

Original Research

Determination of Macronutrients, by Chemical Analysis, of Home-Prepared Milk Feeding Bottles and their Contribution to the Energy and Protein Requirements of Infants from High and Low Socioeconomic Classes

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Key words: infant food, milk, food composition, nutritional requirements

Objectives: To determine the macronutrients composition of home-prepared milk feeding bottles, by chemical analysis, and assess their contribution to the energy and protein requirements of children under two years of age from high (HSE) and low (LSE) socioeconomic classes.

Methods: 72 samples were analyzed for energy density and protein, fat and carbohydrate content: 41 from the LSE group and 31 from the HSE group. The assessment of the percentages of the energy and protein requirements met by the consumption of the milk bottles was calculated as follows: the energy and protein per 100 mL obtained through chemical analysis were multiplied by the volume consumed at each feeding, then by the number of feedings per day, the results divided by the energy and protein requirements and multiplied by 100. Energy and protein requirements were those recommended by the FAO/WHO/UNU Committee and the Food and Nutrition Board. The children's weight-for-age index was assessed.

Results: Unmodified cow's milk was largely consumed by both groups. The addition of sugar and other ingredients to the milk was significantly higher in the LSE group. Moisture, protein and fat content were lower in the LSE group, whereas carbohydrate and energy content were higher. The percentages of energy and protein requirements provided by feeding bottles were higher in the LSE group. Children in the LSE group had lower z-scores for weight-for-age.

Conclusions: Differences in the preparation practices led to differences in the chemical results. The feeding bottles in the LSE group were high in energy, due to the addition of sugar and cereals to the milk in the bottle. The milk feeding bottles were an important weaning food providing more than 50% and 100% of the children's energy and protein requirements, respectively. The children's weight-for-age index was within the normal limits.

INTRODUCTION

The weaning process is a period of particular concern in developing countries because it is often accompanied by malnutrition, most commonly during the second semester of life when foods other than breast milk are added to the diet [1]. Malnutrition can be attributed to high rates of infection, insufficient energy and nutrient intake and poor dietary quality [2]. In developing countries, complementary foods can have energy densities as low as 25–30 Kcal/100 g [2,3]. Bottle-fed porridges prepared with cow's milk and various kinds of cereals are a common complementary food in developing countries [4–7].

Few studies have been made on their actual nutritional composition as defined by chemical analysis [6,7], even though incorrect dilution in home-prepared formula and powdered cow's milk has been found both in developed and in developing countries. Mothers in affluent societies are more likely to over-concentrate [8], whereas mothers in less developed countries usually over-dilute formula and powdered cow's milk [6,7]. The socioeconomic class has been shown to influence feeding-bottles preparation practices [9–11]. Milk and formula play a major role as a source of energy in the infants diet [12–14]. However, in these studies, that role was assessed by food composition tables, not by chemical analysis. Home-prepared foods do not always have an adequate nutritional

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profile, even in developed countries [15,16]. Knowing their chemical composition would allow health professionals to better advise the mothers on the preparation practices of the foods.

The objectives of this study were 1) to determine by chemical analysis the macronutrients composition of home-prepared milk feeding bottles and 2) to assess their contribution to the energy and protein requirements (according to the FAO/WHO/UNU Committee and the Food and Nutrition Board) of children under two years of age from high (HSE) and low (LSE) socioeconomic classes. The contribution of other dietary foods was not taken into account.

MATERIALS AND METHODS

Subjects and Study Design

The study was carried out in São Paulo (a city of approximately 10,300,000 inhabitants in Southern Brazil). The subjects of LSE group were selected from the records of the families living in three slum areas, where the graduate course of nutrition of the Federal University of São Paulo has a field project that provides free primary health care. Mothers attending a private pediatric clinic and selected from the medical appointments of the day were allocated to the HSE group. The final study sample contained 72 subjects, 41 in the LSE group and 31 in the HSE group. For both groups, the children could not exceed two years of age and had to be fully weaned and bottle-fed. The median age of the children in both groups was 12 months. The number of children, according to age was <6 months = 7 (HSE) and 9 (LSE), 6–12 months = 9 (HSE) and 11 (LSE), 12–24 mo = 15 (HSE) and 21 (LSE). The mothers were interviewed and requested to give specific details of how they prepared the feeding bottles, specifically, the type of milk used (formula or unmodified cow's milk, either pasteurized or powdered), other ingredients added and the amount of each one used. All interviews were performed by the first author (T.B.M.). Samples of the contents of the feeding bottles were collected from all 72 subjects in order to perform the chemical analyses. In the LSE group, the samples were collected from bottles at home, whereas in the HSE group they were collected from bottles brought to the pediatric clinic by the mothers. None of the mothers had previously been informed that samples would be collected, thus ensuring that the bottles had been prepared in the normal way. The children's weight-for-age index was assessed in relation to the current international growth reference (NCHS/WHO) and expressed as z-scores. Field constraints in the slum areas prevented the study from obtaining accurate height or length measures of the children. In these areas a two-step evaluation has been used [17]. The index weight-for-age is used for growth monitoring. The children found to be faltering in growth or below a selected cut-off value had their length or height measured to assess their weight-for-height and height-for-age. As none of the children showed

weight-for-age below the cut-off value, length or height measures were not done. This project was approved by the Medical Ethics Committee of the Federal University of São Paulo. Parent/guardian written consent was also obtained.

Energy and Protein Provided by Feeding Bottles as a Percentage of Requirements

In order to calculate the total intake of milk consumed per day, the mothers were also asked how many milliliters of milk the child consumed at each feeding (measured on the scale of the bottles) and the number of feedings per day. The percentages of energy and protein provided by the contents of the feeding bottles was calculated as follows: The energy and protein per 100 mL were obtained through chemical analysis and were multiplied by the volume consumed at each feeding. The results were multiplied by the number of feedings per day, divided by the energy and protein requirements, then multiplied by 100. Energy and protein requirements were those recommended by the FAO/WHO/UNU Committee [18] and the Food and Nutrition Board [19].

Chemical Analyses

The samples were transported to the laboratory at <10°C within two hours of collection and analyzed for moisture, protein, fat and ash [20]. The moisture content was obtained by heating the samples to 102°C until a constant weight was attained. The protein level was obtained by determination of Kjeldahl nitrogen and multiplied by 6.38 [21]. After acid hydrolysis, the fat was extracted by ether using the Soxhlet apparatus. Ash was obtained by incineration at 500–550°C until the ash was carbon-free. Carbohydrate content was determined by difference. The energy density was calculated by multiplying the protein and total carbohydrate content by 4 Kcal and adding the result to the fat content multiplied by 9 Kcal. The results for energy, protein, carbohydrate and fat were compared with the guidelines of the European Society of Pediatric Gastroenterology and Nutrition (ESPGAN) for follow-up formulas [22]: energy: 60–80 Kcal · dL⁻¹, protein: 2.1–3.1 g · dL⁻¹, carbohydrate: 5.7–8.6 g · dL⁻¹, fat: 2.7–4.0 g · dL⁻¹.

Statistical Analyses

The statistical analysis were performed using the softwares EPI INFO 6 ANTHRO (CDC/WHO, 1994) and Sigma Stat for Windows 2.0® (SPSS, 1997). The chi-square test was used to compare the proportions of the addition of sugar and cereals to the milk in the bottle. The Mann-Whitney test was used to compare the two groups' data. The level of significance was $p < 0.05$.

Table 1. Types of Milk Used in the Preparation of Feeding Bottles in the HSE (High Socioeconomic) and LSE (Low Socioeconomic) Groups

	HSE n = 31	LSE n = 41	Total
Unmodified Cow's Milk	24 (77.4%)	40 (97.6%)	64 (88.9%)
Powdered	12 (38.7%)	25 (61.0%)	37 (51.4%)
Pasteurized	12 (38.7%)	15 (36.6%)	27 (37.5%)
Formula	7 (22.6%)	1 (2.4%)	8 (11.1%)

RESULTS

As reported by the mothers and shown in Table 1, unmodified cow's milk was largely consumed by both groups. The HSE group consumed both powdered and pasteurized cow's milk, while the LSE group tended to use powdered cow's milk. The consumption of formula was low in the HSE group and practically non-existent in the LSE group. The addition of sugar and other ingredients to the milk was significantly higher in the LSE group (Table 2).

Macronutrient composition of the feeding bottle contents is given in Table 3. Moisture and fat content were significantly lower in the LSE group, whereas carbohydrate and energy content were significantly higher. Protein content was lower in the LSE group, although the difference did not reach statistical significance.

Table 4 shows the percentages of feeding bottles that fell in the various intervals for energy, protein, carbohydrate and fat proposed in the ESPGAN guidelines. Only 35.5% and 46.3% of the feedings bottles, in the HSE and LSE groups, respectively, had an energy content in the interval of 60–80 Kcal. Furthermore, the HSE group presented a higher percentage of feeding bottles with less than 60 Kcal than the LSE group, while the

Table 2. Addition of Sugar and Other Ingredients in the Preparation of Feeding Bottles in the HSE (High Socioeconomic) and LSE (Low Socioeconomic) Groups

	HSE n = 31	LSE n = 41	χ^2 Test
Sugar			
Yes	4 (12.9%)	37 (90.2%)	$p < 0.001^*$
No	27 (87.1%)	4 (9.8%)	
Other Ingredients			
Yes	13 (42.0%)	28 (68.3%)	$p = 0.046^*$
Cornstarch	2	17	
Corn flour	0	2	
Infant cereals	1	4	
Rice flour	0	2	
Chocolate	3	1	
Oat	1	2	
Sweeteners	2	0	
Sustagen®	2	0	
Honey	2	0	
No	18 (58.0%)	13 (31.7%)	

Table 3. Macronutrient Composition of Feeding Bottles in the HSE (High Socioeconomic) and LSE (Low Socioeconomic) Groups

	HSE n = 31	LSE n = 41	Mann-Whitney Test
Per dL ⁻¹	Median (P25–P75)		
Moisture (g)	87.5 (83.8–87.9)	82.9 (79.7–85.9)	$p < 0.001^*$
Protein (g)	3.4 (2.9–3.9)	3.2 (2.2–3.7)	$p = 0.063$
Fat (g)	3.0 (2.6–3.7)	2.4 (1.8–3.3)	$p = 0.019^*$
Ash (g)	0.6 (0.4–0.6)	0.5 (0.4–0.7)	$p = 0.539$
Carbohydrates (g)	5.7 (4.8–8.2)	11.2 (8.1–13.2)	$p < 0.001^*$
Energy (Kcal)	64.3 (58.6–81.6)	78.1 (64.5–92.7)	$p = 0.017^*$

LSE group presented with a higher percentage of feeding bottles >80 Kcal. Because unmodified cow's milk was used in the majority of the feeding bottles, most of them presented a protein content above 3.1 g · dL⁻¹, although the LSE group presented a higher percentage of feeding bottles with values below 2.1 g · dL⁻¹. Approximately 29% of the feeding bottles for both groups fell within the normal ESPGAN guidelines for carbohydrate (5.7–8.6 g · dL⁻¹). The HSE group had a higher percentage of feeding bottles with a content below 5.7 g · dL⁻¹, while the LSE group presented a higher percentage of feeding bottles with a content above 8.6 g · dL⁻¹. The results for fat were similar in both groups.

As is shown in Table 5, the intake of milk per bottle, the energy per bottle and the total energy per day from feeding bottles were significantly higher in the LSE group. The percentage of energy requirement met by the contents of the feeding bottles was also higher in the LSE group, although it did not reach statistical difference. The percentage of protein

Table 4. Distribution of the Number and Percentage of Feeding Bottles in the HSE (High Socioeconomic) and LSE (Low Socioeconomic) Groups in the Intervals Proposed by ESPGAN

	HSE n (%)	LSE n (%)
Per dL ⁻¹		
Energy (Kcal)		
60–80	11 (35.5%)	19 (46.3%)
<60	12 (38.7%)	7 (17.1%)
>80	8 (25.8%)	15 (36.6%)
Protein (g)		
2.1–3.1	10 (32.3%)	13 (31.7%)
<2.1	1 (3.2%)	7 (17.1%)
>3.1	20 (64.5%)	21 (51.2%)
Carbohydrates (g)		
5.7–8.6	9 (29.0%)	12 (29.3%)
<5.7	15 (48.4%)	1 (2.4%)
>8.6	7 (22.6%)	28 (68.3%)
Fat (g)		
2.7–4.0	13 (42.0%)	17 (41.4%)
<2.7	13 (42.0%)	20 (48.8%)
>4.0	5 (16.0%)	4 (9.8%)

Table 5. Intake Volume and Energy per Bottle, Number of Feedings per Day, Total Energy from Feeding Bottles per Day, Percentages of Energy and Protein Requirements from Feeding Bottles per Day and z-Score (Weight-for-Age) in the HSE (High Socioeconomic) and LSE (Low Socioeconomic) Groups

	HSE n = 31	LSE n = 41	Mann-Whitney Test
	Median (P25–P75)		
Intake (mL)/bottle	200.0 (162.5–232.5)	220.0 (200.0–240.0)	<i>p</i> =0.021*
Energy (Kcal)/bottle	123.6 (103.2–157.5)	162.8 (149.3–197.5)	<i>p</i> =0.002*
Feedings/day	3.0 (3.0–5.0)	3.0 (3.0–5.0)	<i>p</i> =0.950
Total energy (Kcal) from feeding bottles/day	450.7 (362.5–562.9)	599.0 (453.2–773.8)	<i>p</i> =0.010*
% of energy requirements from feeding bottles (RDA: 600–1300 Kcal/day)	46.7 (32.6–74.4)	61.1 (42.7–96.2)	<i>p</i> =0.096
% of protein requirements from feeding bottles (RDA: 13–16 g/day)	160.0 (120.0–220.0)	160.0 (130.0–200.0)	<i>p</i> =0.914
z-Score (weight-for-age)	+0.535 (–0.010/+0.930)	+0.100 (–0.850/+0.595)	<i>p</i> =0.049*

requirement provided by the contents of the feeding bottles were similar in both groups. Children in the LSE group had significantly lower z-scores for weight-for-age, though in both groups they were within the normal limits.

DISCUSSION

Home-prepared weaning foods in developing countries have been regarded as nutritionally poor, with low caloric and protein densities [2,3]. In this study, however, such a pattern was not found. According to the ESPGAN guidelines [22], which were used as a framework to assess the nutritional quality of the macronutrients in the feeding bottles (even though they were intended for cow's milk based follow-up formulas without the addition of other ingredients), most of the feeding bottles, both in the HSE and the LSE group, met or exceeded the recommended values for energy, protein, carbohydrate and fat.

Addition of sugar and other ingredients to feeding bottles was significantly higher in the LSE group. This is a common practice in less developed countries [4–7,9] and in low-income classes in the United States [10,11]. In Brazil, this practice is also a cultural norm in low socioeconomic classes as was reported by Tudisco *et al.* [4] in a study of weaning diet in four Brazilian state capitals. Here, the main complementary food was cow's milk with sugar and cereals, consumed by 68% of 466 children under two years of age. Brazilian pediatric textbooks, however, recommend the addition of sugar and starch to the milk just for malnourished children of low socioeconomic class [23,24]. In this study, even though children in the LSE group had significantly lower z-scores for weight-for-age, they were within the normal limits.

In the HSE group, the percentages of mothers adding sugar (12.9%) and other ingredients (42.0%) to feeding bottles were higher than those observed by Fein *et al.* [25] (2.0% of the mothers added sugar and 15.0% added cereal) and by Skinner *et al.* [26] (33.1% of the mothers added cereal) in middle and upper socioeconomic classes in the United States. The lower

consumption of sugar and cereals in the HSE group might be linked with a concern, on the part of the mothers, of the risk of obesity. Nowadays, obesity in children is a cause of concern in Brazil, affecting about 11.0% of children under four years of age in the high socioeconomic class [27].

The different preparation practices gave rise to differences in the chemical composition of the feeding bottle contents, with the addition of sugar and other ingredients leading to a significantly higher carbohydrate and energy content in the LSE group. The protein content (3.2 g/dL⁻¹) was higher than that found by Hibbert *et al.* [6] (2.8 g/dL⁻¹) in Jamaica and by Dorea *et al.* [7] (2.0 g/dL⁻¹) in the Brazilian capital, Brasília. The energy content (78.1 Kcal/dL⁻¹) was similar to that found in Jamaica (74.0 Kcal/dL⁻¹), lower than in Brasília (96.0 Kcal/dL⁻¹), but higher than other complementary foods consumed in developing countries (25–30 Kcal/100 g) [2,3]. The lower protein and fat contents in the LSE group's feeding bottles could be due to over dilution of the powdered cow's milk and to the relative dilution resulting from the addition of sugar and cereals.

Feeding bottles played a major role as a source of energy and protein in the LSE group, with about 60% and 160% of energy and protein requirements, respectively, being provided by them. These results may explain the normal weight-to-age index of the children of this group. However, as children seem to regulate energy intake [28], the feeding bottles in the LSE group could eventually displace a more varied diet. This leads to a monotonous diet high in calories, poor in micronutrient content and bioavailability, potentially resulting in another set of nutritional problems, such as anemia and obesity. In the state of S. Paulo, anemia has been found in 59% of the children under two years of age attending health care units [29]. According to two large national surveys, underweight in children less than four years old dropped from 20.6% in 1974 to 12.2% in 1989 in the poorest economic stratum. In the intermediate and richest strata, overweight percentages were higher than underweight percentages [27].

Mothers do not always recognize which practices are important for their infants' health, because failure to follow recommendations does not generally have consequences that are obviously linked to feeding practices [25]. The nutritional issues are dynamic, and the professionals working in this area should be prepared to address them.

CONCLUSIONS

Differences in the preparation practices led to differences in the chemical results. The feeding bottles in the LSE group were high in energy, due to the addition of sugar and cereals to the milk in the bottle. The milk feeding bottles were an important weaning food providing more than 50% and 100% of the children's energy and protein requirements, respectively. The children's weight for age index was within the normal limits.

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