Acta Alimentaria, Vol. 31 (3), pp. 297–305 (2002)

Preliminary communications

EVALUATION OF INFANT FORMULA PROTEIN QUALITY

I. COLIĆ BARIĆ*, I. PANJKOTA KRBAVČIĆ and S. PEDISIĆ

Faculty of Food Technology and Biotechnology, University of Zagreb,
Pierottijeva 6, 10000 Zagreb. Croatia

(Received: 20 December 2000; revision received: 12 October 2001; accepted: 3 December 2001)

Infant formulas are designed to simulate not only the content but also the performance of human milk as much as possible in order to be an adequate replacement of human milk. The most common sources of protein in infant's formula are either cow's milk or soy protein (isolate). From this point of view the aim of this study was to determine the nutritive value of these proteins sources in powdered infant formulas present in the Croatian market.

Protein quality has been evaluated in vivo – feeding young growing rats, and in vitro by multienzyme systems.

The results showed that protein digestibility (D) of both formulas are high and not significantly different according to methodology (in vivo and in vitro). Biological value (BV) and net protein utilisation (NPU) of milk protein based formula are lower than that found in the literature. The same bioassays for soy protein isolates based formula are extremely low, while PER and NPR values are higher than that of proteins in milk based formula.

The data indicate that protein qualities of both powdered infant formulas evaluated in vivo are not satisfying and can not provide nutritional support to healthy infants. At this point further investigations should be done in order to identify the factors affecting protein quality.

Keywords: infant formula, protein quality.

Human milk is the ideal food for the human infant. Human milk contains hormones, living cells, active enzymes, immunoglobulins and components with unique molecular structures that can not be replicated in infant formula according to BENSON and MASOR (1994). Human milk protects the infants against certain diseases, infections and allergies. Even so, from two months of age, most infants in North America (USA and Canada) are formula fed according to KLISH and co-workers (1998). Our pilot study showed that 49.4% of investigated mothers breastfeed their infants in first 6 months of life, and 17.3% of infants are only formula fed.

BRUSINCO and co-workers (1998) has drawn attention to a wide variety of formulas that are available to meet the needs of different babies from which milk formula is the

* To whom correspondence should be addressed.
Fax: 385-1-4836083; e-mail: icolic@pbf.hr

first choice, and the second one is soy formula, usually used for infants suffering from lactose intolerance. There is no lactose in soy formula.

The protein content in cow and soy milk is different in amino acid composition. Soy formulas are deficient in sulphur-containing amino acids according to RIGO and co-workers (1994). In some soy formulas the proteins are supplemented with L-metionine, L-carnitine and taurine (SARWAR et al., 1989). Still some authors suggest the pre-digested soybean protein for infant formula (GE & ZHANG, 1993).

The aim of this study was to determine protein quality of two infant formulas from Croatian market based on two different protein sources, cow's milk and soybean protein isolates using in vivo and in vitro methods.

1. Materials and methods

1.1. Materials

All experiments were done on original packed powdered infant formulas taken from Croatian market, which are assigned for infants aged between 0 to 6 months. Sample 1 is an infant formula based on cow's milk, without gluten and crystal sugar. This product contains skimmed milk, demineralised whey, vegetable oil, cornstarch, vitamins and minerals. Vitamin and mineral content of the formula is shown in Table 1.

Sample 2 is an infant formula based on soy protein isolate, without cow's milk, lactose and gluten. This product is specially designed for infants suffering from diarrhoea or lactose intolerance or gluten entheropathiae. The product contains soy protein isolate, vegetable oil, refined milk fat, sucrose, vitamins and minerals, lecithin, L-carnitine and holin. Vitamin and mineral content of the formula shown in Table 1.

The enzymes used in protein digestibility evaluation by in vitro method were trypsin from porcine pancreas (type IX), activity 15200 BAEE units/mg prot. (Sigma Chemical Company, USA); chymotrypsin from bovine pancreas (type II), activity 55 units/mg prot. (Sigma Chemical Company, USA); peptidase from porcine intestinal mucosa, activity 102 units/g solid. (Sigma Chemical Company, USA).

1.2. Methods

Protein quality evaluation of commercially prepared infant formulas, based on cow's milk and soybean protein isolates, was based on biological methods in vivo, on the nitrogen retention in rats' body determined either by direct carcass analyses or by nitrogen balance techniques (FAO, 1970).

Chemical compositions and energy values of infant formulas were determined using A.O.A.C. standards (Table 1) (A.O.A.C., 1995). On that bases two test diets were made (Table 2). As control diets one protein (Ca-casein) and one non-protein diet was prepared (Table 3.) All three protein diets contained the same protein level (10% w/w).

Male Y-59 rats (University of Zagreb, Zagreb, Croatia), 21 days of age were maintained on stock pellets for 7 days in an air-conditioned room at a temperature of 23

±1 °C. All rats received water and food ad libitum. After seven days animals were weighed and divided into four groups with five animals in each group. Difference in mean weights between the groups was not higher than 2 g and between the rats in the same group (n=5) it was less than 5 g (HURT et al., 1975). Thereafter, rats were fed with the test foods for ten days in collective metabolic cages (group of five rats in one cage).

Table 1. Chemical composition, energy values and energy fraction of nutrients in powdered milk-based (sample 1) and soy protein-based (sample 2) infant formulas (mean ± sd)

Parameters	Sample 1 (n=5)	Sample 2 (n=5)
Moisture (%)	3.4 ± 0.65	2.5 ± 0.25
Protein (% d.w.)	11.1 ± 0.15	15.6 ± 0.98
Lipid (% d.w.)	25.4 ± 0.64	28.0 ± 1.74
Carbohydrate (% d.w.)	61.6 ± 2.48	52.8 ± 1.03
Minerals (% d.w.)	1.9 ± 0.31	3.6 ± 0.31
Energy value (kJ/100 g)	2147.0 ± 65.50	2200.0 ± 99.50
Protein (en%)	8.5 ± 0.33	11.9 ± 0.23
Lipid (en%)	44.0 ± 4.03	47.9 ± 1.50
Carbohydrate (en%)	47.5 ± 1.15	40.2 ± 1.31
Minerals:		
Na (mg/100 g)	240	210
K (mg/100 g)	609	510
Cl (mg/100 g)	349	380
Ca (mg/100 g)	370	500
P (mg/100 g)	300	350
Mg (mg/100 g)	66	32
Fe (mg/100 g)	5,7	8
Zn (mg/100 g)	5,9	3.6
Cu (µg/100 g)	245	350
I (µg/100 g)	71	70
Vitamins:		
Vitamin A (µg/100 g)	540	540
Vitamin D (µg/100 g)	9	8,8
Vitamin E (µg/100 g)	2500	10000
Vitamin K1 (µg/100 g)	30	100
Vitamin B1 (µg/100 g)	440	300
Vitamin B2 (µg/100 g)	540	400
Niacin (µg/100 g)	7100	4000
Vitamin B6 (µg/100 g)	440	300
Folic acid (µg/100 g)	36	56
Ca-panthothenate (µg/100 g)	3600	3300
Biotin (µg/100 g)	11	22
Vitamin B12 (µg/100 g)	1,4	2
Vitamin C (µg/100 g)	6400	5500

Using infant formula based diet, one group of animals was fed with cow's milk-based formula, as the source of protein (group 1) and another with soybean isolate-based formula (group 2). The third group was fed with the control protein diet based on Ca-casienate (group 3) and the fourth one with a non-protein diet (group 0).

During the test period the faeces were collected. The faeces, food and animals were weighed during and at the end of the test. At the end of testing period the faeces and the rat tissues were analysed for nitrogen using micro-Kjeldahl method. In order to analyse the nitrogen content of the tissues the rats were killed and the carcass were dried in oven (105 °C/48 h), and homogenised in blender.

Parameters which characterise the quality of proteins were calculated for infant formulas based on cow's milk and soybean protein isolate according to usual methods: digestibility (D) (NJAA, 1977; FAO, 1970), biological value (BV) (MITCHELL, 1924; NJAA, 1977; BENDER & DOELL, 1957a), net protein utilisation (NPU) (BENDER & MILLER, 1953; BENDER & DOELL, 1957b; MILLER & BENDER, 1955) protein efficiency ratio (PER) (NJAA, 1977; STEINKE, 1977) and net protein ratio (NPR) (BENDER & DOELL, 1957a; HACKLER, 1977).

Protein digestibility determination of infant formulas was performed by in vitro enzymatic method according to HSU (1977). Multienzyme system consisting of trypsin, chymotrypsin and peptidase was selected.

All data were statistically analysed (STATSOFT INC., 1995; DIXON & MASSEY, 1975; FISHER & YATES, 1957).

2. Results

The chemical contents and energy values of two powdered, originally closed packages of infant formulas from Croatian market (based on cow's milk (sample 1) and soy protein isolate (sample 2)) used in this test are presented in Table 1. Statistical analyses of the results, using Analysis of Variance (ANOVA), showed that there was a significant difference ($P<0.05$) between the two formulas with regard to protein content. The protein content of soy formula is usually higher than that of milk-based formula due to differences in protein digestibility and amino acid composition (LONNERDAL, 1994).

With regard to chemical composition two testing diets were made as well as one control protein diet with 10% protein content. The chemical compositions and energy values are shown in Table 2. ANOVA showed that there was no significant difference between these three diets with regard to protein, fat and carbohydrate contents and energy values, what was observed as significant parameters for adequate protein evaluation of infant formulas by MITCHELL and JENKINS (1985).

After the ten-day period animals fed with non-protein diet have lost weight (–19.5 g) while the animals fed with protein diets gained weight (Fig. 1 and Table 3). The consumed foods, nitrogen and energy intake as well as the other measured parameters are shown in Table 3.

Table 2. Chemical composition, energy values and energy fraction of test diets containing cow's milk based infant formula (sample 1) and soy protein based infant formulas (sample 2), control protein (Ca-casein) and non-protein rats' diets (mean ± S.D.)

Parameters	Sample 1 (n=5)	Sample 2 (n=5)	Control protein (n=5)	Non-protein (n=5)
Moisture (%)	6.2 ± 1.71	11.2 ± 2.0	12.1 ± 1.23	4.6 ± 0.99
Protein (% d.w.)	10.5 ± 0.55	10.7 ± 1.69	10.5 ± 1.05	0.3 ± 0.08
Lipid (% d.w.)	23.4 ± 2.37	19.8 ± 1.77	20.4 ± 2.74	23.8 ± 3.17
Carbohydrate (% d.w.)	66.2 ± 0.70	69.53 ± 2.42	69.1 ± 0.98	75.9 ± 2.36
Energy value (kJ/100 g)	2162.0 ± 71.02	2068.0 ± 50.65	1894.0 ± 98.72	2079.0 ± 82.35
Protein (en%)	8.1 ± 0.25	8.6 ± 1.22	8.4 ± 0.70	0.2 ± 0.06
Lipid (en%)	40.6 ± 2.86	35.7 ± 2.31	36.5 ± 3.23	41.2 ± 4.04
Carbohydrate (en%)	51.3 ± 3.05	55.6 ± 2.63	55.1 ± 3.12	58.6 ± 4.07

Table 3. Rat bioassays on protein quality evaluation (in vivo) of different protein sources (mean ± S.D.)

Parameters	Sample 1 Milk based (n=5)	Sample 2 Soya based (n=5)	Control protein (n=5)	Non-protein (n=5)
Weight gain (g/10 days)	35.5 ± 1.24	71.0 ± 4.22	81.9 ± 2.47	–19.5 ± 2.11
Weight gain (g/g protein intake)	1.77 ± 0.07	1.92 ± 0.37	3.39 ± 0.29	–
Consumed food (g w.b./day)	20.44 ± 2.19	35.50 ± 7.26	32.52 ± 4.38	26.34 ± 3.79
Consumed food (g w.b./100 g body weight)	85.16 ± 1.29	193.78 ± 3.34	120.35 ± 2.99	177.93 ± 3.22
Energy consumed (kJ/day)	442.3 ± 14.53	734.0 ± 13.59	698.0 ± 28.21	548.0 ± 22.28
Protein intake (mg protein/kJ)	4.55 ± 0.26	5.03 ± 0.78	3.47 ± 0.42	–
N Intake (mg/day)	322.0 ± 0.12	591.0 ± 0.85	387.0 ± 0.41	–
Faecal N (mg/day)	65.0 ± 1.27	101.0 ± 3.97	129.0 ± 2.23	2.7 ± 0.99
Body N (% N on d.w.)	9.22 ± 1.25	7.50 ± 0.99	9.10 ± 1.26	10.14 ± 1.00
Retained N (%)	68.01 ± 0.61	31.3 ± 2.89	78.6 ± 2.47	–
Dried carcass (g)	69.5 ± 2.23	80.9 ± 1.98	79.7 ± 2.12	40.9 ± 1.98

In Table 4 are shown the results of in vivo and in vitro protein quality evaluation.

According to literature data (FAO, 1970; SARWAR et al., 1989) our results showed that digestibility (D) in vivo of cow's milk proteins (88.10%) and soy protein isolates (87.48%) are very high (Table 4). Digestibility of our control protein diet (in vivo) (73.64%) is lower compared with tested formula proteins and literature data (FAO,

1970). Protein digestibility values (D) determined in vitro were high as well as those determined by in vivo method (Table 4). Statistical analysis by *F*-test shows no significant difference between these two methods.

Table 4. Bioassays on protein quality evaluation of infant formulas based on cow's milk (sample 1), soy proteins isolate (sample 2) and control protein (Ca-casein) (mean ± S.D.)

Parameter	Sample 1 Milk based (n=5)	Sample 2 Soya based (n=5)	Control protein (n=5)
In vitro:			
Digestibility (D) (%)	83.71 ± 0.06	78.47 ± 3.65	90.33 ± 1.97
In vivo			
Digestibility (D) (%)	88.10 ± 0.36	87.48 ± 2.14	73.64 ± 1.95
Biological value (BV) (%)	77.11 ± 3.25	35.78 ± 7.99	106.67 ± 10.30
Net protein utilisation (NPU) (%)	68.01 ± 2.72	31.30 ± 6.06	78.55 ± 6.70
Protein efficiency ratio (PER)	1.76 ± 0.10	1.92 ± 0.37	3.39 ± 0.29
Protein efficiency ratio corrected to casein (cPER)	1.30 ± 0.09	1.42 ± 0.33	–
Net protein ratio (NPR)	2.73 ± 0.12	2.45 ± 0.47	4.19 ± 0.36

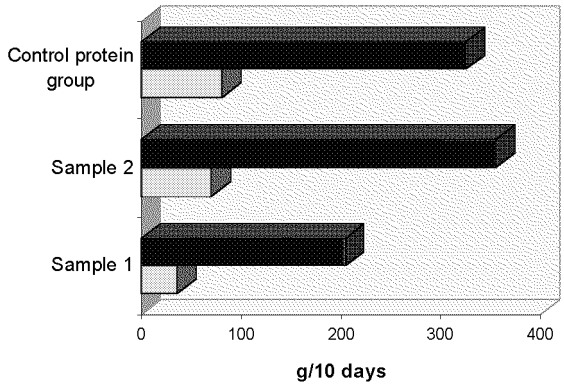

Fig. 1. Weight gain and food intake of tested animals. ■ Consumed food; ▨ Weight gain

The Ca-casein as the control protein has higher BV, PER cPER, NPR and NPU than proteins of both infant formulas (Table 4). Animals fed with control protein consumed less protein and energy than animals fed with soy-based formula but more than animals fed with milk-based formula. The retained nitrogen was the lowest for the soy-based formula fed animals (31.3%) (Table 3).

Biological value (BV) of milk-based formula (77.11%) is lower compared with 90.0% for the same protein (Table 4) (FAO, 1970). Biological value of soy formula

proteins is extremely low (35,78%) (Table 4). This value is cca 40% less than literature data (FAO, 1970; BODWELL, 1977). Such a low biological value of soy formula proteins is unexpected, especially for infant formula (HACKLER, 1977; WOOD et al., 1987).

The control protein has the highest biological value. High correlation also exists between biological value and retained body nitrogen. The highest NPU value is obtained from control protein (Table 4). Soy formula proteins have also very low NPU value, which is not compatible with literature data (FAO, 1970). Such a low protein utilisation is not acceptable for infant formula. BV and NPU for soy formula proteins should be identical to Ca-casein or cow's milk values. According to statistical analysis, net protein utilisation (NPU) is highly correlated ($P<0.05$) to nitrogen intake and retained nitrogen.

The highest PER value is obtained for control protein and the lowest for milk-based formula (Table 4). These data are close to the literature data (2.26%) (FAO, 1970; STEINKE, 1977).

The results obtained of these bioassays showed that PER is highly correlated ($P<0.05$) to weight gain as was expected. On the basis of the PER value criteria, which take into account only weight gain and protein intake, soy formula possesses high quality proteins. Similar results were obtained in research with infants of CHURELLA and co-workers (1994).

Corrected PER values for casein are lower also, and the ratio remained the same as for the uncorrected one (Table 4).

The net protein ratio (NPR) assay is similar to PER (Table 4). Besides weight gain and protein intake observations, the weight loss method of a non-protein control group is sometimes preferred to as NPU, as it is simpler to perform. PER and NPR should be in correlation with the same parameters. Both are highly correlated to digestibility. NPR is also highly correlated ($P<0.05$) with retained nitrogen which means that it depends on protein quality and quantity. In comparison to NPR value, protein quality of infant formula, based on soy protein, corresponds to milk-based formula.

Low values of the parameters, which take into account nitrogen retention for soy formula, are probably due to amino acid composition (SARWAR et al., 1993), the analysis which are not performed in this study. Those analyses are expensive, and for the purpose of easier protein quality control, the producer should declare amino acid composition on label.

High protein quality soy and cow's milk-based formulas effect parameters which take into account only weight gain values and quality of consumed proteins (PER, cPER, NPR). In that case it is good to point that weight gain is not correlated to energy intake.

Even HACKLER (1977) found that different tests show different correlation coefficients, all data can also suggest a lack of accuracy of the in vivo methods as predictors of protein nutritional quality for the rat. MICHELL & GRUNDEL (1986) observed the same.

At this point the research should continue in order to identify the factors affecting quality, thus ensuring constant infant formula quality.

3. Conclusions

Protein quality evaluation of infant formulas based on cow's milk and soy protein isolate have shown in vivo and in vitro very high digestibility (D). There is no statistically significant difference between these two methods. Biological value (BV) of soy protein is extremely low as well as net protein utilisation (NPU) and are not correlated to PER and NPR values. In relation to protein efficiency ratio (PER) and net protein ratio (NPR) both formula have high quality proteins. Soy proteins are even close to control protein (Ca-casein) quality.

From obtained results in vivo, it is evident that all protein quality parameters should be higher. The reason for such low protein quality can be inappropriate amino acid composition or inappropriate method for protein quality evaluation of infant formula. At this point further investigations are needed to achieve this target.

Literature

A.O.A.C (1995) *Official methods of analysis*. 16th ed. A.O.A.C. International, Arlington.

BENDER, A.E. & DOELL, B.H. (1957a): Biological evaluation of proteins: a new aspect. *Br. J. Nutr., 11*, 140–157.

BENDER, A.E. & DOELL, B.H. (1957b): Note on the determination of net protein utilis carcass analysis. *Br. J. Nutr., 11*, 138–139.

BENDER, A.E. & MILLER, D.S. (1953): A new brife method of estimating net protein value. *Biochem. J., 53*, vii.

BENSON, J.D. & MASOR, M.L. (1994): Infant formula development: past. present and future. *Endocrine Regulations*, *28*, 9–16.

BODWELL, C. (1977): Application of animal data to human protein human nutrition: a review. *Cereal Chem.*, *54*, 958–983.

BRUSINCO, L., BRUNO, G. & GIAMPIETRO, P.G. (1998): Soy protein for the prevention treatment of children with cow-milk allergy. *Am J Clin Nutr.*, *68*, (6 Suppl S), 1447S–1452S.

CHURELLA, H.R., BORCHEL, M.W., THOMAS, M.R., BREEN, M. & JACOBS, J. (1994): Growth and protein status of term infants fed soy protein formulas differing in protein content. *J. Am. College Nutr.*, *13*, 262–267.

DIXON, W.J. & MASSEY, F.J. (1975): *Introduction to statistical analysis*. 2nd ed. McGraw and Hill, New York, pp. 23–33.

FISHER, R.A. & YATES, F. (1957): *Statistical tables for biological. agricultural and medical food and research*. 5th ed. Hafner Publishing, New York, p. 59.

FAO (1970): *Amino-acid content of foods and biological data on proteins*. Nutritional Studies No. 24. Food and Agriculture Organisation, Rome.

GE, S.J. & ZHANG, L.X. (1993): Predigestion of soybean proteins with immobilised trypsin for infant formula. *Appl. Biochem. & Biotechnol.*, *43*, 199–209.

HACKLER, L.R. (1977): Methods of measuring protein quality: a review of bioassay procedures. *Cereal Chem.*, *54*, 985–995.

HURT, H.D., FORSYTH, R.H. & KRIEGER, C.H. (1975): Factors which influence the biological evaluation of protein quality by the protein efficiency ratio method. -in: FRIEDMAN, M. (Ed.) *Protein nutritional quality of foods and feeds*. Part I. Marcel Dekker Inc., New York, pp. 87–112.

HSU, H.W. (1977): A multienzyme technique for estimating protein digestibility. *J. Fd Sci*., *42*, 1269–1273.

KLISH, W.J., BAKER, S.S., COCHRAN, W.J., FLORES, C.A., GEORGIEFF, M.K., JACOBSON, M.S., LAKE, A.M., BLUM, D., HARRIS, S.S., HUBBARD, V.S., LEVIN, E., PRENDERGAST, A., SMITH, A.E., YETLEY, E., ZLOTKIN, S. & LAUER, R.M. (1998): Soy protein-based formulas-recommendations for use in infant feeding. *Paediatrics*, *101*, 148–153.

LO, C.W. & KLEINMAN R.E. (1996): Infant formula, past and future: oportunities for improvement. *Am. J. clin. Nutr*., *63*, 646S–650S.

LONNERDAL, B. (1994): Nutritional aspects of soy formula. *Acta Paediatrica*, Suppl. *402*, 105–108.

MITCHEL, H.H. (1924): A method of determining the biological value of protein. *J. biol. Chem*., *58*, 873–903.

MITCHELL, G.V. & GRUNDEL, E. (1986): Nutritional value of proteins in powdered infant formula: *in vitro* and *in vivo* methods. *J. agric. Fd Chem.*, *34*, 650–653.

MITCHELL, G.V. & JENKINS, M.Y. (1985): Assessment of protein quality methodology for infant formulas. *J. A.O.A.C.*, *68*, 680–683.

MILLER, D.S. & BENDER, A.E. (1955): The determination of the net protein utilisation of proteins by a shortened method. *Br. J. Nutr.*, *9*, 382–388.

NJAA, L.R. (1977): Biological methods for the evaluation of protein quality. -in: *Panel proceeding series. Nutrition evaluation of cereal mutants.* International Atomic Energy Agency, Vienna, pp. 59–71.

RIGO, J., SALLE, B.L., PUTET, G. & SENTERRE, J. (1994): Nutrition evaluation of various protein hydrolysate formulae in term infants during the first month of life. *Acta Paediatrica*, Suppl., *402*, 100–104.

SARWAR, G., BOTTING, H.G. & PEACE, R.W. (1989): Amino acid rating method for evaluating protein adequacy of infant formulas. *J. A.O.A.C.*, *72*, 622–626.

SARWAR, G., PEACE, R.W. & BOTTING, H.G. (1993): Effect of amino acid supplementation on protein quality of soy-based infant formulas fed to rats. *Plant Fds for Human Nutr.*, *43*, 259–266.

STEINKE, F.H. (1977): Protein efficiency ratio pitfalls and causes of variability: a review. *Cereal Chem.*, *54*, 949–957.

STATSOFT INC. (1995) Version 5. 1. Tulsa. OK.

WOOD, B.A., PHILLIPS, R.D., EITENMILLER, R.R. & SOLIMAN, A.M. (1987): Determining protein quality of infant formulas using in vivo and in vitro methods. *Nutr.Rep. Int.*, *35*, 1195–1204.