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Whey Predominant, Whey Modified Infant Formula with Protein/energy Ratio of 1.8 g/100 kcal: Adequate and Safe for Term Infants From Birth to Four Months

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ABSTRACT
Background: Protein quality of breast milk is superior to that of formula proteins. To ensure that the protein intake is sufficient, starter formulas with conventional protein composition provide a protein/energy ratio of 2.2–2.5 g per 100 kcal to infants, which is much higher than that supplied with breast milk. Several studies have shown that formula- fed infants have higher plasma or serum urea concentrations than breast-fed infants do. We tested if feeding formulas with improved protein quality and a protein content corresponding to the minimum level that is consistent with international recommendations (1.8 g/100 kcal) allows patients to achieve normal growth and plasma urea concentrations.
Methods: Healthy term infants were enrolled into the study and were either breast-fed or randomly assigned to three formula-fed groups. Formula-fed infants received either a standard formula with a protein/energy ratio of 2.2 g/100 kcal, whereas the two other groups received formulas with a protein/energy ratio of 1.8g/100 kcal differing mainly by their source of protein. Subjects received breast milk or these formulas ad libitum as the sole source of energy from birth to four months of age in a controlled blind design (except for the breast-fed group). Anthropometric measurements (body weight and length) were obtained at birth, at 30, 60, 90, and 120 days. Energy and protein intakes were calculated from three-day dietary records. Blood was collected for biochemical measurements at 30, 60, and 120 days.
Results: No differences were found between the four feeding groups for weight- and length-gains or for body mass indices (BMI). No differences in energy intakes between the formula-fed groups could be found, whereas protein intakes were less in infants fed the 1.8 g/100 kcal formulas. Plasma urea levels of the infants fed the 1.8 g/100 kcal formulas were closer to those found in the breast-fed infants.
Conclusion: Improvement of the amino acid profile permits a whey predominant starter formula with 1.8 g protein per 100 kcal to meet the needs of normal term infants during the first four months of life. ***JPGN 35:275–281, 2002.*** **Key Words:** Infant formula—Protein/energy ratio—Protein requirement.

INTRODUCTION

Human milk is the feeding standard for term infants for the first four to six months of life (1). The adequacy of human milk substitutes is usually ascertained by comparing the growth of infants fed such products with that of breast-fed infants during the same age interval. Recently, attention has been given to the relatively low concentration of nutritionally available protein in mature human milk as compared with the much higher content in conventional infant starter formulas (2).

Protein requirement during the first six months of life has been estimated using breast-fed term infants as a model. The Joint FAO/WHO/UNU Expert Committee Report of 1985 (3) has given intakes of 2.46, 1.93, 1.74, and 1.46 g/kg/day respectively during the monthly intervals from birth to four months. Dewey et al. (4) in a revised estimate from 1996 has presented somewhat lower intakes. The difference in the figures by the two authorities are due to differences in the estimation of the bioavailability of nitrogen from human milk and the difference in growth of breast- and formula-fed infants.

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The United States Food and Drug Administration (FDA) (5) specifies the lower limit of protein in infants formula to be 1.8 g/100 kcal and this is also the lower limit recommended by the European Society for Pediatric Gastroenterology and Nutrition (ESPGAN) (6), by the Codex Alimentarius (7), and by the Committee on Nutrition of the American Academy of Pediatrics (1). Fomon (8) has recommended a minimum level of protein in infant formulas of 2.2 g/100 kcal for infants less than 3 months and a content of 1.6 g/100 kcal for infants over 3 months. This recommendation is very similar to the recommendation of Beaton and Chery (9) of 1.7 g/100 kcal for infants aged 3 to 4 months. Such formula would supply a mean intake of protein of 1.75 g/kg/day and thus be within the safe level of intake (3). Recently, however, Fomon et al. (10) have found that infants fed a casein predominant formula with a protein/energy ratio of 1.7 g/100 kcal receive adequate intakes of protein. However, the authors questioned the safety of such a protein/energy ratio, because ad libitum feeding of such formula may lead to a compensatory increase in food and energy intakes resulting in excessive weight gain and body mass index (10).

The aim of the current prospective, randomized, and blind study was to evaluate the nutritional adequacy and safety of two experimental formulas with 1.8 g protein per 100 kcal, a ratio that is consistent with international recommendations (5–8).

MATERIAL AND METHODS

Subjects

This controlled, blind, parallel, and prospective feeding study enlisted two cohorts of term infants, breast-fed and formula-fed. The study was conducted in the Clinica Ostetrica Ginecologia B dell'Universita di Palermo, Palermo, and in the Maternity Hospital Macedonio Melloni, Milan. All infants studied fulfilled the following inclusion criteria: healthy newborn girls and boys, $\ngeq$37 weeks and $\leqslant$42 weeks gestation with a birth weight $\ngeq$2500 g and $\leqslant$4500 g. Infants with major deformities and/or illness including cardiovascular, gastrointestinal, renal, neurological, or metabolic diseases were excluded. Parents were instructed to exclusively breastfeed or feed the assigned formula up to 120 days of age. In addition, smoking was assessed for mother and in the household separately, which was then combined to create a smoke exposure score: mother + household = 3, mother alone = 2, household alone = 1 and no smoking = 0.

Ethical Considerations

Local institutional ethical committee approval was obtained at both study sites and standards of good clinical practice were followed. The study objectives and design were explained to one of the parent or guardian, including all aspects related to safety. A consent form was signed before any inclusion in the study and parents were informed that they could withdraw their child from the study at any time during the study without any consequences on the quality of the medical care to be provided.

Feedings

The formulas were prepared by Nestec, as powder and the containers marked with color-coded labels. Three isocaloric formulas differing by their protein source and content were studied and compared with breast milk. A conventional whey adapted starter formula with a whey/casein ratio of 60/40 and a protein content of 2.2 g/100 kcal (NAN®) was compared with two experimental formulas with a whey/casein ratio of 70/30 and a protein content of 1.8 g/ 100 kcal. The measured nitrogen concentrations of the formulas were 2366 mg/L, 1985 mg/L and 1974 mg/L for F2.2, F1.8 MSW (Modified Sweet Whey) and F1.8 AW (Acid Whey), respectively. The protein (nitrogen x 6.38) concentration was calculated to be 15.1 g/L (2.3 g/100 kcal) for F2.2 and 12.6 g/L (1.9 g/100 kcal) in the two other formulas. The non-protein nitrogen (NPN) concentration was 10% in the three formulas and when assuming that α-amino nitrogen comprises 40% of the NPN (11), the "true" protein/energy ratio was equivalent to 2.2 g/100 kcal in F-2.2 and 1.8 g/100 kcal in both F-1.8 MSW and F-1.8 AW. To maintain isocaloric feedings, the lactose concentration was increased in the reduced protein formulas. The caloric density of the formulas were targeted at 670 kcal/L and the measured values were 656 kcal/L in F-2.2, 663 kcal/L in F-1.8MSW, and 659.1 kcal/L in F-1.8 AW, as calculated from lipid, protein, and carbohydrate measurements.

The amino acid profiles of the formulas, as measured by standard methods, and that of breast milk adapted from Nayman et al. (12) are given in Table 1. The modification of the protein level and the choice of a 70/30 whey to casein ratio resulted in a decreased concentration of threonine to levels found in breast milk. In the F1.8 AW formula, it was necessary to add free tryptophan to allow for levels comparable to those

TABLE 1. *Amino acid composition of the formulas (g amino acyl/16g N)*

Amino acid	Breast milk	F-2.2	F-1.8 MSW	F-1.8 AW
Aspartic acid	9.0	8.9	9.4	8.9
Threonine	4.6	5.4	4.6	5
Serine	4.6	5.2	4.6	4.7
Glutamic acid	19.9	19.3	17.4	17.2
Proline	9.0	7.8	6.2	6.4
Glycine	2.2	1.9	1.9	1.8
Alanine	3.7	4.0	4.1	4.1
Cystine	2.4	2.0	2.4	1.7
Valine	5.7	5.9	5.4	5.3
Methionine	1.5	2.3	2.4	2.4
Isoleucine	5.4	5.6	5.0	5.0
Leucine	10.3	10.2	10.4	10.8
Tyrosine	4.2	3.2	3.3	3.8
Phenylalanine	3.9	4.0	4.0	3.9
Lysine	6.5	7.5	8.1	8.0
Histidine	2.2	2.3	2.5	2.5
Arginine	3.1	2.9	4.3	4.3
Tryptophan[1]	2.0	1.7	2.0	2.1

Values for breast milk are from ref. 12.

[1] Formula 1.8 AW contained added free tryptophan, whereas formula 1.8 MSW did not.

found in breast milk. In contrast, the F 1.8 MSW formula contained a source of whey protein, modified sweet whey, sufficiently rich in tryptophan to avoid addition of this amino acid in the free form. This was achieved by using a newly patented fractionation process of the whey proteins allowing for the removal of caseino-glyco-macropeptide, a fraction rich in threonin and poor in tryptophan, thereby increasing the α-lactalbumin proportion, a fraction rich in tryptophan (patent: WO 01/22837 A1).

Design

The enrolled infants were either breast-fed or exclusively fed one of three formulas until 120 days of age. Infants who stopped breast-feeding before 28 days of age were randomly assigned to receiving one of the study formulas. Infants in the control group were to be exclusively breast-fed from birth to at least 4 months of age (120 ± 4 days). All subjects in the formula-fed groups were to start formula feeding before 28 days of age, and were then to be fed exclusively their assigned formula to at least 4 months of age. Start of formula feeding in groups F2.2, F1.8 MSW, and F 1.8 AW was 7.2 ± 6.7, 5.3 ± 3.9 and 5.3 ± 4.2, respectively (Table 2). Assignment to one of the three formula groups was randomized by center using a computer-generated randomization table. The study was conducted in a controlled blind design (except for the breast-fed group). Study visits after enrollment took place at 30 (± 2) days, 60 (± 3) days, 90 (± 3) days and 120 (± 4) days of age. Infants and family information was collected at enrollment.

Anthropometrics and Dietary Assessment

Anthropometric measurements (weight and length) were obtained at birth, at enrollment and at 30, 60, 90, and 120 days. An infant measuring board with a built-in millimeter ruler was used to record length and a digital scale accurate to ± 1 g was used to measure weight. Weight and length gains, as well as body mass index (BMI), calculated in the conventional manner as weight in kg over length in meters squared were thus obtained. Results were compared with the recently published Euro-Growth references which provide longitudinal data for term infants between 0 to 4 months of age (13). Dietary assessment was conducted using dietary logbooks. All parents were instructed by the study physicians to complete a dietary logbook and record all formula consumption in ml for three days before the study visits at 30, 60, 90, and 120 days. Formula intakes were calculated per kg body weight and averaged over the three days before the visits. Daily protein and energy intakes were then derived from the volumes consumed and the analyzed values for protein and energy, based on instruction for formula reconstitution (129g powder/l).

Blood Collection and Biochemical Methods

Blood samples for biochemical measures were collected at 30, 60, and 120 days of age during hospital visits, only with parental consent. Blood was collected using a butterfly tubing apparatus. Blood was collected into lithium heparinase sample tubes connected to butterfly needles (Sarstedt, Sevelen, Switzerland). The tubes were centrifuged at 4°C. Electrolytes, iron status, glucose, cholesterol, plasma urea, and albumin were measured by routine laboratory methods on a BM/Hitachi 917 Analyzer (Roche/Boehringer, Rotkreuz, Switzerland). Urea was assayed by the urease-GLDH method using urea SYS reagents (Roche Nr. 1729691) and albumin with the bromcresol green method using the Albumin Plus kit (Roche Nr 1970909) with Roche calibrators (Nr. 759350).

Statistical Analysis

The primary outcome in this study was the increment in anthropometrics parameters from 30 up to 120 days of age (unit/month). In each group 28 subjects were to complete the study protocol, based on the data of Nelson as reported in the AAP/FDA contract (14). Mutual comparison of the three formula-fed groups for increments included a two-way analysis of variance (ANOVA), correcting for sex differences. Differences between the three formula-fed groups and the breast-fed group were evaluated by analysis of covariance with sex, smoke exposure, and mother's year of education as covariates. Daily intakes in protein and energy from the three formula-fed groups were compared using one-way ANOVA after log transformation. Analysis of variance was applied for biochemical parameters. Bonferroni correction for multiple testing was used in all analyses.

RESULTS

One hundred and thirteen infants completed the study (78%) of the 144 infants who were recruited to participate into the study: 28 in the breast-fed group, 29 in the F-2.2 formula group, 29 in the F-1.8 MSW, and 27 in the F-1.8 AW formula groups. The drop-out rate was 22%: 15 parental/physician withdrawals (5 breast-fed, 1 F-2.2, 4 F-1.8 MSW, and 5 F-1.8 AW), 8 non-compliant to the

TABLE 2. *Baseline characteristics (mean ± SD)*

Feeding group	Breast milk	F-2.2	F-1.8 MSW	F-1.8 AW
n	28	29	29	27
Gestational age (wks)	39.1 ± 1.0	39.0 ± 1.1	39.1 ± 1.4	39.3 ± 1.2
Weight at birth (kg)	3.40 ± 0.37	3.28 ± 0.43	3.20 ± 0.30	3.21 ± 0.39
Length at birth (cm)	49.5 ± 1.7	49.2 ± 1.9	48.9 ± 1.6	48.9 ± 1.9
BMI at birth (kg/m^2)	13.9 ± 1.1	13.5 ± 0.9	13.4 ± 1.1	13.4 ± 1.0
Mother's education (y)	11.4 ± 3.9	9.0 ± 2.9	7.7 ± 2.8	10.7 ± 3.8
Smoke exposure (points)	0.7 ± 1.0	0.9 ± 1.1	1.2 ± 1.2	0.9 ± 1.0
Males/females (%/%)	61/39	38/62	5/49	67/32
Start formula feeding (d)	—	7.2 ± 6.7	5.3 ± 3.9	5.3 ± 4.2

diet (6 breast-fed, 1 F-2.2, and 1 F-1.8 AW), 4 failure to complete visits (2 F-2.2, 2 F-1.8) and 4 inclusion criteria not fulfilled (1 breast-fed, 1 F-2.2, and 2F-1.8AW).

Baseline characteristics of the subjects did not differ between groups (Table 2). Subjects in the formula-fed groups started formula feeding at about 6 days of age, and were previously fed either breast milk or a non-study formula. All anthropometric parameters including birth weight and length were somewhat higher in the breast-fed group. This may be explained by differences in life-style between mothers who choose to breastfeed and the others. In addition, breastfeeding mothers had a higher education and their infants were less exposed to cigarette smoking. Since these two variables are associated with both the treatment and the outcome parameter, they may act as cofounders. Data was therefore corrected for smoke exposure and maternal education in an analysis of covariance, only when the breast-fed group is compared to the formula fed groups. The gender distribution across the four groups was not homogenous, a difference taken into account by using z-scores based on the Eurogrowth study or by entering gender as a covariate in the analysis of covariance.

Weight and Length

The primary outcome of the study, growth from 30 to 120 days, is reported in Table 3. There were no differences between the three formula-fed groups for length and weight gains as expressed per unit/day after sex correction. Furthermore, the formula-fed groups did not differ significantly with the breast-fed group for weight and length gains, as found by analysis of covariance using sex, smoke exposure, and maternal education as covariates and after Bonferroni correction for multiple testing.

Weight and length gains were also comparable among the four groups at all time intervals studied. When compared with the Euro-Growth reference data (13), there was also no deviation in the weight for age and length for age changes in the four study groups (Table 4). As reported in Table 5, this resulted in body mass indices comparable among the four groups at all study visits.

Energy and Protein Intakes

The mean daily consumption of formulas at 30 days of age were 181.7 ± 33.2 ml/kg, 183.3 ± 35.4 ml/kg, and 169.5 ± 31.0 ml/kg for formula F-2.2, F-1.8 MSW and F-1.8 AW, respectively (Table 6). At 120 days formula consumption had decreased to 132.8 ± 22.4, 132.9 ± 34.8 and 135.9 ± 22.9 ml/kg in the three groups respectively. Consequently, daily energy intakes per kg body weight also declined from 30 to 120 days of age. There were no significant differences in the daily consumption of formula and energy intakes between the three formula groups, taking into account Bonferroni correction for the multiple testing due to the four visits (Table 6).

As expected, protein intake was significantly higher in the infants fed the formula F-2.2 as compared with those fed the 1.8 g protein/100 kcal formulas F-1.8 MSW and F-1.8AW at all the above-mentioned ages (Table 6).

Plasma Concentrations of Albumin and Urea

Plasma albumin and urea concentrations at 30, 60, and 120 days are presented in Table 7. The albumin concentrations were all within normal range in all feeding groups and there were only small differences between the groups at any of the sampling times.

However, the plasma urea concentrations differed significantly between the feeding groups as would be expected because of the differences in protein intakes between the groups. At 30 days infants fed F-2.2 had significantly higher plasma urea concentrations when compared with both the breast-fed and the formula F-1.8 fed group ($P < 0.001$). The time delay between the end of

TABLE 3. *Weight and length gains (mean ± SD)*

Age interval	Breast-fed n = 28	F-2.2 n = 29	F-1.8 MSW n = 29	F-1.8 AW n = 27	P (ANOVA)
Weight gains (g/d)					
30–120 days	24.77 ± 5.17	27.80 ± 4.70	28.06 ± 6.55	25.06 ± 6.35	NS
Birth–30 days	27.7 ± 9.5	30.9 ± 8.0	25.7 ± 8.5	26.4 ± 8.3	NS
Birth–60 days	30.1 ± 6.7	32.8 ± 6.7	28.8 ± 8.0	28.76 ± 6.7	NS
Birth–90 days	27.8 ± 5.3	30.8 ± 56	28.7 ± 6.5	27.1 ± 5.6	NS
Birth–120 days	25.4 ± 4.6	28.6 ± 4.4	27.5 ± 5.8	25.5 ± 4.8	NS
Length gains (mm/d)					
30–120 days	0.97 ± 0.21	1.04 ± 0.18	0.96 ± 0.19	0.95 ± 0.15	NS
Birth–30 days	1.65 ± 0.50	1.57 ± 0.57	1.44 ± 0.53	1.55 ± 0.40	NS
Birth–60 days	1.35 ± 0.24	1.41 ± 0.25	1.28 ± 0.28	1.37 ± 0.23	NS
Birth–90 days	1.25 ± 0.17	1.26 ± 0.15	1.17 ± 0.27	1.19 ± 0.16	NS
Birth–120 days	1.13 ± 0.13	1.17 ± 0.17	1.08 ± 0.19	1.1 ± 0.13	NS

No statistically significant differences between any of the formula-fed group and the breast-fed group as found by analysis of covariance with sex, smoke exposure, maternal education as covariates and after Bonferroni correction for multiple testing ($\alpha = 0.05/3 = 0.017$).

TABLE 4. *Weight and length for age changes vs Eurogrowth Z-scores) (12)*

ΔWeight for age	Breast-fed n = 28	F-2.2 n = 29	F-1.8 MSW n = 29	F-1.8 AW n = 27	*P* (ANOVA)
30–60 days	0.04 ± 0.46	0.17 ± 0.48	0.06 ± 0.62	−0.03 ± 0.60	NS
30–60 days	−0.13 ± 0.65	0.14 ± 0.67	0.20 ± 0.72	−0.11 ± 0.78	NS
30–120 days	−0.30 ± 0.81	0.11 ± 0.66	0.27 ± 0.90	−0.14 ± 0.94	NS
ΔLength for age					
30–60 days	−0.18 ± 0.55	0.14 ± 0.74	−0.07 ± 0.60	0.00 ± 0.75	NS
30–90 days	−0.07 ± 0.87	0.12 ± 0.74	−0.08 ± 0.97	−0.25 ± 0.69	NS
30–120 days	−0.14 ± 1.02	0.22 ± 0.77	−0.14 ± 0.85	−0.17 ± 0.72	NS

the meal and the time of blood sampling did not differ between the formula groups.

DISCUSSION

Protein/energy ratio of mature human milk ranges from 1.3-1.8 g/100 kcal, whereas for standard commercialized formulas this ratio ranges from 2.2-2.5 g/100 kcal. International recommendations are that the minimum protein content of an infant formula should be 1.8 g/100 kcal. However, previous clinical studies on the adequacy and safety of such formulas from birth are conflicting (15). To evaluate the nutritional adequacy of two experimental formulas with a protein/energy ratio of 1.8g/100 kcal but differing by their protein fraction and the addition or not of free tryptophan, we compared healthy term infants fed these formulas with infants either breast-fed or fed a conventional starter formula (protein/energy ratio of 2.2 g protein/100 kcal) for growth and some indices of protein metabolism. It is generally assumed that the nutritional needs of an infant are met when the infant shows normal growth. Growth performance of healthy infants was shown by Fomon et al. (16) to be a very sensitive indicator of protein and amino acid adequacy in infants in addition to nitrogen balance studies. Ziegler et al. (17,18) recently reported the results of three-days nitrogen balance studies with one of the experimental formulas which were tested in this study (formula F 1.8 with modified sweet whey: 1.8 g protein/100kcal) and the formula with 2.2 g protein/100 kcal (NAN®). Nitrogen retention from the two formulas was the same (117 mg/kg/d). The percent of nitrogen retention from the formula with 1.8g protein/100kcal was higher (39.6%) than from the formula with 2.2g protein/100kcal (32.2%). Urinary nitrogen excretion was significantly lower when the formula with 1.8g protein/100kcal was fed ($P = 0.006$). Those formulas were thus shown to be equivalent in meeting the protein needs of healthy term infants.

In the present study, the growth of infants fed the two experimental formulas was adequate, as demonstrated by weight and length gains that were comparable to those found in the breast-fed and conventional formula-fed infants. The resulting body mass indices were also similar throughout the study in all feeding groups. The volumes of formula consumed and energy intakes at 30, 60, 90, and 120 days did not differ between the two experimental formula fed groups and the conventional formula fed group. The protein intakes, however, were significantly lower in the infants fed the experimental formulas at all times. Picone et al. (19) have estimated daily protein intakes of infants fed breast milk to be 2.1 ± 0.2 g/kg at 4 weeks, 1.7 ± 0.1 g/kg at 8 weeks and 1.5 ± 0.1 g/kg at 12 weeks. These intakes are slightly lower than the intakes we found in the infants fed the experimental formulas having a protein /energy ratio of 1.8 g/100kcal. It can thus be anticipated that the infants fed these formulas did not have protein intakes, which were less than those of breast-fed infants. This finding differs significantly from that reported by Fomon et al. (10) in which infants fed a formula with a protein/energy ratio of 1.7 g/100 kcal consumed significantly more formula, and thus also energy, than reference infants fed a conventional formula with higher protein/energy ratio. This increased formula consumption resulted in greater BMI suggesting an increased fat deposition in the infants fed the reduced protein formula (10). The authors speculated that perhaps the infants were compensating an inadequate protein/energy ratio of the formula by consuming more formula and thereby achieving an adequate protein intake. The authors conclude that a formula with a protein/energy ratio of 1.7 g/100 kcal may not be 'safe'.

TABLE 5. *Body mass index in kg/m^2 (mean ± SD)*

Age	Breast-fed n = 28	F-2.2 n = 29	F-1.8 MSW n = 29	F-1.8 AW n = 27	p (ANOVA)
30 days	14.26 ± 1.01	14.46 ± 1.14	14.01 ± 0.83	13.95 ± 0.85	NS
60 days	15.71 ± 1.17	15.74 ± 1.06	15.39 ± 1.31	15.14 ± 1.10	NS
90 days	16.03 ± 1.35	16.49 ± 1.28	16.39 ± 1.56	15.93 ± 1.26	NS
120 days	16.25 ± 1.31	16.78 ± 1.32	16.98 ± 1.39	16.23 ± 1.43	NS

TABLE 6. *Protein and energy intakes (mean ± SD)*

Age (days)	Feeding group	n	Volume ml/kg/d	*P* (ANOVA)	Protein intake g/kg/d	*P* (ANOVA)	Energy intake kcal/kg/d	*P* (ANOVA)
30	F-2.2	29	181.7 ± 33.2	NS	2.74 ± 0.50[a]	<0.001	119.2 ± 21.8	NS
	F-1.8 MSW	27	183.3 ± 35.4		2.32 ± 0.45[b]		121.5 ± 23.5	
	F-1.8 AW	29	169.5 ± 31.0		2.13 ± 0.39[b]		111.7 ± 20.4	
60	F2.2	29	171.2 ± 37.3	NS	2.58 ± 0.56[a]	<0.001	112.3 ± 24.5	NS
	F-1.8 MSW	27	150.7 ± 27.3		1.91 ± 0.35[b]		99.9 ± 18.1	
	F-1.8 AW	29	149.2 ± 31.6		1.88 ± 0.40[b]		98.3 ± 20.8	
90	F-2.2	29	147.3 ± 36.2	NS	2.22 ± 0.55[a]	<0.001	96.6 ± 23.8	NS
	F-1.8 MSW	27	138.9 ± 26.8		1.76 ± 0.34[b]		92.1 ± 17.8	
	F-1.8 AW	29	140.2 ± 31.9		1.76 ± 0.40[b]		92.4 ± 21.0	
120	F-2.2	27	132.8 ± 22.4	NS	2.00 ± 0.34[a]	<0.001	87.1 ± 14.7	NS
	F-1.8 MSW	27	132.9 ± 34.8		1.68 ± 0.44[b]		88.1 ± 23.1	
	F-1.8 AW	28	135.9 ± 22.9		1.71 ± 0.29[b]		89.5 ± 15.1	

Within each cohort values with different superscripts are statistically different, after Bonferroni correction for multiple testing ($\alpha = 0.05/4 = 0.0125$).

In this study, the formula investigated was a casein predominant formula with unmodified bovine whey and it is thus possible that some of the essential amino acids might have been slightly inadequate in the protein reduced formula. In the present study, the experimental formulas tested had amino acid profiles that were very close to that of breast milk. This was made possible by the addition of free tryptophan in the formula containing acid whey F-1.8 AW. In contrast, formula F-1.8 MSW contained modified sweet whey that displays a high concentration of tryptophan as obtained by a newly patented fractionation process. A protein fraction rich in tryptophan might be a safer means of providing this amino acid in sufficient quantity without leading to an imbalance in plasma amino acid profiles of the infant.

Nutritional adequacy of the two experimental formulas was further confirmed by the plasma concentrations of albumin that were within normal range and did not indicate deficient protein intake in any of the feeding groups. Plasma concentrations of urea depend on one hand on the dietary protein intake and on the other hand on renal perfusion and urinary flow rate; plasma urea increases when the waste nitrogen increases, i.e., when nitrogen intake is higher than that needed for protein synthesis and growth. The utilization of dietary protein depends on amino acid composition and an imbalance will lead to more waste nitrogen. In the present study, plasma urea concentrations of infants fed the improved protein quality formulas were similar to those found in the breast-fed group. This reduces the solute load to the kidneys when compared to the infants fed the conventional formula with higher protein load. Since the total fluid intake (and thus urinary flow rate) did not differ between the groups, our results indicate that the waste nitrogen was lower and thus that the composition of the formulas containing 1.8 g protein/100 kcal is adequate.

Ever since the first infant formula was produced some 87 years ago, the general goal has been to improve formula quality to make it nutritionally and biologically as close to human milk as possible. Furthermore, it has recently been suggested that the breast-fed infant and its internal milieu and not breast milk as such, should be the norm for infant feeding during the critical early months of life when metabolic programming occurs. In the present study the protein-reduced and quality-improved formulations produce metabolic indices in the infant which are very similar to those found in breast-fed infants of the same age.

We conclude that an improved whey predominant formula with a protein/energy ratio of 1.8 g/100 kcal provides adequate intakes of protein from birth to four months without signs of compensatory increased food

TABLE 7. *Plasma albumin and urea*

Age	Breast-fed	F-2.2	F-1.8 MSW	F-1.8 AW	p (ANOVA)
Plasma albumin (g/l)					
30 days	45.1 ± 3.8[a]	42.4 ± 2.5[b]	42.9 ± 1.8[a]	44.0 ± 2.6[a]	$P < 0.05$
60 days	47.0 ± 2.7	46.9 ± 3.14	7.0 ± 3.24	5.8 ± 2.5	NS
120 days	50.6 ± 3.5	48.8 ± 4.04	8.4 ± 1.9	48.8 ± 3.4	NS
Plasma urea (mmol/l)					
30 days	2.97 ± 0.73[a]	3.59 ± 0.55[b]	2.71 ± 0.99[a]	2.35 ± 0.53[a]	$P < 0.001$
60 days	2.16 ± 0.40[a]	3.45 ± 0.76[b]	2.83 ± 0.71[b]	2.70[a] ± 0.61	$P < 0.001$
120 days	2.16 ± 0.82	2.88 ± 0.50	2.88 ± 0.51	2.75 ± 0.56	NS

Values with different superscripts are statistically different.

and energy intakes and that such formulas can be considered safe.

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