

## Dietetic research of infant nutrition and protein efficiency ratio for some infant formulas from Croatian market

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### Summary

The aim of this research was to establish the state of infant nutrition mode in Croatia, with special regard to nutrition quality parameter protein efficiency ratio (PER) of the most used infant formulas. Methods included dietetic survey (n=255 mothers of infants) in urban area and PER (protein efficiency ratio) determination in vivo by rats and infants for the most used infant formulas in Croatian market. Results of dietetic survey showed that almost half of examinees (49.4 %) claim that their infants are exclusively breastfed. Significant amount of examinees (33.3 %) used mixed breast-and-formula feed for their infants. There are also 17.3 % mothers whose infants are exclusively formula fed. According to PER values in infants, Samples 1-4 had very similar quality (PER 2.01-2.36). PER values in rats varied from 1.34 to 3.36. In spite of different PER values for infants and rats, these differences are not statistically significant ( $p < 0.05$ ), therefore it can be presumed that both methods can be used equally.

*Key words:* infant nutrition, infant formulas, PER (protein efficiency ratio)

### Introduction

Newborn and infant nutrition is one of the main factors for normal growth and development of young organisms (Whitney and Rolfes, 1999). Adequate nutrition includes all nutritive needed for good physical, intellectual, emotional and social growth and maturing as well as prevention and decreasing risk of some diseases in older ages of life.

Pediatricians and nutritionists strongly recommend mother's milk as the best way for infant's nutrition. Breast milk by itself fulfils the nutritional requirements of healthy term infants up to 6 months of age (WHO, 2001).

Breastfeeding plays a major role in children's health status and development, and its values have been recognized for a long time. There are also suggestions that it prevents appearance of some chronic diseases later in life (Bergmann et al., 2003; G da-

levich et al. 2001; Owen et al. 2002; Ravelli et al. 2000; Von Kries et al. 1999).

Human milk contains hormones, living cells, active enzymes, immunoglobulins and components with unique molecular structures that cannot be replicated in infant formulas (Benson and Masor, 1994). Because of that, 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 (Klish et al., 1998). Infants who were fed with breast milk more than with infant formula, or who were breastfed for longer periods, had a lower risk of being overweight during older childhood and adolescence (Gillman et al., 2001).

But there are many reasons why mothers do not feed their infants with mother's milk. Attention has been drawn that a wide variety of formulas are available to meet the needs of different babies but milk

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formula is the first choice, and the second one is soy formula, usually used for infant suffering from lactose intolerance (Brusino et al., 1998). There is no lactose in soy formula.

The aim of this research was to establish infant nutrition mode in the capitol of Croatia - Zagreb, with special regard to nutrition quality parameter-protein efficiency ratio (PER) of the most used infant formulas detected by biological methods by infants and rats. PER is based on the weight gain of a test subject divided by its intake of a particular food protein during the test period of 10 to 28 days (Morrison and Campbell, 1960).

## Materials

All experiments were made using six different trademarks of infant formulas from Croatian free-market (age 0-6 months). These formulae were the best sellers in Croatian market.

Five infant formulas used in this research were based on cow's milk (*Sample 1-5*) and one based on soy protein isolate (*Sample 6*). Products were produced by well-known domestic and foreign producers. Energetic value and nutritional composition have shown similar values among chosen cow's milk based infant formulas.

## Methods

### *Dietetic survey*

The survey for this dietetic research includes specially designed semiquantitative questionnaire for establishing infant nutrition mode (to six months of age): exclusively breastfed; mixed breast-and-formula fed; or exclusively formula fed. Six trademarks of infant formula were offered, as well as possibilities to choose option "something else" if none of offered formulae were chosen by infant's mothers. *Something else* included other non offered infant formulas in this questionnaire as well as home made products.

There were also questions about intake of infant formulae per meal and day. This survey was filled up by 255 examinees (mothers of infants) in 3 medical centers in Zagreb area during three weeks in 1999. Examinees were mothers of healthy infants without metabolic diseases and they filled up the survey by themselves. Monitoring infant weight gain during

one month period was established by pediatricians and nurses in medical centers where the survey was conducted.

### *PER determination "in vivo" by rats*

PER was determined by standard in vivo method in rats (AOAC, 1995; Hackler, 1977; Steinke, 1977). 40 rats, Y59, male 21 days of age, were stabilized in metabolic cages for 7 days. The room temperature was 23 +/- 1 °C, humidity 50 % and day/night exchange every 12 hours. Food and water was taken *ad libitum*. Before the test, after adaptation period of seven days, rats were weighted and divided in groups of five according to their body mass. There were 6 test diet groups depending on trademark of infant formula, one control protein group and one control non-protein group. Test diets contained 10 %-level of proteins from tested infant formulas, while control protein test diet contained 10 % protein level of Ca-caseinate. Test diets were made based on chemical composition of each tested infant formula. There was no need for enriching diets with vitamin and mineral supplements because it was already done in production procedure of infant formulas. Cellulose and corn starch were added in diets. Control protein diet contained cellulose, corn oil, vitamin and mineral supplements. Non-protein diet contained the same parameters like protein diets without proteins.

Animals were weighted every second day as well as food was added. Every food addition and body mass changes were notified. Results are expressed as rats weight gain on 100 g of eaten proteins. PER was calculated as change in rats body-weight in g/g protein consumed during the test period of 10 days.

### *Protein determination*

Total nitrogen content in infant formulae was analyzed using Kejdahl method (AOAC, 1995). Protein content was calculated using 6.25 as a conversion factor.

### *PER determination by infants*

Quantity of eaten infant formula and protein intake was established by dietetic survey. Infant weight gains in one-month period were established from dietetic questionnaire and the weight measuring in the clinics. Protein contents of applied infant

Table 1: Infant nutrition mode in Zagreb area (n=255)

Tablica 1: Prehrana dojenčadi na području grada Zagreba (n=255)

Nutrition mode	Examinees (%)	Examinees (n)
Vrsta prehrane	Ispitanici (%)	Ispitanici (n)
Exclusively breast fed	49.4	126
Samo majčino mlijeko		
Mixed breast-and formula fed	33.3	85
Dohrana adaptiranim mlijekom		
Exclusively formula fed	17.3	44
Samo adaptirano mlijeko		

formulas were analyzed. From those data PER was calculated for all applied samples of infant formulas.

#### Statistical analysis

Statistical analysis were made by Student's t test ( $p < 0.05$ ). Means, standard deviation and percentages were used to present descriptive statistic.

#### Results and discussion

Breast milk alone can meet nutrient needs during the first 6 months, with the possible exception of vitamin D in certain populations and iron in infants of relatively low birth weight (Dewey, 2001). The composition of infant formulas, intended to sustain infants during the first 4 to 6 months of life and fully satisfying their nutritional requirements, is regulated within the Croatian Food Law (NN 46/2007).

Results of dietetic research are shown in Tables 1-2. First aim of this research was to establish the mode of infant nutrition in Zagreb area. Results of this research are shown in Table 1. Almost half of examinees (49.4 %) claim that their infants are exclusively breastfed. Significant amount of examinees (33.3 %) used mixed breast-and-formula feed for their infants. There are also 17.3 % mothers whose infants are exclusively formula fed.

Previous researches in Croatia during 1996, shows that 94.6 % of mothers started with breastfeeding which lasted for an average of 3.4 +/- 2.9 months (Zakanj et al., 2000). There is no data about number of exclusively breastfed and mixed breast-and-formula fed infants. Another research shows that after the age of 3 months, 30.7 % of infants were still breast-fed and only 11.0 % after 6 months of age. With regard to the nutritional habits

in the first year of life, 40.0 % of all infants were fed with cow's milk. Supplementary feeding started in 29.2 % of respondents before the end of the third month (Berović, 2003). Literature data shows that in USA (Boston) number of infants exclusively breastfed increased from 5.5 % (1995) to 28.5 % (1998) to 33.5 % (1999) (Philipp et al., 2001).

Table 2 shows the usage of the offered trademarks of infant formulas in nutrition of mixed breast- and-formula fed infants and exclusively formula fed infants (n=129).

The most used formula for mixed or exclusively formula fed infants was formula marked as *Sample 2* (52.7 %). This is infant formula based on cow's milk produced by well-known European producer. About 33 % of examinees used 2 domestic infant formulas marked as *Sample 3* and *Sample 4*. *Sample 1* and *Sample 5* were relatively less used, while *Sample 6* was not used at all. Reason for this is that this research was conducted among healthy infants without metabolic or any other diseases. In Croatia, soy based infant formulas are used just in cases of gastrointestinal disorders like lactose intolerance, celiac disease, diarrhea etc.

Table 3 shows established parameters from this dietetic research. 44 infants were exclusively formulae fed, however 5 of them used infant formulas or home made products (marked as *Something else*). Therefore just 39 infants were exclusively fed with formulae marked as samples 1-4. *Samples 5* and *6* were not use for infant nutrition as exclusive source of energy and nutrients and therefore it was not possible to establish parameters. For other infant formulas or home made products indicated as *Something else* it was not possible to establish parameters as well.

Table 2: Comparison of the offered trademarks of infant formulae in nutrition of mixed breast-and-formula fed infants and exclusively formula fed infants (n=129)

Tablica 2: Upotreba ponuđenih vrsta dječje hrane u prehrani dojenčadi koja se ili dohranjuju ili isključivo hrani adaptiranim mlijekom za dojenčad (n=129)

Infant formula	Examinees (n)	Examinees (%)
Hrana za dojenčad	Ispitanici (n)	Ispitanici (%)
Infant formula based on cow's milk/Hrana za dojenčad na osnovi kravljeg mlijeka:		
Sample/Uzorak 1	7	5.4
Sample/Uzorak 2	68	52.7
Sample/Uzorak 3	26	20.2
Sample/Uzorak 4	16	12.4
Sample/Uzorak 5	5	3.9
Sample/Uzorak 6*	0	0
Something else**	7	5.4
Drugo**		

\*Infant formula based on soy's protein isolate/Hrana za dojenčad na osnovi proteinskog izolata soje

\*\*Something else (infant formulae or home made products which was not included in this research)/Drugo (hrana za dojenčad ili domaći pripravci koji nisu obuhvaćeni ovim istraživanjem)

Table 3: Energy and nutrient intake in exclusively formula fed infants (n = 39) (mean ± sd)

Tablica 3: Unos energije i nutrijenata u dojenčadi hranjene isključivo adaptiranom hranom za dojenčad (n=39) (srednja vrijednost ± sd)

Parameters Parametri	Infant formula/Hrana za dojenčad			
	Sample/Uzorak 1 (n=4)	Sample/Uzorak 2 (n=20)	Sample/Uzorak 3 (n=9)	Sample/Uzorak 4 (n=6)
N intake (g/day)/Uneseni N (g/dan)	2.07±0.54	2.12±0.54	2.35±0.21	2.08±0.29
Protein intake (g/day) Uneseni proteini (g/dan)	12.93±3.39	13.27±3.40	14.70±1.34	12.97±1.81
Protein/energy ratio (g/100 kcal) Proteinsko/energetski omjer(g/100 kcal)	2.21	2.09	2.29	1.97
Fat intake (g/day) Unesene masti (g/dan)	31.05±8.15	30.34±7.78	33.77±3.07	32.43±4.51
Carbohydrate intake (g/day) Uneseni ugljikohidrati (g/dan)	62.10±16.30	73.00±18.69	66.27±6.02	69.50±9.66
Energy intake (kJ/1000 mL) Energetski unos (kJ/1000 mL)	2860	2730	2890	2920
Energy intake (kJ/day) Energetski unos (kJ/dan)	2467±647.32	2588±601.31	2623±239.09	2706±376.75
Energy intake (kcal/day) Energetski unos (kcal/dan)	590±154.71	619±143.72	627±57.14	647±90.05
Energy intake (% RDA) Energetski unos (%RDA)	90.67±23.79	95.11±22.10	96.40±8.79	99.45±13.85
Proteins/day (% RDA) Proteini/dan (%RDA)	99.46±26.08	102.0±26.15	113.08±10.31	99.77±13.92

Table 4: Protein efficiency ratio (PER) and average infant weight gain in exclusively formula fed infants (n=39) (mean  $\pm$  sd)Tablica 4: Omjer proteinske djelotvornosti (PER) i prosječni prirast na masi dojenčadi hranjenih isključivo adaptiranim mlijekom (n=39) (srednja vrijednost  $\pm$  sd)

Infant formulas based on cow's milk Hrana za dojenčad na osnovi kravljeg mlijeka	Average infant's weight gain (g /10 days) Prosječni prirast na tjelesnoj masi u dojenčadi (g/10 dana)	PER*
Sample/Uzorak 1	292.92 $\pm$ 50.15	2.27 $\pm$ 1.48
Sample/Uzorak 2	309.0 $\pm$ 62.83	2.33 $\pm$ 1.85
Sample/Uzorak 3	295.56 $\pm$ 53.72	2.01 $\pm$ 1.15
Sample/Uzorak 4	306.67 $\pm$ 41.30	2.36 $\pm$ 2.28

\*PER-protein efficiency ratio/Omjer proteinske djelotvornosti

The highest fat intake was noticed in infants fed with *Sample 3*, and the highest carbohydrate intake was noticed in infants fed with *Sample 2*. In spite of well-balanced energy a nutritive value of analyzed infant formulas, the highest energy intake (2920 kJ/1000 mL) was observed in infants fed with *Sample 4* (Table 3). Macronutrients and energy share were well-balanced in analyzed infant formulas and met recommended dietary allowances (RDA) values for this population (RDA, 1989, NN 46/2007). Energy values of consumed meals were less than RDA values, but this was not statistically significant. Protein intake for infants fed with *Samples 1-4* was adequate and met RDA values (RDA, 1989). *Samples 1-4* had protein/energy ratio from 1.97-2.29 g/100 kcal which is within the recommended range (1.8 -3 g/100 kcal) defined in Croatian Food Law (NN 46/2007). Today, infant formulas with a protein/energy ratio of 1.8 g/100 kcal are commercially available. These formulas have proven to be safe and nutritionally adequate for term infants (Turck et al, 2006).

Protein efficiency ratio (PER) and average infants weight gain of infant formulas are shown in Table 4. Aim of this research was to establish the infant gain weight related with exclusively used infant formula in their nutrition. During 10-days period, the highest weight gain was in infants fed with *Sample 2* (Table 4). For the same period, the highest weight gain was in rats fed with *Sample 5* (Table 5).

Literature data shows that healthy term breast- and adapted formula-fed infants show similar weight gain and weight gain composition during the first months of life (De Curtis, 2001).

According to PER values in infants, *Samples 1-4* have very similar quality (PER 2.01-2.36). In rats, PER values varies from 1.34 to 3.36 (Table 5). Comparison was possible just between *Samples 1-4* because *Samples 5* and *6* were not used by infants exclusively fed with infant formulae.

Infant PER for *Sample 3* was the lowest, but not statistically significant, while rat PER for the *Sample 3* was the highest among *Samples 1-4*. The highest PER value were found in infants fed with *Sample 4*, while the same sample had the lowest PER value in rats.

Standard method for PER determination is according to AOAC on rats, but this method is very invasive, complicated and significant estimated error can appear. To avoid further research on animals, the aim of this research was to use non invasive dietetic method.

In spite of different PER values for infants and rats, these differences are not statistically significant ( $p < 0.05$ ), so we could presumed that both methods can be used equally.

Dietetic research was conducted in 1999. In recent few years some new brands of infant formulae have appeared on Croatian market, so further

Table 5: Protein efficiency ratio (PER) and average rats weight gain (mean  $\pm$  sd)  
 Tablica 5: Omjer proteinske djelotvornosti (PER) i prosječni prirast na masi štakora  
 (srednja vrijednost  $\pm$  sd)

Infant formulas based on cow's milk Hrana za dojenčad na osnovi kravljeg mlijeka	Average rats weight gain (g /10 days) Prosječni prirast na tjelesnoj masi štakora (g/10 dana)	PER
Sample/Uzorak 1	39.60 $\pm$ 6.21	1.63 $\pm$ 0.01
Sample/Uzorak 2	35.50 $\pm$ 5.97	1.76 $\pm$ 0.01
Sample/Uzorak 3	79.90 $\pm$ 11.88	2.78 $\pm$ 0.02
Sample/Uzorak 4	31.20 $\pm$ 5.34	1.34 $\pm$ 0.01
Sample/Uzorak 5	94.90 $\pm$ 15.22	3.36 $\pm$ 0.02
Sample/Uzorak 6*	71.00 $\pm$ 10.01	1.92 $\pm$ 0.03

PER-protein efficiency ratio/Omjer proteinske djelotvornosti

\*Infant formula based on soy's protein isolate/Hrana za dojenčad na osnovi proteinskog izolata soje

researches should include protein quality of these formulae as well as their implementation in infant nutrition of Croatian infants.

## Conclusions

Results of dietetic survey show that almost half of examinees (49.5 %) claim that their infants are exclusively breastfed. Significant amount of examinees (33.3 %) used mixed breast-and-formula feed for their infants. There are also 17.5 % mothers whose infants are exclusively formula fed.

According to PER values in infants, *Samples 1-4* had very similar quality (PER 2.01-2.36). PER values in rats varied from 1.34 to 3.36.

In spite of different PER values for infants and rats, these differences are not statistically significant ( $p < 0.05$ ), so it can be presumed that both methods can be used equally.

## *Dijetetičko istraživanje prehrane dojenčadi i procjena omjera proteinske djelotvornosti nekih vrsta hrane za dojenčad s hrvatskog tržišta*

### Sažetak

Svrha i cilj ovog rada bili su ustanovljavanje načina prehrane dojenčadi u Hrvatskoj, kao i određivanje jednog od parametara nutritivne kvalitete, omjera proteinske djelotvornosti (PER) nekih vrsta hrane za dojenčad s hrvatskog tržišta. Metode su uključivale dijetetičko istraživanje (anketu) u kojoj je sudjelovalo 255 majki dojenčadi u urbanoj sredini, te određivanje omjera proteinske djelotvornosti (PER) metodom *in vivo* na štakorima. Rezultati ankete pokazuju da je gotovo pola ispitanica (49,4 %) izjavilo kako su njihova dojenčad hranjena isključivo majčinim mlijekom. Znatno udio ispitanica (33,3 %) koristilo je dvovrstu prehranu dojenjem i nekim od industrijski proizvedenih adaptiranih hrana za dojenčad. Samo 17,3 % majki koristilo je isključivo umjetnu prehranu. PER vrijednosti kod dojenčadi, za uzorke od 1 do 4, pokazale su ujednačenu kvalitetu i kretale su se u rasponu od 2,01 do 2,36. Kod štakora, PER vrijednosti su varirale od 1,34 do 3,36.

PER vrijednosti dobivene in vivo kod štakora i djetetičkim istraživanjem kod dojenčadi nisu pokazale statistički značajnu razliku ( $p < 0,05$ ).

**Ključne riječi:** prehrana dojenčadi, hrana za dojenčad, PER (omjer proteinske djelotvornosti)

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