bring benefits; as a result their role in human nutrition has been defined as 'conditionally essential'.[47] These nutrients can become essential as soon as the endogenous levels become insufficient to sustain normal function, even if their absence from the diet does not constitute a classic malnutrition syndrome. Most of the dietary-ingested nucleotides are rapidly metabolized and excreted, although some are incorporated into the tissues, particularly in early life and during conditions of malnutrition.[47] In the case of limited nucleotide intake, rapid growth or some physiological states, dietary nucleotide intake can support *ex novo* synthesis and optimize the maturation of rapidly growing tissues, such as the gastrointestinal epithelium and immune system cells.[47] Breastfed infants receive nucleotides from human milk in the form of nucleic acids, nucleosides, nucleotides and associated metabolites.[48] Nucleotides ingested with the diet have been associated with an increase in the host defences at the gastrointestinal and immune system levels.[47] Infants who receive a cows' milk formula supplemented with nucleotides experienced a reduced risk of diarrhoeal episodes,[49] a greater antibody response following vaccination against *Haemophilus influenzae* type B,[50] and a greater natural killer cell activity compared with infants given a non-supplemented formula.[51]

The hypothesis that nucleotides are conditionally essential nutrients remains to be confirmed, in particular with regard to pre-term infants, intrauterine growth retardation, intestinal illness and states of malnutrition. As no negative effects have been reported for nucleotide-supplemented infant formulae, these compounds are considered safe to be used at concentrations similar to those found in human milk. Further studies are required to define the precise biological role of nucleotides in human milk, and to establish which benefits are associated with the nucleotide supplementation of infant formulae and at what concentrations they should be added.

PROTEIN HYDROLYSATES AND ALLERGY PREVENTION

At least temporarily, 30% of the paediatric population is estimated to be affected by atopic symptoms.[52] The intensity of allergic reactions, their prevalence, the discomfort caused by them and the costs involved mean that allergies are of major clinical interest; consequently, the modification of infant formulae and infant foods in order to make them less likely to be associated with the future development of allergic symptoms is an area that requires further study. The role of breastfeeding in the development of allergies remains controversial.[53,54] Breastfeeding for 4 – 6 months reduced allergic symptoms in high-risk infants (i.e. mother and/or father affected by allergy) compared with infants fed with a cows' milk formula, but recent findings indicate that the concentration of specific defence factors in human milk can affect the development of the allergic response.[55] Extensively hydrolysed proteins derived from cows' milk, in which most of the nitrogen is in the form of free amino acids and peptides < 1500 kDa, have been used in formulae for > 50 years for infants with cows' milk protein allergy.[56] The use of extensively hydrolysed proteins to prevent allergy development when human milk is unavailable remains difficult because of the palatability, costs and long-term nutritional effects of these formulae. In 1985, the first partially hydrolysed formula was introduced to the market as a less expensive and more palatable alternative to standard hydrolysed infant formulae, and one which might offer a preventive, rather than therapeutic, effect.[56] The European Union has accepted that infant formulae can be labelled 'hypoallergenic' (HA) if clinical studies have confirmed that a formula can prevent allergic symptoms. In 1998, a meta-analysis reviewed 15 prospective studies that measured the effect of feeding a partially hydrolysed formula for at least 3 months to infants at risk of allergy. The study looked at the development of allergic symptoms between 6 and 60 months of life[52] and showed that at 6 and 12 months of age, the proportion of infants fed with HA formula who developed allergic symptoms was one-third and one-quarter of the respective proportions of infants receiving standard cows' milk formula.[52] No significant differences were found when comparing the infants fed with HA formula and breastfed infants.[52] According to the recent German Nutrition Intervention Trial, breastfed, HA formula-fed, or extensively hydrolysed formula-fed high-risk infants showed lower incidences of allergic manifestations compared with high-risk infants fed with a standard infant formula.[57] In particular, allergic manifestations were particularly prevented by the use of extensively hydrolysed products, while atopic dermatitis was reduced by either type of hydrolysed formula.[57] A Swiss cohort study showed that feeding infants with HA formula for up to 4 months of life resulted in a normal growth pattern and an improvement in general health status compared with infants receiving a standard infant milk formula.[58]

From these studies there is a consensus view that the extensively hydrolysed infant formulae have less allergic potential when used as alternatives to human milk, but that greater nutritional safety has only been demonstrated by hydrolysed formulae that have a low degree of hydrolysis.[59,60]

A review of the composition of different infant formulae

This review will now discuss the composition of a variety of infant formulae that are available in Italy from several manufacturers.

Table 2 lists the composition of 27 infant formulae in detail.

PROTEIN

Quantity

The following innovations have been used in specific milks to overcome the potential problems associated with protein content, weight gain and obesity without leading to suboptimal amino acid levels (as discussed earlier in this article):

(i) Techniques that increase concentrations of α-lactoalbumin after its isolation from the casein band (formulae 2, 8, 8A and 9);
(ii) Isolation of glycomacropeptide, which results in a reduced threonine concentration: threonine is usually found in excess in whey-protein predominant formulae.

Partial protein hydrolysis

Infant formulae 3B and 8A contain hydrolysed proteins that could prevent both colic and the development of atopy.

LIPIDS

Absorption

Different amounts of β-palmitate, which is present in human milk, have been added to infant formulae 3B, 8A, 10, 10A and 13A, to ensure a greater absorption of lipids and prevent the precipitation of lipids in the form of calcium soaps. The amount of β-palmitate added to infant formulae should always be precisely stated as the percentage of the total palmitate content: this can be the source of important functional differences between infant formulae.

Quality

At present, all of the commercially available formulae contain LA and ALA at a ratio that has been modified to be ≤ 10:1, respectively. In some infant formulae (1, 3, 12, 12A, 13 and 13A), variable amounts of AA and DHA have been added. In formula 10A, γ-linolenic acid instead of AA, together with DHA, has been added.

CARBOHYDRATES

Lactose is the only carbohydrate present in formulae 6, 6A, 7, 11, 12 and 13A, although this is associated with greater fermentability than other carbohydrates. In all other infant formulae, lactose, maltodextrins, polysaccharides (digestible and indigestible) and/or starches have been added:

(i) Maltodextrins (digestible sugars that probably ensure less fermentability): formulae 1, 1A, 2, 2B, 4, 4A, 4B, 5, 7A, 8, 8A, 9, 10, 10A, 12A and 13;
(ii) Digestible polysaccharides (probably have an anti-fermentation effect): formulae 3B and 3C;
(iii) Starch or amylopectin (possibly have an anti-reflux effect): formulae 2A, 2B, 3B, 7A, 8A, 10A and 12A;
(iv) Indigestible polysaccharides (bifidogenic effects, prebiotics): formula 1 (GOS only), formulae 3, 3A and 3B (GOS:FOS ratio 9:1), formula 8A (GOS only), and formula 10 (FOS only). In formulae 4A and 4B indigestible polysaccharides were present as fermentation products.

BIOLOGICAL ACIDIFICATION

The acidification of formulae 4A, 4B and 8A, which aims to prevent local intestinal infections, is obtained using different procedures. In some formulae (2B and 7A), lactic bacteria have been included to stimulate the successive development of acidification.

NUCLEOTIDES

In light of the possible positive effect of nucleotides on the immune system, some

TABLE 2:
The composition of different infant milk formulae that are used as alternatives to human breast milk in Italy

Formula number	1	1A	2	2A	2B*	3	3A	3B	3C
Amounts of ingredients/100 ml									
Calories (kcal)	68	72	67	67	67	67	67	70	72
Protein (g)	1.7	1.4	1.2	1.7	1.6	1.4	1.4	1.7	1.7
Whey:casein ratio	60:40	50:50	70:30	30:70	50:50	60:40	60:40	partially hydrolysed whey protein	60:40
Lipids (g)	3.5	4.1	3.6	3.1	3.2	3.6	3.5	3.3†	3.6
LA/ALA (mg)	532/51	750/70	520/67	490/54	490/62	446/71	395/73	430/83	590/60
AA (mg)	15.0					12.0			
DHA (mg)	8.0					7.0			
Monounsaturates (g)						1.5	1.5	1.4	1.4
Oleic acid (g)									
Cholesterol (mg)						0.004			
Carbohydrates (g)	7.2	7.3	7.5	7.9	7.7	7.4	7.5	8.4	8.3
Lactose (g)	6.6	6.2	6.4	6.0	5.7	7.2	7.1	2.9	7.3
Maltodextrin (g)	0.4	1.1	1.0		0.8				
Glucose (g)	0.2								
Oligosaccharides (g)	0.5					0.4	0.4	0.8	
GOS:FOS ratio	GOS					9:1	9:1	9:1	
Polysaccharides (g)								4.6	1.0
Starch (g) or amylopectin (g)				1.9	1.2			1.5	
Nucleotides (mg)			3.1						
Carnitine (mg)			1.0					1.5	
Taurine (mg)	4.1	4.1	5.4	5.3	5.3	6.3	6.3	5.1	7.0

An explanation of terms and formulae in this table appears on page 607.

TABLE 2 (continued):
The composition of different infant milk formulae that are used as alternatives to human breast milk in Italy

Formula number	4	4A**	4B**	5	6	6A	7	7A*	8
Amounts of ingredients/100 ml									
Calories (kcal)	68	71	69	70	69	69	67	67	69
Protein (g)	1.5	1.5	1.8	1.6	1.4	1.5	1.7	1.6	1.3
Whey:casein ratio	60:40	60:40	20:80	60:40	60:40	60:40	50:50	50:50	50:50
Lipids (g)	3.7	3.5	3.2	3.6	3.7	3.9	3.6	3.2	3.7
LA/ALA (mg)	600/60	620/60	570/50	630/60	610/44	590/60	520/64	490/62	540/49
AA (mg)									
DHA (mg)									
Monounsaturates (g)						1.9			
Oleic acid (g)				1.3					
Cholesterol (mg)									
Carbohydrates (g)	7.1	8.3	8.3	7.9	7.5	6.8	6.9	7.7	7.7
Lactose (g)	6.0	6.4	5.3	6.6	7.5	6.8	6.9	5.7	6.2
Maltodextrin (g)	1.1	2.0	2.5	1.2				0.8	1.5
Glucose (g)									
Oligosaccharides (g)									
GOS:FOS ratio		GOS	GOS						
Polysaccharides (g)									
Starch (g) or amylopectin (g)								1.2	
Nucleotides (mg)		2.5		3.5					2.4
Carnitine (mg)				1.4			1.0	1.0	
Taurine (mg)	5.1	5.0	5.0		6.9	6.8	5.4	5.3	6.8

An explanation of terms and formulae in this table appears on page 607.

TABLE 2 (continued):
The composition of different infant milk formulae that are used as alternatives to human breast milk in Italy

Formula number	8A***	9††	10	10A	11	12	12A	13	13A
Amounts of ingredients/100 ml									
Calories (kcal)	69	68	66	66	68	68	68	71	71
Protein (g)	1.3	1.3	1.8	1.8	1.4	1.4	1.7	1.6	1.6
Whey:casein ratio	50:50 partially hydrolysed whey protein	52:48	60:40	60:40	60:40	60:40	60:40	60:40	60:40
Lipids (g)	3.7†	3.6	3.2†	3.2†	3.7	3.7	3.5	3.6	3.6†
LA/ALA (mg)	540/49	500/45	310/34	300/36	730/80	610/64	580/61	570/50	470/60
AA (mg)				15.3 (GLA)		23.0	23.0	9.1	9.1
DHA (mg)				16.7		11.5	12.0	5.5	5.5
Monounsaturates (g)									
Oleic acid (g)									
Cholesterol (mg)									
Carbohydrates (g)	7.7	7.7	7.5	8.2	7.3	7.0	7.6	8.2	8.2
Lactose (g)	4.7	5.4	3.8	4.4	7.3	7.0	4.6	7.1	8.2
Maltodextrin (g)	0.6	2.3	2.7	1.7			0.9	1.1	
Glucose (g)			0.7						
Oligosaccharides (g)	0.7		0.3						
GOS:FOS ratio	GOS		FOS						
Polysaccharides (g)									
Starch (g) or amylopectin (g)	1.7			2.1			2.1		
Nucleotides (mg)	2.4	2.4	2.8		1.0	2.6		2.7	
Carnitine (mg)			1.3	1.3				2.4	2.4
Taurine (mg)	6.8	6.8	5.1	5.1	4.5	4.1	4.1	4.5	4.5

An explanation of terms and formulae in this table appears on page 607.

Explanation of terms and formulae used in Table 2, which describes the composition of different infant milk formulae that are used as alternatives to human breast milk in Italy

*Enriched with *Bifidobacterium lactis*, fermented with *Lactobacillus helveticus* and *Streptococcus thermophilus*.
**Fermented with *Streptococcus thermophilus* and *Bifidobacterium breve*.
***Pre-treated acidified milk with *Streptococcus thermophilus*.
†β-palmitate.
††Ingredients from biological agriculture.

LA, linoleic acid; ALA, α-linolenic acid; AA, arachidonic acid; DHA, docosahexaenoic acid; GLA, γ-linolenic acid; GOS, galacto-oligosaccharides; FOS, fructo-oligosaccharides.

Formula 1 = Humana 1 (Humana); formula 1A = Humana Plus (Humana); formula 2 = Nidina 1PE (Nestlé); formula 2A = Nidina Confort 1 (Nestlé); formula 2B = Pelargon 1* (Nestlé); formula 3 = Aptamil 1 (Milupa-Numico); formula 3A = Nutrilon 1 (Nutricia-Numico); formula 3B = Conformil 1 (Milupa-Numico); formula 3C = Milumil 1 (Milupa-Numico); formula 4 = Mellin1 (Mellin); formula 4A = Mellin 1 Progress** (Mellin); formula 4B = Pantolac 1** (Mellin); formula 5 = Bebilac 1 (Sicura srl); formula 6 = Miltina 1 (Milte); formula 6A = Bio-miltina 1 (Milte); formula 7 = Nativa 1 (Guigoz); formula 7A = Nativa 1 Bifidus* (Guigoz); formula 8 = Plasmon 1 (Plasmon); formula 8A = Lenilac 1*** (Plasmon); formula 9 = Vivena 1†† (Dieterba); formula 10 = Formulat 1 (Dicofarm); formula 10A = Formulat 1 Pregel (Dicofarm); formula 11 = Similac Formula Plus 1 (Abbott); formula 12 = Enfamil Premium 1 (Mead Johnson); formula 12A = Enfamil Pregel Lipil 1 (Mead Johnson); formula 13 = Blemil Plus forte 1 (Ordesa); formula 13A = Blemil Plus AS 1 (Ordesa).

infant formulae have been supplemented with nucleotides at doses below those found in human milk (formulae 2, 4A, 5, 8, 8A, 9, 10, 11, 12 and 13).

Do infant formulae with bioactive nutrients work?

Considerable research by Italian groups over the past 10 years has evaluated the possible effects of functional nutrients added to infant formulae. Most studies have considered the neurodevelopmental and metabolic effects of LCPUFA,[61 – 66] and the effects of prebiotics and probiotics on intestinal flora, bowel habits and infections (such as necrotizing enterocolitis in infants).[67 – 70] Adding LCPUFA to infant formulae has increased neurodevelopmental performance to levels closer to those achieved in breastfed infants.[61,71] Prebiotics have produced intestinal effects similar to those observed with breastfeeding.[28] The addition of probiotics has not produced favourable effects, however.[31] In general, the considerable efforts made by infant formula and food manufacturers have greatly improved the diets of those who, for several reasons, have not been fed with their mother's own breast milk.

Conclusion

The different types of infant formulae now available aim to provide nutritional alternatives for infants who are unable to receive breast milk. Infant formulae aim to produce the same structural and functional nutritional effects observed in breastfed infants, which are particularly important during the early months of life when human milk should be the only nutrient source. Long-term follow-up data are required for infants fed with the new types of infant formulae. Such data will enable us to assess their possible effects on neurobehavioural performance, prevention of excess weight gain and obesity, and the development of immunoallergic symptoms beyond the favourable findings that have come from short-term studies.

In addition to the qualitative improvement in infant formula composition which aims to provide nutrients of measurable functional value to formula-fed infants, other new

molecules are being studied and characterized with regard to their functional value as infant formulae components, particularly in formulae designed for premature infants. Such molecules include lipases stimulated by biliary salts, insulin, antigenic mixtures that have a 'tolerogenic' aim, and growth factors (e.g. epidermal growth factor). These molecules, when added to infant formulae, could produce formulae with specific functions that would be useful in individual situations, such as infants with faltering growth or those who need allergy prevention and/or therapy. We believe that soon we will be able to identify infant formulae that are tailored to suit individual infants who are unable to receive the best food possible; namely, his or her own mother's milk.

Conflicts of interest

No conflicts of interest were declared in relation to this article.

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