8200958

ABDEL-KADER, MONA KHALIL

AN EVALUATION OF BROAD BEAN, VICIA FABA, AND WHOLE WHEAT COMBINATIONS FOR GROWTH, DEVELOPMENT, AND MAINTENANCE OF RATS

The University of North Carolina at Greensboro PH.D. 1981

University
Microfilms
International 300 N. Zeeb Road, Ann Arbor, MI 48106

AN EVALUATION OF BROAD BEAN, *VICIA FABA*, AND WHOLE WHEAT COMBINATIONS FOR GROWTH, DEVELOPMENT, AND MAINTENANCE OF RATS

by

Mona Abdel-Kader

A Dissertation submitted to
the Faculty of the Graduate School at
The University of North Carolina at Greensboro
in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

Greensboro
1981

Approved by

Aden C. Magee
Dissertation Adviser

APPROVAL PAGE

This dissertation has been approved by the following committee of the Faculty of the Graduate School at the University of North Carolina at Greensboro.

Dissertation Adviser Allen C. Magee

Committee Members Elizabeth Lenz Schiller
William A. Powers
Mildred B. Johnson

June 12, 1981
Date of Acceptance by Committee

June 12, 1981
Date of Final Oral Examination

ABDEL-KADER, MONA KHALIL. An Evaluation of Broad Bean, Vicia Faba, and Whole Wheat Combinations for Growth, Development, and Maintenance of Rats. (1981) Directed by: Dr. Aden C. Magee. Pp. 49

The purpose of this study was to compare growth, development, and maintenance of young and adult rats fed whole-wheat and broad-bean protein combinations. Criteria used to evaluate the nutritive value of the protein combinations for young animals included weight gain, protein efficiency ratio (PER), hemoglobin level, and liver copper and iron deposition. The length of the experimental period with the young rats was four weeks. Weight gain and apparent nitrogen retention were used to evaluate the test protein diets for adult animals over a one-week experimental period. Weight gains of young rats fed whole wheat as the only protein source were markedly lower than the weight gains of rats fed broad beans or whole-wheat and broad-bean combinations. The PER in young rats fed whole-wheat and broad-bean combinations was significantly higher than the PER in young rats fed either whole wheat or broad bean alone. The hemoglobin levels of young rats fed the different diets used in the study were essentially the same. Young rats fed diets containing only whole wheat had liver copper levels which were significantly lower than liver copper levels found in the rats fed the other test diets. The addition of broad bean to the diet was associated with marked increases in liver copper levels. No significant difference between liver iron levels of the animals fed the different diets were observed. The weight gains of adult rats fed whole wheat diets were significantly

lower than the weight gains of rats fed broad bean or whole wheat-broad bean combinations. All adult rats fed the test diets were found to be in positive nitrogen balance.

Combinations of whole wheat and broad bean provided a high quality dietary protein which could support growth and maintenance of young and adult rats. In general, good growth and development of young rats and maintenance of adult animals were observed when about 40 percent of whole wheat protein is replaced by a broad bean protein.

ACKNOWLEDGEMENTS

The author wishes to express her sincere appreciation to Dr. Aden Magee, for his guidance, advising, and patience throughout the direction of this study. Gratitude is also expressed to the members of the advisory committee, Dr. Mildred Johnson, Dr. William Powers, and Dr. Elizabeth Schiller for their helpful comments and to Mrs. Shih-min L-Wu for her technical assistance.

Lastly, I thank my husband, Mohamed and my children, Amr and Alaa, for their patience and unfailing support during the past four years.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF APPENDIX TABLES

APPENDIX A

Composition of the Experimental Diets

APPENDIX B

Protein Efficiency Ratio and Nitrogen Balance Data

APPENDIX C

Statistical Analysis

CHAPTER I

INTRODUCTION

Broad bean, Vicia faba, is an annual pulse which has been cultivated since ancient times in North Africa and the Near East. The plant can be grown in temperate and subtropical zones throughout the world and is used primarily as a source of dry legumes. Although difficult to grow in the tropics, broad beans have been successfully introduced into the highland areas of Central America, where it is now a fairly important crop. With the possible exception of North America, broad beans are grown in all continents in the world, and the production of this pulse ranks fourth in comparison to other pulses. Human consumption of broad beans appears to be highest in the northern countries of North Africa, and broad bean is a particularly important crop in Egypt. The pulse is a major source of protein for several large population groups which are dependent upon plant protein foods because of cultural and/or economic reasons.

The broad bean has a fairly high protein content and contains generous levels of amino acids except cystine, methionine, and tryptophan. The pulse, however, is high in lysine, an amino acid which is usually deficient in many cereal protein sources. A combination of broad beans and certain cereals, such as wheat, would provide a relatively high level of quality protein to the diet.

Legumes and whole grains contain high concentrations of minerals and are found to be excellent sources of both iron and copper. The ability of natural fiber, such as that found in wheat to chelate mineral ions has been known for many years. Iron from plant foods is not well absorbed, and its availability depends more on the influence of other food in the diet than does the availability of heme iron from animal meats. Part of this study was directed toward the comparison of the effect of combining two plant protein foods at different levels, on the utilization of copper and iron, and to determine if hemoglobin levels and the levels of copper and iron in the livers of young rats fed these combinations were significantly affected or not.

The purpose of this study was to determine a combination of broad bean and whole wheat that would promote growth and development in young rats and maintain nitrogen equilibrium in adult animals. Animals were also fed diets containing either broad bean or whole wheat as the total protein source for comparative purposes.

CHAPTER II

REVIEW OF LITERATURE

The use of broad beans, Vicia faba, as a protein source is fairly limited when compared to other legume foods which find widespread use throughout the world. Broad beans are consumed in various forms in fairly large quantities throughout much of North Africa (Altschul, 1974), and are particularly popular food items in Egypt, serving as the basic ingredient in two dishes. A type of stew is made by soaking the beans, changing the water, and gently boiling until the beans are very soft, and most of the water has been removed in cooking. Lemon juice, salt, pepper, and oil are used for seasoning, and the bean stew is served with bread, lettuce leaves, and raw tomatoes. Another popular dish is the Tamia cake (or fallafel) which is prepared by soaking the beans until the skins are loosened. The skins are removed, and the beans are molded into small cakes and fried in hot oil. The cakes are served with bread as a sandwich (Orr, 1964).

The crude protein content of broad beans, on a dry weight basis, averages approximately 30 percent, a value which is higher than many of the other pulses and legumes used as protein sources by humans (Altschul, 1974). In addition to protein, the pulse contains approximately 57 percent carbohydrate (primarily as starch), 2 percent oil, 8 percent crude fiber, and 4 percent minerals. The dried bean has an energy value of approximately 300 kilocalories per 100 grams of edible material (Darwish et al., 1976). The broad bean is deficient in methionine and cystine and is marginal in tryptophan, but it is high in lysine. Like other pulses and legumes, the broad bean can be used to supplement other plant proteins which are low in lysine and adequate in the sulfur-containing amino acids.

Little information is available regarding the effect of supplementing broad beans with other plant proteins to improve nutritive quality. The reason for this is probably because broad beans have not found widespread use as a source of protein for human consumption. Some research, however, has been conducted on the broad bean, and results tend to show the addition of broad beans to diets can improve the total protein quality of the diet.

In one experiment, mice fed soybean supplemented with methionine had a lower weight gain than animals fed casein, whereas mice fed broad beans supplemented with methionine had a higher weight gain compared to casein (Lofquvist, 1969). In both cases, the Protein Efficiency Ratios (PER) for both soybeans and broad beans were lower than the PER observed with casein. Adolph et al. (1955) observed that animals fed diets of cooked parboiled wheat, chick-peas, or broad beans had optimum PER values suggesting that the nutritive value of the broad bean was comparable to the other two proteins tested. Hussein et al. (1974) reported that baladi bread supplemented with a local variety of broad bean resulted in an increase in the Net Protein Utilization (NPU) and the PER of the bread protein. They also reported that the PER for raw or stewed beans used solely as the protein in the diet of young rats was higher than the PER obtained with casein. Hussein et al. (1974) also reported that the best ratio in percentage of bread to bean appeared to be 40/60 for the raw bean and 70/30 for the stewed beans. Fleming and Sasulski (1977) reported that wheat flour bread fortified with soya flour, faba bean, or field pea gave PER values ranging from 1.7, 1.8, and 1.1, respectively, whereas sunflower and wheat bread resulted in PER values of 1.3 and 1.1, respectively.

Since baladi bread, as well as other protein combinations previously used with broad beans, may be restricted to a particular country such as Egypt, it seemed desirable to test the supplementary value of broad bean with other cereal proteins which have a wider consumption. Whole wheat is a protein source which is used widely throughout the world, and a study of the supplemental value of broad bean to this protein could yield information pertaining to broad beans.

Many of the legumes which are possible sources of proteins are known to contain toxic compounds which have adverse effects on humans, particularly if eaten in the raw state. Many of these protein sources, including soybeans, contain trypsin inhibitors which have to be destroyed by cooking before these legumes can be used for humans and other animals. hussein et al. (1974), however, found no trypsin inhibitors in broad beans used in Egypt (Vicia faba and Vicia baladi). The broad bean when ingested fresh or in the uncooked state does, however, cause hemolytic anemia, a characteristic syndrome of favism, in susceptible individuals.

Favism is a common occurrence in some Mediterranean countries, and sporadic cases of favism have been observed in the United States (Dack, 1967). There is evidence that susceptibility to favism is a hereditary trait, and individuals with the trait might be wise to avoid the ingestion of broad beans. Many people do not carry the hereditary traits for favism, and thus, broad beans could be a good source of protein for many population groups. Like other toxic plant components, cooking apparently destroys or inactivates the compound responsible for the clinical manifestations of favism.

Trace mineral availability has been shown to differ in various protein sources, and these differences have been attributed to various dietary components such as fiber, phytates, and organic acids. Several researchers (Widdowson & McCance, 1942; McCance & Widdowson, 1942; Sharpe et al., 1950; and Hussein & Patwardhan, 1959) reported that humans fed foods high in phytates had decreased levels of iron absorption. Others (Walker et al., 1948; Callender & Warner, 1970; Cook et al., 1973), however, have reported that the presence of phytate in a food had no marked influence on iron absorption or availability. Elvehjem et al. (1933) reported wheat to be a moderately available iron source, but other researchers (Rose & Vahlteich, 1932; Vahlteich et al., 1936; Free & Bing, 1940; Pye & MacLeod, 1946) reported that wheat iron was as available as ferric chloride. Welch and Van Campen (1975) found that iron of immature soybeans was less available than iron in mature soybeans, even though the phytate content of the immature soybeans was lower than that of the mature soybeans. Morris and Ellis (1976) reported that whole wheat had iron largely in the form of monoferric phytate and that the iron in this form was easily available for use by rats.

Ganapathy et al. (1981) reported that the ingestion by an adult man of 46 gm. protein per day, primarily as bread made from white wheat flour, over a period of 74 days, resulted in positive balances for copper, iron, molybdenum, selenium, and zinc. Iron balances were significantly decreased by the isonitrogenous substitution of pinto beans or peanut butter for 20% of the nitrogen supplied by white bread. Freeland et al. (1980) found that the copper content of legumes was high and may be as much as two to thirty-three times the quantity found in muscle meat. No evaluation was available on the utilization of iron and copper in broad beans or whole-wheat broad-bean combinations.

The objectives of this study were to compare the growth and development of young rats fed broad beans, whole wheat flour, and combinations of broad bean and whole wheat and to compare the nitrogen status of adult rats fed the various protein combinations.

CHAPTER III

EXPERIMENTAL PROCEDURES

The primary objectives of this study were to compare (a) the growth and development of young rats fed broad beans, whole wheat, or combinations of broad beans and whole wheat and (b) the nitrogen status of adult rats fed broad beans, whole wheat, or broad-bean and whole-wheat combinations. Criteria used to determine the effectiveness of the various diets with young rats included weight gains, Protein Efficiency Ratios (PER), hemoglobin levels, and copper and iron deposition in the liver. The criterion used for determining the effectiveness of the test diets on nitrogen status was apparent nitrogen retention. Phase I of the study pertained to the evaluation of the test diets with young rats, while Phase II involved that part of the study involving adult animals.

Whole broad beans[1] (*Vicia faba*) were soaked in tap water for 14 hours in a stainless steel saucepan. The beans were heated gradually and boiled for one hour after the boiling temperature of the water reached. The water was drained from the beans, and the beans were allowed to cool to room temperature. The seed coats were removed, and the beans were ground in a steel grinder. The ground material was spread in ½ inch layers on enamel trays, dried overnight in an oven at 60° C, and further ground to a finer powder.

---

[1]Mira International Foods, Inc., Hoboken, New Jersey.

Six test diets were used for both phases of the study. One diet (Diet I) consisted only of whole wheat[2] as the protein source, and a second diet (Diet V) consisted solely of broad beans as the protein source. The other test diets, based on combinations of whole wheat to broad bean ratios (in percentage of total protein) of 60/40 (Diet II), 50/50 (Diet III), and 30/70 (Diet IV) were included. A diet (Diet VI) with casein[3] as the protein source was used for comparative purposes. The level of protein for all diets used in Phase I was 10 percent, and estimates of protein content of the protein sources reported in the literature were used in calculating the amounts of each protein required for a particular diet. In addition to 10 percent protein, the diets contained 20 percent vegetable shortening[4] (with the exception of the diet containing only the whole wheat), 4 percent mineral mix,[5] 2 percent vitamin mix,[6]

---

[2]ICN Nutritional Biochemicals, Cleveland, Ohio.

[3]Vitamin-test casein, ICN Nutritional biochemicals, Cleveland, Ohio.

[4]Krogo, Krogo Corporation, Cincinnati, Ohio.

[5]Wesson Salt Mixture, ICN Nutritional Biochemicals, Cleveland, Ohio. The composition of this mixture is listed (in percent) $CaCO_3$ 21.000; $CuSo_4 \cdot 5H_2O$, 0.039; $FePO_4 \cdot 2H_2O$, 1.470; $MnSO_4$, 0.020; $MgSo_4$, 9.000; $KA1(SO_4)_2 \cdot 12H_2O$, 0.009; KC1, 12.000; HK_2PO_4, 31.000; KI, 0.005; NaC1, 10.500; NaF, 0.057, and $Ca_3(PO_4)_2$, 14.900.

[6]Each 100 grams of vitamin mix contained: (in mg) Vitamin B_{12} 0.1; biotin, 1; folic acid, 5; thiamin·HC1, 25; pyridoxine·HC1, 25; 2 methyl-napthoquinone, 50; riboflavin, 50; nictonic acid, 50; Ca pantothenate, 150; p-amino-benzoic acid, 500; (in gm) inositol, 5; choline chloride, 7.5, and corn starch 86.6. All vitamins were products of ICN Nutritional Biochemicals, Cleveland, Ohio.

oleum percomorphum[7] (24 drops per kilogram of diet), and 0 to 64 percent corn starch[8] (depending upon the amount of protein source required for a 10 percent level). In order to formulate a 10 percent protein diet with whole wheat, it was necessary to decrease the level of fat from 20 percent to 3 percent so that the necessary amount of whole wheat required could be added. The particular diet was calculated to contain approximately 0.5 calories per gram less than the other diets used in Phase I. Compositions of the individual test diets used in Phase I are given in Table 1, Appendix A.

Nitrogen determinations were made on 0.1 gm. samples of each diet by standard micro-Kjeldahl techniques (AOAC, 1975), and the nitrogen values were multiplied by the factor of 6.25 to calculate the amount of protein in each diet. The actual analyses of Diets I-VI used in Phase I revealed percent protein estimates of 14.0, 12.6, 11.8, 10.9, 12.6, and 10.1, respectively. Since these final levels of protein were generally higher than anticipated, nitrogen determinations were made of 0.1 gm. samples of the broad beans, beans, and whole wheat used for the study. The protein content of the broad beans was approximately 31.6 percent, while the level

---

[7]Mead Johnson and Company, Evansville, Indiana. The composition of this product is listed as 1250 USP units of Vitamin A and 180 USP units of vitamin D per drop.

[8]Powdered cornstarch 105-A, Clinton Corn Processing Company, Clinton, Iowa.

of protein in the whole wheat was approximately 14.8 percent. Since these values were different from the literature values used to calculate the diets in Phase I, the diets used for Phase II were formulated with the analyzed protein figures and not the literature figures.

Copper and iron contents of each experimental diet were determined on an aliquot (4-5 grams) by wet ashing and using the methods of Matrone et al. (1947), which were modifications of copper and iron methods developed by Parks et al. (1943) and Kitzes et al. (1944), respectively. The amount of iron and copper of diets I-VI used in Phase I were found to contain approximately 124.8, 111.2, 113.2, 109.3, 115.5, 80.2 and 4.7, 4.3, 5.1, 6.5, 9.0, 3.6 (expressed in mcg./gm. of diet), respectively.

The composition of the diets used for Phase II of the study were similar to that used in Phase I, except that the level of fat in each diet was dropped to 5 percent and the starch level was increased by 15 percent in each diet. The fat level was lowered because animals in Phase I experiment tended to reject the diets with 20 percent fat. A level of 5 percent dietary fat has also been used in a similar study in Egypt (Hussein et al., 1974). Compositions of the individual test diets used in Phase II are given in Table 2, Appendix A. Although analyzed nitrogen values of the protein sources were used to prepare diets for Phase II which would contain 10 percent protein levels, nitrogen determination of these diets, when converted to protein using the 6.25 conversion factor, revealed percent protein levels of 10.9, 12.6, 11.8, 10.1, and 10.1 for Diets I-VI, respectively.

Forty-two weanling Sprague Dawley rats,[9] averaging 60 grams in weight initially, were used for Phase I of the study. The animals were housed in individual stainless-steel, wire-bottom cages for four weeks, the length of the experimental period. Animals were randomized into seven replications according to initial body weights, and the six test diets were assigned at random in a randomized block design. The animals had free access to distilled water and food. Individual food records were kept, and the total food consumption per animal for the experimental period was determined. The animals were weighed at the end of the experimental period, and the total weight gain per animal was determined. The Protein Efficiency Ratio (PER) for each animal was determined by dividing the total weight gain by the total protein consumed for the experimental period.

Hemoglobin determinations by the method of Shenk et al. (1934) were made on blood samples taken from the tails of the animals at the end of the experimental period, and the animals were sacrificed.

Livers of all animals were removed at the time of sacrifice and weighed. A small amount (0.5-1 gram) of each liver was removed and dried in an oven at 60°C to provide dry weight data. The remainder of each liver was prepared for mineral analyses by ashing

[9]Holtzman Company, Madison, Wisconsin.

with hot nitric and perchloric acids. The ashed liver samples were dissolved in 3 ml of 0.6N HC1 and diluted to 25 ml with redistilled water. Copper and iron contents of each liver were determined on appropriate aliquots using the methods previously mentioned.

Twenty-four adult rats of the Sprague-Dawley strain, averaging 265 grams in weight initially, were used in Phase II of the study. The animals were housed in individual stainless-steel metabolism cages and were assigned at random into four replications according to initial body weights. The six test diets were also assigned at random within each replication. The animals were maintained on experiment for one week, and individual food consumption records were maintained. Urine from each animal was collected in 100 ml beakers, and the total urine excreted was collected in covered bottles containing two drops of toulene and stored in a refrigerator. Individual fecal material was removed from the retaining screens of each cage every two days, and the total feces collected per animal was kept in small sealed bottles in the refrigerator. The total fecal collection for each animal was dried in an oven at 60°C overnight and allowed to come to equilibrium with the moisture in the air before being weighed. The weighed feces were ground and bottled for analysis. Nitrogen determinations were made on duplicate samples of diets and on the feces and urine of each animal by the micro-Kjeldahl method previously mentioned. The apparent nitrogen retention of each animal was determined.

All data were analyzed by standard analysis of variance techniques (Snedecor, 1962). Significant means differences were compared by Duncan's Multiple Range Test (Agricultural Research Service, USDA, 1957).

CHAPTER IV

RESULTS AND DISCUSSION

Detailed data obtained from this study are presented in Appendix B. A summary of the parameters observed in young rats fed whole wheat, whole-wheat and broad-bean combinations, broad bean, or casein is shown in Table 1. Protein copper and iron analyses of the diets fed to young animals are presented in Table 2. Results of the nitrogen balance study with adult animals are given in Table 3. Statistical analyses of all data are given in Appendix C.

Responses of Young Rats (Phase I)

Growth

The weight gains of young rats fed whole wheat as the only protein source were markedly lower ($p \leq 0.01$) than the weight gains of rats fed broad beans, whole-wheat and broad-bean combinations, or casein (Table 1). There was no difference between the weight gain of animals fed broad beans only and those fed casein, but when broad beans were combined with whole wheat, the weight gains of animals fed these combinations were significantly higher ($p \leq 0.05$) than the weight gains of rats fed either broad beans or casein. The weight gains of the animals fed each combination protein diet were essentially equal in magnitude.

Table 1

Responses of Young Rats Fed Whole Wheat, Broad Bean, and Casein (Phase I)

Diet	Type of Protein	Weight[1] ain	PER[1]	Hemoglobin[1]	Liver Mineral Constituent[2]	
					Cu	Fe
		gm.		gm./100 ml.	mcg./gm. dry weight	
1	Whole wheat	45[3a]	1.13[a]	12.41[a]	4.28[a]	519.95[a]
2	Whole wheat/broad bean (60/40)	99[b]	2.36[b]	13.22[a]	10.42[b]	355.15[a]
3	Whole wheat/broad bean (50/50)	109[b]	2.60[b]	13.07[a]	14.08[b]	420.15[a]
4	Whole wheat/broad bean (30/70)	101[b]	2.81[b]	14.78[a]	13.68[b]	477.95[a]
5	Broad bean	82[c]	2.29[b]	12.50[a]	9.49[b]	509.20[a]
6	Casein	71[4c]	2.50[4b]	14.38[4a]	10.65[b]	547.77[a]

[a]Each value is the mean of seven animals unless otherwise indicated.

[b]Each value is the mean of four (4) animals.

[3]Means not sharing a common superscript letter are significantly different ($p \leq 0.05$).

[4]Mean of six (6) animals.

Protein Efficiency Ratio

Analysis of the PER data revealed that a significantly lower ($p \leq 0.05$) amount of protein from broad beans was required to produce a gram of weight gain in young rats than was required when the protein came from whole wheat (Table 1). Broad beans appear to improve the quality and utilization of whole wheat when these two protein sources were fed in combinations. Based on the results, the protein qualities of broad beans and casein appear to be essentially equal. Although the apparent utilization of the protein tends to increase as the level of broad beans in the protein mixture increases, good growth and protein utilization were obtained in young rats fed the combination with the minimum amount of broad beans. These data are in agreement with previous reports of increased PER values when wheat diets were supplemented with various types of legumes and pulses (Narayanaswamy et al., 1972; Abrahamsson et al., 1974; Hussein et al., 1974).

Hemoglobin

There were no significant differences in the hemoglobin levels of young rats fed the various protein diets used in the study (Table 1). Apparently, sufficient copper and iron (Table 2) were provided by each of these diets to allow for acceptable levels of hemoglobin to be maintained.

Liver Copper

Young rats fed diets containing only whole wheat had liver copper levels which were significantly lower ($p \leq 0.05$) than liver

Table 2

Protein and Mineral Analysis of Diets used in Phase I

Diets

1. Whole wheat
2. Whole wheat/broad bean (60/40)
3. Whole wheat/broad bean (50/50)
4. Whole wheat/broad bean (30/70)
5. Broad bean
6. Casein

Diets	Protein Level	Iron	Copper
	percent	mcg./gm-dry weight	
1	14.0	124.8	4.7
2	12.6	111.2	4.3
3	11.8	113.3	5.1
4	10.9	109.3	6.5
5	12.6	115.5	9.0
6	10.1	80.2	3.6

copper levels found in the rats fed the other test diets (Table 2). The addition of broad beans to the diet was associated with marked increases in liver copper levels. Part of this increase in liver copper in rats fed broad-bean and whole-wheat diets could have been due to the increase in dietary copper level associated with broad beans (Table 2). There is also the possibility that the total copper level of the diet was better utilized when the diet contained broad beans.

Liver Iron

Although there were wide variations in the mean liver iron levels of young rats fed different levels of broad beans and whole wheat, statistical analysis of this data revealed no significant difference between liver iron levels of the animals fed the different diets (Table 1). A difference in dietary iron levels apparently was not a factor in this study because the levels of iron found in the broad bean, whole wheat, or whole-wheat and broad-bean combinations were approximately the same (Table 2).

Responses of Adult Rats (Phase II)

Growth

The weight gains of adult rats fed whole wheat diets were significantly lower ($p \leq 0.05$) than weight gains of rats fed broad bean or whole-wheat and broad-bean combinations. There was no difference between the weight gains of animals fed casein and those fed either whole wheat only or broad bean. The differences between weight gains of the animals fed the protein combination diets were not statistically different.

Nitrogen Retention

All adult rats fed the test diets in Phase II were found to be in positive nitrogen balance, and the amounts of nitrogen retained by animals on the various test diets were essentially the same magnitude with the exception of animals fed the 60/40 combination of whole wheat and broad beans. Animals fed the 60/40 combination of whole wheat and broad beans had nitrogen retention levels which were significantly higher ($p \leq 0.05$) than animals fed any of the other diets. The reason for this difference, however, is not apparent from the data obtained in this study.

Table 3

Growth and Nitrogen Balance of Adult Rats fed Whole Wheat, Broad Bean, and Casein for One Week (Phase II)[1]

Diet	Type of Protein	Weight Gain	Total Nitrogen Intake	Total Nitrogen Output		Apparent Nitrogen Retention[2]
				Feces	Urine	
		gm.	gm.	gm.	gm.	gm.
1	Whole wheat	18[a]	2.40	0.35	0.53	+1.51[a]
2	Whole wheat/broad bean (60/40)	42[bc]	2.90	0.50	0.22	+2.17[b]
3	Whole wheat/broad bean (50/50)	48[b]	2.78	0.45	0.80	+1.52[a]
4	Whole wheat/broad bean (30/70)	36[bc]	2.37	0.42	0.82	+1.12[a]
5	Broad bean	46[bc]	2.32	0.48	0.29	+1.53[a]
6	Casein	26[ac]	1.81	0.07	0.30	+1.44[a]

[1]Each value is the mean of four (4) animals

[2]Means not sharing a common superscript are significantly different ($p \leq 0.05$).

General Discussion

The nutritional quality of the protein is generally dependent upon the amino acid composition and the chemical configuration of the protein. A partial deficiency of one or more essential amino acids can result in a lower biological value. The biological value of such a protein can be improved, however, if the protein is furnished the missing amino acid by means of another protein. Mutual supplementation of legumes and cereals, each providing missing amino acids of the other, can result in nutritionally complete and well balanced protein mixtures (Bressani et al., 1962; Bressani et al., 1966; Patwardhan and Ramachardran, 1966; Bressani et al., 1967; Daniel et al., 1967; Panomangalor et al., 1967; Jaya & Venkataraman, 1979).

Results obtained from this study showed that combinations of whole-wheat and broad-bean protein sources provided a high quality dietary protein which could support growth and maintenance of young and adult rats. When used separately, broad beans were found to be a much better source of protein than whole wheat, a finding which was not surprising since most legumes and pulses generally have a higher protein qualities than cereals. The study did indicate, however, that a whole wheat-broad bean combination provided a higher quality protein diet than broad bean alone which would suggest that broad bean does not contain the optimal amino acid pattern necessary for growth and maintenance of young and adult rats. Many of the legumes and pulses are deficient in the sulfur-containing amino acids, such as methionine, and cereals usually have relatively high proportions of these types of amino acids.

Considering all parameters used in this study to compare the nutritive values of broad beans, whole wheat, and combinations of the two sources, it would appear that a 50/50 ratio of whole-wheat and broad-bean combination is the best. If economics is a factor to be considered, the results also show that a 60/40 whole-wheat and broad-bean ratio is acceptable for growth and development of young rats and the maintenance of adult animals. Some investigators, however, have emphasized that optimum supplementary effects are observed when about 25 percent of a legume protein is replaced by a cereal protein (Adolph et al., 1955; Phansalkar et al., 1957; Bressani et al., 1961; Bressani et al., 1962).

Hemoglobin levels of young rats were approximately the same magnitude, regardless of the protein source, and were comparable to levels of young rats fed similar diets in a previous study (Motsinger & Magee, 1980). Although sufficient copper and iron from either whole wheat or broad beans could be utilized for the maintenance of hemoglobin levels which could be considered adequate, the results suggest the availability of these two minerals for liver deposition is dependent on the type of protein. Significantly less copper was deposited in the livers of animals fed only whole wheat, and the addition of broad beans to the diet markedly improved liver-copper deposition. There is the possibility that this difference could be attributed to the fiber content of the various diets. In contrast, the highest level of liver-iron deposition of animals fed the plant protein diets occurred in those fed whole wheat. The iron levels of rats fed whole wheat were not significantly different from the iron levels of animals fed casein. The addition of broad beans to the protein mixture resulted in decreases in liver-iron deposition in young rats even though the liver-iron deposition of rats fed only broad beans were not significantly different from the iron levels of animals fed whole wheat. Previous researchers have suggested that iron from whole wheat is easily available to laboratory animals such as the rat (Patwardhan, 1937; Pileggi, 1959; Morris & Ellis, 1976). The results of this study would suggest that some type of substance which interferes with iron utilization and is not present in either whole wheat or broad beans is formed when these two protein sources are combined. Additional information is needed before this observed phenomenon can be fully explained.

Although the adult animals fed only whole wheat gained less than animals fed broad beans or whole-wheat and broad-bean combinations during the experimental period, the apparent nitrogen retention of animals fed either whole wheat or broad beans was essentially equal. The apparent nitrogen retention of rats fed either protein source was slightly higher, but not statistically significant, than the apparent nitrogen retention of animals fed casein. Thus, it would appear that for the maintenance of an adult animal, whole wheat is adequate. This is not surprising since it has been reported that the adult man can be maintained in a nitrogen balance on a wheat protein diet which provides 46 grams of protein per day (Edwards et al., 1971).

CHAPTER V

SUMMARY AND RECOMMENDATIONS

Summary

Young and adult rats were fed diets containing whole wheat, broad beans, or whole-wheat and broad-bean mixtures for the purpose of comparing the nutritive value of each protein, as well as combinations of the two proteins. Animals were also fed diets containing casein as the protein source for comparative purposes. Criteria used to evaluate the two protein sources for use with young animals included weight gain, protein efficiency ratio, hemoglobin level, and liver copper and iron deposition. Maintenance of weight and nitrogen balance were criteria used to evaluate the utilization of the proteins by adult animals.

Young rats fed the broad beans or whole wheat and broad-bean combinations had significantly higher ($p \leq 0.05$) weight gains than rats fed whole wheat. A combination of whole wheat and broad-bean actually gave better growth than that observed in animals fed only broad beans. Both sources of protein allowed for adequate maintenance of hemoglobin levels in young rats. Copper deposition was lower in rats fed whole wheat, and copper utilization apparently was improved when broad beans were added to the diet. Liver-iron levels, however, appeared to be higher in rats fed whole wheat than in rats fed broad beans.

Both whole-wheat and broad-bean protein supported states of positive nitrogen retention, and the results suggested that whole wheat is just as good for an adult maintenance diet as one consisting of broad beans.

Recommendations

Since the level of dietary protein used for the growth study was kept at approximately 10 percent, it was not possible to test whole-wheat and broad-beans ratios between 100/0 and 60/40 since the resulting level of dietary protein from such combinations would have been less than 10 percent. Additional information concerning the supplemental value of broad beans to a cereal such as whole wheat could be obtained if the protein level were disregarded and whole wheat were replaced in diets by broad beans on a gram-for-gram basis. Another possibility would be to use broad beans in combination with a cereal source which provides more total protein to the diet than does whole wheat.

Recent results[10] have indicated that levels of 7.5 percent dietary protein can promote nitrogen retention in adult rats fed several protein sources. There is the possibility that lower levels of whole wheat and/or broad beans may also promote a state of nitrogen retention in adult animals.

Since zinc availability appears to be dependent on the type and level of protein, studies involving the availability of zinc from

---

[10]Magee, A. C., 1981. Unpublished data.

broad beans would be appropriate. Previous studies (Oberleas et al., 1966; O'Dell, 1968; O'Dell et al., 1972; Sandstead, 1973; Hardie-Muncy and Rassmussen, 1979; Magee and Grainger, 1979) have indicated the utilization of several proteins is improved if these proteins are supplemented with zinc. Little information concerning the availability of zinc from broad beans or broad beans-cereal protein combinations is found in the literature.

BIBLIOGRAPHY

Abrahamsson, L., Farsum, E., & Hambraeus, L. Nutritional evaluation of emergency food mixtures based on wheat supplemented by different protein concentrations. Nutr. Reports Inter., 1974, 9, 169.

Adolph, W. H., Shammas, E. I., & Hallaby, S. H. The nutrition value of legumes proteins and legume wheat mixed protein in Near Eastern diets. Food Research, 1955, 20, 31.

Altschul, A. M. New protein foods. New York: Academic Press, 1974.

Association of Official Agricultural Chemists. Official methods of analysis (12th ed.). Washington, D.C.: AOAC, 1975.

Agricultural Research Service. Mean separation by the functional analysis of variance and multiple comparison. Washington, D.C.: United States Department of Agriculture, 1957.

Bressani, R., & Elias, L. G. All vegetable protein mixture for human feeding. The development of INCAP vegetable mixture 14 based on soybean flour. J. Food Science, 1966, 31, 626.

Bressani, R., Elias, L. G., Aguirre, A., & Scrimshaw, N. S. All vegetable protein mixture for human feeding III. The development of INCAP vegetable mixture 9. J. Nutr., 1961. 74, 201.

Bressani, R., Elias, L. G., Braham, J. E., & Erales, M. Vegetable protein mixtures for human consumption (INCAP. 15). Arch Latin Amer. Nutrition, 1967, 17, 177.

Bressani, R., & Valients, A. T. All vegetable protein mixture for human feeding VII. Protein completion between polished rice and cooked blackgram. J. Food Science, 1962, 27, 401.

Bressani, R., Valients, A. T., & Jejada, C. E. All vegetable protein mixtures for human feeding. VI: The value of combinations of lime-treated corn and cooked black beans. J. Food Science, 1962, 27, 394.

Callender, S. T. & Warner, C. T. Iron absorption from brown bread. Lancet, 1970, 1, 546.

Cook, J. D., Minnich, V., Moore, C. V., Rassmussen, A., Bradley, W. B., & Finch, C. A. Absorption of fortification iron in bread. Am. J. Clin. Nutr., 1973, 26, 861.

Dack, G. M. F. Food poisoning. Chicago: The University of Chicago Press, 1967.

Daniel, V. A., Venkata Rao, S., Swaminathan, M., & Parpia, H. A. B. Amino acid supplementation of proteins III. The effect of supplementing rice and wheat and diets based on them with L-Lysine and DL-threonine on the nutritive value of their proteins. J. Nutr. & Deitet., 1967, 5, 278.

Darwish, N. M., El-Nahry, F., & Tharwat, S. Effect of preparation and cooking on the nutritive value of broad beans. Qualitas plantarum, 1976, 25, 331.

Edwards, C. H., Booker, L. K., Rumph, H. C., Wright, W. G., & Ganapathy, S. N. Utilization of wheat by adult man: Nitrogen metabolism, plasma amino acids and lipids. Am. J. Clin. Nutr., 1971, 24, 181.

Elvehjem, C. A., Hart, E. B., & Sherman, W. C. The availability of iron from different sources for hemoglobin formation. J. Biol. Chem., 1933, 103, 61-70.

Fleming, S. E., & Sosulski, F. W. Nutritive value of bread fortified with concentrated plant proteins and lysine. Cereal Chemistry, 1977, 54, 1238.

Free, A. H. & Bing, F. C. Wheat as a dietary source of iron. J. Nutr., 1940, 19, 449.

Freeland, J. H., Lavone, M., & Boxzy, P. W. Zinc and copper content of foods used in vegetarian diets. J. Am. Dietet. Assoc., 1980, 77, 648.

Ganapathy, S. N., Booker, L. K., Craven, R., & Edwards, C. H. Trace minerals, amino acids, and plasma proteins in adult men fed wheat diets. J. Am. Dietet. Assoc., 1981, 78, 490.

Hardie-Muncy, D. A., & Rassmussen, A. I. Interrelationships between zinc and protein level and source in weanling rats. J. Nutr., 1979, 109, 321.

Hussein, L., Gabrial, G. N., & Morcos, S. R. Nutritional value of mixtures of baladi bread and broad beans. J. Science, Food, Agri., 1974, 25, 1433.

Hussein, R., & Patwardhan, V. N. The influence of phytate on the absorption of iron. Ind. J. Med. Res., 1959, 47, 676.

Jaya, T. V. & Venkataraman, L. V. Effect of germination on the supplementary value of chickpea and greengram protein to those of rice and wheat. Nutr. Reports Inter., 1979, 19, 777.

Kitzes, G., Elvehjem, C. A., & Schuette, H. A. The determination of blood plasma iron. J. Biol. Chem., 1944, 155, 653.

Lofquvist, B. Legume seeds as raw material for production of protein-rich foods. Swedish J., 1969, 79, 231.

Magee, A. C. & Grainger, F. P. Zinc-protein interrelationships in young rats. Nutr. Reports Inter., 1979, 20, 771-776.

Matrone, G., Peterson, W. J., Baxley, H. M., & Grinnells, C. D. Copper and iron in the blood serum of dairy cows. J. Dairy Sci., 1947, 30, 121.

McCance, R. A. & Widdowson, E. M. Mineral metabolism of healthy adults on white and brown bread dietaries. J. Physiol., 1942, 101, 44.

Morris, E. R. & Ellis R. Isolation of monoferric phytate from wheat bran and its biological value as an iron source to the rat. J. Nutr., 1976, 106, 753.

Motsinger, B. M. & Magee, A. C. Zinc supplementation of optimal and suboptimal protein diets. Nutr. Reports Inter., 1980, 22, 581.

Narayanaswamy, D., Kurien, S., Daniel, V. A., Swaminathan, M., & Parpia, H. A. B. Effect of incorporation of a low cost protein food (Bal. AHAR) in poor rice and ragi diets on their overall nutritive value. Ind. J. Nutr. and Dietet., 1972, 9, 73.

Oberleas, D., Muhrer, M. E., & O'Dell, B. L. Dietary metal-complexing agents and zinc availability in the rat. J. Nutr., 1966, 90, 56.

O'Dell, B. L. Effect of dietary components upon zinc availability. A review with original data. Amer. J. Clin. Nutr., 1968, 22, 1315.

O'Dell, B. L., Burpo, C. E., & Savage, J. E. Evaluation of zinc availability in food stuffs of plant and animal origin. J. Nutr., 1972, 102, 653.

Orr, M. L., & Watt, B. K. Broad bean. In W. R. Aykrogd, & J. Doughty (Eds.), Legumes in human nutrition. Rome: United Nations Food and Agriculture Organization, 1964.

Panomangalore, M., Guttikar, M. N., Narayano Rao, M., Rajalakshmi, I., & Swaminathan, M. The relative efficiency of protein foods based on blends of groundnut, bengalgram, soybean and sesame flours in meeting protein needs of protein depleted albino rats. J. Nutr. & Dietet., 1967, 4, 178.

Parks, R. Q., Hood, S. L., Hurwitz, C., & Ellis, G. H. Quantitative chemical microdeterminations of twelve elements in plant tissue. Ind. Eng. Chem. (Anal. Ed.), 1943, 15, 527.

Patwardhan, V. N. The occurrence of a phytin-splitting enzyme in the intestines of albino rats. Biochem. J., 1973, 31, 560.

Patwardham, V. N. & Ramachandran, M. Nutrition in India. Ind. J. Med. Sciences, 1966, 65, 14.

Phansalkar, S. V., Ramachandran, M., & Patwardham, V. N. Nutritive value of vegetable proteins. Part I: PER cereals and the supplementary effect of the addition of a leafy vegetable. Ind. J. Med. Res., 1957, 45, 11.

Pileggi, V. J. Distribution of phytase in the rat. Arch. Biochem. Biophys., 1959, 80, 1.

Pye, O. F., & MacLeod, G. The utilization of iron from different foods by normal young rats. J. Nutr., 1946, 32, 677.

Rose, M. S. & Vahlteich, E. M. Factors in food influencing hemoglobin regeneration. I. Whole wheat flour, white flour, prepared bran, and oatmeal. J. Biol. Chem., 1932, 96, 593.

Sandstead, H. Zinc nutrition in the United States. Amer. J. Clin. Nutr., 1973, 26, 1251.

Sharpe, L. M., Peacock, W. C., Cooke, R., & Harris, R. S. The effect of phytate and other food factors on iron absorption. J. Nutr., 1950, 41, 433.

Shenk, J. H., Hall, J. L., & King, H. H. Spectrophotometric characteristics of hemoglobins. I. Beef blood and muscle hemoglobins. J. Biol. Chem., 1934, 105, 741.

Snedecor, G. W. Statistical methods (5th Ed). Ames: Iowa State University Press, 1962.

Vahlteich, E. M., Rose, M. S., & Macleod, G. The effect of digestibility upon the availability of iron in whole wheat. J. Nutr., 1936, 11, 31.

Walker, A. R. P., Fox, F. W., & Irving, J. T. Studies in human metabolism. I. The effect of bread rich in phytate phosphorus on the metabolism of certain mineral salts with special reference to calcium. Biochem. J., 1948, 42, 452.

Welch, R. M. & Van Campen, D. R. Iron availability to rats from soybeans. J. Nutr., 1975, 105, 253.

Widdowson, E. M., & McCance, R. A. Iron exchanges of adults on white and brown bread diets. Lancet, 1942, 1, 588.

APPENDIX A

Table 1

Composition of the Experimental Diets

(Phase I)

Constituents	Diets					
	1	2	3	4	5	6
	gm./2 kg. of diet					
Broad bean	--	332	420	610	869	--
Casein	--	--	--	--	--	200
Whole wheat	1820	1090	900	544	--	--
Corn starch	--	58	160	326	611	1280
Mineral mix	80	80	80	80	80	80
Vitamin mix	40	40	40	40	40	40
Vegetable oil	60	400	400	400	400	400
Oleum percomorphum	48 drops/2 kg.					

Table 2

Composition of the Experimental Diets

(Phase II)

Constituents	Diets					
	1	2	3	4	5	6
	gm./2 kg. of diet					
Broad bean	--	252	316	444	632	--
Casein	--	--	--	--	--	200
Whole wheat	1420	856	714	428	--	--
Corn starch	360	672	750	908	1148	1580
Mineral mix	80	80	80	80	80	80
Vitamin mix	40	40	40	40	40	40
Vegetable oil	100	100	100	100	100	100
Oleum percomorphum	48 drops/2 k.g.					

APPENDIX B

Table 1

Growth, PER, Hemoglobin, and Liver Mineral Data

Growth Data

Diets

1. Whole wheat
2. Whole wheat/broad bean (60/40)
3. Whole wheat/broad bean (50/50)
4. Whole wheat/broad bean (30/70)
5. Broad bean
6. Casein

Rep.	Diets					
	1	2	3	4	5	6
	4 weeks weight gain (gm.)					
1	59	113	70	87	89	65
2	40	83	124	98	98	78
3	43	100	106	95	82	58
4	47	103	130	103	83	86
5	46	106	116	115	73	(76)[a]
6	43	90	122	106	68	80
7	37	100	99	101	83	62
Total	315	695	767	705	576	429
Mean	45	99	109	101	82	71

[a]( ) indicates calculated missing plot value.

Table 2

Food Consumption Data

Diets

1. Whole wheat
2. Whole wheat/broad bean (60/40)
3. Whole wheat/broad bean (50/50)
4. Whole wheat/broad bean (30/70)
5. Broad bean
6. Casein

Rep.	Diets					
	1	2	3	4	5	6
	4 weeks food consumption (gm.)					
1	324	333	282	284	321	275
2	260	312	357	298	260	281
3	288	325	349	309	270	299
4	237	298	362	328	314	287
5	288	337	350	342	239	(281)[a]
6	267	314	353	324	221	252
7	244	312	309	306	293	239
Total	1908	2231	2362	2191	1918	1633
Mean	272	319	337	313	274	272

[a]( ) indicates calculated missing plot value.

Table 3

PER DATA

Diets

1. Whole wheat
2. Whole wheat/broad bean (60/40)
3. Whole wheat/broad bean (50/50)
4. Whole wheat/broad bean (30/70)
5. Broad bean
6. Casein

Rep.	Diets					
	1	2	3	4	5	6
1	1.24	2.57	2.01	2.69	2.10	2.24
2	1.04	2.02	2.81	2.88	2.86	2.64
3	1.01	2.33	2.46	2.70	2.30	1.83
4	1.35	2.62	2.91	2.75	2.00	2.84
5	1.09	2.38	2.68	2.95	2.31	(2.54)[a]
6	1.19	2.17	2.79	2.87	2.33	3.01
7	1.03	2.43	2.59	2.89	2.14	2.46
Total	7.95	16.52	18.25	19.73	16.04	15.02
Mean	1.13	2.36	2.60	2.81	2.29	2.50

[a]( ) indicates calculated missing plot value

Table 4

Hemoglobin Data

Diets

1. Whole wheat
2. Whole wheat/broad bean (60/40)
3. Whole wheat/broad bean (50/50)
4. Whole wheat/broad bean (30/70)
5. Broad bean
6. Casein

Rep.	Diets					
	1	2	3	4	5	6
	mg./100 ml. blood					
1	13.6	12.4	12.7	14.5	11.9	12.3
2	10.5	15.2	13.4	15.4	12.5	15.0
3	12.7	14.8	13.6	17.1	12.1	14.9
4	12.0	11.7	14.0	14.5	14.4	15.0
5	12.9	12.2	11.9	12.8	13.0	(13.6)[a]
6	11.1	13.8	13.5	13.2	11.4	13.6
7	14.1	12.5	12.4	16.0	12.2	15.5
Total	86.9	92.6	91.5	103.5	87.5	86.3
Mean	12.4	13.2	13.1	14.8	12.5	14.4

[a]( ) indicates calculated missing plot value.

Table 5

Liver Iron Data

Diets

1. Whole wheat
2. Whole wheat/broad bean (60/40)
3. Whole wheat/broad bean (50/50)
4. Whole wheat/broad bean (30/70)
5. Broad bean
6. Casein

Rep.	Diets					
	1	2	3	4	5	6
	mcg./gm. dry weight					
1	317.7	456.4	498.8	378.5	478.5	649.0
2	578.0	324.4	405.3	515.3	445.3	554.9
3	516.0	330.3	403.0	528.8	747.3	554.3
4	614.1	309.1	373.5	489.2	365.7	432.9
Total	2079.8	1420.2	1680.6	1911.8	2036.8	2191.1
Mean	519.9	355.0	420.1	477.9	509.2	547.8

Table 6

Liver Copper Data

Diets

1. Whole wheat
2. Whole wheat/broad bean (60/40)
3. Whole wheat/broad bean (50/50)
4. Whole wheat/broad bean (30/70)
5. Broad bean
6. Casein

Rep.	Diets					
	1	2	3	4	5	6
	mcg./gm. dry weight					
1	3.69	11.33	13.52	12.44	7.26	10.05
2	5.04	13.02	13.57	13.87	10.68	3.73
3	1.31	11.12	15.32	13.54	7.08	9.25
4	7.07	6.21	13.93	14.90	12.93	19.57
Total	17.11	41.68	56.34	54.75	37.95	42.60
Mean	4.28	10.42	14.08	13.69	9.49	10.65

Table 7

Apparent Nitrogen Retention of Adult Rats fed Diets used in Phase II

Growth Data

Diets

1. Whole wheat
2. Whole wheat/broad bean (60/40)
3. Whole wheat/broad bean (50/50)
4. Whole wheat/broad bean (30/70)
5. Broad bean
6. Casein

Rep.	Diets					
	1	2	3	4	5	6
	7 days weight gain (gm.)					
1	16	26	34	36	36	22
2	41	42	39	33	43	39
3	19	49	57	23	56	16
4	-5	52	61	52	48	26
Total	71	169	191	144	183	103
Mean	17	42	48	36	46	26

Table 7 - Continued

Item	Rep.	Diets					
		1	2	3	4	5	6
		Total amount per collection period (gm.)					
Nitrogen intake	1	2.59	2.84	2.73	2.39	2.33	1.98
	2	2.55	2.96	2.71	2.22	2.27	1.68
	3	2.55	2.90	2.83	2.31	2.32	1.82
	4	1.92	2.92	2.88	2.56	2.36	1.79
Total		9.61	11.62	11.15	9.48	9.28	7.27
Mean		2.40	2.90	2.78	2.37	2.32	1.81
Fecal Nitrogen	1	0.33	0.55	0.58	0.39	0.46	0.05
	2	0.42	0.54	0.31	0.37	0.60	0.07
	3	0.41	0.49	0.45	0.55	0.46	0.09
	4	0.27	0.45	0.46	0.38	0.43	0.08
Total		1.43	2.03	1.80	1.68	1.95	0.29
Mean		0.35	0.50	0.45	0.42	0.48	0.07
Urinary nitrogen	1	0.08	0.02	0.37	0.51	0.39	0.42
	2	1.20	0.77	1.10	0.47	0.12	0.01
	3	0.70	0.02	1.11	0.35	0.58	0.14
	4	0.14	0.09	0.65	1.95	0.09	0.64
Total		2.12	0.90	3.23	3.28	1.18	1.21
Mean		0.53	0.22	0.80	0.82	0.29	0.30

Table 7 - Continued

Item	Rep.	Diets					
		1	2	3	4	5	6
Apparent Nitrogen	1	2.18	2.27	1.78	1.49	1.48	1.51
Retention	2	0.93	1.65	1.29	1.38	1.55	1.59
	3	1.44	2.39	1.27	1.41	1.28	1.59
	4	1.51	2.38	1.77	0.22	1.84	1.07
Total		6.05	8.69	6.11	4.50	6.15	5.76
Mean		1.51	2.17	1.52	1.12	1.53	1.44

APPENDIX C

Table 1

Analysis of Variance of Data from the Experiment of Protein Efficiency Ratio of Casein and Whole Wheat/ Broad Bean Combination (Phase I)

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares
	Weight gain		
Total	40	24905.84	
Replication	6	758.74	126.45
Treatments	5	19598.51	3919.70**
1, 5, 6 vs 2, 3, 4	1	14153.36	14153.36**
1, 5 vs 6	1	337.17	337.17
1 vs 5	1	4865.78	4865.78**
3 vs 4	1	274.57	274.57
3, 4 vs 2	1	160.09	160.09
Error	29	4548.59	156.84
	PER		
Total	40	14.70	
Replication	6	0.52	0.08
Treatments	5	12.16	2.43**
1, 5, 6 vs 2, 3, 4	1	3.99	3.99**
1, 5 vs 6	1	2.95	2.95**
1 vs 5	1	4.67	4.67**
3 vs 4	1	0.1564	0.16*
3, 4 vs 2	1	0.1650	0.58**
Error	29	2.02	0.06

*Significant ($p \leq 0.05$)
**Highly Significant ($p \leq 0.01$)

Table 1 - Continued

Source of Variance	Degrees of Freedom	Sums of Squares	Mean Squares
		Hemoglobin	
Total	40	84.26	
Replications	6	11.93	1.98
Treatments	5	20.99	4.19
Error	29	51.34	1.77
		Liver Copper	
Total	23	453.39	
Replications	3	32.46	10.82
Treatments	5	250.43	50.08*
1, 5, 6 vs 2, 3, 4	1	126.55	126.55**
1, 5 vs 6	1	37.85	37.85*
1 vs 5	1	54.29	54.29**
3 vs 4	1	0.32	0.32
3, 4 vs 2	1	32.04	32.04
Error	15	170.50	11.37
		Liver Iron	
Total	23	280184.33	
Replication	3	20445.63	6815.12
Treatments	5	103301.43	20660.29
Error	15	156437.27	10429.15

*Significant ($p \leq 0.05$).

**Highly Significant ($p \leq 0.01$).

Table 2

Analysis of Variance of Data from the Experiment of Apparent Nitrogen Retention of Casein and Whole Wheat/Broad Bean Combination (Phase II)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares
	Weight gain		
Total	23	5766.63	
Replications	3	482.47	16.82
Treatments	5	2840.88	568.18*
1, 5, 6 vs 2, 3, 4	1	900.37	900.37*
1 vs 5	1	1568.00	1568.00**
1, 5 vs 6	1	3.37	3.37
3 vs 2, 4	1	198.37	198.37
3 vs 6	1	968.00	968.00*
Error	15	2443.28	162.88
	Apparent Nitrogen Retention		
Total	23	5.174	
Replications	3	0.514	0.171
Treatments	5	2.320	0.464*
1, 5, 6 vs 2, 3, 4	1	0.073	0.073
1, 5 vs 6	1	0.019	0.019
1 vs 5	1	0.001	0.001
3 vs 4	1	0.328	0.328
3, 4 vs 2	1	1.904	1.904**
Error	15	2.340	0.156

*Significant ($p \leq 0.05$)
**Highly Significant ($p \leq 0.01$)