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Age Dependence of the Utilization of Different Quality Proteins in Animal Experiments

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Summary

Male rats aged 45, 85, 145 and 270 days (daily body mass increments on an optimal diet containing casein were 6.73, 2.88, 0.53 and 0.31 g respectively) were fed 15 days *ad libitum* on a diet with a nutrient content physiological for their age, in which the protein source was milk casein (ratio of essential to nonessential amino acids E/N=0.79, compensation coefficient K=14) or wheat gluten (E/N=0.30, K= -8). In the case of gluten, net protein utilization (NPU) fell markedly in rapidly growing animals aged 45 and 85 days (33 and 30 % more than with casein), indicating that without essential amino acid compensation, gluten is inadequate for animals of this age, whose organism requires fully ensured proteosynthesis for growth and development. In adolescence and adulthood (145 and 270 days), the utilization of proteins is not dependent on their quality (the decrease in NPU 13 and 12 % – is nonsignificant). That means that a smaller amount of essential amino acids, including the limiting amino acid in uncompensated protein, is sufficient for the maintenance and renewal of organs and tissues, i.e. for proteosynthesis. The activation of gluconeogenesis (phosphoenolpyruvate carboxykinase activity in the liver) after the intake of plant protein confirms the effect of proteins on catabolic processes.

Key words

Optimal diet – Fast-growing rats – Adolescence – Adulthood – Net protein utilization – Milk casein – Wheat gluten – Protein quality – Gluconeogenesis

Introduction

The primary function of proteins in the organism is the utilization of alimentary proteins or their amino acids for synthesis of body proteins in the growing phase and for the renewal of tissues and organs in adulthood. Biological methods for determining the nutritional value of proteins express growth and maintenance processes in the organism and draw attention to the extent of the utilizability of proteins for anabolic processes in relation to age (Bressani *et al.* 1973, Krajčovičová and Dibák 1980, Krajčovičová-Kudláčková and Dibák 1986), the quality of the proteins (Young and Pellet 1987, Krajčovičová-Kudláčková 1990a) and the composition of food (Lynch and Jackson 1985, Krajčovičová-Kudláčková 1990b, Tanaka *et al.* 1991).

Evaluation of the quality of proteins from the proportion of essential amino acids, their ratio to non-essential amino acids and comparison with a reference protein (FAO/WHO 1973), supplemented by elucidation of the role of the limiting amino acid (Mitchell 1964), lacked numerical expression of the extent of the organism's ability to utilize the aminogram of food proteins for proteosynthesis at the expense of the essential amino acid pool and the corresponding decrease in the metabolism of these acids. Chernikov (1986) eliminated this deficiency in the evaluation of the quality of proteins by introducing the compensation coefficient.

We evaluated milk casein and wheat gluten according to the above criteria. In the following experiment we determined how the quality of proteins affects their utilization under optimal nutritional conditions differentiated in relation to the animal's age. We used rats in the phase of rapid growth (45 and 85 days), animals at the end of adolescence (145 days) and adult rats (270 days), i.e. rats with metabolic processes of different intensities.

Material and Methods

The experimental animals were male rats (Wistar strain, Velaz, Prague) aged 45, 85, 145 and 270 days – the mean age group values according to the

Table 1
Correlation of body mass to age in animals fed on an optimally composed diet with casein as the source of protein

Age	30-60 days	y=6.7268x - 141.5955	n=53	r=0.9371	p<0.001
	61-105 days	y=2.8795x + 91.8236	n=25	r=0.9775	p<0.001
	106-180 days	y=0.5296x + 329.5539	n=29	r=0.9937	p<0.001
	181-360 days	y=0.3131x + 368.3796	n=12	r=0.9699	p<0.001

Table 2
Composition of the optimal diet for casein and gluten for the means of the age ranges (age groups) given in Tab. 1

	Casein				Gluten			
	Age (days)							
Composition of diet (g/1500 g)	45-60	85-100	145-160	270-285	45-60	85-100	145-160	270-285
Proteins	15.0	11.5	10.0	7.0	21.8	16.7	14.5	10.2
Fats	20.0	11.0	10.0	9.0	20.0	11.0	10.0	9.0
Saccharide	41.0	51.0	51.0	46.0	41.0	51.0	51.0	46.0
Salt mixture	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Agar	20.0	22.5	25.0	34.0	13.2	17.3	20.5	30.8

rate of growth on the optimal diet differentiated according to age and containing casein as the source of protein (daily body mass increments 6.73, 2.88, 0.53 and 0.31 g respectively, see Tab. 1). The rats were given a diet of the optimal nutrient composition (for the relevant age group) containing casein and wheat gluten as the source of protein, for 15 days, as shown in Tab. 2. Up to the given age they were fed on a standard (Larsen) diet containing 24 % protein, 9 % fat and 46 % saccharides.

In our experiments, the optimal nutrient values were determined first of all on the basis of the maximum net protein utilization values (Krajčovičová-Kudláčková and Dibák 1986, 1989, Krajčovičová-Kudláčková 1990b) and gluconeogenesis (not yet activated by a mounting fat intake and no longer activated by a rising saccharide intake) (Krajčovičová and Dibák 1983). The amino acid content of the protein sources (milk casein – Laktos, Prague; wheat gluten – Slovenské škrobárne, Trnava) was determined by the automatic chromatographic method of Spackman *et al.* (1958). In previous experiments (Krajčovičová-Kudláčková 1990b), the dose found to be optimal for casein was modified for gluten (Tab. 2) by the multiple of the mean value of the ratio of the amino acid content of casein and gluten (excepting lysine, the limiting amino acid of gluten), i.e. by multiplying the optimal dose of casein by a factor of 1.45 (taken from Tab. 4). The fat source was margarine containing saturated and unsaturated fatty acids in the ratio 1 : 4 and the saccharide source was sucrose and wheat starch in the physiological ratio 1 : 6.2 (Krajčovičová-Kudláčková and Dibák 1989). The food also included Osborne's salt mixture and a vitamin mixture was added to it every day before being given to the experimental animals (Henry 1965).

The animals (six for every age and each type of food) were placed singly in cages with a mesh floor. For each age there was an additional group of six animals fed on a protein-free diet in which the energy represented by protein was replaced by an equivalent amount of saccharide (wheat starch). The rats were allowed food and drinking water *ad libitum*, except on the last day of the experiment, when they were deprived of food and 16 h later were killed by ether anaesthesia. Their liver was then quickly removed, cooled and samples were taken for the determination of enzymatic activity. After cleaning out their gastrointestinal tract and determining their total dry mass (at 105 °C to constant mass), the carcases were homogenized and body nitrogen was determined by Dumas's method (Vondenhof and Schulte 1979). Net

protein utilization (NPU; Miller and Bender 1955) was determined from the amount of retained nitrogen in relation to nitrogen intake during the given observation period.

$$\text{NPU} \frac{\text{retained nitrogen}}{\text{nitrogen intake}} \times 100 =$$

$$= \frac{\text{body nitrogen of animals on the test diet (g)} - \text{body nitrogen of animals on the protein-free diet (g)} \times 100}{\text{nitrogen intake (g)}}$$

Phosphoenolpyruvate carboxykinase (PEPCK, E.C.4.1.1.32) activity in the liver was determined by UV spectrophotometry after Flores and Alleyne (1971). For computing specific activity, the proteins in liver homogenates were determined by the biuret reaction according to the Laboratory Handbook, using the standard protein from the Bio-test (Lachema).

The nitrogen content of the casein, gluten and food was determined by Dumas's method. The protein content equalled N x 6.25. The fat content of the margarine and food was determined by the Soxhlet extraction method on a Soxtec automatic fat analyser. The saccharide content of the starch, sucrose, casein, gluten and food was determined according to Schoorl (Příbela 1978). The amount of nutrients in the food varied within permissible limits (error 0.3 to 0.6 %),

Results

Tab. 3 shows the essential amino acid content (+ cystine and tyrosine) of casein and gluten compared with the reference protein (whole hen's egg). The proportion of essential amino acids is 94 % in casein and 52 % in gluten. The amino acid score for every amino acid has been computed, together with its difference in relation to the limiting amino acid, in both protein values; for casein they are amino acids containing sulphur and for gluten lysine. The mean value of differences in the amino acid score is 24 for casein and 33 for gluten, while the respective potential biological values are 76 and 67. The differences between the experimental and potential biological value yields a compensation coefficient (K) of 14 for casein and of -8 for gluten.

Tab. 4 shows the composition of the optimal dose of gluten. It gives the essential and non-essential amino acid content of casein and gluten. The ratio of these amino acids is 0.79 for casein and 0.30 for gluten. The ratio of the casein and gluten amino acid content (the mean value with the exception of lysine) is 1.45; the gluten amino acid values are multiplied by this factor. After adjustment of the gluten amino acid content, the mean value of the amino acid ratio in the two proteins is 1.01.

Tab. 1 explains the choice of the four given age categories. Animals fed on the optimal diet containing casein and aged 30-360 days were divided according to their growth rate. Expression of the linear course of the animals body mass according to age by means of regression equations allowed their division into four groups. The mean body mass increment from 30 to 60 days was 6.73 g/day (the slope of the regression line), for the second age category (61-105 days) it was 2.88 g, for the third group (106-180 days) it was 0.53 g/day and from 181 days onwards it was only 313 mg/day. Tab. 2 gives the optimal composition of the food (for both casein and gluten) for the mean values of the above four age ranges.

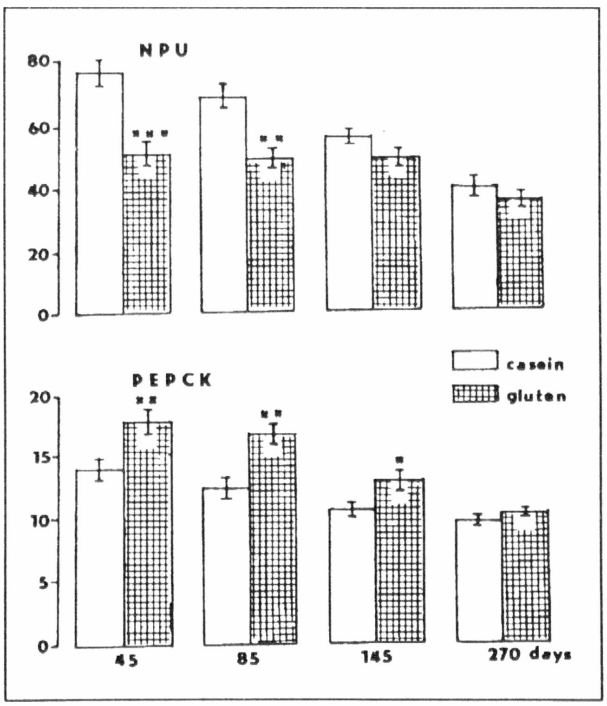

Fig. 1

Net protein utilization and specific phosphoeniolpyruvate carboxykianse (PEPCK) activity (μmol PEP/min/g protein) in rats aged 45, 85, 145 and 270 days on a diet with the optimal nutrient composition and with milk casein or wheat gluten as the source of protein $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

Table 3

Essential amino acids, cystine and tyrosine values (μmol.g^{-1} protein) in whole egg, milk casein and wheat gluten, the amino acid scores for the individual (AS_x) and total (AS) amino acids in relation to egg, the AS difference in relation to the limiting amino acids (DAS), the coefficient of the AS difference (CDAS), the potential and the experimental biological value (BV_p, BV_e) and the compensation coefficient (K)

Amino acid	Egg*	Casein n=6	ASx	DAS	Gluten n=6	ASx	DAS	
His	155	168±26	108	39	99±6	64	46	
Ile	503	374±43	75	6	247±20	49	31	
Leu	671	757±26	113	44	539±19	80	62	
Lys	438	514±16	118	49	80±4	18	0	
Met	208	179±16			80±5			
Cys/2	96	30±2				75±3		
Met+Cys/2	304	209.0		69	0	155.0	51	33
Phe	351	338±13			265±16			
Tyr	237	317±10			145±8			
Phe+Tyr	588	655.0	112	43	410.0	70	52	
Thr	428	302±23	71	2	152±9	36	18	
Val	632	312±22	81	12	259±16	41	23	
AK	3719	3491			1941			
AS		94			52			
CDAS		24			33			
100-CDAS-BVp		76			67			
BV_e**		90			59			
K		14			-8			

* – FAO/WHO 1973, ** – Chernikov 1986

Fig. 1 shows the net protein utilization (NPU) values and specific phosphoenolpyruvate carboxykinase (PEPCK) activity in the liver after 15 days' administration of the optimal diet. The casein NPU value is highest at the age of 45 days and then gradually falls – only slightly in the next age group, by 28 % in the third group (in which growth is much slower) and by 49 % in the 270-day-old group (adult animals whose growth is virtually completed). Gluten utilization in the first three age groups (i.e. animals with a steadily diminishing growth rate) is almost the same, whereas it falls by 29 % in adult animals compared with the 145-day-old group. The casein NPU and gluten NPU values display significant differences at 45 and 85 days; differences in the last two age groups are not significant. The utilizability of gluten is 33 and 30 % lower than that of casein at 45 and 85 days respectively, and 13 and 12 % lower in animals with slower and almost completed growth.

The course of gluconeogenesis is recorded in the lower part of Fig. 1. It shows that PEPCK activity is significantly higher for gluten in 45- and 85-day-old animals and that it borders on significance at 145 days and is nonsignificantly raised in the last age group.

Discussion

To be able to synthesize body proteins, the organism needs to be supplied with all the essential amino acids in the same amount and same ratio to the non-essential amino acids as in the reference protein (a hen's egg or cow's milk) (FAO/WHO 1973, FAO/WHO/UNU 1985). This is the only way in which amino acids can be utilized adequately for proteosynthesis. The ratio of essential to non-essential amino acids in a protein or a mixture of proteins should be close to one; the aminogram of the protein mixture is then balanced and can be utilized by the alimentary tract in full for protein synthesis.

Another step forwards in the evaluation of the value of proteins since Thomas (1909) introduced the concept of their nutritional value was the elucidation of the role of the limiting amino acid (the one present in the smallest amount) in proteosynthesis (Mitchell 1964). The first deficient amino acid determines the

Table 4
Amino acid content of casein and gluten (mg.g^{-1} protein), adjustment of the amino acid content of gluten and the ratio of essential to non-essential amino acids (E/N)

Amino acid n=6	Casein (K)	Gluten (G)	K/G	G x 1.45	K/G x 1-45
His	28±1	15±1	1.86	22	1.27
Ile	42±3	33±3	1.27	48	0.88
Leu	107±4	71±3	1.51	103	1.04
Lys	81±3	12±1	6.75	17	4.76
Met	29±1	12±1	2.41	17	1.71
Phe	56±2	44±3	1.27	64	0.88
Thr	34±3	18±1	1.89	26	1.31
Val	65±3	30±2	2.16	44	1.48
Tyr	64±4	26±2	2.46	38	1.68
Cys	7±1	18±1	0.39	26	0.27
Arg	35±2	31±2	1.13	45	0.78
Glu	190±7	478±33	0.40	693	0.27
Pro	120±5	124±9	0.97	180	0.67
Ser	51±3	38±3	1.34	55	0.93
Ala	26±1	23±1	1.13	33	0.79
Asp	62±4	24±1	2.58	35	1.77
Gly	15±1	29±2	0.52	42	0.36
		Mean K/G = 1.45 (-Lys)		Mean K/Gx l.45 = 1.01 (-Lys)	
E/N	0.79	0.30		0.30	

degree of the productive utilization (for plastic, anabolic processes) of all the other essential amino acids. The amount of certain essential amino acids in proteins of vegetable origin is low (e.g, lysine in cereals, methionine and cystine in pulses), with resultant amino acid imbalance (a low E/N ratio) and low proteosynthesis, since according to Mitchell (1964) it is the limiting amino acid which initiates synthesis of the peptide chain.

In this study, a comparison of casein and gluten showed the ratio of essential to non-essential amino acids to be 0.79 and 0.30 respectively and the proportion of the limiting acid lysine in gluten to be only 15 % of its proportion in casein. After adjusting the dose of gluten in the food for the various age groups to conform to the optimal casein value by multiplying it by 1.45, the mean value of the casein/gluten amino acid ratio (the optimal dose of gluten), the proportion of lysine rose to 21 % and the gluten aminogram became generally more compensated in relation to casein, with an increase of 106 mg in the amount of essential amino acids *per* gramme protein. This did not, however, abolish the lysine debt, which was manifested in lower utilization of gluten. Moreover, according to the latest evaluation, this amino acid, together with threonine, is absolutely indispensable (Jackson 1983). Adjustment of the dose of gluten in relation to casein by the factor 1.45 corresponds to the results of biological experiments on rats of different ages (Dibák *et al.* 1984, 1985, 1986). In these reports, the authors used mounting doses of alimentary proteins according to the peak of linearity between changes in body mass or body nitrogen in relation to protein intake and age, and found a casein/gluten ratio of 1.43.

The intake of lower quality gluten markedly reduced utilization at 45 and 85 days (by 33 and 30 %), i.e. in rapidly growing animals with a daily body mass increment of 6.73 and 2.88 g on the optimal diet containing animal proteins, which require a balanced amino acid mixture for full proteosynthesis, needed for the building and development of the organism. Milk proteins (casein) have this type of amino acid mixture. The casein utilization values for young animals are high and correspond to the experimental biological value (FAO/WHO 1973, Chernikov 1986).

In rats at the end of adolescence (aged 145 days, body mass increment 530 mg/day) and in adult, fully grown animals (270 days, 313 mg/day), the decrease in the gluten NPU value is nonsignificant (13

and 12 %). Animals of this age need alimentary proteins primarily for body maintenance and for the renewal of tissues and organs (Krajčovičová-Kudláčková and Dibák 1988, 1989). In adulthood, therefore, protein utilization is practically independent of the quality of the protein under optimal nutritional conditions, i.e. proteosynthesis takes place on a smaller scale at this age, so that the essential amino acid values – including the limiting amino acid – in uncompensated proteins are sufficient.

The elevated gluconeogenesis values found in the presence of a plant protein intake confirm that these proteins are used for catabolic processes (reduced NPU values), i.e. that they are broken down by way of glucose formation by a raised glucoplastic amino acid content (30 % higher than for casein - Krajčovičová-Kudláčková 1990a).

Chernikov (1986), using Mitchell's principle, introduced a new way of evaluating the quality of proteins by the compensation coefficient K, preceded by the coefficient of amino acid score difference, CDAS. The latter is the mean value of differences in the eight essential amino acids (including cystine and tyrosine) compared with the reference protein. The lower the CDAS value, the higher the quality of the protein from the aspect of its primary function in the organism (24 – casein, 33 – gluten), because CDAS represents the essential amino acid residue which is not utilized for the plastic needs of the organism and is utilized for protein catabolic processes. The degree of the productive utilization of essential amino acids (for proteosynthesis) or the potential (theoretical) biological value, BV_p, equals 100 – CDAS, The difference between the experimental and the theoretical biological value is the protein compensation coefficient, which numerically expresses the degree to which the organism is capable of utilizing the aminogram of the alimentary proteins at the expense of the essential amino acid pool and the corresponding decrease in their metabolism. In Chernikov's classification, group 1 comprises reference proteins with a high K (20-35) and group 2 the remaining animal proteins, together with soya, further pulses and potatoes (K close to zero, but a high BV_p value), while group 3 include cereal proteins with essential amino acid imbalance, with a low BV_p and low K values (in the region of zero in both a positive and a negative direction). The compensation coefficient for casein (14) and for gluten (-8) confirms the values of the biological experiment. The results also document the application of this classification of proteins to the young, developing organism. In adulthood, when a lower degree of proteosynthesis is sufficient the differences between the various groups of protein classes are obliterated.

In conclusion, it should be noted that, in the phase of simultaneous development of "alternative" nutrition, it must be borne in mind that for essential proteosynthesis – and hence for construction – the developing organism needs a full complement of amino acids.

References

BRESSANI R., VITERI F., ELUAS L.G.: The usefulness of results with human being in standardizing rat assays. In: PORTER J.W.C., ROLLS A.B. (eds) Protein in Human Nutrition. London-NewYork, Academic press 1973, 560 pp.

CHERNIKOV M.P.: On rational classification of food proteins (in Russian), *Vop. Pitan.* **55**: 68-70, 1986.

DIBÁK O,, KRAJČOVIČOVÁ M., KOPEC Z., GRANČIČOVÁ E.: Physiological casein and gluten protein requirements of growing rats. *Physiol. Bohemoslov.* **33**: 320-335, 1984.

DIBÁK O., KRAJČOVIČOVÁ M., GRANČIČOVÁ E, KOPEC Z.: Determination of physiological casein and wheat gluten protein requirements of adult rats aged 75-89 days. *Physiol. Bohemoslov.* **34**: 161-171, 1985.

DIBÁK O., KRAJČOVIČOVÁ-KUDLÁČKOVÁ M., GRANČIČOVÁ E., JANKOVIČOVÁ M.: Body composition and physiological casein and wheat gluten protein requirements of 180-day-old rats. *Physiol. Bohemoslov.* **35**: 71-79, 1986.

FAO/WHO: Energy and Protein Requirements. Rep. joint FAO/WHO *ad hoc* committee. Geneva, FAO/WHO 1973, 118 pp.

FAO/ WHO/UNU: Energy and Protein Requirements. Rep, joint FAO/WHO/UNU meeting. Geneva, WHO 1985.

FLORES H., ALLEYNE G.A.O.: Phosphoenolpyruvate carboxykinase of kidney. *Biochem. J.* **123**: 35-39, 1971.

HENRY K.P.: A comparison of biological method with rats for determining the nutritive value of proteins. *Brit. J. Nutr.* **19**: 125-135, 1965.

JACKSON A.A.: Amino acids – essential and non-essential? *Lancet* **1**: 1034-1037, 1983.

KRAJČOVIČOVÁ-KUDLÁČKOVÁ M.: Utilization of protein of different quality and fat intake in animal experiments (in Slovak), *Čs. Gastroent. Výž.* **44**: 498-505, 1990a.

KRAJČOVIČOVÁ-KUDLÁČKOVÁ M.: The age dependence of protein utilization on the intake of fats of different quality. *Veter. Med.* **35**: 365-372, 1990b (in Slovak).

KRAJČOVIČOVÁ M., DIBÁK O.: Protein utilization in correlation to protein intake. *Physiol. Bohemoslov.* **29:** 37-47, 1980.

KRAJČOVIČOVÁ M., DIBÁK O.: Protein utilization and gluconeogenesis under different nutritional conditions (in Slovak), *Bratisl. lék. Listy* **79:** 211-221, 1983.

KRAJČOVIČOVÁ-KUDLÁČKOVÁ M., DIBÁK O.: The relationship between protein retention and energy requirements in rats of different ages. *Physiol. Bohemoslov.* **35:** 243-250, 1986.

KRAJČOVIČOVÁ-KUDLÁČKOVÁ M., DIBÁK O.: Protein retention in relation to age (in Slovak), *Veter. Med.* **33:** 55-63, 1988.

KRAJČOVIČOVÁ-KUDLÁČKOVÁ M., DIBÁK O.: Protein utilization with regard to age and diet (in Slovak), *Čs. Gastroent. Výž.* **43:** 244-250, 1989.

LYNCH G.P., JACKSON C.JR.: Serum amino acids as indicators of protein status in gestating ewes. *Growth* **49:** 306-317, 1985.

MILLER D.S., BENDER A.E.: The determination of the net utilization of proteins by a shortened method. *Brit. J. Nutr.* **9:** 382--388, 1955.

MITCHELL H.H.: Comparative nutrition of man and domestic animals. New York, Academic Press 1964, pp. 595-604.

PRÍBELA A.: Analysis of natural substances in foodstuffs (in Slovak), Bratislava, Alfa 1978, pp. 430.

SPACKMAN D.H., STEIN W.H., MOORE S.: Automatic recording apparatus for use in the chromatography of amino acids. *Anal. Chem.* **30:** 1190-1206, 1958.

THOMAS K.: Uber die biologische Wüertigkeit der Stickstoffsubstanzen in verchiedenen Nahrungsmitteln. *Arch. Anat. Physiol.* 1909, 219-302.

TANAKA H., NAKATOMI Y., TAKAHASHI K., OGURA M.: Metabolism of lysine and tryptophan in growing rats at varioua dietary protein levels, *Agr. Biol. Chem.* **55:** 531-537, 1991.

VONDENHOF T., SCHULTE K.: Bestimmung des Stickstoffs bei Fleischerzeugnissen nach dem Kjeldhal- und Dumaa-Verfahren. *Dtach. Lebensm.-Rundsch.* **75:** 187-189, 1979.

YOUNG V.R, PELLET P.L.: Protein intake and requirements with reference to diet and hea1th. *Am. J. Clin. Nutr.* **45:** 1323-1343, 1987.

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