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EVALUATION OF PROTEIN QUALITY USING MICE VS. RATS

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EVALUATION OF PROTEIN QUALITY USING MICE VS. RATS

by

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As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Zafrallah Taha Cossack

entitled Evaluation of Protein Quality Using Mice Vs. Rats

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

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Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

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ABSTRACT

Experiments were designed to investigate the possibility of using mice as model animals for the evaluation of protein quality, and to compare it with rats under the same conditions. Parameters measured were protein efficiency ratio, net protein ratio, protein digestion and relative protein value. Six sources of protein were tested at three dietary levels for each. Measurements were taken at four different periods of feeding, thus, the effects of dietary level of protein and the length of the feeding period were studied.

Results on mouse experiment indicated that the highest PER values were obtained at 6 and 8% levels of dietary protein with significantly lower values when 11% levels were fed. The highest NPR values were obtained at 6% level of dietary protein then declined when 8 or 11% levels were fed. The highest efficiency regarding PER and NPR were obtained when mice were fed for 10 days than when they were fed for 14, 21, or 28 days of experiment. High quality protein sources were needed at lower levels in the diet for shorter periods of time to obtain the maximum efficiency when compared to poor quality sources of protein. Results for protein digestion did not correlate with those of PER,

NPR or RPV indicating that protein digestion is a poor measurement. However, PER, NPR, and RPV were highly correlated.

Results of rat experiment were in agreement with what was reported in the numerous works for rat bioassay in the literature review. Rate of body weight gain increased with increasing levels of dietary protein and the PER reached a maximum value, then decreased. Values of NPR tended to fall with increasing levels of dietary protein. PER values tended to increase gradually with prolonged period of feeding, then decline. The maximum PER values were obtained when 10% level of dietary protein was fed for a period of 15 days. Likewise mice experiment, PER, NPR, and RPV correlated highly while protein digestion correlated poorly with the other methods used.

In general mice appeared to be influenced by the same factors as rats when used for the evaluation of protein quality. Mouse could be used as a model animal for protein quality evaluation with the advantages of small animal size, lower feed intake, shorter period of feeding, plus is highly desirable for experiments involving the use of isotopes or whole carcass analysis. A dietary protein level of 8% for a feeding period of 10 days would be suitable for use with mice instead of a 10% dietary level for a 28 day feeding experiment in rats. Whole egg could be used as a suitable reference standard protein for mouse bioassay.

CHAPTER 1

INTRODUCTION

Techniques for the biological evaluation of proteins were quickly developed in the early years of this century; these were based on nitrogen balance (Munro, 1964; Mitchell 1924) and on rat growth (Osborne, Mendel, and Ferry (1919). It took until the 1940's, however, before analytical techniques were sufficiently advanced for amino acids data to be used in the prediction of protein quality (Block and Mitchell, 1946). The most extensively used method for the determination of nutritive quality of proteins for growth is the protein efficiency ratio (PER) method of Osborne, Mendel, and Ferry (1919), where rats have been the most widely used laboratory model, and its amino acids requirements for maintenance and growth have been well established; however, the assessment of the nutritive value of proteins by growing rats has progressed little beyond the contribution of Osborne and co-workers.

With the advent of nutritional labeling regulations, the food industry and the compliance and regulatory agencies have a real need for a rapid and easy means of predicting protein quality. Since the growth rate of the mouse is

approximately twice as much as that of the rat, using mice for the evaluation of protein quality will decrease the time necessary for growth measurements. This, coupled with smaller feed requirements, would result in saving time, labor, and dietary ingredients.

The mouse likewise lends itself to studies involving expensive materials or those difficult to prepare such as isotopically labeled compounds. For studies involving the whole carcass, the size of the mouse lends itself to simplified procedures for total carcass analysis.

Less is known about the nutritional requirements of mice than of rats, probably because mice have been employed less extensively in nutritional research. One of the objectives of this work is to study the possibility of employing mice in protein evaluation studies using different protein sources and trying to establish a comparison between employing mice vs. rats in protein evaluation. Protein efficiency ratio (PER), net protein ratio (NPR), relative protein value (RPV), and protein digestion were used for comparison between rats and mice where high quality protein sources, e. g., lactalbumin and low quality protein sources, e.g., white beans were evaluated. Although the absolute maximal values are not necessarily the same for both mice and rats, proteins should bear the same relative nutritional value for each other when assayed by rats or by mice.

CHAPTER 2

LITERATURE REVIEW

Bioassay Procedures for Protein Quality Evaluation

Protein Efficiency Ratio (PER)

Osborne et al. (1919) proposed this method for expressing numerically the growth-promoting value of protein sources. The PER is calculated by the formula:

$$\text{PER} = \frac{\text{weight gain (g)}}{\text{protein consumed (g)}} .$$

Protein efficiency ratio is considered as the official procedure for regulatory purposes within the U. S. The standard AOAC (1975) procedure requires the use of at least ten weanling rats, crude protein level of 10%, four weeks duration of growth period, and casein is used as a standard protein for this method. Despite its long history, wide usage, and official status, PER is not a good assay procedure (Hegsted and Chang, 1965; Hegsted and Samonds, 1978). It does not specify the strain of rat to be used in the bioassay since growth rates and metabolic patterns are known to vary among the genetic strain in rats, Morrison and Campbell (1960). The level of protein would influence the efficiency by which it was utilized by growing animals (Barnes et al., 1945).

Morrison and Campbell (1960) also noted that the PER of casein and plant protein (85% wheat - 15% soy) were dependent upon the quantity of protein fed. Moreover, the PER of the lower quality plant protein was lower when fed at the 7% protein level than what was determined when the diets were fed at the 10% and 15% protein level. Chapman, Castillo, and Campbell (1959) demonstrated that the age of the animal at the time of initiation of the study would influence the protein efficiency ratio. However, the differences tended to be reduced with prolonged time of experimentation. PER determination makes no allowance for maintenance. The method assumes that the gain in body weight is an indicator of protein synthesized by the body. Of the criteria defined for a valid bioassay, i.e., precision, reproducibility, statistical validity, proportionality, simplicity, and low cost (Hegsted and Samonds, 1978), PER could be considered as meeting only the criterion of simplicity.

In spite of these factors, protein efficiency ratio is still a method of choice for evaluating protein quality by many researchers and in the food industry.

Net Protein Ratio (NPR)

Some improvement can be made on the protein efficiency ratio by inclusion of a group of animals consuming a non-protein diet for similar periods of time. When modified

in this way the procedure is called Net Protein Ration NPR (Bender and Doell, 1957). The assay becomes somewhat more precise and reproducible but still falls short of most of the criteria defined for a valid bioassay (Hegsted and Samonds, 1978). The net protein ratio can be expressed as follows:

$$NPR = \frac{\text{gain in wt of test grp + loss of wt of nonprotein grp}}{\text{protein intake}}$$

This procedure allows for maintenance requirements and also permits the evaluation of poor proteins which do not promote growth and for which, consequently, PER cannot be measured. The NPR method gives constant values with varying levels of protein. Most criticism on this method is that it overestimates the poor quality proteins compared to high quality proteins and also the fact that animals on protein free diet do not consume as much food as those on protein diets.

A modification of NPR called Relative NPR (Rel NPR) is recommended by the recent National Academy of Sciences-National Research Council Committee on Protein Evaluation (Young and Pellette,1978) as a useful procedure if a multiple point assay cannot be performed. Relative NPR is performed in an identical manner as NPR, but the results are expressed relative to the value obtained with an 8% lactalbumin diet taken as 1.00 or 100.

Relative Protein Value (RPV)
or Slope Ratio Assay

This method was proposed by Hegsted and Chang (1965) and Said and Hegsted (1969), in which gain is used as the response and nitrogen intake or protein intake as the measure of the dose. It is a multi-dose assay which necessitates feeding of at least three levels of protein in order to obtain a straight line. The assay utilizes lactalbumin as a reference standard. A relative growth index or a relative protein value can then be calculated for each protein source, based on lactalbumin as the reference standard.

Only values falling on the linear portion of a curve are used in computation of the slope assay value. The necessity for feeding several dietary protein levels is a major fault of the procedure, since much labor is involved. Also, the mixing of three to five diets increases the chance for error. Additionally, lysin deficient proteins may not yield a valid slope ratio; in other words the linear portion of the curve is flatter, while threonine deficient diet tends to yield a steeper curve (McLaughlan, 1972). McLaughlan and Keith (1974) fed several diets considered to be marginally deficient in threonine to rats at protein levels ranging from 3-9% crude protein. They observed increased growth at low protein levels with threonine supplementation and little or no extra growth at high protein levels, which resulted in decreased slopes in the RPV assay. Thus, threonine

supplementation apparently decreased RPV for threonine deficient diets (3-4% protein level), but PER and RNU were not affected. McLaughlan and Keith (1974) concluded that RPV assay may overestimate the protein quality of threonine deficient proteins.

The major difficulties with the RPV assay is the selection of the linear portion of the curve in some cases. In addition, the protein sources should have a common point of origin (Hackler, 1977). Some researchers, however, favor the RPV procedure since they feel it may more accurately characterize the usefulness of poorer proteins (Hegsted and Chang, 1965; Said and Hegsted, 1969; Hegsted and Juliano, 1974).

Moreover, Hegsted and Samonds (1978) studied a collaborative assay using seven sources of proteins assayed by seven different laboratories (Table 1). Results indicated that the slope ratio assay (RPV, relative to lactalbumin) was superior to both PER and NPR at accurately discriminating between proteins with minimal between lab variations. They concluded that the slope ratio assay (RPV) meets many of the criteria of a valid bioassay, particularly, in the relation to the criteria of precision, reproducibility, statistical validity, and proportionality.

Table 1. ANOVA (Hegsted and Samonds, 1978).

S.O.V.	DF	F VALUES[a]		
		PER	NPR	RPV[b]
Proteins[c]	6	68.8	65.5	108.9
Laboratories	6	6.7	5.5	2.3*
Interaction	36	9.7	7.4	2.1

[a]F-values all significant, $P < 0.001$ except where shown.

[b]Lactalbumin as reference.

[c]Tested proteins were; Latalbumin, soyflour, de-fatted meat, ANRC casein, soy conic., wheat gluten and white flour.

*Not significant.

Protein Digestion and the Use of Indicators

The potential value of a food for supplying a particular nutrient can be determined by chemical analysis, but the actual value of the food to the animal can be arrived at only after making allowances for the inevitable losses that occur during digestion, absorption and metabolism. The first tax imposed on foods is that represented by the part of it which is not absorbed and is excreted in the feces.

The digestibility of food is most accurately defined as that portion which is not excreted in the feces and which

is, therefore, assumed to be absorbed by the animal. It is commonly expressed in terms of dry matter and as a percentage, the digestibility coefficient.

Chromic oxide (Cr_2O_3) as an external (added) indicator is extensively used in nutritional studies of digestibility, utilization, and retention (Edin, 1918; Whitson et al., 1943). An ideal indicator should be insoluble in the digestive tract, be indigestible, pass through the tract at the same rate as ingested food, have no undesirable pharmacological effect on the animal (such as diarrhea), and be suitable for chemical analysis (Church, 1969). The following equations are used for determination of digestibility (Edwards and Gillis, 1959):

$$\% \text{ dry matter digestibility} = 100 - \frac{\%Cr_2O_3 \text{ in food}}{\%Cr_2O_3 \text{ in feces}} \times 100$$

$$\% \text{ of digestible nutrient} = 100 - \frac{\%Cr_2O_3 \text{ in food}}{\%Cr_2O_3 \text{ in feces}} \times \frac{\%\text{nutrient in feces}}{\%\text{nutrient in food}} \times 100 .$$

The Use of Mice for the Measurements of Protein Quality

While a considerable amount of work has been done on the amino acid requirements of mice (Bauer and Berg,1943; Sauberlich and Baumann, 1946; Pearce, Sauberlich, and Baumann, 1947; Moddy and Elvehjem, 1949), relatively very little work has been done on the use of this animal for

protein quality evaluation. Of the earliest work, Bosshardt et al. (1946) found that the maximum protein efficiency for mice fed casein was obtained when the level of casein in the diet was 10%, and the maximum growth was obtained with diets containing between 15 and 20% casein, which is in agreement with the work of Fenton and Carr (1951). Mice have the problem of numerous genetic strains available which have different reported growth rates (Poily, 1972). A great spread in food consumption, weight gain and a wide range of protein efficiency ratios were observed when mice of different strains were studied under identical conditions (Fenton and Carr, 1951). Earlier, Bosshardt et al. (1946) conducted the first trial to establish a rational procedure for the use of mice for protein efficiency determinations and to investigate the effect of such factors as pre-test standardization of test animals, the duration of the feeding period, and the level of the test protein in the diet. Their results indicated that the albino mouse is a suitable test animal for determination of nutritive quality of proteins for growth. They concluded that a reliable growth result may be obtained in ten days. Thus a marked saving of time is realized since the conventional test period for the rat method is twenty-eight days. Approximately one-seventh the amount of test

protein and other dietary ingredients are needed because of the saving in time and the smaller food consumption of the mouse as compared with the rat.

Feeding Weanling mice four different protein sources; dried whole egg, isolated soybean supplemented with methionine, Brewer's yeast and fish meal at various levels in the diet, where protein levels were plotted against the weight gain for the mice (Weber, Cossack, and Thompson, 1979). Protein requirements were determined as follows: whole egg as 10.4%, isolated soybean supplemented with methionine of 12.0%, Brewer's yeast of 14.2% and fish meal of 11.6%. It was also concluded that the NRC protein requirement was too high for a high quality protein fed to weanling mice, thus the good quality protein source has a lower requirement than that listed in N.R.C. (1978) or by several investigators (Bing, Adams, and Bowman, 1932; and Goettsch, 1960).

Weight changes and food consumption for the mouse are considerably less than for the rat, so that measurements must be made more accurately. It is not expected that absolute efficiency figures are the same for both rats and mice, but the results of a large number of assays indicate that proteins and protein hydrolysates fall in the same order of classification by the two methods.

CHAPTER 3

EXPERIMENTAL PROCEDURE

Mouse Experiment No. 78-5

Animals

Nineteen groups of male Weanling mice of the Charles River CD-1 strain were employed in this experiment. Each group consists of ten males of an approximately equal average initial body weight between and within the groups. Mice were housed in stainless wire mesh cages. Stainless feeders with wire screens and narrow tops were used to prevent scattering the feed. They were housed one mouse per cage.

Diets

Six sources of protein, at three levels each, were tested, i.e., whole egg, casein, skim milk, white beans, soybean protein isolate, and lactalbumin at levels of approximately 6, 8, and 11% dietary crude protein. The allocation of the dietary treatments, and its levels of for nineteen groups of mice is shown in Table 2. Therefore, this will result in the total number of treatments being nineteen with one of the groups on a protein-free diet as recommended for the measurements of the net protein ratio (Bender and Doel, 1957). The dietary composition is shown in Table 3

Table 2. The allocation of treatments for Mouse Exp. No. 78-5.

Treatment No. (Group No.)	No. of Mice	Dietary Treatment		
		Protein Source	% Dietary Protein	(Actual % Diet Protein*)
1	10	Whole Egg	6%	6.19
2	10	Whole Egg	8%	8.04
3	10	Whole Egg	11%	11.03
4	10	Casein	6%	6.36
5	10	Casein	8%	8.25
6	10	Casein	11%	11.42
7	10	Milk, Skim dry	6%	6.37
8	10	Milk, Skim dry	8%	7.92
9	10	Milk, Skim dry	11%	10.95
10	10	Beans, white	6%	6.44
11	10	Beans, white	8%	8.04
12	10	Beans, white	11%	11.15
13	10	Soybean Isolate	6%	5.89
14	10	Soybean Isolate	8%	7.9
15	10	Soybean Isolate	11%	10.82
16	10	Lactalbumin	6%	6.31

Table 2, Continued.

Treatment No. (Group No.)	No. of Mice	Dietary Treatment		
		Protein Source	% Dietary Protein	(Actual % Diet Protein*)
17	10	Lactalbumin	8%	8.31
18	10	Lactalbumin	11%	11.06
19	10	PROTEIN FREE DIET	0.0	0.0

*Analysis, on dry matter basis.

Table 3. Composition of diets (Mouse Exp. No. 78-5).

Treatment No. or Diet No.	% Dietary Protein	Ingredients as % of the Diet						
		Whole Egg	Casein	Milk, Skim	Beans, White	Soy Protein	Lactal-bumin	Corn Oil
1	6% Whole Egg	13.34	-	-	-	-	-	5.41
2	8% Whole Egg	17.78	-	-	-	-	-	3.91
3	11% Whole Egg	24.44	-	-	-	-	-	3.5
4	6% Casein	-	6.38	-	-	-	-	10
5	8% Casein	-	8.51	-	-	-	-	10
6	11% Casein	-	11.7	-	-	-	-	10
7	6% Milk, Skim	-	-	17.65	-	-	-	12.97
8	8% Milk, Skim	-	-	23.53	-	-	-	13.99
9	11% Milk, Skim	-	-	32.35	-	-	-	15.50
10	6% Beans, White	-	-	-	24.01	-	-	8.97
11	8% Beans, White	-	-	-	32.03	-	-	8.65
12	11% Beans, White	-	-	-	44.04	-	-	8.15
13	6% Soy Protein	-	-	-	-	7.32	-	10.89
14	8% Soy Protein	-	-	-	-	9.76	-	11.20
15	11% Soy Protein	-	-	-	-	13.41	-	11.66
16	6% Lactalbumin	-	-	-	-	-	7.32	9.80
17	8% Lactalbumin	-	-	-	-	-	9.76	9.75
18	11% Lactalbumin	-	-	-	-	-	13.41	9.67
19	N-FREE DIET	-	-	-	-	-		9.96

Table 3, Continued.

Treatment No. or Diet No.	% Dietary Protein	Ingredients as % of the Diet						
		Cerelose	Choline Chloride	Cr_2O_3	Min. Mix	Vit. Mix	Cellulose	Bentonite
1	6% Whole Egg	73.35	0.2	0.2	3.5	1.0	3.0	-
2	8% Whole Egg	70.41	↓	↓	↓	↓	↓	-
3	11% Whole Egg	61.55	↓	↓	↓	↓	↓	2.61
4	6% Casein	75.64	↓	↓	↓	↓	↓	0.08
5	8% Casein	73.50	↓	↓	↓	↓	↓	0.09
6	11% Casein	70.29	↓	↓	↓	↓	↓	0.11
7	6% Milk, Skim	61.48	↓	↓	↓	↓	↓	-
8	8% Milk, Skim	54.58	↓	↓	↓	↓	↓	-
9	11% Milk, Skim	44.25	↓	↓	↓	↓	↓	-
10	6% Beans, White	59.12	↓	↓	↓	↓	↓	-
11	8% Beans, White	51.42	↓	↓	↓	↓	↓	-
12	11% Beans, White	39.91	↓	↓	↓	↓	↓	-
13	6% Soy Protein	73.89	↓	↓	↓	↓	↓	-
14	8% Soy Protein	71.14	↓	↓	↓	↓	↓	-
15	11% Soy Protein	67.03	↓	↓	↓	↓	↓	-
16	6% Lactalbumin	74.98	↓	↓	↓	↓	↓	-
17	8% Lactalbumin	72.59	↓	↓	↓	↓	↓	-
18	11% Lactalbumin	69.02	↓	↓	↓	↓	↓	-
19	N-FREE DIET	82.14	↓	↓	↓	↓	↓	-

where all diets contain the same level of: 1% vitamin mix, 3.5% mineral mix, 0.2% choline chloride, 3% cellulose, and 0.2% of chromic oxide (Cr_2O_3) as an external marker as recommended for the determination of digestibility (Church, 1969), plus varying levels of cerelose, corn oil, bentonite, and the protein source, to bring the dietary crude protein up to the level required for each treatment with the energy level constant in all the treatments, i.e. (3520 cal/gm dry matter). The composition of both vitamin mix and mineral mix are shown in Tables 4 and 5 respectively.

Experimental

Initial weight of each mouse was recorded and the average initial weight for each group was computed. Mice were weighed and fed (ad Libitum) twice a week; feed was initially weighed with the residual oven dried and weighed again in order to compute the amount of feed intake. Ingredients of each diet were well mixed and offered in a powdered form. Feces was collected, screened to clean it from the feed residual, oven dried, weighed, and a portion of 50% from each collection kept for chemical analysis. Deionized water was offered free choice. Each feeding period lasted for twenty-eight days at which point the animals were sacrificed.

Table 4. Composition of vitamin mix.*

Ingredient	Per Kg. Mixture	Per Kg. Diet
Thiamin HCl	600 mg	6 mg
Riboflavin	600 mg	6 mg
Pyridoxine HCl	700 mg	7 mg
Nicotinic acid	3 g	0.03 g
D-Calcium pantothenate	1.6 g	0.02 g
Folic Acid	200 mg	2 mg
D. Biotin	20 mg	0.2 mg
Cyanocobalamin	1 mg	0.01 mg
Retinyl palmitate	800 mg	8 mg
dl-a-Tocopheryl acetate	20 g	0.2 mg
Cholecalciferol	2.5 mg	0.03 mg
Menaquionine	5.0 mg	0.05 mg
Sucrose, powdered	972.9 g	9.73 mg

*10663 AIN Vitamin Mixture 76 - American Institute of Nutrition. U. S. Biochemicals.

Table 5. Composition of mineral mix.*

Ingredient	% of Mix	% of Diet
Calcium Phosphate ($CaHPO_4$)	50	1.750
Sodium Chloride (NaCl)	7.4	0.259
Potassium Citrate	22	0.770
Potassium Sulfate	5.2	0.182
Magnesium Oxide	2.4	0.084
Manganese Carbonate (43.48% Mn)	0.35	0.012
Ferric Citrate (16-17% Fe)	0.60	0.021
Zinc Carbonate (70% ZnO)	0.16	0.006
Capric Carbonate (53-55% Cu)	0.03	0.001
Potassium Iodate	0.001	0.4 ppm
Sodium Selenate	0.001	0.4 ppm
Chromium Potassium Sulfate	0.055	0.002
Sucrose, Powdered	11.80	0.413
TOTAL	100%	3.5%

*10664 AIN mineral mixture 76 - American Institute of Nutrition. U. S. Biochemicals.

Nitrogen and Crude Protein Determination

Nitrogen and crude protein determinations were done by the standard Macro Kjeldahl method and crude protein was calculated as N × 6.25. Diets were analyzed for protein after the preparation and before feeding. The Kjeldahl method was also applied to the collected feces at the end of each feeding experiment.

Chromic Oxide Determination

Spectrophotometric method using Coleman Junior II was employed to determine the percent of chromic oxide in the experimental diets and the feces collected in order to calculate the digestibility of protein. Duplicate samples were weighed into 125 ml erlenmeyer flasks, stored overnight after adding 10 ml of conc. HNO_3 and then digested for about thirty minutes or until 2-4 ml of the liquid remained. Samples were then further digested, after adding 15 ml of 60% perchloric acid HClO, until the color turns into orange. Samples were cooled, a small amount of deionized H_2O added and then transferred into a 100 ml volumetric flask using additional deionized H_2O to bring the volume up to 100 ml. Samples then were allowed to stand overnight, read on Coleman Junior II spectrophotometer at 444 mu using a deionized H_2O as a reference. The following equation was used:

$$\% \ Cr_2O_3 = \frac{O.D \times K \ (standard)}{\text{sample weight (g)} \times 100} .$$

Protein Efficiency Ratio

PER was determined as described by Osborne et al. (1919). Casein was used as the standard. The equation used was as follows:

$$PER = \frac{\text{weight gained}}{\text{protein consumed}} .$$

Net Protein Ratio

NPR was determined according to Bender and Doel (1957), using the equation:

$$NPR = \frac{\text{weight gain of test group + weight loss of protein free diet group}}{\text{protein consumed}}$$

Protein Digestibility

The following equation was used for the determination of protein digestibility using chromic oxide (Cr_2O_3) as an external indicator. The equation of Edwards and Gillis (1959) was applied;

$$\% \text{ protein digestibility} = 100 - \left[\frac{\% \ Cr_2O_3 \text{ in feed}}{\% \ Cr_2O_3 \text{ in feces}} \times \frac{\% \text{ protein in feces}}{\% \text{ protein in feed}} \times 100\right]$$

Relative Protein Value

RPV or slope ratio assay was determined as described by Hegsted and Chang (1965), using gain as the response and

nitrogen intake as the measure of dose. It should be noted that three dietary levels of protein were used as mentioned previously.

$$RPV = \frac{\text{slope of protein source}}{\text{slope of lactalbumin as std}} .$$

Rat Experiment No. 78-1

Animals

Nineteen groups of male Weanling rats of the Sprague Dawley strain were employed in this experiment. Each group consists of six males with an average initial body weight of 55 gms.

Diets

The same six sources of protein previously used in Mouse Exp. No. 78-5, but with varying dietary protein levels, were tested in this experiment. Dietary protein levels employed in this experiment were 7, 10, and 13% crude protein. Table 6 shows the allocation of treatments in the rat groups and diets composition is shown in Table 7. Energy level was the same in all experimental diet and around 3520 cal/g. Group on protein free diet was also employed for the calculation of net protein ratio. The same mineral and vitamin mixtures were used as described in Tables 4 and 5.

Table 6. Allocation of treatments for Rat Experiment No. 78-1.

Treatment No. (Group No.)	No. of Rats	Dietary Treatment		
		Protein Source	% Dietary Protein	(actual % Diet. Protein*)
1	6	Whole Egg	7%	7.12
2	6	Whole Egg	10%	10.06
3	6	Whole Egg	13%	13.36
4	6	Casein	7%	7.08
5	6	Casein	10%	10.37
6	6	Casein	13%	13.42
7	6	Skim milk, dried	7%	7.13
8	6	Skim milk, dried	10%	10.13
9	6	Skim milk, dried	13%	13.47
10	6	Beans, white	7%	7.33
11	6	Beans, white	10%	10.48
12	6	Beans, white	13%	13.30
13	6	Soybean, isolate	7%	7.22
14	6	Soybean, isolate	10%	10.53
15	6	Soybean, isolate	13%	13.33
16	6	Lactalbumin	7%	7.04

Table 6, Continued.

Treatment No. (Group No.)	No. of Rats	Dietary Treatment		
		Protein Source	% Dietary Protein	(actual % Diet Protein*)
17	6	Lactalbumin	10%	10.48
18	6	Lactalbumin	13%	13.82
19	6	PROTEIN FREE DIET	0.0	0.0

*Analysis, on dry matter basis.

Table 7. Composition of diets (Rat Exp. No. 78-1).

Treatment No. or Diet No.	% Dietary Protein	Ingredients as % of the Diet						
		Whole Egg	Casein	Milk, Skim	Beans, White	Soy Protein	Lactal-bumin	Corn Oil
1	7% Whole Egg	15.56	-	-	-	-	-	4.66
2	10% Whole Egg	22.22	-	-	-	-	-	3.50
3	13% Whole Egg	28.89	-	-	-	-	-	3.5
4	7% Casein	-	7.45	-	-	-	-	10.00
5	10% Casein	-	10.64	-	-	-	-	9.97
6	13% Casein	-	13.83	-	-	-	-	9.93
7	7% Milk, Skim	-	-	18.64	-	-	-	13.44
8	10% Milk, Skim	-	-	26.63	-	-	-	14.4
9	13% Milk, Skim	-	-	34.62	-	-	-	15.89
10	7% Beans, White	-	-	-	28.02	-	-	9.00
11	10% Beans, White	-	-	-	40.03	-	-	8.32
12	13% Beans, White	-	-	-	52.04	-	-	7.83
13	7% Soy Protein	-	-	-	-	8.54	-	11.05
14	10% Soy Protein	-	-	-	-	12.20	-	11.51
15	13% Soy Protein	-	-	-	-	15.85	-	11.97
16	7% Lactalbumin	-	-	-	-	-	8.54	9.77
17	10% Lactalbumin	-	-	-	-	-	12.20	9.69
18	13% Lactalbumin	-	-	-	-	-	15.85	9.61
19	N-FREE DIET	-	-	-	-	-	-	9.96

Table 7, Continued.

Treatment No. or Diet No.	% Dietary Protein	Ingredients as % of the Diet						
		Cere-lose	Benton-ite	Cellu-lose	Vit.Mix AIN	Min.Mix AIN	Cr_2O_3	Choline Chloride
1	7% Whole Egg	71.88	-	3.0	1.0	3.5	0.2	0.2
2	10% Whole Egg	64.82	1.56	↓	↓	↓	↓	↓
3	13% Whole Egg	54.98	4.73					
4	7% Casein	74.56	0.09					
5	10% Casein	71.49	-					
6	13% Casein	68.34	-					
7	7% Milk, Skim	60.33	-					
8	10% Milk, Skim	51.00	-					
9	13% Milk, Skim	41.59	-					
10	7% Beans, White	54.81	0.27					
11	10% Beans, White	43.75	-					
12	13% Beans, White	32.23	-					
13	7% Soy Protein	72.51	-					
14	10% Soy Protein	68.39	-					
15	13% Soy Protein	64.28	-					
16	7% Lactalbumin	73.79	-					
17	10% Lactalbumin	70.21	-					
18	13% Lactalbumin	66.64	-					
19	N-FREE DIET	82.14	-	↓	↓	↓	↓	↓

Experimental

Initial weight of each rat was recorded and the average initial weight for each group was computed. Rats were weighed and fed (ad Libitum) twice a week; feed was initially weighed with the residual oven dried and weighed again in order to compute the amount of feed intake. Ingredients of each diet were well mixed and offered in a powdered form. Feces was collected, screened to clean it from the feed residual, oven dried, weighed, and a portion of 50% from each collection kept for chemical analysis. Deionized water was offered free choice. Each feeding period lasted for twenty-eight days at which point the animals were sacrificed.

CHAPTER 4

RESULTS AND DISCUSSION

Mouse Experiment No. 78-5

The objectives of this series of experiments were to study the possibility of using mice in protein quality evaluations and to establish a rational procedure for the use of mice for protein quality evaluation. Growth, protein efficiency ratio (PER), net protein ratio (NPR), protein digestion and relative protein value (RPV) from slope ratio assay were used as parameters in this study. The length of feeding period, source of protein, level of dietary protein, and their interactions were studied as factors affecting protein quality when employing the parameters mentioned above.

Growth

Results on growth rate, expressed as daily body weight gain, food intake, and protein intake are presented in Tables 8 and 9. Mice were found to consume similar amounts of food daily from the different sources tested in this experiment, except from beans containing diet. Animals on beans containing diet had lower daily food intake and protein intake than animals fed the other protein sources. Beans protein is known to be of low quality and deficient

Table 8. Growth rate, food intake, and protein intake in mice fed six sources of protein.

Source	Dietary Protein %	Protein Intake g/day	Mean, Daily Body Wt. Gain g/day	Mean, Daily Food Intake g/day
Whole egg	6.19	0.192	0.584	3.105
	8.04	0.253	0.801	3.152
	11.03	0.363	1.008	3.288
Casein	6.36	0.182	0.362	2.869
	8.25	0.255	0.487	3.092
	11.42	0.415	0.893	3.635
Milk, Skim	6.37	0.172	0.397	2.701
	7.92	0.252	0.604	3.194
	10.95	0.411	0.761	3.752
Beans, white	6.44	0.173	0.131	2.689
	8.04	0.141	0.111	1.759
	11.15	0.254	0.186	2.278
Soybean Isolate	5.89	0.162	0.255	2.745
	7.90	0.246	0.403	3.118
	10.82	0.366	0.547	3.110
Lactalbumin	6.31	0.196	0.678	3.099
	8.13	0.243	0.719	2.983
	11.06	0.333	0.854	3.013

Table 9. Means, growth rate, food intake, and protein intake in mice fed six sources of protein.

Source	Protein Intake g/day	Food Intake g/day	Body Weight Gain g/day
Whole egg	0.269 ± 0.087	3.182 ± 0.095	0.798 ± 0.021
Casein	0.284 ± 0.119	3.199 ± 0.394	0.581 ± 0.278
Milk, skim	0.278 ± 0.122	3.216 ± 0.525	0.587 ± 0.183
Beans, white	0.189 ± 0.058	2.242 ± 0.466	0.143 ± 0.039
Soybean Isolate	0.248 ± 0.087	2.991 ± 0.213	0.402 ± 0.146
Lactalbumin	0.257 ± 0.07	3.032 ± 0.06	0.750 ± 0.092

in methionine (FAO/WHO, 1973). Daily food intake was not noticeably influenced by the level of dietary protein, however, variations were observed in mice on different levels of dietary protein from beans and skim milk, but no trend could be established. The most efficient growth, expressed as daily weight gain, was observed in mice consuming whole egg (.798 g/day) followed by lactalbumin with a slight difference (.750 g/day) (Table 9). Casein and skim milk gave moderate efficiency with no significant differences, being .581 and .587 g/day respectively. Isolated soybean and white beans were the least efficient in promoting weight gain, being .402 and .143 g/day, respectively. Within each source of protein tested (Table 8), the growth rate increased gradually with the increase in the level of dietary protein or protein intake (Goettsch, 1960, and Barnes et al., 1945).

Protein Efficiency Ratio and Net Protein Ratio

Results on protein efficiency ratios and net protein ratios of the six sources of protein tested are shown in Tables 10, 11, 12, and analysis of variance is shown in Table 13. Sources of protein fed in this experiment differed significantly in their protein efficiency ratio (PER) and net protein ratio values (Table 13). A multiple range test (Duncan, 1955) showed that differences among all sources tested were significant (Table 12) except for whole egg and

Table 10. Protein efficiency ratios of six protein sources fed to mice at three different levels of protein in diet.

Sources	Dietary Protein %	Days[1] 10	14	21	28
Whole egg	6.19	3.384	3.179	3.038	2.731
	8.14	3.755	3.345	2.980	2.566
	11.03	3.398	3.041	2.545	2.123
Casein	6.36	2.021	1.967	1.941	1.982
	8.25	2.082	1.980	1.819	1.753
	11.42	2.645	2.216	2.020	1.730
Milk, skim	6.37	2.694	2.441	2.052	2.046
	7.92	3.019	2.489	2.132	1.922
	10.95	2.122	1.928	1.726	1.638
Beans, white	6.44	0.618	0.654	0.911	0.839
	8.04	0.638	0.702	1.050	0.757
	11.05	0.738	0.703	0.706	0.780
Soybean Isolate	5.89	1.615	1.642	1.540	1.490
	7.90	1.827	1.564	1.582	1.564
	10.82	1.913	1.657	1.515	1.423
Lactalbumin	6.31	3.901	3.772	3.361	2.827
	8.13	3.320	3.208	2.900	2.435
	11.06	2.942	2.712	2.468	2.139

[1]Measurements taken at the end of four different periods of feeding experiments; 10, 14, 21, and 28 days.

Table 11. Summary results on net protein ratios of six protein sources fed to mice at three levels of protein in the diet.

Source	Dietary Protein %	Day[1] 10	14	21	28
Whole egg	6.19	4.523	4.039	3.642	3.234
	8.04	4.627	3.951	3.399	2.911
	11.03	3.951	3.426	2.858	2.355
Casein	6.36	2.946	2.881	2.642	2.528
	8.25	2.822	2.639	2.312	2.125
	11.42	3.170	2.600	2.289	1.936
Milk, skim	6.37	2.772	3.368	2.748	2.579
	7.92	3.769	3.133	2.583	2.267
	10.95	2.642	2.350	2.001	1.852
Beans, white	6.44	1.702	1.598	1.683	1.502
	8.04	1.887	1.774	2.005	1.449
	11.15	1.541	1.378	1.234	1.203
Soybean Isolate	5.89	2.826	2.616	2.349	2.106
	7.90	2.653	2.233	2.059	1.943
	10.82	2.519	2.120	1.873	1.701
Lactalbumin	6.31	4.928	4.631	3.967	3.277
	8.13	4.415	3.913	3.396	2.805
	11.06	3.602	3.224	2.826	2.368

[1]Measurements taken at the end of four different periods of feeding experiment; 10, 14, 21, and 28 days.

Table 12. Means[1], protein efficiency ratios and net protein ratios for six sources of protein fed to mice.

Source	Protein Efficiency Ratio[2]	Net Protein Ratio[2]
Whole egg	3.007[a]	3.576[a]
Casein	2.013[b]	2.574[b]
Milk, skim	2.184[c]	2.755[c]
Beans, white	0.758[d]	1.580[d]
Soybeans Isolate	1.611[e]	2.250[e]
Lactalbumin	2.999[a]	3.613[a]

[1]Grand means; average of all variables involved.
[2]Means not having common letter superscripts are significantly different at the 0.05 level of probability.

Table 13. Analysis of variance[1] on protein efficiency ratio and net protein ratio data in mice fed six sources of protein.

Sources of Variation	DF	Means of Squares PER	NPR
Sources (S)	5	88.4542*	73.7751*
Levels (L)[3]	2	3.9079*	24.5303*
Periods (P)[2]	3	9.8369*	33.8802*
Reps.	9	0.1503	0.4414
SL	10	1.9872*	1.8785*
SP	15	1.2591*	1.4542*
LP	6	0.2419*	0.1323*
SLP	30	0.1623*	0.1725*
Error A	162	0.1765	0.3997
Error B	486	0.0234	0.0604
Remainings	369	0.6394	1.4592
TOTAL	719		

[1]Significant at 0.05 level of probability.
[2]Length of feeding period.
[3]Dietary protein (%).

and lactalbumin differences were not significant, ranking whole egg as the highest in its PER value (3.007) and lactalbumin (PER = 2.999). Diets containing beans were the least efficient among all sources tested (PER = 1.611). This could be due to its amino acids deficiency, particularly sulfur containing amino acids failure to meet the requirements of the mouse. It should be noted that results on protein efficiency ratios for the protein sources tested (Table 12) were in agreement with the data on growth rate (Table 9).

The effects of level of dietary protein on PERs and NPRs have been investigated (Tables 14, 15, 16; and Figs. 1, 2, 3, 4, and 5). Mice are known to have lower protein requirements than rats (Goettsch, 1960; John and Bell, 1976; and Weber, Cossack, and Thompson, 1979), which led to the use of dietary levels of 6, 8, and 10% for mice, and 7, 10, and 13% protein for rats. Analysis of variance indicated a significant difference in PER and NPR values when calculated using different levels of dietary protein (Table 13). Differences were not significant when using 6 or 8% levels of dietary protein (Table 14), while using a level of 11% gave significantly lower values. When sources of protein were considered (Table 15), it was found that the level of test protein in the diet at which the maximal protein efficiency ratio was obtained varies with the different protein sources

Table 14. Effect of the level of protein in diet on protein efficiency ratios[1] and net protein ratios[2] in mice fed six sources of protein.

Dietary Protein %	PER[2]	NPR[3]
6	2.194[a]	3.004[c]
8	2.141[a]	2.795[d]
11	1.951[b]	2.376[e]

[1]Values for PER and NPR are the grand means for each level mentioned.

[2,3]Means not having common letter superscripts are significantly different at the 0.05 level of probability.

Table 15. Effect of the level of protein in diet on protein efficiency ratios[1] in mice fed six protein sources.[2]

Source	Dietary Protein (%)		
	6[a]	8[b]	11[c]
Whole egg	3.038^{e}	3.161^{e}	2.777^{f}
Casein	1.978^{jk}	1.909^{k}	2.153^{ij}
Milk, skim	2.308^{hi}	2.390^{gh}	1.854^{k}
Beans, white	0.755^{m}	0.787^{m}	0.731^{m}
Soybean, Isolate	1.572^{l}	1.634^{jl}	1.627^{jl}
Lactalbumin	3.465^{d}	2.966^{e}	2.565^{g}

[1]Each PER value is the mean of four PER values taken from four different periods of feeding experiment; 10, 14, 21, and 28 days.

[2]Means not having common letter superscripts are significantly different at the 0.05 level of probability.

Table 16. Effect of level of protein in diet on net protein ratios[1] in mice fed six sources of protein.[2]

Source	Dietary Protein (%) 6	8	11
Whole egg	3.859^{b}	3.722^{b}	3.147^{c}
Casein	2.749^{d}	2.474^{edf}	2.499^{df}
Milk, skim	3.117^{c}	2.938^{cd}	2.111^{fg}
Beans, white	1.621^{i}	1.779^{hi}	1.339^{j}
Soybean, Isolate	2.474^{df}	2.222^{fg}	2.053^{gh}
Lactalbumin	4.201^{a}	3.632^{b}	3.005^{cd}

[1]Each NPR value is the mean of four NPR values obtained from four different periods of feeding experiment 10, 14, 21, and 28 days.

[2]Means not having common letter superscripts are significantly different at the 0.05 level of probability.

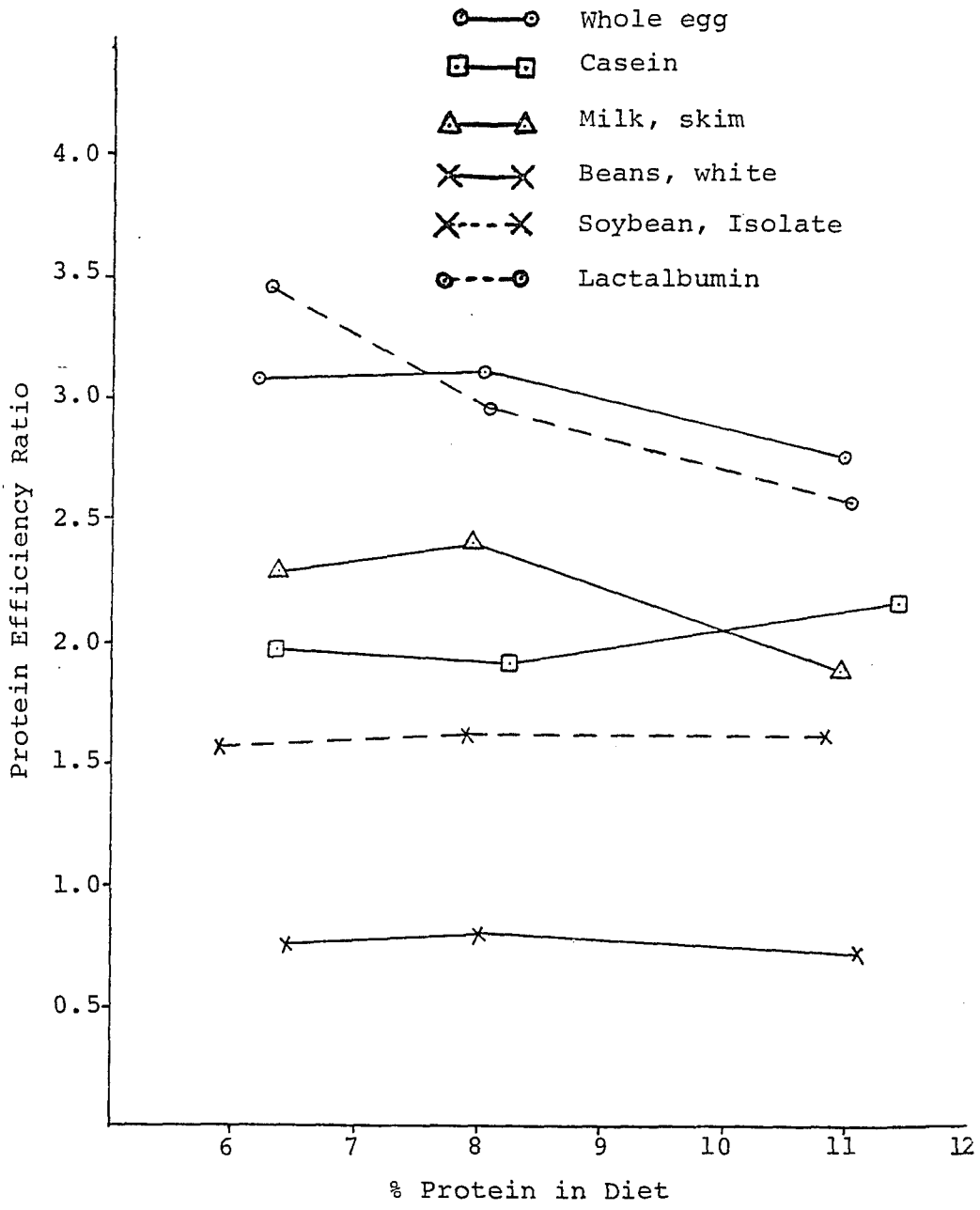

Fig. 1. Effect of the level of protein in the diet on protein efficiency ratio.

PER as the average value of four measurements on four different periods of feeding (10, 14, 21, and 28 days).

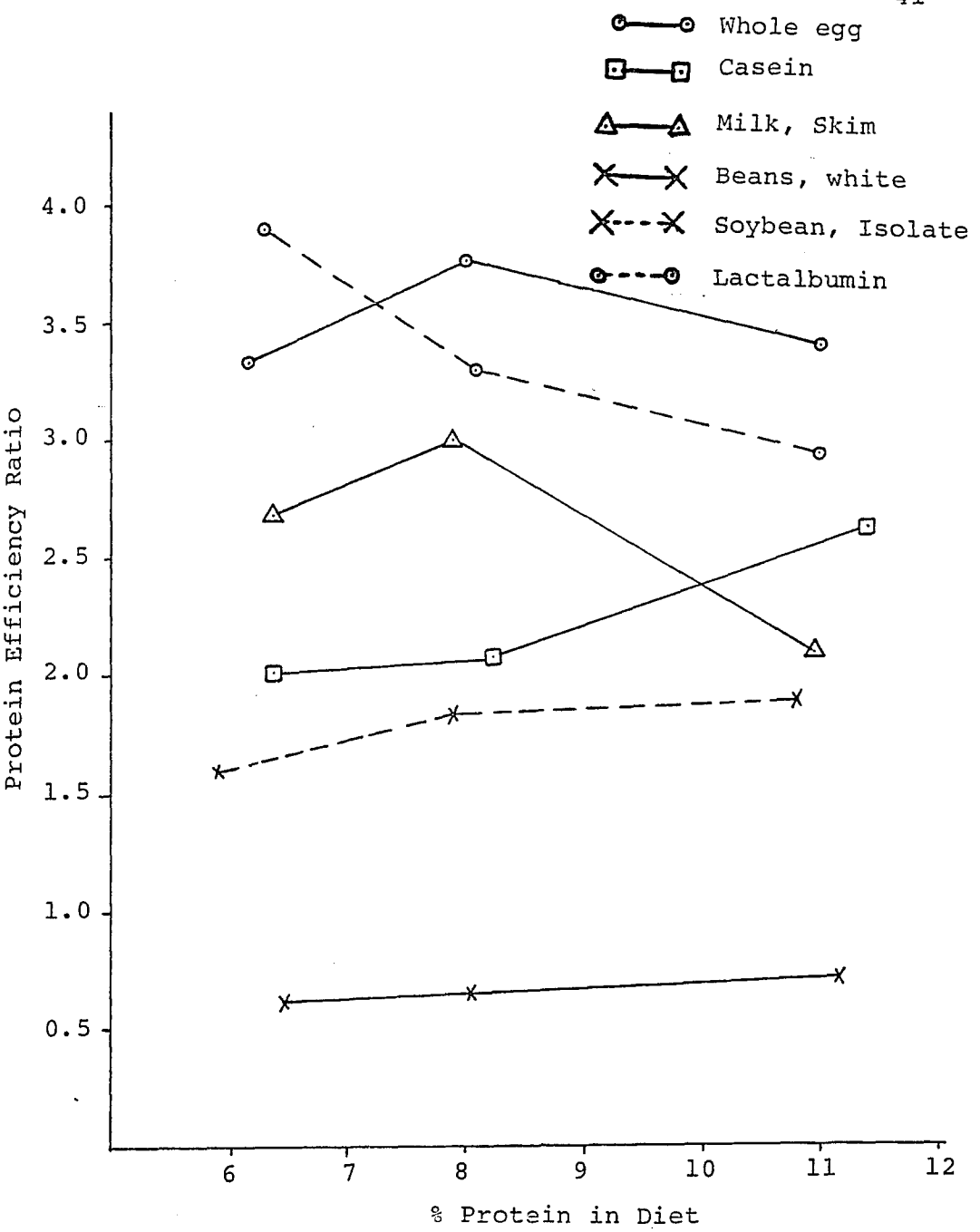

Fig. 2. Effect of protein level in the diet on protein efficiency ratios at the end of a 10-day feeding experiment.

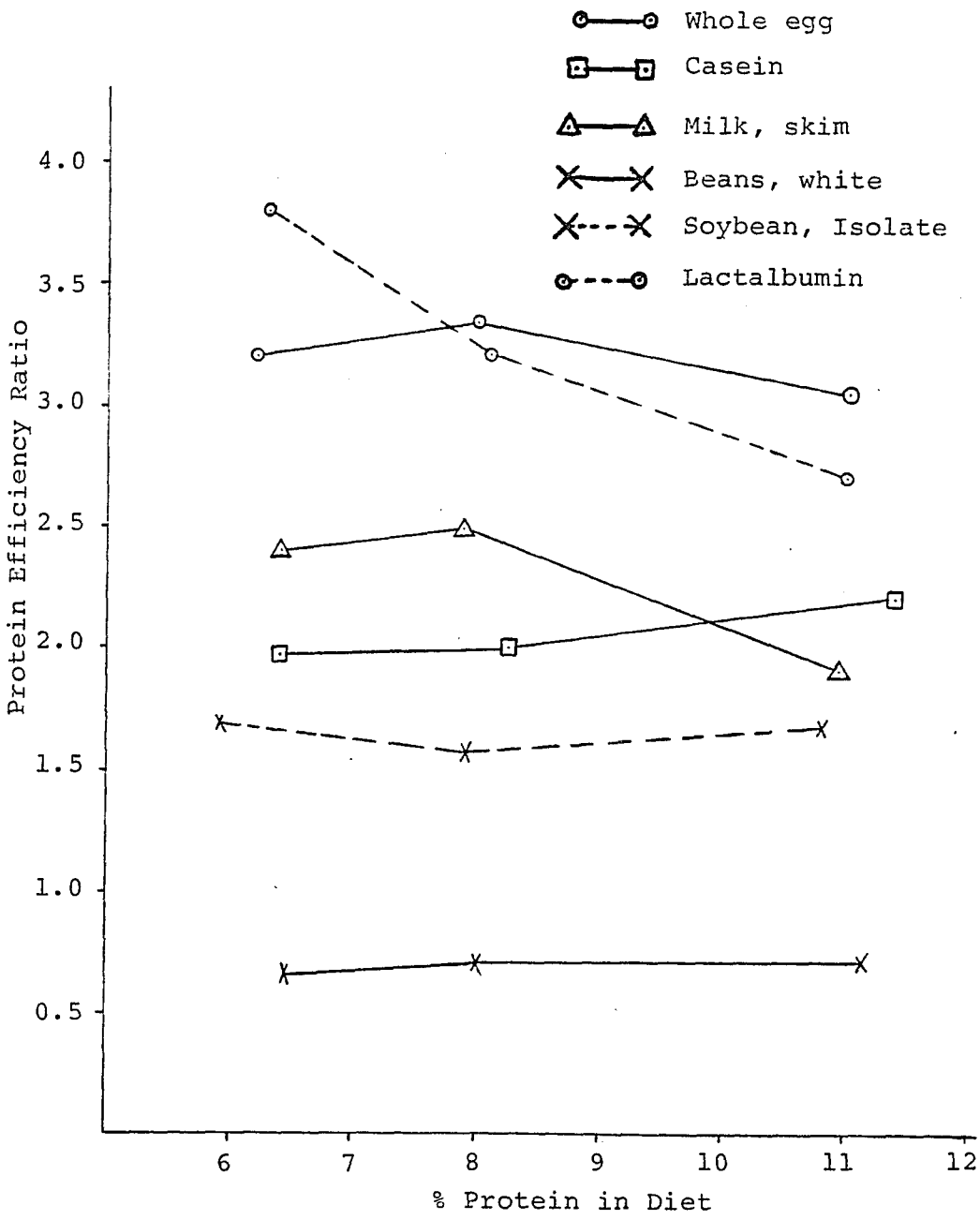

Fig. 3. Effect of protein level in diet on protein efficiency ratios at the end of a 14-day feeding experiment.

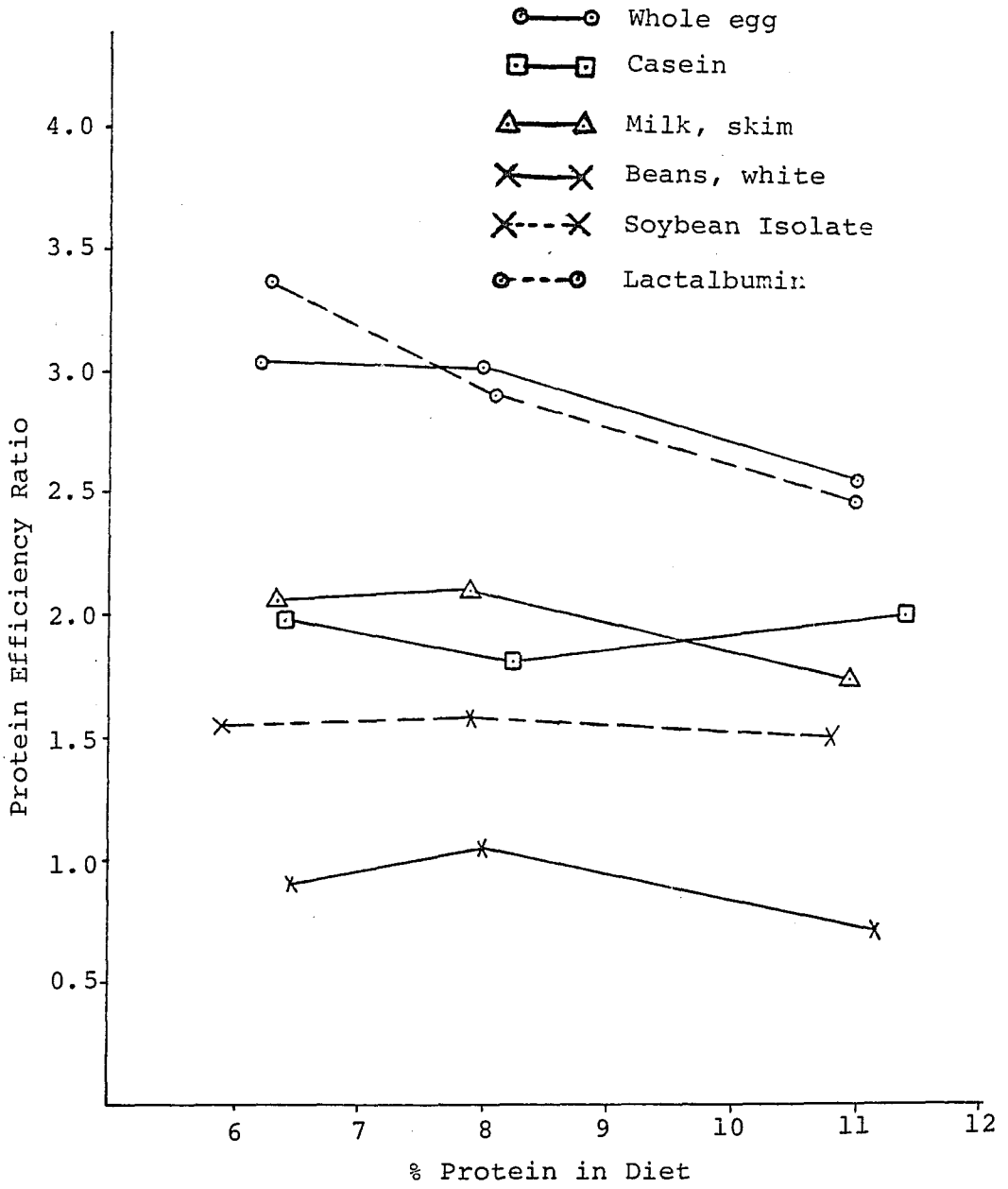

Fig. 4. Effect of protein level in diet on protein efficiency ratios at the end of a 21-day feeding experiment.

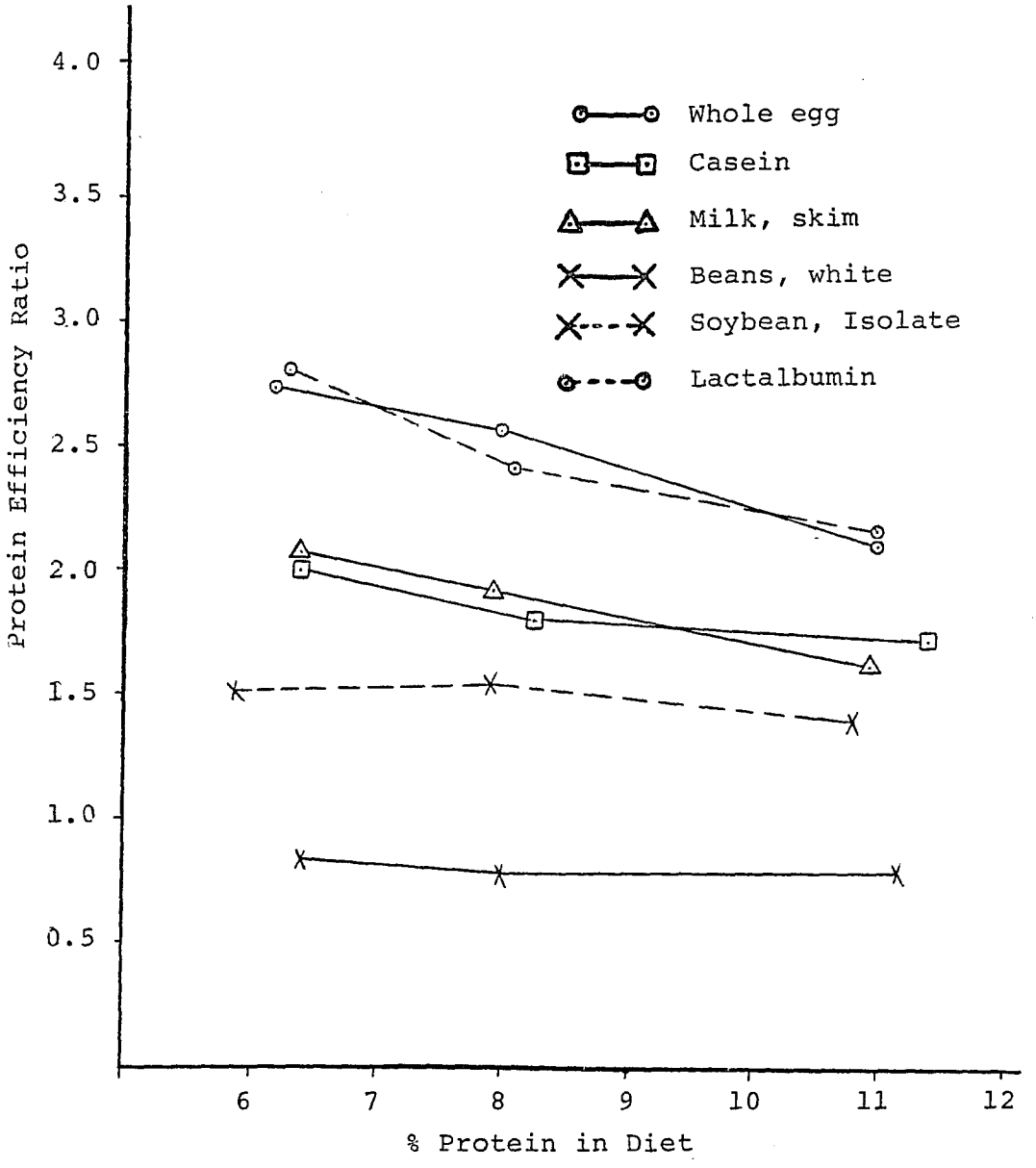

Fig. 5. Effect of protein level in diet on protein efficiency ratios at the end of a 28-day feeding experiment.

being fed. This is in accord with results obtained by Barnes et al. (1945) and Osborne, Mendel, and Ferry (1919). Maximum PER values were obtained at 6% level of dietary protein for lactalbumin, 8% with no significant difference with the 6% level for whole egg and skim milk, 8% with no significant difference with 11% for soybean and beans, and 11% for casein (Table 15). This confirms the fact that poor quality proteins must be fed at relatively high levels to obtain the maximal protein efficiency ratio (Barnes et al. (1945). This could also be explained by the finding that when raising the dietary protein level from 6 to 11% in soybean and white beans, the PER values did not increase significantly (Table 15) while it increased significantly in the case of high quality proteins of whole egg and lactalbumin when their dietary level of protein increased from 6 to 8%. Net protein ratio values (Table 16) followed the same trend but with higher numerical values particularly for poor quality proteins, i.e., soybean and white beans, since NPR method is known to upgrade the poor quality proteins (Pellette, 1978; Hackler, 1977; McLaughlan and Keith, 1974). Tables 10, 11, and Figs. 2, 3, 4, and 5 show the effect of the level of dietary protein on PER values when measurements were taken at the end of four different periods of feeding experiments. It could be concluded that maximum PER values were obtained at low levels of dietary protein

with prolonged periods of feeding experiment (Fig. 5). This was well demonstrated with low quality proteins, e.g., soybean and white beans, containing diets where the highest efficiency was obtained at 11% dietary protein when feeding experiment lasted 10 to 14 days (Figs. 2 and 3), at 8% level when feeding lasted for 21 days (Fig. 4), and at 6% level when feeding experiment lasted for 28 days (Fig. 5). The present data also indicated that the dietary levels at which maximum utilization for growth obtained was very definite for better quality proteins, i.e., whole egg and lactalbumin. This point was not so definite for poor quality proteins, e.g., soybean and white beans, when measurements were taken on different periods up to 21 days. This is in accord with results of Bosshardt et al. (1946).

The effects of the length of feeding period on PER and NPR values are shown in Tables 17, 18, 19, and Figs. 6, 7, 8, and 9. The maximum protein efficiency ratio and net protein ratio occurred in mice fed the protein sources for 10 days feeding experiment, with significantly higher values than if measurements were taken at the end of 14, 21, or 28 days of feeding experiment (Table 17). This is in agreement with the results of Bosshardt et al. (1946) using whole egg, casein and wheat gluten. Protein efficiency ratios and net protein ratio, generally, tended to decrease gradually with prolonged periods of feeding. However, this was not true in case of poor quality proteins, i.e., beans (Table 18, Fig. 6).

Table 17. Effect of the length of feeding period on protein efficiency ratios and net protein ratios in mice fed six sources of protein.[1]

Length of Feeding Period (days)	PER[2]	NPR[3]
10 days	2.368[a]	3.239[e]
14 days	2.178[b]	2.882[f]
21 days	2.016[c]	2.548[g]
28 days	1.819[d]	2.230[h]

[1]Means not having common letter superscripts are significantly different at the 0.05 level of probability.

[2,3]Grand means for each period of experiment.

Table 18. Effect of the length of feeding period on protein efficiency ratios[1] in mice fed six sources of protein.[3]

Source	Days[2] 10	14	21	28
Whole egg	3.512^{a}	3.188^{c}	2.854^{d}	2.473^{g}
Casein	2.249^{h}	2.054^{i}	1.927^{jk}	1.822^{l}
Milk, skim	2.612^{f}	2.286^{h}	1.970^{j}	1.869^{kl}
Beans, white	0.665^{t}	0.686^{t}	0.889^{r}	0.792^{p}
Soybean Isolate	1.785^{l}	1.621^{m}	1.546^{mn}	1.492^{n}
Lactalbumin	3.388^{b}	3.231^{c}	2.910^{d}	2.467^{g}

[1]Each PER value is the mean of three values obtained from feeding three different levels of dietary protein at the same period of feeding experiment.

[2]Length of feeding period.

[3]Means not having common letter superscripts are significantly different at the 0.05 level of probability.

Table 19. Effect of length of feeding period on net protein ratios[1] in mice fed six sources of protein.[2]

Source	Days[3] 10	14	21	28
Whole egg	4.367^{a}	3.805^{b}	3.300^{c}	2.833^{de}
Casein	2.979^{cd}	2.707^{def}	2.414^{efgh}	2.196^{ghi}
Milk, skim	3.394^{c}	2.950^{cd}	2.444^{efgh}	2.233^{ghi}
Soybean Isolate	1.710^{jhk}	1.583^{hk}	1.641^{hk}	1.385^{k}
Beans, white	2.666^{defg}	2.323^{fghi}	2.094^{hij}	1.917^{ijh}
Lactalbumin	4.315^{a}	3.923^{b}	3.396^{c}	2.817^{de}

[1]Each NPR value is the mean of three values obtained from feeding three levels of dietary protein at the same period of feeding experiment.

[2]Means not having common letter superscripts are significantly different at the 0.05 level of probability.

[3]Measurements taken at the end of four periods of feeding experiments; 10, 14, 21, and 28 days.

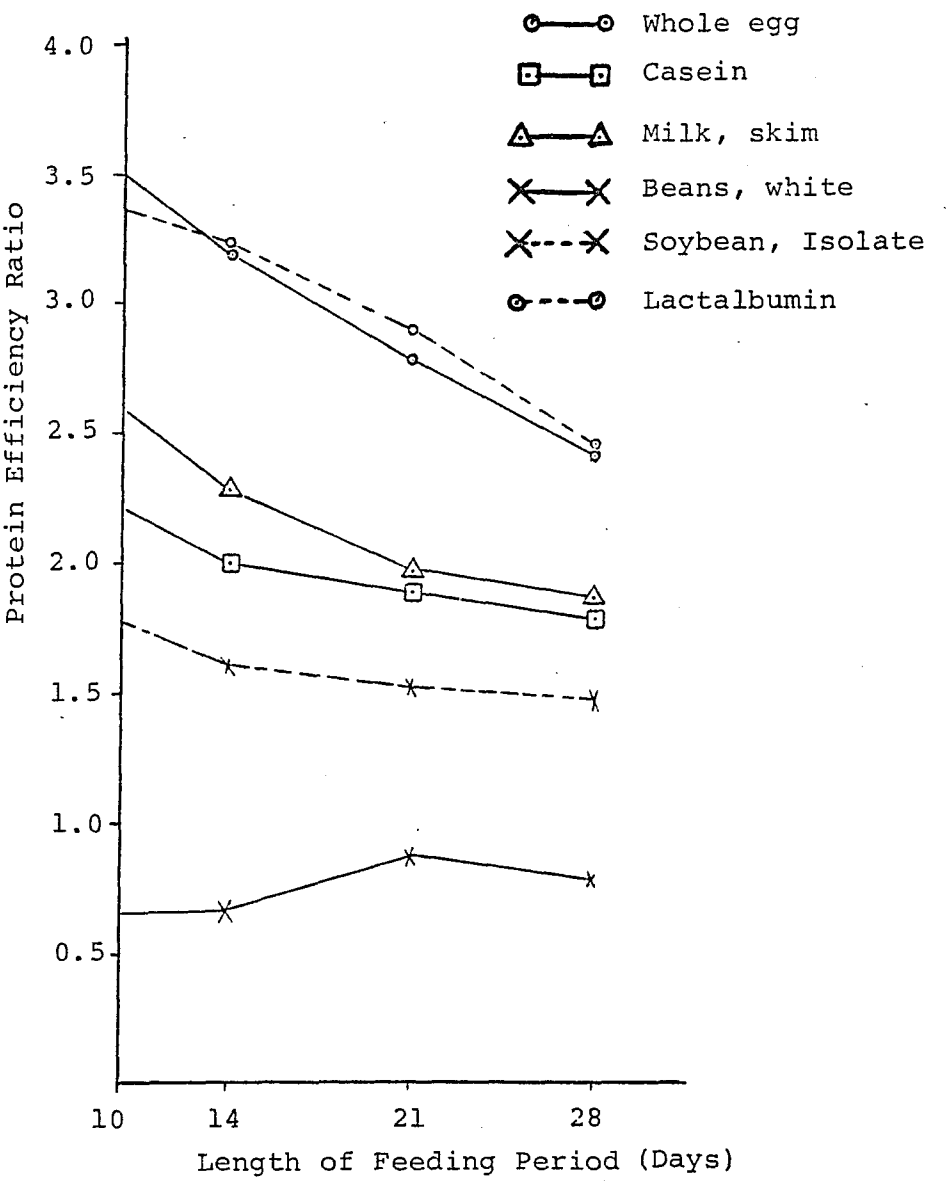

Fig. 6. The relation between the length of the feeding period and protein efficiency ratios.

Each PER value is the average PER value of the three levels used (6, 8, and 11% protein in diet).

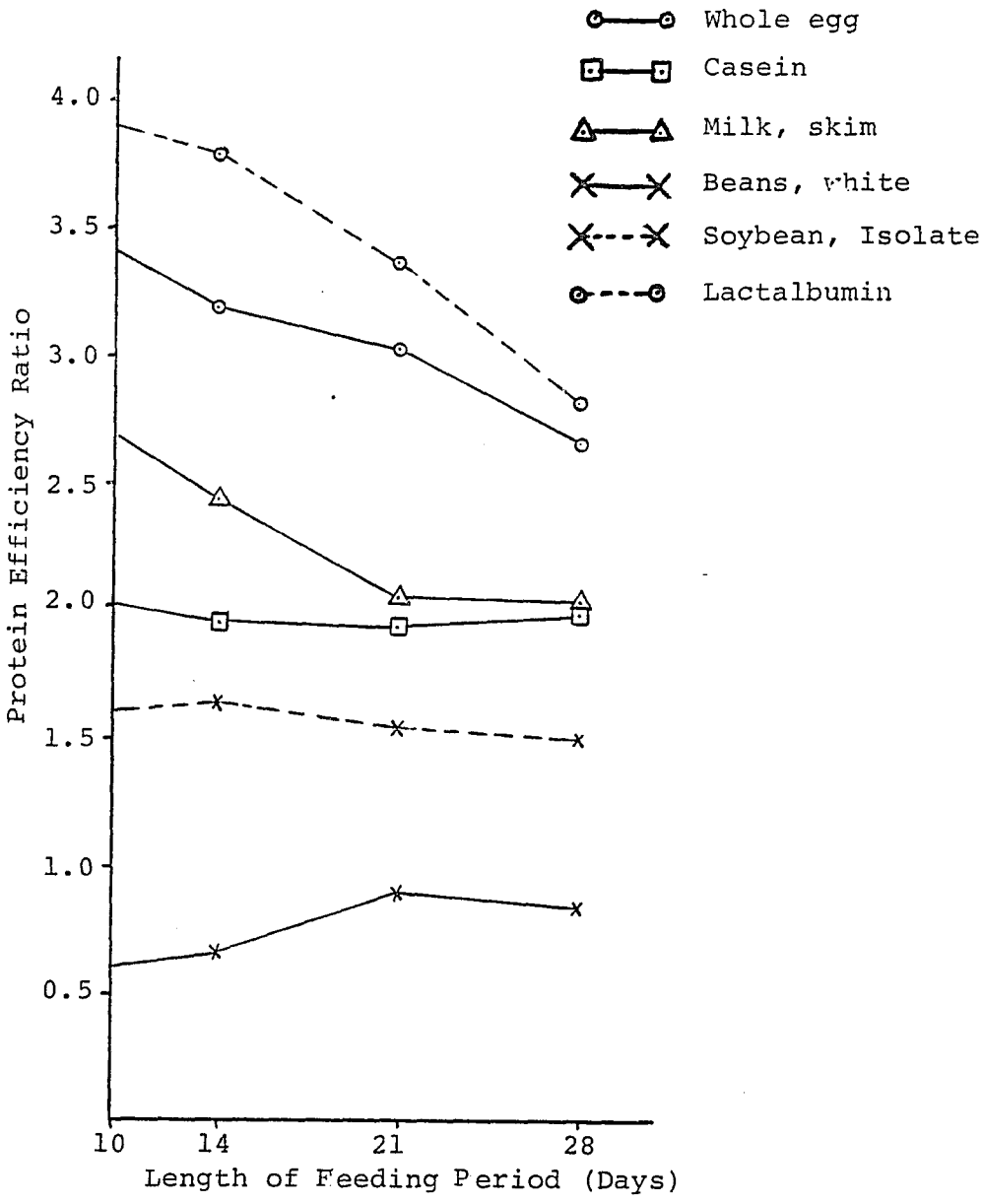

Fig. 7. The relation between the length of the feeding period and protein efficiency ratios at 6% level of protein in diet.

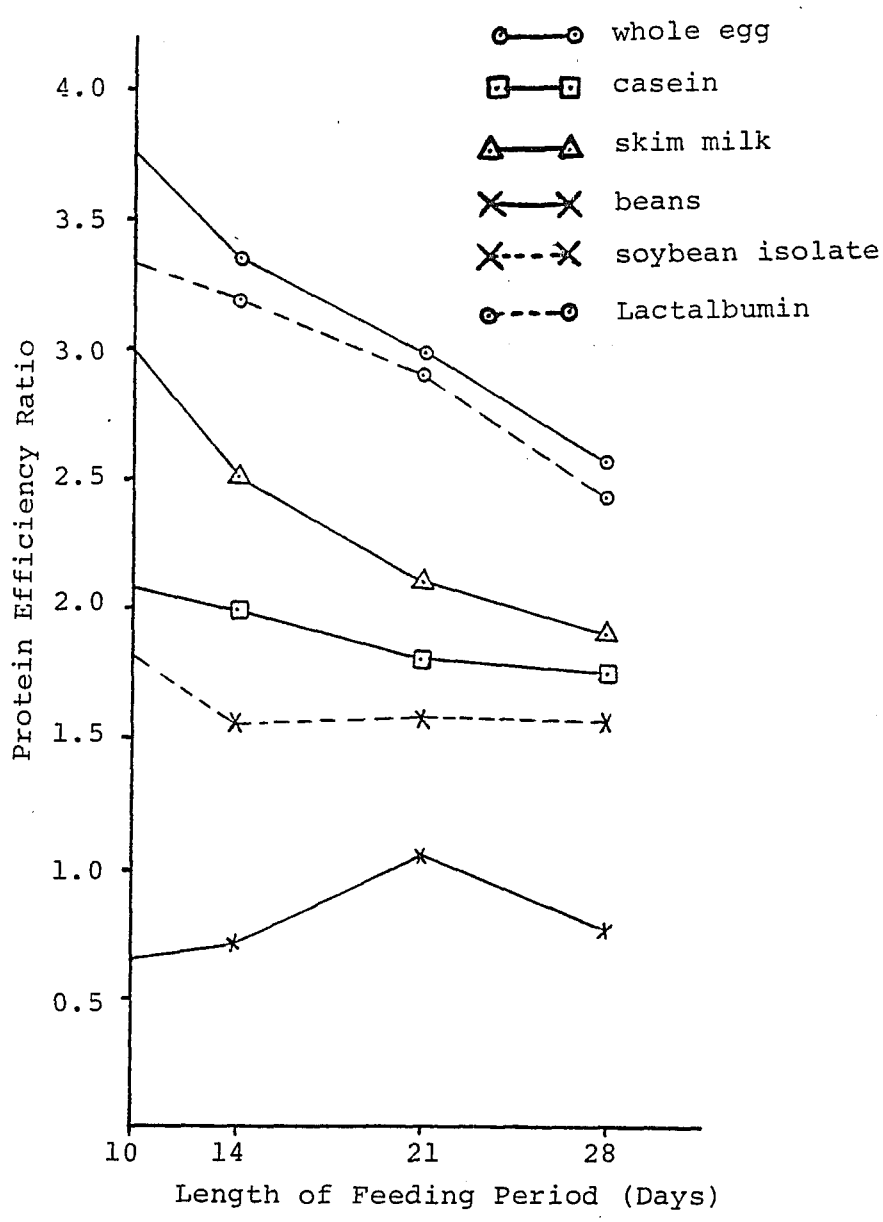

Fig. 8. The relation between the length of feeding period and protein efficiency ratio at 8% level of protein in diet.

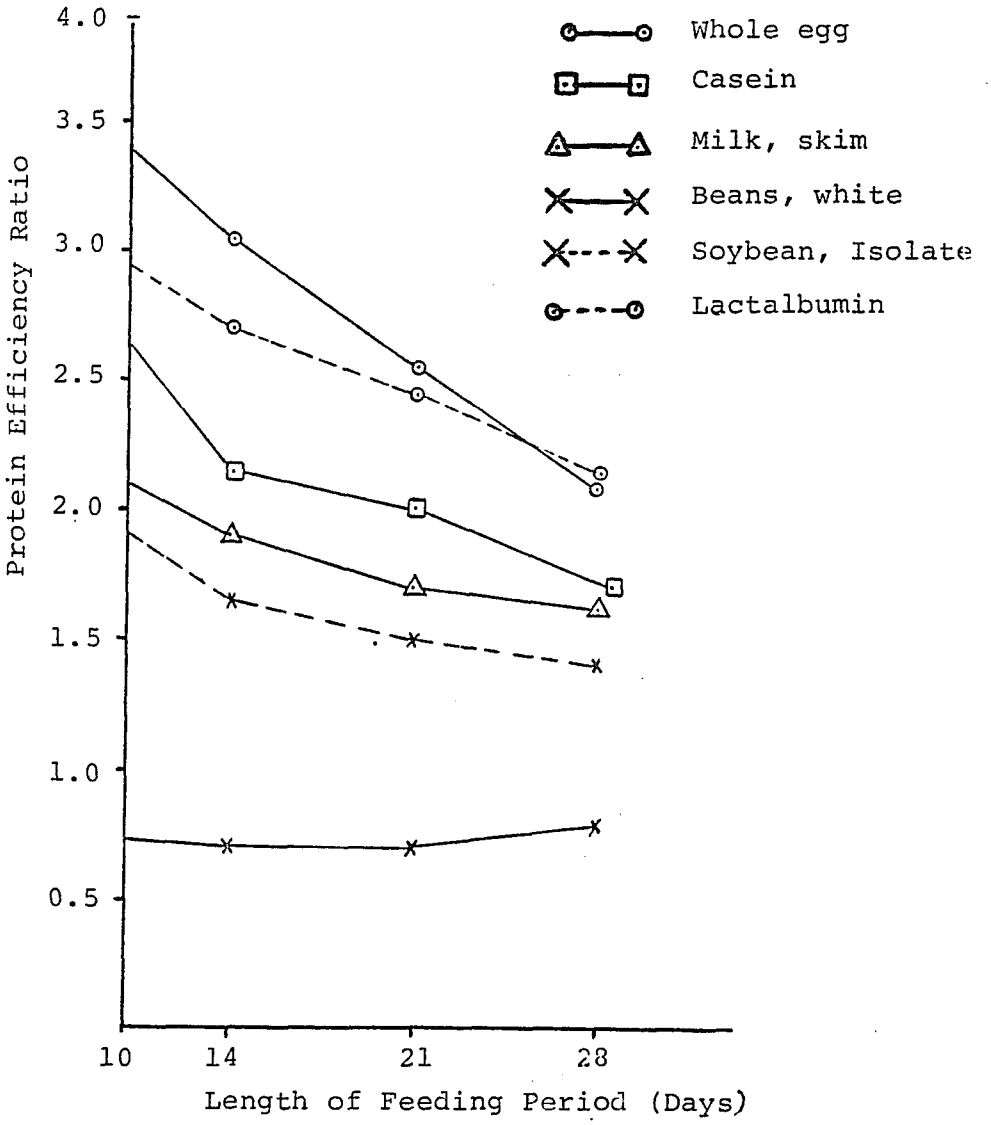

Fig. 9. The relation between the length of feeding period and protein efficiency ratio at 11% level of protein in diet.

Maximum PER and NPR values for poor sources of protein were obtained at 21 days of feeding period. This could be attributed to an adaptation mechanism to the poor quality and deficient proteins which needs a longer period than when a good quality protein source is fed. The relation between the length of the feeding period and protein efficiency ratios for the six sources of proteins were investigated individually at each level being fed (Figs. 7, 8, and 9). Results indicated that all sources of protein tested followed the same trend regardless of the levels being fed. Net protein ratios (Table 19) obtained at four different periods of feeding followed the same trend in all sources tested, as the PER values (Table 18). The maximum NPR values were obtained at the end of a 10-day feeding period then declined gradually with prolonged period of feeding giving the minimum values at 28-day feeding periods.

In order to establish a rationale for the use of mice in protein efficiency ratio and net protein ratio methods for the evaluation of protein quality, it was necessary to study the interaction between the level of dietary protein and the length of the feeding period and its effect on PER and NPR values (Tables 20, 21, and Fig. 10). It was found that PER values taken at 6% level of dietary protein were significantly higher than those taken at 8% level with the exception of the measurement taken at 8% level for a 10-day feeding experiment was the highest among all values (Table

Table 20. Effect of the interaction between the level of protein in the diet and length of feeding period on PER values of six sources of protein fed to mice.[1]

	Dietary Protein (%)		
Day[2]	6	8	11
10	2.372^{b}	2.440^{a}	2.293^{c}
14	2.276^{c}	2.215^{d}	2.042^{f}
21	2.140^{e}	2.077^{f}	1.830^{h}
28	1.986^{g}	1.833^{h}	1.639^{i}

[1]Means not having common letter superscripts are significantly different at the 0.05 level of probability.

[2]Length of feeding period (days).

Table 21. Effect of the interaction between the level of protein in diet and length of feeding period on net protein values of six sources of protein fed to mice.[1]

	Dietary Protein (%)		
Day[2]	6	8	11
10	3.449[a]	3.362[a]	2.904[c]
14	3.189[b]	2.940[c]	2.516[c]
21	2.838[c]	2.626[d]	2.180[f]
28	2.538[de]	2.250[f]	1.902[g]

[1]Means not having common letter superscripts are significantly different at the 0.05 level of probability.

[2]Length of feeding period (days).

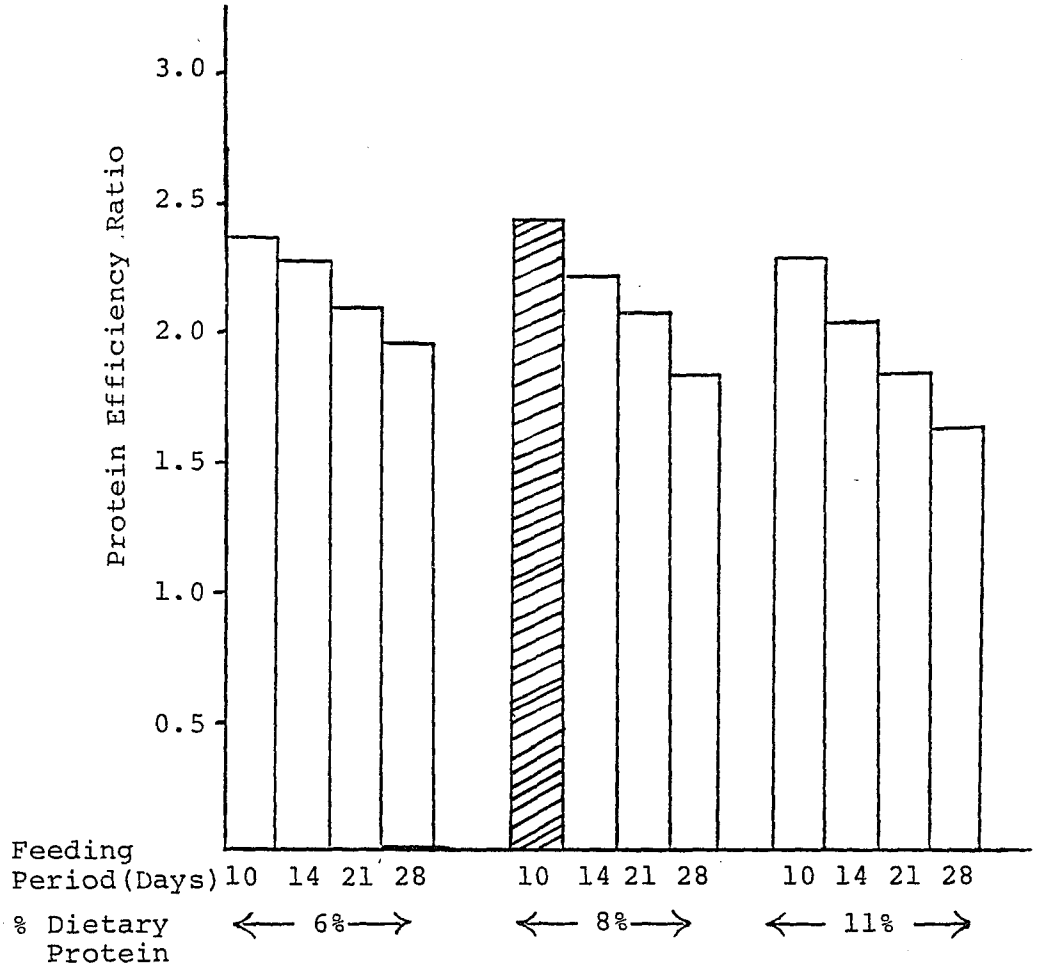

Fig. 10. The relation between the length of the feeding period and the level of dietary protein on protein efficiency ratio.

20 and Fig. 10). It is noteworthy to indicate that whole egg gave the highest protein efficiency ratio when fed at 8% level of dietary protein for a 10-day feeding experiment (Table 10). If we could define the reference standard protein as the protein source which possess a high nutritive value (Hegsted and Worcester, 1966), therefore it would be recommended, within the limits of this study, to use whole egg as the reference standard in the evaluation of protein quality in mice using either the PER or NPR methods. These findings are in agreement with early data from Bosshardt et al. (1946) using whole egg at approximately 8% level. Moreover, casein as a protein standard, for rat bioassay, has been criticized by several laboratories because of its poorer growth than whole egg or lactalbumin (Weber, Cossack, and Thompson, 1979). This was also observed in this study when employing mouse bioassay, as previously described in several sections in this discussion.

Protein Digestion

Digestibility coefficients of the six sources of protein were also determined in this study in order to correlate these results, with those of PER, NPR, and relative protein value (RPV). It should be noted that digestibility coefficients were determined only once from samples that were collected at the end of the feeding experiments that

lasted for 28 days. Results on protein digestion are presented in Table 22. There was a lack of trend that could be applied to all protein sources tested in terms of the effect of the levels of dietary proteins being fed. Differences in the levels of dietary protein may not be sufficient to influence digestibilities significantly in the limits of this experiment. However, it could be concluded from the present data that protein digestibility declined slightly with the increasing level of dietary protein, being 86.14, 85.26, and 83.26% as averages for the levels 6, 8, and 11%, respectively. Digestibility coefficients of whole egg and soybean isolate were in agreement with those reported by Weber, Cossack, and Thompson (1979), using weanling mice, with slight differences, which could be related to the differences in the length of feeding period between both studies, i.e., 21 days versus 28 days in this study.

Relative Protein Value

The method of slope ratio assay (Hegsted and Chang, 1965) was employed to calculate the relative protein value for the six sources of protein used in this experiment. Slopes and relative protein values were calculated at the end of four different periods of feeding experiment, 10, 14, 21, and 28 days (Table 23), using the same groups of mice previously employed in PER, NPR, and digestion

Table 22. Digestion coefficients* of protein in mice fed six sources of protein.

Source	Dietary Protein %	Protein Digestion Coefficient %	Mean ± S.E.
Whole egg	6.19	88.12	
	8.04	87.35	
	11.03	85.36	86.94 ± 1.42
Casein	6.36	91.11	
	8.25	93.04	
	11.42	92.80	92.35 ± 1.05
Milk, skim	6.37	84.76	
	7.92	81.07	
	10.95	80.45	82.09 ± 2.33
Beans, white	6.44	70.72	
	8.04	66.63	
	11.15	58.69	65.35 ± 6.12
Soybean Isolate	5.89	91.91	
	7.90	92.70	
	10.82	92.85	92.49 ± 0.51
Lactalbumin	6.31	92.04	
	8.13	90.74	
	11.06	91.27	91.35 ± 0.65

*Conducted on the basis of 28 days of feeding experiment.

Table 23. Slopes and relative protein value of six sources of protein fed to mice at three different levels.[1]

Source	Slopes[2]				Relative Protein Value[3]				Mean ± S.E.	
	10	14	21	28	10	14	21	28		
Whole egg[4]	4.01	3.48	2.93	2.42	100	100	100	100	100	0.00
Casein	3.10	2.58	2.25	1.91	77	74	77	79	78	2.06
Milk, skim	2.78	2.32	2.04	1.87	69	67	70	77	71	4.35
Beans	1.52	1.39	1.23	1.22	38	40	42	50	43	5.26
Soybean, Isolate	2.52	2.11	1.88	1.71	63	61	64	71	65	4.35
Lactalbumin	3.74	3.34	2.93	2.43	93	96	100	100	97	3.40

[1]Levels fed were 6, 8, 11% and non-protein diet.

[2,3]Measured at the end of four different periods of feeding experiment; 10, 14, 21, and 28 days.

[4]Standard reference protein.

experiments. The slopes of all protein sources followed the same trend, e.g., being the highest at 10-day feeding experiments then declined gradually with prolonged periods of feeding (Table 23). This trend was observed previously in protein efficiency ratios (Fig. 6) and net protein ratios (Table 9). However, this trend was lacking when relative protein value (RPV) was calculated (Table 23). It was suggested by Hegsted and Chang (1965) that a minimum of three levels of dietary protein to be fed from each source of protein in order to obtain a linear line. Levels of dietary protein used in this study were the same as those used in PER and NPR experiment, i.e., 6, 8, and 11%. An additional point was added; zero dose (protein free diet) in order to gain the practical effect of adding an additional data point (Hackler, 1977). In satisfactory slope-ratio assays, the regression lines relating dose to response must be linear over the dosage utilized; the regression lines for the standard and the unknown must intersect at the same point, and this should correspond to the response of the animals given zero dosage (Hegsted and Worcester, 1966). Within the limits of this experiment, considering the levels of dietary protein used, failure to obtain linear lines was observed with both high and poor quality sources of protein used in this study (Fig. 11). It is speculated that levels of dietary protein chosen for the slope ratio assay in this

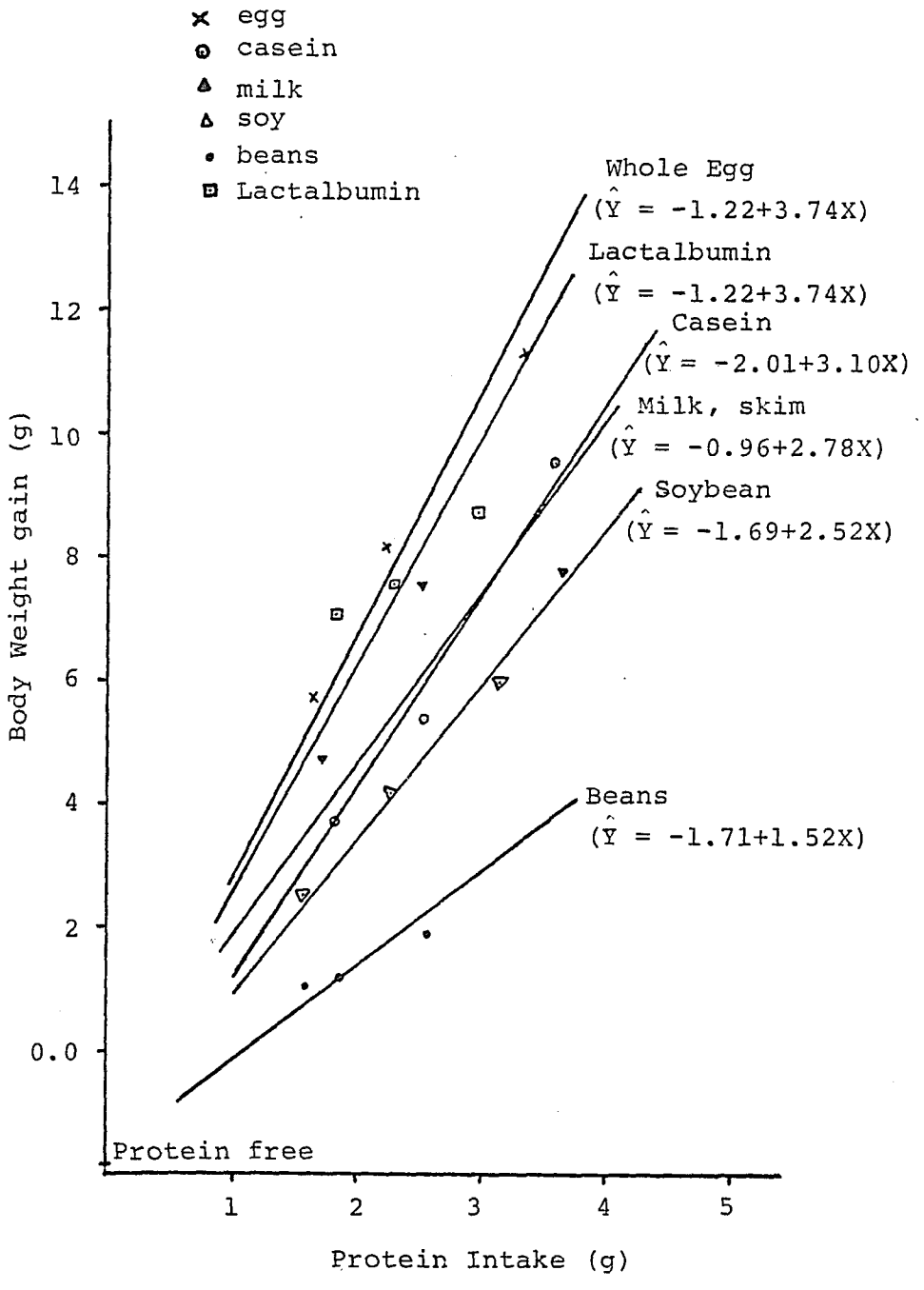

Fig. 11. Slopes for six sources of protein at the end of a 10-day feeding experiment in mice.

experiment were not sufficient in number and not carefully selected since only for a small range can a straight line by assumed; at high levels more and more protein would be deaminated and used for energy, while at low levels, especially for low quality proteins, deviation from linearity could occur because of such phenomena as adaptive responses, which consequentially result in a more efficient utilization of amino acids. This is especially true when the limiting amino acid in lysine (Pellette,1978). When the quality of dietary protein is high, e.g., whole egg and lactalbumin, maximum gain will be reached at lower levels of intake and curvature begins at lower levels of intake than when dietary protein is of lesser quality (Hegsted and Neff, 1970). When rats were fed diets containing three different proteins, lactalbumin, casein and soy protein each fed at levels from zero to over 50% of the diet (Hegsted and Neff, 1970), it was found that no significant curvature occurred when rats were fed lactalbumin ranging from zero to 10.02% levels of dietary protein, but when data from groups fed 12.33% protein and above were included, departure from linearity was observed. With casein as the dietary protein, data were linearly related for the first groups on dietary levels ranging from zero to 10.9%, but the inclusion of groups consuming casein at levels 15% protein and above caused the departure from linearity.

When the soy protein isolate was fed the data were linearly related for levels from zero to 12.9% and departure from linearity occurred when levels of 18.1% and above were included (Hegsted and Neff, 1970). In the light of these findings along with the consideration of the fact that the mouse protein requirements are much less than those of the rat (Goettsch, 1960; and Weber, Cossack, and Thompson, 1979), it could be concluded that levels of dietary protein from lactalbumin, whole egg, and milk, used in this experiment, were thought to be high enough to cause the departure from linearity. It is important to re-state that the maximum efficiency for utilization of protein sources expressed as PER occurred at the 6% level for lactalbumin and 8% for whole egg and skim milk (Fig. 2). It is recommended that protein levels to be used for the slope ratio assay method must be lower than the level of dietary protein which promotes the maximum PER value.

It is noteworthy to indicate that the slope ratio assay ranked the protein sources in the same fashion as PER and NPR except for casein and milk which were reversed. This could be explained by the sharp drop in the response of weight gain to the increasing level of dietary protein from skim milk over the 8% (Table 15) while it increased

in case of casein as a poorer quality than skim milk. These changes are best predicted by the slope ratio bioassay.

Table 24 shows data from the present study on PER, NPR, digestibility and RPV for the six sources of protein used in the mice study. Correlation coefficients for these four bioassays have been computed and are shown in Table 25. With this particular set of data, the correlation coefficients were high except with digestibility. However, correlation coefficients between digestibility and PER were higher than what has been reported by FAO/WHO (1973) using rats, r = .65 and .48 respectively. Other data could be obtained, from other experiments, and would show different correlation coefficients, but the main point that each procedure is different and comparisons, as shown (Table 25) are mainly academic and tend to rank proteins nearly in the same order in the mouse.

Rat Experiment No. 78-1

Rat bioassay has been long recognized by the National Research Council and the food quality regulatory agencies as the official method for protein quality evaluation. The standard for the PER method of evaluation has been well established (NRC, 1978) several years ago. Rat bioassays have been extensively reviewed (Hackler, 1977; McLaughlan and Keith, 1974; Pellette, 1978; and Steinke, 1977).

Table 24. Comparison of four bioassays for measuring protein quality in mice fed six sources of protein.

Source	Protein Efficiency Ratio[1]	Net Protein Ratio[2]	Relative Protein Value[3]	Protein Digestibility Coefficient[4]
Whole egg	3.512	4.367	100	86.94
Casein	2.249	2.979	77	92.35
Milk, skim	2.612	3.394	69	82.09
Beans, white	0.665	1.710	38	65.35
Soybean Isolate	1.785	2.666	63	92.49
Lactalbumin	3.388	4.315	93	91.35

[1,2]Protein efficiency ratio and net protein ratio both measured at the end of a 10-day feeding experiment.

[3]Relative protein value measured at the end of a 10-day feeding experiment.

[4]Measured on the basis of 28 days feeding experiment (mean value of the three levels being fed).

Table 25. Comparisons of four bioassays, correlation coefficients[1] in mice.

	r	Level of Significance
D[2] : PER[3]	0.65	0.16271
D : RPV	0.71	0.11147
PER : RPV[4]	0.97	0.00120
D : NPR[5]	0.61	0.2008
PER : NPR	1.00	0.00003
NPR : RPV	0.96	0.00183

[1]Calculated from data in Table 24.

[2]Digestibility measured on the basisof 28-day feeding period.

[3]PER measured at the end of a 10-day feeding experiment.

[4]Relative protein value measured at the end of a 10-day feeding experiment.

[5]Net protein ratio measured at the end of a 10-day feeding experiment.

Rat experiment was conducted under the same conditions as the mice experiment discussed previously, in order to justify the comparison between both bioassays. The same protein sources were fed but with different levels of dietary protein which represents a high, moderate, and low level i.e., 13, 10, and 7% dietary protein.

Results on protein efficiency ratios and net protein ratios for the six sources of protein are presented in Tables 26, 27, and 28, and analysis of variance in Table 29. Differences among all protein sources regarding their PER and NPR values were significant (Tables 28 and 29). Employing the multiple range test (Duncan, 1955), lactalbumin had the highest PER and NPR values as an indication of being the highest quality source, PER = 3.9 and NPR = 4.8 compared to whole egg, PER = 3.7 and NPR = 4.57.

The effects of the level of dietary protein on PER and NPR are shown in Tables 30, 31, 32, and Fig. 12. Barnes et al. (1945) confirmed the original findings of Osborne et al. (1919) that the level of protein fed would influence the efficiency by which it was utilized by the growing animal. These researchers noted that as the rate of body weight gain increased with increasing levels of dietary protein, the calculated protein efficiency reached a maximum value then decreased. This was demonstrated in the results of the present study (Tables 30, 31, and Fig. 12).

Table 26. Protein efficiency ratios of six sources of protein fed to rats at three different levels for four different periods of feeding experiment.

Source	Dietary Protein %	Days[1] 10	14	21	28
Whole egg	7.12	3.138	4.202	4.068	4.243
	10.06	3.340	3.942	3.763	3.682
	13.36	3.375	3.885	3.530	3.440
Casein	7.08	3.448	3.997	3.370	3.273
	10.37	3.527	3.775	3.530	3.460
	13.42	3.505	3.640	3.190	3.212
Milk, skim	7.13	3.580	3.343	3.110	3.873
	10.13	3.497	3.233	3.213	2.958
	13.47	3.240	3.038	2.992	2.833
Beans, white	7.33	0.102	0.117	0.188	0.277
	10.48	0.380	0.515	1.063	0.960
	13.30	0.808	0.890	1.238	1.272
Soybean, Isolate	7.22	2.230	2.113	2.248	2.048
	10.53	2.715	2.610	2.493	2.260
	13.33	2.902	2.595	2.498	2.257
Lactalbumin	7.04	4.212	3.847	3.862	3.832
	10.48	4.497	4.132	4.002	3.705
	13.82	4.370	3.855	3.562	3.315

[1]Measurements taken at the end of four different periods of feeding experiment, 10, 14, 21, and 28 days.

Table 27. Net protein ratios of six sources of protein fed to rats at three different levels for four different periods of feeding experiment.

Source	Dietary Protein %	Days[1] 10	14	21	28
Whole egg	7.12	4.913	5.457	5.082	5.087
	10.06	4.493	4.737	4.333	4.133
	13.36	4.208	4.562	4.018	3.823
Casein	7.08	5.648	5.687	4.750	4.400
	10.37	4.725	4.625	4.218	3.985
	13.42	4.413	4.257	3.655	3.562
Milk, skim	7.13	5.118	4.562	4.143	3.827
	10.13	4.415	3.970	3.830	3.483
	13.47	3.980	3.600	3.440	3.208
Beans, white	7.33	1.945	1.745	1.920	1.822
	10.48	2.100	1.832	2.113	1.902
	13.30	2.010	1.818	1.992	1.897
Soybean, Isolate	7.22	3.987	3.520	3.423	3.103
	10.53	3.667	3.362	3.135	2.760
	13.33	3.623	3.145	2.970	2.643
Lactalbumin	7.04	6.160	5.315	5.527	5.005
	10.48	5.562	4.907	4.592	4.202
	13.82	5.002	4.312	3.918	3.587

[1]Measurements taken at the end of four different periods of feeding experiment, 10, 14, 21, and 28 days.

Table 28. Means[1], protein efficiency ratios and net protein ratios of six sources of protein fed to rats.

Source	Protein Efficiency Ratio[2]	Net Protein Ratio[3]
Whole egg	3.717[b]	4.570[g]
Casein	3.494[c]	4.495[g]
Milk, skim	3.159[d]	3.965[h]
Beans, white	0.651[f]	1.925[k]
Soybean Isolate	2.414[e]	3.278[l]
Lactalbumin	3.932[a]	4.841[m]

[1]Grand means.

[2,3]Measurements not having common letter superscripts are significantly different at the .05 level of probability.

Table 29. Analysis of variance[1] on protein efficiency ratio and net protein ratio data in rats fed six sources of protein.

Source of Variation	D.F.	Mean of Squares PER	NPR
Sources (S)	5	107.2570*	85.8602*
Level (L)[3]	2	0.7790*	21.6579*
Period (P)[2]	3	0.9009*	11.2364*
Reps.	5	0.3321	0.6886
SL	10	1.4123*	1.6618*
SP	15	1.1514*	1.0563*
LP	6	0.1701*	0.0158
SLP	30	0.1215*	0.1014*
Error A	90	0.2026	0.4522
Error B	270	0.0501	0.0691
Remainings	355	0.8911	1.7413
TOTAL	431	-	-

[1]Significant at the 0.05 level of probability.

[2]Length of feeding period.

[3]% dietary protein.

Table 30. Effect of level of protein in the diet on protein ratios[1] and net protein ratios[2] in rats fed six sources of protein.[3]

Dietary Protein %	PER	NPR
7	2.822^{b}	4.256^{d}
10	2.969^{a}	3.795^{e}
13	2.893^{ab}	3.485^{f}

[1,2]Grand means for each level fed.

[3]Means not having common letter superscripts are significantly different at the .05 level of probability.

Table 31. Effect of the level of protein in diet on protein efficiency ratios[1] in rats fed six sources of protein.

Source	Dietary Protein % 7	10	13
Whole egg	3.913^{abc}	3.682^{bcde}	3.558^{cdef}
Casein	3.522^{def}	3.573^{cdef}	3.387^{ef}
Milk, skim	3.227^{fg}	3.225^{fg}	3.026^{g}
Beans, white	0.171^{k}	0.730^{k}	1.052^{k}
Soybean Isolate	2.160^{i}	2.520^{h}	2.563^{h}
Lactalbumin	3.938^{ab}	4.084^{a}	3.775^{abcd}

[1]Each PER value is the mean of four different values obtained from four different periods of feeding experiment 10, 14, 21, and 28 days.

[2]Means not having common letter superscripts are significantly different at the .05 level of probability.

Table 32. Effect of the level of protein in diet on net protein ratios[1] in rats fed six sources of protein.[2]

	Dietary Protein (%)		
Source	7	10	13
Whole egg	5.135^{ab}	4.424^{cd}	4.152^{d}
Casein	5.121^{ab}	4.388^{cd}	3.974^{de}
Milk, skim	4.412^{cd}	3.925^{de}	3.557^{ef}
Beans, white	1.858^{g}	1.987^{g}	1.929^{g}
Soybean Isolate	3.508^{ef}	3.231^{f}	3.095^{f}
Lactalbumin	5.502^{a}	4.815^{bc}	4.205^{d}

[1]Each NPR value is the mean of four values obtained from four different periods of feeding experiment, 10, 14, 21, and 28 days.

[2]Means not having common letter superscripts are significantly different at the .05 level of probability.

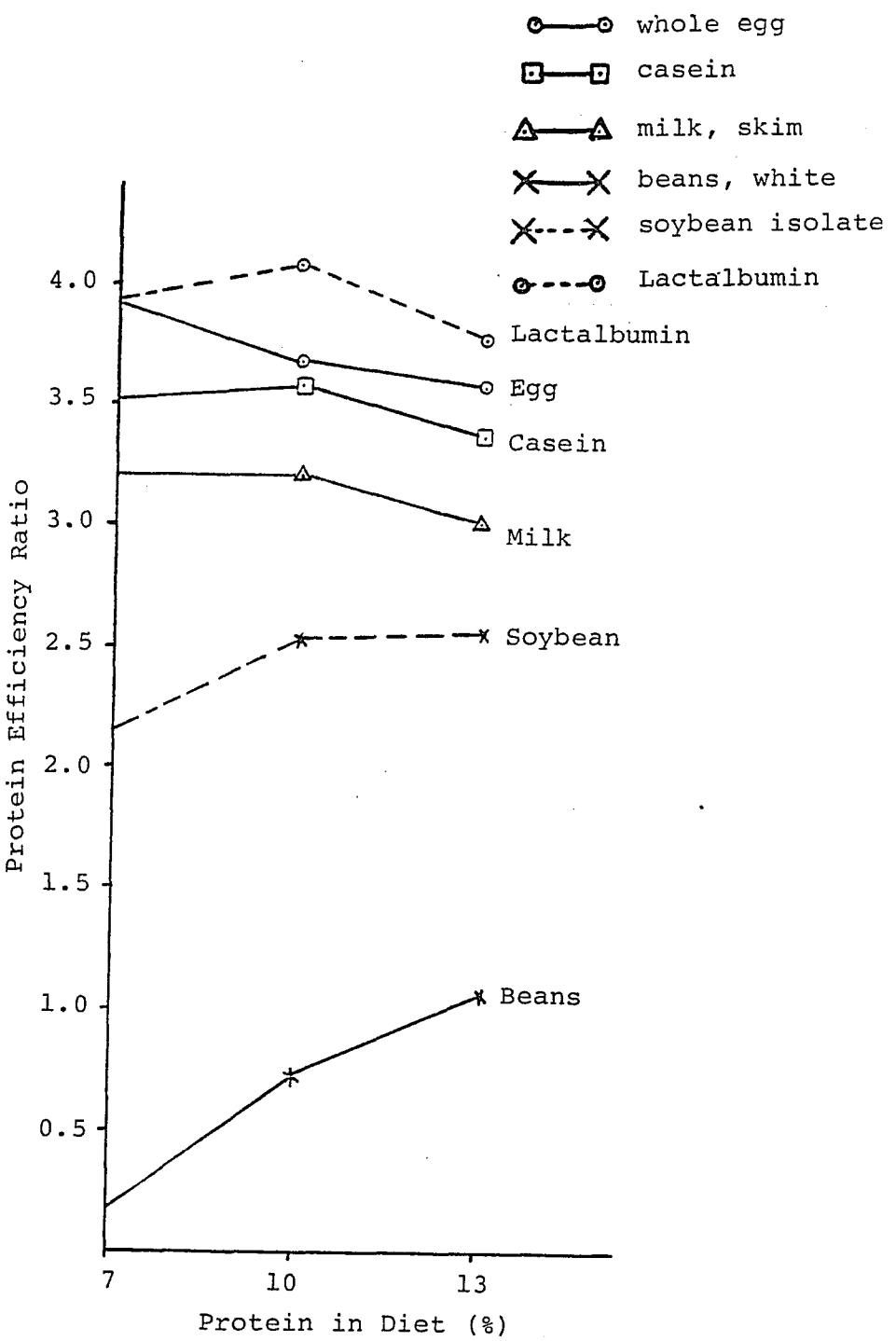

Fig. 12. Effect of level of protein in diet on protein efficiency ratio in rats.

Protein efficiency ratio was the highest at 10% level then declined at 13% or 7% level. However, when a foodstuff of lower quality protein was fed, both weight gain and efficiency of protein utilization increased in direct relationship to the level of protein fed (Morrison and Campbell, 1960). This finding was best demonstrated with soybean isolate and white beans fed in this experiment (Table 31 and Fig. 12). The PER of soybean isolate and beans were lower when fed at the 7% protein level than was determined when the diets were fed at 10% and 13% protein level. Conversely, the PERs of a high quality protein, e.g., lactalbumin, milk, whole egg, and casein were high when fed at 7% and 10% protein level (Table 31 and Fig. 12) than what was determined when diets were fed at the 13% level (Morrison and Campbell, 1960; and Hurt, Forsythe, and Krieger, 1974).

The effect of the level of dietary protein on NPR is shown in Table 32. It was demonstrated by McLaughlan and Keith (1974) that values of NPR tend to fall with increasing level of dietary protein, which is in accord with results from this experiment (Table 32). Values of NPR tended to fall more sharply in case of high quality proteins, e.g., whole egg, lactalbumin, casein and milk while it falls slightly in case of lower quality proteins, e.g., soybean and beans. This could be attributed to the larger increase

in protein intake from high quality protein than the lower quality sources.

Hackler (1974) and Hegarty (1975) concluded that PER measured at the end of a 2 week feeding period were higher than values obtained at the end of a 4 week period of feeding, suggesting that the PER assay could be shortened to 2 weeks without loss in accuracy. This was in accord with results from the present work (Tables 33, 34, 35, and Fig. 13) on PER and NPR except for poor quality sources, e.g., beans (Table 34). PER values for beans tended to increase gradually with prolonged periods of feeding which could be attributed to an adaptation phenomena (Pellette, 1978), while for whole egg, lactalbumin and milk the PER and NPR values were higher at 10 or 14 days versus 21 and 28 days.

Results on protein digestion are presented in Table 36. Same as the mouse experiment, the digestibility coefficients were determined only once after the end of the 28 day feeding period for each level of protein being fed from each source. Slight increase in digestibility coefficients with increasing level of dietary protein was observed. This increase was the highest in case of beans, 59, 65, and 70% digestion coefficients for the levels 7, 10, and 13% of dietary protein, respectively (Table 36).

Table 37 and Fig. 14 show the slopes and relative protein values for each source of protein fed, according to the method of Hegsted and Chang (1965). It would appear

Table 33. Effect of the length of the feeding period on protein efficiency ratios and net protein ratios in rats fed six sources of protein.[1]

Length of Feeding Period (Days)	PER[2]	NPR[3]
10	2.937^{ab}	4.220^{a}
14	2.985^{a}	3.968^{b}
21	2.885^{b}	3.726^{c}
28	2.772^{c}	3.468^{d}

[1]Means not having common letter superscripts are significantly different at the .05 level of probability.

[2,3]Grand means for each period of feeding experiment.

Table 34. Effect of the length of feeding period on protein efficiency ratios[1] in rats fed six sources of protein.[2]

Source	Days[3] 10	14	21	28
Whole egg	3.284^{gh}	4.009^{b}	3.787^{cd}	3.788^{cd}
Casein	3.493^{ef}	3.804^{cd}	3.363^{fgh}	3.315^{fgh}
Milk, skim	3.439^{fg}	3.205^{hi}	3.105^{i}	2.888^{k}
Beans, white	0.430^{r}	0.507^{r}	0.830^{p}	0.836^{p}
Soybean, Isolate	2.616^{l}	2.439^{m}	2.413^{m}	2.188^{n}
Lactalbumin	4.359^{a}	3.944^{bc}	3.808^{cd}	3.617^{de}

[1]Means of the three levels of dietary protein being fed from each source.

[2]Means not having common letter superscripts are significantly different at the .05 level of probability.

[3]Length of feeding period.

Table 35. Effect of length of feeding period on net protein ratios[1] in rats fed six sources of protein.[2]

Source	Days[3] 10	14	21	28
Whole egg	4.537^{de}	4.918^{b}	4.478^{de}	4.348^{ef}
Casein	4.929^{b}	4.859^{bc}	4.208^{fg}	3.982^{h}
Milk, skim	4.504^{de}	4.044^{gh}	3.804^{i}	3.506^{k}
Beans, white	2.018^{p}	1.798^{p}	2.008^{p}	1.873^{p}
Soybean Isolate	3.759^{i}	3.342^{km}	3.176^{m}	2.836^{n}
Lactalbumin	5.574^{a}	4.844^{bc}	4.679^{cd}	4.264^{f}

[1]Means of the three levels of dietary protein being fed from each source.

[2]Means not having common letter superscripts are significantly different at the .05 level of probability.

[3]Length of feeding period.

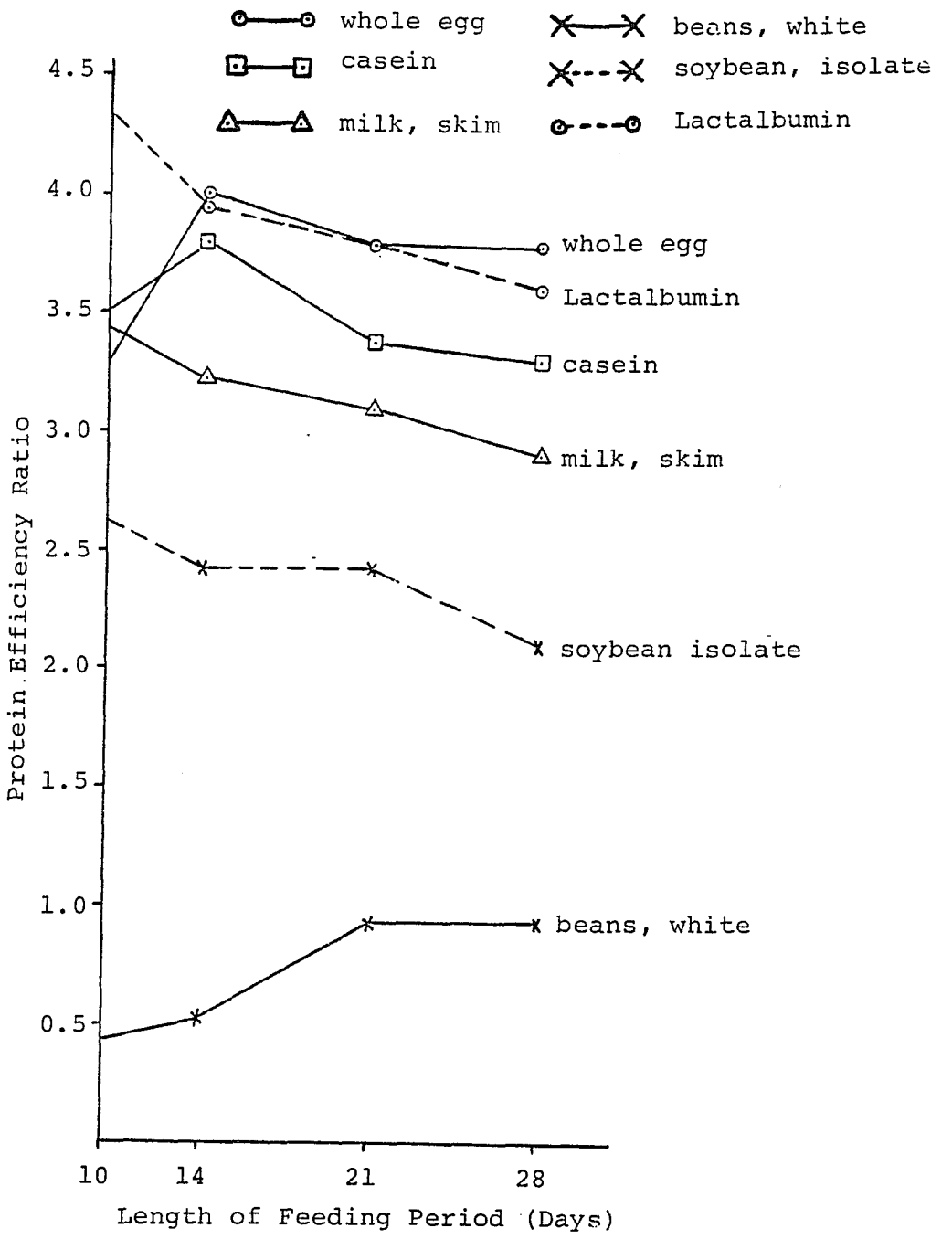

Fig. 13. Effect of the length of the feeding period on protein efficiency ratio in rats.

Table 36. Digestion coefficients of protein in rats fed six sources of protein.

Source	Dietary Protein %	Protein Digestion Coefficient %	Mean ± S.E.
Whole egg	7.12	89.20	
	10.06	90.76	
	13.36	89.62	89.86 ± 0.807
Casein	7.08	92.79	
	10.37	94.49	
	13.42	95.59	94.29 ± 1.411
Milk, skim	7.13	84.36	
	10.13	85.08	
	13.47	86.19	85.21 ± 0.922
Beans, white	7.33	59.35	
	10.48	65.19	
	13.30	70.18	64.91 ± 5.421
Soybean Isolate	7.22	90.16	
	10.53	91.85	
	13.33	92.78	91.60 ± 1.33
Lactalbumin	7.04	91.57	
	10.48	92.48	
	13.82	92.85	92.30 ± 0.66

Table 37. Slope ratios[1] and net protein values[2] of six sources of protein fed to rats.

Source	Slope	Relative Protein Value[3]
Whole egg	3.84	107
Casein	3.56	99
Milk, skim	3.18	89
Beans, white	1.95	54
Soybean Isolate	2.63	73
Lactalbumin[4]	3.59	100

[1]Sources fed at three levels of dietary protein, 7, 10, and 13%. Zero dose was considered in computing the slopes.

[2]According to the standardized method of Hegsted and Chang (1965).

[3]Relative protein value = $\frac{\text{slope of source}}{\text{slope of standard (Lactalbumin)}}$

[4]Reference standard protein

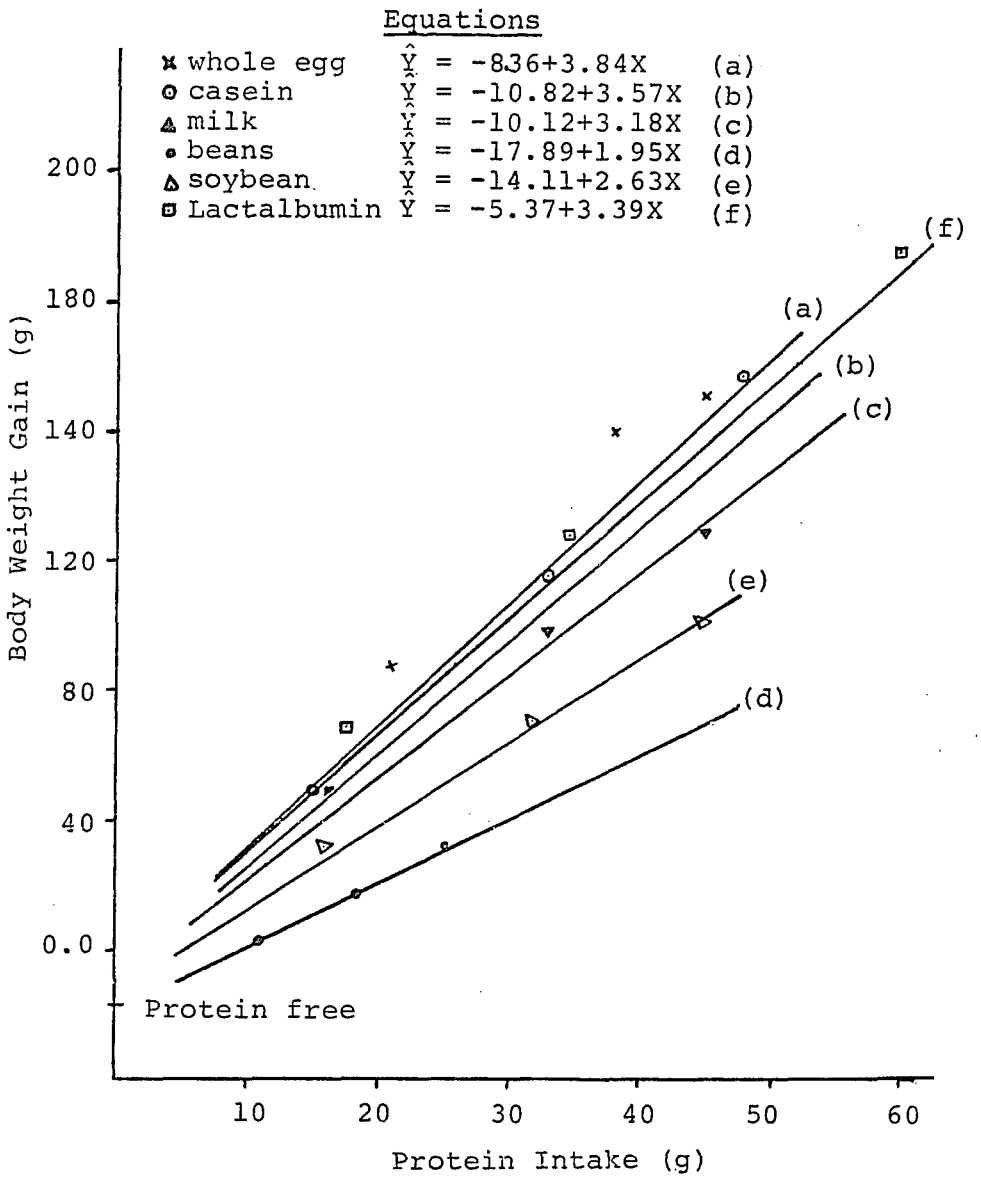

Fig. 14. Slopes for six sources of protein fed to rats.

from the present data that levels of dietary protein used in this experiment are within the limits that give a linear line from the three points with the exception of whole egg (Fig. 14). It should be noted that the maximum efficiency for whole egg to promote growth was at the 7% level of dietary protein (Table 31), while it appeared that the 13% level was beyond the limit which is necessary to give a linear line (Fig. 14).

Similar results were reported by Hegsted and Neff (1970) but they used lactalbumin, casein and soybean isolate. From the present data, whole egg appeared to be superior to lactalbumin in its relative protein value. This is in agreement with data reported by Hackler (1977) and Hegsted and Chang (1965).

It could be concluded from the present investigation that PER, NPR, and RPV methods had ranked the protein sources in the same order (Table 38) except for whole egg and lactalbumin which was reversed in RPV method. No significant differences between whole egg and lactalbumin were observed regarding their PER and NPR values. However, RPV correlated highly with both PER and NPR, $r = .99$ for both (Table 39). Protein digestion method did not follow any of the previous methods in ranking the protein sources. This is obvious since protein digestion is a poor method for evaluation of protein quality and does not express the

Table 38. Comparison of four bioassays for measuring protein quality in rats fed six sources of protein.

Source	Protein Efficiency Ratio[1]	Net Protein Ratio[2]	Relative Protein Value[3]	Protein Digestibility Coefficient[4]
Whole egg	3.682	4.133	107	89.86
Casein	3.460	3.985	99	94.29
Milk, skim	2.958	3.483	89	85.21
Beans, white	0.960	1.902	54	64.91
Soybean Isolate	2.260	2.760	73	91.6
Lactalbumin	3.705	4.202	100	92.3

[1 2 3]Values calculated according to the standard method of the AOAC (1975).

[4]Measured on the basis of 28 day feeding experiment (mean of the three levels being fed.

Table 39. Comparisons of four bioassays, correlation coefficient[1] in rats.

	r	Level of Significance
D[2] : PER[3]	0.86	0.02956
D : RPV[4]	0.80	0.05685
D : NPR[5]	0.81	0.04971
PER : NPR	1.00	0.00003
PER : RPV	0.99	0.00017
NPR : RPV	0.99	0.00013

[1]Calculated from data in table

[2]Digestibility coefficient

[3]Protein efficiency ratio

[4]Relative protein value

[5]Net protein ratio

availability of the protein source to satisfy the tissue needs (McDonald, Edwards, and Greenhalgh, 1971). However, the correlation coefficients for protein digestion to the other method used were lower, i.e., 0.86, 0.80, 0.81 for Dig.:PER, Dig.:RPV and Dig.:NPR respectively.

General Discussion

Data from the mice experiment indicated that there are certain factors such as level of dietary protein and length of feeding period that affects the results of PER, NPR, protein digestion and RPV measurements when the mouse is used as the test animal. Similar effects, under the same conditions, were observed with rats. Figures 15 and 16 are scattergrams showing the effect of the interactions between the factors, i.e., length of feeding experiment (P), level of dietary protein (L), and source of protein (S) on values of PER (Fig. 15) and NPR (Fig. 16) using both rats and mice. It appeared that the interaction between the factors PxLxS exerted similar effects on both rats and mice for PER values (R = .857, significance level = .00001), and for NPR values (R = .862, significance level of .00001).

Correlation coefficients between the four methods of evaluation protein quality employing both mice and rats are summarized in Table 40. In both species, digestibility appeared to be a poor method of evaluation and had a poor

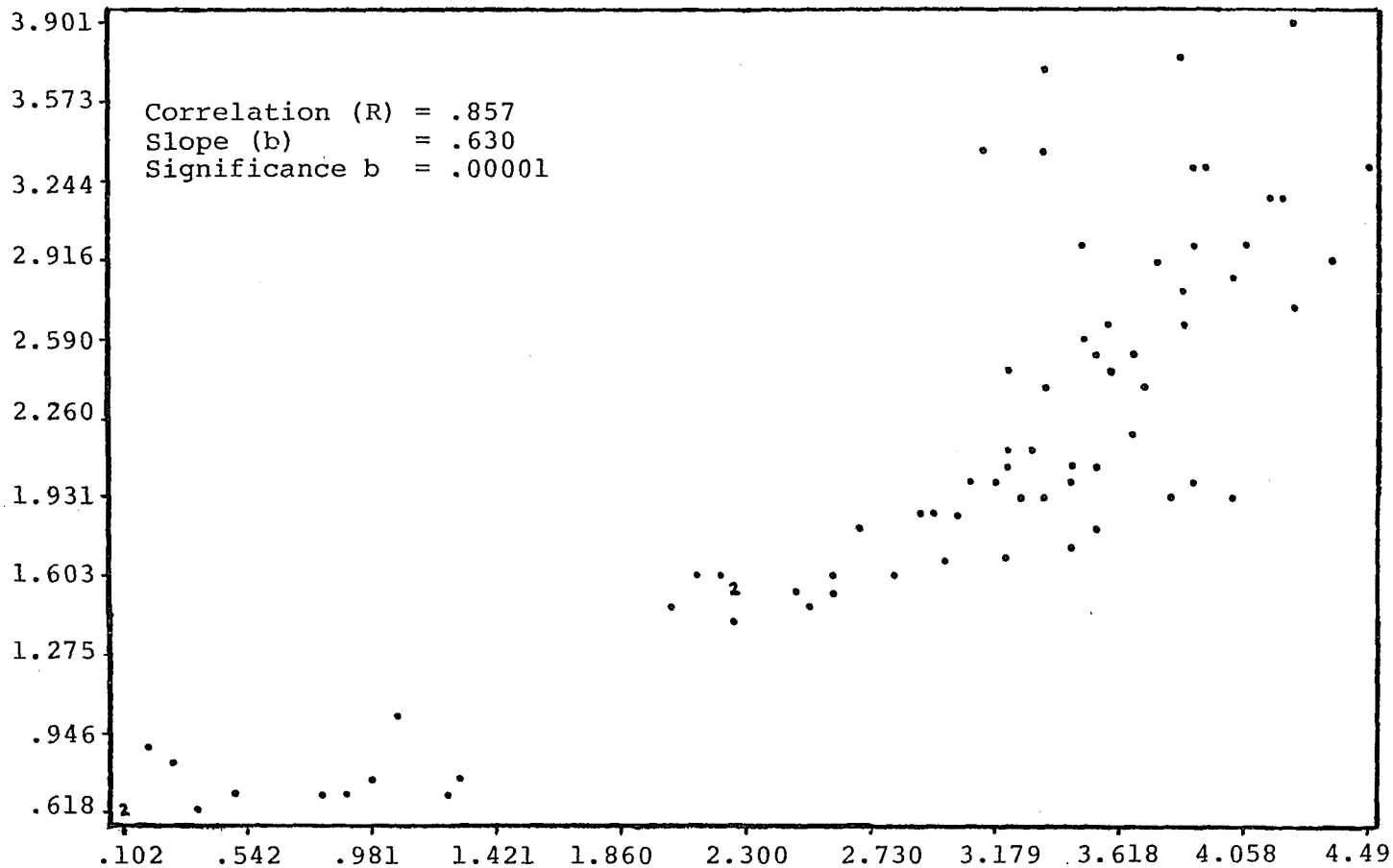

Fig. 15. Scattergram, PER values as affected by P xL xS in rats versus mice.

P = length of feeding period; L = level of dietary protein;
S = source of protein.

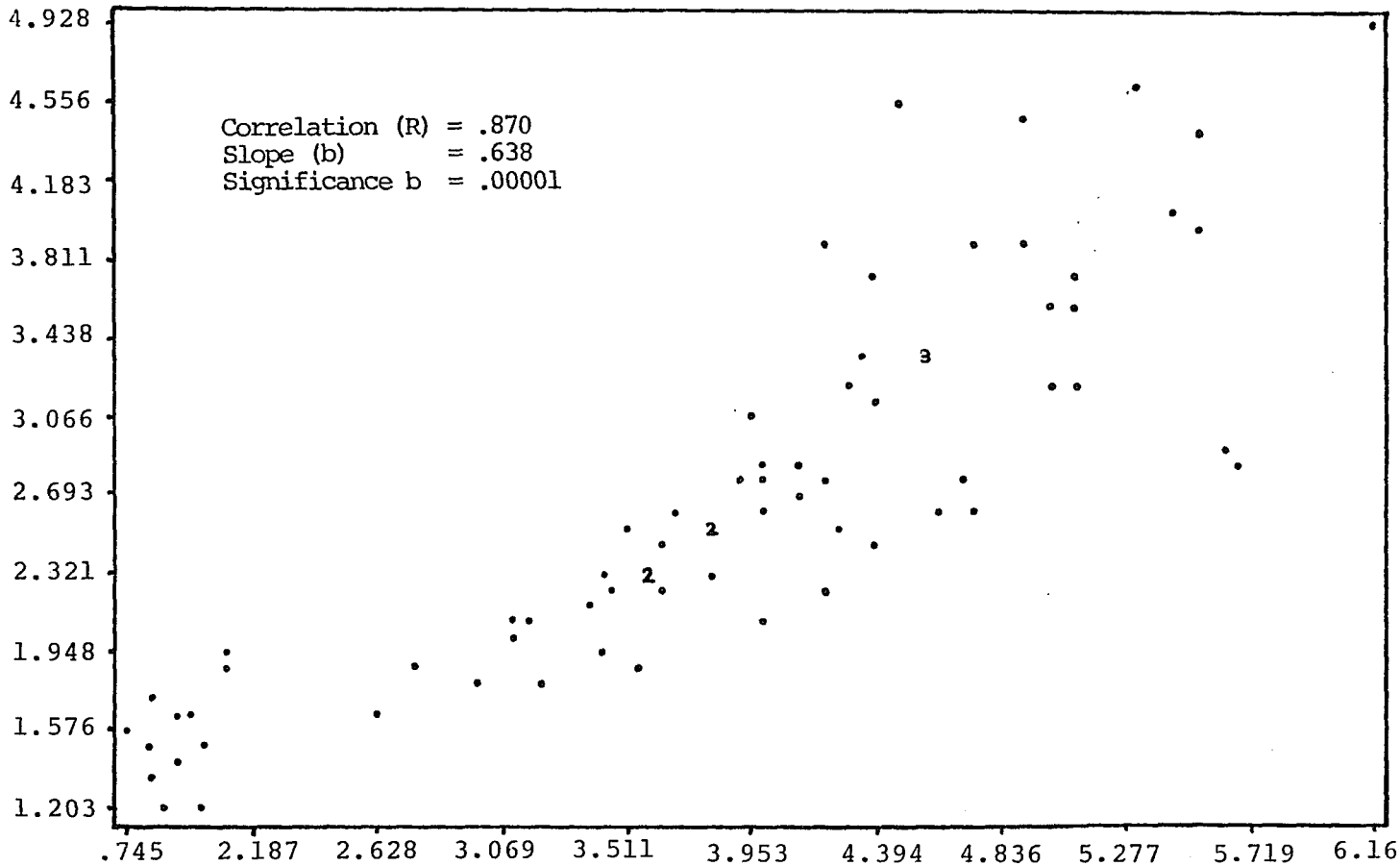

Fig. 16. Scattergram, NPR values as affected by P xL xS in rats versus mice.

P = length of feeding period; L = level of dietary protein;
S = source of protein.

Table 40. Correlation coefficients between four methods of evaluation of protein quality using mice versus rats.

Methods[1]	r mice	r rats
D : PER	.65	.86
D : RPV	.71	.80
D : NPR	.61	.81
PER : NPR	1.00**	1.00**
NPR : RPV	.96**	.99**
PER : RPV	.97**	.99**

*Significant at the level .05 of probability

[1]D = protein digestibility, PER = protein efficiency ratio, NPR = net protein ratio, RPV = relative protein value.

correlation with the other methods used. However, PER, NPR, and RPV correlated highly in both rats and mice.

Employing the correlation coefficient test on mice versus rats (Table 41), it could be concluded that values of PER, NPR, protein digestion, and RPV for the six sources of protein fed to either mice or rats are highly correlated between the two species (R = 0.94 to 0.99).

Mice appeared to be good experimental animals to use to test protein quality evaluation with advantages of smaller animal size, lower food consumption and shorter test period. Some of the disadvantages in using mice are that measurements must be made more accurately since weight changes and food consumption for the mouse are considerably less than for the rat. Mice have the problem of wide range of strains, which necessitates further studies for specifications of the strains if mice are to be used for the evaluation of protein quality.

Table 41. Correlation coefficients between rats and mice data on the four methods of protein evaluation.[1]

Method	r*	Level of Significance
RPV	0.96	0.00272
PER	0.93	0.00568
NPR	0.91	0.01357
Digestion, protein	0.99	0.00020
PER (standard)[2]	0.94	0.0050

[1]Calculated from data in Tables 24 and 40.

[2]Measurements taken according to the standard procedure; 10% dietary protein, 28 days for rats, and 8% dietary protein for 10 days in mice.

*All are highly significant at the level of .05 of probability.

CHAPTER 5

SUMMARY

A series of experiments were carried out in order to investigate the possibility of using mice for the evaluation of protein quality and to compare the results with the rat bioassay. Six sources of protein were tested, i.e., whole egg, casein, white beans, Isolated soybean and Lactalbumin. Each source was fed at three levels, i.e., 6, 8, and 11% for mice and 7, 10, and 13% for rats. Measurements were taken for protein efficiency ratio (PER), net protein ratio (NPR), protein digestion, and relative protein value (RPV) by the slope ratio assay method. Measurements were taken at four experimental periods, i.e., 10, 14, 21, and 28 days in order to study the effects of the length of feeding period, levels of dietary protein and their interactions on values obtained from the four parameters mentioned previously.

Results of mice experiment indicated that the level of dietary protein at which maximum protein efficiency ratio is obtained varies with the different protein sources being fed. Maximum PER values were obtained at 6% level of dietary protein for lactalbumin, 8% with no significant difference than 6% for whole egg and skim milk, 8% with no significant difference with 11% for soybean and beans, and

11% for casein. Values of net protein ratio followed the same trend but with higher numerical values particularly for poor quality proteins, i.e., soybeans and white beans.

The maximum PERs for poor quality proteins were obtained when the proteins were fed for prolonged time at low dietary level. This was well demonstrated with soybeans and white beans containing diets where the highest efficiency was obtained at 11% dietary protein when feeding experiment lasted for 10-14 days, at 8% level when feeding lasted for 21 days, and at 6% level when feeding experiment lasted for 28 days. Data indicated that the dietary levels of protein at which maximum utilization for growth could be obtained was very definite for high quality proteins, i.e., whole egg and lactalbumin but was not so definite for poor quality proteins, i.e., soybeans and beans. Maximum PER and NPR occurred in mice fed the protein sources for a 10-day feeding experiment with significantly higher values being obtained at this protein level than if measurements were taken at the end of 14, 21, or 28 days. PER and NPR generally tended to decrease gradually with prolonged period of feeding. However, for poor protein sources, i.e., beans, maximum PER was obtained at 21 days of feeding period. Studying the effect of the interaction between the level of dietary protein and the length of feeding period it was found that maximum PER and NPR values were obtained when

feeding 8% dietary level of protein for 10 days feeding experiment.

No consistent trend could be found and applied to all protein sources in terms of the effect of the level of dietary protein versus protein digestion coefficients. However, generally, values of protein digestion declined slightly with increasing levels of dietary protein, being 86.14, 85.26, and 83.26% as averages for the levels 6, 8, and 11% respectively.

It appeared that levels of dietary protein used in the mice experiment were higher than what is necessary to obtain a straight line when employing the slope ratio assay. This explains the failure to obtain a straight line in the limits of the present study. However, values of slopes calculated followed the same trend as PER and NPR in terms of the effect of the length of feeding experiment being highest at 10-days then decline gradually.

Rat experiment was conducted under the same conditions. It could be concluded that rats as well as mice were affected by the same factors, i.e., level of dietary protein and length of feeding period,in the same fashion. Recommendations are made to use mice as a model animal in protein quality evaluation studies. This will be most beneficial for the food industry where a level of 8% of dietary protein being fed for a 10 day feeding experiment

will result in saving time and dietary ingredients compared to the rat bioassay where 10% dietary level is fed for a 28 day feeding period. Mouse is smaller in size and renders itself for experiments involving the use of isotopes or carcass studies. Whole egg proved to be a better reference standard than casein which recommends its use as a standard source of protein for mouse bioassay.

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