

ABSTRACT

EARLY GENERATION SELECTION IN TRITICALE
FOR FACTORS CONTRIBUTING TO
PROTEIN QUALITY

By

Charles Joseph Knoblauch

The feasibility of selection for desired protein efficiency indices in the early generations of a breeding program was studied with winter triticale lines. Prediction of protein efficiency indices was also attempted from various chemical analyses procedures.

Weanling vole (Microtus pennsylvanicus) bioassays of seed harvested from F_3 plants along with several F_4 single plant selections from each F_3 plant row indicate that selection for protein efficiency indices was practical as early as the F_3 generation. Although results showed that heritability was sufficiently high for effective selection, limited numbers of lines used in this study discouraged computation of heritability estimates.

Several chemical determinations of triticale meals have shown promise for predicting protein efficiency indices (PEI). The percent water-soluble protein, total trypsin units inhibited (adjusted for the percent water-soluble protein), and the extracted dye-binding capacity (indicative of lysine content after removal of water-soluble proteins) have shown the highest correlations with PEI. A group of 20 F_3 single plants were selected for widely varying PEI values

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with low standard errors. Their simple correlations were -0.71, -0.79, and 0.66, for percent water-soluble protein, total trypsin units inhibited, and extracted dye-binding capacity with PEI, respectively. The multiple correlation coefficient for the regression of extracted dye-binding capacity and total trypsin units inhibited on PEI was $r = 0.8642$ (significant at the $P < 0.01$ level), indicating that 75% of the variation in PEI was explained by the two independent variables.

A number of spring triticale lines were used to determine the predictability of PEI by the previously described chemical analyses. A group of five spring triticale lines selected to give low PEI and a group of five spring triticale lines selected to give high PEI averaged 2.03 and 3.09, respectively. Indicating that extracted DBC and total TUI can be used to select effectively for PEI. This knowledge makes it possible to use the costly bioassays only as a final selection criteria, using the chemical analyses approach to screen large numbers of lines.

EARLY GENERATION SELECTION IN TRITICALE
FOR FACTORS CONTRIBUTING TO
PROTEIN QUALITY

By

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INTRODUCTION

Undernutrition and malnutrition are currently widespread in many areas of the world. Cereals, the principal source of plant protein, supply approximately 70% of the total world protein supply. Further, it is anticipated that more and more of the world's need for protein will have to be supplied by plant proteins.

At present wheat contributes a substantial proportion of the world food supply. However, rapidly increasing populations in many countries have increased the need for higher yielding cereal grains with higher protein quality than wheat. Triticale, a cereal produced by chromosome doubling of wheat-rye F_1 's, has been suggested to meet these requirements. Knowledge of the inheritance of protein quality and the factors contributing to protein quality are very limited in this new cereal.

The purpose of this study was to (1) determine the feasibility of selection for protein quality in early segregating generations from crosses of triticale lines, (2) identify factors contributing to protein quality by chemical analyses, (3) determine the association of these factors to protein quality as determined in weanling vole growth assays, and (4) test the relationship of the chemical analyses to determine if they do predict protein quality with reasonable accuracy.

REVIEW OF LITERATURE

I. Triticale: General Considerations

Rapidly increasing populations in many countries with an inherent increase in undernutrition point out the need for development and utilization of protein sources with higher yields and better protein quality. Triticale, especially hexaploid types, offer an important potential protein source for both human consumption and animal feed.

A. Historical Background of Triticale

The production of a wheat-rye cross is a very old endeavor. The first report of artificially produced wheat-rye crosses were made by Wilson in 1876, however, these crosses were found to be sterile. Spontaneous occurrences of partially fertile wheat-rye hybrids were described by Rimpau in 1891. These partially fertile hybrids were apparently derived from a doubled sector of an otherwise sterile hybrid. Rimpau's description of these plants left little doubt that they were true hybrids and in all probability represented the first wheat-rye amphiploid (triticale) to be observed.

The next significant period of interest in triticale occurred early in the 20th century when a number of hybrids were observed by different researchers [Leighty 1915, Meister 1921, and Leighty and Sando 1928]. However, attempts at artificial hybridization were met by sterility and variability in seed setting depending on the

background of the wheat parent. Some wheats were shown to possess a very high crossability with rye [Backhouse 1916], although invariably the development of the seed was incomplete.

A major occurrence in the production of triticales occurred with the discovery of the chromosome doubling properties of colchicine [Eigsti 1938 and Blackeslee 1937]. This discovery enabled scientists to produce fertile hybrids in far greater numbers than ever before. A great number of studies involving triticales and other crops were conducted during this period. Recently, Tsuchiya [1974] has reviewed the cytology and cytogenetics of triticales.

The first large scale breeding program in the North American Continent was initiated at the University of Manitoba at Winnipeg in 1954 under the direction of Drs. L. H. Shebeski and B. C. Jenkins. The initial stages of this program were devoted to the introduction, synthesis and evaluation of primary hexaploid triticales under conditions at Winnipeg [Jenkins 1965]. Intercrossing and selection within promising lines was begun in 1958. By 1967, lines were developed which equalled the yield of the recommended Canadian bread wheat varieties of that time. Limited commercial production of triticales was under way by this time for distilling purposes.

The initiation of a triticales program by the Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT) in 1965 under the direction of Dr. F. J. Zillinsky, provided the necessary input to escalate the triticales breeding effort on this continent to one of international status. Through the screening of the University of Manitoba's triticales and under Mexico's short-day conditions, lines were isolated which were highly fertile and day-length insensitive. These fertile

lines were designated as the "Armadillo" selections. The Armadillos represented a major break-through in triticale improvement work and rapidly became the building blocks of triticale breeding programs throughout the world.

B. Uses and Nutrition Aspects of Triticale

Triticale has been shown to have superior nutritional qualities over its wheat parent. A comparison of the protein nutritive value for adult humans of triticale grain and wheat grain indicated a higher nutritional value for triticale [Kies et al. 1970]. In addition, when the protein qualities of triticale, rye, and wheat were evaluated with growing rats, the protein efficiency ratio (P.E.R.) of triticale was found to be equal to that of rye and significantly higher than that of wheat [Knipfel 1969]. This higher value was believed due to the higher content of lysine and sulfur amino acids. Villegas [1970] showed the lysine content of triticale to average 20% above that of durum and spring wheat.

Though average yields of triticale do not approximate those of wheat, certain triticale varieties have been developed to considerably increase yield [Lorenz 1972 and Zillinsky 1974]. Triticale has been fed in rations to chick, hogs, sheep, beef and dairy cattle, and, in general, can successfully replace wheat, corn, barley, and sorghum as an energy source [Moody 1973 and listed references].

Triticale also offers potential for a wheat replacement in bread making. Though earlier reports [Rooney et al. 1969 and Unrau 1964] indicated that triticale compared unfavorably with bread wheat in both milling and baking characteristics, more recent reports have

demonstrated that certain varieties can be successfully used in the production of breads, rolls, and noodles [Lorenz 1972 and Tsen 1973].

II. Protein Evaluation Methods

A. Protein Determination

The determination of protein content in foods is largely based on an estimation of its nitrogen content. A wide variety of procedures have been developed over a period of time and are available for protein measurement [Munro and Fleck 1969; NAS-NRC 1963]. The Dumas method, based on the combustion of nitrogen, and the Kjeldahl method, based on an organic oxidation to release nitrogen as ammonia, are the two main methods for nitrogen estimation. However, the Kjeldahl method is more widely used for biological material and is carefully standardized [AOAC 1970]. The amount of protein has traditionally been estimated by multiplying the amount of nitrogen present by 6.25. This constant, 6.25, is based on the assumption that protein contains 16 percent nitrogen. However, this value has been corrected in certain cereals (triticale included) to 5.7 where the amount of nitrogen per gram of protein has been found to vary (more nitrogen per gram of actual protein).

Other protein estimation methods have been based on the different chemical and physical properties of proteins [Munro and Fleck 1969]. Most of the methods involve colorimetric reactions involving specific amino acids, amino groups, peptide bonds, or intact proteins.

B. Lysine Estimation

Lysine has been determined as the first limiting amino acid (that amino acid supplied in lowest amount in proportion to the

requirement of a particular species) in triticale grain as well as in wheat and rye. Since lysine availability is adversely affected by heat (particularly in the presence of carbohydrates), special analytical problems are encountered [Boctor 1968; Rice 1953; Evans 1948; and Meade 1973].

A variety of tests have been developed for lysine, an essential amino acid, which contains a reactive E-amino group. Lysine determination methods can be divided into three groups; micro-biological, biological, and chemical methods.

1. Microbiological Methods

The microorganism Leuconostoc mesenteroides, is commonly used to measure the lysine content of proteins [Cohen-Chouhami 1963; Horn et al. 1947]. The response of the organism was measured by potentiometric titration. The use of Aspergillus nidulans has been used for evaluating normal and opaque-2 maize for methionine, lysine, and phenylalanine [Lucio de Azevedo et al. 1970]. A strain of A. Nidulans, deficient in one amino acid, was used for each of the three amino acid studies. The microbiological determinations showed close agreement with other results.

2. Biological Methods

The nature of amino acid alteration in milk processed by four commonly used operations was studied by Mauron et al. [1955]. The experimental procedure involved comparing the total amino acid content of the acid or papain hydrolyzed fresh and processed milks with the amino acid liberated enzymatically from the fresh or processed

milk. The four processes investigated were a spray-dried milk powder, a roller-dried milk powder, an evaporated milk, and a sweetened condensed milk. The in vitro hydrolyses were carried out in a continuous digestion-dialysis apparatus involving 15 hours of pepsin digestion and 24 hours of pancreatin digestion. Of the three heat-labile essential amino acids tested (tryptophan, methionine, and lysine) only lysine was significantly affected by processing treatments. Mauron et al. [1955] indicated that available lysine in industrially processed milks could be estimated from the amount of lysine destroyed using a regression equation.

Later Mauron and Mottu [1958] reported the relationship between in vitro and in vivo availability of lysine in milk powders. The same procedures as described previously were used for the in vitro lysine availability determination. The protein efficiency ratio (PER) was used as a measure of the growth rate of rats fed on the milk powders. Methionine addition to the diets increased the rat assay sensitivity to lysine deterioration. Thus, a high correlation was observed between in vivo decrease in protein efficiency and in vitro lysine deterioration ($r = 0.991$).

Gupta et al. [1958] estimated the availability of lysine from three cereal proteins, two milk powders and seven purified proteins using the growth of weanling rats as the index of availability. The availability was calculated from weight gains and from weight gain per unit of food consumed, the latter calculations being more accurate.

3. Chemical Methods

Several chemical methods for measuring lysine content have been considered to indicate 'available' lysine. The most widely known is the 1-fluoro-2, 4-dinitrobenzene (FDNB) method developed by Carpenter [Carpenter 1960; Bruno and Carpenter 1957; Carpenter and Bjarnason 1970]. The lysine with reactive E-amino groups was converted to the yellow E-dinitrophenyl (E-DNP) lysine by treatment with FDNB, followed by acid hydrolysis. The ether-soluble interfering substances were extracted and the aqueous phase was measured at 435 nm. Carpenter [1960] pointed out that heat treated samples which had lower nutritional values also showed lower "available lysine."

Bujard et al. [1967] reported four procedures (total lysine value after acid hydrolysis, lysine available by enzymatic procedure, FDNB available lysine, and Carpenter's corrected available lysine procedure) were all highly correlated but the values were different. The enzymatic procedure and Carpenter's chemical method produced similar results for available lysine. Mottu and Mauron [1967] also noted the enzymatic procedure and the corrected FDNB method correlated best with the biological evaluation by young rats. The biological availability and lysine availability in heat treated milk was lowered due to the reaction of the E-amino group lysine into an enzyme-resistant form [Mottu and Mauron 1967; Roach et al. 1967]. Boctor and Harper [1968] reported some of the lysine determined by the FDNB method as available may not have been since it appeared as though some of it were excreted in the feces as undigestible residue.

Blom et al. [1967] and Ostrowski et al. [1970] noted that samples

high in carbohydrate tended to cause higher values in the FDNB method. Subsequent modifications of the method were free from carbohydrate interference.

Further alterations of the FDNB method were made by Nielsen and Weidner [1966] involving the isolation of the E-DNP-lysine on a column of Amberlite CH-120 with a citrate buffer at pH 8.0. The method related well to in vivo available lysine determinations and high carbohydrate content did not cause interference. The method was reported to be more rapid than Carpenter's method.

Selim [1965] modified the FDNB method by converting the amino acids to their copper salts prior to treatment with FDNB. Thus, lysine was the only amino acid which would yield the yellow colored dinitrophenyl derivative. This modification was used to determine the concentration of added lysine in fortified wheat and bulgur [Finley et al. 1970].

Kakade and Liener [1969] standardized conditions for using 2, 4, 6, -trinitrobenzenesulfonic acid (TNBS) to determine available lysine. The TNBS is specific for primary amino groups. After a one hour acid hydrolysis the A-TNP-amino acids were extracted with ether and the A-TNP amino acids remained in the aqueous phase to be measured at 346 nm. The procedure was more rapid due to the one hour as compared with the 16 to 17 hour FDNB acid hydrolysis. The TNBS results were similar to the FDNB results for available lysine. Recently, Finley [1973] has summarized the chemical methods for measuring lysine availability.

In plant breeding, especially for quality characters, there is always a great need for fast inexpensive methods to screen large

numbers of samples. For determinations of amino acid composition, the methods described above are too elaborate, time consuming and ultimately expensive. However, Laible et al. [1969] reported a highly significant correlation between lysine content and the protein content determined by the dye-binding procedures involving the Udy [1971] colorimeter. Mossberg, [1969] observed the dye-binding capacity was more correlated with basic amino acid (histidine, arginine, and lysine) content than with nitrogen or crude protein content in the analysis of barley, wheat, oats, rye, triticale, and maize. Both reports recommended the dye-binding method as a rapid, efficient screening technique in breeding programs for the estimation of lysine.

C. Biological Assay Procedures

Bioassay procedures for protein evaluation have been well documented [Eggum, 1969; 1970; McLaughlan and Campbell 1969; NAS-NRC 1963]. Under defined conditions, the rate of growth of an animal is a simple way of measuring protein value. The most widely used and simplest method is that involving the estimation of protein efficiency ratio (PER, Osborne and Mendel 1919]. PER is defined as the grams gain in weight per gram of protein consumed. Most PER studies (using rats) are standardized at 10 percent protein. Some disadvantages of PER are: (1) the questionable validity of weight gain as an index of protein quality, (2) the results vary with food intake, and (3) the assumption that all the protein is used for growth with no allotment for maintenance use.

Nitrogen balance is the sum of gains and losses of nitrogen from the body, the smaller the amount of protein required to maintain

N-balance, the greater the protein value. The biological value (BV) of a protein is the percentage of absorbed nitrogen retained in the body. A similar method, net protein utilization (NPU), measures the percentage of the nitrogen intake for the test sample. NPU is equal to biological value (BV) multiplied by digestibility. Both of these methods (BV and NPU) are laborious and time consuming.

Results comparing the weight gain methods with the nitrogen utilization methods have been conflicting. Some reports concluded the NPU and BV methods were more precise than the PER method. However, other reports did not confirm these findings. As a result, each method is considered useful within its limitations. The methods are not measuring the same factors but all the methods provide essentially the same crude relative rating of nutritive value of proteins. This indicates that the methods are measuring some critical function of the protein quality.

The most widely used animal in nutritional evaluation methods is the rat. The requirements of different rat strains have been well documented allowing more complete interpretation of results. However, for our purposes, traditional or modified rat assays are impractical because of limitations in dietary materials, time, ultimate expense and the coprophagy practiced by the rats. While each animal and avian species are unique in their nutritional requirements, the protein quality requirements for growth of each are sufficiently similar to allow their use in preliminary screening assays. Meadow Vole (Microtus pennsylvanicus) weanlings have been suggested [Elliott 1974] to adequately meet these requirements for preliminary screening

assays. The potential for voles as laboratory animals was first emphasized by Ransom in 1934. Elliott in 1963 reported their usefulness as test animals for individual alfalfa plants. Vole nutrition studies with semisynthetic diets were reported by Shenk et al. in 1970. The employment of voles in preliminary assays of protein efficiency in triticale and other cereals was summarized by Elliott in the Texas Tech. Univ. Centennial Symposium on Triticale. Because of their small size, fast growth rate, ability to survive on high fiber diets and small daily food intake, they are ideal for evaluating nutritional quality of cereals in early generations where only a small amount of seed is available.

D. Role of Resorcinol

Resorcinol, a toxic organic compound which commonly exists in rye and therefore, in appreciable amounts in triticale, has been found to adversely affect the growth rate of rats [Wieringa 1967]. Zillinsky [1971] also postulated that resorcinol has an antimetabolite role in triticale grain. However, Zillman et al. [1974] has recently reported that resorcinol extracted from rye grain and added artificially to the feeding rations of experimental mice strains did not significantly affect the growth rate even at levels of a ten-fold increase over normally encountered levels.

III. Seed Protease Inhibitors

Proteolytic inhibitors in plants and seeds have been reviewed by Ryan [1973], Dechary [1970], and Ambrose [1966]. The relationship of levels of proteolytic inhibition to survival during dormancy and germination are not well established, although Ryan [1973] included in

their functions the regulation of endogenous proteases, a storage role, and plant protection against microorganisms and insects.

A. Trypsin Inhibitor

The presence of trypsin inhibitor in barley, wheat, and rye, in both the embryo and endosperm, was shown by Mikola and Kirsi [1972]. Rye exhibited the greatest quantity and activity of trypsin inhibitor followed by barley and wheat. Triticale could then be expected to show great variability in amount and activity of trypsin inhibitor present due to differential selection since it is a wheat-rye cross. Mikola and Kirsi [1972] reported that trypsin inhibitors were present in concentrations of 5 to 10 percent of the water-soluble protein fraction of wheat, rye, and barley. The presence of trypsin inhibition properties of triticale has specifically been shown by Madl and Tsen [1974]. Although Shyamala and Lyman [1964] reported that a trypsin inhibitor they isolated from whole wheat was heat labile, Madl and Tsen [1974] found that the trypsin inhibitor isolated from triticale did not lose its activity even after one hour in a boiling water bath. Thus, the procedure of heating to inactivate the trypsin inhibitor would not be effective in triticale.

1. Electrophoretic Identification

The heterogeneity of the purified trypsin inhibitor in soybean, lima bean, and triticale has been reported by several researchers [Obara and Watanabe 1964; Haynes and Feeney 1967; Roy 1972; Madl and Tsen 1974]. These heterogenous groups of proteins were found to contain as many as five or six different proteins all with active trypsin inhibitory properties. Triticale specifically showed eight

protein bands, at least four of which exhibited significant levels of inhibitor activity [Madl and Tsen 1974].

2. Molecular Weight Determination

According to Madl and Tsen [1974] the molecular weight of the triticale inhibitor fraction was in the range of 10,000 to 14,000, with the highest specific activity at 10,000 units. Hochstrasser and Werle [1969], who isolated trypsin inhibitors from wheat and rye germs, reported that the inhibitors consisted of one protein of 17,000 and three smaller proteins of about 13,000 units and that all four proteins exhibited trypsin inhibitor activity.

3. Methods for Determination of Trypsin Inhibitor Activity

The most common method employs casein as a substrate for measuring the trypsin inhibitor activity of natural trypsin inhibitors [Kunitz 1947]. However, as pointed out by several investigators [Jacobsson 1955; Bundy and Mehl 1958], the rate of hydrolysis of casein by trypsin does not follow zero order kinetics. This problem has been resolved in the utilization of the synthetic substrate benzoyl-DL-arginine-p-nitroanilide (BAPA), first introduced by Erlanger et al. [1961], which does follow a zero order reaction. This reaction, within broad limits, produces a linear relationship between quantity of p-nitroaniline released and the concentration of the active enzyme. The use of BAPA has since been well established by several researchers [Mole and Horton 1973; Kakade et al. 1969]. Kakade et al. [1969] has defined one trypsin unit (TU) as an increase of 0.01 absorbance units at 410 nm per 10 ml of total reaction mixture under prescribed conditions. Trypsin inhibitor activity was defined as the

number of trypsin units inhibited (TUI) under those same conditions.

B. *Aspergillus oryzae* Protease Inhibitor

In 1955, Matsushima reported that extracts of barley grains inhibit the proteolytic activity present in culture filtrates of *Aspergillus oryzae*. Similar inhibitor activity was found to be present in other cereals, some other seeds, and some tubers. In later papers, [Matsushima 1957; 1959] it was demonstrated that the inhibitors present in barley, broad bean, kidney bean, and potato were proteins, and inhibit only the alkaline protease of *A. oryzae*, with the neutral and acid proteases not being affected. Mikola and Suolinna [1971] have since observed that in barley, resting grains also contain inhibitors which affect the endopeptidases present in germinating grains. The inhibitory activity was determined to be a group of 'isoinhibitors,' which were found to be of a protein nature.

Mikola and Suolinna [1971] outline an assay procedure utilizing a crude extract of *A. oryzae* with casein as a substrate to measure the inhibitory properties of extracts from cereal meals.

MATERIALS AND METHODS

I. Origin and/or Development of Experimental Triticale Lines

A. Lysine-Resorcinol Study

The spring triticale lines used in this study were supplied by Dr. F. Zillinsky from the CIMMYT program in Mexico. This group was made up of 300 spring triticale lines in which the percent protein, percent resorcinol, and the percent of lysine in each line had been determined by Dr. E. Villegas of the CIMMYT program. These lines were harvested at Navajoa, Mexico in the 1970-71 growing season.

The triticale lines were first plotted according to their lysine and resorcinol levels and five groupings were chosen for use in the study: (1) high lysine, low resorcinol; (2) high lysine, high resorcinol; (3) low lysine, high resorcinol; (4) low lysine, low resorcinol; and (5) mean lysine, mean resorcinol (shown in Figure 1). The selected samples were milled to pass through a 1/2 mm screen and diets made up at a protein level of 7%. One triticale line from each of the five groups described above plus a casein control was fed to individual weanling vole litter mates in each of 12 replications (three voles per assay) to determine what effect resorcinol and lysine level had upon protein efficiency indices (PEI).

B. Early Generation Selection for Protein Efficiency Indices

The parental lines used in this study were from two sources:

(1) from Dr. B. C. Jenkins while he was at Winnipeg in 1966--selection

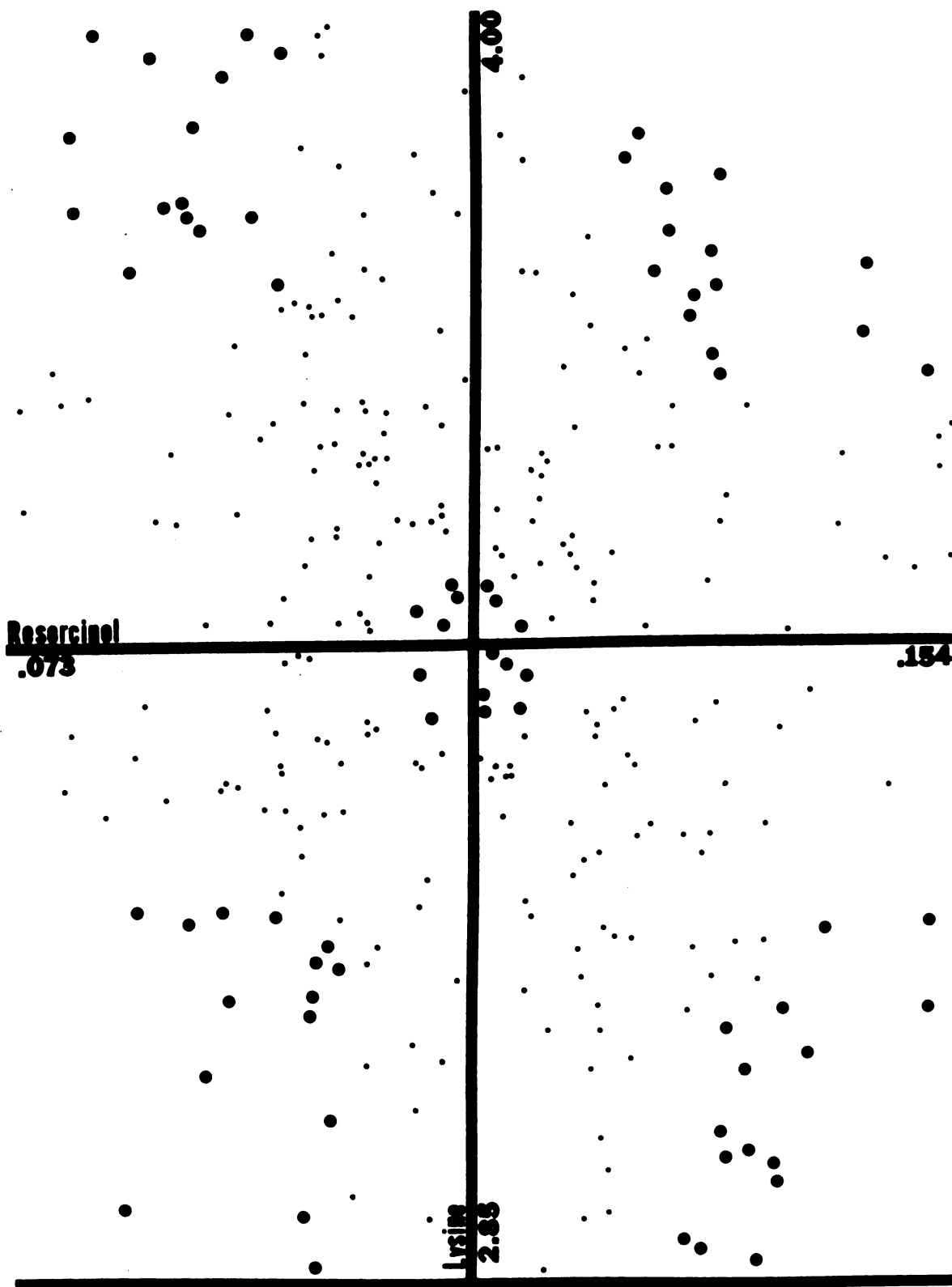


Figure 1. Graphical representation of the 300 spring triticale lines used in the Lysine-Resorcinol Study (large dots indicate lines used in study, small dots indicate lines not used in study).

numbers 358, 320, and 193; and (2) 27Y (Armadillo Strain) from the CIMMYT program. Selection 358, 320, and 193 were grown at Michigan State University since 1966, bulk harvested, brine treated and replanted each year by Dr. F. C. Elliott. The spring-type triticale 27Y, was the first breakthrough for high fertility from the CIMMYT program.

The Armadillo variety (27Y) was crossed to 358, 320, and 193 and the winter types (358, 320, and 193) were intercrossed. The F_2 generation material was planted at East Lansing for the 1969-70 season. Seventy-two superior F_2 plants were selected and replanted at East Lansing (1970-71 season) in F_3 plant rows. Of the 72 plant rows grown, only 26 contained plants of acceptable agronomic value. Up to 11 plants (80 plus grams of seed) were selected from each of the selected plant rows and the remaining plants bulked. Seed of selected F_3 plants and F_2 remnant seed were space planted at East Lansing for the 1972-73 season. Weanling vole bioassays were used to determine PEI on the seed of approximately 250 selected F_3 plants and also bulked F_3 material (three bioassays per selection). Of the 250 PEI's, three groups were constructed: (1) high PEI, 3.00 and above; (2) average PEI, 2.40 to 2.60; and (3) low PEI, 1.50 and below, all with the lowest possible standard errors.

Seven high yielding, fertile F_4 plants were selected from each of six chosen F_3 plant rows (two each from the high, average, and low classes). Weanling vole bioassays were used to determine PEI values.

C. Prediction of Protein Efficiency Indices Through
Chemical Analyses Study of Winter Triticale

Three principle groups of material were utilized in the completion of this study: (1) the 42 F_4 plants (14 high, 14 average, and 14 low PEI) selected in the previous study, (2) the F_3 bulk lines grown in both the 1971-72 and 1972-73 season, and (3) ten of the highest PEI F_3 plants (H) and ten of the lowest PEI F_3 plants (L).

In this study, the following chemical determinations were completed on all selections: percent protein, percent water-soluble protein, percent lysine (by dye-binding technique) before and after extraction of the water-soluble protein, and trypsin inhibition determination. The H and L plants also were analyzed for Aspergillus oryzae protease inhibition. The methods for determination of the above mentioned procedures are described in the Biological and Chemical Analyses section.

D. Prediction of Protein Efficiency Indices Through
Chemical Analyses Study of Spring Triticale

The spring triticale lines used in this study were originally screened from a group of 191 selections from CIMMYT material in 1969. Three lines were found to be superior with respect to PEI (106, 165, and 59). These three selections were intercrossed and the F_1 grown in Arizona in 1969-70. The F_2 and F_3 generations were grown in North Dakota with the F_4 being grown in Arizona. In the F_2 and F_3 generations selection pressure was applied for general superior plant types with good fertility. The F_4 seed received the chemical determinations described in the previous study, then ten lines were selected and weanling vole bioassays were used to determine PEI values.

II. Biological and Chemical Analyses

A. Protein Determination

The nitrogen content of the triticale samples was carried out using two methods; (1) a Technicon Auto-Analyzer and (2) a Micro-Kjeldahl procedure [Humphries 1956]. Protein content was estimated by multiplying the nitrogen content by the factor of 5.7. Thirty mg of triticale seed was used for each analysis determined on the Technicon Auto-Analyzer and 100 to 110 mg was used for the Micro-Kjeldahl procedure.

B. Moisture Content Determination

The moisture content of the whole kernel ground triticale samples was determined following the A.O.A.C. [1970] procedure. Approximately 2g of whole grain triticale was used for each analysis.

The moisture content of the prepared vole diets were determined by drying in an oven at 120 F for 24 hours.

C. Percent Water-Soluble Protein

Percent water-soluble protein was determined by two methods: (1) directly from the extract with a Phenol Reagent procedure modified from Lowry [1951], and (2) indirectly by a Micro-Kjeldahl procedure on the freeze-dried residue meal after the water-soluble proteins were extracted. The percent water-soluble protein was calculated by difference on a dry weight basis.

D. Lysine Estimation

Lysine was estimated colorimetrically in this study by the dye-binding capacity (DBC) method outlined by Mossberg [1969]. The most

suitable method used the acid diazo dye Acilane Orange G (acid orange 12), which is chemically the monosodium salt of 1-phenylazo-2-naphthol-6-sulfonic acid. At pH 2.6, the anionic sulfonic acid dye binds quantitatively with the cationic imidazol, guanidine and amino groups corresponding to histidine, arginine, but mainly to lysine. The reaction occurs in a suspension in which the dye precipitates the protein. Following a decolorization of the original solution, the optical density was measured in a short-path cuvette. Low molecular weight peptides, etc. are not precipitated [Munck 1972]. Non-specific binding can occur owing to the starch or even calcium ions. Since the binding is due to weak ionic forces, the procedure is founded on a dynamic, partly reversible reaction with an equilibrium varying for different proteins.

It was demonstrated by Mossberg [1969] that a stronger correlation was found between DBC and basic amino acid (BAA) than between DBC and crude protein. This relationship was tested in cereal material, with variable amino acid composition, consisting of barley, oats, rye, wheat, triticale, and maize varieties [Munck 1968; Mossberg 1969; 1970]. The different DBC to crude protein relationships were demonstrated to be due to the varying content of the basic amino acids. Further, it was shown that lysine, histidine and arginine were positively correlated. Therefore, the DBC screening method was shown to be reliable for lysine estimation.

In a normal analysis situation, 120 mg of finely ground sample was added to 40 ml of dye solution and shaken vigorously for three minutes (using Udy apparatus, Golden, Colo.). The dye-meal mixture was then filtered and its transmission percent recorded. This was used

directly (because of a straight line relationship with absorbance) or the absorbance computed.

E. Trypsin Inhibition Determination

In the past several years a number of procedures have been developed for determining relative trypsin inhibitor content of whole grain meals. The procedure utilized in this study was modified from Kakade et al. [1969] and is shown in detail in Appendix Table 24.

F. *Aspergillus oryzae* Protease Inhibitor Determination

The inhibition of casein hydrolysis by crude *Aspergillus oryzae* protease was outlined by Mikola and Suolinna [1971]. The determination was carried out at a pH of 10.3 with 10 mM ethylene-diaminetetracetic acid (EDTA) to inactivate the neutral *Aspergillus* protease which is not affected by triticales inhibitors. The modified procedure utilized in this study is outlined in Appendix Table 25.

G. Weanling Vole Bioassays

The vole was selected for the bioassay portion of this study because of their rapid growth and the small amount of material needed to complete each study. Diets were made up according to methods described by Elliott [1963] and Shenk et al. [1969]. The composition of the experimental and control diets are shown in Table 1. Whole grain triticales is ground in a Wiley Mill to pass a 1/2 mm screen and stored in air tight containers until used. The experimental (triticales) diets were constructed at approximately seven percent protein and the remaining ingredients were similar to the standard casein diet.

In the feeding experiments, weanling voles were used ranging in

Table 1. Formulation of experimental and casein control diets for weanling vole bioassays.

Ingredient	Experimental Diet (gms)	Control Diet (gms)
Triticale meal ^a	36-60	-
Casein ^b	-	7.5-9.5
Vitamin mix ^c	2.0	2.0
Mineral mix ^d	3.0	3.0
Corn oil	-	2.0
Alpha-cellulose ^e	20.0	20.0
Carbohydrate mix ^f	$\frac{15-39}{100}$	$\frac{64-66}{100}$

a 36-60 grams of triticale depending on the amount of protein (%N X 5.7) in the meal.

b "Vitamin Free" casein - Hammersten Quality, Nutritional Biochemicals Corp., Cleveland, Ohio.

c Vitamin Diet Fortification Mixture, Nutritional Biochemicals Corp., Cleveland, Ohio.

d Salt Mixture W, Nutritional Biochemicals Corp., Cleveland, Ohio.

e Alpha-cellulose, Nutritional Biochemical Corp., Cleveland, Ohio.

f Carbohydrate mixture: 2 parts corn starch, 1 part dextrin and 1 part sucrose.

age from 12 to 14 days and from 12.5 to 16.0 grams in weight. The voles were housed individually in plastic bottom cages with corncob bedding and nonabsorbent cotton for nesting. Food and water were available ad libitum during the experimental period. The animals were observed closely during the experimental period for general appearance

and health. During the 6 day test (1-day "break in," and 5-day experimental) a vole ate approximately 20-25 grams of diet. Experimentally, a block in the designs used were made up of single litters, with each individual vole as a replication. This allows for adjustment of the treatment means for litter effects. At the end of the experimental period, gain and intake were noted and protein efficiency indices (PEI) were computed.

RESULTS AND DISCUSSION

I. Lysine-Resorcinol Study

The protein content, percent lysine in protein, resorcinol content in total meal, and protein efficiency index (PEI) are given in Table 2 for the 60 spring triticale lines selected for this study. In the feeding trial one triticale line from each class: (1) average lysine, average resorcinol (AVG); (2) low lysine, low resorcinol (LL); (3) high lysine, low resorcinol (HL); (4) low lysine, high resorcinol (LH); and (5) high lysine, high resorcinol (HH), were randomly chosen and assigned to each block. These five selections, along with a casein control, were fed to three different litters of weanling voles of six voles each. An example of the analysis of variance performed on each test is shown (test #1) in Table 3.

The analysis of variance showed that in only one case was there a significant difference between litters (Table 4). This lack of significant litter effect on PEI indicates that using litters as blocks is unnecessary. In no case was there a significant difference of treatment means. Even where the resorcinol was at a high level (.152), PEI responses are not significantly different from those at a low level (.066). This was also found to be true with total lysine. There was not a significant difference between high (4.00%) and low (2.70%) lysine with respect to the level of PEI values obtained.

Table 2. Data of selected spring triticale lines used in the lysine-resorcinol study.

Sample Identity	% Lysine of protein	% Resorcinol of meal	% Protein	Protein Efficiency Index
S-1089-AVG	3.48	.110	13.22	2.31
S-1433-LL	3.14	.096	14.02	1.88
PC-302-HL	3.87	.074	15.73	1.88
PC-712-LH	2.88	.135	16.64	1.76
PC-324-HH	3.83	.132	14.88	2.18
PC-422-AVG	3.50	.116	15.16	2.41
PC-813-LL	3.21	.085	16.53	2.56
S-1578-HL	3.92	.095	14.02	1.31
S-1438-LH	3.12	.166	13.45	1.58
S-1338-HH	3.91	.128	14.82	1.97
PC-711-AVG	3.43	.112	16.02	1.04
S-1675-LL	3.21	.093	14.65	1.41
S-1360-HL	3.85	.085	16.87	1.95
PC-811-LH	3.00	.136	15.96	1.78
S-1107-HH	3.62	.121	14.02	2.52
S-37-AVG	3.46	.109	18.47	2.66
S-1613-LL	3.06	.086	16.36	1.79
PC-202-HL	3.84	.086	16.42	2.49
PC-606-LH	2.97	.136	16.81	2.20
S-1475-HH	3.71	.136	13.22	1.80
S-38-AVG	3.41	.107	18.18	1.19
S-1314-LL	2.92	.096	14.36	1.66
PC-102-HL	3.86	.074	15.28	2.14
PC-714-LH	2.71	.140	18.07	2.02
S-1444-HH	3.80	.151	12.88	2.32
S-1247-AVG	3.48	.113	15.79	2.37
PC-623-LL	3.18	.098	17.27	2.87
PC-423-HL	3.85	.091	15.05	3.27
S-1424-LH	3.20	.147	16.87	2.99
S-1117-HH	3.72	.152	15.05	2.97
S-372-AVG	3.47	.114	14.71	2.15
PC-614-LL	2.94	.100	15.28	1.97
PC-721-HL	4.08	.094	14.93	2.26
S-1611-LH	2.97	.142	16.13	1.27
PC-114-HH	3.78	.136	15.33	2.61

Table 2. (continued)

Sample Identity	% Lysine of protein	% Resorcinol of meal	% Protein	Protein Efficiency Index
PC-307-AVG	3.48	.112	16.07	2.50
PC-319-LL	3.12	.096	16.99	2.60
PC-303-HL	3.87	.085	16.02	2.43
PC-716-LH	2.96	.142	15.85	2.60
PC-308-HH	3.87	.132	14.71	2.44
PC-313-AVG	3.51	.119	15.89	1.70
S-53-LL	3.12	.100	17.61	2.29
S-330-HL	3.76	.095	13.57	2.34
PC-418-LH	3.18	.140	16.36	1.81
S-1332-HH	3.93	.126	13.74	2.24
PC-217-AVG	3.51	.114	15.39	2.01
S-1090-LL	3.21	.099	13.45	3.03
S-289-HL	3.75	.096	14.14	2.16
S-1331-LH	3.18	.137	14.48	2.25
PC-416-HH	3.73	.130	15.28	2.65
S-1059-AVG	3.45	.120	11.86	3.92
PC-609-LL	3.17	.102	15.16	2.02
PC-224-HL	4.05	.096	14.82	3.30
S-1688-LH	3.15	.135	15.22	3.34
S-1256-HH	3.66	.140	13.11	1.44
PC-524-AVG	3.39	.106	15.05	3.08
PC-122-LL	3.23	.094	15.16	3.27
PC-621-HL	3.73	.089	16.36	2.76
S-1584-LH	3.18	.133	13.85	1.45
PC-309-HH	3.63	.186	15.96	2.14

AVG - average lysine, average resorcinol

LL - low lysine, low resorcinol

HL - high lysine, low resorcinol

LH - low lysine, high resorcinol

HH - high lysine, high resorcinol

Table 3. Sample analysis of variance of lysine-resorcinol classes and litter effects (test #1).

Source	d.f.	Sum of squares	Mean square	F-ratio
Treatment	4	0.6482	0.1621	0.2361 n.s.
Litter	2	5.5220	2.7610	4.0224 n.s.
Error	8	5.4913	0.6864	
Total	14	11.6615		

n.s. - nonsignificant

Table 4. Significance of F-tests for litter and treatment effects of the 12 tests completed for spring triticale lines.

Test #	F-test significance values	
	Litter effect	Treatment effect
1	.062	.910
2	.723	.413
3	.138	.262
4	.535	.571
5	.017	.137
6	.582	.270
7	.583	.478
8	.077	.997
9	.079	.785
10	.060	.073
11	.892	.302
12	.347	.363

The simple correlations of all 60 treatments (5 for each test) for percent lysine in protein, percent protein, percent resorcinol in meal, and PEI, are given in Table 5.

Table 5. Simple correlations for percent lysine, percent resorcinol, percent protein, and protein efficiency indices (PEI) for the lysine-resorcinol study.

	% Lysine	% Resorcinol	% Protein	PEI
% Lysine	1.0000			
% Resorcinol	-.1979	1.0000		
% Protein	-.2335	-.1272	1.0000	
PEI	.1258	-.0849	-.0729	1.0000

The simple correlations given in Table 5 show clearly that neither percent lysine in protein nor percent resorcinol in the grain when considered alone, have significant effects on the level of PEI obtained using weanling voles as test animals with whole triticales grain meals. Further, even in diets where the amount of resorcinol is twice as great as in others, the PEI is not affected significantly. The spread in lysine concentration in this study was (in actual percentage points), 2.71 to 4.08. Some of the higher PEI values were obtained in this study at levels of lysine in protein around 3.10%, indicating, that 25% of the lysine in some lines of triticales was not being utilized, or was in a form unavailable to the weanling voles.

II. Early Generation Selection for Protein Efficiency Indices

Several plants were selected on the basis of good agronomic characteristics from each of 19 F_2 plant rows. The remaining F_2 plants in the rows were combined producing 19 unselected bulks. These unselected and selected (made up of equal portions of seed from each selected plant) bulks were milled and made into diets containing 7% protein on a dry weight basis. These diets were fed to weanling voles in a partially balanced incomplete block design (P.B.I.B.) containing two associate classes [Bose et al. 1954]. The analysis of variance for the PEI's is shown in Table 6.

The analysis of variance showed significant differences between the PEI's of the F_3 bulk triticales lines. The unadjusted, adjusted with intrablock information, and adjusted with intrablock plus interblock information treatment means (PEI values) are given in Table 7. Of the adjustments made, the changes were generally not significant, indicating that in a preliminary trial such as used here, a highly refined statistical feeding design is probably not necessary.

A graphical representation of selected and unselected F_3 bulk PEI values are shown in Figure 2. It is evident from this graph that selection of plants for agronomic characters usually raise the PEI in this particular set of materials. This can be clarified by looking at the protein percents of the bulks involved (Table 8). Generally, increased PEI was accompanied by a decreased percent protein. This was probably due to improvements of the seed type (decrease in the amount of shrivelling) which reflect some internal changes in structure or protein content. This will be discussed in more detail in part III of the Results and Discussion.

Table 6. The analysis of variance for 1971-72 F_3 selected and unselected bulks.

Source	d.f.	Sums of squares	Mean square	F-ratio
Treatments				
unadjusted	39	24.5696		
adjusted	39	18.2531	.4680	1.4993*
Litters				
unadjusted	39	47.5033		
adjusted	39	41.1868	1.0561 E(B)	3.3828 n.s.
Error	81	25.2850	.3122 E(E)	
Total	159	91.0414		

	Intrablock	Intrablock
F-ratio (treatment effects	1.4993*	-Interblock
Chi-square		55.9707*
Standard error of treatment differences		
between first associates	.4504	.4324
between second associates	.4676	.4441
average	.4623	.4405

*significant at $P < .05$.

Table 7. Table of treatment means for protein efficiency indices (PEI) of selected and unselected F_3 bulks from the 1971-72 growing season.

Bulk No.	Unadjusted PEI	Adjusted with intra-block information (PEI)	Adjusted with intra- block and interblock information (PEI)
2 sel	2.80	2.92	2.88
2 unsel	2.88	2.89	2.88
3 sel	3.04	2.75	2.85
3 unsel	1.90	2.71	2.43
6 sel	2.38	2.31	2.33
6 unsel	2.47	2.73	2.65
9 sel	2.53	2.72	2.65
9 unsel	3.12	2.62	2.79
10 sel	2.44	2.49	2.47
10 unsel	2.63	2.36	2.45
12 sel	2.75	2.41	2.53
12 unsel	2.67	3.05	2.92
17 sel	2.76	3.13	3.01
17 unsel	2.45	2.51	2.50
19 sel	2.79	2.78	2.78
19 unsel	2.77	2.83	2.81
22 sel	2.56	2.34	2.42
22 unsel	2.23	2.18	2.20
23 sel	2.97	2.71	2.80
23 unsel	2.30	2.38	2.35
24 sel	2.97	3.33	3.21
24 unsel	2.83	2.85	2.84
25 sel	2.20	2.52	2.41
25 unsel	2.39	2.49	2.46
28 sel	3.47	3.74	3.65
28 unsel	2.80	2.33	2.49
31 sel	2.84	2.63	2.71
31 unsel	3.11	3.12	3.12
47 sel	3.56	3.67	3.63
47 unsel	2.57	2.44	2.48
48 sel	2.49	2.98	2.81
48 unsel	2.72	2.15	2.35
49 sel	2.52	2.99	2.83
49 unsel	2.82	2.77	2.79
50 sel	3.00	2.70	2.80
50 unsel	2.36	2.58	2.51
69 sel	3.20	2.70	2.87
69 unsel	3.02	2.78	2.86

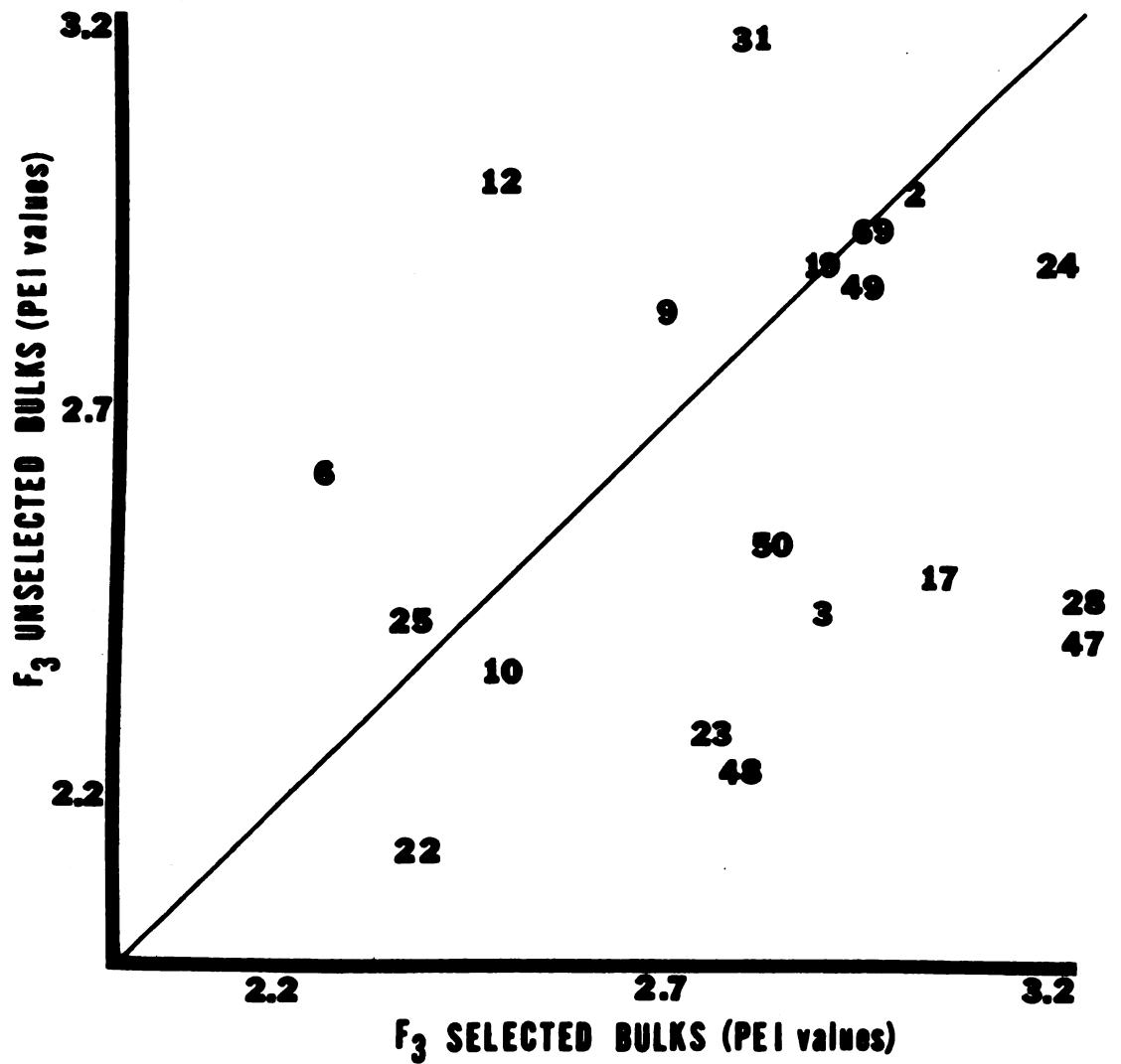


Figure 2. Relationship between F_3 selected and unselected bulk lines of triticale with respect to protein efficiency indices.

Table 8. Percent protein of F_3 selected and unselected 1971-72 bulk lines of triticale.

Bulk no.	Selected % protein	Unselected % protein
2	17.22	16.69
3	17.58	18.29
6	16.87	17.04
9	16.33	15.98
10	18.29	17.22
12	16.69	17.22
17	18.82	18.82
19	16.33	16.69
22	17.22	16.87
23	18.29	18.82
24	18.64	18.82
25	18.82	19.71
28	16.33	18.29
31	16.87	16.33
47	15.80	17.04
48	15.45	15.98
49	15.27	17.04
50	16.33	17.76
69	15.45	15.98

Of the 250 F_3 plants on which PEI was determined, a number were chosen which fit into the three classes of PEI outlined earlier (high PEI, average PEI, and low PEI) with relatively low standard errors (see Table 9).

The 1972-73 growing season was not conducive for high yield per plant. Even though the planting was replicated three times, there was difficulty in obtaining plants in the selected F_3 plant rows with sufficient seed yields for PEI assays. Over 80 grams of seed is necessary for the determination of a PEI with three replications. For this reason six (two high, two average, and two low) F_3 plant rows were chosen where at least seven plants in each produced enough seed yield

Table 9. High, average, and low protein efficiency indices (3 voles per assay) with relatively low standard errors from 250 F_3 selected triticale plants harvested in the summer of 1972.

Low PEI		Average PEI		High PEI	
Plant no.	PEI	Plant no.	PEI	Plant no.	PEI
17-7	1.54 $\pm$.19	6-8	2.42 $\pm$.30	17-1	3.31 $\pm$.14
27-4	1.83 $\pm$.47	10-6	2.59 $\pm$.17	22-2	3.52 $\pm$.11
28-11	.49 $\pm$.20	19-8	2.48 $\pm$.13	31-9	3.65 $\pm$.07
47-9	.79 $\pm$.48	25-2	2.55 $\pm$.13	49-2	3.87 $\pm$.28
49-10	1.32 $\pm$.33	27-1	2.57 $\pm$.22	50-7	3.22 $\pm$.19
49-11	1.44 $\pm$.39	48-4	2.39 $\pm$.13	50-10	3.76 $\pm$.06
50-9	1.51 $\pm$.46	50-11	2.51 $\pm$.37	69-8	3.23 $\pm$.10

for all necessary determinations. These were: high PEI, 68-9 and 50-10; average PEI, 48-4 and 50-11; and low PEI, 17-7 and 47-9. Diets were made up to contain 7% protein on a dry weight basis as previously described and PEI values determined. The design used was a partially balanced incomplete block with two associate classes with three replications and 45 treatments (three casein control diets). The analysis of variance of the selected high, average, and low F_4 triticale lines is given in Table 10.

The analysis of variance showed that there were significant differences between the PEI's for the different triticale selections. However, to determine if the PEI's for the F_3 plant selections resulted in any useful information we have to look at the table of treatment

Table 10. The analysis of variance table for the partially balanced incomplete block design of selected F_4 triticales plants for protein efficiency indices.

Source	d.f.	Sums of squares	Mean square	F-ratio
Treatments				
unadjusted	44	55.7227		
adjusted	44	36.6354	.8326	1.0098*
Litters				
unadjusted	26	44.2652		
adjusted	26	25.1779	.9684 E(B)	1.1745*
Error	64	52.7704	.8245 E(E)	
Total	134	133.6710		

	Intrablock	Intrablock -Interblock
F-ratio (treatment effects)	1.0098*	
Chi-square		60.9931*
Standard error of treatment differences		
between first associates	.6197	.5722
between second associates	.6561	.5575
average	.6463	.5560

*significant at $P < .05$.

means (Table 11). From this table several bits of information are evident. Selection for agronomic characters (mainly yield and fertility) tends to drive the PEI's toward the mean. Of the low group (7 from 47-9 and 7 from 17-7) where the F_3 parents (PEI) were 1.54 and 0.79, the means of the PEI for the 7 selected F_4 plants were 2.26 and 2.15, respectively. This shows a definite increase in PEI. However, taking the high group, whose F_3 PEI values were 3.76 and 3.23, the F_4

Table 11. Table of treatment means for protein efficiency indices (PEI) of high, average, and low F_4 winter triticales selections.

F_3 Parent no.	F_4 Selection no.	Unadjusted PEI	Adjusted with intrablock data (PEI)	Adjusted with intrablock and interblock data (PEI)
L-17-7	-	-	-	1.54 $\pm$.19
	L-17-7-1	2.55	2.21	2.51 $\pm$.10
	L-17-7-2	2.17	2.01	2.15 $\pm$.48
	L-17-7-3	1.80	2.26	1.86 $\pm$.74
	L-17-7-4	1.97	2.78	2.08 $\pm$.69
	L-17-7-5	3.16	3.19	3.16 $\pm$.57
	L-17-7-6	2.55	2.11	2.49 $\pm$.45
	L-17-7-7	1.63	1.88	1.66 $\pm$.47
L-47-9	-	-	-	0.79 $\pm$.48
	L-47-9-1	1.99	2.33	2.03 $\pm$.94
	L-47-9-2	2.65	2.74	2.66 $\pm$.86
	L-47-9-3	1.21	1.41	1.24 $\pm$.42
	L-47-9-4	2.52	2.39	2.50 $\pm$.28
	L-47-9-5	1.46	1.44	1.45 $\pm$.40
	L-47-9-6	2.12	2.06	2.11 $\pm$.13
	L-47-9-7	3.08	2.66	3.03 $\pm$.41
M-48-4	-	-	-	2.39 $\pm$.13
	M-48-4-1	2.88	2.49	2.83 $\pm$.46
	M-48-4-2	2.06	2.19	2.08 $\pm$.58
	M-48-4-3	2.13	2.24	2.14 $\pm$.86
	M-48-4-4	3.22	2.83	3.17 $\pm$.33
	M-48-4-5	3.18	3.17	3.18 $\pm$.56
	M-48-4-6	1.72	1.71	1.72 $\pm$.42
	M-48-4-7	2.49	2.33	2.47 $\pm$.83
M-50-11	-	-	-	2.51 $\pm$.37
	M-50-11-1	3.07	3.06	3.06 $\pm$.37
	M-50-11-2	1.58	1.37	1.55 $\pm$.66
	M-50-11-3	3.34	3.48	3.36 $\pm$.36
	M-50-11-4	3.16	3.64	3.22 $\pm$.71
	M-50-11-5	2.88	2.39	2.81 $\pm$.66
	M-50-11-6	1.64	2.12	1.71 $\pm$.56
	M-50-11-7	2.22	2.48	2.26 $\pm$.75
H-50-10	-	-	-	3.76 $\pm$.06
	H-50-10-1	2.04	2.02	2.04 $\pm$.23
	H-50-10-2	2.48	2.80	2.52 $\pm$.82
	H-50-10-3	3.95	3.65	3.91 $\pm$.38
	H-50-10-4	3.16	3.45	3.20 $\pm$.29
	H-50-10-5	2.38	1.93	2.32 $\pm$.20
	H-50-10-6	3.36	3.04	3.32 $\pm$.30
	H-50-10-7	1.85	2.18	1.89 $\pm$.62

Table 11. (continued)

F ₃ Parent no.	F ₄ Selection no.	Unadjusted PEI	Adjusted with intrablock data (PEI)	Adjusted with intrablock and interblock data (PEI)
H-69-8	-	-	-	3.23 + .10
	H-69-8-1	3.42	3.15	3.39 + .36
	H-69-8-2	2.45	2.83	2.50 + .17
	H-69-8-3	2.20	2.87	2.29 + .13
	H-69-8-4	2.52	2.31	2.49 + .33
	H-69-8-5	3.54	3.35	3.52 + .46
	H-69-8-6	3.25	2.48	3.15 + .98
	H-69-8-7	3.40	2.99	3.35 + .19
Casein	-	-	-	2.80 + .06

M = average triticales selections

PEI values (the average of 7 PEI's, and 3 replications each) were 2.75 and 2.97 respectively. Conversely, this shows a definite decrease in PEI.

Averages of the 14 high, 14 average, and 14 low selections for weanling vole bioassays resulted in PEI's of 2.86, 2.54, and 2.21, respectively. This is a difference of 0.32 for the high and average groups, 0.33 for a difference of the average and low groups, with an overall difference of 0.65 for the high and low groups. The average difference is significantly different according to the standard error of the treatment mean differences. Therefore, from an overall standpoint, PEI values determined in the F₃ generation are meaningful and it appears that quite a reduction can be made in the amount of material needfully carried from generation to generation.

It was noted by Dr. F. C. Elliott that voles on experimental diets containing high amounts of cereals had a tendency to accumulate material in their gastro-intestinal tracts. It was found [Elliott unpublished] that with triticale the average cecum weight after one day on an experimental diet could be imitated if a vole underwent a 2-hour evacuation (lack of diet, but water ad libitum) period. This was explored to determine if the accumulation of weight resulted in unequal bias to the treatment means. The 2-hour evacuation PEI's are shown in Table 12.

The evacuation period reduces the PEI's appreciably, however, only minor changes in ranking are shown. The relative difference between the overall classes is maintained; the mean of the high, average, and low classes are 2.00, 1.80, and 1.57 respectively. Giving an overall difference of 0.20 between high and average classes and an overall difference of 0.23 between the average and the low classes. Thus, the magnitude of the difference remained the same even after the 2-hour evacuation period. However, in most cases, the 2-hour evacuation period did reduce the standard error of PEI treatment means.

III. Prediction of Protein Efficiency Indices Through Chemical Analyses Study of Winter Lines

A. Interrelationships of Protein Efficiency Indices and Various Chemical Analyses

It has been known for many years that the nutritive value of proteins in raw soybeans and other crops can be improved by cooking. This knowledge has generated considerable research interest to identify the growth inhibitor and to explore the nutritional significance of

Table 12. Treatment means for 2-hour evacuation protein efficiency indices (PEI) of high, average, and low F_4 winter triticale selections.

F_3 Parent no.	F_4 Selection no.	PEI mean	Standard error	Reps.
L-17-7		.64	.01	3
	L-17-7-1	1.97	.11	3
	L-17-7-2	1.62	.60	3
	L-17-7-3	1.00	.48	3
	L-17-7-4	1.43	.56	3
	L-17-7-5	2.34	.60	3
	L-17-7-6	1.84	.53	3
	L-17-7-7	1.04	.63	3
L-47-9		.47	.27	3
	L-47-9-1	1.44	.66	3
	L-47-9-2	1.97	.77	3
	L-47-9-3	.51	.12	3
	L-47-9-4	2.20	.09	3
	L-47-9-5	.90	.35	3
	L-47-9-6	1.71	.06	3
	L-47-9-7	1.90	.33	3
M-48-4		1.39	.51	3
	M-48-4-1	2.07	.43	3
	M-48-4-2	1.37	.64	3
	M-48-4-3	1.36	.78	3
	M-48-4-4	2.21	.41	3
	M-48-4-5	2.43	.56	3
	M-48-4-6	.91	.33	3
	M-48-4-7	2.07	.85	3
M-50-11		1.76	.44	3
	M-50-11-1	1.98	.42	3
	M-50-11-2	.90	.62	3
	M-50-11-3	2.56	.42	3
	M-50-11-4	2.30	.78	3
	M-50-11-5	2.21	.74	3
	M-50-11-6	1.08	.62	3
	M-50-11-7	1.82	.63	3
H-50-10		3.12	.15	3
	H-50-10-1	1.25	.19	3
	H-50-10-2	1.72	.75	3
	H-50-10-3	3.15	.34	3
	H-50-10-4	2.21	.10	3
	H-50-10-5	1.82	.22	3
	H-50-10-6	2.41	.27	3
	H-50-10-7	.83	.43	3

Table 12. (continued)

F_3 Parent no.	F_4 Selection no.	PEI mean	Standard error	Reps.
H-69-8		2.37	.15	3
	H-69-8-1	2.62	.24	3
	H-69-8-2	1.41	.26	3
	H-69-8-3	1.59	.17	3
	H-69-8-4	1.60	.56	3
	H-69-8-5	2.80	.54	3
	H-69-8-6	2.23	.75	3
	H-69-8-7	2.36	.19	3
Casein		2.07	.04	9

M = average triticales selections

the trypsin inhibitor. Madl et al. [1974] has established the presence and a number of properties of the trypsin inhibitor in triticales grain. In the present study the relationship of the trypsin inhibitor and other factors contributing to nutritional quality of the triticales grain were explored.

Three sets of triticales lines were used to determine the correlations between percent protein, percent water-soluble protein, dye-binding capacity (DBC, a lysine estimation), extracted DBC (a DBC completed after the water-soluble protein has been extracted), trypsin inhibitor content, and PEI. The first set used was made up of F_3 bulk material grown in two growing seasons, 1971-72 and 1972-73. Results of chemical analyses performed on the F_3 bulk lines are given in Table 13. The simple correlations for the above data are given in Table 14.

Table 13. The protein efficiency indices (PEI), percent water-soluble protein, weight per seed (100), trypsin units inhibited (TUI), total trypsin units inhibited (total TUI), dye-binding capacity (DBC), and extracted dye-binding capacity (Ex. DBC) for the F_3 bulks grown in two years.

Year grown	Bulk no.	PEI	% Prot.	% Water-soluble protein	Weight per seed	TUI	Total TUI	DBC	Ex. DBC
1972	2	2.88	16.69	7.67	4.35	38.5	29.3	67.00	55.25
1972	6	2.65	17.04	3.81	4.76	37.4	14.1	62.00	51.25
1972	9	2.79	16.69	1.96	4.71	43.6	8.8	68.25	49.25
1972	10	2.45	17.22	7.81	4.26	42.4	33.1	62.00	49.00
1972	12	2.92	17.22	8.40	4.26	37.4	18.0	61.25	49.25
1972	17	2.49	18.82	9.19	4.71	39.7	36.8	54.75	49.75
1972	19	2.81	16.69	6.20	4.60	30.1	18.6	59.50	49.75
1972	22	2.20	16.87	8.40	4.76	42.0	35.3	57.75	45.50
1972	24	2.84	18.82	7.93	4.21	32.4	25.3	54.00	46.00
1972	28	2.49	18.29	10.12	4.04	38.0	38.4	52.50	49.75
1972	31	3.12	16.33	5.64	4.35	34.0	19.0	60.25	49.00
1972	47	2.48	15.98	3.57	5.06	41.1	14.8	62.25	46.50
1972	48	2.35	17.04	6.74	5.00	40.7	27.5	54.25	46.25
1972	49	2.79	17.04	5.36	4.21	44.6	24.3	57.75	47.75
1972	50	2.51	17.76	5.86	4.60	43.7	25.6	54.00	48.00
1972	69	2.86	15.98	2.04	4.40	39.4	7.8	58.00	48.50
1973	2	2.19	14.85	6.10	5.19	39.1	23.9	61.50	51.75
1973	6	2.24	15.89	8.50	4.40	38.4	32.6	68.50	50.25
1973	9	2.52	14.85	4.10	4.55	52.3	21.3	70.25	53.50
1973	10	2.32	13.99	4.70	4.35	53.1	24.9	56.00	49.50
1973	12	2.15	15.02	6.00	4.40	37.3	22.3	56.75	43.50
1973	17	2.49	17.27	4.80	4.88	33.8	16.2	60.00	47.00
1973	19	2.47	14.51	16.80	3.92	37.3	25.4	56.75	57.25
1973	22	1.97	17.10	7.80	4.82	41.3	32.0	49.75	46.25
1973	24	2.13	19.69	7.10	4.94	41.4	29.1	51.50	41.25
1973	28	2.62	18.13	10.00	4.00	32.0	32.0	55.75	52.25
1973	31	3.49	15.02	5.40	5.26	36.1	19.4	64.75	53.50
1973	47	2.64	13.99	4.30	4.82	38.3	16.3	58.25	46.75
1973	48	2.93	15.89	4.20	5.26	37.2	15.5	59.75	47.75
1973	49	2.37	13.99	6.70	5.13	44.8	30.2	52.25	44.00
1973	50	2.16	14.51	9.20	5.41	41.9	38.6	59.00	43.25
1973	69	1.90	15.54	7.90	4.76	38.0	31.0	54.75	41.50

Table 14. Simple correlation coefficients among all pairs of analyses for all triticales lines, upper diagonal is the 42 high, average, and low lines and the lower diagonal is the F_3 bulk material.

	PEI	% Prot.	% Water-soluble protein	Seed no.	TUI	Total TUI	DBC	Ex. DBC
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
(1)		-.18	-.45	-.10	-.02	-.53	.09	.66
(2)	.06		.56	.42	-.52	.23	-.69	-.12
(3)	-.27	.23		.40	-.55	.78	-.50	-.22
(4)	.15	.26	.43		-.22	.28	-.15	.08
(5)	-.33	-.32	-.24	-.13		.07	.50	-.13
(6)	-.53	.21	.68	.12	.11		-.21	-.33
(7)	.39	-.29	-.33	-.04	.08	.40		.01
(8)	.49	-.12	.21	.42	-.07	-.16	.52	

Although no strong simple correlations exist for the prediction of PEI from the above chemical analyses, several interesting associations are evident. Since total trypsin units inhibited was the trypsin units inhibited adjusted for the amount of water-soluble protein extracted, it is interesting to note that the largest share of the correlation of total TUI to PEI was due to the correlation of percent water-soluble protein and PEI. The association between TUI and PEI is negligible, however, the simple correlations of the percent water-soluble protein and TUI are additive. Thus, for a rapid determination of total TUI (where the percent protein and percent water-soluble protein is unknown), extracting whole meal to determine TUI will result in a fairly accurate

ranking of estimated total TUI values. Then, the highest values can be more accurately determined by the determination of percent protein and percent water-soluble protein following the methods described earlier.

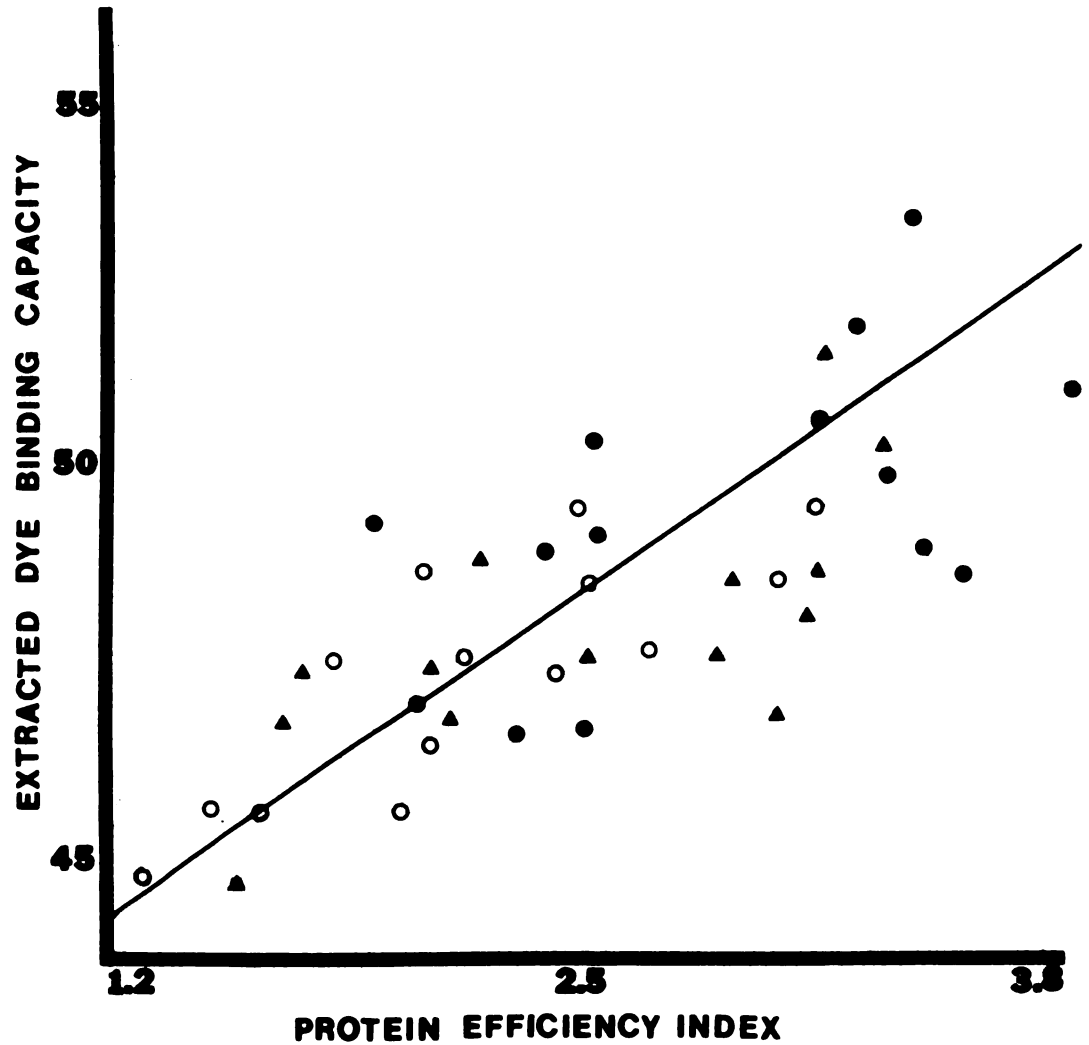
The analysis of variance for the regression of the independent variables, total TUI, and extracted DBC on the dependent variable PEI is given in Table 15. The regression about the mean is significant at below the one percent level. The multiple correlation coefficient for the regression is $r = 0.6770$ indicating that extracted DBC and total TUI explains 46% of the variation in PEI. This does not appear significant but if you consider that the average standard error of a PEI is in the range of 0.20 to 0.60 it explains a great deal of the discernable variance.

Figure 3 and 4 show the relationship between extracted DBC and PEI and total TUI and PEI respectively. The numbers on these two graphs represent the F_3 bulk numbers and the 1972-73 season F_3 bulks have a small dot beneath their numbers. There exists a clear trend in that

Table 15. The analysis of variance for the overall regression of total trypsin units inhibited and extracted dye-binding capacity for protein efficiency indices for the F_3 bulk winter triticale lines.

Source	d.f.	Sum of squares	Mean square	F-ratio
Regression (about mean)	2	1.7127	.8564	.212658**
Error	29	2.0247	.0698	
Total (about mean)	31	3.7374		

**significant $P < .01$



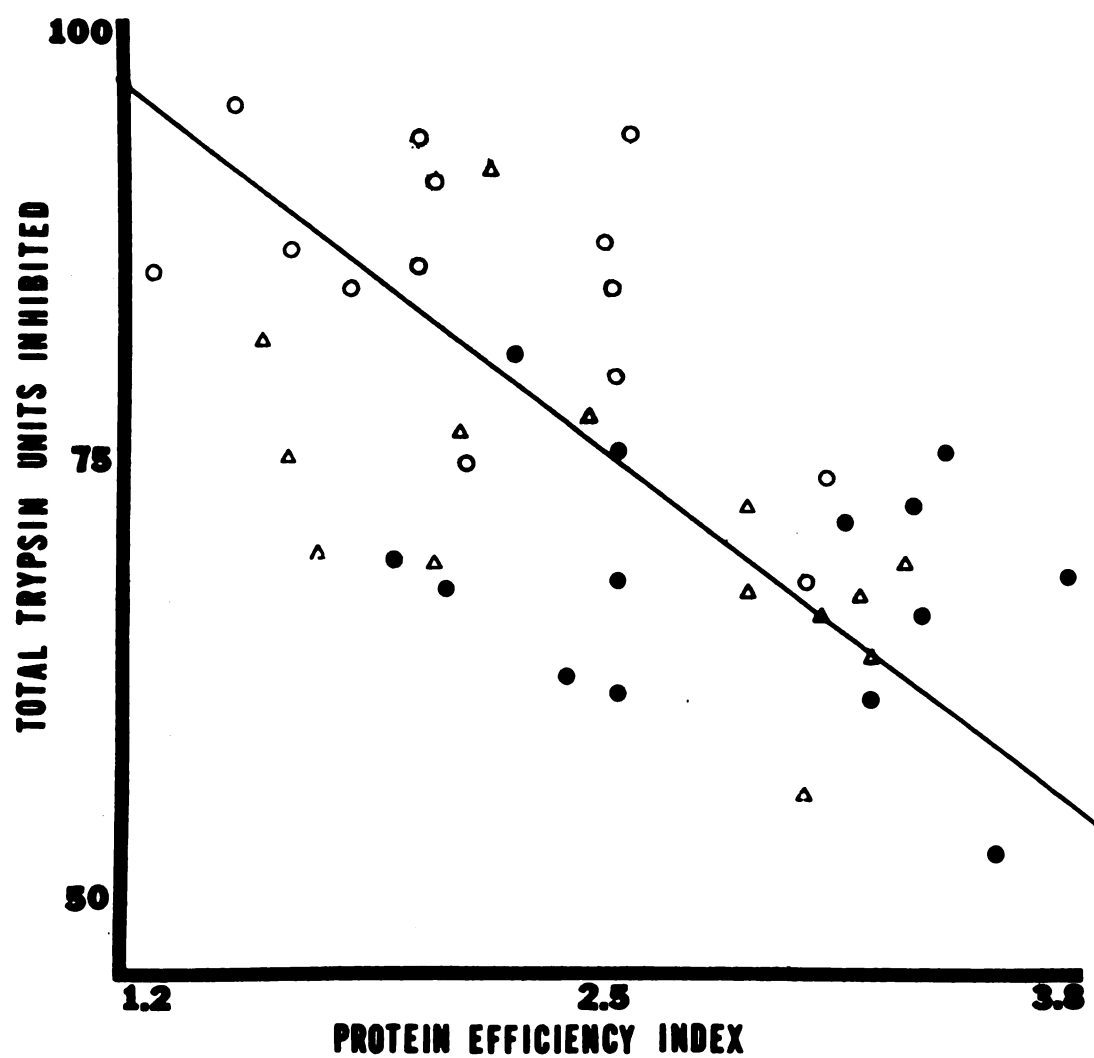


Figure 4. The relationship of total trypsin units inhibited to protein efficiency index of the 14 high (dots), 14 average (triangles), and 14 low (circles) triticale lines selected from F_3 plant rows for protein efficiency index levels.

the bulks with the lowest PEI have the highest total TUI estimates while the lower TUI bulks usually have higher PEI's. Also, the lower the PEI the lower the extracted DBC estimate.

To more clearly show the relationships between PEI and extracted DBC and between PEI and total TUI, the 42 selected F_4 plants from the previous study are presented. Table 16 shows the various analyses which were performed on these 42 selections and Table 17 gives the analysis of variance for the overall regression, which is significant below the one percent level. The multiple correlation coefficient for the regression is $r = 0.7441$. Thus, 55% of the variation in PEI is explained by the variation in extracted DBC and total trypsin units inhibited. The graphs (Figures 5 and 6) show this relationship clearly. The solid dots represent the high PEI group, the triangles the average group and the circles the low PEI selections. The correlation between the extracted DBC and PEI is high and there are no selections varying radically from the regression line.

It is interesting to note that the DBC on the whole meal does not correlate with PEI. Only after the water-soluble proteins are extracted does a correlation exist between the PEI and the DBC (extracted). This means that the lysine in the water-soluble protein adds unequal bias. The voles ability to utilize this lysine varies appreciably, most likely due to the different enzymes or other proteins in which the lysine exists. In the high, average, and low set of data (Table 16) there was no correlation between DBC and extracted DBC, indicating that most of the 'unavailable lysine' had been extracted. This is supported by the increased correlation between extracted DBC and PEI (0.66) and lack of correlation between PEI and DBC. On the other hand, with the

Table 16. The trypsin units inhibited (TUI), protein efficiency index (PEI), dye-binding capacity (DBC), percent water-soluble protein, weight per seed (100), percent protein, extracted dye-binding capacity (Ex. DBC), and total trypsin units inhibited (total TUI) for the 42 selected high, average, and low F_4 triticale lines.

Selection no.	TUI	PEI	DBC	% Water-soluble protein	Weight per seed (100)	% protein	Ex. DBC	Total TUI
H-69-8-1	54.2	3.39	50.50	14.8	4.71	15.60	49.25	79.9
H-69-8-2	57.6	2.50	54.25	10.8	5.19	14.60	47.00	62.6
H-69-8-3	58.1	2.29	54.50	14.8	5.25	14.75	47.00	85.8
H-69-8-4	48.1	2.49	51.75	14.7	4.88	15.10	49.75	70.6
H-69-8-5	45.3	3.52	53.00	11.9	4.59	15.55	48.75	53.6
H-69-8-6	49.0	3.15	53.25	15.4	4.42	14.95	51.25	75.5
H-69-8-7	52.0	3.35	51.50	13.4	3.70	15.80	54.25	69.7
H-50-10-1	68.0	2.04	50.50	10.3	4.52	16.20	47.50	70.0
H-50-10-2	56.0	2.48	51.25	14.1	4.76	16.40	51.00	79.0
H-50-10-3	54.3	3.95	53.25	13.3	4.62	15.15	51.50	71.8
H-50-10-4	60.2	3.16	54.00	10.6	4.63	15.95	52.50	63.6
H-50-10-5	54.1	2.38	52.25	11.9	4.35	16.10	49.50	64.3
H-50-10-6	53.7	3.36	51.75	14.2	4.87	16.80	50.25	76.7
H-50-10-7	46.3	1.85	53.00	15.5	5.31	15.40	50.00	71.8
M-48-4-1	55.8	2.83	51.75	12.5	4.31	15.60	48.00	69.8
M-48-4-2	53.2	2.08	54.50	13.4	4.57	15.70	47.75	71.0
M-48-4-3	60.1	2.14	57.75	13.2	4.33	14.05	47.25	79.3
M-48-4-4	60.0	3.17	56.25	11.6	4.41	14.00	48.75	69.6
M-48-4-5	57.8	3.18	57.00	11.7	4.20	14.05	52.25	67.9
M-48-4-6	60.6	1.72	56.00	11.8	4.66	14.60	48.25	72.0
M-48-4-7	57.6	2.47	52.75	14.2	4.35	16.00	48.00	82.4
M-50-11-1	62.6	3.07	55.50	9.0	4.91	15.90	47.00	56.7
M-50-11-2	61.4	1.58	55.25	14.0	4.86	15.70	45.25	86.0
M-50-11-3	68.2	3.34	55.50	10.6	4.34	15.00	50.75	72.1
M-50-11-4	57.5	3.16	53.50	12.0	4.74	15.40	48.50	69.0
M-50-11-5	65.8	2.88	56.00	11.4	4.28	15.40	48.75	75.2
M-50-11-6	55.9	1.64	55.00	13.9	4.80	14.70	47.50	77.8
M-50-11-7	69.4	2.22	56.00	14.2	4.55	15.60	49.75	98.5

Table 16. (continued)

Selection no.	TUI	PEI	DBC	% Water-soluble protein	Weight per seed (100)	% protein	Ex. DBC	Total TUI
L-47-9-1	54.7	2.03	57.50	18.1	3.96	14.10	49.50	99.6
L-47-9-2	57.3	2.66	50.25	18.7	4.80	15.80	48.00	106.4
L-47-9-3	54.3	1.24	51.25	17.0	3.42	15.75	45.75	91.8
L-47-9-4	57.1	2.50	48.25	16.2	3.97	16.40	49.00	92.5
L-47-9-5	58.0	1.45	50.00	17.9	4.87	16.00	46.50	103.8
L-47-9-6	67.3	2.11	58.00	14.6	3.88	14.75	47.50	97.8
L-47-9-7	49.4	3.03	56.00	14.1	4.50	15.80	49.00	69.1
L-17-7-1	52.2	2.55	56.25	16.1	3.76	17.40	48.25	83.7
L-17-7-2	44.9	2.17	51.50	17.0	3.55	18.50	48.75	76.5
L-17-7-3	45.8	1.80	47.75	19.6	3.95	19.20	48.00	89.8
L-17-7-4	47.4	1.97	49.50	19.3	4.13	18.55	46.75	91.4
L-17-7-5	42.3	3.16	50.00	16.4	3.93	17.75	50.75	77.1
L-17-7-6	49.6	2.55	50.75	18.0	3.90	17.35	49.75	90.0
L-17-7-7	47.1	1.63	50-25	19.5	3.85	18.35	46.00	91.7

M = average triticales selections

Table 17. The analysis of variance for the overall regression of the 42 selected F_4 triticales plants for protein efficiency index.

Source	d.f.	Sums of squares	Mean square	F-ratio
Regression (about mean)	2	9.6745	4.8372	23.8966**
Error	39	7.8945	.2024	
Total (about mean)	41	17.5690		

** significant $P < .01$

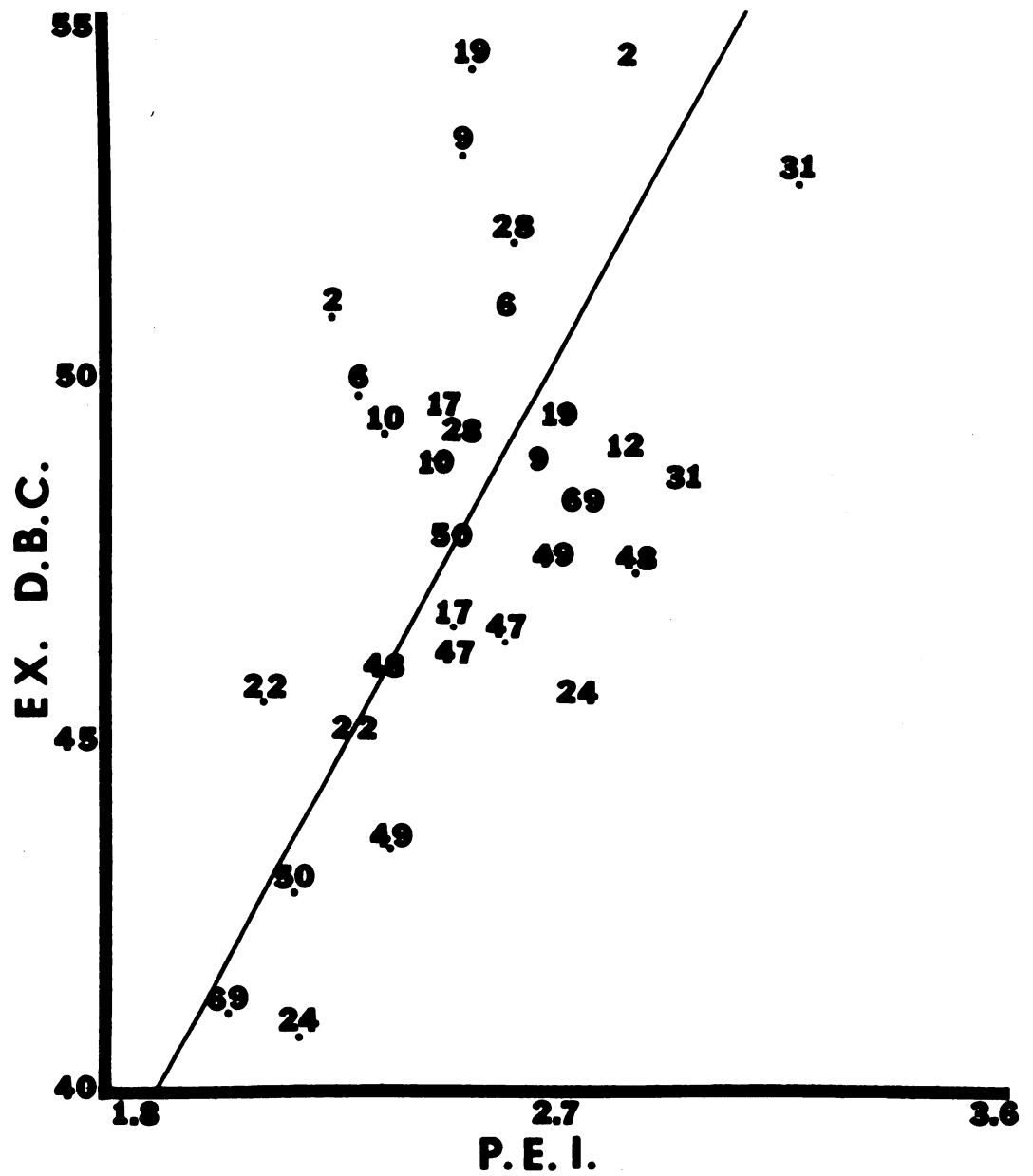


Figure 5. The relationship of extracted dye-binding capacity (Ex. DBC) to protein efficiency index (PEI) of F_3 bulk triticale lines.

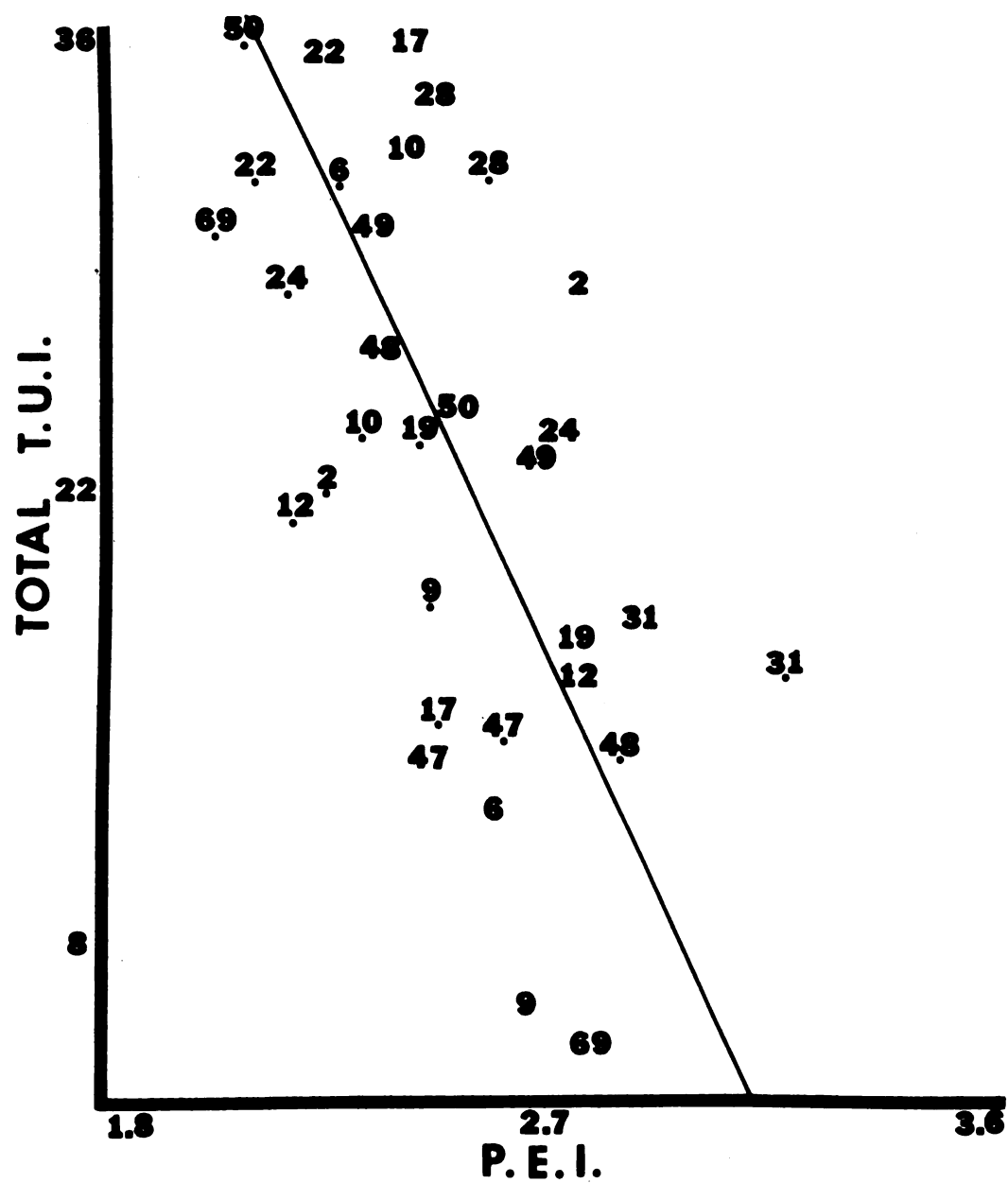


Figure 6. The relationship of total trypsin units inhibited (total TUI) to protein efficiency index (total TUI) to protein efficiency index (PEI) of F₃ bulk triticales lines.

F_3 bulks, a correlation between DBC and extracted DBC (0.52) does exist, indicating that not all of the 'unavailable lysine' was extracted. This is supported by a lower correlation between PEI and extracted DBC (0.49). Furthermore, the average water-soluble protein extracted from the high, average, and low F_4 triticale lines was appreciably higher than the average of the F_3 bulk triticale lines.

B. Interrelationships Between Percent Protein, Percent Water-Soluble Protein, and Weight Per 100 Seeds in the Various Triticale Lines Studied

Triticale inherently has a special problem in that many selections have shrunken seeds. The degree of shrunken seeds may be greatly affected by the environment so that it will vary from year to year within a specific line. This can affect nutritional quality in a number of ways. One is increasing the percent protein per unit weight of seed. However, the quality of protein is also affected. If protein is increased in this manner the percent water-soluble protein will also increase, which is shown in Table 14. This will increase the total trypsin units inhibited (0.78) which has a negative affect on PEI. This will be true if the seed number per unit weight is positively correlated with percent protein. Therefore, when seed shrivelling is the main factor increasing percent protein, percent water-soluble protein is increased which increases total trypsin units inhibited (per gram of protein) which, decreases PEI (which is determined on a per unit protein basis). In the upper diagonal of Table 14, this appears to be the case. However, the the lower diagonal, percent protein is not elevated specifically by increased weight per seed. Although the PEI vs total TUI correlation is the same in both cases,

in the case of the F_3 bulks much more of the correlation is coming from the unadjusted TUI factor.

C. Evaluation of Predicted Correlations Through Selected Triticale Lines

Ten lines of the lowest PEI and ten lines of the highest PEI with low standard errors were chosen to find if the correlations determined would support earlier trends. Each of the lines trace to an individual F_3 plant with the widest possible background (from the original 250 F_3 selected plants). If the correlations on the previously tested material were meaningful, they should be increased with this material. Also a test for Aspergillus oryzae protease inhibitor was included to determine its usefulness in predicting PEI. The chemical determinations were completed and are presented in Table 18. The simple correlations of the data and the analysis of variance for selected determinations which best predict PEI are shown in Table 19 and 20 respectively.

The overall regression was again significant below the one percent level. The multiple correlation coefficient for the regression of total TUI and extracted DBC on PEI is $r = 0.8642$, or that 75% of the variation in the dependent variable PEI is explained by the independent variables extracted DBC and total TUI. The remaining variation in PEI can be due to either random variation or other important variables not included in the regression. However, considering: (1) 75% of the variation is already explained, (2) PEI values have rather large standard errors, and (3) it is a rapid preliminary screening procedure without extreme precision, improvement would be difficult.

The Aspergillus oryzae protease inhibitor determination appears

Table 18. The trypsin units inhibited (TUI), percent water-soluble protein, percent protein, extracted dye-binding capacity (Ex. DBC), total trypsin units inhibited (total TUI), Aspergillus oryzae protease units inhibited (AUI), and total Aspergillus oryzae protease units inhibited (total AUI) analyses for the high and low selected F_3 winter triticale plants.

Selection no.	TUI	PEI	% Water-soluble protein	% protein	Ex. DBC	Total TUI	AUI	Total AUI
H-9-4	65.3	31.1	7.19	19.72	32.9	46.8	31.0	22.3
H-17-1	48.6	3.31	4.50	22.97	30.9	21.9	39.0	17.6
H-19-5	67.3	3.18	5.70	19.74	32.3	38.2	25.1	14.3
H-22-2	56.8	3.52	4.83	19.55	31.2	27.4	35.8	17.3
H-24-4	48.1	3.44	8.70	20.05	31.1	41.8	58.5	50.8
H-27-3	45.2	2.90	4.90	20.20	33.1	22.1	37.1	18.2
H-31-1	36.7	3.12	4.62	18.45	32.0	17.0	52.1	24.1
H-47-6	56.3	3.19	5.16	17.61	32.3	29.1	31.5	16.3
H-48-2	60.5	3.12	5.95	17.93	31.3	36.6	25.0	14.9
H-49-2	64.8	3.87	5.10	17.88	31.2	33.2	36.5	18.6
L-10-5	62.2	1.27	11.07	20.98	30.0	68.8	20.0	22.1
L-20-3	61.1	1.20	11.75	18.71	31.0	72.0	29.0	34.1
L-22-5	70.5	1.63	9.87	20.63	31.0	70.2	24.5	24.2
L-24-1	68.0	1.11	9.97	23.04	29.2	67.8	35.5	35.4
L-25-4	64.7	1.72	9.26	21.88	29.8	59.9	52.0	48.2
L-27-4	62.9	1.83	14.35	19.93	31.7	90.7	37.0	53.1
L-47-8	65.4	1.65	12.78	16.99	31.0	83.6	30.0	38.3
L-49-10	71.3	1.44	6.74	17.28	30.0	47.6	26.0	17.5
L-49-11	67.7	1.31	7.60	18.64	29.6	52.7	40.0	30.4
L-50-9	75.0	1.51	7.99	19.45	30.1	60.0	26.2	20.9

Table 19. Simple correlations among protein efficiency index (PEI), percent protein, percent water-soluble protein, trypsin units inhibited (TUI), total trypsin units inhibited (total TUI), extracted dye-binding capacity (Ex. DBC), Aspergillus oryzae protease units inhibited (AUI), and total Aspergillus oryzae protease units inhibited (total AUI) for selected F_3 triticales plants.

	PEI (1)	% Prot. (2)	% Water-soluble protein (3)	TUI (4)	Total TUI (5)	Ex. DBC (6)	AUI (7)	Total AUI (8)
(1)	-	-	-	-	-	-	-	-
(2)	-.13	-	-	-	-	-	-	-
(3)	-.71	.10	-	-	-	-	-	-
(4)	-.56	-.07	.40	-	-	-	-	-
(5)	-.79	.07	.96	.62	-	-	-	-
(6)	.66	-.26	-.35	-.48	-.43	-	-	-
(7)	.31	.21	-.14	-.59	-.29	.01	-	-
(8)	-.37	.22	.73	.01	.61	-.28	.55	

Table 20. The analysis of variance for overall regression of extracted dye-binding capacity and total trypsin units inhibited on protein efficiency index for selected F_3 winter triticales plants.

Source	d.f.	Sum of squares	Mean square	F-ratio
Regression (about mean)	2	13.1131	6.5566	25.0782**
Error	17	4.4446	.2614	
Total (about mean)	19	17.5577		

**singificant $P < .01$

to be positively correlated with PEI. However, after the Aspergillus oryzae protease units inhibited (AUI) were adjusted for percent water-soluble protein, the total AUI becomes negative. It apparently reflects the strongly negative (percent water-soluble protein vs PEI) characteristic of the percent water-soluble protein. In this case then measurement of Aspergillus oryzae protease inhibitor content does not aid in the prediction of PEI.

IV. Prediction of Protein Efficiency Indices Through Chemical Analyses Study of Spring Triticale Lines

Chemical analyses were completed on 62 2nd cycle spring triticale lines giving the following: percent protein; percent water-soluble protein; extracted DBC; TUI; total TUI; and weight per 100 seeds (Table 21). Using the described data, ten lines were chosen to determine if the PEI's could be predicted (Table 22). Five of the spring triticale lines were chosen which were predicted to give low PEI's (numbers 1-5) because of the following reasons: lines 1 through 4 because of lower than average extracted DBC and higher than average TUI; and line 5 because of high percent water-soluble protein (giving a very high total TUI) even though the extracted DBC was average and the TUI was quite low. Five lines were selected expecting to give high PEI's (numbers 5-10) because of the following reasons: lines 6 through 8 because of high extracted DBC and low TUI; line 9 because of very low TUI; and line 10 because of very low percent water-soluble protein even though the TUI per unit of water-soluble protein was high.

The PEI's which were determined are shown in Table 23. Totally, the prediction of the PEI value was quite effective in that the average of the low selections was 2.03 and the average of the high selections

Table 21. The percent protein, percent water-soluble protein, weight per seed (100), trypsin units inhibited (TUI), total trypsin units inhibited (total TUI), and extracted dye-binding capacity (Ex. DBC) of selected 2nd cycle spring triticale lines.

Selection no.	% Protein	% Water-soluble protein	Weight per seed (100)	TUI	Total TUI	Extracted DBC
165/106-1	12.3	6.69	4.71	32.1	21.4	62.75
" -2	14.1	7.09	4.00	32.8	23.4	58.75
" -5	12.3	3.48	4.40	43.2	15.1	53.25
" -6	12.7	4.95	4.40	48.0	24.0	55.50
" -7	14.8	2.00	4.82	44.8	9.0	47.25
" -8	13.1	4.80	4.82	49.3	23.5	51.75
" -9	12.5	6.14	4.17	54.8	33.6	50.75
" -10	12.7	11.24	4.88	49.5	56.0	61.00
" -11	13.3	3.22	4.60	50.0	16.0	53.25
" -12	12.7	9.67	4.60	56.1	54.3	61.25
" -13	14.0	6.53	4.04	45.7	29.9	53.50
" -14	13.8	9.59	4.08	45.4	43.2	60.25
" -15	12.3	8.36	5.00	49.6	42.0	63.50
" -16	13.1	13.95	5.00	47.9	67.2	57.50
" -17	14.2	13.55	3.96	44.9	61.2	60.75
" -18	13.1	6.32	4.35	45.7	29.0	55.75
" -19	13.3	4.72	4.40	52.4	24.4	57.25
" -20	15.2	13.91	4.82	48.4	67.5	60.75
" -21	13.1	10.90	4.60	49.4	53.4	61.75
" -22	12.3	8.36	4.44	49.2	41.2	62.25
" -23	14.0	8.03	4.65	51.7	41.6	59.75
" -24	12.3	11.53	4.94	57.5	66.7	67.75
106/59-3	16.1	12.42	4.00	56.7	70.7	50.75
" -4	15.2	7.21	4.04	47.5	34.6	52.75
" -5	13.3	6.23	3.67	54.1	33.5	50.25
" -6	16.5	8.19	3.64	41.0	33.6	47.50
" -7	14.8	10.23	3.96	59.9	61.2	55.75
" -8	13.1	4.80	4.26	50.1	24.0	53.00
" -9	14.2	12.14	5.33	58.0	70.2	55.75
" -10	14.6	13.18	3.85	58.1	76.6	57.50
" -11	13.8	13.94	4.40	57.0	79.2	62.75
" -12	14.2	15.70	4.82	60.6	95.8	65.00
" -13	16.7	14.29	4.44	49.6	71.5	57.75
" -14	13.1	13.92	4.60	56.5	79.2	63.00
" -15	16.7	15.54	4.26	40.8	63.6	55.75
" -16	16.9	21.39	5.00	53.0	113.4	61.00
" -17	14.2	10.73	4.08	53.6	57.8	56.00
" -18	15.2	17.92	4.71	62.4	111.0	63.75
" -19	14.2	19.18	4.94	62.9	121.0	63.50
" -20	15.4	15.03	4.76	52.6	79.5	60.50

Table 21. (continued)

Selection no.	% Protein	% Water-soluble protein	Weight per seed (100)	TUI	Total TUI	Extracted DBC
106/59-21	16.1	20.02	4.49	57.8	116.0	61.00
" -22	14.6	14.55	4.49	40.6	59.8	60.50
" -23	13.3	7.70	4.49	64.5	50.1	56.25
" -24	15.9	16.44	4.35	52.6	86.9	57.75
" -25	16.5	10.71	4.26	43.1	46.0	50.00
" -26	14.4	10.58	4.71	64.6	68.9	58.25
" -27	13.8	13.94	4.12	55.5	77.8	55.25
" -28	16.1	12.39	4.88	53.4	65.7	55.25
" -29	13.1	6.29	4.94	58.5	37.2	54.25
" -30	17.6	15.10	4.21	56.0	84.6	54.50
" -31	16.1	13.64	4.55	52.1	70.7	54.50
" -32	14.2	12.14	4.44	45.0	54.5	58.50
" -33	12.7	8.06	4.40	62.6	51.0	54.75
" -34	15.2	8.52	4.94	58.7	49.3	51.00
" -35	15.2	4.45	4.04	52.9	23.9	47.75
" -36	14.2	3.55	4.21	55.5	20.2	50.25
" -37	14.6	6.20	4.71	50.6	31.6	52.75
" -38	16.7	6.10	4.30	59.4	23.9	45.50
" -39	18.4	7.25	3.67	38.6	28.5	48.00
" -40	13.3	4.65	4.49	66.9	31.5	50.00
" -41	15.2	3.07	4.55	51.0	15.8	48.50
" -42	18.4	4.92	3.67	52.4	25.5	46.25

was 3.09. However, there are significant differences within the high and low groups (based on significant differences of 0.45 from previous studies). For example, in the high group, selection number 8 has appreciably higher water-soluble protein and total TUI than selection number 9. However, selection number 9 has a much lower extracted DBC in relation to selection number 8. In all of the other studies the extracted DBC estimation was the most stable predictor of a significant portion of variation in PEI.

Triticale lines numbered 5 and 10 indicate the importance of

Table 22. Percent water-soluble protein, extracted dye-binding capacity (Ex. DBC), trypsin units inhibited (TUI), weight per seed (100), percent protein, and total trypsin units inhibited (total TUI) for selected 2nd cycle spring triticale lines for protein efficiency index (PEI) determinations.

Selection no.	% Water soluble protein	Extracted DBC	TUI	Weight per seed (100)	% protein	Total TUI
(1) L-165/106-9	6.14	50.75	54.8	4.17	12.5	33.6
(2) L-106/59-29	6.29	54.25	58.5	4.94	13.1	37.2
(3) L-106/59-33	8.06	54.75	62.6	4.40	12.7	51.0
(4) L-106/59-34	8.52	51.00	58.7	4.94	15.2	49.3
(5) L-106/59-15	<u>15.54</u>	<u>55.75</u>	<u>40.8</u>	<u>4.26</u>	<u>16.7</u>	<u>63.6</u>
Average	8.91	54.50	55.8	4.51	14.0	46.9
(6) H-165/106-17	13.55	60.75	44.9	3.96	14.2	61.2
(7) H-106/59-22	14.55	60.50	40.9	4.49	14.6	59.8
(8) H-106/59-32	12.14	58.50	45.0	4.44	14.2	54.5
(9) H-106/59-39	7.25	48.00	38.6	3.67	18.4	28.5
(10) H-106/59-40	<u>4.65</u>	<u>50.00</u>	<u>66.9</u>	<u>4.49</u>	<u>13.3</u>	<u>31.5</u>
Average	10.43	55.55	47.2	4.18	14.9	46.9
Overall mean (of 62 lines)	9.96	56.11	51.5	4.41	14.4	51.3

Table 23. Protein efficiency indices for the ten selected 2nd cycle spring triticales lines.

Selection no.	Protein efficiency index
(1) L-165/106-9	2.08 $\pm$.69
(2) L-106/59-29	2.30 $\pm$.30
(3) L-106/59-33	1.89 $\pm$.24
(4) L-106/59-34	2.45 $\pm$.10
(5) L-106/59-15	1.44 $\pm$.16
average	2.03
(6) H-165/106-17	3.30 $\pm$.39
(7) H-106/59-22	2.88 $\pm$.28
(8) H-106/59-32	3.47 $\pm$.42
(9) H-106/59-39	2.90 $\pm$.05
(10) H-106/59-40	2.89 $\pm$.45
average	3.09

the percent water-soluble protein in the prediction of PEI. Line number 5, even though it has an average extracted DBC and a low TUI, still results in a low PEI. Line number 10, with a lower extracted DBC and higher TUI, results in a good PEI. This difference (between PEI's of lines 5 and 10) is the direct result of the large difference in the effect of the percent water-soluble protein on the amount of total TUI per unit of protein in the diets fed to the weanling voles.

SUMMARY AND CONCLUSIONS

The present study was undertaken to determine the feasibility of selection for protein efficiency indices in the early generations of a breeding program. Predictability of protein efficiency indices was also determined from various chemical analyses procedures.

The first limiting amino acid in cereals with respect to most monogastric species, has been found to be lysine. Resorcinol has been shown to be an antinutritional material found in appreciable quantities in rye and also in triticale. The present study showed that neither percent lysine in protein nor percent resorcinol in whole meal affect the protein efficiency indices determined by weanling meadow voles. Rat protein efficiency ratios have been shown to correlate well with percent lysine. However, rats have developed a feces recycling system (coprophagy) whereby lysine from normally unavailable forms are made available. Thus, monogastrics which do not practice coprophagy should correlate to a higher degree with the vole bioassay.

Weanling vole bioassays of F_3 plants (seed yield) along with several F_4 plant selections from each F_3 plant row indicate that selection for protein efficiency indices was practical as early as the F_3 generation. Selection as early as the F_3 generation means that the amount of material that must be carried from generation to generation can be greatly reduced, thus increasing the speed at which genes that effect properties which increase PEI can be fixed.

Several chemical determinations of triticale meals have shown promise of predicting PEI values. The percent water-soluble protein, total trypsin units inhibited (adjusted for the percent water-soluble protein), and the extracted dye-binding capacity (after water-soluble proteins have been extracted) have shown the highest correlations with PEI. A group of 42 F_4 winter triticale plants; 14 from high PEI F_3 plants, 14 from average PEI F_3 plants, and 14 from low PEI F_3 plants, gave simple correlations of -0.45, -0.53, and 0.66 for percent water-soluble protein, total TUI, and extracted DBC respectively. The multiple correlation coefficient for the above regression was found to be $r = 0.7421$ (utilizing extracted DBC and total TUI only), significant at the $P < .01$ level.

A group of 20 F_3 plants selected for widely varying PEI with low standard errors gave similar responses. Their simple correlations were -0.71, -0.79, and 0.66 for percent water-soluble protein, total TUI and extracted DBC respectively. The multiple correlation coefficient for the regression of extracted DBC and total TUI on PEI was $r = 0.8642$, indicating that 75% of the variation in PEI was explained by the two independent variables.

The above chemical determinations were performed on 62 spring triticale selections. Ten lines were chosen, five were selected with the above information to give low PEI's and five were chosen to give high PEI's. The five 'low' selected lines averaged a PEI of 2.03 (average of 3 voles per assay) and the five 'high' lines averaged 3.09 (average of 3 voles per assay). The results indicate that extracted DBC and total TUI are correlated with PEI and can be used to select effectively for PEI. This knowledge makes it possible

to effectively screen large numbers of selections for protein quality using a bioassay as a final selection criteria on a relatively few selected lines. This would reduce the cost and time required to screen large numbers of lines solely using a bioassay approach.

APPENDIX

Table 24. Benzoyl-DL-arginine-p-nitroanilide (BAPA) substrate method for measuring trypsin inhibitory activity of triticales samples.

Reagents

1. Tris buffer (0.054, pH 9.0) containing 0.02M CaCl_2 :
 - a) 6.05 g tris-(hydroxymethyl) aminomethane (Sigma Chemical Co., St. Louis, Mo.)
 - b) 2.94 gm $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$
 - c) above chemicals are added to 900 ml distilled water and the pH adjusted to 9.0 with 1M HCl and brought to one liter in volume of H_2O .
2. BAPA solution:
 - a) 30 mg BAPA HCl (Nutritional Biochemical Corp.) is dissolved per ml of dimethylsulfoxide (DMSO) and diluted to 100 ml with tris buffer solution prewarmed to 37 C.
3. Trypsin solution:
 - a) 5 mg trypsin (2X crystallized, salt-free, Worthington Biochemical Corp., Freehold, N.J.) is dissolved in 100 ml 0.001M HCl trypsin solution is quite stable and can be stored at 5 C for several days without appreciable loss of activity.
4. Preparation of triticales sample:
 - a) 2 g [300 mg of protein (N X 5.7)] of finely ground triticales meal is suspended in distilled water (300 mg protein/5 ml), and the suspension is then adjusted to pH 4.9 by the addition of 1M acetic acid.
 - b) The extraction is allowed to continue for two hours without agitation, and the extract is then separated by centrifugation (approximately 3,000 g.).
 - c) 2 ml of the extract is then pipetted into 10 ml of distilled water, mixed well and used in the inhibitor assay.
 - d) The amount of soluble protein in the extraction preparation can be determined with a Phenol Reagent procedure modified from Lowry [1951].

Procedure

1. Trypsin Standard Curve (Figure 7)

- a) 0.2 to 1.0 ml of the stock trypsin solution is pippetted to a triplicate set of test tubes, and the volume made up to 2 ml with distilled water. To one set of the triplicate, add 1 ml of 30% acetic acid to serve as a blank.
- b) The tubes are then placed in a water bath warmed to 37C. Then to each tube add 7 ml of BAPA solution, previously warmed to 37 C, and exactly 10 minutes later, terminate the reaction by the addition of 1 ml 30% acetic acid to the experimental tubes.
- c) After thorough mixing, the absorbance of each solution is measured at 410 mu against the appropriate blank.

2. Trypsin Inhibitor Activity

- a) 0.2 to 1.0 ml of triticales extract is pippetted into a triplicate set of test tubes and the volume adjusted to 1 ml with distilled water.
- b) One ml of stock trypsin solution is then added to each tube and then assayed as described for the standard trypsin curve.
- c) Example:

Volume of triticales extract		0.2	0.5	1.0
absorbance	Rep. I	.544	.373	.129
	Rep. II	.534	.380	.128
average		.539	.377	.129

Note: For each new batch of BAPA solution a check to determine maximum activity of your particular trypsin stock should be completed. In the case of the example the absorbance was .645.

3. Expression of Activity

One trypsin unit is arbitrarily defined as an increase in absorbance of 0.01 at 410 mu per 10 ml of reaction mixture under the previously described conditions. Trypsin inhibitor activity is defined as the number of trypsin units inhibited (TUI).

<u>Trypsin Units (TU)</u>	<u>TUI</u>	<u>TUI/ml extract</u>	
64.5			
55.9	10.6	53.0	
37.7	26.8	53.6	
12.9	51.6	51.6	52.6 TUI/ml extract

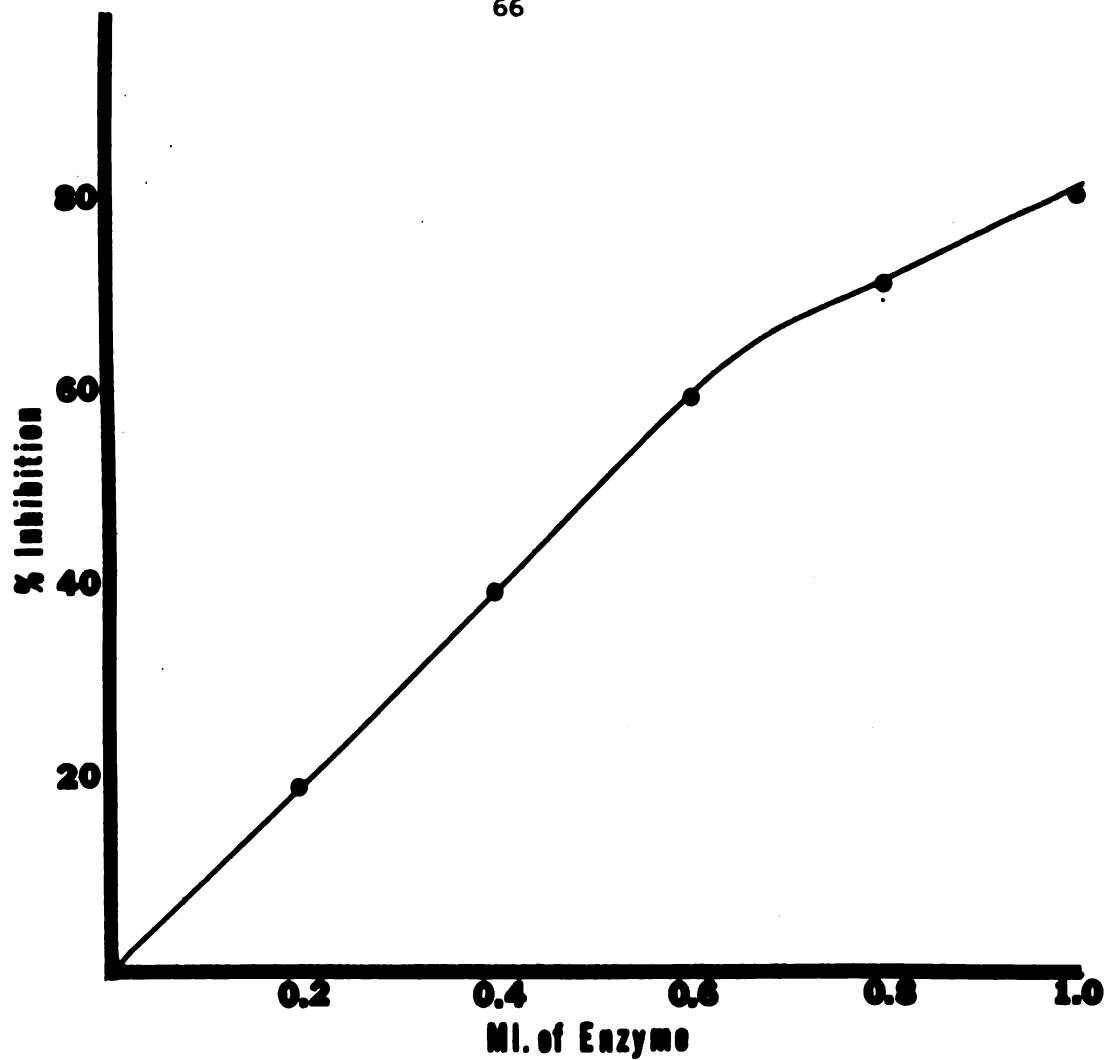


Figure 7. Trypsin inhibition standard curve.

<u>Vol. of Enzyme Soln.</u>	<u>Absorbance</u>		<u>average</u>
	<u>I</u>	<u>II</u>	
0.2	.202	.200	.201
0.4	.401	.408	.405
0.6	.621	.611	.616
0.8	.721	.741	.731
1.0	.803	.822	.811

As long as total inhibition does not exceed 65% (which would require a different dilution of initial extract) a ranking of triticales lines can be made from the 1.0 ml extract or the average of the 0.5 and 1.0 ml extract (TUI) means.

Table 25. Casein substrate method for determining Aspergillus oryzae protease inhibitory activity of triticales samples.

Reagents

1. Sodium glycinate buffer:
 - a) dissolve 0.05M glycine in 900 ml distilled water, add 1M NaOH until the solution is at a pH of 10.3. Then add 10mM EDTA and readjust the pH to 10.3.
2. Casein substrate solution:
 - a) dissolve 1% casein in 0.05M sodium glycinate buffer solution.
3. Aspergillus protease
 - a) 60 mg Aspergillus protease/100 ml
4. Preparation of triticales sample:
 - a) the sample is handled the same as in the trypsin assay, except the pH is not adjusted and distilled water is used for extraction.

Procedure

1. Aspergillus oryzae protease standard curve (Figure 8):
 - a) 0.6 to 3.0 ml (into 5 test tubes) of A. oryzae protease solution is pipetted into the first group of a triplicate set of test tubes. The volume of this group of tubes is adjusted to 6 ml, mixed well, and then with a clean pipette 4 ml is withdrawn and 2 ml is pipetted into the other two tubes of each triplicate set. Six ml of 5% trichloroacetic acid (TCA) is added to one set of the triplicate to serve as a blank.
 - b) The entire set of test tubes is then placed in a water bath warmed to 35 C. Then 2 ml of stock casein solution is added to all tubes, and, 30 minutes later, 6 ml of 5% TCA is added to the experimental tubes to terminate the reaction.
 - c) After thorough mixing, the tubes are left for 30 minutes, filtered, and read at 280 mu against the appropriate blank.

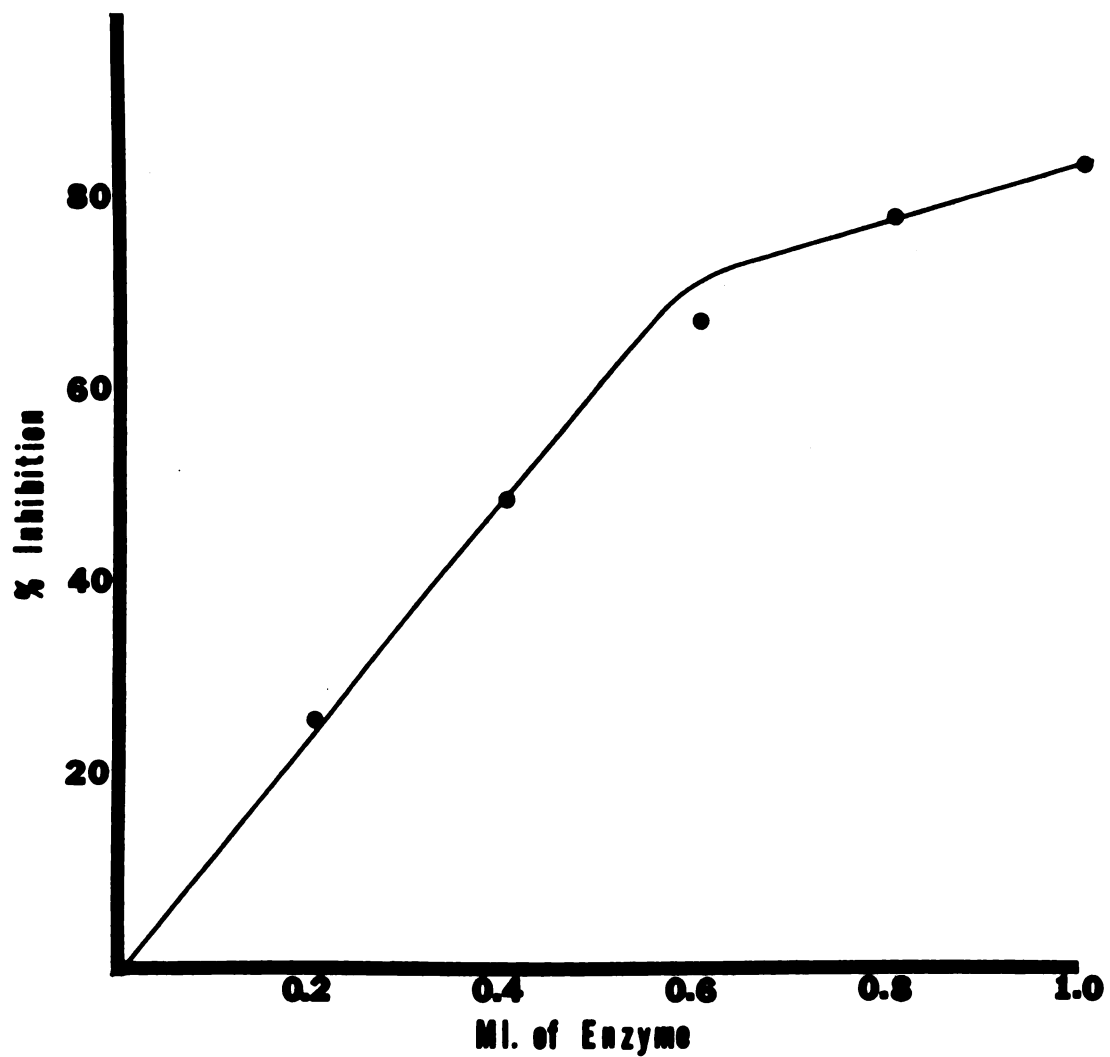


Figure 8. Aspergillus oryzae protease inhibition standard curve.

Vol. of Enzyme Soln.	absorbance		average
	I	II	
0.2	.273	.273	.273
0.4	.501	.517	.510
0.6	.688	.694	.691
0.8	.808	.814	.814
1.0	.861	.874	.868

2. Aspergillus oryzae inhibitor activity

- a) Three ml of inhibitor solution is pippetted into one group of a triplicate set of test tubes along with 3 ml of stock A. oryzae protease solution. The test tubes are then allowed to stand for 10 minutes. Then 2 ml of reaction mixture is placed in each of the other two tubes of the triplicate with 6 ml of 5% TCA added to one group to serve as a blank.
- b) The triplicate set of test tubes is then placed in a heated water bath at 35 C. Two ml of stock casein solution is added to each tube and allowed to react for 30 minutes. Then 6 ml of 5% TCA is added to each experimental tube to terminate the reaction.
- c) After thorough mixing the tubes are left for 30 minutes before filtering and reading at 250 mu against the appropriate blank.
- d) A unit of inhibition is defined as 0.01 absorbance units per 10 ml of reaction mixture under the previously described conditions.

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LITERATURE CITED

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