

EFFECT OF PROCESSING ON THE PROTEIN QUALITY
OF WHEAT GERM

by

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TABLE OF CONTENTS

INTRODUCTION	1
REVIEW OF LITERATURE	2
Wheat and Wheat Germ	2
Production and use of wheat	2
Milling of wheat	3
Composition and nutritive value of wheat and wheat germ	4
Processing of wheat germ	7
Use of wheat germ as a food supplement	7
Importance of Protein in the Diet	8
Proteins for growth and maintenance	8
Labile protein reserves	9
Liver proteins	10
Measurements of Quality	10
Utilization of protein	10
Growth	10
Nitrogen retention	11
Amino acid composition	13
Liver composition	13
EXPERIMENTAL PROCEDURE	14
Wheat Germ and Soybean Oil Meal	14
Diets	15
Animals and Their Care	16
Analyses	18
Liver composition	18

Carcass nitrogen	19
Calculations and Statistical Analysis	19
RESULTS AND DISCUSSION	20
Food Consumption	20
Growth	22
Weight gain	22
Protein efficiency ratio	25
Nitrogen Retention	27
Amino Acid Composition	29
Liver Composition	31
Pancreas Weight	31
SUMMARY	34
ACKNOWLEDGMENTS	35
LITERATURE CITED	36
APPENDIX	40

INTRODUCTION

An increasing shortage of nutritious foodstuffs is apparent in the less developed countries of the world where cereals constitute a large portion of the diets. As world population increases, it is likely that there will be even more dependence upon cereal products.

Mast (1964) stated that in many parts of the world, people now live on a diet composed largely of cereals. The pattern of consumption of cereals varies from region to region and within regions. Among regions, consumption of cereal is highest in the Near East, followed by the Far East, and lowest in North America (F.A.O., 1963). The Near East countries depend heavily on wheat and to a lesser extent on barley, maize and millet. In the Far East, rice is usually the preferred cereal; however, in North China and parts of India and Japan, wheat, maize and millet are more important and in Indonesia, maize is the common cereal. The North African countries depend heavily on wheat and barley as their staple foods whereas in other areas of Africa, millet, maize and rice are of greater importance.

Inasmuch as land suitable for agriculture is already near maximum usage, more efficient utilization of existing food is necessary. Mast (1964) emphasized that investigations must be undertaken to determine to what extent common and special wheat foods can supply the protein needs of human beings. With large quantities of wheat being milled each year, ways of utilizing mill fractions for both human and animal consumption are

important. Millfeed, which is commonly used as an animal feed, is a combination of several wheat mill streams which include red dog, bran, shorts and germ. Some wheat germ is available for human consumption, but much more could be utilized for such purposes. More information is needed about the effect of processing on protein quality of wheat germ. The purpose of this investigation was to determine the effect of processing on the utilization of wheat germ protein by the rat.

REVIEW OF LITERATURE

Wheat and Wheat Germ

Production and use of wheat. Kent-Jones and Amos (1957) stated that wheat is the most important of the cereals because it can grow in almost any kind of soil and in any fairly temperate climate. The cultivation of wheat dates back to ancient times in such areas as Persia, Egypt, Greece and Europe.

Production of wheat in areas around the world is related to water supply, intensity of cultivation and type of wheat sown. Today some of the world's principal producers of wheat include the United States, U.S.S.R., Europe, Canada, India and Pakistan. Total world production of wheat in 1963 was approximately 8.27 billion bushels (U.S.D.A., 1964). Of the 1.13 billion bushels of wheat produced in the United States in 1963, approximately 495 million bushels were used for human consumption.

Winter wheats are planted in the fall in moderate regions and harvested in the late spring. Spring wheats are planted in

the spring in colder regions and harvested in the late summer. Winter wheat, with a longer growing period than spring wheat, normally produces a higher yield per acre than spring wheat. Pyler (1952) stated that hard red winter and hard red spring wheats are used primarily for bread. Soft red winter wheat is used for cakes, hard and soft white wheat for pastry and crackers and durum for macaroni, noodle and spaghetti products.

Milling of wheat. Kent-Jones and Amos (1957) stated that since the wheat kernel is a seed, it contains a germ or embryo which produces the new plant, a starchy endosperm which provides food for the new plant when the embryo first starts to grow and an outer covering or bran which serves as protection for the seed. The germ is located at the base of the grain and occupies only a small portion of the kernel. It is composed of 2 parts, the embryonic axis which develops into the seedling and the scutellum whose function is to secrete enzymes during germination and to carry food material from the endosperm. The endosperm makes up the largest part of the kernel. It is made up of cells containing many starch granules that are embedded in a matrix of proteinaceous material rich in gluten-forming properties. The bran is made up of an outer pericarp which is thin and papery, an inner pericarp and a thin seed coat. The aleurone layer is next to the endosperm but is removed with the bran during the milling process.

Griswold (1962) stated that the purpose of the milling process is to separate the endosperm from the bran and germ and then to pulverize it into flour. This separation is possible

because the endosperm is more easily crushed than the bran and germ. The average composition of the wheat grain is approximately 2% germ, 85% endosperm and 13% bran.

Several workers (Griswold, 1962; Meyer, 1961; and Pyler, 1952) have outlined the various steps in the milling process which include blending, cleaning, tempering and grinding. Clean wheat is tempered (conditioned) before grinding by treatment with water so that the bran will be tough and easy to separate from the endosperm. The endosperm is separated from the bran and germ by a complex process which varies in detail and with the mill and products desired. The first grinding, called the first break, is separated by sieving into very fine particles (flour), intermediate particles (middlings) and coarse particles (chop or stock). The process of sending the chop or stock through the break rolls may continue 5 or 6 times. With each break, the rolls have finer corrugations and are set closer so that more endosperm can be removed. Material reaching the fifth break rolls is high in bran and germ. It is at this stage that the germ is separated from the flour by being flattened into flakes upon passage between the rolls and is removed by bolting.

Composition and nutritive value of wheat and wheat germ.

The composition of wheat and its mill streams will vary, depending on the nature of the grain, environment and fertilizer treatment. The proximate composition of wheat and wheat germ is shown in table 1. Kent-Jones and Amos (1957) reviewed various publications on composition of a variety of wheats to compile the range of values for wheat. Grewe and LeClerc (1943) used 19

TABLE 1
Proximate composition of wheat and wheat germ

	Wheat ¹	Wheat germ ²
	%	%
Moisture	9-18	7.4-11.5
Protein (N x 5.7)	8-15	18.3-35.3
Cellulose (fiber)	2-2.5	2.1
Fat	1.5-2	5.2-15.0
Mineral matter	1.5-2	4.1
Carbohydrate	62-71	25.8-49.4

¹Kent-Jones and Amos, 1957

²Grewe and LeClerc, 1943

samples of wheat germ, including 2 to 6 samples from each of the 5 classes of wheat grown in the United States, to determine the proximate composition of wheat germ. Protein content of wheat germ is more than twice that of whole wheat. Fat and mineral matter is much higher in wheat germ than in whole wheat.

Hepburn et al. (1960) determined 18 commonly occurring amino acids in 2 blends of hard red spring and hard red winter wheat and in each of the final products (including wheat germ) produced by commercial milling. Values for wheat and wheat germ are given in table 2.

Mitchell and Block (1946) assessed the nutritive value of the proteins of whole wheat and wheat products by comparing their amino acid content with that of whole egg proteins which are known to be almost completely utilized by the rat. They found that wheat protein was most deficient in lysine, isoleucine,

TABLE 2
Concentration of amino acids in wheat and wheat germ¹

Amino acid	Wheat	Wheat germ
	g per 16 g nitrogen	
Alanine	3.34-3.37	5.08-5.23
Arginine	4.71-4.88	6.88-7.04
Aspartic acid	4.85-5.09	7.19-7.48
Cystine	1.66-1.80	1.04-1.19
Glutamic acid	29.30-31.60	14.0-17.3
Glycine	3.79-3.94	4.94-5.22
Histidine	1.91-2.12	2.08-2.26
Isoleucine	3.78-3.86	3.28-3.48
Leucine	6.52-6.58	5.72-5.75
Lysine	2.67-2.76	4.78-5.28
Methionine	1.73-1.74	1.73-1.91
Phenylalanine	4.43-4.66	3.38-3.68
Proline	9.94-10.24	5.03-6.09
Serine	5.22-5.25	4.60-4.60
Threonine	2.76-2.96	3.42-3.44
Tryptophan	1.13-1.17	0.98-1.10
Tyrosine	3.19-3.25	2.84-2.85
Valine	4.69-4.79	4.90-4.91

¹Hepburn et al., 1960

methionine, valine, arginine and threonine, in this order, as compared to whole egg protein. In comparing wheat to the FAO Reference Pattern, Howe (1961) found that lysine was the most deficient amino acid and tryptophan the next. Hepburn et al. (1960) found a greater concentration of alanine, arginine, aspartic acid and glycine in the wheat germ than in the whole wheat. Wheat germ was found to be lower in glutamic acid, phenylalanine and proline as compared to wheat. The proportion of the remaining amino acids was less affected by milling.

Processing of wheat germ. Mitchell et al. (1945) stated that the most important factor modifying nutritive value of protein in foods during processing is heat. The sensitivity of cereal proteins to heat has been known since the experiments of Morgan (1931). While working with young rats, Morgan (1931) showed that proteins of wheat products subjected to dry heat or toasting were not well utilized for growth. Hutchinson et al. (1964) observed that heat treatment of wheat impaired the nutritional value of the protein by partially destroying or making less available certain essential amino acids in the protein.

In contrast, Willingham et al. (1961) concluded that autoclaving and mild acid hydrolysis of wheat and its by-products improved the growth response of young chicks. In a long term study at the University of Maryland, workers (Creek et al., 1961; Creek and Vasaitis, 1962; Attia and Creek, 1965) observed that the inclusion of raw wheat germ meal in the diet of chicks was found to depress growth and interfere with feed efficiency. However, growth depressant effects were minimized by autoclaving the wheat germ. Rand and Collins (1958), working with young chicks, observed that mild heat treatment such as steaming and light toasting enhanced the protein quality of wheat germ.

Use of wheat germ as a food supplement. Rand and Collins (1958) found that supplementing cereal diets of chicks with 10-15% defatted wheat germ which had been lightly toasted or mildly steamed produced an increase in nutritive value of enriched wheat flour, rice, barley and oats. Westerman et al. (1952, 1954) used

defatted wheat germ that had been toasted to supplement cereal, flour and bread. Their results indicated that the addition of wheat germ was significantly beneficial in promoting growth in rats when added to enriched flour. The addition of wheat germ to enriched flour produced an increase in growth rate, better reproduction and lactation performance and an increase in the storage of several vitamins in the liver.

Importance of Protein in the Diet

Proteins for growth and maintenance. Proteins occupy a key position in the structure and functioning of living matter (White et al., 1964). Every kind of cell contains its own special proteins. Since proteins are nitrogenous compounds that cannot be synthesized by the animal organism from atmospheric nitrogen or inorganic nitrogenous salts, protein foods from animal and/or vegetable sources are needed to build living tissue. Osborne and Mendel (1914) reported that the primary function of protein is maintenance or repair of tissues of the body, whereas the secondary function is growth.

During digestion, proteins are broken down into units for absorption. These units are amino acids, the "building stones" from which proteins are structured (Kleiner and Orton, 1962). Proteins are made up of more than 21 amino acids, 8 of which are considered essential (indispensable) for human beings and 10 for rats. The essential amino acids must be ingested for they cannot be synthesized by the animal organism. They must be present in

the body in the proper proportions in order to be used to build body protein.

Labile protein reserves. The body does not store protein in the same manner that carbohydrate and fat are stored. The cellular proteins are in a dynamic state and the "metabolic pool" is a means for transferring amino acids from one tissue to another (Whipple, 1948). The protein pool permits rapid and continuous interchange of nitrogenous compounds between the cells and surrounding fluids which then insures a balance between synthesis and breakdown of amino acids (Allison, 1959). These tissue proteins have been called the labile "protein reserves" or "protein stores" (Allison, 1953).

Several workers disagreed with the concept of protein stores. Halac (1960) suggested that the term "labile nitrogen" be used instead of "reserve protein." Halac (1962) stated that from his studies with growing rats fed different levels of protein, it appeared more likely that the body maintained a constant nitrogen make-up in the presence of an increased protein intake by increasing the rate of protein turnover; therefore, he suggested the term "labile nitrogen." Holt et al. (1962) indicated 2 misconceptions in interpreting nitrogen retention as "protein reserves" on the basis of a) when an individual's protein intake is below his minimum requirement, he subsists on his own tissue and b) nitrogen loss is not the loss of protein that has been stored, but rather the loss of all the components of the tissue; therefore, he suggested the term "tissue protein" instead of "reserve protein."

Liver proteins. The protein content of the liver is influenced by the intake of protein. A deficiency in quantity or quality of protein affects the liver by causing a decrease in protein and an increase in fat deposition. Kosterlitz (1947) studied the effects of changes in protein on the structure and composition of liver cells. He stated that liver weights increased in rats fed a high protein diet and decreased on a protein deficient diet. Harrison and Long (1945) studied the effect of various dietary proteins on regeneration of depleted liver proteins. Vegetable proteins produced less regeneration of liver proteins than milk proteins. Allison et al. (1963) stated that the rise and fall of liver proteins are influenced by increase or decrease of dietary proteins.

Measurements of Quality

Utilization of protein. Quality of protein can be measured by analysis of utilization of the protein by the body. Mitchell (1944) reported that determination of the nutritive value of a protein is a study of the nitrogen economy of animals that are fed the protein to be tested. Two methods for determining the nutritive value or utilization of protein are the measurement of growth and nitrogen retention.

Growth. Weight gain is often used but is not always a good measure of growth of new tissue protein. Gain in body weight can be the result of other constituents such as fat and water.

Osborne et al. (1919) introduced the concept of protein efficiency ratio (PER) as a means of measuring growth. PER is

defined as the grams gain in weight per gram of protein (measured as nitrogen) ingested. PER values are usually measured in growing rats. Osborne et al. (1919) determined the levels of different proteins to be ingested that produced the greatest gains and found that the better the quality of protein, the lower the level in the diet required to produce the highest PER.

Bender and Doe11 (1957) modified the PER to protein retention efficiency (PRE). PRE is defined as the value obtained by converting net protein ratio (NPR) into a percentage scale by use of an experimentally determined factor. NPR is defined as gain in weight of a test group of animals plus loss of weight of a non-protein group divided by protein intake of the test group. PRE allows for maintenance requirements and also permits evaluation of poor quality proteins which do not promote growth.

Nitrogen retention. The biological value of a protein represents that part of the absorbed protein that is retained by the body for growth and maintenance (Mitchell, 1924). The nitrogen balance technique of determining dietary intake and excretion in the urine and feces is used to measure biological value. Usually a correction is made for metabolic and endogenous losses by feeding a non- or low-protein diet previous to feeding the test diets.

Bender and Miller (1953a) defined net protein value (NPV) as:

$$NPV = \frac{B_f - B_k + I_k}{I_f}$$

where B_f and I_f denote carcass nitrogen and nitrogen intake of animals fed the test diets, respectively,

and B_k and I_k denote carcass nitrogen and nitrogen intake with the nitrogen-free control diet. They fed one group of rats a diet containing test protein at the 10% level and another group of litter-mates a non-protein diet for 10 days. Then the animals were sacrificed and body nitrogen was determined by Kjeldahl digestion of the whole carcass.

In a second paper Bender and Miller (1953b) proposed eliminating the carcass nitrogen analysis and relying upon a dry weight determination of the animal. These workers found that the ratio of nitrogen/water of the body was constant at any given age and therefore, the nitrogen content of the body could be derived from the water content. They suggested this method would permit estimation of body nitrogen on the animal before and after feeding test diets, i.e. nitrogen balances, without sacrificing the animal by measuring the body water by isotope dilution or antipyrine. Forbes and Yohe (1955) used Bender and Miller's method (1953a, 1953b) to compare the NPV of commercial blood fibrin fed to rats with the nitrogen balance technique and concluded that carcass nitrogen can be derived from the ratio of nitrogen/water of the body.

In later work Miller and Bender (1955) referred to net protein utilization of protein (NPU), but used the same equation as they had used for NPV (Bender and Miller, 1953a). Groups of 4 rats were given a 10% protein test diet and a similar group of rats were given a non-protein diet. Body water was determined after sacrificing the animals and the nitrogen content of the bodies was calculated from water content. Nitrogen also was

determined by the Kjeldahl method. No significant difference was found between the determinations made using actual body nitrogen analyses and those calculated from nitrogen to body water ratio. The NPU value calculated by Miller and Bender (1955) for wheat germ by the NPU method was 67% whereas the biological value calculated by Block and Mitchell (1946) was 71%.

Amino acid composition. The National Research Council (1963) stated that the nutritive value of a dietary protein depends on the quantity and pattern of essential amino acids it presents to the body after absorption from the intestine. Assuming that chemical analysis of a dietary protein reveals the pattern of amino acids liberated in and absorbed from the gastrointestinal tract, a calculation of the nutritive value can be made by comparing the amino acid pattern with a reference pattern. A number of methods for making such comparisons that use reference patterns such as those derived from egg or milk protein have been suggested.

Liver composition. A deficiency in quantity and quality of protein affects the liver. Kosterlitz (1947) observed that liver cells contain more fat than usual in the first few days of protein deficiency. Harper et al. (1954) found that the amount of threonine, tryptophan, serine and glycine may affect the deposition of fat in the livers of rats fed a low protein diet. The Food and Nutrition Board (1959) reported that a deficiency of tryptophan, lysine and threonine leads to fatty infiltration. Williams (1961) formulated a theory that liver cells contain more fat than usual after rats were placed on a protein deficiency

diet. The ratio of liver weight to body weight of rats on a protein deficient diet increased markedly. Winje et al. (1955) reported that fat which accumulated in livers of rats fed low protein diets was reduced when dietary protein was increased.

EXPERIMENTAL PROCEDURE

Wheat Germ and Soybean Oil Meal

The wheat germ used in this study was milled from a mixture of hard red winter wheat varieties by the Kansas State University Flour and Feed Milling Department. The raw wheat germ was divided into 3 portions of 1500 grams each which were treated in the following manner: raw wheat germ (RWG)--no additional treatment; autoclaved wheat germ (AWG)--raw wheat germ was spread evenly on 4 flat tins (9 in x 13 in x $\frac{1}{2}$ in) and placed in an autoclave for 30 minutes at 121°C and 16 lb pressure and then dried in a vacuum oven for 30 minutes at 100°C; vacuum-toasted wheat germ (VTWG)--raw wheat germ was spread evenly on 3 flat tins (9 in x 13 in x $\frac{1}{2}$ in) and placed in a vacuum oven for 1 hour at 140°C.

The soybean oil meal (SBOM) had been previously steam treated, cracked and defatted, therefore no additional treatment was employed. It was used as a control source of protein for comparison with the wheat germ. Altschul (1958) reported that heated soybean oil meal is nutritionally adequate as the sole protein source for the growing rat.

The proximate composition of the three types of wheat germ and the soybean oil meal as determined by A.O.A.C. methods (1965) is presented in table 3. Samples of raw wheat germ and soybean oil meal were analyzed for their amino acid content with a Beckman Model 120 amino acid analyzer by the Kansas State University Flour and Feed Milling Department.

TABLE 3

Proximate composition of wheat germ and soybean oil meal

Nutrient	SBOM	RWG	AWG	VTWG
	%	%	%	%
Protein ¹	50.03	21.37	21.61	23.58
Fat ²	2.07	2.20	2.84	2.70
Moisture	6.50	10.56	9.48	4.04
Ash	4.94	4.20	4.29	4.56
Carbohydrate (by difference)	36.46	61.67	61.78	65.12

¹Factor used for conversion of nitrogen into protein:
Soybean oil meal - 6.25
Wheat germ - 5.70

²Ether extract

Diets

The percentage composition of the experimental diets is given in table 4. The wheat germ and soybean oil meal were used as the sources of protein for the experimental diet groups as follows: I, soybean oil meal; II, raw wheat germ; III, autoclaved

TABLE 4
Percentage composition of experimental diets

Ingredients	Diets			
	I SBOM	II RWG	III AWG	IV VTWG
	%	%	%	%
Protein source	19.99	46.80	46.27	42.41
Cottonseed oil	7.59	6.97	6.69	6.86
U.S.P. XVI salt	4.01	3.04	3.02	3.07
Vitamin mixture	1.00	1.00	1.00	1.00
Water	3.70	0.06	0.61	3.30
Cornstarch	63.71	42.13	42.41	43.36

wheat germ; IV, vacuum-toasted wheat germ. The experimental diets were planned as a modification of the method of the A.O.A.C. (1965) to contain protein, 10%; fat, 8%; ash, 5%; vitamin mixture, 1%; water, 5%; and cornstarch to make 100%.

The supplementary vitamins as listed by the A.O.A.C. (1965) were weighed on a Mettler analytical balance, combined and added to the diets before mixing. The diets were mixed in a 20 quart Hobart electric mixer for one-half hour at low speed and then stored in tightly-covered jars in a household type refrigerator.

Animals and Their Care

Twenty-four weanling male albino rats (41 to 51 g) of the Sprague-Dawley strain were divided into four groups with similar total weights. The animals were randomly distributed (table 12,

Appendix) into individual metabolic cages in a room maintained between 24-25°C. Water bottles and feeders were provided at the back of each cage. To minimize food spillage in the cage, the feeders were adjusted by movable walls to animal size. Food and water were given ad libitum. The feed cups were filled twice a day, morning and evening. Every seventh day water bottles were washed and refilled. The arrangement of baffle and feces cup in the removable collection funnel provided at the bottom of each cage separated the urine from the feces. Fecal and urine analyses were not done in this study. Urine was discarded and fecal material was collected and stored for amino acid analysis at a later date.

The animals were weighed on a Toledo balance to the nearest gram at the beginning of the experiment and thereafter every seventh day. Records were kept of food intake, water consumption and weight gain on the basis of the following periods:

Adjustment period	2 days
Period I	7 days
Period II	7 days
Period III	7 days
Period IV	7 days

At the end of the experimental period, the animals were fasted for 24 hours before being sacrificed. They were then anesthetized with ethyl ether. The pancreas was removed, placed on a weighed square of aluminum foil and tightly wrapped. The wrapped pancreas was weighed on a Mettler analytical balance and then frozen with the carcass. There was difficulty in assessing whether the pancreas had been removed intact. The liver was removed, placed on a weighed square of aluminum foil and tightly

wrapped. The wrapped liver was weighed on a Mettler analytical balance, frozen and stored in a freezer at -10°C . The animal carcass was placed in a weighed jar and tightly covered. The jar and contents were weighed on a Toledo balance and frozen and stored in a freezer at -10°C .

Three animals were sacrificed at the beginning of the experimental period. Their livers and carcasses were handled in a similar manner to that used for the experimental animals. The amount of carcass nitrogen/body weight of these 3 animals was 1.254 g/45 g, 1.244 g/44 g and 1.320 g/49 g. Their average nitrogen content was 0.028 g nitrogen/g body weight.

Analyses

Liver composition. Each solidly frozen liver was removed from the freezer, sliced in approximately 1/16 to 1/32-inch slices as rapidly as possible and placed in a covered weighing bottle. Duplicate samples were placed on the interior surface of opened cotton pads of known weight (1.2 to 2.9 g) and their weight obtained. The cotton had previously been ether extracted and dried. The liver samples on the cotton pads were dried in a vacuum oven at 110°C for 72 hours to constant weight. The dried samples were extracted with ethyl ether for 20 to 24 hours on low heat in a Goldfish extractor. The ether was reclaimed after extraction and the beakers were placed in the vacuum oven at 110°C for 1 hour, then cooled in a desiccator for 30 minutes and weighed. Weight of fat in the samples was obtained by difference in beaker weights before and after extraction. The percentage of

fat was calculated on the wet basis. The duplicate fat-free liver samples in the cotton were analyzed for total nitrogen by the macro-Kjeldahl method (A.O.A.C., 1965).

Carcass nitrogen. Each jar containing a frozen carcass was removed from the freezer and held under hot water for 1 minute. The carcass was transferred to a wide-mouth 500 ml Erlenmeyer flask and 200 ml of concentrated sulfuric acid was added. The flask was lightly stoppered and the digestion mixture allowed to stand for one week with occasional swirling. Then 200 ml of cold distilled water was added to the flask. The solution of sulfuric acid, water and tissue was transferred to a 2 liter volumetric flask and made to volume with hot distilled water. This mixture was inverted 50 times and a portion was transferred to a 250 ml pharmaceutical bottle. Duplicate 15 ml samples were taken immediately and analyzed for total nitrogen by the macro-Kjeldahl method (A.O.A.C., 1965).

Calculations and Statistical Analysis

From the data obtained, the following measurements of protein quality were calculated:

$$\text{Percent weight gain} = \frac{\text{g at end of period} - \text{g at beginning of period}}{\text{g at beginning of period}}$$

$$\text{Protein efficiency ratio} = \frac{\text{g gain in body weight}}{\text{g protein ingested}}$$

$$\text{Increment in body N} = \text{g final carcass N} - \text{g initial carcass N}$$

Initial carcass nitrogen was determined by multiplying average grams of nitrogen per gram body weight of the animals

sacrificed at the beginning of the study by the initial weight of each animal in grams.

Analysis of variance was performed on all measurements of protein quality to find the differences attributed to diet group or period (Snedecor, 1950). Fisher's least significant difference at the 5% level was used to note significant differences attributable to specific diets or periods.

RESULTS AND DISCUSSION

The food consumption, weight and percent weight gain during the 4 collection periods for each of the animals are presented in tables 13 and 14 (Appendix). The protein efficiency ratio of the diets during each collection period for each animal is found in table 15. Initial and final carcass nitrogen, increment in carcass nitrogen and carcass nitrogen/body weight for each animal are given in table 16 (Appendix). Liver weight, liver weight/body weight and percent fat, nitrogen and moisture in the liver of each animal are given in table 17 (Appendix).

Food Consumption

The means, F-values and least significant differences at the 5% level for food consumption for each diet group during each period are given in table 5. Animals in diet group II (RWG) consumed significantly less food than animals fed any of the other 3 diets. There were no significant differences in food consumption among diet groups I (SBOM), III (AWG) and IV (VTWG).

TABLE 5

Means, F-values and least significant differences for food consumption of rats fed various diets

Diet group	Periods				Grand mean
	1	2	3	4	
I, Soybean oil meal	57.7	83.5	91.0	80.8	78.2*
II, Raw wheat germ	47.5	60.8	54.8	62.3	56.2*
III, Autoclaved wheat germ	60.8	81.0	92.8	85.3	80.0*
IV, Vacuum-toasted wheat germ	56.2	79.5	86.7	87.2	77.4
Grand mean	33.3	* 76.2	* 81.2	78.9	
F values ¹					
Diets					54.03***
Periods					59.63***
Diets x Periods					3.46**
Animals:Diets					2.77**
Lsd* ²					
Diets					4.317
Periods					4.317

¹*** Significant at 0.001 level

** Significant at 0.01 level

²Lsd* Least significant difference at 5% level

Food consumption during period 1 was significantly lower than during the other 3 periods. In addition, food consumed during period 2 was significantly less than food consumed during period 3. In general, there was a sharp increase in food consumption between periods 1 and 2 for all 4 diet groups which was followed by further increases in period 3 for diet groups I (SBOM), III (AWG) and IV (VTWG) but a decrease for diet group II (RWG). During period 4, food consumption increased for diet group II (RWG), decreased for diet groups I (SBOM) and III (AWG) and remained approximately the same for diet group IV (VTWG).

Growth

Weight gain. Figure 1 illustrates the cumulative mean weight gain for each of the four diet groups during each 7 day period. Animals in diet groups I (SBOM), III (AWG) and IV (VTWG) displayed similar growth rates. Growth of the animals in diet group II (RWG) was sharply depressed. Creek et al. (1961, 1962) reported that raw wheat germ was extremely deleterious to the growth of young chicks and that the growth depressant effects were minimized by autoclaving.

The means, F-values and least significant differences at the 5% level for percent weight gain for each diet group during each period are given in table 6. Percent weight gain varied significantly among the diets and periods. Weight gain of diet group II (RWG) was significantly lower than those of the other three diet groups which did not differ significantly. Significantly greater growth of the animals occurred during periods 1 and 2

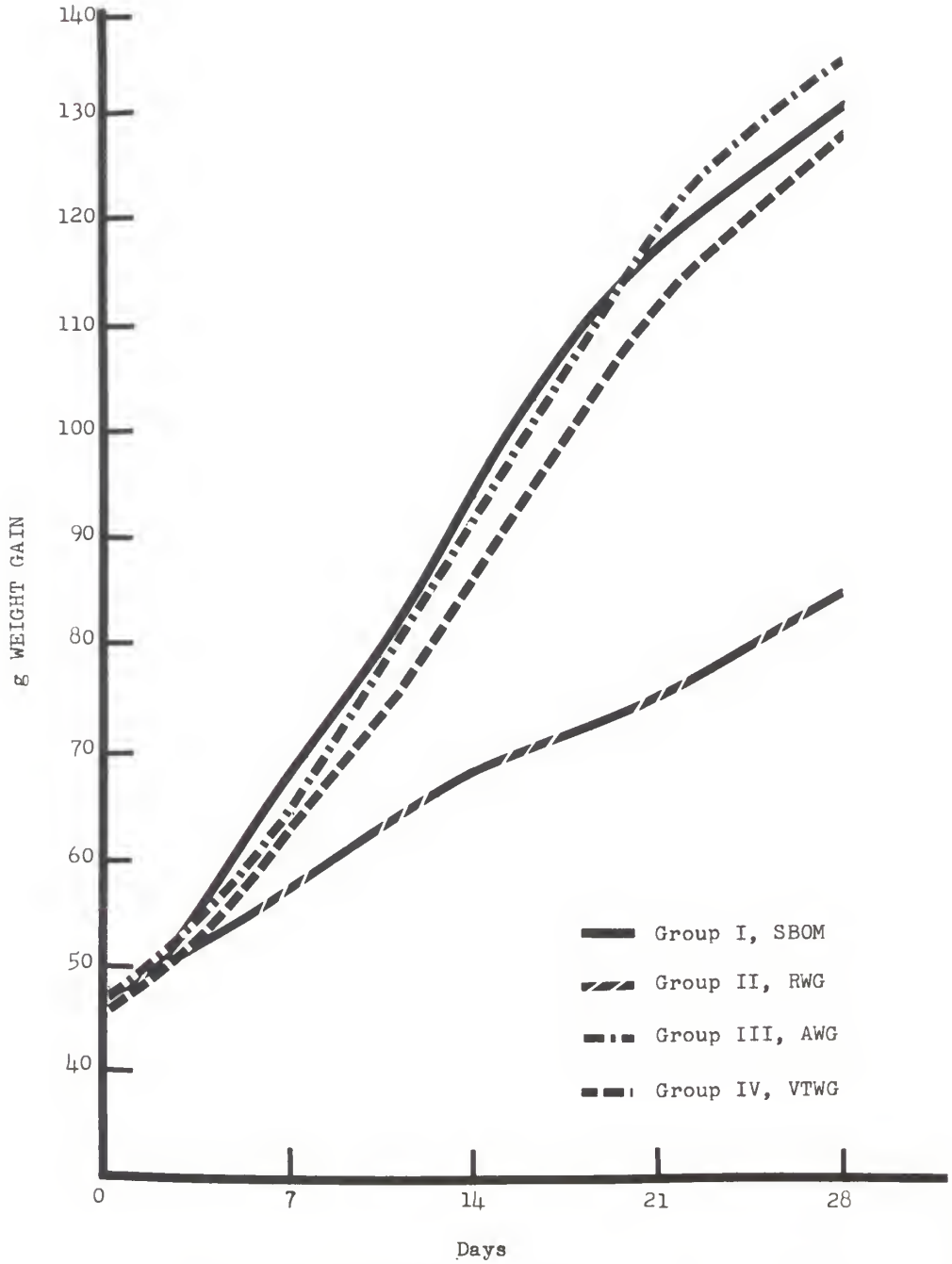


Fig. 1 Cumulative mean weight gain for each diet group during 4 periods.

TABLE 6

Means, F-values and least significant differences for percent weight gain of rats fed various diets

Diet group	Periods				Grand mean
	1	2	3	4	
I, Soybean oil meal	40.7	38.7	24.8	11.5	28.1*
II, Raw wheat germ	16.8	20.2	9.7	12.3	14.7*
III, Autoclaved wheat germ	36.8	40.7	30.8	12.7	30.2*
IV, Vacuum-toasted wheat germ	33.3	36.2	28.3	15.3	28.3
Grand mean	31.9	33.9*	23.4*	12.9	
F values ¹					
Diets					
Periods					
Diets x Periods					
Animals:Diets					
Lsd* ²					
Diets					
Periods					

¹*** Significant at 0.001 level, ns-not significant

²Lsd* Least significant difference at 5% level

than during period 3. Moreover, growth during period 3 was significantly greater than that during period 4, with the exception of diet group II (RWG). No explanation is known for the decreases in growth during periods 3 and 4. Overall food consumption was significantly more during period 3 than periods 1 and 2 and decreased during period 4 but not to the level of period 2.

Protein efficiency ratio. The means, F-values and least significant differences at the 5% level for PER for each diet group during each period are given in table 7. The PER for diet group II (RWG) was significantly lower than those for diet groups I (SBOM), III (AWG) and IV (VTWG). No significant difference in PER was found among diet groups I (SBOM), III (AWG) and IV (VTWG), but that of diet group IV (VTWG) was slightly lower than the other two. There was no significant difference in PER between periods 1 and 2, but there was a significant decrease in PER during period 3, followed by another significant decrease during period 4. The patterns for PER and percent weight gain were similar.

Hove et al. (1945) used mill fractions of hard red spring wheat and reported a range of PER values for wheat germ of 2.70-3.14. In this study PER for the wheat germ diet groups were: II (RWG), 1.55; III (AWG), 2.79; and IV (VTWG), 2.65. These PER values of processed wheat germ were comparable to the lower range of the values (2.6-3.8) reported for whole egg (Barnes et al., 1945).

TABLE 7

Means, F-values and least significant differences for protein efficiency ratio (PER) of various diets

Diet group	Periods				Grand mean
	1	2	3	4	
I, Soybean oil meal	3.53	3.29	2.66	1.71	2.80 *
II, Raw wheat germ	1.66	1.88	1.21	1.47	1.55 *
III, Autoclaved wheat germ	2.95	3.35	3.11	1.76	2.79 *
IV, Vacuum-toasted wheat germ	2.85	2.92	2.80	2.02	2.65 }
Grand mean	2.75	2.86 *	2.44 *	1.74	
F values ¹					
Diets		42.71***			
Periods		29.97***			
Diets x Periods		4.03***			
Animals:Diets		0.97 ns			
Lsd*2					
Diets		0.26			
Periods		0.26			

¹*** Significant at 0.001 level, ns-not significant

²Lsd* Least significant difference at 5% level

Nitrogen Retention

Means, F-values and least significant differences for carcass nitrogen/body weight and increment in body nitrogen for each diet group are shown in table 8. No significant difference was found among the diet groups for carcass nitrogen/body weight indicating that protein composition of the body was similar for the 4 diet groups.

Increments in body nitrogen for diet groups I (SBOM) and III (AWG) were not significantly different. Increment in body nitrogen was significantly less for diet group II (RWG) than for the other 3 diet groups. In addition, it was significantly less for diet group IV (VTWG) than for diet groups I (SBOM) and III (AWG).

The raw wheat germ was inferior in protein quality to both the autoclaved and vacuum-toasted wheat germ as indicated by significantly lower percent weight gain, PER, and increment in body nitrogen. Since the amino acid composition was the same for the raw and processed wheat germ, the conclusion may be drawn that heat processing had made the amino acids more available for utilization by the animal body. Moreover, the values for percent weight gain, PER and increment in body nitrogen indicated a slight superiority in protein utilization for the autoclaved over the vacuum-toasted wheat germ.

TABLE 8

Means, F-values and least significant difference for carcass nitrogen/body weight and increment in body nitrogen of rats fed various diets

Diet group	Carcass nitrogen/ body weight		Increment in body nitrogen	
	%		g	
I, Soybean oil meal	2.500		2.164	*
II, Raw wheat germ	2.572		0.725	*
III, Autoclaved wheat germ	2.330		2.027	*
IV, Vacuum-toasted wheat germ	2.682		1.676	*
F-value ¹				
Diets	2.105	ns		
Lsd* ²				
Diets				0.169

¹** Significant at 0.01 level, ns-not significant

²Lsd* Least significant difference at 5% level

Amino Acid Composition

The amino acid composition of wheat germ and soybean oil meal used in this study and published values for whole egg are shown in table 9. Similar values for the distribution of amino acids in wheat germ (Hepburn et al., 1960) and soybean oil meal (Kent-Jones and Amos, 1957) have been reported.

Whole egg is used as a reference because it is more nearly completely utilized by man and the rat than any other protein food and is not enhanced in biological value by supplementation with any amino acid. The wheat germ contained a lower percentage of 10 of the 18 amino acids than whole egg. In ascending order of percentage, these included methionine, isoleucine, tryptophan, valine, phenylalanine, lysine, leucine and threonine which are the 8 amino acids considered essential for both man and the rat and serine and tyrosine, which are nonessential. Wheat germ was higher than whole egg in 8 amino acids--arginine and histidine which are considered essential for the rat and alanine, aspartic acid, glutamic acid, glycine, half-cystine, and proline which are nonessential amino acids.

The soybean oil meal contained a lower percentage of 11 of the 18 amino acids than whole egg. It contained less of the 8 amino acids considered essential for both man and the rat than the high quality whole egg but more of 7 of them than did the wheat germ. Thus, the amino acid composition values indicate that soybean oil meal should be superior in protein quality to wheat germ. However, no significant differences were found in

TABLE 9

Amino acid composition of wheat germ¹ and soybean meal² as compared to whole egg³

Amino acid	Wheat germ		SBOM		Whole egg		Wheat germ/ whole egg		SBOM/ whole egg	
	%		%		%		%		%	
Essential										
Methionine	1.46		2.00 ⁴		3.14		46		64	
Isoleucine	3.19		4.04		6.64		48		61	
Tryptophan ³	1.00		1.60		1.65		61		97	
Valine	4.77		4.10		7.42		64		56	
Phenylalanine	4.16		4.91		5.78		72		85	
Lysine	4.73		6.15		6.40		74		96	
Leucine	6.68		7.47		8.80		76		85	
Threonine	3.79		4.13		4.98		76		83	
Histidine ⁵	2.82		2.64		2.40		118		110	
Arginine ⁵	8.20		7.43		6.56		125		113	
Nonessential										
Serine	5.15		5.67		8.40		61		68	
Tyrosine	3.14		3.59		4.30		73		83	
Aspartic acid	7.79		12.38		7.01		111		176	
Half cystine	2.71		2.14		2.34		116		92	
Proline	6.42		5.05		4.24		151		119	
Glycine	5.67		4.09		3.54		160		116	
Glutamic acid	20.66		19.19		12.37		167		155	
Alanine	6.66		4.14		-		-		-	
Ammonia	1.98		1.61		-		-		-	
Protein (of sample)	21.50		50.05		12.80					

¹Analyzed by Mary Ann Lambert, Dept. Flour and Milling, Kansas State University; ²Orr and Watt, 1957; ³Tryptophan value from Kent-Jones and Amos, 1957; ⁴Methionine value for SBOM from Kent-Jones and Amos, 1957; ⁵Essential for rat.

percent weight gain, PER and increment in body nitrogen between the SBOM diet and the AWG and VTWG diets with one exception - the VTWG diet resulted in a significantly lower increment in body nitrogen than did the SBOM diet.

Liver Composition

Means and F-values for liver weight/body weight and percent fat, nitrogen and moisture in the livers of rats fed various diets are shown in table 10. Creek et al. (1961) noted marked hypertrophy of the liver when chicks were fed a raw wheat germ diet, but in this study there was no significant difference in liver weight/body weight of rats due to diets. Williams (1961) also reported that the ratio of liver weight to body weight of rats on a protein deficient diet increased. No significant differences were found among diet groups for percent fat, nitrogen and moisture in the liver.

Pancreas Weight

Means and F-value for pancreas weights of rats fed various diets are shown in table 11. Attia and Creek (1965) noted that raw wheat germ caused pancreatic hypertrophy in chicks. This finding was not noted in this study where no significant difference was found for pancreas weights of the animals fed the various diets.

TABLE 10

Means and P-values for liver weight/body weight, percent fat, percent fat, nitrogen and moisture in the liver of rats fed various diets

Diet group	Liver weight/ body weight		Fat	Nitrogen		Moisture
	%	%		%	%	
I, Soybean oil meal	4.8	2.5	19.7	75.4		
II, Raw wheat germ	4.6	2.0	18.4	76.6		
III, Autoclaved wheat germ	4.7	1.7	16.4	76.8		
IV, Vacuum-toasted wheat germ	5.1	2.9	18.8	76.2		
P-values ¹	0.56 ns	1.31 ns	2.87 ns	0.97 ns		

¹ns Not significant

TABLE 11
Means and F-values for pancreas weight of rats
fed various diets

Diet group	Pancreas weight
I, Soybean oil meal	0.986
II, Raw wheat germ	0.133
III, Autoclaved wheat germ	0.110
IV, Vacuum-toasted wheat germ	0.174
F-value ¹	0.086 ns

¹ns - Not significant

SUMMARY

The purpose of this investigation was to determine the effect of processing on the utilization of wheat germ protein by the rat. Soybean oil meal was used as a control source of protein. The proximate composition and amino acid composition of the wheat germ and soybean oil meal were determined. When compared to whole egg, both wheat germ and soybean oil meal were low in the 8 amino acids considered essential for man. However, soybean oil meal was higher than wheat germ in 7 of them.

Twenty-four weanling male albino rats were randomly distributed in individual metabolic cages and fed a 10% protein diet ad libitum for an experimental period of 28 days. The source of protein in the 4 experimental diets was: soybean oil meal, raw wheat germ, autoclaved wheat germ and vacuum-toasted wheat germ.

Weight and food intake of each animal were measured at the end of each 7-day period. At the end of the experimental period, weights were obtained for the pancreas, liver and total carcass of each animal. The carcass of each animal was analyzed for nitrogen. The liver was analyzed for percent fat, nitrogen and moisture. Percent weight gain, protein efficiency ratio and increment in body nitrogen were calculated. The data were submitted to analysis of variance and least significant differences were obtained.

The raw wheat germ diet group was significantly lower than the other 3 diet groups in food consumption, weight gain, protein efficiency and increment in body nitrogen. The only other significant finding was a lower increment in body nitrogen for the vacuum-toasted wheat germ group than for the autoclaved wheat germ and soybean oil meal groups.

It was concluded that the raw wheat germ was inferior in protein quality to both the autoclaved and vacuum-toasted wheat germ. Since the amino acid composition was the same for the raw and processed wheat germ, the heat processing may have made the amino acids more available for utilization by the animal body. In comparing the two methods of processing, the values for percent weight gain, protein efficiency ratio and increment in body nitrogen indicated a slight superiority in protein utilization for the autoclaved over the vacuum-toasted wheat germ.

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APPENDIX

TABLE 12

Random distribution of animals and diets in cages

Cage and rat No.	1	2	3	4	5	6	7	8
Diet group	II	III	I	IV	III	III	IV	IV
Initial weight (g)	45	45	43	44	47	41	51	42
Cage and rat No.	9	10	11	12	13	14	15	16
Diet group	I	II	II	II	I	IV	II	II
Initial weight (g)	43	45	47	46	45	49	42	49
Cage and rat No.	17	18	19	20	21	22	23	24
Diet group	IV	IV	III	III	I	I	I	III
Initial weight (g)	45	43	45	50	49	46	48	46

TABLE 13

Food consumption of rats fed various diets during collection periods

Rat No.	Period 1	Period 2	Period 3	Period 4
	g	g	g	g
<u>Group I, Soybean oil meal</u>				
3	55	76	81	78
9	56	81	90	80
13	53	74	91	77
21	63	95	97	90
22	56	76	79	76
23	63	99	108	84
<u>Group II, Raw wheat germ</u>				
1	44	69	54	58
10	55	69	58	67
11	43	53	56	56
12	50	65	57	57
15	44	55	49	67
16	49	54	51	69
<u>Group III, Autoclaved wheat germ</u>				
2	64	64	89	84
5	52	75	98	83
6	51	77	81	87
19	66	87	96	97
20	66	89	93	78
24	66	94	100	83
<u>Group IV, Vacuum-toasted wheat germ</u>				
4	55	90	94	82
7	44	66	78	79
8	63	81	93	89
14	63	80	80	128
17	53	75	81	71
18	59	85	94	74

TABLE 14

Weight and percent weight gain of rats fed various diets at the end of the adjustment period and each collection period

Rat No.	Adjustment period		Period 1		Period 2		Period 3		Period 4	
	g	%	g	%	g	%	g	%	g	%
<u>Group I, Soybean oil meal</u>										
3	44	50	66	39	92	22	112	22	130	16
9	46	43	66	36	90	24	112	24	126	13
13	50	32	66	33	88	25	110	25	122	11
21	52	38	72	44	104	25	130	25	146	12
22	46	43	66	36	90	20	108	20	120	11
23	52	38	72	44	104	33	138	33	146	6
<u>Group II, Raw wheat germ</u>										
1	42	38	58	24	72	8	78	8	86	10
10	50	20	60	20	72	11	80	11	92	15
11	50	8	54	15	62	13	70	13	78	11
12	50	20	60	27	76	11	84	11	92	10
15	48	4	50	16	58	10	64	10	72	13
16	56	11	62	19	74	5	78	5	90	15
<u>Group III, Autoclaved wheat germ</u>										
2	44	41	62	32	82	37	112	37	130	16
5	46	30	60	50	90	33	120	33	132	10
6	42	38	58	41	82	32	108	32	124	15
19	50	40	70	37	96	31	126	31	150	19
20	54	37	74	38	102	25	128	25	138	8
24	52	35	70	46	102	27	130	27	140	8
<u>Group IV, Vacuum-toasted wheat germ</u>										
4	44	45	64	34	86	35	116	35	130	12
7	54	26	68	26	86	26	108	26	130	20
8	44	36	60	40	84	33	112	33	130	16
14	52	35	70	40	98	18	116	18	136	17
17	50	28	64	34	86	23	106	23	124	17
18	46	30	60	43	86	35	116	35	128	10

TABLE 15

Protein efficiency ratio of various test diets fed to rats
during each collection period

Rat No.	Period 1	Period 2	Period 3	Period 4
<u>Group I, Soybean oil meal</u>				
3	4.133	3.535	2.551	2.384
9	3.690	3.062	2.526	1.808
13	3.119	3.072	2.498	1.610
21	3.280	3.481	2.770	1.837
22	3.690	3.263	2.354	1.631
23	3.280	3.340	3.253	0.984
<u>Group II, Raw wheat germ</u>				
1	3.592	2.004	1.098	1.362
10	1.796	1.718	1.362	1.769
11	0.919	1.491	1.411	1.411
12	1.975	2.431	1.386	1.386
15	0.449	1.437	1.209	1.179
16	1.209	2.195	0.775	1.718
<u>Group III, Autoclaved wheat germ</u>				
2	2.859	3.177	3.426	2.178
5	2.737	4.066	3.112	1.470
6	3.189	3.168	3.263	1.869
19	3.080	3.038	3.177	2.515
20	3.080	3.198	2.842	1.303
24	2.772	3.460	2.846	1.225
<u>Group IV, Vacuum-toasted wheat germ</u>				
4	3.609	2.426	3.167	1.694
7	3.158	2.707	2.799	2.764
8	2.520	2.940	2.988	2.007
14	2.835	3.473	2.233	1.551
17	2.621	2.911	2.450	2.516
18	2.355	3.036	3.167	1.609

TABLE 16

Initial and final carcass nitrogen, increment in carcass nitrogen and carcass nitrogen/body weight of rats fed various diets

Rat No.	Carcass nitrogen		Increment in carcass nitrogen	Carcass nitrogen/body weight
	Initial	Final		
	g	g	g	%
<u>Group I, Soybean oil meal</u>				
3	1.218	3.476	2.258	2.997
9	1.274	3.485	2.211	3.168
13	1.385	-----*	-----*	-----*
21	1.440	3.434	1.994	2.704
22	1.274	3.202	1.929	3.109
23	1.440	3.870	2.430	3.000
<u>Group II, Raw wheat germ</u>				
1	1.163	2.184	1.021	2.730
10	1.385	2.167	0.782	2.580
11	1.385	2.004	0.619	1.805
12	1.385	2.304	0.920	2.880
15	1.329	1.797	0.468	2.852
16	1.551	2.093	0.542	2.584
<u>Group III, Autoclaved wheat germ</u>				
2	1.218	3.330	2.111	2.921
5	1.274	3.154	1.880	2.673
6	1.163	2.576	1.413	2.342
19	1.385	3.737	2.353	2.920
20	1.495	3.873	2.378	3.123
24	1.440	-----*	-----*	-----*
<u>Group IV, Vacuum-toasted wheat germ</u>				
4	1.218	2.482	1.264	2.256
7	1.495	3.166	1.670	2.827
8	1.218	2.997	1.779	2.676
14	1.440	3.109	1.669	2.727
17	1.385	3.220	1.836	2.850
18	1.274	3.114	1.840	2.756

* Not analyzed

TABLE 17

Liver weight, liver weight/body weight and percent fat, nitrogen and moisture in the liver of rats fed various diets

Rat No.	Liver weight g	Liver weight/ body weight %	Fat %	Nitrogen %	Moisture %
<u>Group I, Soybean oil meal</u>					
3	6.01	5.18	4.90	18.64	72.82
9	5.66	5.15	1.63	19.63	76.39
13	5.03	4.75	1.22	20.17	76.71
21	5.73	4.51	2.54	19.54	75.05
22	4.81	4.67	2.21	20.48	75.62
23	6.08	4.71	2.33	19.68	75.79
<u>Group II, Raw wheat germ</u>					
1	3.32	4.15	2.71	18.88	76.19
10	4.51	5.37	2.19	16.34	77.24
11	3.91	3.52	1.56	17.17	78.74
12	4.32	5.40	1.34	19.01	75.76
15	3.65	5.79	1.37	18.94	78.37
16	2.84	3.51	2.57	20.18	73.14
<u>Group III, Autoclaved wheat germ</u>					
2	5.61	4.92	2.27	19.18	75.23
5	6.25	5.30	1.73	19.35	79.48
6	4.44	4.04	1.26	20.07	76.60
19	6.04	4.72	1.97	19.27	76.48
20	5.30	4.27	1.20	20.74	76.33
24	6.28	5.02	1.75	-----*	76.49
<u>Group IV, Vacuum-toasted wheat germ</u>					
4	6.20	5.64	2.47	18.37	76.14
7	5.29	4.72	1.12	18.98	77.67
8	4.20	3.75	5.85	17.74	74.72
14	6.27	5.50	1.99	19.22	76.46
17	5.48	4.85	2.21	19.46	76.57
18	7.18	6.35	3.51	19.28	75.74

*Not analyzed

EFFECT OF PROCESSING ON THE PROTEIN QUALITY
OF WHEAT GERM

by

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The purpose of this investigation was to determine the effect of processing on the utilization of wheat germ protein by the rat. Soybean oil meal was used as a control source of protein. The proximate composition and amino acid composition of the wheat germ and soybean oil meal were determined. When compared to whole egg, both wheat germ and soybean oil meal were low in the 8 amino acids considered essential for man. However, soybean oil meal was higher than wheat germ in 7 of them.

Twenty-four weanling male albino rats were randomly distributed in individual metabolic cages and fed a 10% protein diet ad libitum for an experimental period of 28 days. The source of protein in the 4 experimental diets was: soybean oil meal, raw wheat germ, autoclaved wheat germ and vacuum-toasted wheat germ. Weight and food intake of each animal were measured at the end of each 7-day period. At the end of the experimental period, weights were obtained for the pancreas, liver and total carcass of each animal. The carcass of each animal was analyzed for nitrogen. The liver was analyzed for percent fat, nitrogen and moisture. Percent weight gain, protein efficiency ratio and increment in body nitrogen were calculated. The data were submitted to analysis of variance and least significant differences were obtained.

The raw wheat germ diet group was significantly lower than the other 3 diet groups in food consumption, weight gain, protein efficiency and increment in body nitrogen. The only other significant finding was a lower increment in body nitrogen for

the vacuum-toasted wheat germ group than for the autoclaved wheat germ and soybean oil meal groups.

It was concluded that the raw wheat germ was inferior in protein quality to both the autoclaved and vacuum-toasted wheat germ. Since the amino acid composition was the same for the raw and processed wheat germ, the heat processing may have made the amino acids more available for utilization by the animal body. In comparing the two methods of processing, the values for percent weight gain, protein efficiency ratio and increment in body nitrogen indicated a slight superiority in protein utilization for the autoclaved over the vacuum-toasted wheat germ.