THE EFFECT OF LEGUME SUPPLEMENTATION ON SOME NUTRITIONAL AND PHYSICAL CHARACTERISTICS OF COUSCOUS

by 632

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INTRODUCTION

Adequate nutrition must be considered one of the most important problems facing the world population at the present time. For the people of the world to have maximum growth and mental development, there must be an adequate supply of high quality protein in their diets.

In many parts of the world the supply of high quality protein is short. Many of the countries which have this problem, have cereal grains as the staple food, which are deficient in some of the essential amino acids needed for human growth. Countries in the Near East and Africa only use meat and meat products in 2.0% of their diets, whereas meat in the United States takes up to 31% of the diet (2). In these countries cereals and legumes may be 42% of the diet in comparison to 29% in the United States.

One method for improving nutrition would be to supplement the cereal foods with other foods that would yield a nutritionally balanced intake. Horsebean (Vicia faba) is a legume common in the Near East. If added to a major cereal food, to yield a product acceptable to the people, it could aid in improving their nutrition.

LITERATURE REVIEW

Agglomeration

Agglomeration occurred when dry, powdery flour base materials were dispersed in a humid atmosphere to wet the surface and form adhesive gluten in the flour particles. The dispersion was then mechanically agitated to cause random collision and agglomeration, with the agglomerates having a loose bulky density not less than that of the starting material (27). Armstrong, Bushuk, and Dunne (7) stated that agglomerates were produced by suspending fine particles in a fluidized bed with heated gas, such as air, and atomizing a fluid-agglomerating agent directly into the fluidized bed, the fluid wetting and agglomerating the particles. Other methods included the spraying of the flour particles with an edible, sticky, cohesive, fluid agglutinant. The particles were moved slightly and mixed with the sticky fluid, forming non-sticky free running agglomerates (28). High-protein cereal flour was first moistened, then transferred to a mixing zone where it was agglomerated. The agglomerates were then dried (26). Swanson and Finske (54) used a method for the agglomeration of small particles of flour. The moisture content in the flour particles was lowered by pre-drying below the equilibrium value at a given temperature in air of suitable relative humidity. The flour particles were then exposed to air at a given temperature and then agglomerated.

In the macaroni industry, water was sprayed from a pipe with a multiple of small holes during the agglomeration of semolina (36). It was not important how the water was added as long as the water was added evenly on the semolina and not on the surrounding metal mixing parts.

Effect of Heat Treatment

Heat processing can improve the nutritional value of proteins, wherein the limiting factor was usually lysine (40). A new lysine linkage was

produced which was either not digestible by the enzymes or was so slowly digested that part of the lysine entered the blood stream. This linkage was a condensation reaction between the free epsilon amino acid of the lysine residues and the free carboxyl groups of aspartic or glutamic acid.

At least three types of reactions were found to be involved in the inactivation of amino acids by prolonged autoclaving of a sucrose-containing food (24). Lysine, aspartic, and glutamic acids combined with some constituents of the protein, probably the free carboxyl with the free amino groups to form enzyme resistant linkages. Destruction of the amino acids occurred when free amino groups reacted with sucrose. Resistant linkages to enzymatic hydrolysis resulted from bound methionine, cysteine, and histidine with sucrose.

The nutritional values of raw and autoclaved wheat germ were compared using the chick as an experimental animal (8). Raw wheat germ was found to depress growth and fat utilization, and also to induce pancreatic hypotrophy. A hemaggutinin factor detected in raw wheat germ was destroyed in the autoclaved sample.

Stevens and McGinnis (53) studied the effect of autoclaving lysine in the presence of carbohydrate on utilization by the chick. Lysine autoclaved alone for four hours at 120 C, supplemented overheated soybean oil meal for chick growth as effectively as unheated lysine. Lysine autoclaved with cerelose for four hours at 120 C, was either destroyed or rendered unavailable for chick growth. Supplementary methionine and lysine did not improve the growth of chicks fed a diet containing soybean meal which was autoclaved at 100 C for thirty minutes. The decreased nutritive value of overheated soybean meal was almost fully corrected by supplementary methionine and lysine. Methionine alone had little supplementary value to overheated soybean oil meal. Moran, et al.(44) found that toasting wheat germ meal significantly improved

net protein utilization (NPU), but autoclaving had no effect. Further autoclaving reduced NPU. Toasting or autoclaving for twenty minutes destroyed cysteine. Processing beyond twenty minutes destroyed lysine, cysteine, and arginine.

It was found that steam-pelleting of wheat germ increased the metabolizable energy value for both chicks and roosters, whereas steam pelleting reduced the metabolizable energy value of the middlings and the shorts (12). The toasting of wheat germ caused an increase in metabolizable energy for roosters but not for chicks. Autoclaving wheat germ caused increases in this value for roosters but decreases for chicks.

Autoclaved wheat germ produced significantly higher weight gain in chicks as compared to diets containing unautoclaved wheat germ(21). Horsebean, fed to rats as the sole source of protein at a 12% level of protein, was improved by autoclaving through the inactivation of the trypsin inhibitor (15). It was found that parching increased the biological value of the proteins of some cereals and pulses in rat feeding experiments. In many cases the digestibility coefficient was improved (1).

By prolonged heating of soybean meal, the liberation of lysine, arginine, and tryptophan by hydrolysis was reduced (47). The amount of each of the essential amino acids liberated by pancreatic hydrolysis was increased by proper heat treatment and decreased by excessive heat treatment.

Autoclaving casein at 15 psi for four minutes had no effect on the amino acid composition as measured by microbiological determination after acid hydrolysis (29). Autoclaving for 20 hours reduced the cysteine content but had no effect on the amount of the other amino acids.

Evans, et al. (23) found that part of the lysine was destroyed when soybean meal was mixed with sucrose and autoclaved for four hours at 121 C.

When isolated proteins of soybean were autoclaved, there was little loss of lysine.

Nutrition Studies

The use of rats and mice for nutritional studies has been widely adopted because of the short, reliable growth rate that can be established with these animals (31) (16). Njaa (45) studied weight maintenance and protein intake of young rats. The protein levels required for the maintenance of body weight at 22 C, room temperature, of young rats weighing 50-60g, were determined on groups of five or six rats with eight protein sources of widely differing chemical scores. Each protein source was given under ad libitum feeding conditions at four protein levels, of which at least the lowest was assumed to be below the maintenance level. The maintenance protein level was about 2% for egg albumin, between 2-3% for casein, about 3% for soybean meal and linseed meal, about 3.5% for wheat gluten, and about 5% for pea meal. Zein fortified with L-lysine and tryptophan to give a chemical score of about 30 did maintain body weight when included at protein levels of up to 10%. The daily intake of ideal protein (actual intake·chemical score) was reasonably constant at about 190mg. N/kg. $^{0.73}$. when rats maintained their body weight on the seven protein sources. The corresponding calorie intake was about 200 kcal./kg. $^{0.73}$. This quantity was not appreciably higher in growing rats. The type of carbohydrates in the diet and the practice of giving the diet wet or dry did not significantly influence the protein utilization of the egg albumin diet at the 2.3% level. When egg albumin protein was given at 2.1 and 4.0% levels at 22 and 28 C, the body weight was maintained at the 2.1% level at both temperatures. The food intake at both levels was lower at 28 than at 22 C. Thus, the maintenance requirement of protein was lowest

at the higher temperature. The rats on the 4.0% protein diet grew faster at 22 than 28 C, the protein utilization measured as grams extra gain in weight per gram. Extra protein eaten above maintenance was slightly better at 22 C. Importance of starting temperature on protein utilization studies was stressed.

The effect of dietary protein upon amino acid excretion by rats and mice was studied by Sauberlick and Bauman (50). The rats and mice were fed semisynthetic diets containing 10-50% of casein and the amounts of each of 16 amino acids in the urine were determined microbiologically. The amount excreted in microbiologically available form depended upon the amount ingested as protein. Rats excreted an average of 0.1-1.6% of the amount ingested, mice 1.0-5.5%. No significant differences were observed between the amounts and percentages of the nonessential amino acids excreted and those of the essential amino acids. Chemical determinations of alpha-amino N yielded significantly higher values than those obtained microbiologically. The presence of peptides in urine was demonstrated by large increased in both alpha-amino N and in microbiologically available amino acids after hydrolysis.

Michell and Block (43) studied some relationships between the amino acid contents of proteins and their nutritive values for the rats. The relationships of the amino acid composition of a protein to its nutritive value for the growing rat was best revealed by computing for each protein, the percent deviations of the contents of each essential amino acid, expressed per 16g. of N, from the corresponding content of a protein mixture that was completely digestible by the rat and utilizable in adolescent metabolism. From these computations, the essential amino acid limiting the nutritive efficiency of the protein was revealed as the one whose percent deficit from that of the standard protein was the greatest.

Hepburn, et al. (19) (31) studied the biological availability of essential amino acids in wheat, flour, bread, and gluten. Rat feeding studies were conducted and the availability values of the amino acids were determined by the proportion of sample amino acid excreted in the feces and by equating the increase in carcass nitrogen to the ingestion of the amino acids. The increase in total N content varied directly with the amount of available amino acid consumed when that amino acid was limiting in the diet. Availabilities tended to be highest in the gluten and lowest in the whole wheat.

Campbell (20) determined the protein efficiency ratio (PER) of foods which were fed, at 10% protein level, to rats in a standardized four-week rat growth study. The determination of the amino acids lysine, methionine, and cysteine was used as a basis for the chemical score which correlated well with PER. The PER was found to be the most generally applicable procedure for evaluating protein quality.

Nutritional studies using durum wheat macaroni supplemented with lysine and milk protein were used to determine the protein efficiency ratio (PER) (10). Tests with rats showed that the mean PER of plain wheat macaroni made from semolina at a level of 10% protein was 1.11 compared to 2.48 for enriched macaroni prepared from a blend of semolina 90, casein 7, and skim-milk solids 3%. Supplementation of both macaroni products with lysine monohydrochloride (0.25 g./10g. protein) increased the PER values to 2.11 and 2.63 respectively.

Growth was obtained from weanling rats in which increasing levels of lysine were added to a diet containing 90% dried commercial white bread supplemented with fat, minerals, and vitamins (including B12) (48). This basal diet contained 12.5% protein (N 6.25) and, by microbiological analysis, about 0.3% lysine. Additions of DL-lysine·HCl equivalent to levels of L-lysine ranging from 0.1-0.8% were studies, and with each increment up to 0.4 or 0.5%

L-lysine, there was noted an increased growth response in the young rats and a marked increase in food efficiency. Higher levels produced a plateau in the curve of the response. It was also reported that if sufficient lysine was added to the bread diet, growth was obtained equivalent to that produced by the stock colony diet (21.5% protein from both plant and animal sources), and on this basis it was concluded that lysine was the only important amino acid deficient in commercial bread containing 3% non-fat milk solids.

Other work in which lysine was added to diets, which contained 13.4% protein at a level of 0.25% and fed to male weanling rats, showed increasing weight gains as the lysine was increased in increments of 0.06% (37).

Howe (34) found that when the food intake of rats was restricted, lysine supplementation of a wheat gluten diet was equally effective in supporting growth. There was no significance as to whether it was incorporated in the diet or given by gavage six hours after feeding.

Rosenberg, Rohdenbury, and Baldini (49) tested methionine, valine, and threonine as possible secondary amino acid deficiencies in a basal bread diet. None of their combinations with lysine improved on the results produced by lysine alone. The same was found to be true when both valine and threonine were added along with lysine.

Four diets containing wheat farina as the sole source of protein (8%) were supplemented with zero and 0.31% of L-lysinehydrochloride and with salt mixtures containing zero and 0.41% of potassium and fed ad libitum to four groups of weanling rats (56). The amount of carcass protein synthesized per gram of protein eaten was significantly improved by the additions of lysine to the diet, but not by the amount of potassium.

Hegsted and Chang (30) studied protein utilization in growing rats at different levels of intake. Four different proteins, lactalbumin, casien,

soy protein, and wheat gluten were fed to groups of weanling rats by diets which supplied seven different levels of each protein for a three week period. These workers concluded that the net nitrogen utilization was essentially constant for each of the proteins at all levels of intake up to those which support maximum growth.

It was found necessary in amino acid supplementation studies to consider the effect of excesses of some amino acids, besides the adequacy of the amount of each amino acid (18). Maximum response was obtained only when a correlation was made in the amount of the most limiting amino acid in such a way as to improve the balance of the other essential amino acids.

Vegetable Protein

Research has shown that, for the cereal eating population of the world, the shortage of protein is one of quality rather than quantity (35). Results indicated that under the most adverse conditions, subsistence entirely upon cereal grains, with no intake of animal protein, the protein consumed when caloric requirements were satisfied would be adequate both for infants and adults if it were of a quality comparable to the animal proteins. Amino acid supplementation involving only lysine, tryptophan, and threonine increased the quality of the proteins of the staple cereals to equal that of casein. Data from rat feeding studies indicated that lysine supplementation of whole wheat increased weight gain and protein efficiency ratio.

Prier and Derse (46) studied the nutritional value of a vegetable protein mixture. They found that receiving one gram protein per day from either casein or the vegetable mixture grew at a slow but equal rate. The protein efficiency ratios were approximately equal. INCAP Vegetable Mixture 9 is an example of a vegetable protein mixture formulated for human feeding (17).

It contains 28% corn, 28% sorghum grain, 38% cottonseed flour, 3% dehydrated leaf meal, and 3% Torula yeast. Baptist and DeMel (11) concluded that a suitable mixture of vegetable proteins can support growth in children.

Measurements of the protein efficiency ratio (PER) were carried out on the common legumes used in the Near East and on various legume-containing mixtures (3). Broadbean (Vicia faba), which was used extensively in Egypt, yielded low PER. When broadbeans were cooked they had a PER of 1.17. When the broadbeans were combined with parboiled wheat a PER of 1.62 was achieved.

Adolph, et al. (3) studied the method of cooking legumes commonly used in the Near East. Most commonly water is added to the legumes which were then cooked till soft, which requires two hours. Broadbeans are first soaked in water overnight before cooking.

Everson and Heckert (25) tested for biological value of leguminous sources of protein. Heating for 45 minutes at 15 psi improved the nutritional value of many legumes. They found an appreciable difference in the growth promoting qualities of various legumes, and that each source of leguminous protein varied in biological value according to the condition of the legume and the method of preparation with relation to the application of heat.

Gelatinization

When starch was mixed with cold water, no changes occurred, but as the water was heated, the viscosity of the mixture increased (41). If the concentration of the starch was at a high enough level, a paste formed. At times when the starch did not form a thick paste at the high temperature, a gel was formed when the temperature of the suspension was cooled.

Woodruff and Wilson (32) observed the first changes of heated wheat starch occurring at 55 C. The larger granules swelled slightly and lost

their birefringence. As the temperature was increased, these changes were observed in an increasing number of granules. In 1928 Katz (14) found that gelatinization of starch took place in steps and that for some starches these steps occurred at widely differing temperatures.

Crossland and Favor (22) reported that the various stages of swelling occurring during gelatinization of certain starches could be shown by viscosity measurements in the amylograph, if a viscous water-binding dispersion medium with proper temperature-viscosity characteristics was employed. Other research showed that large and small granule wheat starch preparations gave curves essentially similar in character (14). This indicated that the two types of wheat starch granules were similar in gelatinization characteristics.

Work done with the modes of swelling of various starches, evaluated over a range of pasting temperatures, showed that the extent of starch solubilization during pasting paralleled the swelling of the starch granules (39). Other studies indicated that starches of hard wheats gave higher viscosities than starches of soft wheats (5) (38).

The rate of heating was found to have an effect on the degree of swelling in starch (9). When starch was heated slowly the degree of swelling was reduced, a phenomenon suppose to be due to the additional hydrogen bonds formed during retrogradation.

Anderson, et al.(4) studied the gelatinization of corn grits by the use of an amylograph test. Five hundred grams of a 9% (dry basis) suspension of the sample (less than 60 mesh) was heated from 29 to 95 C in 44 minutes. The temperature was then held at 95 C for 16 minutes, then cooled to 50 C in 30 minutes. The final cooked paste viscosity served as the criterion of suitability for the product.

Absorption

The absorption of water by grain or products milled from grain products refers to the water being taken into the material (33). During absorption several different forces act to keep the water within the grain. 'Free water' is held in the intergranular and intercellular spaces and within the constituents (such as starch granules) of the material, in which case the absorbing substance acts as a supporting structure. Water is also held to the substance by hydrogen bonds as a result of the interaction between the water molecules and those of the substance. The absorbed water may also combine chemically with the supporting substance.

Becker (13) studied the absorption of liquid water by the wheat kernel. He found that the average moisture gain due to the absorption was 0.045 grams per gram of kernel (dry basis) at 22 C, but decreased with an increase in temperature.

Anderson, et al. (4), while working with the gelatinization of corn grits, developed a Water Absorption Index (WAI) and a Water Solubility Index (WSI) from a modification of a method for the measurement of the swelling power of starch. A 2.5-gram sample of ground product (less than 60 mesh) was suspended in 30 ml of water at 30 C in a 50-ml tared centrifuge tube. The suspension was stirred intermitently over a 30 minute period, and then centrifuged at 3000 x g for 10 minutes. The supernatant was poured carefully into a tared evaporating dish. The remaining precipitate or gel was then weighed and the WAI was calculated from its weight. The WSI was calculated from the amount of dried solids recovered by evaporating the supernatant from the WAI test and was expressed as percent of dry solids in the 2.5-gram sample.

Semolina Milling

The flour from Durum wheat is often known as 'semolina' since its granular character is different from the flours of other wheats (55). The granulation usually varies from coarse (840-540u), to medium (540-350u), to fine (350-250u) (52).

Semolina used in macaroni products is usually milled from hard wheat only (Amber Durum) (36). A fine semolina should not be of irregular granulation or from a high milling fraction. The semolina should not contain flour, or have a particle size less than 250u (51). The size of semolina particles should not be as important as their regularity. When mixed with water, fine particles absorb more than their share of water.

PURPOSE OF THIS STUDY

This study was conducted to investigate the effects of legume supplementation on some nutritional and physical characteristics of couscous. The physical tests were conducted with supplemented couscous processed in one of three different ways. The purpose of the physical tests was to find a reliable method for determining the absorption, solubility, and gelatinization characteristics of couscous. Rat feeding studies were conducted to determine the effect of horsebean flour supplementation on nutritional characteristics of couscous.

MATERIALS AND METHODS

Material

The wheat and semolina used in this study were obtained from the Peavey Company. The wheat was Durum Wheat. The wheat and semolina were stored in poly bags in metal drums during the time preceding the experiment. The storage was under cold room conditions. The horsebeans were also stored in the same manner prior to milling.*

Methods

Various physical and biological methods were used in the evaluation of the product at different stages of supplementation with horsebean flour. These methods were used to determine the effect of supplementation and physical properties of the product.

Milling of Raw Material

Commercial semolina and semolina milled in Kansas State University's experimental milling laboratory were used for experimentation and in the diets used in the rat feeding studies. These two products were blended to produce a uniform product. The semolina and 85% extraction durum flour were prepared from clean, sound wheat of the class Durum. Foreign material was removed by use of the Carter Dockage Tester. The cleaned Durum wheat was stored in poly bags under cold room conditions until milled into semolina or 85% extraction durum flour. The milling flow used for the production of semolina in the experimental milling laboratory is shown in Figure 1. The particle size of the experimentally milled semolina compared with that of the commercial semolina, and is shown in Table 1.

*Horsebeans were obtained from Mr. Paul E. Robinson, 421 Nichols Rd, K.C., Mo.

The 85% extraction durum flour and horsebean flour were milled using the experimental mills that were used for the milling of semolina. The respective flows are shown in Figures 2 and 3. The accepted milling fractions of the 85% extraction durum flour were blended together in a ribbon blender for twenty minutes. This procedure was also followed in blending the fractions from the semolina and horsebean flour flows.

Table 1. Sieving Analysis Using 100 Grams of Semolina.

U. S. Standard Sieves	Commercial Semolina (g.)		Experimentally Milled Semolina (g.)		
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 3
14	---	---	---	---	---
18	---	---	---	---	---
45	48.0	48.5	49.0	50.0	49.0
80	48.0	48.0	37.0	36.5	36.5
PAN	4.0	3.5	14.5	13.5	14.5

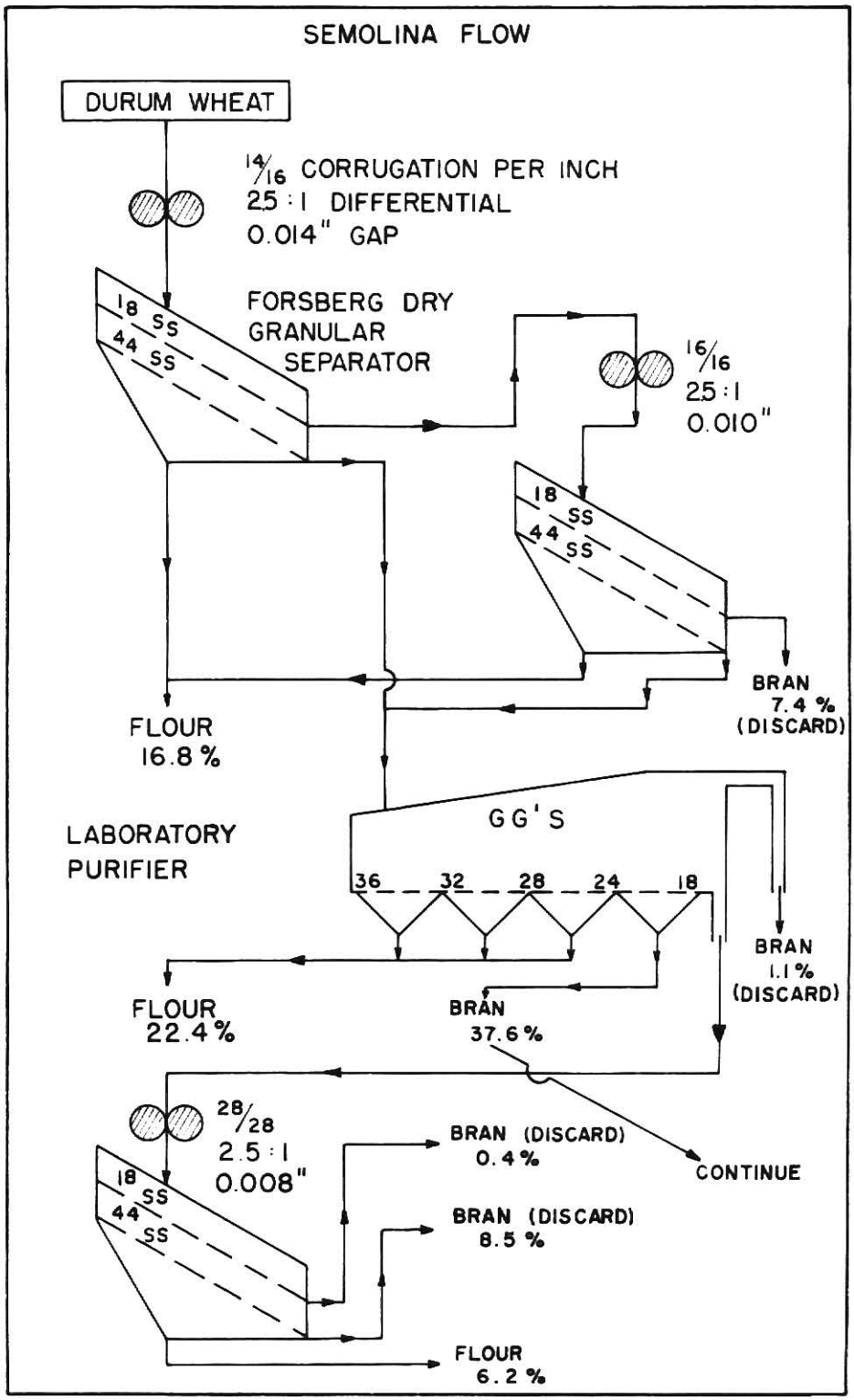
SEMOLINA FLOW
DURUM WHEAT
14/16 CORRUGATION PER INCH
2.5 : 1 DIFFERENTIAL
0.014" GAP
FORSBERG DRY
GRANULAR
SEPARATOR
18 SS
44 SS
16/16
2.5 : 1
0.010"
18 SS
44 SS
BRAN
7.4 %
(DISCARD)
FLOUR
16.8 %
LABORATORY
PURIFIER
GG'S
36 32 28 24 18
BRAN
1.1 %
(DISCARD)
FLOUR
22.4 %
BRAN
37.6 %
28/28
2.5 : 1
0.008"
BRAN (DISCARD)
0.4 %
CONTINUE
18 SS
44 SS
BRAN (DISCARD)
8.5 %
FLOUR
6.2 %

Figure 1.

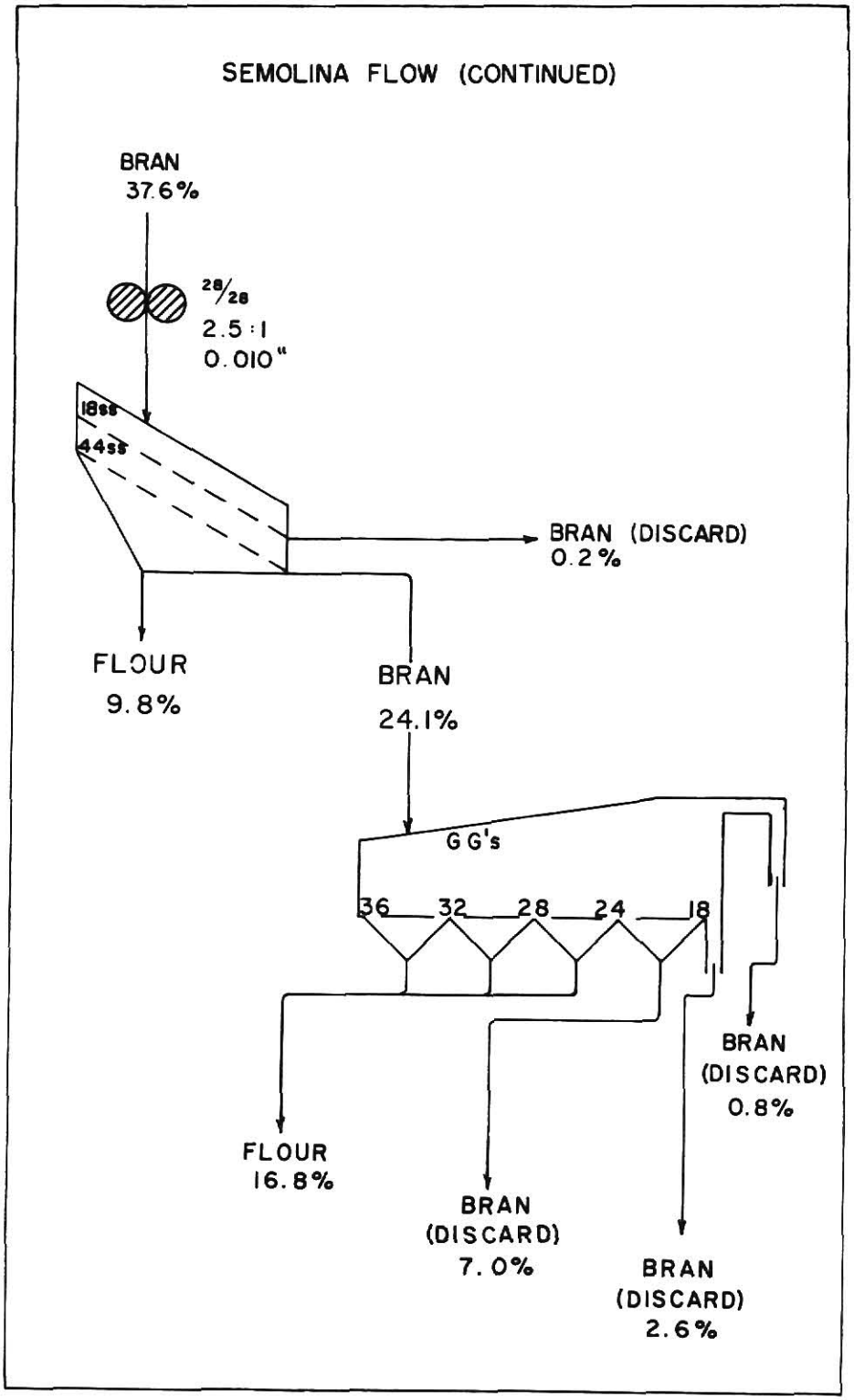

Figure 1.(cont'd).

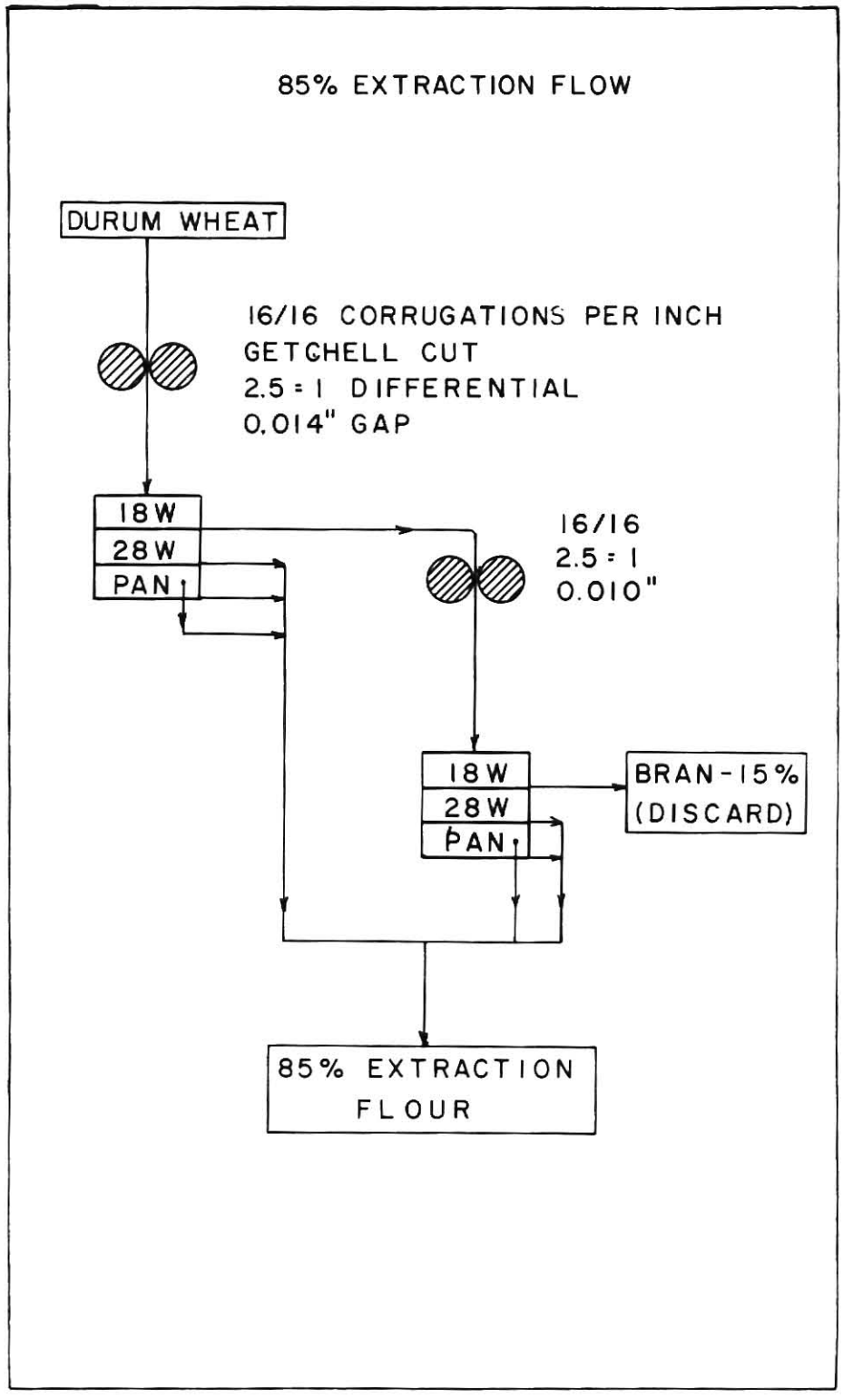
85% EXTRACTION FLOW
DURUM WHEAT
16/16 CORRUGATIONS PER INCH
GETCHELL CUT
2.5 : 1 DIFFERENTIAL
0.014" GAP
18W
28W
PAN
16/16
2.5 : 1
0.010"
18W
28W
PAN
BRAN - 15%
(DISCARD)
85% EXTRACTION
FLOUR

Figure 2.

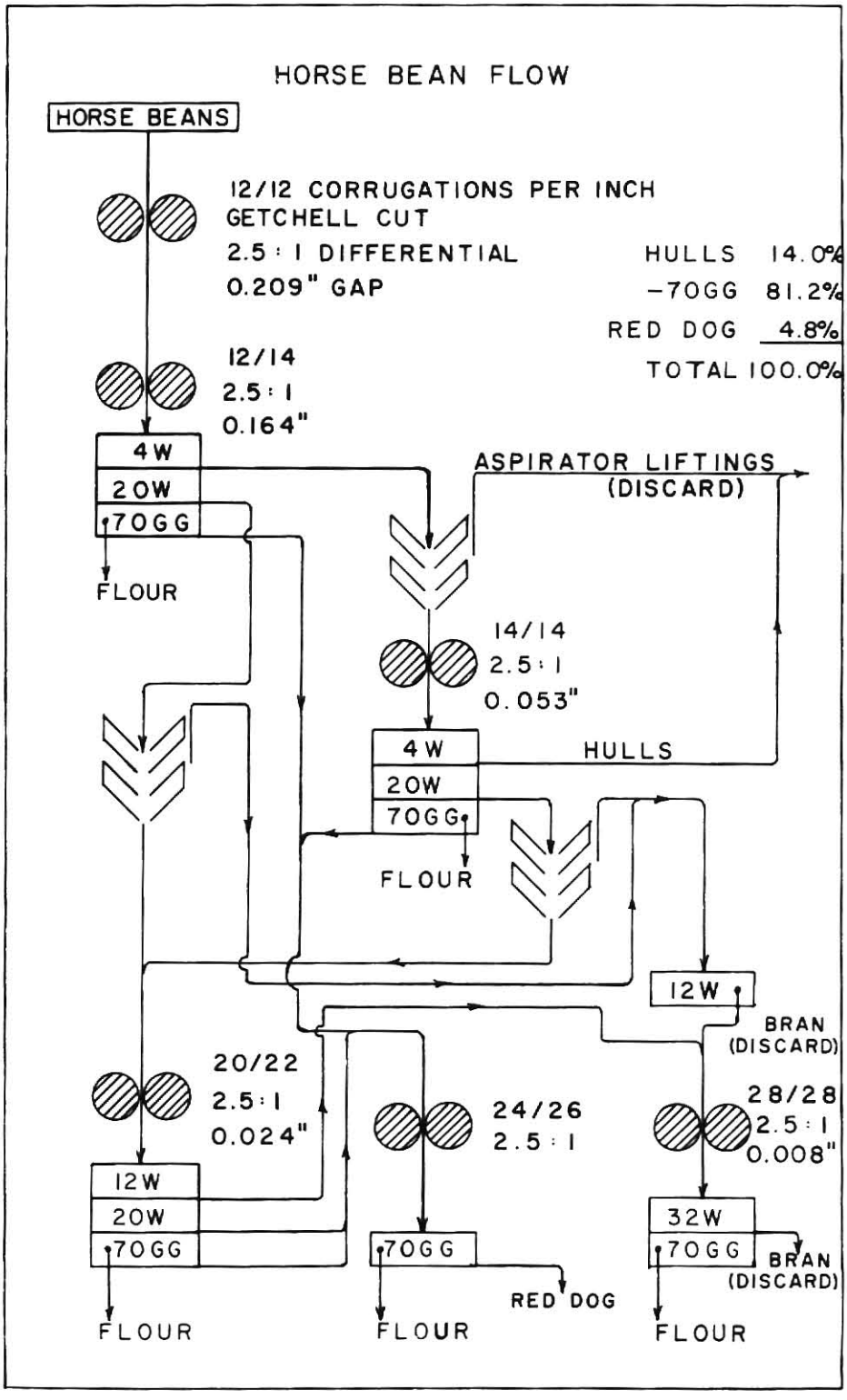
HORSE BEAN FLOW
HORSE BEANS
12/12 CORRUGATIONS PER INCH
GETCHELL CUT
2.5 : 1 DIFFERENTIAL
0.209" GAP
HULLS 14.0%
-70GG 81.2%
RED DOG 4.8%
TOTAL 100.0%
12/14
2.5 : 1
0.164"
4W
20W
70GG
FLOUR
ASPIRATOR LIFTINGS
(DISCARD)
14/14
2.5 : 1
0.053"
4W
20W
70GG
FLOUR
HULLS
12W
BRAN
(DISCARD)
20/22
2.5 : 1
0.024"
24/26
2.5 : 1
28/28
2.5 : 1
0.008"
12W
20W
70GG
FLOUR
70GG
RED DOG
FLOUR
32W
70GG
BRAN
(DISCARD)
FLOUR

Figure 3.

The Preparation of Couscous

The flour from durum wheat was used as the raw material for the preparation of couscous. In this particular study semolina (72.0% extraction durum flour) and 85% extraction durum flour were used individually as the raw materials.

The raw material was agglomerated with water, then sieved to separate the agglomerated particles. Following the sieving, the particles were either dried, or dried following steaming or autoclaving. The couscous particles are steamed prior to comsumption.

The steps involved in the preparation of couscous are listed below:

1. The raw material was agglomerated in 3000 gram batches.
2. The flour was placed into the mixing bowl of a Hobart (A-200) mixer (with wire ship).
3. The mixer was set to speed number two and turned on.
4. Two hundred seventy-five grams distilled water was added slowly and continuously over a two-minute period.
5. The mixing was continued for an additional two minutes after the addition of the water.
6. Following the second two-minute period, the mixer was stopped and the sides of the mixing bowl were scrapped down.
7. The mixer was then started again at speed number two and 275 grams of distilled water was added over a two-minute period.
8. After the addition of the water, the mixing was continued for two minutes.
9. The mixer was then stopped, and the sides of the bowl were scraped down.

10. The mixer was started again at speed two and 80 grams of water were added over a two-minute period.
11. Following the addition of the water, the mixing has continued for two minutes.
12. The mixer was then turned off and the agglomerated particles were sifted through a nest of sieves consisting of U.S. Standard number eight (2.38mm), twelve (1.68mm), and eighteen wire (1.00mm).

The overs of the number eight wire were considered too large and were discarded. The overs of the number twelve wire were designated as large to medium couscous, and the overs of the eighteen wire were considered fine couscous. The throughs of the eighteen wire (pan) were considered too fine and were subject to further agglomeration. Following sieving, the couscous particles were processed by one of three different methods.

One method was to dry the couscous particles in a forced air oven at 50 C for 20 hours. The second method was to steam the couscous for 7 minutes, then dry in an air oven at 50 C for 20 hours. The third method used was to autoclave the couscous for 4 minutes at 20 psi and 120 C, then dry the couscous in the air oven as stated above. Figures 4 and 5 show couscous made with semoline and 85% extraction flour.

During the addition of water, the agglomerated particles were increased in size, to approximately 2mm. When too much water was added, the agglomerated particles would stick together and could not be separated through sieving. The amount of water was regulated by the development of the agglomerated particles. When less than the stated amount of water was added to the flour, which had a moisture percentage of approximately 14.5 for semolina and 15.0 for 85% extraction durum flour, the flour would agglomerate slowly and would not attain the desirable size. When the water

Figures 4. and 5. Semolina Couscous is shown on the left and 85% Extraction Couscous is shown on the right.

added exceeded the desired amount, the agglomerated particles produced were too large and would stick together. The rate at which the water was added also was important.

If the water was added at a rate faster than stated above, the water would not be evenly distributed among the flour particles, resulting in dry, unagglomerated flour particles, and large, moist masses of unseparatable agglomerated particles. Adding the water at a slower rate than stated had no undesirable results with the exception of the longer time needed to prepare the couscous. The rate used for the addition of water was the fastest rate that would yield a desirable product.

At the end of preparation and drying the particle size of the couscous was determined in the same manner as that of wet couscous.

For consumption the procedure was to place the dried couscous in cheesecloth and place in the top section of a couscous steamer. The material was then steamed for 20 minutes. The couscous was then removed and turned and aerated, then was allowed to stand for several minutes. This procedure was then repeated two more times, resulting in a palatable couscous product. The above procedure was used for couscous which had been agglomerated or agglomerated and steamed prior to drying. When the couscous was autoclaved prior to drying, additional steps were added to the procedure. The couscous was placed in a strainer and dipped in water for ten seconds, allowed to drain for thirty seconds, then allowed to stand for several minutes prior to steaming. The desired final product was composed of particles which were soft and moist. The couscous particles would have a tendency to adhere to each other but would retain their individual identities.

Water Absorption and Solubility Tests

Water absorption and water solubility tests were conducted with semolina couscous which was agglomerated, agglomerated and steamed, and agglomerated and autoclaved. These samples were also supplemented with horsebean flour at levels of 5, 10, and 15%. The purpose of the tests was to evaluate the effect of method and processing and degree of supplementation with horsebean flour on the water absorption and water solubility characteristics of the couscous.

A dried 2.5-gram couscous sample, ground to pass through a 60 mesh screen was placed in suspension in a 50 ml tared centrifuge tube which contained 30 ml of distilled water. The centrifuge tube was placed in a 30 C water bath, and stirred continously for 30 minutes at 600 rpm. The suspension was then centrifuged at 3000 x g for 10 minutes. Following the centrifuging, the supernatant liquid was decanted into a tared evaporating dish and dried for 10.5 hours in a forced air oven at 60 C. The weight of the gel obtained per gram of dry sample was taken as the Water Absorption Index (WAI). The percentage of dry solids remaining, following the evaporation of the supernatant liquid represented the Water Solubility Index (WSI).

Amylograph Tests

Amylograph tests were carried out on semolina couscous and 85%-extraction couscous samples which were processed in different manners and supplemented at varying levels with horsebean flour. The tests were carried out with 500 grams of 9% (dry basis) suspension in water of the sample which had been ground to pass through a 60 mesh screen. The slurry was then heated from 29 to 95 C in 44 minutes. The temperature was then held at 95 C for 16 minutes. The slurry was then cooled to 50 C in 30 minutes.

Rat Feeding Study I

In order to determine nutritional differences in couscous prepared from two different flours with and without horsebean flour supplementation, a nutritional study using male albino rats was initiated. Nine different blended diets, which were assayed, contained added minerals, vitamins, and oil. The diets were prepared from couscous and from flour samples identical to those used for the preparation of couscous.

Proximate analysis was made on each component of the diets prior to the calculation of the diet formulas. The formulation of each diet was based on the results of the proximate analysis. The results of the proximate analysis are presented in Table 2.

Table 2. The Proximate Analyses Determinations of the Ingredients in 9 Different Blended Diets.

Ingredients	Moisture %	Protein* %
Casein	4.3	87.6
Semolina	14.7	12.2
Semolina Couscous	5.8	13.3
Semolina with 10% Horsebean flour	14.2	13.6
Semolina Couscous with 10% Horsebean flour	5.7	14.8
85% Extraction Durum flour	15.2	12.8
85%-Extraction couscous	5.6	13.9
85% Extraction Durum flour with 10% Horsebean flour	14.7	14.2
85%-Extraction couscous with 10% Horsebean flour	5.2	16.0

*Percentage Protein = N X 6.25

(6)

The mineral premix used in blending the diets contained calcium diphosphate, sodium chloride, potassium chloride, magnesium oxide, all of which were of food grade. The vitamin premix contained vitamins A, D_3, E, and K; thiamin hydrochloride, riboflavin, pyridoxine, calcium D-pantothenate, niacin, inositol, PABA, biotin, Folic acid, and Vitamin B_{12}.

Diet Formulations

Nine different blended diets were studied for protein efficiency and feed efficiency. The analytical results given in Table 2 were used in calculating the diets. Diet 1, the control diet, was a blend of casein (ANRC casein), cornstarch, oil, vitamins, and minerals. Diet 2 was a blend of semolina, cornstarch, oil, vitamins, and minerals. The third diet was prepared the same way with semolina couscous substituted for semolina. Diet 4 contained semolina, horsebean flour added at a level of 10% of the semolina, cornstarch, oil, minerals, and vitamins. The fifth diet contained semolina couscous and horsebean flour added at a level of 10% of the semolina couscous, with the remaining part of the diet the same as above. Diet 6 contained 85% extraction durum flour, while Diet 8 contained the 85% extraction flour with horsebean flour at the 10% level of the durum flour. Diet 7 contained 85%-extraction couscous, whereas Diet 9 contained 85% extraction couscous with horsebean flour at the 10% level. The remaining part of the diet was the same as above.

The diets were blended in a V-type liquid-solids blender using a minimum blending time of five minutes per blend once all of the ingredients were added. Seven kilograms of each diet were blended in order to have a quantity that would last the length of the rat feeding study. The capacity of the blender made it necessary to blend the diets in 3,500 gram quantities. Once blended,

the diets were stored in poly bags in metal containers under cold room conditions during the feeding study to maintain the nutritional level of the diets.

The individual diets and formulations are contained in Table 3. The blended diets were analyzed for moisture, protein, ash, crude fat, and crude fiber. The proximate analysis is shown in Table 4.

Table 3. Formulas for Diets Used in Rat Feeding Study, Percentage, Amounts, and Calculated Percentage Protein.

Diet No.	Ingredient	Percent	Amount/7kg.(gm.)
1	Casein (vitamin free)	11.40	798.6
	Cornstarch	65.40	4580.4
	Oil	7.97	558.0
	Mineral Mix	3.38	236.0
	Vitamin Mix	0.01	0.8
	Choline	0.28	20.0
	Water	11.51	805.8
	Calculated % Protein = 10.8		
2	Semolina	82.30	5761.3
	Cornstarch	6.09	426.5
	Oil	7.93	555.0
	Mineral Mix	3.38	236.4
	Vitamin Mix	0.01	0.8
	Choline	0.28	20.0
	Water	0.00	0.0
	Calculated % Protein = 11.6		

Table 3.(cont'd)

Diet No.	Ingredient	Percent	Amount/7kg.(gm.)
3	Semolina Couscous	75.19	5263.2
	Cornstarch	5.28	370.0
	Oil	7.97	558.0
	Mineral Mix	3.38	236.4
	Vitamin Mix	0.01	0.8
	Choline	0.28	20.0
	Water	7.89	551.6
	Calculated % Protein = 11.6		
4	Semolina with Horsebean flour	72.72	5090.9
	Cornstarch	14.10	987.4
	Oil	7.92	555.0
	Mineral Mix	3.38	236.4
	Vitamin Mix	0.01	0.8
	Choline	0.28	20.0
	Water	1.56	109.5
	Calculated % Protein = 12.6		
5	Semolina Couscous with Horsebean flour	68.26	4778.2
	Cornstarch	11.81	826.5
	Oil	7.97	558.0
	Mineral Mix	3.38	236.4
	Vitamin Mix	0.01	0.8
	Choline	0.28	20.0
	Water	8.29	580.1
	Calculated % Protein = 12.6		

Table 3.(cont'd)

Diet No.	Ingredient	Percent	Amount/7kg.(gm.)
6	85% Extraction durum flour	77.82	5447.5
	Cornstarch	10.47	732.8
	Oil	7.92	555.0
	Mineral Mix	3.38	236.4
	Vitamin Mix	0.01	0.8
	Choline	0.28	20.0
	Water	0.18	12.5
	Calculated % Protein = 11.5		
7	85%-Extraction Couscous	70.92	4964.5
	Cornstarch	9.18	642.4
	Oil	7.97	558.0
	Mineral Mix	3.38	236.4
	Vitamin Mix	0.01	0.8
	Choline	0.28	20.0
	Water	8.26	577.9
	Calculated % Protein = 11.5		
8	85% Extraction durum flour with Horsebean flour	68.58	4801.1
	Cornstarch	17.95	1257.0
	Oil	7.84	549.0
	Mineral Mix	3.38	236.4
	Vitamin Mix	0.01	0.8
	Choline	0.28	20.0
	Water	1.94	135.7
	Calculated % Protein = 12.2		

Table 3.(concluded)

Diet No.	Ingredient	Percent	Amount/7kg.(gm.)
9	85%-Extraction couscous with Horsebean flour	62.42	4369.5
	Cornstarch	14.12	1198.1
	Oil	7.97	558.0
	Mineral Mix	3.38	236.4
	Vitamin Mix	0.01	0.8
	Choline	0.28	20.0
	Water	8.82	617.2
	Calculated % Protein = 11.8		

Table 4. Proximate Analyses of 9 Different Blended Diets.

Diet No.	Moisture %	Protein %	Ash %	Crude Fat %	Crude Fiber %
1	19.8	10.4	2.3	4.8	2.4
2	12.3	11.6	1.9	5.2	2.3
3	11.7	12.0	2.0	4.7	2.7
4	13.4	12.7	2.4	4.6	2.9
5	13.1	12.6	1.9	4.8	2.7
6	11.9	11.1	2.1	5.1	3.0
7	12.2	11.5	1.8	4.9	2.5
8	13.1	11.9	1.8	4.8	2.8
9	12.0	11.3	2.0	4.8	3.0

Rat Feeding Study Design

The rat study was designed for statistical analysis in a randomized complete block design. Each of the nine experimantal diets was fed to eight rats; a total of 72 rats were thus fed for the study. Weanling male albino rats purchased from the Charles River Breeding Laboratories were used in the experiment. At the start of the experiment the rats were three weeks old and ranged in weight from 51 to 57 grams.

The individual rats were weighed on the day they were received and then placed in individual wire-bottomed cages. The rats were watered and fed and given two days to adjust to the environment. At the end of two days the rats were weighed and feed consumption recorded. The actual beginning of the experiment started at this time. The rats consumed their feed ad libitum. Each rat was given sufficient amounts of feed so that its consumption would not be limited by the amount of feed present. Each rat was also given demineralized water ad libitum. Papers, with dimensions slightly larger than those of the wire-bottoms of the cages were placed under each cage to catch the fecal material and wasted feed of each rat. This material was removed daily. The wasted feed was then sifted from fecal material, weighed, and the amount recorded. The rats were weighed every seven days and the amount of feed consumed by each rat was determined on the same day.

Records were maintained for weekly feed intake. The amount of feed needed per rat per week was estimated and weighed at the beginning of each week, and the rat was fed from this supply. At the end of the 7 day period, the unused feed offered to the rat was recombined with the remaining original supply. This feed was weighed and the weight was recorded. The uncorrected feed consumption was determined by substracting the grams of

feed remaining at the end of the week from the grams of feed at the beginning of the period. This amount would represent the amount of feed offered to the rat over the seven-day period. The amount of feed which the rat wasted over the seven-day period was subtracted from the amount of feed offered to the rat. The resulting amount was recorded as weekly feed consumption.

The experiment was continued for 28 days. This time period was considered necessary for the rats to reach maturity. At the end of the experiment, the rats were sacrificed.

The total weight gained and the total feed consumed were calculated for each rat from the weekly data. These data were then used to calculate the average weekly gain and the average weekly feed consumption. Next, the feed efficiency (FE) was calculated by dividing weight gain into the feed consumption. The protein efficiency ratio (PER) was then calculated by first determining the amount of protein consumed by the rat, which was done by multiplying total feed consumption by the percentage protein as determined by the proximate analysis. The protein efficiency ratio (PER) was then found by dividing the total weight gained by the protein intake. The calculated average weekly gain, average weekly consumption, feed efficiency (FE), and protein efficiency ratio (PER), were analyzed statistically to determine significance. The calculations made from the collected data of each rat are included in Table 5.

Table 5. Summary and Averages of Weight Gain, Feed Consumption, Feed Efficiency, and Protein Efficiency Ratios of Rats Fed 9 Different Blended Diets for 28 Days.

Diet No.	Rat No.	Total Gain (g.)	Total Consum. (g.)	Average Weekly Gain (g.)	Average Weekly Con.(g.)	Feed* Effic.	Protein** Efficiency Ratio
1	1	153	426	38.2	106.5	2.78	3.33
	2	189	549	47.2	137.2	2.90	3.19
	3	155	492	38.8	123.0	3.17	3.15
	4	174	552	43.5	138.0	3.17	2.92
	5	160	484	40.0	121.0	3.02	3.06
	6	148	522	37.0	130.0	3.53	2.62
	7	170	517	42.5	129.2	3.04	3.04
	8	152	511	38.0	127.8	3.36	2.75
2	1	34	269	8.5	67.2	7.91	1.09
	2	20	200	5.0	50.0	10.00	0.86
	3	29	267	7.2	66.7	9.21	0.96
	4	32	252	8.0	63.0	7.88	1.10
	5	30	251	7.5	62.8	8.37	1.03
	6	28	304	7.0	76.0	10.86	0.79
	7	32	263	8.0	65.8	9.78	0.05
	8	28	274	7.0	68.5	9.78	0.88

Table 5.(con't.)

Diet No.	Rat No.	Total Gain (g.)	Total Consum. (g.)	Average Weekly Gain (g.)	Average Weekly Con.(g.)	Feed* Effic.	Protein** Efficiency Ratio
3	1	40	222	10.0	55.5	5.55	1.55
	2	32	283	8.0	40.8	8.84	0.96
	3	56	381	14.0	95.2	6.80	1.27
	4	41	294	10.2	73.5	7.17	1.20
	5	19	140	4.8	42.5	8.95	0.90
	6	39	260	9.8	65.0	6.67	1.29
	7	56	381	14.0	95.2	6.80	1.27
	8	26	251	6.5	62.8	9.65	0.89
4	1	71	375	17.8	93.8	5.28	1.50
	2	105	395	26.2	98.8	3.76	2.11
	3	97	389	24.2	97.2	4.01	1.98
	4	96	377	24.0	94.2	3.93	2.02
	5	73	376	18.2	94.0	5.15	1.54
	6	99	431	24.8	104.8	4.35	2.30
	7	68	340	17.0	85.0	5.00	1.59
	8	71	349	87.2	87.2	4.92	1.61

Table 5.(con't.)

Diet No.	Rat No.	Total Gain (g.)	Total Consum. (g.)	Average Weekly Gain (g.)	Average Weekly Con.(g.)	Feed* Effic.	Protein** Efficiency Ratio
5	1	78	375	19.5	93.8	4.81	1.65
	2	79	395	19.8	79.0	5.00	1.58
	3	116	389	29.0	97.2	3.35	2.37
	4	87	377	21.8	94.2	4.33	2.21
	5	97	376	24.2	94.0	3.88	2.05
	6	83	431	20.8	107.8	5.19	1.56
	7	90	340	22.5	85.0	3.78	2.10
	8	104	349	26.0	87.2	3.36	2.59
6	1	50	356	12.5	89.0	7.12	1.22
	2	65	412	16.2	103.0	6.36	1.36
	3	40	502	10.0	125.5	12.55	0.69
	4	57	468	14.2	117.0	8.21	1.06
	5	45	465	11.2	116.2	10.33	0.84
	6	34	389	8.5	97.2	11.44	0.76
	7	42	435	10.5	108.8	10.36	0.84
	8	65	392	16.2	98.0	6.03	1.44

Table 5.(con't.)

Diet No.	Rat No.	Total Gain (g.)	Total Consum. (g.)	Average Weekly Gain (g.)	Average Weekly Con.(g.)	Feed* Effic.	Protein** Efficiency Ratio
7	1	70	411	17.5	102.8	5.87	1.48
	2	71	391	17.8	97.8	5.51	1.58
	3	39	255	9.8	63.8	6.58	1.33
	4	80	396	20.0	99.0	4.95	1.66
	5	62	350	14.5	87.5	5.64	1.54
	6	74	393	18.5	23.2	5.31	1.64
	7	70	385	17.5	96.2	5.50	1.58
	8	76	408	19.0	102.0	5.87	1.62
8	1	142	476	35.5	119.0	3.35	2.44
	2	111	441	27.8	110.2	3.97	2.06
	3	135	464	33.8	116.0	3.44	2.38
	4	122	519	30.5	129.8	4.25	1.93
	5	139	496	34.8	124.0	3.57	2.74
	6	134	459	33.5	114.8	3.42	2.39
	7	145	490	36.2	122.5	3.38	2.43
	8	185	488	46.2	122.0	2.64	3.11

Table 5.(concluded)

Diet No.	Rat No.	Total Gain (g.)	Total Consum. (g.)	Average Weekly Gain (g.)	Average Weekly Con.(g.)	Feed* Effic.	Protein** Efficiency Ratio
9	1	134	525	33.5	131.2	3.92	2.16
	2	120	449	30.0	112.2	3.74	2.26
	3	136	441	34.0	117.8	3.46	2.45
	4	97	408	24.2	102.0	4.21	2.02
	5	147	571	36.8	142.8	3.88	2.18
	6	132	531	33.0	132.8	4.02	2.11
	7	141	533	35.2	133.2	3.48	2.24
	8	114	456	28.5	114.0	4.00	2.12

* Feed Efficiency = Feed Consumption/Weight Gained

** Protein Efficiency Ratio = Weight Gained/Protein Intake

Rat Feeding Study II

A second nutritional study using weanling male albine rats was made in order to evaluate the diet formulations on a more equal basis, to determine the sparing effect of having more than sufficient protein for growth and development, and to determine the protein efficiency of diets at 18% protein. Fourteen blended diets were formulated using semolina as the basic source of protein. The duplication of the diets at 18% protein was done to see if an increased level of protein would have an effect on the hair loss observed in Rat Feeding Study I. Supplementation with horsebean flour was done at levels of 5, 10, and 15% of the semolina. Seven different blended diets were formulated at 10% protein and then 7 at 18% protein. Three of the diets at each protein level contained semolina couscous supplemented with horsebean flour. Each level also contained a diet composed of semolina couscous plus lysine at a level equivalent to horsebean flour supplemented at the 10% level The remaining diets at each level consisted of a casein control with the other diet containing semolina. The diets were calculated according to the proximate analysis of the ingredients given in Table 6. Since lysine is the limiting amino acid in wheat, it was incorporated in two of the diets with semolina couscous, to see if it would have any effect on hair loss. Diammonium citrate was used to raise the level of nitrogen in the diets to a level of 18% protein.

The diets were blended in the same manner as Rat Study I. The specific diets and formulation with the calculated percentage protein are shown in Table 7. The 14 blended experimantal diets were analyzed for moisture, protein, ash, crude fat, and crude fiber. The results of the proximate analysis are given in Table 8.

Design of Rat Feeding Study II

The rat feeding design was the same as used for Study I. The fourteen diets were fed with eight rats per diet. The rats used for the study were received from the Charles River Breeding Laboratories three weeks after birth. The rats were acclimated to the environment for two days and ranged in weight from 42 to 59 grams.

Rat Study II was carried out in the same manner as Study I, with the exception of the diets being different. The rats were fed and watered ad libitum, and the physical facilities of the first study were used. The wasted feed was collected daily and the amount was recorded for use in the feeding records. The weekly weight gain and weekly feed consumption records were maintained at the start of the third day after the rats were received. The data were recorded for 28 days. These records were used to calculate the total weight gained and the total feed consumed by each rat. These data were then used to calculate the average weekly weight gain, the average weekly feed consumption, the feed efficiency (FE), and the protein efficiency ratio (PER), as was done in Study I. These data are shown in Table 9.

The data for average weekly weight gain, average weekly consumption, feed efficiency, and protein efficiency ratio by rats fed the 14 experimental diets were analyzed statistically. The protein levels of the seven diets in the 10% protein series ranged from 6.9, for the casein control, to 11.6 for the semolina diet. The protein levels of the 18% protein series varied from 13.9 for the casein control to 18.0 for Diet 9 which contained semolina.

Table 6. Proximate Analysis Determinations of the Protein Sources Used in 14 Blended Experimental Diets.

Ingredient	Moisture %	Protein %	Ash %	Crude Fat %	Crude Fiber %
Casein	4.4	88.8	---	---	---
Semolina	14.0	12.3	1.2	1.4	1.4
Semolina couscous	4.5	13.9	1.2	1.4	1.3
Semolina couscous with 5% horsebean flour	4.2	14.9	1.3	1.5	1.6
Semoline couscous with 10% horsebean flour	4.7	15.6	1.1	1.4	1.4
Semolina couscous with 15% horsebean flour	4.7	16.4	1.3	1.3	1.7
Semolina couscous with lysine**	3.6	14.1	1.2	1.0	1.9

(6)

* Percent protein = N x 6.25

**The lysine used was L-lysine monohydrochloride

Table 7. Formulas for Diets used in Rat Feeding Study: Percentage, Amount, and Calculated % Protein.

Diet No.	Ingredient	Percent	Amount/7 kg.
1	Casein (vitamin free)	11.1	798.0
	Cornstarch	77.0	5386.6
	Mineral premix	3.4	236.4
	Vitamin premix	0.1	4.0
	Choline	0.3	20.0
	Oil	7.6	555.0
	Calculated % Protein	6.9	
2	Semolina	81.3	5690.0
	Cornstarch	7.1	494.6
	Mineral premix	3.4	236.4
	Vitamin premix	0.1	4.0
	Choline	0.3	20.0
	Oil	7.9	555.0
	Calculated % Protein	11.6	
3	Semolina couscous	71.9	5036.0
	Cornstarch	16.4	1148.6
	Mineral premix	3.4	236.4
	Vitamin premix	0.1	4.0
	Choline	0.3	20.0
	Oil	7.9	555.0
	Calculated % Protein	10.8	

Table 7. (con't.)

Diet No.	Ingredient	Percent	Amount/7 kg.
4	Semolina couscous with broad bean flour (5%)	66.8	4678.0
	Cornstarch	21.5	1506.6
	Mineral premix	3.4	236.4
	Vitamin premix	0.1	4.0
	Choline	0.3	20.0
	Oil	7.9	555.0
	Calculated % Protein	10.8	
5	Semolina couscous with broad bean flour (10%)	63.3	4432.0
	Cornstarch	25.0	1752.6
	Mineral premix	3.4	236.4
	Vitamin premix	0.1	4.0
	Choline	0.3	20.0
	Oil	7.9	555.0
	Calculated % Protein	10.6	
6	Semolina couscous with broad bean flour (15%)	60.2	4212.0
	Cornstarch	28.2	1972.6
	Mineral premix	3.4	236.4
	Vegetable premix	0.1	4.0
	Choline	0.3	20.0
	Oil	7.9	555.0
	Calculated % Protein	10.6	

Table 7.(con't.)

Diet No.	Ingredient	Percent	Amount/7 kg.
7	Semolina couscous with lysine	70.0	4972.0
	Cornstarch	17.3	1212.6
	Mineral premix	3.4	236.4
	Vitamin premix	0.1	4.0
	Choline	0.3	20.0
	Oil	7.9	555.0
	Calculated % Protein	10.9	
8	Casein (vitamin free)	11.4	798.0
	Cornstarch	66.6	4663.2
	Mineral premix	3.4	236.4
	Vitamin premix	0.1	4.0
	Choline	0.3	20.0
	Oil	7.9	555.0
	Diammonium citrate	10.3	723.4
	Calculated % Protein	15.2	
9	Semolina	78.0	5460.0
	Cornstarch	----	----
	Mineral premix	3.4	236.4
	Vitamin premix	0.1	4.0
	Choline	0.3	20.0
	Oil	7.9	555.0
	Diammonium citrate	10.3	723.4
	Calculated % Protein	18.0	

Table 7.(con't.)

Diet No.	Ingredient	Percent	Amount/7kg.
10	Semolina couscous	71.9	5036.0
	Cornstarch	6.1	425.2
	Mineral premix	3.4	236.4
	Vitamin premix	0.1	4.0
	Choline	0.3	20.0
	Oil	7.9	555.0
	Diammonium citrate	10.3	723.4
	Calculated % Protein	16.7	
11	Semolina couscous with broad bean flour (5%)	66.8	4678.0
	Cornstarch	11.2	783.2
	Mineral premix	3.4	236.4
	Vitamin premix	0.1	4.0
	Choline	0.3	20.0
	Oil	7.9	555.0
	Diammonium citrate	10.3	723.0
	Calculated % Protein	16.6	
12	Semolina couscous with broad bean flour (10%)	63.3	4432.0
	Cornstarch	14.7	1029.2
	Mineral premix	3.4	236.4
	Vitamin premix	0.1	4.0
	Choline	0.3	20.0
	Oil	7.9	555.0
	Diammonium citrate	10.3	723.0
	Calculated % Protein	17.2	

Table 7. (concluded)

Diet No.	Ingredient	Percent	Amount/7 kg.
13	Semolina couscous with broad bean flour (15%)	60.2	4212.0
	Cornstarch	14.7	1029.2
	Mineral premix	3.4	236.4
	Vitamin premix	0.1	4.0
	Choline	0.3	20.0
	Oil	7.9	555.0
	Diammonium citrate	10.3	723.0
	Calculated % Protein	16.8	
14	Semolina couscous with lysine	71.0	4972.0
	Cornstarch	7.0	489.2
	Mineral premix	3.4	236.4
	Vitamin premix	0.1	4.0
	Choline	0.3	20.0
	Oil	10.3	723.0
	Diammonium citrate	10.3	723.0
	Calculated % Protein	17.0	

Table 8. Proximate Analysis of 14 Different Blended Diets.

Diet No.	Moisture %	Protein* %	Ash %	Crude Fat %	Crude Fiber %
1	18.9	6.9	2.1	6.0	2.5
2	12.6	11.6	2.4	7.1	2.8
3	12.4	10.8	2.3	5.2	3.7
4	13.3	10.8	2.8	5.6	2.6
5	12.8	10.5	2.6	6.9	3.5
6	13.6	10.5	2.8	7.4	2.4
7	12.4	10.9	2.5	9.7	1.9
8	12.8	13.9	2.0	8.2	1.8
9	12.6	18.0	2.2	13.1	3.1
10	12.6	16.7	2.9	10.3	3.1
11	12.8	16.5	2.7	8.7	2.8
12	13.2	17.0	2.4	5.8	2.7
13	13.2	16.6	2.6	8.3	3.0
14	13.3	17.0	2.6	5.9	2.2

* Percent protein = N x 6.25 (6)

Table 9. Summary and Averages of Weight Gain, Feed Consumption, Feed Efficiency, Protein Efficiency Ratio of Rats Fed 14 Different Diets for 28 days.

Diet No.	Rat No.	Total Gain (g.)	Total Consum. (g.)	Average Weekly Gain (g.)	Average Weekly Con. (g.)	Feed* Effic.	Protein** Efficiency Ratio
1	1	108	336	17.0	84.0	3.11	4.25
	2	103	337	25.8	84.2	3.27	4.84
	3	146	403	36.5	100.8	2.76	4.79
	4	122	354	30.5	88.5	2.90	4.55
	5	---	---	----	----	----	----
	6	88	314	22.0	78.5	3.57	3.70
	7	105	370	26.2	92.5	3.52	3.75
	8	155	425	36.2	106.2	2.74	4.82
2	1	33	245	8.2	61.2	7.42	1.16
	2	30	232	7.5	58.0	7.73	1.12
	3	42	255	10.5	63.8	6.07	1.42
	4	36	238	9.0	59.5	6.61	1.30
	5	31	241	7.8	60.2	7.77	1.11
	6	33	245	8.2	61.2	7.42	1.16
	7	44	268	11.0	67.0	6.09	1.42
	8	39	237	9.8	59.2	6.08	1.42

Table 9.(con't.)

Diet No.	Rat No.	Total Gain (g.)	Total Consum. (g.)	Average Weekly Gain (g.)	Average Weekly Con. (g.)	Feed* Effic.	Protein** Efficiency Ratio
3	1	27	268	6.8	67.0	9.93	0.93
	2	15	166	3.8	41.5	11.07	0.84
	3	15	159	3.8	39.8	10.60	0.87
	4	23	214	5.8	53.5	9.30	1.00
	5	20	200	5.0	50.0	10.00	0.93
	6	26	245	6.5	61.2	9.42	0.98
	7	15	175	3.8	43.8	11.67	0.79
	8	19	181	4.8	45.2	10.05	0.97
4	1	45	240	11.2	60.0	5.33	1.73
	2	66	329	16.5	82.2	4.98	1.85
	3	41	257	10.2	64.2	6.27	1.47
	4	38	268	9.5	67.0	7.05	1.31
	5	67	344	16.8	86.0	5.13	1.80
	6	58	315	14.5	78.8	5.43	1.70
	7	58	292	14.5	73.0	5.03	1.83
	8	53	267	13.2	66.8	5.04	1.83

Table 9.(con't.)

Diet No.	Rat No.	Total Gain (g.)	Total Consum. (g.)	Average Weekly Gain (g.)	Average Weekly Con. (g.)	Feed* Effic.	Protein** Efficiency Ratio
5	1	61	294	15.2	73.5	4.82	1.96
	2	41	217	10.2	54.2	5.29	1.78
	3	60	295	15.0	73.8	4.92	1.92
	4	58	310	14.5	77.5	5.34	1.76
	5	63	301	15.8	75.2	4.78	1.98
	6	57	267	14.2	66.8	4.68	2.01
	7	94	271	23.5	92.8	3.95	2.39
	8	51	265	12.8	66.2	5.19	1.82
6	1	104	388	26.0	97.0	3.73	2.52
	2	89	378	22.2	94.5	4.25	2.21
	3	84	345	21.0	86.2	4.11	2.29
	4	67	323	16.8	80.8	4.82	1.95
	5	74	358	18.5	89.5	4.84	1.94
	6	68	282	17.0	70.5	4.15	2.26
	7	62	267	15.5	66.8	4.31	2.18
	8	70	298	17.0	74.5	4.26	2.21

Table 9.(con't.)

Diet No.	Rat No.	Total Gain (g.)	Total Consum. (g.)	Average Weekly Gain (g.)	Average Weekly Con. (g.)	Feed* Effic.	Protein** Efficiency Ratio
7	1	60	307	15.0	76.8	5.12	1.79
	2	--	---	----	----	----	----
	3	57	328	14.2	82.0	5.75	1.59
	4	85	364	21.2	91.0	4.28	2.14
	5	79	331	19.8	82.8	4.19	2.19
	6	57	272	14.2	68.0	4.77	1.92
	7	61	286	15.2	71.5	4.69	1.96
	8	73	334	18.2	83.5	4.57	2.00
8	1	61	282	15.2	70.5	4.62	1.42
	2	51	271	12.8	67.8	5.31	1.24
	3	24	229	6.0	57.2	9.54	0.69
	4	21	218	5.2	54.5	10.38	0.63
	5	26	258	6.5	64.5	9.92	0.66
	6	14	201	3.5	50.2	14.35	0.46
	7	27	236	6.8	59.0	8.74	0.75
	8	51	216	12.8	54.0	4.23	1.55

Table 9.(con't.)

Diet No.	Rat No.	Total Gain (g.)	Total Consum. (g.)	Average Weekly Gain (g.)	Average Weekly Con. (g.)	Feed* Effic.	Protein** Efficiency Ratio
9	1	23	187	5.8	46.8	8.13	0.68
	2	20	139	5.0	34.8	6.95	0.80
	3	18	158	4.5	39.5	8.78	0.63
	4	19	171	4.8	42.8	9.00	0.62
	5	15	178	3.8	44.5	11.87	0.47
	6	5	112	1.2	28.0	2.20	0.25
	7	13	148	3.2	37.0	11.38	0.49
	8	17	181	4.2	45.2	10.68	0.52
10	1	16	196	4.0	49.0	12.25	0.49
	2	18	228	4.5	57.0	12.67	0.47
	3	16	156	4.0	39.0	9.75	0.61
	4	12	178	3.0	44.5	14.83	0.40
	5	9	145	2.2	36.2	16.11	0.37
	6	18	192	4.5	48.0	10.67	0.56
	7	8	183	2.0	45.8	22.87	0.26
	8	16	201	4.0	50.2	12.56	0.48

Table 9.(con't.)

Diet No.	Rat No.	Total Gain (g.)	Total Consum. (g.)	Average Weekly Gain (g.)	Average Weekly Con. (g.)	Feed* Effic.	Protein** Efficiency Ratio
11	1	40	263	10.0	65.8	6.57	0.92
	2	25	224	6.2	56.0	8.96	0.67
	3	28	253	7.0	63.2	9.04	0.67
	4	22	155	5.5	38.8	7.05	0.86
	5	21	263	5.2	65.8	12.52	0.48
	6	28	241	7.0	60.2	8.61	0.70
	7	19	189	4.8	47.2	9.95	0.61
	8	22	201	5.5	50.2	9.14	0.66
12	1	29	237	7.2	59.2	8.17	0.71
	2	30	267	7.5	66.7	8.90	0.65
	3	30	245	7.5	61.2	8.17	0.71
	4	30	218	7.5	54.5	7.27	0.80
	5	23	191	5.8	37.8	8.30	0.70
	6	34	199	8.5	49.8	5.85	1.00
	7	25	220	6.2	55.0	8.80	0.66
	8	20	171	5.0	42.8	8.55	0.68

Table 9.(concluded)

Diet No.	Rat No.	Total Gain (g.)	Total Consum. (g.)	Average Weekly Gain (g.)	Average Weekly Con. (g.)	Feed* Effic.	Protein** Efficiency Ratio
13	1	31	246	7.8	61.5	7.94	0.75
	2	27	215	6.8	53.8	7.96	0.75
	3	24	216	6.0	54.0	9.00	0.66
	4	44	259	11.0	64.8	5.89	1.01
	5	32	206	8.0	51.5	6.44	0.92
	6	32	169	8.0	42.5	5.28	1.12
	7	25	198	6.2	49.5	7.92	0.75
	8	22	218	5.5	54.5	9.91	0.60
14	1	25	187	6.2	46.8	7.48	0.79
	2	17	155	4.2	38.8	9.12	0.64
	3	30	232	7.5	58.0	7.73	0.76
	4	22	129	5.5	32.2	5.86	1.00
	5	33	212	8.2	53.0	6.42	0.92
	6	26	158	6.5	39.5	6.08	0.97
	7	--	---	---	----	----	----
	8	28	220	7.0	55.0	7.86	0.75

RESULTS AND DISCUSSION

Moisture Content

During the preparation of couscous the moisture content varied greatly. The flour used had a moisture content of between 14.5 and 15.5%. After the final agglomeration with water, the moisture content increased to approximately 32.0%. Steaming and autoclaving had little effect on the moisture percentage. When dried at 50 C, the moisture content decreased to approximately 4.0%. During the steaming of the dried couscous prior to consumption, the moisture content must be raised to the level attained following agglomeration.

Supplementation with horsebean flour at 5, 10, and 15% levels was carried out by replacing an equivalent percentage of semolina. Such supplementation decreased the amount of water needed to obtain optimum agglomeration. When horsebean flour replaced 5% of the weight of the semolina, then agglomerated, the amount of water needed to attain optimum agglomeration was decreased by 10.8%. When the supplementation was increased to 10 and 15%, the water needed to be decreased by 18.2 and 29.4% respectively. These results are shown in Table 10.

Table 10. Effect of Horsebean Flour Supplementation on Amount of Water Needed for Optimum Agglomeration of Couscous.

Sample	Wt. of water added in 1 st mixing (g.)	Wt. of water added in 2 nd mixing (g.)	Wt. of water added in 3 rd mixing (g.)	Total weight of water added (g.)	Decrease in added water (%)
Semolina	275	275	80	630	----
Semolina with horsebean flour (5%)	245	245	72	562	10.8
Semolina with Horsebean flour (10%)	225	225	65	515	18.2
Semolina with horsebean flour (15%)	195	195	55	445	29.4

Water Absorption and Solubility Tests

The method of processing couscous and the degree of supplementation with horsebean flour had an effect on the Water Absorption Index (WAI). Semolina couscous, which was only agglomerated, yielded a WAI value of 0.85. When steaming or autoclaving was added to the processing of couscous, the WAI values were 3.29 and 3.47 respectively. When semolina couscous, supplemented with horsebean flour at the 5% level, was only agglomerated, the WAI value increased to 0.99. When semolina couscous, which was supplemented with horsebean flour at the 5% level, was agglomerated, then steamed, the WAI value decreased to 3.21. When the supplemented couscous was autoclaved, the WAI value increased to 3.62. When semolina couscous was supplemented with horsebean flour at the 10% level, the agglomerated sample had a WAI value of 0.96, and the steamed and autoclaved samples had values of 2.86 and 3.76 respectively. With 15% horsebean flour, the value for the agglomerated sample was 0.99, that for the steamed sample was 2.75, and that for the autoclaved sample was 3.91. A summary of these results is shown in Figure 6.

Steaming or autoclaving the couscous after agglomeration yielded higher WAI values than when the couscous was only agglomerated. The degree of supplementation with horsebean flour had little effect on the value for the agglomerated couscous, but had major effects on that for the steamed or for the autoclaved samples. The WAI values, for the autoclaved couscous increased with increasing supplementation, while couscous which was steamed yielded lower WAI values.

Semolina couscous which was agglomerated, yielded a high Water Solubility Index (WSI) of 8.50%. When the semolina couscous was agglomerated and then steamed, the WSI value decreased to 6.74%. When agglomerated, then

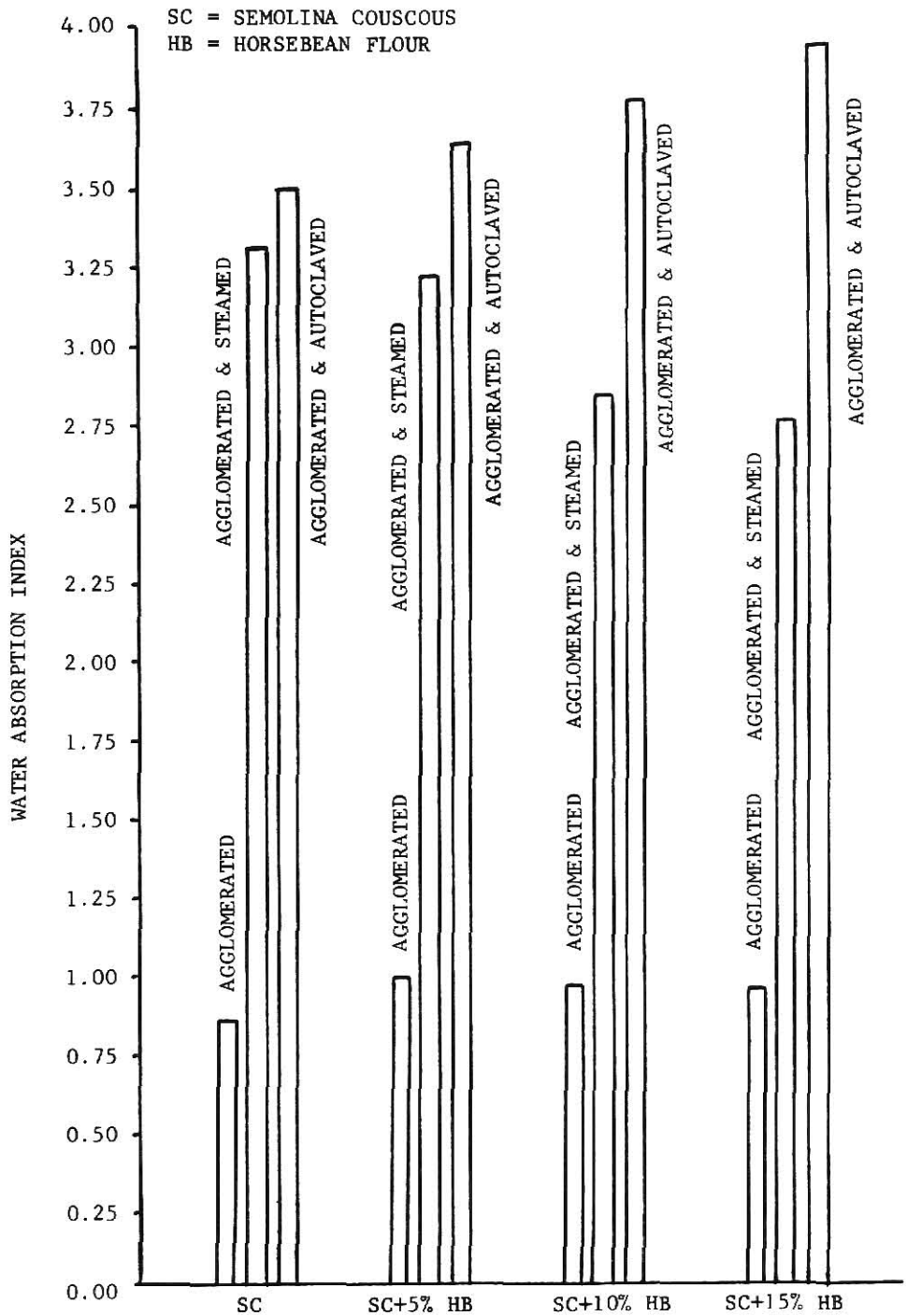

Figure 6. Graph of Water Absorption Index (WAI).

autoclaved, the WSI value was decreased to 3.94%. Supplementation of agglomerated semolina couscous with horsebean flour at levels of 5, 10, and 15%, increased the WSI value to 9.40, 10.00, and 10.90% respectively. The agglomerated and steamed couscous, when supplemented in the same manner, had WSI value of 6.00, 7.14, and 8.91%, respectively. Supplementation at the above three levels with horsebean flour also increased the WSI values for semolina couscous which was agglomerated and autoclaved to 3.20, 4.40, and 5.13%, respectively. The results are shown in Figure 7.

The method of processing the couscous had a definite effect on the Water Solubility Index. Steaming agglomerated couscous lowered the WSI value as compared to agglomerated couscous. When the couscous was agglomerated and then autoclaved, the WSI value was lowered greatly. In general, the degree of supplementation with couscous was directly related to the WSI value. The severity of the treatment tended to decrease the WSI value. Agglomerated couscous which was steamed for seven minutes yielded lower WSI values than couscous which was agglomerated. When the couscous was autoclaved, the WSI values were greatly lower than those obtained for agglomerated couscous.

Amylograph Tests

The type of processing of couscous had a great effect on the Amylograph curve. Semolina couscous which was agglomerated had a final amylograph value of 235 Brabender Units (BU). When semolina couscous was agglomerated, then steamed for seven minutes, the amylograph value was increased to 340 BU. The reverse effect was observed when semolina couscous agglomerated, then autoclaved for 4 minutes. The amylograph value decreased to 55 BU. The method of processing also had a similar result when 85%-

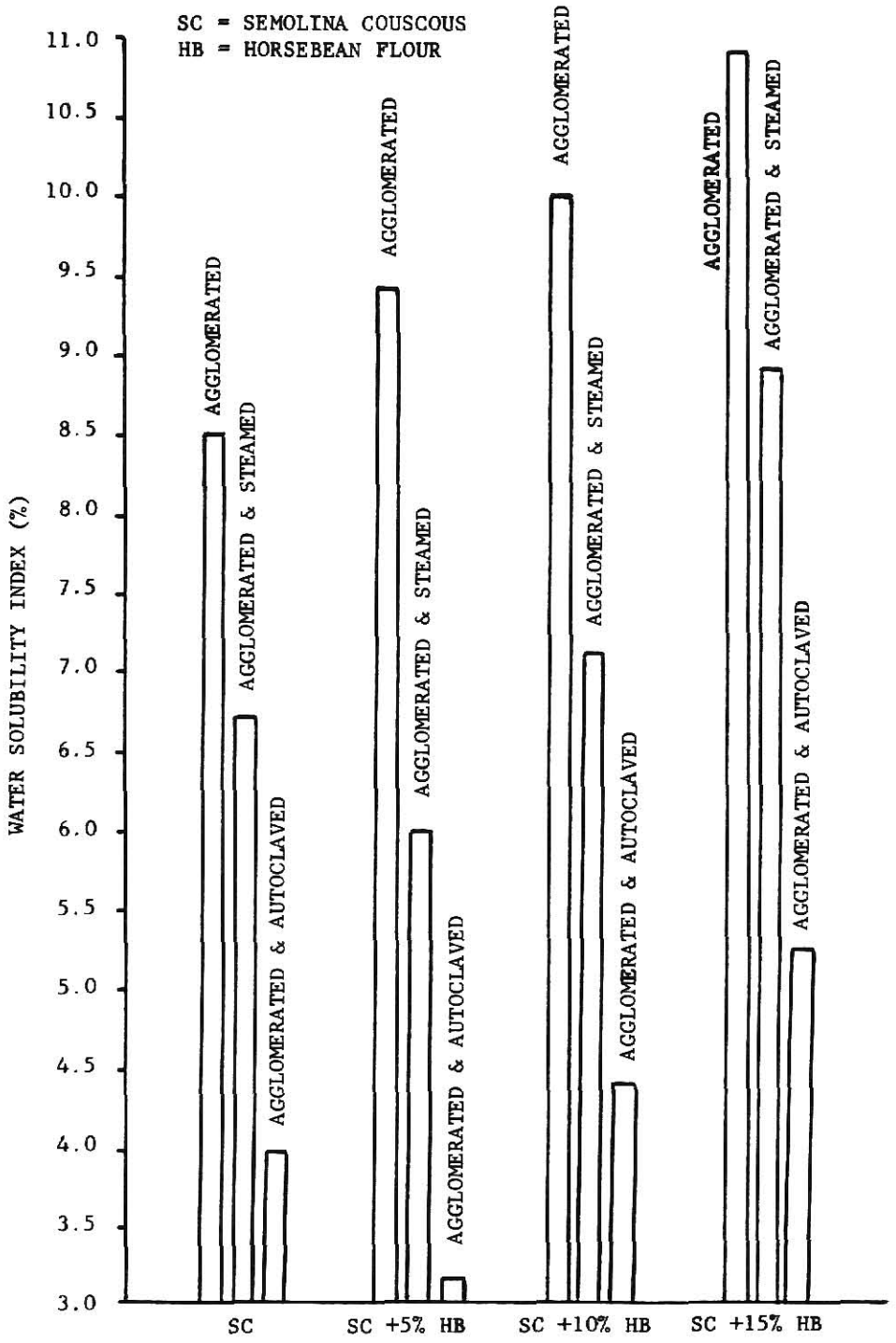

Figure 7. Graph of Water Solubility Index (WSI).

extraction couscous was tested. Agglomerated 85%-extraction couscous yielded amylograph values of 375 BU, which were higher than those observed for semolina couscous which was agglomerated or agglomerated and steamed. The steamed 85%-extraction couscous gave a high amylograph value of 460 BU. Autoclaving 85%-extraction couscous yielded lower values, 38 BU, than the agglomerated or agglomerated and steamed samples of 85%-extraction couscous. Representation of these results is shown in Figure 8.

During the autoclaving process, much of the starch in the couscous is gelatinized. Under autoclaving conditions, the structure of the starch granules is broken down. As a result, further swelling of the starch granules during the amylograph test would occur at a very low degree. The steaming process will gelatinize the starch but will not break down the granule structure. The higher amylograph values for steamed couscous over those of agglomerated couscous could be related to the larger starch granules in the steamed couscous. This would be the result of gelatinization of the starch during steaming.

Supplementation with horsebean flour had a depressing effect on the amylograph value. The addition of horsebean flour at the 5% level decreased the value from 235 to 155 BU. Further supplementation at the 10 and 15% level decreased the amylograph value to 115 and 100 BU respectively (Figure 9). Supplementation with horsebean flour at 5, 10, and 15% of semolina couscous which was agglomerated, then steamed, decreased the value from 340 to 315, 239, and 200 BU respectively. When autoclaved semolina couscous was supplemented at the above three levels with horsebean flour, the amylograph value decreased from 59, to 45, 25, and 15 BU. The results are shown in Figures 10 and 11.

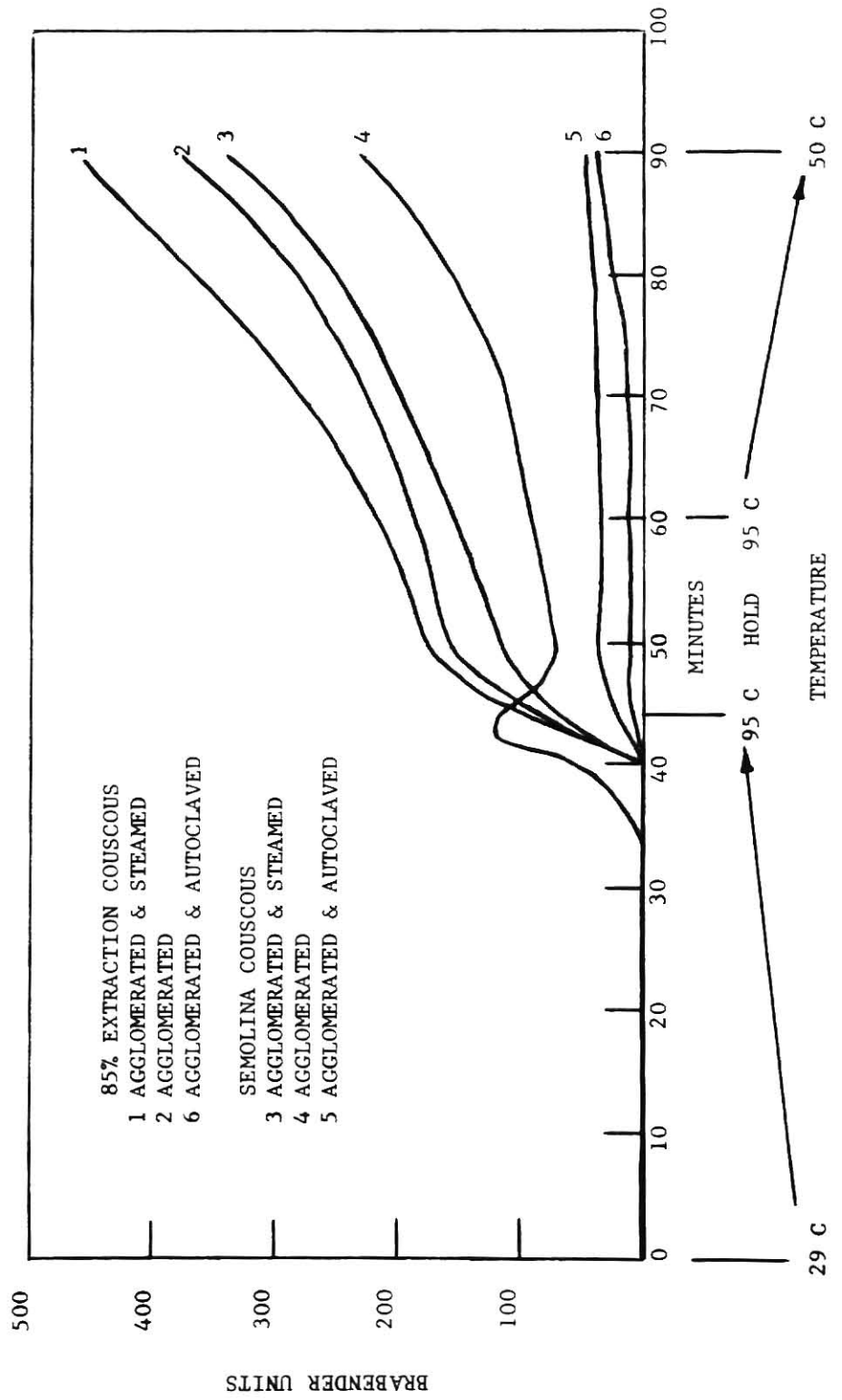

Figure 8. Amylograph curves for Semolina Couscous and 85% Extraction Couscous.

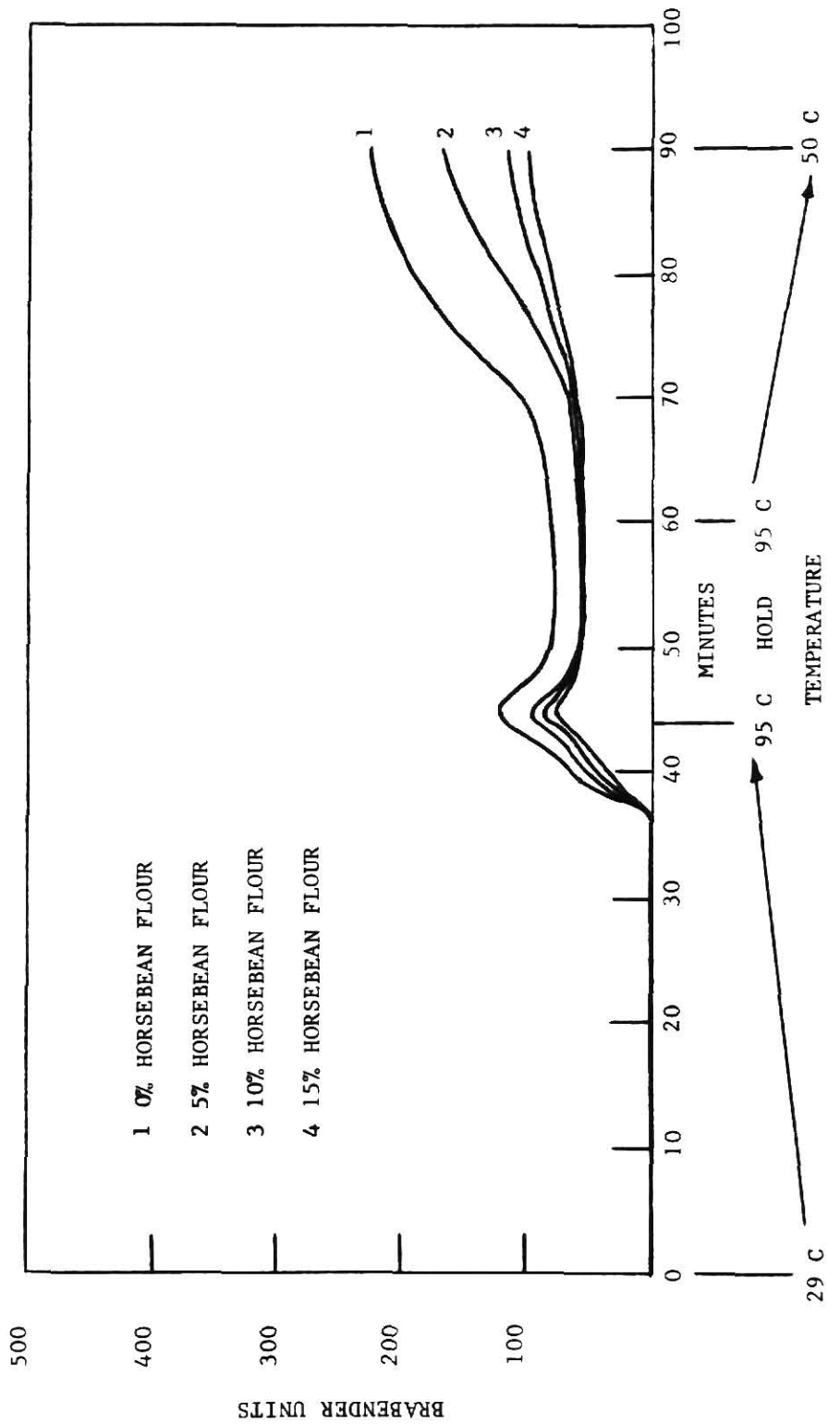

Figure 9. Amylograph curves for Semolina Couscous which was agglomerated and supplemented at three different levels with Horsebean Flour.

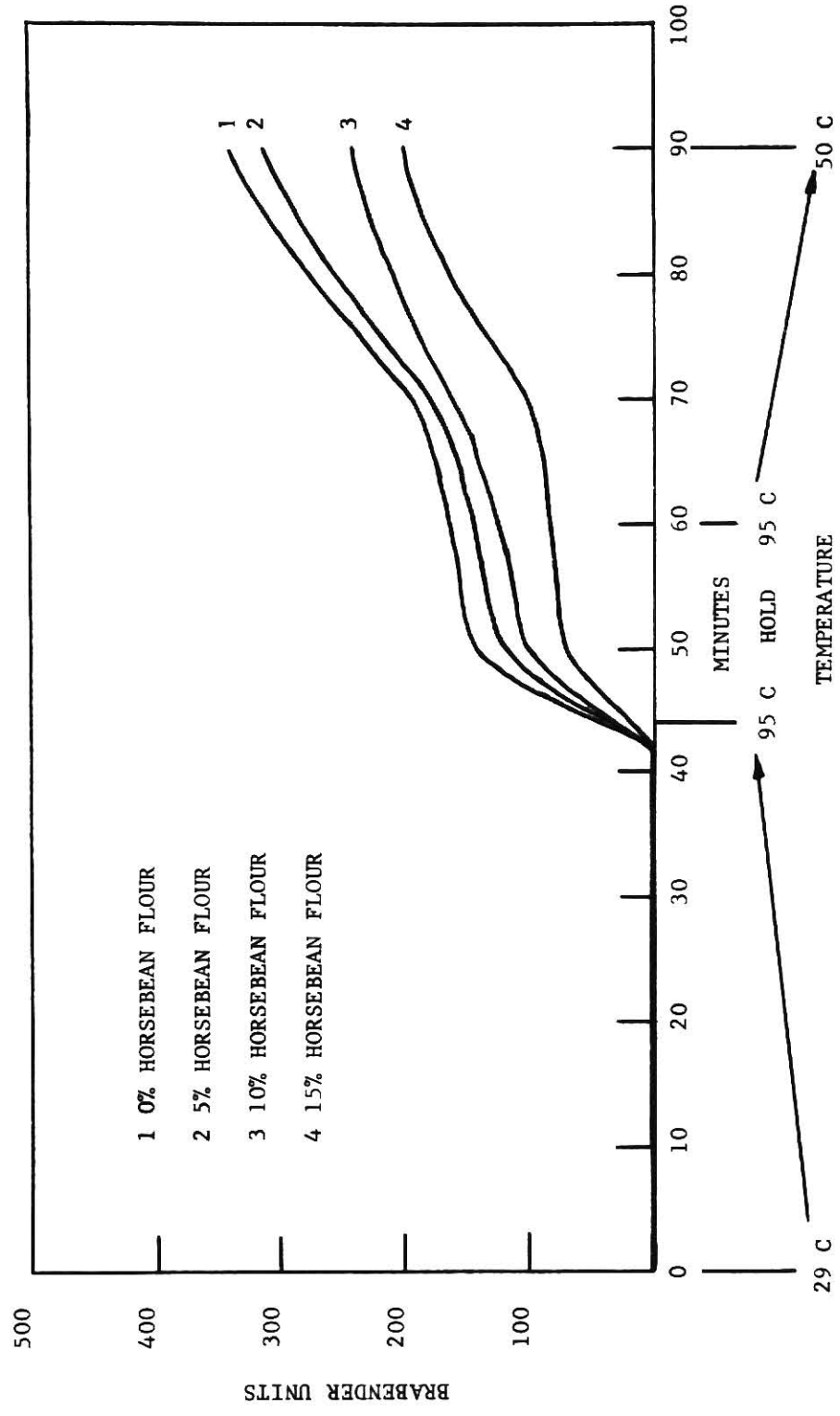

Figure 10. Amylograph curves for Semolina Couscous which was agglomerated and steamed, and supplemented at three different levels with Horsebean Flour.

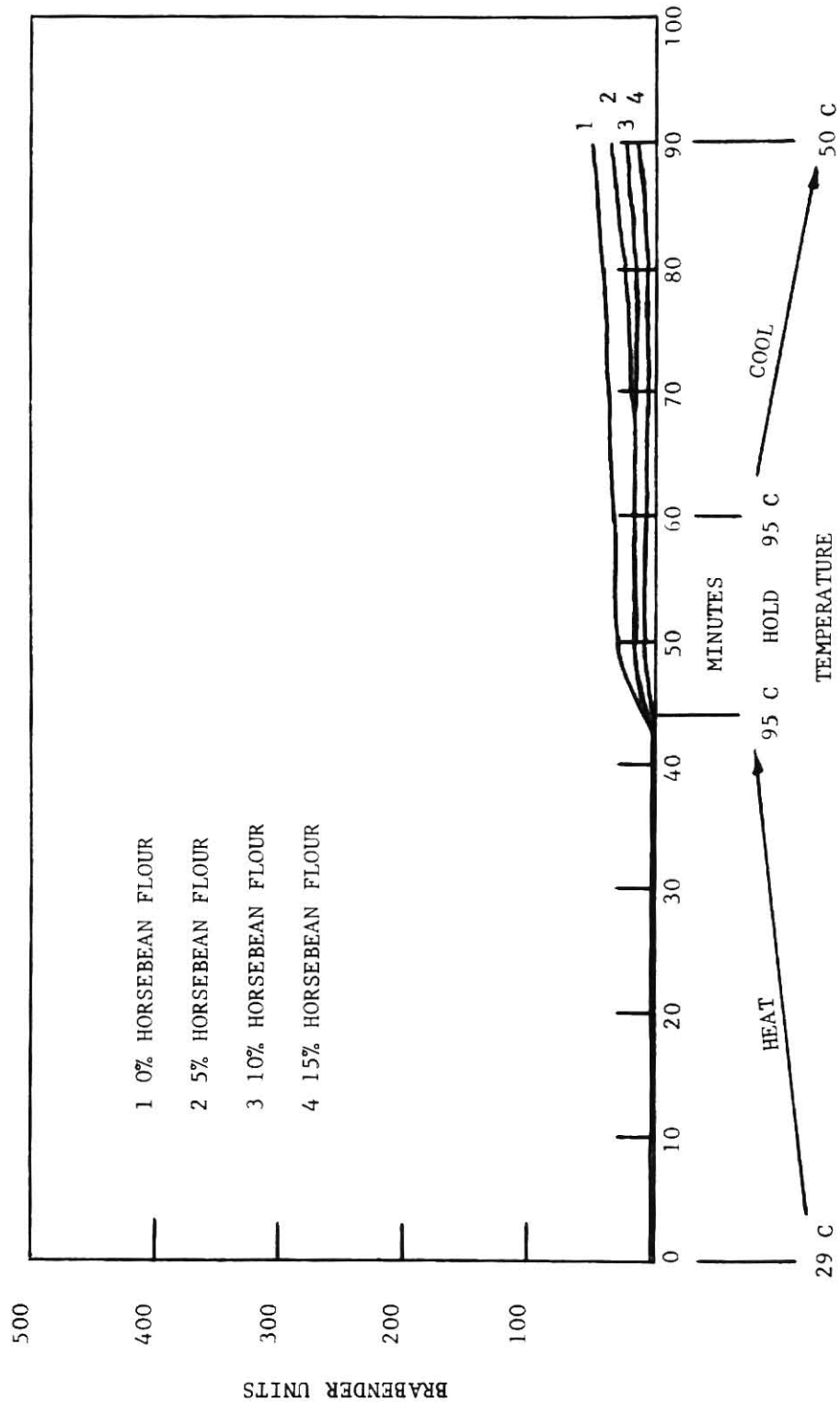

Figure 11. Amylograph curves for Semolina Couscous which was agglomerated and autoclaved, and supplemented at three different levels with Horsebean Flour.

Horsebean flour had a similar effect on 85%-extraction couscous. Supplementation with horsebean flour at the 10% level decreased the amylograph value of agglomerated 85%-extraction couscous from 375 to 300 BU, and the values of agglomerated and steamed couscous from 460 to 360 BU (Figure 12 and 13).

When, the percentage of horsebean flour supplementation was increased, the total percent of couscous starch in the slurry decreased. This factor could be responsible for the lower amylograph values and lower increase in viscosity exhibited by the supplemented couscous.

Statistical Analysis
Rat Feeding Study I

The data for average weekly weight gain, average weekly consumption, and feed efficiency by rats fed the nine different experimental diets were analyzed statistically. Tables 11 to 14 contain the results of analysis of variance, and values for the least significant differences (LSD) for average weekly weight gain and average weekly consumption. Table 15 contains the values for the least significant differences (LSD) for feed efficiency. The protein levels of the nine blended experimental diets ranged from 10.8 to 12.6% with the control diet the lowest and diets four and five being the highest.

The means, variances, and standard deviations were determined for the average weekly weight gain, and average weekly consumption. Bartlett's test for homogeneity of variance showed significant inequalities between the variances obtained for average weekly weight gain and average weekly consumption. These inequalities violated the assumption that all variances were equal and indicated that the treatment could affect the variance. The least significant differences (LSD) for the average weekly weight gain,

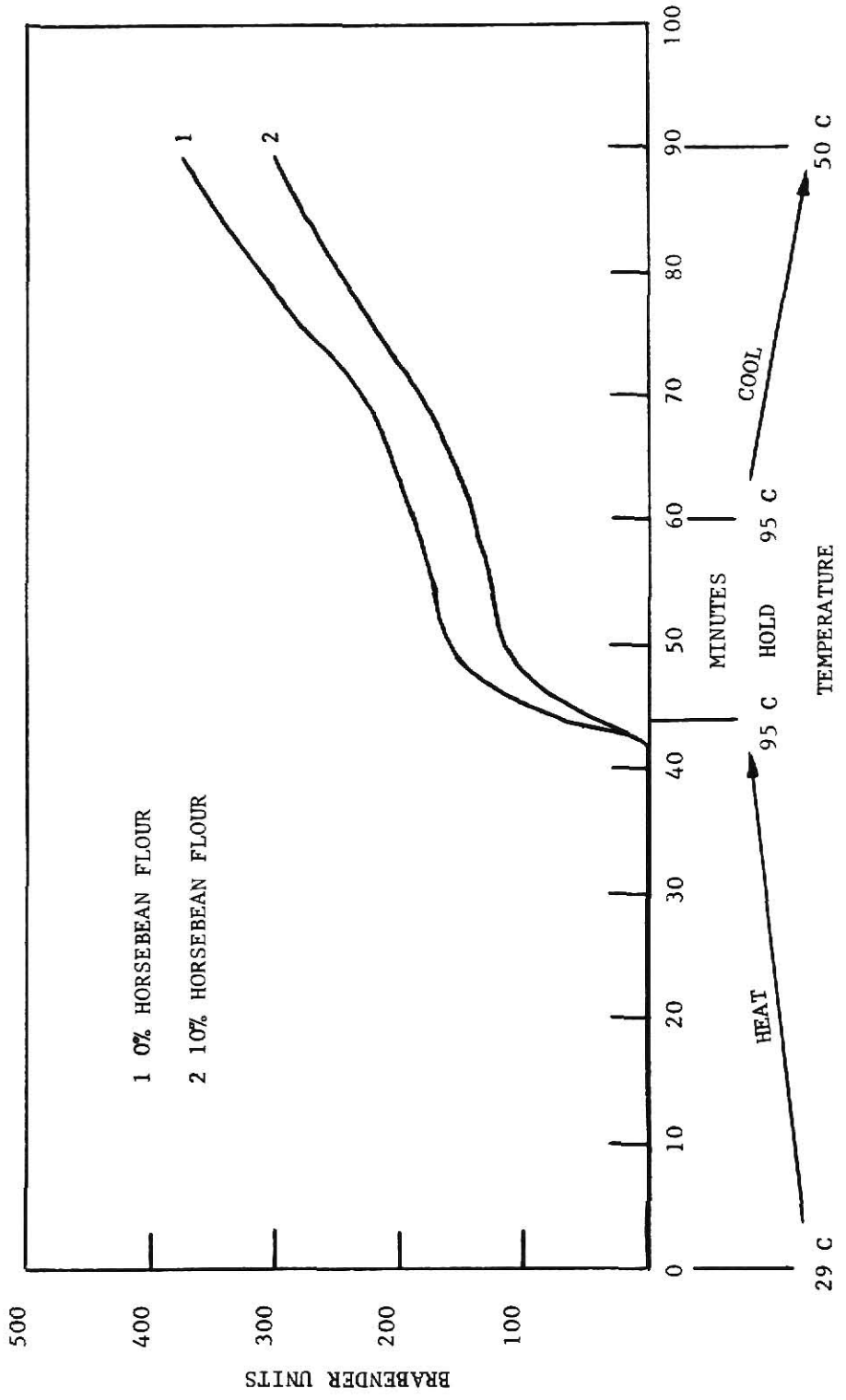

Figure 12. Amylograph curves for 85% Extraction Couscous which was agglomerated and supplemented with Horsebean Flour.

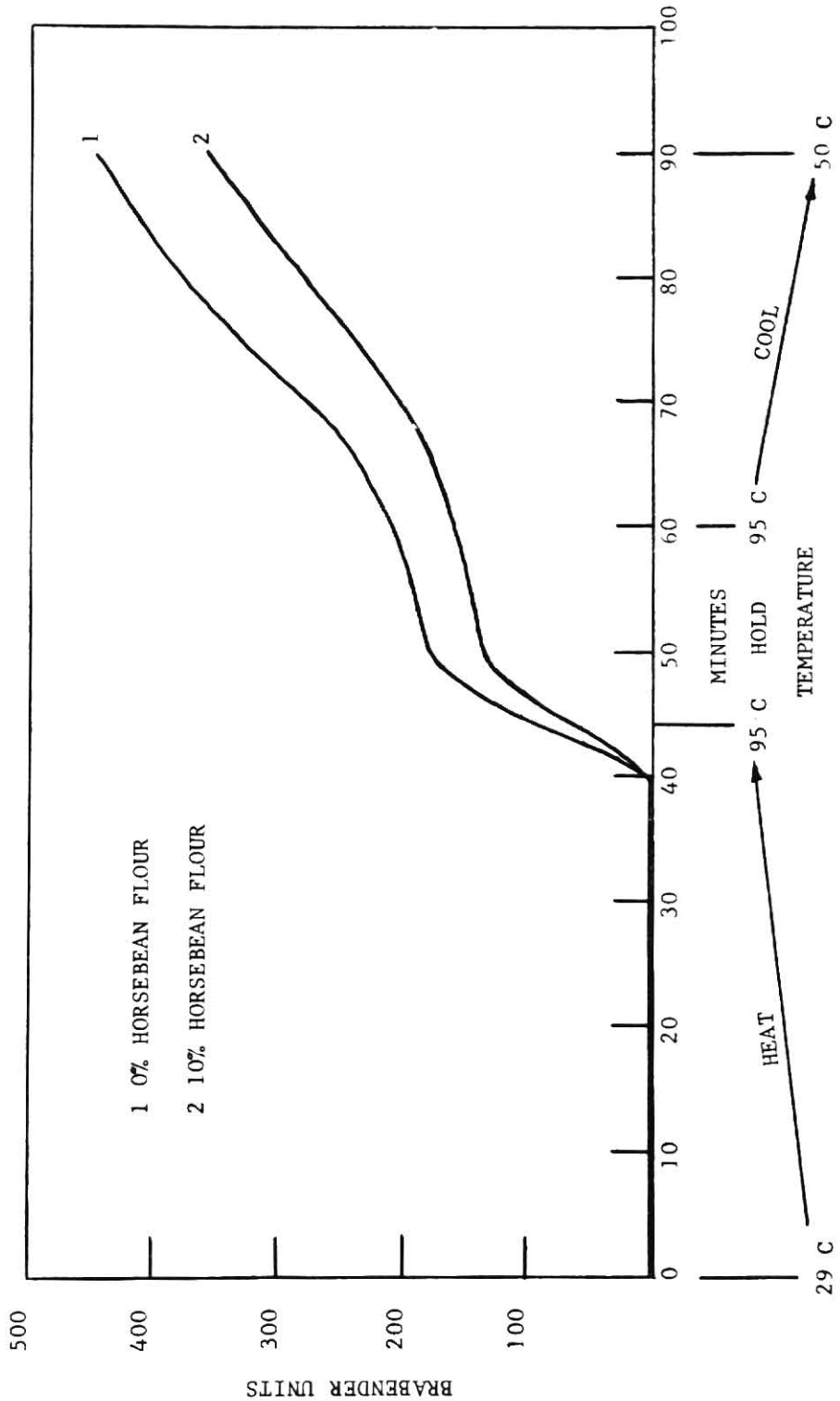

Figure 13. Amylograph curves for 85% Extraction Couscous which was agglomerated and steamed, and supplemented with Horsebean Flour.

average weekly consumption, and feed efficiency were calculated to determine which means were unequal. The diets are listed in an ordered array and the diet numbers which lie above the horizontal lines are not significantly different.

Table 11. Analysis for Average Weekly Weight Gain by Rats Fed 9 Different Blended Diets.

Level	Sample Size	Means	Variances	Standard Deviations
1	8	40.6	12.2	3.49
2	8	7.3	1.1	1.06
3	8	9.6	10.7	3.27
4	8	29.9	549.3	23.44
5	8	22.9	11.4	3.87
6	8	12.4	8.4	2.90
7	8	16.8	10.7	3.27
8	8	34.7	29.2	5.40
9	8	31.9	16.1	4.01

Bartlett's Test of Homogeneity of Variance

Chi-Square = 93.848 D.F. = 8 X^2_8 (0.05) = 15.507

Table 12. Analysis of Variance for Average Weekly Weight Gain by Rats Fed 9 Different Experimental Diets.

Source	D.F.	S.S.	M.S.	F-Test	Prob
Treatments	8	9216.219	1152.027	15.972	0.0000
Error	63	4543.984	72.127		
Total	71	13760.203			

LSD = 8.486

Diet No.	1	8	9	4	5	7	6	3	2

Table 13. Analysis for Average Weekly Consumption of Feed by Rats Fed 9 Different Blended Diets.

Level	Sample Size	Means	Variations	Standard Deviations
1	8	126.6	101.6	10.08
2	8	57.4	472.8	21.74
3	8	70.4	328.0	18.11
4	8	205.6	99862.0	316.01
5	8	92.3	75.4	8.68
6	8	106.8	149.5	12.23
7	8	84.0	764.6	24.65
8	8	119.8	37.2	6.10
9	8	123.2	189.0	13.75

Bartlett's Test of Homogeneity of Variance

Chi-Square = 206.794 D.F. = 8 $X^2_8 (0.05) = 15.507$

Table 14. Analysis of Variance for Average Weekly Feed Consumption By Rats Fed 9 Different Experimental Diets.

Sources	D.F.	S.S.	M.S.	F-Test	Prob.
Treatments	8	120078.875	15009.859	1.324	0.000
Errors	63	713861.875	11331.141		
Total	71	833970.750			

LSD = 106.448

Diet No.	4	1	9	8	6	5	7	3	2

Table 15. Analysis of Variance for Feed Efficiency of Rats Fed 9 Different Blended Diets.

Sources	D.F.	S.S.	M.S.	F-Test	Prob
Treatments	8	351.882	43.985	36.928	0.0000
Error	63	75.039	1.191		
Total	71	426.921			

LSD = 1.080

Diet No.	6	2	3	7	4	5	9	8	1

The average weekly weight gain (Table 12) was highest for Diet 1, the casein control diet, and lowest for Diet 2, which contained semolina as the source of protein. At the 5% level, there was no significant difference between Diet 2, and Diet 3 which contained semolina couscous and Diet 6 which contained 85% extraction flour. Diet 7, which contained 85%-extraction couscous was significantly higher in average weekly weight gain than Diet 2, but not significant from Diet 3, 6, and 5 which contained semolina couscous with horsebean flour. Diet 5, was not significantly lower then Diet 4 which contained semolina with horsebean flour, but at the 5% level, was significantly lower than Diets 1, 8, and 9, which contained casein, 85% extraction flour with horsebean flour, and 85%-extraction couscous with horsebean flour respectively. There was no significance between Diets 8, 9, and 4. Diet 1 was not significantly higher than Diet 8. At 10.8% protein, the weight gain by rats fed Diet 1 was significantly higher than the gain by rats fed Diets 4 and 5 which had 12.6% protein. Supplementation with horsebean flour caused a significant increase in average weekly weight gain.

The average weekly consumption of feed (Table 14) by rats was not necessarily the highest by the those being fed the diets which gave the greatest average weekly weight gain. Rats which were fed Diet 2 had the lowest consumption of feed, and those fed Diet 4 had the most. The average weekly consumption by rats fed Diets 4, 1, 9, 8, and 6 were not significantly different, with Diet 4 giving the higher feed consumption and Diet 6 the lowest. There was also no significant difference between the average weekly consumption by rats fed Diets 1, 9, 8, 6, 5, 7, 3, and 2, with Diet 8 being the higher and Diet 2 yielding the lowest feed consumption. Rats which were fed Diets 4, 1, 9, 8, and 6 gave significantly higher average weekly consumptions than the rats which were fed Diets 5, 7, 3, and 2.

The feed efficiency (FE) was calculated by dividing the weight gained into the feed consumption (Table 15). A feed efficiency value of 1 would represent a perfect conversion, whereas a high FE value would represent a poorer conversion. The most desirable feed efficiencies were produced by Diets 1, 8, and 9 with no significant differences between them at the 5% level. Diet 1 gave the most efficient conversion. The most undesirable conversion feed efficiencies were given by Diets 6, and 2 with Diet 6 yielding the most undesirable FE value. There was a significant difference between Diets 2, 3, and 7; 7, 5, and 1. There were no significant differences between the diets which were supplemented with horsebean flour, with the diets being significantly more desirable than the diets with no horsebean flour.

The protein efficiency ratios (PER) were analyzes statistically separate from the other data, and results are shown in Table 16. The effect of horsebean flour supplementation and processing on PER is shown in Table 17. The results from individual, one degree of freedom comparisons showed that supplementation with horsebean flour generally increased the PER by a factor of one-half, 85% extraction flour produced higher PER than semolina, and Diet 1, which contained casein as the source of protein produced the highest PER.

Table 16. Protein Efficiency Ratio (PER) for Rats Fed 9 Different Blended Diets.

Diet	Average PER
1	2.979
8	2.814
9	2.192
4	1.720
5	1.708
7	1.566
6	1.454
3	1.177
2	0.967

Table 17. Effect of Horsebean Flour and Processing (Couscous) on PER.

Treatment	Horsebean flour	No Horsebean flour	Means
Processing	1.950	1.371	1.660
Raw	2.017	1.211	1.614
Means	1.983	1.291	

During the latter stages (third week) of the rat feeding study, hair loss was observed in several of the rats. A mild case of hair loss consisted of a samll, bare patch across the belly and is shown in Figure 15. The control is shown in Figure 14. As the hair loss increased, the bareness would spread over the entire underside as far forward as the throat and also to the thighs and legs (Figures 16 and 17). In the more severe cases, the rats would retain a strip of hair along the center back between the two bare thighs (refer to Figures 18 and 19). There was a large variation in hair loss within each dietary group of rats. The group of rats fed Diet 1, casein, had no hair loss in any animal; group 2 had no hair loss with the exception of one rat which had extreme hair loss. Group 3 showed all degrees of hair loss, while group 4 had 2 rats showing hair loss, one extreme, and one moderate. Group 5 had 3 rats showing hair loss, two of which were extreme. There was extreme hair loss in all eight rats in group 6, and all degrees of hair loss in group 7. Group 8 and 9 had several with mild hair loss.

Three rats, one each from groups 1, 2, and 5, and examined for parasites by the Department of Parasitology at Kansas State University. The examination showed no evidence of mites or pinworms. Due to the randomness in the occurance and severity of hairloss among the diets, no correlation

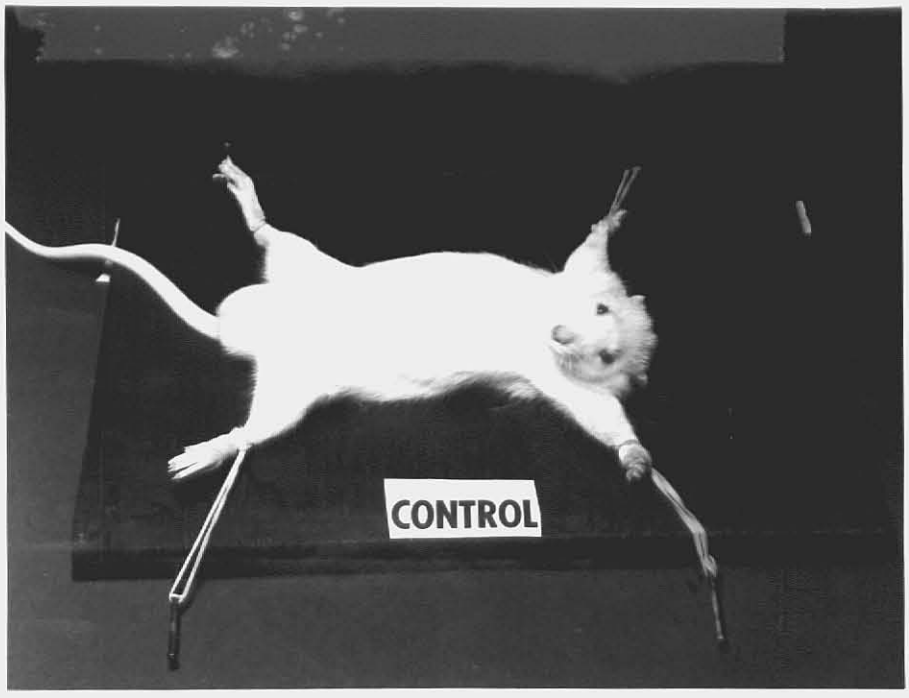

Figure 14. Control rat
showing no hair loss.

Figure 15. Rat 7 from Diet 8
showing mild hair loss across belly.

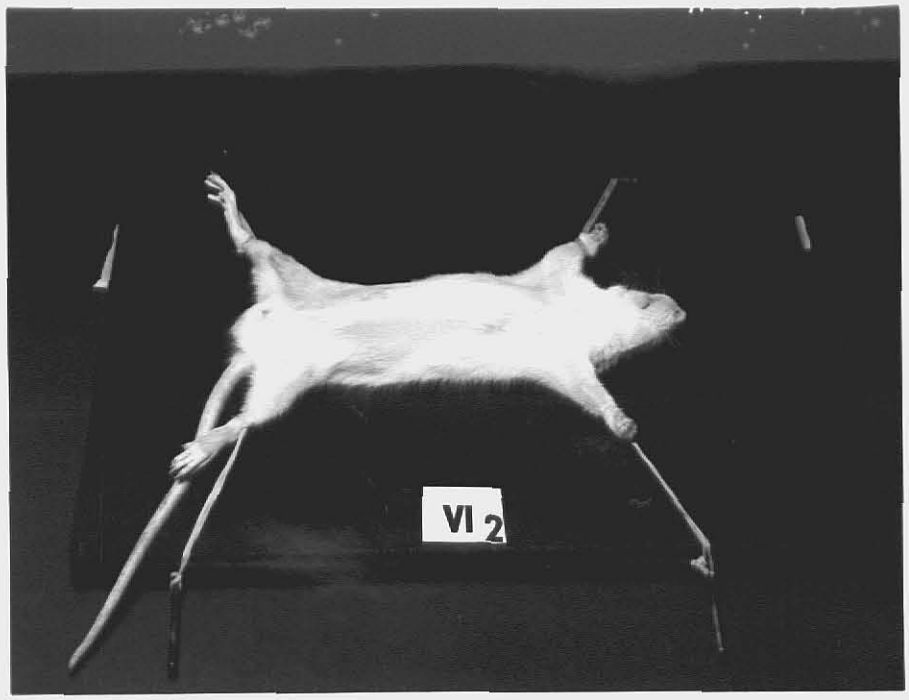

Figure 16. Rat 2 from Diet 6
showing hair loss extending from the belly.

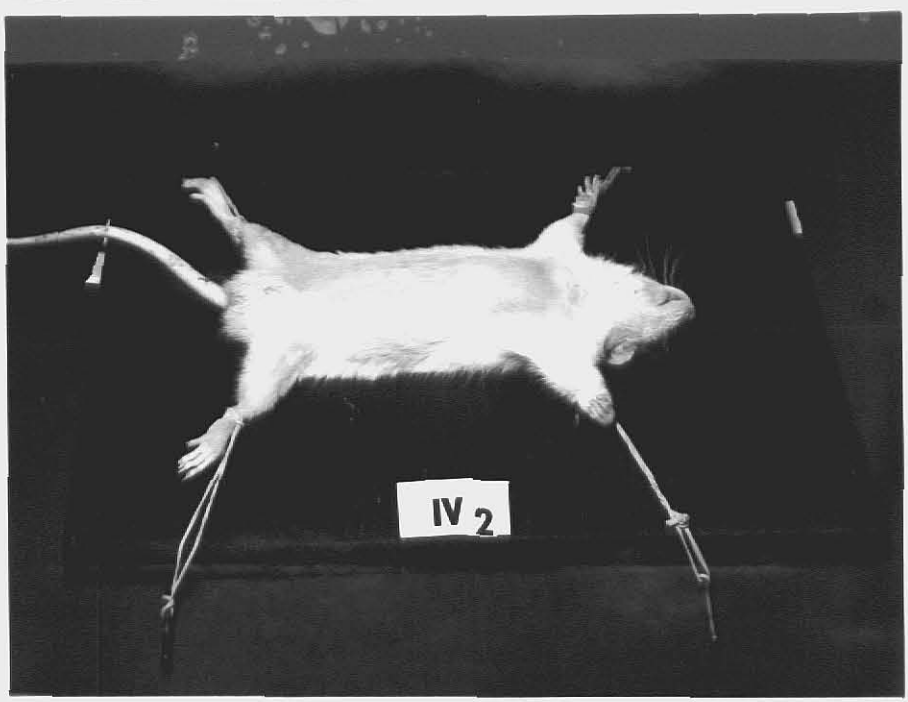

Figure 17. Rat 2 from Diet 4 showing expansion of hair loss to chest and thighs.

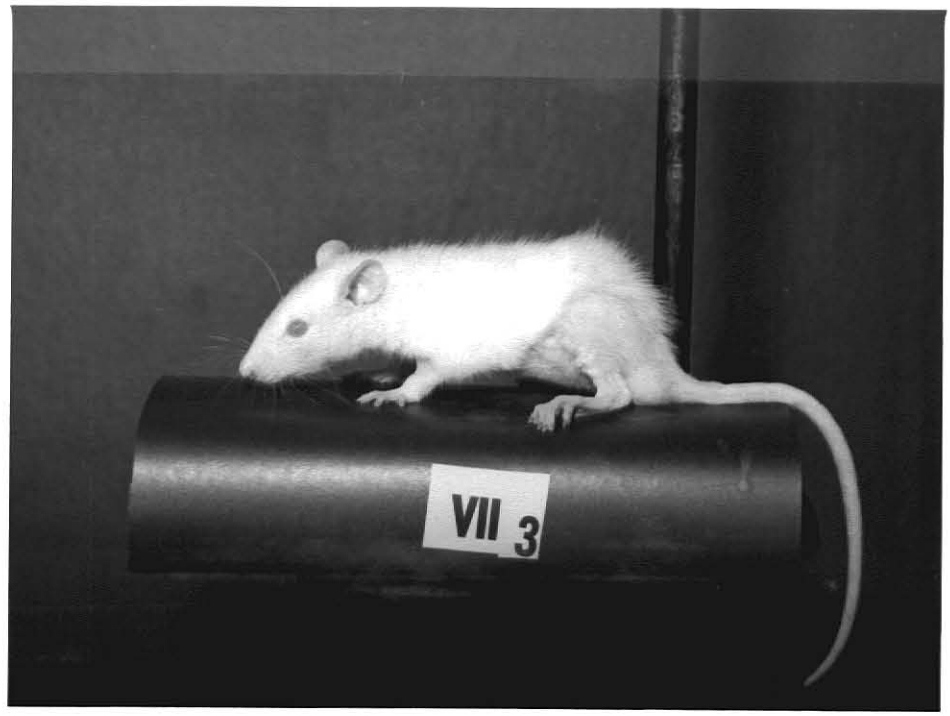

Figure 18. Rat 3 from Diet 7 showing expansion of hair loss to entire thigh.

Figure 19. Rat 7 from Diet 5 showing expansion of hair loss to the back.

was found between hair loss and the experimental diets.

Statistical Analysis
Rat Feeding Study II

The statistical analysis of Rat Feeding Study II was conducted in the same manner as in Study I.

Tables 18 to 21 contain the results of analysis, one way analysis of variance, and values for the least significant differences (LSD) for average weekly weight gain and average weekly consumption. Tables 22 and 23 contain the values for the least significant differences (LSD) for feed efficiency and protein efficiency ratio (PER).

Diet 1, the casein control at 10% protein gave the highest average weekly weight gain (Table 19) and was significantly different from all other diets. Diet 10 which contained semolina couscous at 18% protein gave the lowest. With the exception of Diet 3, semolina couscous, the 10% protein series yielded higher average weekly weight gains than the 18% protein series. Diet 6, semolina couscous with 15% horsebean flour was significantly higher than Diet 5, which contained semolina couscous with 10% horsebean flour. Neither of these diets was significantly different from Diet 7, which contained semolina couscous with lysine. There were no significant differences among the equivalent diets, Diets 12 to 14, in the 18% protein series. Diet 4, semolina couscous with 5% horsebean flour, was not significantly different from Diet 5, but was significantly lower in average weekly weight gain than Diet 7 and higher than Diet 3 which contained semolina couscous and Diet 2 which contained semolina. In the 18% protein series, Diet 11, semolina couscous with 5% horsebean flour, was significantly higher than Diet 9 and 10 which contained semolina and semolina couscous respectively. Diets 14, 13, and 12, which contained

Table 18. Analysis for Average Weekly Weight Gain by Rats Fed 14 Blended Experimental diets.

Level	Sample Size	Means	Variations	Standard Deviations
1	7	29.2	30.3	5.507
2	8	9.0	1.7	1.296
3	8	5.0	1.5	1.232
4	8	13.3	7.6	2.751
5	8	15.2	14.4	3.792
6	8	19.2	12.7	3.558
7	7	16.8	8.2	2.864
8	8	8.6	18.6	4.318
9	7	3.8	1.6	1.280
10	8	3.5	1.0	0.986
11	8	5.4	2.8	1.663
12	8	6.9	1.3	1.141
13	8	7.4	3.0	1.737
14	7	6.5	1.7	1.318

Bartlett's Test of Homogeneity of Variance

Chi-Square = 52.801 D.F. = 13 $X^2_{8\ (0.05)} = 15.507$

Table 19. Analysis of Variance for Average Weekly Weight Gain for Rats Fed 14 Blended Experimental Diets.

Sources	D.F.	S.S.	M.S.	F-test	Prob.
Treatments	13	4994.035	384.156	51.363	0.000
Error	94	703.020	7.479		
Total	107	5697.055			

LSD = 5.471

Diet No.	1	6	7	5	4	2	8	13	12	14	11	3	9	10

Table 20. Analysis for Average Weekly Consumption of Feed by Rats Fed 14 Blended Experimental Diets.

Level	Sample Size	Means	Variations	Standard Deviations
1	7	90.7	97.7	9.884
2	8	61.3	8.3	2.877
3	8	50.2	95.2	9.756
4	8	72.2	86.2	9.287
5	8	72.5	121.5	11.022
6	8	82.5	125.2	11.189
7	7	79.4	61.4	7.888
8	8	59.7	51.4	7.169
9	7	38.8	37.7	6.144
10	8	46.2	42.6	6.525
11	8	55.9	95.4	9.768
12	8	54.6	60.6	7.787
13	8	54.0	48.1	6.935
14	7	46.2	93.0	9.645

Bartlett's Test for Homogeneity of Variance

Chi-square = 14.712 D.F. = 13 $X^2_{8\ (0.05)} = 15.507$

Table 21. Analysis of Variance for Average Weekly Consumption of Rats Fed 14 Blended Experimental Diets.

Sources	D.F.	S.S.	M.S.	F-Test	Prob.
Treatments	13	22832.008	1756.308	23.992	0.000
Error	94	6881.184	73.204		
Total	107	29713.191			

LSD = 8.560

Diet No.	1	6	7	5	4	2	8	11	12	13	3	10	14	9

Table 22. Analysis of Variance for Feed Efficiency of 14 Blended Experimental Diets.

Sources	D.F.	S.S.	M.S.	F-Test	Prob.
Treatment	13	904.500	69.577	13.807	0.000
Error	95	478.746	5.039		
Total	108	1383.246			

LSD = 2.248

Diet No.	10	9	3	11	12	8	13	14	2	4	5	7	6	1

Table 23. Analysis of Variance for Protein Efficiency Ratios of 14 Blended Experimental Diets.

Sources	D.F.	S.S.	M.S.	F-Test	Prob.
Treatment	13	96.642	7.434	155.816	0.000
Error	95	4.532	0.048		
Total	108	101.174			

LSD = 0.216

Diet No.	1	6	5	7	4	2	8	3	14	13	12	11	9	10

semolina couscous with lysine, semoline couscous with 15% horsebean flour, and semolina couscous with 10% horsebean flour; were not significantly higher than Diet 11. Supplementation with horsebean flour tended to increase the average weekly weight gain. The use of diammonium citrate to increase the level of nitrogen of the 18% series, tended to decrease the average weekly weight gain.

Average weekly feed consumption had the highest results with the casein control diet of the 10% protein series, and the lowest results with the semolina diet of the 18% protein series. Diet 1 was significantly higher than all other diets with the exception of Diet 6, which was significantly higher than Diet 5, but not higher then Diet 7. There were no significance differences between Diets 7, 5, and 4, or Diets 2, 8, 11, 12, and 13. Diet 4 was significantly higher than Diet 2. There was also no significance among Diets 11, 12, 13, and 3, or Diets 12, 13, 3, 10, and 14; or 10, 14, and 9. Horsebean flour supplementation tended to increase the average weekly feed consumption, with the 10% protein series generally higher than the 18% protein series.

The casein control diet of the 10% protein series gave the most desirable feed efficiency value, while Diet 10, containing semolina couscous, of the 18% protein series gave the most undesirable feed efficiency. There were significant differences between Diets 1 and 2, 7 and 14, 4 and 13, 2 and 11, 14 and 3, 13 and 9, and 11 and 10. The feed efficiency values showed more significant differences between the diets of the 10% protein series than the average weekly feed consumption values. Generally the diets of the 10% protein series gave more desirable FE values than those of the 18% protein series.

All diets of the 10% series were significant with relation to protein efficiency ratio (PER). Diet 1 gave the highest protein efficiency ratio whereas Diet 10 yielded the lowest. There was no significance between Diets 5 and 7; 8, 3, 14, 13, and 12; 14, 13, 12, and 11; 12, 11, and 9; and 9 and 10. Diets 8 and 3 were significantly higher than Diet 11. Diet 13 was significantly higher than Diet 9, and Diet 11 was higher than 10. Supplementation with horsebean flour tended to improve the PER. Generally the 10% protein series tended to have higher PER's than the diets of the 18% series.

Varying degrees of hair loss was observed in all of the 14 diets. The type of hair loss and the distribution of rats with hair loss among the diets was similar to those observations in Study I. The section of the feeding study involving the rats fed with Diets 1 through 7 was extended two weeks to bring the length of the study to six weeks. The purpose for the extension of the study was to see if the rats that had not shown hair loss at the end of four weeks, would have hair loss at the end of five or six weeks. The rats that showed loss of hair in the early stages of the study (third week) were showing signs of new hair growing in at the end of four weeks. By extending the study for two extra weeks, it was hoped that the new hair would have time to grow.

The data collected at the end of the sixth week were analyzed for feed efficiency (FE) and protein efficiency ratio (PER) by the same method used for the data collected at the end of four weeks and is given to Tables 24 and 25.

At the end of six weeks Diet 1 gave the most desirable feed efficiency with Diet 3 yielding the most undesirable value. Diet 1 was significantly higher in feed efficiency than Diet 6, whereas in the data taken at the end

Table 24. Analysis of Variance for Feed Efficiency by Rats Fed 7 Different Experimental Diets for 6 Weeks.

Sources	D.F.	S.S.	M.S.	F-Test	Prob.
Treatment	6	432.708	72.118	41.330	0.000
Error	46	80.268	1.745		
Total	52	512.976			

LSD = 1.318

Diet No.	3	2	4	5	7	6	1

Table 25. Analysis of Variance for Protein Efficiency Ratio by Rats Fed 7 Different Experimental Diets for 6 Weeks.

Sources	D.F.	S.S.	M.S.	F-Test	Prob.
Treatments	6	42.863	7.144	154.196	0.000
Error	47	2.177	0.046		
Total	53	45.041			

LSD = 0.215

Diet No.	1	6	7	5	4	2	3

of four weeks, there was no significance between these diets. The ralationship of the other diets to one another was unchanged from the results obtained at the end of four weeks.

The casein control, Diet 1, gave the highest protein efficiency ratio while Diet 3 gave the lowest. At the end of four weeks, all of the diets in the 10% protein series had been significantly different with the exception of Diet 5 and 7. The significance was decreased at the end of six weeks. The PERs of diets 6, 7, and 5, and 7, 5, and 4 did not have significant differences between them. Diet 1 remained significantly higher in PER than the other diets, and Diets 2 and 3 remained significantly lower. The extension of the rat feeding study two weeks tended to increase the significance between feed efficiency values and decrease the significance between protein efficiency ratios.

Hair loss continued to occur at the end of the sixth week of the feeding study. Rats from all of the dietary groups continued to lose hair, while some rats from each diet showed signs that the new growth of hair was permanent. The distribution of the rats showing hair loss and those showing hair loss and then hair gain, was in a manner that could not be related to the individual diets.

The rats fed Diets 8 to 14, the 18% protein series, showed lower average weekly weight gains, average weekly feed consumption, protein efficiency ratios, and higher feed efficiency values, than the rats fed the 10% protein diets, 1 to 7. Eighteen percent protein is considered optimum for the growth of rats, but the data from Study II did not support this theory. The 18% protein diets contained diammonium citrate which may have influenced the acceptability of the diets to the rats.

SUMMARY AND CONCLUSIONS

The purpose of this study was to investigate the effects that legume supplementation had on some nutritional and physical characteristics of couscous. Different levels of horsebean flour supplementation were investigated for nutritional value.

The couscous which was made from the flour of durum wheat, semolina or 85% extraction flour, was agglomerated with water in 3000 gram batches. Following the agglomeration, the particles were sieved to separate and size. The overs of the 8 wire were considered too large and were discarded. The overs of the 12 wire were designated as large to medium couscous, and the overs of the 18 wire were considered fine couscous. The throughs of the 18 wire were considered too fine in size and were reagglomerated.

The agglomerated particles were then processed in one of three ways. They were dried at 50 C for 20 hours, or steamed 7 minutes and then dried at 50 C for 20 hours, or autoclaved 4 minutes and then dried.

In order to determine the ability of the couscous to support growth and to compare the nutritional qualities of supplemented autoclaved couscous, two nutritional studies using male albino rats were executed. From the two studies, which were continued for 28 days, the average weekly weight gain, average weekly feed consumption, feed efficiency, and protein efficiency ratio were calculated. The 10% protein series of Study II was extended to 42 days.

Study I assayed 9 blended diets at a 10% protein level. The diets consisted of semolina and 85% extraction flours and couscous prepared from these flours. These ingredients were also supplemented at the 10% level with horsebean flour. The diets also contained starch, oil, vitamins and minerals. The diets of Study I are listed below.

Diet No.	Major Source of Protein
1	Casein (vitamin free)
2	Semolina
3	Semolina couscous
4	Semolina with horsebean flour (10%)
5	Semolina couscous with horsebean flour (10%)
6	85% extraction durum flour
7	85%-extraction couscous
8	85% extraction durum flour with horsebean flour (10%)
9	85%-extraction couscous with horsebean flour (10%)

All of the diets of Study I supported growth and development of the rats. With the exception of hair loss observed in many cases, the rats grew in a satisfactory manner.

Generally, the rats fed with the 85% extraction diets produced higher weight gains, and higher protein efficiency ratios than the rats with the semolina flour. The average weekly feed consumption was also higher for the rats fed the 85% extraction diets. There were slight differences in feed efficiency between the two kinds of diets.

With the exception of Diet 1, the casein control, Diet 8, 85% extraction flour with 10% horsebean flour, appeared to support the growth and development of the rats better than the other diets. Diet 9, 85%-extraction couscous with 10% horsebean flour, was not significantly different from Diet 8 in nutritional quality, but was lower. The poorest diet was Diet 2, which contained semolina.

Study II contained 14 diets, 7 at 10% protein and 7 at 18% protein. Semolina and semolina couscous were used. The autoclaved couscous was supplemented at 5, 10, and 15% with horsebean flour. Two of the couscous diets were supplemented with lysine at a level equivalent to that of 10% horsebean flour. Diammonium citrate was used to increase the nitrogen level of the diets in the 18% protein series to 18% protein. The diets of Study II are listed below.

10% Protein Series

Diet No.	Major Source of Protein
1	Casein (vitamin free)
2	Semolina
3	Semolina couscous
4	Semolina couscous with horsebean flour (5%)
5	Semolina couscous with horsebean flour (10%)
6	Semolina couscous with horsebean flour (15%)
7	Semolina couscous with lysine

18% Protein Series

Diet No.	Major Source of Protein
8	Casein (vitamin free)
9	Semolina
10	Semolina couscous
11	Semolina couscous with horsebean flour (5%)
12	Semolina couscous with horsebean flour (10%)
13	Semolina couscous with horsebean flour (15%)
14	Semolina couscous with lysine

As in Study I, the diets of Study II supported growth and development of the rats. Hairloss was also observed in the same manner as in Study I. In general, the rats fed diets from the 10% protein series showed higher weight gain, feed consumption, feed efficiency, and protein efficiency ratio than the rats fed from diets of the 18% protein series.

Diet 6, semolina couscous with horsebean flour at a level of 15% in the 10% protein series, supported the growth and development of the rats better than the other diets, with the exception of the casein control, Diet 1. Diet 7, which contained semolina couscous with lysine, was significantly lower in protein efficiency ratio than Diet 6, but not significantly different with relation to weight gain, feed consumption, and feed efficiency. Diet 3, containing semolina couscous as a source of protein, was the poorest of the diets in the 10% protein series. Diet 10, of the 18% protein series and containing semolina couscous, generally yielded the poorest results of all the diets in

Study II.

In Study I, the supplementation of the flour and couscous diets with horsebean flour improved the diets ability, as a source of protein, to support the growth and development of the rats. By increasing the quantity and quality of the protein through the addition of horsebean flour, the limiting effect of lysine on utilization of the wheat protein is diminished. The 85% extraction flour constitutes a greater percentage of the wheat kernel than semolina. Since the endosperm represents approximately 83% of the kernel, the 85% extraction flour contains a small percentage of bran, which is rich in vitamins, and would yield a higher nutritional value than the lower extraction semolina. Generally, the processing of the flour into couscous increased the nutritional value. This could be due to the destruction of enzyme inhibitors. The processing of couscous also decreased the effect of horsebean flour supplementation as compared to the effect of supplementation of unprocessed flour. The results of heating horsebean flour may be related to the reaction of protein with carbohydrates, leading to the destruction of amino acids and an alteration in the digestibility of the protein by proteolytic enzymes. The reaction of protein in the absence of carbohydrate, which results in a decrease in the availability of some amino acids, may also affect the horsebean flour (42).

In Study II, diets supplemented at levels of 10 and 15% yielded results which compared more favorably with the casein control than the other diets. In contrast to Study I, the processing of the flour into couscous decreased the nutritional value. The nutritional value of the diets increased with increased supplementation with horsebean flour. The diets of the 18% protein series of Study II, which contained diammonium citrate, yielded results that were inferior to those of the 10% protein series. The diammonium citrate gave a very bitter and unpleasant taste and aroma to the diets that resulted in

their rejection by the rats. In future studies of this nature, diammonium citrate should not be used to increase the nitrogen level of the diet.

Based on the results of the rat feeding studies, it can be concluded that supplementation of couscous with horsebean flour can have positive nutritional qualities. In some instances the processing of the flour into couscous decreased the nutritional value, but this was compensated for by the increase in nutritional value resulting from the horsebean flour supplementation.

The method of processing and level of supplementation affected the Water Absorption Index (WAI) and Water Solubility Index (WSI). The suspension was then centrifuged, with the remaining gel used to calculate the WAI. The supernatant from the centrifuging was evaporated and the percentage of dry solids remaining was used to calculate the WSI.

Couscous which was agglomerated, then steamed or autoclaved had higher WAI values than couscous which was only agglomerated. Horsebean flour supplementation had little effect on agglomerated couscous, but tended to decrease the WAI value of the steamed couscous and increase the WAI value of autoclaved smaples. The degree of supplementation generally increased the solubility index. These results may be due to the degree of gelatinization of the starch in the samples.

Amylograph studies were conducted to study the gelatinization of couscous processed in the three different manner. The studies showed that couscous which was agglomerated and then steamed, yielded higher amylograph values than agglomerated or agglomerated and autoclaved samples. The agglomerated and autoclaved couscous yielded results that were much lower than those of agglomerated couscous. Generally supplementation with horsebean flour tended

to decrease the amylograph values.

The WAI, WSI, and amylograph tests could be of importance in the commercial preparation of couscous. Since couscous is an agglomeration product, tests to determine absorption and solubility would be of importance in maintaining the quality control of couscous. The absorption and solubility characteristics of couscous are affected by the degree of gelatinization incurred during processing. The ability to make a uniform couscous product of high quality and to determine the proper method of preparation of couscous for consumption will depend on the proper knowledge of these factors. These three tests are reliable methods for determining the absorption, solubility, and degree of gelatinization of couscous.

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THE EFFECT OF LEGUME SUPPLEMENTATION ON SOME NUTRITIONAL AND PHYSICAL CHARACTERISTICS OF COUSCOUS

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THE EFFECT OF LEGUME SUPPLEMENTATION ON SOME NUTRITIONAL AND PHYSICAL CHARACTERISTICS OF COUSCOUS

AN ABSTRACT

The purpose of this study was to investigate the effects that legume supplementation had on some nutritional and physical characteristics of couscous. Different levels of horsebean flour were investigated for nutritional value.

The couscous was made from the flour of durum wheat, semolina or 85% extraction flour, which was agglomerated with water in 3000 gram batches. Following the agglomeration, the particles were sieved to separate and size. The overs of the 8 wire were considered too large and were discarded. The overs of the 12 wire were designated as large to medium couscous, and the overs of the 18 wire were considered fine couscous. The throughs of the 18 wire were considered too fine in size and were reagglomerated.

The agglomerated particles were then processed in one of three ways. They were dried at 50 C for 20 hours, or steamed 7 minutes and then dried at 50 C for 20 hours, or autoclaved 4 minutes and then dired.

In order to determine the ability of the couscous to support growth and to compare the nutritional qualities of supplemented autoclaved couscous, nutritional studies using male albino rats were executed. Study I assayed 9 blended diets at a 10% protein level. The diets consisted of semolina and 85% extraction flours, and couscous prepared from these flours. These ingredients were also supplemented at the 10% level with horsebean flour. The diets also contained starch, oil, vitamins, and minerals.

Study II contained 14 diets, 7 at 10% protein and 7 at 18% protein. Semolina and semolina couscous were used. The autoclaved couscous was supplemented at 5, 10, and 15% with horsebean flour. Two of the couscous

diets were supplemented with lysine at a level equivalent to that of 10% horsebean flour. From the two studies, which were continued for 28 days, the average weekly weight gain, average weekly feed consumption, feed efficiency, and protein efficiency ratio were calculated. The 10% protein series of Study II was extended to 42 days.

All diets supported growth and development with significant differences between some diets. Diets supplemented with horsebean flour gave higher nutritional values than unsupplemented diets.

The method of processing and level of supplementation affected the Water Absorption Index (WAI) and the Water Solubility Index (WSI). Ground couscous samples were suspended in water at 30 C for 30 minutes. The suspension was then centrifuged, with the remaining gel used to calculate the WAI. The supernatant from the centrifuging was evaporated and the percent of dry solids remaining was used to calculate the WSI.

Couscous which was agglomerated, then steamed or autoclaved had higher WAI values than couscous which was only agglomerated. Horsebean flour supplementation had little effect on agglomerated couscous, but tended to decrease the WAI value of steamed couscous and increase the WAI value of autoclaved couscous. Couscous which was agglomerated showed a higher WSI value than the steamed or autoclaved samples. The degree of supplementation generally increased the solubility index.

Amylograph studies were conducted to study the gelatinization of couscous processed in the three different manners. The studies showed that couscous which was agglomerated then steamed yielded higher amylograph values than agglomerated or agglomerated and autoclaved samples. Generally supplementation with horsebean flour tended to decrease the amylograph values.