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AN INVESTIGATION OF THE PROTEIN QUALITY OF SEVERAL PROTEIN MATERIALS

by Marvin E. Buck

A Thesis Submitted to the Faculty of the School of Graduate Studies in partial fulfillment of the Degree of Master of Arts

Western Michigan University Kalamazoo, Michigan July 1967

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I wish to pay special thanks to Dr. Jean M. Lawrence, Dr. Imy V. Holt, and Dr. Gian C. Sud for serving as members of my graduate committee. I wish to give thanks to General Foods Corporation for furnishing the necessary raw materials and laboratory facilities.

Marvin E. Buck

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MASTER'S THESIS

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INTRODUCTION

Presentation of the Problem

Protein is a vital component of the diet of animals because of its role in tissue growth and maintenance. The different dietary protein sources vary in quality as a result of their origin and/or the commercial processing procedures to which they have been subjected. The quality of a particular protein is determined by its capacity to support tissue growth and maintenance.

The purpose of this investigation was to evaluate the quality of six different protein materials which are commonly used as ingredients in animal feeds manufactured in this country. One protein source was of milk origin, one of vegetable origin, and four were derived from animal tissues. Of the four proteins from animal sources used in this study, three were derived from identical raw materials, namely beef trimming byproducts from the head and cheek. These three materials were subjected to different temperature, solvent extraction, and dehydration methods during the manufacturing processes.

The evaluation of the quality of these six proteins were made, in vivo, by means of the Protein Efficiency Ration (PER) method. By this method, defined and accepted by the Association of Official Analytical Chemists in 1960 (1), the ratio between the amount of protein consumed and the weight gained was determined.

The Syrian Hamster (<u>Mesocricetus auratus</u>) was selected as the test animal because of its small size, ease of handling, and the advantage offered by its short breeding cycle. A further reason for selecting this animal was the lack of specific knowledge regarding its nutrition.

Literature Review

The term "quality" as applied to protein in this paper is used as an indication of its ability to promote growth of tissue, i.e. new protoplasm, in hamsters. This quality is dependent not only upon the supply of amino acids specific to that protein material, but the minimum levels of amino acids are also critical to metabolic utilization (2). As further explained by Baumgarten, Mather, and Stone in 1945, the amino acid requirements of different animals determines the protein quality of that specific protein material for that specific animal (2). Among the first studies in amino acid nutrition, was an experiment with chickens by Osborne and Mendel in 1914, where they fed wheat, corn, and soy grains to chickens (3). The protein utilization was determined by the Protein Efficiency Ratio method. Hegsted and Worcester has stated that protein nutrition is influenced by amino acid levels, and when the supply of an essential amino acid is exhausted, protein formation ceases (4). In the same study, it was found that relative levels of amino acids may restrict protein metabolism by inter-reactions between amino acids, even though adequate levels of each are present. This impairment may take place in the form of binding between amino acids or by having active sites obstructed by improper amino acids.

The quality of a given protein material can also be greatly influenced by the treatment to which it is subjected prior to its use in the diet. During commercial preparation the protein is often exposed to excessive heat, and/or harsh chemical treatments (5). In one such study of the

effect of heat treatment on protein quality, Renner and Hill compared the growth of chickens fed a soybean diet, which had been subjected to varying levels of heat processing (5). It was found that heat treatment of soybeans were tested as raw soybeans (no heat treatment), as toasted soybeans (300°F for 15 minutes), and as autoclaved soybeans (350°F for 8 hours). The sovbeans were then fed as the sole protein component of the chicken's diet to evaluate the relative protein quality. It was found that the raw soybean diet would not sustain normal growth, and the chickens lost weight and declined in health. The toasted soybean diet was found to sustain body weight, and promoted some growth. The authors proposed that the toasting process had inactivated an enzyme system which inhibits the utilization of raw soybean protein. The autoclaved soybean diet resulted in poor weight gains in the chickens, and general loss in health. The authors' explanation of this result was that the sustained high heat had denatured the protein in the autoclaved soybeans in such a way as to render it unavailable to the chickens.

Nutritive loss by other protein materials during exposure to high heat has been shown to occur in several cases (6). A. M. Altschel has described the process which takes place during heat denaturization. The application of excessive heat is associated with profound changes in the protein molecule itself and interactions with nearby carbohydrates occur. Under such conditions, the protein molecules lose their normal spatial configuration and may have active sites blocked by foreign molecules. This author further states that these proteins are less susceptible to trypsin activity and thus several basic amino acids are rendered less available.

Chemical reactions during manufacturing processes also reduce the protein quality of a given material. An example would be the use of solvents to remove the large amounts of oil often found in animal byproducts. As much oil as possible is removed from the raw material because the value of the oil for commercial use is about five times greater than the residual protein material (6). As a result, the manufacturer is usually more concerned with complete oil removal, than in preserving the protein quality of the residual by-product.

The type of raw material and the equipment used often necessitate the use of different solvents, such as hexane, di-ethylene chloride or diethylchloride. K. A. Kuiken has shown that the protein quality is influenced by the type of solvent used and the associated processing (7). In his study of solvent effect, Kuiken fed a meat meal which had been treated by hexane, ether, and diethylene chloride. The different meat meals were fed to rats and the protein quality determined by the Protein Efficiency Ratio method. Hexane was shown to produce the meat meal with the best PER.

The method of using a Protein Efficiency Ratio (PER) to describe protein quality and nutritive value was originally proposed by Osborne in 1919 (8). The PER method consists of feeding diets of known protein levels for given periods of time. Weight gain records were kept, and protein efficiency was calculated by dividing the weight gain of the animal by the weight of actual protein consumed during the test period as determined by feeding diets of known protein levels. This method of protein quality evaluation has been widely used in rat nutrition studies

to evaluate the relative effects of different protein diets. In one such study by Hegsted and Worcester, it was shown that the PER method was consistent and when tests were repeated, similar results were obtained (4). Mitchell and Beadles also used the PER method to conduct comparative tests of different protein diets (9). Albino rats were used in their study to determine the quality of the various proteins. In 1960, the Association of Official Analytical Chemists accepted and endorsed the Protein Efficiency Ratio as a valid and reliable test procedure for evaluating Protein Quality (1).

Cravens and Holpin used the PER method to evaluate the protein quality of four protein materials using chickens as test animals (10). They stated that the PER provided an excellent basis to compare protein diets when a precise control diet is not used since comparison between diets was based upon the standard of weight gain versus no weight gain.

According to Adler, the Syrian Hamster, used as the test animal in this study, was first discovered as recently as 1930 (11). A lone female was captured near Jerusalem in the desert, and found to be pregnant. Her offspring provided the original breeding stock for all hamsters now found in Europe and America. Their natural diet in their native habitat has not been determined (12). In addition, very little work has been done on the nutritional requirements of hamsters in general. This lack of nutritional data is mentioned in <u>The Nutrient</u> <u>Requirements of Laboratory Animals</u>, published by the National Academy of Sciences (12). Some isolated studies have been made to determine the dietary levels of protein which maintain body tissues and permit

growth of the hamsters. In one such study, Schweigert found that purified diets containing 20-24 per cent protein were considered adequate to meet the needs of growing hamsters (13). Casein was the protein used in this experiment: Work done by Hamilton and Hogan showed that a casein diet using 20-24 per cent protein in a purified diet appeared adequate for growth in hamsters (14). Their diet was composed of 24 per cent protein, 62 per cent carbohydrate, 5 per cent fat, with some added cellulose, salt, animal liver and vitamins.

MATERIALS AND METHODS

Test Diets: Their Composition and Formulation

The six protein materials selected for this study represented a range of protein products used in the preparation of commercial animal feeds. Each is described below. In these brief descriptions the origin and manufacturing process to which each had been subjected are summarized. The processes differed in the choice of solvents, amount of heat exposure, and methods of drying the product. Reference data on these materials is provided by Product Data Sheets provided by the manufacturer, and a reference to these data sheets is included in Appendix A.

The four animal protein materials were derived from the same raw material, namely head and cheek trimming of beef cattle. The 52 per cent meat meal product has been altered by the addition of bone meal to control the protein content; and cannot be considered identical to the other three diets. The Viobin Meat Meal, Vita Pro 90, and Fresh Beef By-Products did utilize identical raw materials before processing. The use of the identical raw materials was confirmed by personal communications by letter with the supplying manufacturers (See Appendix B).

1. Promine R

This was the brand name for a soybean vegetable protein processed by Central Soya Company, Chicago, Illinois. The product was the result of a refining process which includes 250°F heat treatment, solvent extraction with hexane, and final dehydration by spray

drying. A protein content of 90 per cent was claimed by the manufacturer.

2. Viobin Meat Meal

Viobin was the brand name of a meat meal product produced by Armour and Company, Food Research Division, Oak Brook, Illinois. This product utilized beef by-products of the meat-packing industry. The product was subjected to 160° F heat treatment, and solvent extraction and drying with diethylchloride solvent. The manufacturer claimed 90 per cent protein content. The beef by-products raw material was the same as is used in the Vita Pro 90 diet, and the fresh beef by-products diet (Appendix A).

3. Vita Pro 90

This product was a meat protein concentrate refined by using 300° F heat treatment, solvent extraction by diethylchloride, and by dehydration by spray-drying at 450°F. It was produced by the Rath Packing Company, Waterloo, Iowa. The final protein level was claimed to be 90 per cent. The raw materials consisted of fresh beef by-products as in the Viobin Meat Meal and the fresh beef by-products diet below (Appendix A).

4. 52 Per Cent Meat Meal

This product was secured from Darling and Company, Detroit, Michigan. It was a by-product of their rendering operation, and has been subjected to a 350° F heat treatment, pressing to remove most of the oil, and finally a solvent extraction with hexane. This product was then dried at 400° F. Protein level

was claimed to be 52 per cent. Some bone meal has been added to this product, unlike the other three animal protein materials.

5. Fresh Beef By-Products

These beef by-products were secured from the Vogt Packing Company of Flint, Michigan. They were processed by using a steam jacketed mixer and the water was decanted during a cook cycle of 212°F for 30 minutes. The product had a final protein level of 50 per cent. The beef by-products used in this diet were the same as used in the Viobin meat meal and the Vita Pro 90 diets above (Appendix A).

6. Sodium Caseinate

This product was the sodium salt of casein which was produced by a sodium hydroxide precipitation reaction with milk. It was ordered from Sheffield Chemicals, Norwich, New York. The manufacturer claimed a 93 per cent protein level.

The above protein materials were used to formulate six test diets, each with a final protein content of 25 per cent. Due to the different levels of protein from these various sources, the amount of this ingredient was necessarily varied between diets in order to make the final protein content of each diet similar. The level of total carbohydrate added to each diet varied also, since sources with a lower protein level also contained more carbohydrate (Table I). Final carbohydrate levels of the test diet are similar. These diets all include salts and vitamins as recommended by Hamilton and Hogan (14) (Table II). Minor amounts of

protein are contributed by other ingredients in the test diet, such as the corn flour and fresh liver used in all diets. Thus the percentage of raw materials may vary due to the protein levels of each protein material. The final products were very similar in total protein, fat, carbohydrate, etc. (Table III, page 13). The slight differences in final analysis were not considered important since only the actual protein level of each diet was used to calculate the amount of pure protein consumed by each animal. Hamilton and Hogan's results indicated that when the total protein is above 13 per cent, minor differences between diets were compensated for by the computation of actual protein consumed (14).

The six diet formulations are summarized in Table I. The selt and vitamin mixtures are shown in Table II. Some water was added to facilitate processing and handling. This water was later removed by drying the final product. The analytical data presented in Table III, page 13, was obtained by submitting actual final test diets to the analytical laboratory of the Research Department of General Foods Corporation. The analysis of the test diets included levels of protein, fat, fiber, ash, and moisture.

	Promine R	<u>Viobin</u>	Vita Pro 90	52 Per Cent <u>Meat Meal</u>	Fresh <u>Meats</u>	Sodium Caseinate
Protein Source	23.0	23.0	23.0	43.0	43.0	23.0
Corn Flour	37.0	37.0	37.0	28.0	28.0	37.0
Sucrose	25.0	25.0	25.0	12.0	12.0	25.0
Cellulose	3.0	3.0	3.0	3.0	3.0	3.0
Salt Mixture	4.0	4.0	4.0	4.0	4.0	4.0
Animal Fat (as Beef Tallow)	5.0	5.0	5.0	7.0	7.0	5.0
Fresh Liver	1.8	1.8	1.8	1.8	1.8	1.8
Vitamin Mixture	0.2	0.2	0.2	0.2	0.2	0.2
B rewers Yeast	1.0	1.0	1.0	1.0	1.0	1.0

Table I. A Summary of Diet Formulations as Used in this Investigation. All data are expressed as percentages.

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Table II. A Summary of Salt and Vitamin Mixtures as Used in this Investigation (Batch Formulations).

Salt Mixture

Dicalcium Phosphate	4,000.0	gms
Magnesium Sulfate	460.0	gms
Magnesium Carbonate	150.0	gms
Ferrous Sulfate	155.0	gms
Zinc Chloride	3.0	gms
Manganese Chloride	30.0	gms
Potassium Iodide	5.0	gms
Cupric Sulfate	2.5	gms
Sodium Chloride	1,000.0	gms

5,805.5 gms

Vitamin Mixture

Choline Chloride	6.00 gms
Vitamin A	0.50 gms
Vitamin D ₂	0.50 gms
Vitamin E ²	2.10 gms
Riboflavin	0.50 gms
Niacin	1.50 gms
Pyridoxine Hydrochloride	0.50 gms
Thiamine Hydrochloride	0.50 gms
Vitamine B ₁₂	0.05 gms
Menadione Sõdium Bisulfite	0.05 gms

12.20 gms

		(<u>Test Diet</u> By Per Cent	t)		
	Promine <u>R</u>	<u>Viobin</u>	Vita Pro 90	<u>Meat Meal</u>	Fresh <u>Meats</u>	Sodium <u>Caseinate</u>
Protein	22.9	23.7	23.0	26.9	27.8	25.2
Fat	7.1	6.6	6.6	7.1	7.4	7.0
Fiber	4.7	4.5	3.1	5.5	5.1	5.0
Ash	3.7	3.8	4.0	6.0	5.1	3.8
Moisture	3.1	7•4	10.8	4.4	4 .3	3.0
Carbohy- drate	58.5	54.0	52.5	50.1	50.3	56.0

Table III. A Summary of Test Diet Analyses as Performed on the Six Diets as Fed to the Animals in the Investigation. Each of the six diets were prepared and processed in the following manner:

- The protein source material was added to the processing water and animal fat portion, and this mixture was brought to a boil at 212°F. The cooking was done in a steam jacketed mixer at 50 psi for 20 minutes.
- 2. The vitamin and salt mixtures were added to the mixer and allowed to mix for one minute.
- 3. Remaining ingredients were added to the mixer.
- 4. The total product was cooked until the temperature reached 200°F.
- 5. The cooked product was then cooled to a temperature of 100° F.
- The cooled product was extruded into donut shaped pieces,
 1/2" in diameter, 1/8" thick, with a 1/4" center void.
- The pieces were then dried to a moisture level that ranged from
 5 to 8 per cent at a temperature of 200°F using forced air.

Experimental Procedure

The original breeding stock of Syrian Hamsters was purchased from Con Olson, Madison, Wisconsin. A breeding colony of 8 mature females, and 4 mature males was established and the offspring of this colony comprised the sole source of test animals. Records were kept of all matings and all litters. When the young animals were 28 days old, they were assigned to one of the six experimental diets which were formulated from the protein materials from six different sources. Each animal was assigned to a particular diet by the use of a table of random numbers. In this manner all diets were fed on a random basis, so that litter differences would not be a variable.

Each animal was caged separately, and fed the test diet ad libitum for a period of 28 days. An ample supply of water was always available. Test room temperatures ranged from 68° to 74°F during the experiment, and the humidity ranged from 30 to 65 per cent relative humidity. Initial animal weights were recorded, and final weights were taken after the 28 day test feeding period. The difference between initial and final weights was defined as the weight gain and was used in the calculation for the Protein Efficiency Ratio (PER). Records were also kept of the amount of food consumed by weighing the food put into the cage, and weighing all spillage from the cage and food which was not consumed. From the known amount of protein in each diet, the amount of actual protein consumed was determined. The PER was computed for each animal on the test diets by dividing the weight gain (in grams) by the weight (in grams) of protein consumed.

After the 28 day feeding program, all animals were sacrificed individually, digested in sulfuric acid, and then analyzed for total carcass protein. The total protein of each animal was determined by means of the standard Kjeldahl method of protein analysis (15). This was done to evaluate the quality of various proteins by means of the PER method. It must be determined if the weight gain was due to actual tissue growth, and not to excess fat deposition or to excess liver glycogen. The protein content of all animals was then compared. It was hypothesized that if the protein content of all animals were similar, then the weight gain was due to actual tissue growth and that no unusual fat deposition had taken place in any one group (1). Further, if a weight gain were due to unusual fat deposition, then the percentage of total body protein would be lower, and the weight gain would be considered to be due to reasons other than protein utilization.

By accepted PER procedures, animals which died during the experiment were discarded from the data analysis (1). Such animals were considered to have been influenced by factors other than the protein diet. In fact, the several animals which died during the course of this experimental work showed the symptoms of a hamster disease referred to as "wet tail" (11). Such animals were deleted in compliance with PER procedures.

Statistical Analysis of Results

The PER values and carcass protein values were subjected to an analysis of variance in order to determine if significant differences did exist. A confidence level of 0.05 was selected to compare the PER values between groups of animals. The Duncan multiple range test was also used to evaluate the differences between the groups. The procedure used was based upon Cooley and Lohnes (16).

RESULTS AND DISCUSSION

The mean PER values derived for each group of animals on each test diet are presented in Table IV. Tables V through X present the experimental data obtained from the feeding experiments. Each group of animals was fed a particular diet during the 28 day feeding period. Each table provides the total protein consumed, the weight gained, and PER for each individual animal on a particular diet, as well as group mean values.

Table IV. A Summary of Protein Efficiency Ratios for the Six Experimental Diets Used in this Study.

	Diet	Mean Protein Efficiency Ratio
1.	Promine R	• 331
2.	Viobin Meat Meal	• 339
3.	Vita Pro 90	. 292
4.	52 Per Cent Meat Meal	.413
5.	Fresh Beef By-Products	. 680
6.	Sodium Caseinate	• 328

Arranged in order according to PER Values:

1.	Fresh Beef By-Products	.680
2.	52 Per Cent Meat Meal	.413
3.	Viobin Meat Meal	• 339
4.	Promine R	• 331
5.	Sodium Caseinate	• 32 8
6.	Vita Pro 90	• 292

Table V. A Summary of Weight Gain During the 28 Day Feeding Program, Total Protein Consumption (Computed from total food consumption) and Resultant Protein Efficiency Ratio for the Promine R Test Diet.

	Promi	ne R Diet	
Animal <u>Number</u>	Total Protein <u>Consumption</u> (gms)	Weight Gain (28 Days) (gms)	Protein Efficiency Ratio
1	29.4	2.0	.068
7	24.0	0.9	•038
13	26.5	10.1	• 381
19	25.7	18.4	.716
25	38.1	10.8	.283
31	25.7	9.6	• 374
37	24.5	10.1	.412
43	22.8	7.4	• 325
49	24.3	8.6	•354
55	27.5	9.8	• 356

Mean 26.85

Mean 8.77

Mean .331

(N = 10)

Table VI. A Summary of Weight Gain During the 28 Day Feeding Program, Total Protein Consumption (Computed from total food consumption) and Resultant Protein Efficiency Ratios for the Viobin Meat Meal Diet.

	<u>Viobin Me</u>	at Meal Diet	
Animal <u>Number</u>	Total Protein <u>Consumption</u> (gms)	Weight Gain <u>(28 Days)</u> (gms)	Protein Efficiency Ratio
2	28.8	0.5	.017
14	22.7	12.9	• 394
20	30.3	11.5	• 380
26	35•3	13.9	• 394
32	30.9	10.6	• 343
3 8	27.3	9.8	• 359
44	33.8	13.1	• 388
50	29.8	11.1	• 372
56	30.5	12.4	•407
	Mean 29.9	Mean 10.64	Mean .339

(N = 9)

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Table VII. A Summary of Weight Gain During the 28 Day Feeding Program, Total Protein Consumption (computed from total food consumption) and Resultant Protein Efficiency Ratios for the Vita Pro 90 Diet.

Animal <u>Number</u>	Total Protein <u>Consumption</u> (gms)	Weight Gain <u>(28 Days)</u> (gms)	Protein Efficiency Ratio
3	36.9	5.5	•149
15	41.3	21.1	•511
33	37•5	6.4	•171
3 9	39.5	12.7	• 322
45	40.3	13.6	•337
51	32.6	9.8	• 301
57	30.0	7.6	.253
	Mean 36.87	Mean 8.52	Mean .292
			(N = 7)

Vita Pro 90 Diet

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Table VIII. A Summary of Weight Gain During the 28 Day Feeding Program, Total Protein Consumption (computed from total food consumption) and Resultant Protein Efficiency Ratios for the 52 Per Cent Meat Meal Diet.

Animal <u>Number</u>	Total Protein <u>Consumption</u> (gms)	Weight Gain (28 Days) (gms)	Protein Efficiency Ratio
4	57.6	24•5	•425
10	53.9	12.5	•232 -
16	47.9	15.3	•319
22	22.7	19.1	•841
28	53.4	17.9	•335
34	58.0	22.6	• 390
40	56.7	24.4	•430
46	53•7	20.1	• 374
52	55•5	21.9	• 395
58	60.5	23.7	• 392

52 Per Cent Meat Meal Diet

Mean 52.0

Mean 20.2

Mean .413

(N = 10)

A Summary of Weight Gain During the 28 Day Feeding Program, Total Protein Consumption (computed from total food con-Table IX. sumption) and Resultant Protein Efficiency Ratios for the Fresh Beef By-Products Diet.

	Fres	h Beef By-Products	Diet
Animal Number	Total Protein <u>Consumption</u> (gms)	Weight Gain <u>(28 Days)</u> (gms)	Protein Efficiency Ratio
5	52.3	27.5	•526
11	42.9	27.9	.650
17	41.8	30.7	•734
23	39•3	32.5	.827
2 9	41.8	30.5	.730
35	38.5	32.1	.834
41	42.0	28.0	.666
47	44.8	33.2	•741
53	44.6	25.0	• 561
59	51.6	27.3	•529
	Mean 43.96	Mean 29.47	Mean .680* (N = 10)

* Statistical test of variance indicates significance from the other five diets at the 0.05 level of confidence.

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Table X. A Summary of Weight Gain During the 28 Day Feeding Program, Total Protein Consumption (computed from total food consumption) and Resultant Protein Efficiency Ratios for the Control Sodium Caseinate Diet.

	Sodium	Caseinate Diet (Co	ntrol)
Animal Number	Total Protein <u>Consumption</u> (gms)	Weight Gain (28 Days) (gms)	Protein Efficiency Ratio
12	32.1	11.2	• 349
18	25.9	9.7	• 375
24	36.2	10.2	•282
30	26.2	9•3	• 355
36	36.7	10.1	•275
42	33.1	12.0	• 302
48	29.0	9.6	•331
54	37.1	12.0	• 323
60	25.1	9.1	• 363
	Mean 31.27	Mean 10.36	Mean .328
			(N = 9)

The Protein Efficiency Ratio data were subjected to an analysis of variance in order to determine if significant differences between diets did exist. The results of the statistical analysis are summarized as follows:

Degr Fre	ees of edom	Sum of the Squares of PER Values	Mean of the Squares of PER Values	F <u>Value</u>
Between Groups	5	0,9921	0.1984	11.02
Within G rou ps	<u>49</u>	0.8806	0.0180	
Total	54			

Based upon this F Value, significant difference does exist between group PER values.

Employing the Duncan Multiple Range test, and a confidence level of 0.05, the PER data was analyzed to locate the ranges of significant difference. It was found that the only significant differences were between the fresh beef by-products diet, and all other diets. No other diets were significantly different from each other. The basic data used in calculating the significant differences between diets are summarized in Table XI.

PER Value Comparisons	Actual Range Difference	Minimum Range For Significances	Significant Difference
.680 vs .292	• 388	.1430	x
.680 vs .328	.352	.1406	x
.680 vs .331	• 349	.1377	x
.680 vs .339	• 341	.1334	x
.680 vs .413	.267	.1267	x
.413 vs .292 .413 vs .328 .413 vs .331 .413 vs .339	.121 .085 .082 .074	.1406 .1377 .1344 .1267	
.339 vs .292	.047	.1377	
.339 vs .328	.011	.1344	
•339 vs •331	.008	.1267	
•331 vs •292 •331 vs •328	.039 .003	•1334 •1267	
.328 vs. 292	.036	.1267	

Table XI. A Summary of the Statistical Data resulting from an analysis of PER values of six protein diets.

Carcass protein values are presented in Table XII. The test animals were grouped according to test diets, as in the PER data. The test for variance was used on these data, and the results were as follows:

Deg: Fre	rees of eedom	Sum of the Squares of Carcass Values	Mean of the Squares of Carcass Values	F <u>Value</u>
B etween G rou ps	5	0.269	0.0538	0.44
Within Groups	<u>49</u>	5.925	0.1209	
Tota:	1 54			

Examination of the carcass protein data did not show significant differences between the groups of animals on the six different protein diets at the 0.05 level of confidence. Thus, the use of the PER data gathered in this study would be valid, as this carcass protein data indicated that the weight gain was not due to deposition of fat or liver glycogen in any group of animals. If any of the diets had produced a pronounced increase in deposition of fat or liver glycogen, then protein values would have shown lower values for those animals on that diet.

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1.	Promine	R	3.	<u>Vita Pr</u>	<u>o 90</u>	5.	Fresh B	eef
	Animal <u>Number</u>	Per Cent <u>Protein</u>		Animal <u>Number</u>	Per Cent <u>Protein</u>		Animal Number	Per Cent Protein
N	1 7 13 19 25 31 37 43 49 55 = 10 Me	18.2 18.3 17.6 18.4 18.8 18.2 18.5 18.2 17.9 18.5 an 18.26	N	3 15 33 39 45 51 57 = 7 Me	17.5 17.8 18.1 18.2 18.6 18.0 18.0 18.0 an 18.03	N	5 11 17 23 29 35 41 47 53 59 = 10 Me	18.4 17.9 18.0 18.0 18.0 17.9 18.3 18.2 18.0 18.3 an 18.10
2.	Viobin <u>Meat Me</u> Animal <u>Number</u>	al Per Cent <u>Protein</u>	4.	52 Per <u>Meat Me</u> Animal <u>Number</u>	Cent <u>al</u> Per Cent <u>Protein</u>	6.	Sodium <u>Caseina</u> Animal <u>Number</u>	<u>te</u> Per Cent Protein
2. N	Viobin <u>Meat Me</u> Animal <u>Number</u> 2 14 20 26 32 38 44 50 56 = 9 Me	al Per Cent <u>Protein</u> 17.9 18.3 18.5 18.4 18.2 18.4 18.2 18.4 18.2 18.1 17.6 an 18.18	4.	52 Per <u>Meat Me</u> Animal <u>Number</u> 4 10 16 22 28 34 40 46 52 58	Cent <u>al</u> <u>Per Cent</u> <u>Protein</u> 18.6 18.1 17.9 17.8 17.8 17.7 18.3 18.4 18.2 18.2	6. N	Sodium Caseina Animal Number 12 18 24 30 36 42 48 54 60 = 9 Me	te Per Cent Protein 18.2 19.1 18.1 18.1 16.9 18.1 18.3 18.4 18.1 an 18.14

Table XII. A Summary of Carcass Protein Values Obtained by Protein Analysis of the Entire Body of the Test Animal.

It was found that all diets appeared to satisfy the minimal nutritional needs of the hamsters because in all instances, the weight gain occurred was due to tissue growth. The quality of the protein in the Vita Pro 90, Viobin Meat Meal, and Promine R diets were similar to that of the sodium caseinate diet, using PER values as the basis. The sodium caseinate diet could be considered as an indirect control in this experiment, since it has served as control diet in studies conducted by Cravens and Halpin (10). Although the 52 per cent Meat Meal diet showed a slightly higher average PER value, this difference was not statistically significant. Only the Fresh Beef By-Products diet showed a significant level of improved protein quality when compared with the other five diets. The PER of the Fresh Beef By-Products diet was twice that of the lowest four diets, and 50 per cent higher than the 52 per cent Meat Meal diet.

The results of this study therefore show_that the protein quality of the Fresh Beef By-Products diet was significantly higher than that of all other diets tested on the hamsters in this investigation, because it was capable of supporting a greater amount of tissue growth. Differences in the quality of various protein sources may be partly due to the kinds of amino acids present and/or their relative levels (2, 3, 4). However, insufficient data in regard to amino acid content were available for these six proteins to draw any conclusions along these lines.

The treatment to which proteins are subjected in preparing them for use in diets can also affect their quality (5). Excessive heat and/or chemical treatment have been shown to greatly influence their ability to support tissue growth (6, 7). The processing methods applied to the six

proteins used in the investigations consisted of varying amounts of heat, and the use of various solvents to extract the oils. Few, if any, definite conclusions can be drawn by analyzing the manufacturing methods used for all six proteins because of differences in the original protein.

However, three of these proteins materials consisted originally of the same raw material, namely, beef head and cheek trimmings. Thus, between these three materials the effects of processing can be evaluated. The three proteins of identical origin are the Viobin Meat Meal, Vita Pro 90, and the Fresh Beef By-Products (Appendix A). The 52 Per Cent Meat Meal consisted of the same original raw materials but bone meal had been added, so that this protein cannot be included in this evaluation of processing effect.

The three diets which used identical raw materials did show different processing procedures during their manufacture. The quality of the protein contained in the Fresh Beef By-Products diet proved to be significantly better than the Viobin Meat Meal and the Vita Pro 90 diets. The Fresh Beef By-Products were never exposed to temperature exceeding 212° F during its entire processing, and no solvents were used to remove oils. The processing of Vita Pro 90 included heat at $400^{\circ} - 450^{\circ}$ F, particularly during a spray-drying step, and the solvent Hexane was used to remove as much oil as possible. Viobin Meat Meal was subjected to solvent extraction of oil using diethylen-chloride at 200° F for about 4 hours. The product was then dried at $400^{\circ} - 450^{\circ}$ F to produce the commercial protein material. All three products were then incorporated into the

test diet formulation and received identical processing from that point on.

The differences shown between these three processes indicate that processing conditions exert a definite effect upon the protein quality of a given protein material. The use of excessive heat and/or the solvents (hexane and diethylene-chloride) reduced overall protein quality as shown in this investigation.

SUMMARY

The purpose of this study was to evaluate the protein quality of six protein materials commonly used in the animal feed industry. The ability of any protein to support tissue growth is an indicator of its quality. The amount of tissue growth in this study was determined by means of the Protein Efficiency Ratio (PER).

Six experimental diets were prepared using these six sources as the protein ingredient. Protein levels in the test diets were kept similar, as were all other components of the test diets, such as fat, carbohydrates and moisture.

Hamsters were used as the experimental animals and were fed for 28 days on one of the experimental diets. By PER procedures, weight gain data was compared to protein consumption to determine Protein Efficiency Ratios.

Statistical analysis of PER data showed that the protein of the Fresh Beef By-Products diet was of a significantly higher quality than the other five diets. Carcass protein data indicated that the total body protein content of all groups was similar and showed no statistical differences. This indicated that no undue deposition of body fat or liver glycogen had taken place. Thus the weight gains represented true tissue growth.

Three of the protein materials which were tested for protein quality originated from the same source, namely beef head and cheek trimmings. These three were Vita Pro 90, Viobin Meat Meal, and Fresh Beef By-Products. The Fresh Beef By-Products proved to have a significantly higher quality of protein than the other two materials.

The processes to which these three materials were subjected in preparation for dietary use were compared. It was concluded that high heat and the use of solvents for fat extraction significantly affected the protein quality of a given protein material.

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APPENDIX A

Product Data Sheets

- 1. Central Soya Company, 1966. <u>Product Data Sheet for Promine R</u> soy protein, Chicago, Illinois.
- 2. Armour and Company, 1966. <u>Product Data Sheet for Viobin Process</u> <u>Meat Meal</u>, Oak Brook, Illinois.
- 3. Rath Packing Company, 1966. <u>Product Data Sheet for Vita Pro 90</u>, Waterloo, Iowa.
- 4. Darling and Company, 1965. <u>Product Data Sheet for 52 Per Cent Meat</u> <u>Meal</u>, Detroit, Michigan.
- 5. Vogt Packing Company, 1966. <u>Product Data Sheet for Deef By-Products</u>, Flint, Michigan.
- 6. Sheffield Chemical Company, 1966. <u>Product Data Sheet for Sheftene</u> (Sodium Caseinate), Norwich, New York.

APPENDIX B

Personal Communications

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- 1. Letter from George T. Green, General Foods Corporation, dated March 27, 1967.
- 2. Letter from R. J. Smith, Armour and Company, dated March 30, 1967.
- 3. Letter from Eugene K. Lubbs, The Rath Packing Company, dated April 11, 1967.