Iran. J. Agric. Res. Vol. 3, No. 1, 1975

BIOLOGICAL AND ORGANOLEPTIC PROPERTIES OF MILK PROTEIN AS AFFECTED BY DILUTION WITH NON-ESSENTIAL NITROGEN SOURCES[1]

S. Mozayani and S.S. Akrabawi[2]

ABSTRACT

Three experiments were conducted to study the effects of 10 and 20% isonitrogenous replacement (dilution) of milk protein in the diet with four non-essential nitrogen sources namely, L-alanine, L-glutamic acid, glycine and urea on the growth rate and operative net protein utilization (NPUop) of rats. The diluted milk samples were also evaluated organoleptically.

The results indicated that 10% dilution of milk protein with L-alanine slightly reduced the growth of rats and the overall acceptability of milk. Significant ($P<0.05$) reduction in growth rate of rats, flavor and overall acceptability occurred when L-alanine was substituted for 20% of the milk protein. The growth rate, NPUop, flavor and overall acceptability were adversely affected with each level of L-glutamic acid substitution. Glycine improved significantly the flavor and overall acceptability of the diluted milk. The NPUop utilization was not affected with the 10 or 20% glycine dilution of milk protein. Growth, however, in 2 out of the 3 experiments, was reduced with 10% dilution. Urea was the only source which did not bring about any significant change in the growth rate as well as the NPUop at the level of 10% dilution. Furthermore, such level of dilution did not cause any change in the overall acceptability of liquid milk.

---

1. Contribution from the Department of Food Technology and Nutrition, Facully of Agricultural Sciences, American University of Beirut, Beirut, Lebanon. Part of thesis submitted by the senior author in partial fulfillment of the reguirements for the M.S. degree.

2. Graduate student and Assistant Professor of Nutrition, respectively, Department of Food Technology and Nutrition, American University of Beirut, Beirut, Lebanon. Present addresses: Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Mass., USA and Department of Nutrition and Biochemistry University of Tennessee, Nashville, USA, respectively.

INTRODUCTION

The 1965 FAO/WHO (4) Expert Group on Protein Requirement stated that the essential amino acids content per g nitrogen in most food proteins of high biological value is higher than that required by children and young adults. It is then possible that some of the protein in milk can be isonitrogenously replaced with non-specific N sources without altering the retention and utilization of N by human as well as experimental animals (7,8,9). However, another group of investigators (2,3) reported that the isonitrogenous replacement of protein with some of these non-specific N sources produced significant decrease in gorwth rate and protein efficiency ratio in the rat and also a drop in the biological value of the protein.

This study was initiated because of the discrepancies in the literature on the effect of the dilution of milk protein with non-essential N sources. The purpose of this study was to investigate the biological and organoleptic properties of skim milk protein as affected by dilution with L-alanine, L-glutamic acid, glycine or urea. The parameters used for judging the effects of this dilution were growth rate of rats and operative net protein utilization (NPUop). Organoleptic properties of the diluted liquid milk samples were judged by a laboratory taste panel.

MATERIALS AND METHODS

Twenty-two-day old albino rats of the Sprague-Dawley strain were used throughout the experiments. The rats were given a stock diet for one week. They were divided into groups of similar average weight. Each rat was housed individually in a stainless steel cage located in a temperature-controlled room with the temperature set at 25 $\pm$ 4C. Water and food was provided *ad libitum*. The feeding trials lasted for 10 to 12 days. Food consumption, spillage and weight gain were recorded daily.

The percentage composition of the basal 15% protein and the protein-free diet is shown in Table 1. Dried skim milk (Regilait, France-Lait Company, France) provided the protein in the basal diet. The composition of the experimental diet in which L-alanine , L-glutamic acid, glycine or urea replaced isonitrogenously 10% and 20% of the protein of the skim milk in the basal diet is shown in Table 2. Diets 1,2,3,4,5,6,7 and 8 were modified from the basal diet in that 10 and 20% of the N was replaced with L-alanine in diets 1 and 2, L-glutamic acid in 3 and 4, glycine in 5 and 6 and urea in 7 and 8. To replace isonitrogenously 10% of the N in the basal diet, 1.52, 2.25, 1.28 and 0.52% of L-alanine, L-glutamic acid, glycine and urea were used, respectively. The amounts were doubled for

Table 1. Percentage composition of the basal and protein-free diets.

Ingredients	Basal	Protein–free
Skim milk – Regilait*	45	–
Corn oil	10	10
Salt mixture †	05	05
Vitamin mixture**	02	02
Corn starch	19	83
Dextrine	19	83

* Regiliat milk, N x 6.25 = 34.93% protein.

† USP XIV Salt Mix., General Biochemical (CBI), Chagrin Falls, Ohio.

** Obtained from Nutritional Biochemicals Corporation (NBC), Cleveland, Ohio, and provided the following in mg per kg of diet: vitamin A concentrate (200,000 units per g), 90.0; vitamin D concentrate (400,000 units per g), 5.0; alpha tocopherol, 100; ascorbic acid, 900; inositol 100; choline chloride, 1,500; menadione, 45.0; P-amino-benzoic acid, 100.0; niacin, 90; riboflavin, 20; pyridoxine hydrochloride, 20; thiamin hydrochloride, 20; calcium pantothenate, 60.0; biotin, 0.4; folic acid, 1.8; and vitamin B-12, 0.027 mg.

Table 2. Percentage compostition of experimental diets.

Ingredients	Diets							
	1	2	3	4	5	6	7	8
Skim milk	40.50	36.00	40.50	36.00	40.50	36.00	40.50	36.00
L-alanine	1.52	3.04	–	–	–	–	–	–
L-glutamic acid	–	–	2.52	5.04	–	–	–	–
Glycine	–	–	–	–	1.28	2.56	–	–
Urea	–	–	–	–	–	–	0.52	1.04
Corn oil	10	10	10	10	10	10	10	10
Salt mixture *	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin mixture *	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Corn starch	20.49	21.98	19.99	20.98	20.61	22.22	20.99	22.98
Dextrine	20.49	21.98	19.99	20.98	20.61	22.22	20.99	22.98

* For composition see Table 1.

the 20% replacement.

For the determination of NPUop, rats in groups of 5 were randomized among the experimental treatments so that the groups had the same average weight and weigh distribution. They were fed the basal experimental or the protein-free diets for 10 days and were killed by chloroform anesthesia according to the procedure of Miller (5). The basal experimental diets, however, contained 15% protein rather than the suggested 10%. The N of the diets and the dry carcasses was determined by the macro-Kjeldalh method (1).

For the organoleptic testing of the undiluted milk sample (control), 45 g of the dried skim milk were dissolved in 450 ml of chilled (5 C) water and served to panelists. For the diluted samples, 1.52 and 3.04 g of L-alanine were added to 40.5 and 36.0 g of the dried skim milk to formulate the 10 and 20% diluted milk samples, respectively. Each mixed sample was also dissolved in 450 ml of chilled water and served to panelists. The same procedure was followed in the case of the other N sources with their respective amounts added to the dried skim milk for the formulation of the 10 and 20% dilution. The panelists tested each time two levels of diluted milk against a non-diluted control. The samples were evaluated according to a 10 point hedonic scale. The factors evaluated were color, flavor, odor and overall acceptability.

Results from all experiments were analyzed statistically by analysis of variance (10). Difference among mean values were assessed using the Duncan's Multiple Range Test (12). All results were reported at the 5% level of probability. The t-test was used for comparing the differences of means obtained from NPUop in Experiments II and III.

RESULTS AND DISCUSSION

Data from Experiments I, II and III are shown in Table 3. In Experiment I, rats fed the basal diet gained an average of 81.8 g in 12 days. This weight gain was not significantly different from that of groups receiving 10 and 20% dilution with L-alanine, 10% with glycine and 10% with urea. Ten percent dilution with L-glutamic acid affected the growth of rats adversely. This is in agreement with Daniel (2) who stated that the addition of L-glutamic acids to diets containing 5 to 8.5% milk protein decreased growth rate. In Experiment II, all the groups receiving the diluted milk protein diets gained less weight than those receiving the basal diet. This is in agreement with the report of Young and Villarreal (13). The net protein utilization (NPUop) of the basal diet, however, was not affected by

Table 3. Mean values per group for weight gain (Wt.), food consumption (FC) and operative net protein utilization (NPUop) in Experiments I, II and III.

Variable*		Diets									
		Basal	1	2	3	4	5	6	7	8	S.E.
Wt.,g	I	81.8ab†	77.5abc	67.7bc	62.8c	72.3abc	86.0a	68.8abc	–	–	5.62
	II	59.4a	–	–	47.2b	43.0b	46.8b	41.8b	–	–	3.38
	III	64.4a	–	–	53.0bc	44.8c	52.0bc	51.4bc	59.4ab	57.8ab	3.57
FC, g	I	165.2a	177.0a	166.2a	160.5a	171.5a	186.7a	175.3a	–	–	8.72
	II	128.8a	–	–	119.2ab	114.8ab	110.8b	109.2b	–	–	5;27
	III	135.0a	–	–	130.6a	128.2a	131.2a	135.8a	140.0a	145.0a	7.17
NPUop	II	66.7	–	–	68.1	57.0	58.5	64.6	–	–	–
	III	58.8	–	–	60.8	49.5	55.3	50.8	58.8	54.8	–
	Average	62.8a	–	–	64.4a	53.2b	56.9a	57.7a	–	–	–

* Experimental period for Experiment I was 12 and for Experiments II and III 10 days. Mean intitial weight in Experiment I was 138 g, in Experiment II, 75 and in Experiment III, 80 g. Number of rats/treatment in Experiment I was 6 and in Experiments II and III, 5.

† Means not followed by the same letter in each group are significantly different (P<.05).

the 10% L-glutamic acid and the 10 or 20% glycine dilution but was decreased drastically when the milk protein was diluted with 20% L-glutamic acid.

Experiment III was a duplicate of Experiment II except for adding two more test diets namely, Nos. 7 and 8. Similar results to those of Experiment II were obtained with respect to weight gain. All levels of dilution except the 10 and 20% urea affected weight gain adversely. This may suggest that in the case of rats as well as ruminants (11,6), urea is easily utilized for synthesis of body proteins. Net protein utilization of urea diluted milk protein was quite similar to that of the undiluted protein. The 10% dilution of milk protein with any non-specific N source did not affect the NPUop as much as the 20% diet. Only the diet with 20% L-glutamic acid dilution had a significantly lower NPUop than the basal undiluted one. Statistical analysis on the effects of urea dilution could not be carried out. Results on urea, however, indicated strongly that dilution with urea does not affect NPUop at the levels used.

The organoleptic properties of the diluted milk samples were evaluated by a taste panel against the undiluted milk (Table 4). No change in the milk color and odor resulted from either level of dilution regardless of the source of N. Ten or 20% dilution with L-glutamic acid resulted in the curdling of milk. The 10% dilution with L-alanine did not alter the flavor and overall acceptability of milk although 20% did. Ten or 20% dilution with L-glutamic acid resulted in extremely strong rejection by all panelists. Since weight gain and NPUop were also adversely affected with each dilution of L-glutamic acid, it can be concluded that this amino acid is not suitable for the purpose of dilution.

In the case of human feeding, glycine could be a good source of N replacement (7,8) because it gave a sweet flavor to milk and was more preferred by the panel than the undiluted milk. Twenty percent dilution with this simple amino acid gave the highest scores of flavor and overall acceptability which were significantly higher than the undiluted control. Further, the 10% glycine diluted milk scored higher with respect to flavor than the control. It is unfortunate that in 2 out of the 3 experiments, the 10% dilution with glycine caused a significant reduction in weight gain in rats. The results in the case of glycine were encouraging with respect to NPUop, flavor and overall acceptability but not with respect to support of weight gain.

Twenty percent dilution of milk protein with urea caused a significant reduction

Table 4. Effect of 10 or 20% dilution of skim milk protein with L-alanine (Ala), L-glutamic acid (Glu), glycine (Gly) or urea (Ure) on color, odor, flavor and overall acceptability.

Samples	Mean scores*			
	Color	Odor	Flavor	Overall acceptability
Control	7.4a†	7.2a	7.3a	7.3a
10% Ala	6.9a	6.5a	6.0a	6.4a
20% Ala	7.2a	5.9a	4.3b	5.1b
Control	7.0a	6.6a	6.4a	6.4a
10% Glu	7.0a	5.8a	2.4b	2.9b
20% Glu	6.9a	6.1a	1.2b	2.4b
Control	7.5a	6.5a	6.6b	6.8b
10% Gly	7.7a	6.6a	7.8a	8.0a
20% Gly	7.8a	6.8a	7.9a	8.0a
Control	7.5a	7.5a	8.1a	8.0a
10% Ure	7.4a	7.4a	7.1b	7.3a
20% Ure	7.5a	7.2a	5.7c	6.8b

* Mean of 10 scores.

† Scores not followed by the same letter are significantly different ($P < .05$).

in the flavor scores and overall acceptability. Overall acceptability of the 10% urea dilution milk protein, however, was similar to the undiluted control. These results as well as the NPUop results for urea in Experiment III suggest that 10% dilution of milk protein with urea could be established without adversely affecting the biological and organoleptic values of milk protein. Urea/ g N is the cheapest source of N (0.006 US $/g) and therefore the dilution is economically feasible and highly preferable.

LITERATURE CITED

1. A.O.A.C. 1965. Official Methods of Analysis. 10th ed. Association of Official Agricultural Chemists, Washington, D.C.
2. Daniel, V.A., B.L.M.Desai, S. Venkat Rao, M. Swaminathan and H.A.B. Parpia. 1968. Dilution of cow's milk and egg proteins with glutamic acid and the effect on the protein efficiency ratio. Food Sci. 33: 432-435.
3. Daniel, V.A., T.R. Doraiswamy, M. Swaminathan and D. Rajalakshmi. 1970. The effect of dilution of milk proteins with non-essential amino acids (L-alanine and L-glutamic acid) on nitrogen retention and biological value of the proteins in children. British Nutr. 24: 741-747.
4. F.A.O. 1965. Protein Requirements. Joint F.A.O./W.H.O. Report Series No. 37 of Expert group on Protein Requirements. F.A.O. Nutrition Meetings. F.A.O. of the U.N., Rome.
5. Miller, D.S. 1963. A procedure for determination of NPU using rats body N technique. In Evaluation of Protein Quality. Nat. Acad. Sci. Publ. No. 1100, Washington, D.C.
6. Reid, J.T. 1953. Urea as a protein replacement for ruminants: A review. Dairy Sci. 36: 955-991.
7. Scrimshaw, N.S. and M.J. Forman. 1971. Extending protein concentrates for child feeding by adding of inexpensive simple nitrogen sources. Final Report. Contract No. AID/csd - 1441.
8. Scrimshaw, N.S., V.R. Young, P.C. Haung, C. Thanangkul and B.V. Cholakos. 1969. Partial dietary replacement of milk protein by non-specific nitrogen in young men. J. Nutr. 98: 9-17.
9. Scrimshaw, N.S., V.R. Young, R. Schewartz, M.L. Piche and J.B. Das. 1966. Minimum dietary essential amino acid to total nitrogen ratio for whole egg protein fed to young men. J. Nutr. 89: 8-18.
10. Snedecor, G.W. and W.G. Cochran. 1967. Statistical Methods. 6th ed. Iowa State

Univ., Ames, Iowa.

11. Watson, C.J., W.M. Davidson and J.W. Kennedy. 1949. The nutritive value of nitrogenous compounds for ruminants. II. The formation of body protein from urea labelled with isotope N^{15}. Sci. Agric. 29: 185-199.
12. Weiner, B.J. 1962. Statistical Principles in Experimental Designs. 3rd ed. McGraw Hill Book Co.,New York and London.
13. Young, V.R. and A. Villarreal. 1970. Effect in rats of partial replacement of cow's milk protein by supplementary nitrogen. J. Food Sci. 35: 170-174.