International Development Research Centre

NUTRITIVE VALUE of TRITICALE PROTEIN

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Wheat × Rye = Triticale

"that it may give seed to the sower, and bread to the eater" [*Isaiah*]

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Nutritive Value

of

Triticale Protein

(and the proteins of wheat and rye)

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Abstract

Triticale promises higher yields of grain than the most productive bread wheats. Advanced triticale lines are at least equal in protein content and superior in biological quality to wheat. Triticale has a higher content of the essential amino acid, lysine, which is first limiting for triticale, wheat, and rye. It is expected that further improvements in biological value will follow, particularly from a more intensive search for genetically superior varieties of rye.

In addition to increasing protein quality by genetic control, lysine content of triticale foods can be improved by supplementation with other materials comparatively rich in lysine, the food legumes offering the most attractive source for the poorer nations. The considerable literature devoted to the processing and supplementation of wheat and wheat flour is largely relevant to triticale.

While more reliable data based upon advanced triticale lines are needed, present evidence suggests that sound, healthy triticale is equal, and possibly superior, to wheat in most farm animal diets. Since triticale will tolerate environments unsuitable for wheat, it promises a significant addition to the world's cereal crops for both human food and animal feed.

Triticale's ultimate contribution cannot be fully realized without a closer collaboration than is customary among plant scientists, nutritionists, and food scientists and technologists. In particular, there is an urgent need for well-defined and universally accepted standards and methods of chemical analysis and biological evaluation by which to select those triticale lines which are genetically superior in protein quality.

Résumé

Les rendements en grain du triticale semblent devoir être plus élevés que ceux des céréales panifiables les plus productives. Les lignées de multiplication du triticale ont une teneur en protéines au moins égale à celle du blé et lui sont supérieures sur le plan des qualités biologiques. La teneur du triticale en lysine, l'acide aminé essentiel qui constitue le premier des facteurs limitants du triticale, du blé et du seigle, est plus importante que chez les autres céréales. L'avenir devrait amener une nouvelle amélioration de sa valeur sur le plan biologique, en particulier à la suite de recherches plus poussées sur des variétés de seigle génétiquement supérieures.

En plus d'une amélioration des qualités protéiques du triticale grâce à la maîtrise de sa génétique, il est possible d'améliorer la teneur en lysine des aliments qui en sont tirés en les complémentant avec d'autres matières premières comparativement riches en cet élément: les légumineuses alimentaires constituent dans ce domaine la source la plus intéressante pour les pays défavorisés. Une très grande partie de la masse de textes consacrés à la transformation et à la complémentation du blé et de la farine de blé est applicable au triticale.

Bien que l'on ait besoin de données plus sûres fondées sur l'étude des lignées de pointe du triticale, les données dont on dispose déjà laissent à penser que pour la plupart des régimes alimentaires des animaux d'élevage, un triticale de bonne qualité est un composant au moins égal sinon supérieur au blé. Etant donné que le triticale tolère des conditions de milieu que ne supporterait pas le blé, il constitue un élément riche de promesses pour un accroissement des disponibilités mondiales en céréales destinées à l'alimentation des hommes et des animaux.

Il ne sera pas possible d'établir pleinement la contribution finale que peut apporter le triticale, sans un renforcement de la collaboration dans ce domaine entre les phytotechniciens, les nutritionnistes et les spécialistes et les techniciens de l'industrie alimentaire. Il est nécessaire notamment de définir rapidement et avec précision des normes et des méthodes universellement acceptées d'analyse chimique et d'évaluation biologique permettant de sélectionner des lignées de triticale génétiquement supérieures sur le plan des qualités protéiques.

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Authors' Preface

Cereal grains are the main source of calories and protein for many people in the developing world. Triticale is a man-made cereal grain, a cross between wheat (*Triticum* spp.) and rye (*Secale* spp.). This publication comprises a review of the literature, a commentary, and a bibliography, relative to the biological value of triticale, and its parents wheat and rye. It concerns itself predominantly with the proteins of these cereals. It is hoped that it will prove interesting and useful to plant scientists, nutritionists, food scientists, and technologists, and others, particularly in developing countries, where triticale may be grown and eaten.

The present state of triticale development is largely the outcome of the Triticale Project, a scientific cooperative venture between the International Maize and Wheat Improvement Centre (CIMMYT) and the University of Manitoba, supported by the Canadian International Development Agency and the International Development Research Centre. The authors wish to acknowledge, with admiration, the work of many plant scientists, particularly those at CIMMYT and the University of Manitoba, whose imaginative curiosity and scientific skill have made possible the advanced state of development of this remarkable new cereal grain.

In the preparation of this manuscript particular thanks are due to Drs J. M. McLaughlan and J. A. Campbell who kindly contributed Chapter 2, Methodology For Evaluation of Plant Proteins For Human Use. The help and facilities extended by Dr C. T. Greenwood, Director of the Flour Milling and Baking Research Association, Dr P. C. Spensley and his colleagues at the Tropical Products Institute, and Prof. J. B. M. Coppock are also acknowledged with appreciation. The helpful editorial contribution of Mr R. L. MacIntyre, and the assistance of Miss Wanda Huff, Mrs Margaret Kovesi, Miss Caroll Rathwell, and Mrs Helen Gay, who typed the manuscript are gratefully acknowledged.

November 1973

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Acres of triticale growing at CIMMYT, El Batan, Mexico

Chapter 1

BACKGROUND AND SUMMARY

Triticale: Its Nature and History

Dr. Norman E. Borlaug, Nobel Peace Prize Winner in 1970 and head of the international wheat research program at the International Maize and Wheat Improvement Center (CIMMYT), Mexico stated in a paper published in October 1973 "... it appears certain that triticales will soon add to the world's food production potential. Because of their good protein and amino acid properties, they may also play a role in correcting protein malnutrition among cereal-eating nations."

Triticale is an artificial genus synthesized by combining the genomes of wheat (genus *Triticum*) and rye (genus *Secale*). If the wheat parent is a tetraploid (*T. durum*), the resultant triticale will be hexaploid (*Triticale hexaploide*); if the wheat is hexaploid (*T. aestivum*), the resultant triticale will be octaploid (*Triticale octaploide*), the rye parent in each case being diploid. So far the hexaploid triticales have proved more stable than the octaploids. It should be emphasized that triticale is not a single species. Triticale is a genus and, like wheat and rye, it embraces many cultivars of widely variable characteristics.

The early history of triticale research and development was described by Zillinsky and Borlaug (1971a), and Zillinsky (1973) has presented a more recent review. A description of progress from many parts of the world, based upon the proceedings of a workshop sponsored by the International Development Research Centre held at CIMMYT in October 1973 will be produced as an IDRC publication.

This publication seeks to bring together what is known about the biological value of the protein of triticale, and as much as appears relevant concerning the biological quality of the proteins of its parents wheat and rye. It is hoped that the publication will be helpful to all plant scientists, food and nutrition scientists, and cereal technologists interested in triticale, and in particular to both scientists and agricultural policy-makers in developing countries where triticale might beneficially join the existing spectrum of established cereal grains.

Triticale is not as new as is sometimes suggested. It has existed as an academic curiosity since the first naturally occurring triticale was described in a report to the Botanical Society of Edinburgh in 1875. When a natural cross is made between wheat and rye the first generation (F_1) plant is normally sterile. It was discovered in 1937 however that when an F_1 seedling was treated with colchicine its chromosome makeup was doubled and the resultant triticale plant was partially fertile. This discovery opened the door to the genetic improvement which has since taken place. Colchicine is an alkaloid present in certain lilaceous plants of the genus *Colchicum* which includes the autumn crocus.

Since triticale is a cross between wheat and rye, it would be expected to inherit characteristics from both of its parents. While this is so, triticale is, without question, a unique plant, and the main purpose of this publication is to review what is known of the factors which influence the biological value of its protein. Since triticale may derive varying degrees of genetic influence from its parents, it was considered necessary to review as much of the literature relating to the biological value of wheat and rye as appeared relevant and potentially useful to the future nutritional improvement of triticale.

The CIMMYT—Manitoba Project

Triticale research began in Canada at the University of Manitoba where in 1954 the Rosner research chair was endowed by the Samuel and

Saidye Bronfman Family Foundation. In 1963 the Manitoba triticale program was extended into a nursery at the CIANO Research Station at Ciudad Obregon, Mexico. In 1965, using the University of Manitoba's material as initial breeding stock, a substantial triticale program was initiated by the Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT) in Mexico, which subsequently has grown into a fruitful cooperative research program between CIMMYT and the University of Manitoba.

This cooperative effort has flourished for several reasons. First, the triticale program benefited from CIMMYT's highly organized, intensive and successful bread and durum wheat research. The genetic base of triticale was diversified and its qualities improved both through a planned program of crossing to CIMMYT's immense pool of wheat varieties and also by an adventitious, promiscuous outcrossing which gave rise to the fertile triticale progeny known as Armadillo.

Second, two generations of triticale could be grown each year, one in Mexico, one in Canada. Third, alternate generations could be grown under widely different environmental conditions, thus enabling the identification of lines with day-length insensitivity and wide adaptability.

The close working relation between CIMMYT and the University of Manitoba gave rise to the Triticale Project now supported by the International Development Research Centre and the Canadian International Development Agency. The objective of the Triticale Project is to develop triticale varieties that will complement wheat, barley, rye and other cereal grains and to provide a new crop well adapted to a wide variety of conditions, a crop capable of substantially increasing food production in the developing countries. Because the primary objective of the Triticale Project is to improve the nutritional welfare of the developing countries, serious attention is being given to the grain's biological value.

While both CIMMYT and the University of Manitoba are cooperating in plant breeding research, the primary responsibility for the development of improved breeding lines of triticale rests with CIMMYT, where, under the direction of Dr. Norman E. Borlaug, the high yielding dwarf varieties of wheat were developed and disseminated to many parts of the world. The scientists at the University of Manitoba are addressing themselves to fundamental studies in cytogenetics, biochemistry, plant physiology and pathology, and cereal technology.

It is doubtful if any group of plant scientists is better aware than the CIMMYT team of the potential for increased productivity in cereal grains. The Food and Agriculture Organization (1970a) data indicate that the yields of wheat in Mexico increased from 8.8 to 28.4 kg per hectare between 1948–52 and 1970. Dalrymple (1972) estimates that the area under high yielding varieties outside Mexico rose from 9,000 to more than 10 million between 1965/66 and 1970/71.

Cereal Grains in Human Nutrition

Wheat is grown over a wide area of the world's arable land but is generally most successful between the latitudes 30° and 60° North and 27° and 40° South. It has been known to grow at altitudes from sea level up to 15,000 feet and at precipitations between 275 and 760 mm. It grows best in temperate and subtropical climates and prefers heavy loam and clay soils (Kent 1966).

Close to 220 million hectares of the earth's surface are given over to the growing of wheat, the total world production being of the order of 343 million metric tons per annum. Roughly 20 million hectares are under rye for a total world production of about 31 million metric tons. No official figures for the commercial production of triticale are readily available but it is estimated that about 500,000 hectares were planted for harvesting in 1971 (Zillinsky and Borlaug 1971b). Triticale testing nurseries are to be found in 52 different countries.

The number of known wheat varieties is very large indeed, more than 16,000 in the World Collection having been screened for protein and lysine content (Johnson and Mattern 1972). The principal wheats grown are the hexaploid *Triticum aestivum* used mostly in bread and baked products, and the tetraploid *Triticum durum* which, in the western world, is particularly popular for alimentary pastes such as noodles and macaroni. The most agronomically successful varieties of triticale so far developed are the hexaploid derivatives of *Triticum durum* crossed with the diploid rye *Secale cereale*.

Considerably fewer varieties of rye than wheat are documented. Rye will grow in roughly the same latitudes as wheat but is much more tolerant of sandy soils and soils of lower fertility (Shands 1969). Consequently, it is hoped the triticale

TABLE 1. Proportion of daily per caput nutritional supply from different sources, 1962 and proposed 1985 levels (data from Food and Agricultural Organization 1969a).

	Africa S. of Sahara		Asia		Latin America		Near East	
	Calories[a]	Proteins[a]	Calories	Proteins	Calories	Proteins	Calories	Proteins
				1962				
Cereals	51.2	53.2	65.9	59.9	40.7	40.0	65.9	67.0
Pulses[b], nuts, seeds	8.9	18.3	9.4	20.0	6.7	15.7	3.7	7.1
Vegetable oils	4.6	–	4.1	–	5.2	–	5.2	–
Other food crops	29.7	12.8	14.3	6.7	30.5	8.0	15.0	3.1
Total plant products	94.4	84.3	93.7	86.6	83.1	63.7	89.8	79.7
				1985				
Cereals	51.3	51.4	61.0	57.5	39.7	39.4	62.6	64.5
Pulses, nuts, seeds	9.4	19.3	9.7	21.4	6.7	16.1	4.0	8.0
Vegetable oils	4.9	–	6.0	–	6.5	–	5.9	–
Other food crops	28.4	12.1	17.3	7.3	30.2	8.1	17.0	6.0
Total plant products	93.9	82.2	94.0	86.2	83.1	63.6	89.5	78.5

[a]All data are estimated average percentages of total diet.
[b]Pulses are dried legumes. It is possible that in this table the term "pulse" includes food legumes in general.

hybrid will demonstrate a greater environmental adaptability than either of its parents and, in particular, that it will grow successfully on what may be described as marginal lands.

As will be detailed later in the text (Table 3), the protein of rye is generally higher in the essential amino acid lysine than is wheat and therefore triticale varieties superior in nutritional value to the common wheat varieties can be synthesized. Wheat, on the other hand, is much more widely consumed than rye in human diets. In Asia, for example, based upon data given in the FAO Production Yearbook 1970, per capita production of wheat increased from 26 kg to 38 kg. The world production and consumption of rye is of a much lower order of magnitude.

The Triticale Project seeks to offer a cereal grain technologically and gustatorily as attractive as wheat, with a biologically superior protein derived from its rye ancestry.

In "Food and the New Agricultural Technology", Palmer (1972) writes about "The final divorce of agriculture and nutrition through the agency of the 'Green Revolution'." Until recently, the working relations between plant scientists and nutrition scientists might better be described as casual transitory affairs rather than durable productive unions, close cooperation between the two having been a rare phenomenon. Within the Triticale Project, however, a close working partnership does exist between the plant scientist and the nutritional biochemist. It is hoped therefore that, in addition to its technical output, the Triticale Project will serve as a model for other future plant improvement projects.

The Triticale Project is designed primarily for the benefit of people in the less developed countries of the world, particularly those who are below, or close to, the margin of dietary calorie and protein sufficiency. One cannot overstate the importance of cereal grains and grain legumes in human diets, particularly in those of the world's poor and malnourished. Cereals, generally, make a larger single contribution to both energy (calories) and protein in the human diet than any other single commodity group. Consequently any significant improvement in the biological value of cereal protein will add significantly to the quality of the diet. Table 1, taken from the FAO Provisional Indicative World Plan for Agricultural Development (Food and Agriculture Organization 1969a), states the approximate proportion of calories and protein supplied by cereals, food legumes and other crops to the average diets of the peoples of

Africa, Asia, Latin America and the Near East. The data include estimates for the year 1962 and the forecast for 1985.

The predominance of cereal grains and grain legumes as sources of both calories and protein is readily evident. It is equally evident that cereal and food legumes are expected to provide most of the calories and protein through the year 1985 and beyond. The authors of the Indicative World Plan forecast that between 1965 and 1985 the population of the poor countries is expected to increase by about 60%. The population pressure, added to other demand pressures, suggest to the authors of the Indicative World Plan that the production of cereal grains and other crops needs to increase by no less than four per cent per annum. At its 59th meeting in December 1972, the Council of FAO expressed "grave concern" over the trend in agricultural production which showed a global increase of only one to two percent in 1971 against a target increase of four percent.

The advent and introduction of the high-yielding varieties of wheat and rice gave cause for cautious optimism that, given reasonably favourable climatic conditions, the total production of cereal grains in the developing countries could be significantly increased. However, the tragic events which occurred in 1973 in West Africa and other parts of the world serve to emphasize how tenuous is the present balance between human need and the availability of the major subsistence crops.

In addition to this precarious calorie balance, there is little evidence to suggest that the overall nutritional quality of the diet, and in particular of its protein content, is improving to any marked degree among the developing nations. The data quoted in Table 1 are overall averages covering large regional populations; it is probable that the poorest and the most malnourished people of these regions derive a significantly higher proportion of their calories and protein from cereal grains and grain legumes than the means quoted.

Cereal protein and legume protein, when eaten together, provide a more nutritious diet than either eaten separately. There is evidence to suggest that because of the higher cash return per hectare from cereals, the areas allocated to grain legumes, particularly in Southeast Asia, are contracting. Palmer (1972) quoting data calculated from FAO Production Year Book 1970, indicates that in Asia between 1948–52 and 1970 per capita chickpea production declined from 6 to 3 kg, soybeans from 1.2 to 0.9 kg and lentils from 0.6 to 0.4 kg. Dalrymple and Jones (1973) state that "The drop in pulses [production in India] reflects a long term decline in production which preceded the 'green revolution' and which is a function of low yields and low prices."

If these trends persist, one can anticipate that the poor people of the developing countries may have to depend upon cereals for an even higher proportion of their future protein calories. Consequently, concern must be given both to the qualitative and the quantitative aspects of cereal protein production in the less developed countries. While it is not suggested that a nutritionally superior triticale plant will solve the protein problem of all or even most of the world's malnourished, triticale can, and hopefully will, provide an additional valuable source of good quality protein and calories.

Any protein source must be considered in relation to the total diet of the specific population for which it is intended. Conclusions based upon average data for large populations should be viewed with caution since significant variations in both the quantity and quality of the protein in the diet are known to occur among different groups within any given population, among families within a group, and even among members within a family.

Differing solutions will be needed for different population groups and, therefore, this review addresses itself not only to the biological improvement of the triticale plant but also to the beneficial and detrimental influences of processing and supplementation. The Triticale Project is in fact as much concerned with how triticale will be used as with how it will be grown. While little is recorded about the processing of triticale, the literature on wheat and rye processing and supplementation is extensive and relevant. It is hoped, therefore, that this aspect of the review will be helpful to the improved future use of triticale.

Nature and Composition of Cereal Protein

The name "protein" is etymologically derived from the Greek *πρωτειοσ* meaning "primary" and is so named because protein is the primary and fundamental material of most living organisms. "Protein" is the name given to a class of organic compounds made up of linked amino acids composed of nitrogen and other elements.

Amino acids are the essential building materials of which all animal tissues and organs are composed and maintained.

Protein is essential to the growth and restoration of body tissue and is particularly important in the diets of young children, pregnant and lactating women, and to aid recovery after serious illness or injury. There is evidence to suggest that serious protein deficiency in early childhood can impede brain development and learning ability (Winick and Rosso 1969, 1970).

Protein is an essential component of a balanced diet which must also be adequate in calorie sources. In a diet deficient in energy sources, the protein present may be used as a source of calories thus leading to an aggravated state of protein malnutrition (Carpenter 1970). The point of Carpenter's comment is that where both dietary calorie and protein deficiencies occur, the protein will be consumed as an energy source thus aggravating the organism's protein deficiency as indicated by body nitrogen balance. It should be noted however that while an inadequate intake of protein can be seriously detrimental to health, no harmful effects have been demonstrated from protein intakes in excess of probable need apart, of course, from the hazards associated with excessive overeating.

The protein content of wheat has been shown to vary widely among different varieties grown under different environmental conditions. As early as 1889 it was reported that protein contents ranged from 7% in wheat grown in Scotland to more than 24% in wheats grown in the Caucasus. Though the precise accuracy of the data is doubtful in the light of modern analytical methods, the wide variability in total wheat nitrogen content is supported in more recent publications. Johnson and Mattern (1972) in their analysis of samples of bread wheat from the World Collection found protein contents to vary from 6.0% to 23.0% with a mean of 12.9% on a dry weight basis. The protein content of rye is believed to vary from about 6.5% to close to 14.5%, the higher values originating mainly in North America (Kent-Jones and Amos 1967).

As is discussed below, both the total nitrogen content and protein composition vary significantly among the endosperm, seed coats (bran) and embryo (germ) of cereal grains. From the data in Table 72 in Chapter 5, calculated from data published by Hinton (1953) for wheat it is evident that while the germ and scutellum fractions contain more protein than any other fraction, they represent so small a proportion (2.5%) of the whole grain that their contribution to the total protein content is indeed small. On the other hand the endosperm plus the surrounding aleurone layer provide approximately 88% of the total protein present in the whole grain. Consequently any research aimed at either increasing total protein or improving the biological quality of the protein should concentrate upon the storage proteins in the endosperm and aleurone. Milling techniques, in particular, should strive to include as much of the aleurone layer as possible. A labelled diagram of a sectioned grain appears in Chapter 5 (Fig. 2).

The early lines of triticale, in common with the progeny of other wide genetic crosses, demonstrated a high frequency of shrivelled grains in which the embryo and bran represented a relatively high proportion of the grain. Since variability in the ratios of endosperm to bran and germ may, in themselves, make for variability in grain nitrogen content and biological value, it is important in reporting protein and amino acid compositions in whole cereal grains to describe the overall physical characteristics of the grain under discussion.

Different proteins are composed of different combinations of amino acids. Some of these amino acids can be synthesized by living organisms from other nitrogenous material, the amino acids which can be synthesized varying among different higher animals. Other amino acids essential to the diet cannot be synthesized in vivo but must be ingested as such. These are known as the "essential" or "indispensible" amino acids and must all be provided by the food eaten. Where all essential amino acids appear together in appropriate proportions in a single protein source, that source is sometimes described as an "ideal" or "perfect" source of protein. As is described in greater detail below, where a particular protein is quantitatively deficient in one or more amino acids, its nutritional or biological value can be improved by the addition of the amino acid in which it is most deficient.

Of the eighteen amino acids most commonly found in natural edible protein, according to Kasarda et al. (1971), ten are generally described as essential for human infants though only nine may be essential for adults. Chickens and turkeys need a total of twelve amino acids. Rats are

TABLE 2. Pattern of amino acid requirements as milligrams per gram protein compared with milk protein (data from World Health Organization 1973).

Amino acid	Suggested patterns of requirement			Reported composition	
	Infant	Schoolchild 10-12 Years	Adult	Human Milk	Cow's Milk
Histidine	14	–	–	26	27
Isoleucine	35	37	18	46	47
Leucine	80	56	25	93	95
Lysine	52	75	22	66	78
Methionine + Cystine	29	34	24	42	33
Phenylalanine + Tyrosine	63	34	25	72	102
Threonine	44	44	13	43	44
Tryptophan	8.5	4.6	6.5	17	14
Valine	47	41	18	55	64
Total	373	326	152	460	504

believed to require the same ten essential amino acids as human beings and are therefore generally used as test animals in determining the biological efficiency of protein sources intended for humans. Altschul (1965) has reviewed the amino acid-compositions of proteins as they relate to biological values, and the FAO/WHO Ad Hoc Expert Committee on Protein and Energy Requirements (World Health Organization 1973) has suggested patterns of ten essential amino acid requirements for school children and adults. It is recommended by the Expert Committee that the additional amino acid, histidine, is required in infant diets. The FAO/WHO recommended levels appear in Table 2 wherein the higher concentration of essential amino acids per gram of nitrogen required in the diets of infants and children is immediately evident.

The biological value of a protein depends upon its content of essential amino acids in relation to the requirement for these same essential amino acids in the species of animal for which the protein is intended. The essential amino acid in which a protein is most seriously deficient is known as the first limiting amino acid.

In addition to the essential amino acids, humans and other animals require non-specific sources of nitrogen from which to synthesize the "non-essential" amino acids needed for body building and maintenance.

Table 3 quotes the average amino acid contents of wheat, wheat products, rye and triticale, this data being derived from an FAO publication based upon a review of a large number of analyses (Food and Agriculture Organization 1970b). The table also cites what the FAO compilers have stated to be the first and second limiting amino acids for wheat, wheat products and rye. In common with wheat and rye the first limiting amino acid in triticale is lysine (Knipfel 1969; Kies and Fox 1970b). Consequently where plant breeding, processing and supplementation research have been related to an improved biological quality, greatest attention has been given to increasing the proportion of lysine.

Scope of the Text and Summary

Following this introductory chapter, the text consists of a review of the relevant literature under four chapter headings. The remainder of this chapter comprises a summary of and commentary upon each of the four succeeding chapters.

Evaluation of Plant Proteins

Chapter 2 has been kindly contributed by Dr. J. M. McLaughlan and Dr. J. A. Campbell of Health and Welfare, Canada. Dr. Campbell was the Chairman and Dr. McLaughlan a member of IDRC's Working Group on Biological Evaluation and both are internationally recognized authorities on the nutritional evaluation of proteins.

TABLE 3. Average amino acid content (*milligram per gram total nitrogen*), (ranges in parentheses) of wheat, wheat products, rye and triticale, determined by column chromatography (data from Food and Agriculture Organization 1970b).

Amino acid	Wheat meal	Wheat germ	Wheat bran	Wheat flour (*70–80% extraction rate*)	Rye whole meal	Triticale (*one sample only*)	Triticale[a] (*1972–73*)
Isoleucine	204 (188–214)	225 (210–252)	209 (193–226)	228 (200–272)	219 (200–242)	239	187
Leucine	417 (371–450)	433 (408–490)	415 (400–432)	440 (376–643)	385 (361–406)	402	450
Lysine	179 (131–249)	407 (369–517)	270 (257–312)	130 (108–194)	212 (151–281)	192	196
Methionine	94 (63–156)	122 (111–164)	102 (89–127)	91 (63–122)	91 (59–181)	87	60
Cystine	159 (111–212)	130 (110–172)	168 (145–189)	159 (123–208)	119 (85–156)	–	79
Phenylalanine	282 (234–338)	257 (239–283)	263 (255–279)	304 (263–346)	276 (250–300)	286	286
Tyrosine	187 (86–225)	194 (185–219)	197 (178–211)	145 (74–218)	120 (76–175)	187	195
Threonine	183 (148–222)	265 (253–294)	223 (164–254)	168 (143–211)	209 (191–231)	169	196
Tryptophan[b]	68 (51–136)	66 (56–78)	80 (85–113)	67 (65–70)	46 (34–88)	–	63
Valine	276 (228–325)	314 (293–342)	315 (233–371)	258 (221–312)	297 (206–343)	274	242
Arginine	288 (234–344)	513 (471–528)	490 (461–540)	221 (162–337)	286 (184–344)	286	382
Histidine	143 (125–163)	180 (165–210)	195 (175–217)	130 (114–151)	138 (125–165)	140	133
Alanine	226 (188–314)	410 (373–430)	347 (319–371)	192 (160–250)	266 (235–302)	227	258
Aspartic Acid	308 (263–338)	576 (537–631)	513 (474–556)	257 (217–337)	447 (383–511)	297	416
Glutamic acid	1866 (1581–2019)	1102 (954–1287)	1282 (1144–1433)	2184 (1512–2733)	1511 (1356–1676)	1860	1528
Glycine	254 (188–275)	393 (375–440)	405 (375–412)	222 (183–271)	271 (250–302)	245	265
Proline	621 (550–736)	343 (290–426)	395 (371–450)	726 (556–849)	586 (517–738)	705	521
Serine	287 (256–319)	303 (262–359)	304 (260–342)	294 (251–377)	270 (250–306)	233	250
First limiting[c] amino acid	Lysine	Isoleucine	Isoleucine	Lysine	Tryptophan		
Second limiting[c] amino acid	Isoleucine	Tryptophan	Lysine	Threonine	Isoleucine		

[a]Calculated by the authors from data provided by Dr. E. Villegas and representing the means of three advanced triticale lines (6387a, 11453a and 1142a) (tryptophan 2 samples only) produced at CIMMYT in 1972-73.

[b]Microbiological assay.

[c]The first and second limiting amino acids are as stated in the FAO publication.

Perhaps the most important general conclusions to be drawn from McLaughlan and Campbell's Chapter 2 is the urgent need for:

(*a*) an internationally standardized and accepted methodology by which to evaluate the biological quality of cereal and legume proteins;

(*b*) a clear differentiation between (i) approximate methods proposed for early identification of promising plant materials, and (ii) more precise biological methods by which to make a final and definitive evaluation;

(*c*) a standardized terminology and universal system of recording, computing and evaluating analytical results related to protein content and quality, and amino acid composition;

(*d*) improved micro and preferably non-destructive methods of analysis for protein and essential amino acids in early selections of cereal grains and legumes, where only very small quantities of seed are available.

Equally important is the need for a much closer working relationship in the future than in the past between the plant scientist and the nutrition scientist and, also, between the food scientist and the nutrition scientist. While it is fully recognized that the currently available methodology is inadequate and has many shortcomings, little is to be gained by introducing new, seemingly more convenient, methods, if these new methods are inaccurate, indiscriminating and irrelevant to the analytical purpose intended.

The need for international standardization of both methodology and terminology will be apparent to anyone who studies in depth the literature reviewed. Some authors see fit to mention neither the methods employed nor the basis upon which their results are expressed. It need hardly be emphasized that protein determined by N × 6.25 and expressed on a dry weight basis will give a much higher result than N × 5.7 at 14% moisture. For example let us assume, on a dry weight basis, that N, by Kjeldahl, equals 2.50:

N × 6.25 (at zero moisture) = 15.62% protein

N × 5.70 (at zero moisture) = 14.25% protein

N × 5.70 (at 14% moisture) = 12.00% protein

The highest result (15.62%) is 30% higher than the lowest (12.00%).

There appears to be some measure of disagreement, even among knowledgeable analysts, concerning appropriate nitrogen conversion factors. For example, Tkatchuk (1969) proposes conversion factors for wheat (N × 5.61) and rye (N × 5.64) somewhat lower than those recommended by WHO (*see* Table 5). Tkatchuk recommends a factor of N × 5.76 for triticale.

A universally accepted convention of expressing the amounts of individual amino acids present is also needed. Different authors quote, for example, lysine in terms of (i) % of protein (ii) % of total dry matter (iii) gram (or mg) per gram N (iv) gram per 16 g N (v) micromoles per gram N (vi) micromoles per gram protein.

Convention (iv), g/16 g N, assumes a constant protein conversion factor of N × 6.25 for all foods (6.25 × 16 = 100). For cereals, however, a conversion factor between 5.7 and 5.8 is more appropriate hence lysine should be expressed as g/17.25 or g/17.5 g N. This is obviously a clumsy convention and therefore g (or mg) lysine/g N is to be preferred.

McLaughlan and Campbell refer to several other methods requiring universal standardization including, for example, the method of protein hydrolysis before amino acid determination. This need for a standardized methodology is referred as a matter of urgency to the International Union of Pure and Applied Chemistry, the International Union of Nutrition Sciences, and the United Nations Protein Advisory Group.

The need for micro non-destructive methods of analysis has been repeatedly urged by plant breeders seeking to develop cereals and legumes of improved nutritional quality. The plant breeder has available only a very small quantity of material from his early lines, consequently methods of analysis and selection which call for only a few seeds are a primary requisite. The methods will be of considerable merit if they can be applied without damage to the seed's viability.

This point again emphasizes the need for the closest possible working relation between the plant scientist and the nutritional biochemist through the whole sequence of new varietal development. A deeper and continuing understanding of the plant breeder's difficulties might serve to obviate some of the unreasonable requests made of him by nutritional scientists who, in some instances, do not appear to appreciate the complexity of changing the biochemical characteristics of a plant without prejudice to its other essential qualities. At the same time, a close cooperation would discourage plant breeders, motivated by a justifiable sense of frustration, from adopting

analytical and biological methods of selection which, though economic and convenient, may prove misleading and in the long run counter-productive. Much scientific effort may well be wasted if the nutritional biochemist waits until the final stages of varietal development before becoming actively interested in plant breeding programs.

McLaughlan and Campbell review both chemical and biological methods of evaluation pointing out the virtues and deficiencies of each method discussed. They describe the underlying principles and the factors which affect alternative methods of biological evaluation including, among others, Protein Efficiency Ratio (PER), Net Protein Utilization (NPU), Net Protein Ratio (NPR) and Biological Value (BV). They emphasize that the final evaluation of a cereal or legume must be by a biological (animal feeding) method, at more than one level of protein intake, as described in their discussion of the Growth Slope assay method.

This point is further emphasized by Scrimshaw and Young (1973) who state that in well-nourished young men the utilization of wheat gluten nitrogen is relatively high at low levels of intake but falls off at higher levels even though these higher levels are still within the body nitrogen sub-maintenance range. They refer to other studies of wheat gluten utilization which support the conclusion that, for proteins limiting in lysine, the efficiency of nitrogen utilization drops markedly with increased protein intake well before requirement levels are reached. Scrimshaw and Young state "It is clear that more information about the ability of a protein to meet protein needs is obtained from the slope ratio approach than from the assay of a protein at any single level of intake." They go on to state that while it may not be necessary to abandon entirely single protein level biological assay methods, the differences between the nutritive values of proteins of high and low quality are more clearly demonstrated with the slope ratio method.

The points made by McLaughlan and Campbell and by Scrimshaw and Young emphasize the plant breeder's need for several standardized methods: first, those which can be used for early sorting of new lines; second, methods which are more precise and comprehensive to be used as sufficient plant material becomes available; and finally, biological methods by which to determine definitively the overall biological quality and suitability for use of the cereal or legume variety developed. Wherever possible the final definitive evaluation should be made using the animal for which the cereal is intended.

McLaughlan and Campbell review several methods of determining protein nitrogen and specify the conversion factors appropriate to different plant protein sources. They discuss amino acid analysis by chemical and microbiological methods, the calculation of Chemical Score and the means of identifying limiting amino acids. They make reference to amino acid availability, pointing out that some of the amino acids recorded by ion exchange chromatography may be "unavailable" nutritionally when the protein is fed to an animal.

The biological methods of protein evaluation described include those which depend upon body weight gain and those which depend upon nitrogen retention in test animals. The slope assay referred to in Chapter 2 depends upon body weight gain; the slope assay described by Scrimshaw and Young (1973) depends upon carcass nitrogen retention. It would appear that the controversy among nutritional biochemists and physiologists concerning the relative merits of biological methods based upon weight gain versus those based upon nitrogen retention may continue for some time.

This clearly emphasizes the need for an internationally recognized body such as the Protein Advisory Group of the United Nations, in consultation with the appropriate international scientific unions, to recommend and publish, for the immediate guidance of plant breeders, acceptable and applicable methods of biological selection and evaluation.

At the conclusion of Chapter 2, McLaughlan and Campbell, recognizing the plant breeders' need, present a series of recommended procedures by which to determine the biological value of cereal and legume proteins. Briefly, where small samples of grain are available from early lines, they recommend the following determinations:

(i) Total Kjeldahl nitrogen.

(ii) Amino acid content by ion exchange chromatography or amino acid auto-analyzer.

(iii) The limiting amino acids, based upon FAO/WHO reference patterns.

(iv) Net Protein Ratio assay using 5 rats for 10 days.

For a more complete biological evaluation where larger samples are available, they recommend a Growth Slope assay at no less than three levels of protein intake.

The remainder of the text covered in the review falls into two broad categories:

A. Research by plant scientists.

B. Research by food and nutrition scientists.

Even a cursory review of the subsequent text will reveal a lack of uniformity in content, considerably more having been published on certain aspects of the subject than others. Since most of the research published has been undertaken in North America and the developed nations of Europe, the literature heavily emphasizes bread wheats. Furthermore, greater attention has been given to those factors which influence economic value (including yield capability of the grain, and water absorption and "baking strength" of the derived flours), than to nutritional values of wheat and rye proteins. This review deals almost exclusively with the nutritional aspects of the subject.

Though plant scientists have striven for many years to increase yield potential and total protein content, since both are factors of economic importance, until very recently they have shown little interest in amino acid composition as it relates to the biological value of the cereal grains. The food scientists and cereal technologists have occupied themselves largely with the functional properties of cereal grains and with the development of labour saving and more profitable methods of breadmaking and other processing technologies. Those nutritionists who have explored the biological value of cereals appear to have been more interested in fortification and supplementation than in the possible influence of genetic background, agronomic and environmental history upon nutritional value.

Varietal and Environmental Factors

Chapter 3 describes the influence of genetic, varietal, environmental and agronomic factors upon the biological value of the protein of triticale and its parents. Both wheat and rye are used as food and feed grains and as forage crops, and it is probable that triticale will be similarly used. The review specifically related to triticale attempts to cover comprehensively its protein content and biological value for humans and other animals. In reviewing the wheat and rye literature, emphasis has been placed upon those protein factors which influence biological value in human nutrition since the literature relating to varietal and environmental influence upon nitrogen content, particularly in wheat, and its use in feed and forage, is so vast as to be virtually unmanageable in a review of this kind. Consequently, this aspect of the text has been confined to a few basic references.

GENETIC INFLUENCE

The chapter begins with a brief discussion of the complexities of plant breeding and genetic influence as they relate to cereal protein composition. Since the precise composition of the embryo protein is vital to the plant's survival, any intended genetic manipulation must concentrate upon the storage proteins of the endosperm. The discovery in maize of certain genetic mutations which depress the prolamin content of the protein and thereby increase the proportion of lysine and other essential amino acids, together with the advent of new processing technologies which are less dependent upon high gluten contents, encourage the continued search for high lysine (prolamin depressant) genetic mutations in wheat and rye.

Protein content and composition vary among different fractions of the cereal grain seed. In selecting for high protein genotypes it would appear more meaningful to select on the basis of protein content per seed rather than protein as percent dry matter. Protein as percent dry matter is influenced by seed weight and the relative proportions of the various seed fractions present. These in turn are influenced by environment and agronomic conditions.

The literature reviewed clearly indicates that while the potential to deposit a high proportion of protein nitrogen in the endosperm of triticale, wheat and rye is controlled genetically, the amount actually deposited, (i.e. whether the grain achieves its full potential for protein production) is greatly influenced by environment and agronomic management. Because of these strong environmental and agronomic influences, any potentially superior genetic material must be widely tested and confirmed before a final judgment is pronounced.

While there appears to be some difference of opinion, there is evidence to indicate that the protein of triticale possesses its own unique

characteristics and its spectrum cannot be regarded as a simple addition of the protein spectra of its two parents. Nevertheless, all relevant factors being equal, the analysable protein nitrogen content of triticale appears to fall between the protein contents of its two immediate parents. This appears to be also true of the proportion of essential amino acids present, the lysine content of triticale being generally higher than in wheat but lower than in rye.

One of the most interesting observations comes from Riley and Ewart (1970) who report a significant interchromosomal interaction when different pairs of rye chromosomes were added separately and in turn to a wheat genotype. The content of several amino acids in triticale appeared to be influenced by interchromosomal interaction whereas others were not. The chromosomes in homoeologous group 5 appeared to be of particular interest in promoting a higher lysine content.

LYSINE IN TRITICALE

As is detailed more precisely for wheat, the lysine content of triticale expressed as percent of total protein is generally inversely correlated with protein as percent dry matter. Lysine as percent total dry matter is positively correlated with protein expressed on the same basis.

From the outset of the Triticale Project, triticale lines have demonstrated lysine contents superior to wheat. This superiority is evident again in the preliminary screening of CIMMYT's 1972/73 selections where lysine (as percent of protein) reportedly ranged in sixteen samples from 3.72 (13.0%) to 4.35 (13.0%), the figures in parenthesis being protein (Kjeldahl N × 5.7) contents. Villegas (Zillinsky 1973) reports advanced (1972-73) triticale lines at CIMMYT equal in lysine content but significantly superior in protein content to opaque-2 maize.

BIOASSAYS OF TRITICALE

The biological value of triticale varieties has been assessed in a number of animals. In human feeding experiments, in terms of nitrogen retention, triticale appeared superior to wheat at two levels of protein intake. Lysine was established as being the first limiting amino acid. Lysine was also first limiting in rat feeding experiments in which, as assessed by PERs, triticale appeared superior to wheat. On the other hand, in laboratory mice, methionine was reported as first limiting both for triticale and for wheat.

At the University of Manitoba, triticale was compared with wheat and several other cereal grains in feeding tests with rats, laboratory mice and the meadow vole (*Microtus pennsylvanicus*). Though the standard error was lowest with the rats, both rats and mice clearly indicated the superior biological value of triticale over wheat at two protein levels. The results from the voles appeared totally unreliable, the standard error being of the order of 50% of the means. Furthermore, the voles rated triticale and other cereal grains biologically superior to casein, the standard protein of comparison.

TRITICALE FED TO FARM ANIMALS

In trials with chicks, the results are variable among several reported experiments, the variability being probably attributable in some degree to the varying composition and proportion of dietary ingredients other than triticale and the cereals with which it was being compared. In general, however, it appeared that triticale was at least equal to, if not better than, wheat and that lysine was the first limiting and methionine the second limiting amino acid. The difficulty of evaluation arose, in some instances, since soya protein was used to bring all test diets to the same nitrogen level. It is probable in such instances that any differences between triticale and other cereals are obscured by differences in soya protein content. Also, as a general comment on animal feeding trials, too little attention appears to have been given, or at least recorded, concerning the characteristics of the triticale used, particularly with regard to the size and condition of the kernels, and to what degree the triticale was free from ergot contamination. From the data derived from early triticale lines it would appear that the utilization of triticale and wheat protein by chicks are roughly equivalent. In general, and in the light of some recent data, triticale, if free from ergot, appears to be a satisfactory cereal for inclusion in the diets of broilers, laying hens and turkey poults.

The earlier results of hog feeding trials suggest that triticale is equal to barley for heavier animals but inferior in the diet of lighter pigs. Some authors report reduced palatability and loss of appetite when hogs were fed large proportions of triticale. Again, several authors do not indicate whether the triticale used was clean or infected. Where ergot infection was clearly demonstrated,

marked reductions in feed intake and weight gain were recorded. Some authors report triticale as being satisfactory as a feed for growing-finishing swine but one author recommends that triticale be restricted to 25% of the ration for growing pigs, to 50% of the ration for finishing pigs, and that it be not recommended in hog starter rations or for breeding stock. Others report triticale, when fed as 95% of the diet of hogs, as being equal to wheat and better than barley. There appears general agreement that lysine is the first limiting amino acid in triticale for swine.

Triticale when fed to dairy calves resulted in small reductions in feed intake and weight gain compared with barley-urea and barley-soya. The relative feed efficiencies were reported as not significantly different. Again, no reference was made to possible ergot contamination. Several trials with steers demonstrated lower feed consumption with triticale though the protein digestibility and feed efficiency of triticale appeared superior to both wheat and sorghum. Feed intake improved when the triticale was steam-rolled. Some authors reported a higher incidence of liver abscesses in steers fed triticale. Steam-rolled triticale appeared equal to steam-rolled barley for lactating Holsteins. In general, triticale was reported at least equal to wheat in sheep rations.

It must be borne in mind that many of the animal feeding studies reviewed are based upon early triticale lines most of which originated from breeding trials and yield nurseries. Furthermore there is a marked paucity of information concerning the overall quality and characteristics of the triticales tested. It is therefore desirable that many of the animal feeding studies be repeated using advanced genotypes of known history and identification.

ADVANCED TRITICALE LINES

Considerable progress has been made during the recent past in increasing triticale yields, in reducing the incidence of grain shrivelling, and in improving kernel characteristics. Yields of triticale in excess of 8000 kg/ha, significantly higher than the highest yielding bread wheats grown under comparable conditions, are reported from CIMMYT. As reported above, the CIMMYT advanced lines continue to demonstrate comparatively high lysine contents.

However as kernel characteristics have improved, the ratio of endosperm to bran has increased and the average protein content has noticeably declined. During the late 1960s protein contents (N × 5.7) of hexaploid triticale lines grown at CIMMYT ranged from 11.7% to 22.5% with an average of 17.5%. These early lines included a large proportion of shrivelled kernels. The advanced hexaploid lines grown at CIMMYT during 1972 ranged in protein from 10.9% to 19.1% with an average of 13.4%. It should be noted, however, that in the earlier lines the lysine content (percent of protein) ranged from 2.5% to 3.7% with an average of 3.2%. Samples from 1972 trials ranged in lysine from 2.6% to 3.9% with an average of 3.4% (Zillinsky 1973). As stated above, preliminary screening of 1972-73 materials indicates a continued improvement in lysine content.

While there is still much to be learned about the precise influence of genetic background upon the protein content and composition of triticale, there is adequate evidence to predict with confidence that triticale will take its place as a high yielding, high protein cereal crop of comparatively superior amino acid composition and of satisfactory digestibility and feed efficiency.

WHEAT PROTEIN

The literature on wheat is so vast that neither time nor space permit a comprehensive review. Consequently, the text consists of a review of certain important publications drawn from as many geographical areas as possible.

From the extensive literature relating to wheat, certain clearly defined patterns and relationships are evident. For example, among wheats containing protein content (N × 5.7) up to about 14%, lysine expressed as percent protein is significantly negatively correlated with protein expressed as percent dry matter. Above 14% protein the negative correlation is not significant and disappears entirely above 16% protein content. Lysine expressed as percent of total dry matter in the grain is positively correlated with grain protein content. The highest lysine value reported from the World Wheat Collection is about 4.3% of protein.

The total protein nitrogen deposited in the endosperm is greatly influenced by environmental and agronomic conditions, therefore, as stated above, it is necessary to grow any apparently "high protein" wheats over several years in a variety of locations in order to establish a truly genetic high protein capability. Threonine, generally considered the second limiting amino

acid in wheat, and leucine, appear to be positively correlated with lysine.

Within the World Wheat Collection, protein contents on a dry weight basis range from 6 to 23% and lysine as percent of protein from 2.2 to 4.3%. While no high lysine gene in wheat comparable to the mutations found in maize has yet been discovered, it is important to note that in terms of essential amino acids expressed as percent of total dry weight matter, high protein wheats provide more of all essential amino acids than low protein wheats.

Neither protein nor lysine contents in wheat appear influenced by the size of the kernel provided it is plump, healthy and adequately filled. Shrivelled and deformed kernels with a high bran and germ to endosperm ratio tend to give high protein and high lysine analyses.

The influence of environmental conditions upon wheat protein nitrogen content is well established. Significant varietal differences in response to nitrogen fertilizer, indicating genetic control of total protein content, have been recorded in many publications. In general the response to increased nitrogen fertilizer is an increase in the prolamin fraction. Several authors have reported a linear relation, given the necessary genetic potential, between protein nitrogen content and the rate of nitrogen fertilizer applied. Though increase in fertilizer increases the proportions of proline and glutamic acid which predominate in the prolamin fraction, neither environment in general nor fertilizer application in particular appears to influence the relative proportions of the essential amino acids present in wheat protein.

Some evidence is presented that certain herbicides lead to an increase in total protein nitrogen but they do not appear to affect essential amino acid composition.

RYE PROTEIN

Several authors have described how the biological value of rye protein is superior to wheat protein. Since rye is of minor significance in world dietary patterns compared with wheat, rice and maize, comparatively little research has been devoted to improving its agronomic and biological properties. A more thorough examination of varietal influence upon protein content and quality in rye might well prove fruitful in breeding nutritionally superior triticale varieties.

The response of rye to nitrogen fertilizer appears to parallel that of wheat, in that grain nitrogen increases with the addition of nitrogenous fertilizer, the main increase being in the prolamin fraction. In general, the literature indicates higher lysine values, expressed as percent of protein, in rye than in wheat, the highest encountered in this review being 5.3%.

Nutritional Inhibitors and Toxic Factors

Chapter 4 describes toxic substances and nutritional inhibitors naturally present and acquired in the cereal grains under discussion. In general, too little scientific attention has been given to such substances to permit a clear understanding of the mechanism of their physiological action in humans and other animals or to the overall importance of their influence upon human and animal nutrition.

Resorcinol derivatives have been reported in rye, triticale and wheat, generally being found in highest concentration in rye and lowest in wheat. They appear to be concentrated in the pericarp and therefore appear in higher proportion in shrivelled than in well-formed, plump grains. There is some, but by no means complete, evidence that their presence is influenced both genetically and environmentally.

The symptoms of resorcinol action appear to be a depression of appetite and a lowered food intake. Resorcinols have been shown to be present in both rye bran oil and wheat bran oil and to be significantly reduced in activity during baking and steam-rolling. In Chapter 5, which deals with processing, it is suggested that some attention might be given to removing the pericarp by controlled abrasion milling. This might also serve to remove undesirable microorganisms attached to the outer grain surface.

Phytic acid and its derivatives are not known to be of importance in protein metabolism but, since phytic acid is associated with the higher protein fractions of wheat and rye, it deserves mention. Phytic acid forms insoluble salts with the dietarily important elements calcium, iron, magnesium and zinc, thus rendering them unavailable to the organism. Phytic acid is enzymatically destroyed during dough fermentation and during certain stages of cooking and baking. Its importance should not be overlooked in those less developed countries where the phytic acid present in high

extraction cereal flours may seriously affect the absorption of calcium, iron, magnesium and zinc present in infant diets.

Trypsin inhibitors are present in a wide variety of edible plant seeds including wheat, rye and probably triticale. While their action may be of significance in raw cereals fed to animals, it is not unlikely that they are of minor importance in cooked cereals.

Factors other than resorcinol derivatives which depress growth have been reported present in rye. Their action appears to be offset by antibiotics and by autoclaving in water or mild acid. It is possible that these unknown substances may stimulate the growth of undesirable intestinal microflora but their nature and mode of action have not been positively identified. There is some evidence to suggest that, unlike the resorcinols, these unknown growth depressants are located in greater concentration in the endosperm than in the bran.

Ergot is one of the most serious contaminants to which triticale appears susceptible. This susceptibility is inherited from its rye parent and also from the tetraploid *T. durum* from which most existing triticales are derived and which is more susceptible to ergot than the hexaploid *T. aestivum*. Ergot is considerably influenced by environment and is virtually unknown in some of the regions where triticale might be grown. In several reported instances, both feed intake and weight gain have been inversely related to the concentration of ergot.

A considerable volume of literature has appeared in recent years concerning aflatoxins. Apart from the serious acute and chronic toxicity symptoms attributable to the presence of aflatoxins, the presence in grain of the mould fungi responsible has been shown to reduce significantly the proportion of total amino acids and of certain essential amino acids originally present. Other mould fungi may cause a loss in protein quality in cereal grains and grain legumes by selectively destroying some essential amino acids.

In addition to the mycotoxins reviewed in Chapter 4 there may be others as yet unidentified. A recent verbal communication to the authors indicates that some species of the genus *Fusarium* known to infect triticale, are able to produce mycotoxins.

Tolerance limits for ergot and mycotoxins are extremely difficult to prescribe since the extent of their physiological action is influenced by the animal species, its size, age and general health and by the total quantity of food consumed. Where tolerance limits are recommended it is essential that the required methods of sampling, inspection and analysis also be precisely defined.

Processing and Supplementation

In Chapter 5, which deals with the technologies of processing and supplementation, very little is to be found on how the biological value of triticale is influenced by milling, baking, other processing, or by supplementation with additional protein sources. On the other hand, there is a substantial literature relating to the biological value of processed wheat and rye products, with and without nitrogenous supplements, much of which is relevant and applicable to triticale. Since virtually all cereals are processed before being eaten, and since the supplementation of cereal flours when processed into bread, other baked products or alimentary pastes, is neither novel nor technically difficult, the influence of both processing and supplementation is believed to be relevant and germane to this review.

Once again, the task would be less difficult if all of the authors reported could have employed a standardized methodology and terminology and shown a greater attention to detail in describing both the cereals used and the methods by which they were processed and analyzed. One might suggest that if the research is worth doing, it is worth the effort to determine the nature and history of the cereal grains and other raw materials used and to specify the conditions of processing. The importance of describing the physical characteristics of cereal grains used in nutritional studies, and in particular whether they are plump or shrivelled has already been emphasized.

MILLING OF TRITICALE

There is insufficient data on which to base any definitive conclusions concerning the milling qualities of triticale. Though triticale appears to respond satisfactorily to conventional laboratory milling technologies, the comparatively high proportion of inadequately filled kernels among early lines has tended to give lower milling yields. Villegas (Zillinsky 1973) refers to milling tests using a laboratory mill in which flour yields ranged from 51.7% to 59.0%, compared with the control wheat INIA 66 which yielded 83.7% flour.

In the light of the considerable improvement evident in the kernel characteristics of advanced triticale lines it is recommended that more attention now be given to milling and other technological characteristics. Both CIMMYT and the University of Manitoba are examining the technological and functional properties of triticale. In addition, in cooperation with the International Union of Food Science and Technology, an international working group has been formed to explore how triticale might best be processed and used in cereal foods in various countries throughout the world.

It is recommended in the text that triticale be subjected to abrasion milling to remove the seed coats of the pericarp and thus reduce the resorcinol content together, hopefully, with most of the undesirable microorganisms present on the surface of the grains.

Of particular interest are a few results obtained from air classification experiments in which triticale lines displayed significantly higher protein shifts than either wheat or rye. No evidence has been presented to suggest that the amino acid composition of high protein, air classified flour is essentially different from triticale flours processed by conventional milling.

WHEAT AND RYE FLOURS

Flours from low protein soft wheats tend to be higher in lysine, arginine and several other essential amino acids than high protein strong wheat flours. The lysine content of wheat flours, and probably triticale flours, decreases with decreasing extraction rate since the inner bran and, in particular, the aleurone layers are richer both in total protein and lysine than the inner endosperm. Lysine is the first limiting amino acid in both wholemeal and white (endosperm) flours and in the unsupplemented bread made from them. From the point of view of protein content and biological quality, 85% extraction is significantly superior to 75% extraction flour.

The addition of bran to white flours increases body weight gain and biological value in rats though the addition of bran alone has not, as judged by PERs, been shown to equal the optimum supplementation of white flour with synthetic lysine and threonine.

While there is some variation among other essential amino acids, the availability of lysine in wheat and milled flour are not greatly different. The digestibility of uncooked wheat and rye flours decreases with increasing extraction rate. Consequently, the biological superiority of high extraction versus low extraction endosperm flours is somewhat offset by reduced digestibility. In rye, as in wheat, the percentage protein content and the lysine both as percent protein and as percent total dry matter, increase with extraction rate. Similar trends are evident in triticale. To what degree the improved protein pattern of higher extractions flours is offset by decreased digestibility, appetite-depressant and other inhibiting factors deserves serious attention among the more advanced triticale lines. The nutritional value as influenced by processing conditions needs to be examined in the diets of a variety of animals.

BAKING WITH TRITICALE

A few authors have reported that satisfactory bread can be baked from flours containing high proportions of triticale flour though none of the research reported gives any account of the biological value of the bread produced. Since most of the triticales available are hexaploid and derived from a durum wheat parentage, in addition to which alpha-amylase activity tends to be higher in triticale than in wheat, it is not to be expected that triticale will, by conventional fermentation methods, produce bread equivalent to flour from bread wheats. Nevertheless some results suggest that by adopting new breadmaking technologies, including those based upon mechanical development, satisfactory bread is possible from triticale flours.

Villegas (Zillinsky 1973) reports that acceptable tortillas and chapatis can be made from 100% triticale flour. A private communication indicates that triticale flour has been found acceptable as a 75% replacement for teff and other grains in traditional Ethiopian cereal foods.

Though little is published on the processing of triticale, the effect of baking upon its nutritional value would not be expected to differ greatly from rye and wheat. Baking of both wheat and rye flours reduces the available lysine content, the loss, all other things being equal, being a function of time and temperature. In bread, therefore, the greatest reduction occurs in the crust, and lysine losses are highest in bread in which the crust to crumb ratio is highest. Rye crisp breads, for example, suffer greater damage to protein biological value during baking than do "soft" rye breads. Bread baked by

microwave heating is biologically superior to oven baked bread in that the time of microwave baking is extremely short and no crust is formed.

According to several authors, there is no significant decline in protein value when high extraction wheat flour (atta) is baked into chapatis. It is suggested that any lysine loss is compensated by improved palatability and digestibility. It may also be that chapati doughs are proportionally lower in reducing sugars than fermented bread doughs in which maltose is known to be formed. During baking, reducing sugars react with, and render, lysine partially unavailable. Puris, which are fried in oil, were lower in PER than chapatis baked from the same atta.

OTHER PROCESSED CEREAL FOODS

Both the available lysine and biological value of pastas (macaroni, spaghetti, etc.) decline with increasing drying temperature. In breakfast cereals the biological value does not appear damaged by cooking in water but may be lowered by toasting, puffing and high temperature extrusion. There is evidence that steam or hot water cooking of wheat followed by drying, as in the production of bulgur, significantly increases the metabolizable energy and may improve the biological value of the protein, though some authors report no improvement in protein quality between bulgur and the original wheat.

Fermentation of cooked wheat and mixtures of wheat and soybeans by *Rhizopus* organisms found in Indonesian tempeh, significantly increased the protein value, fermentation of wheat flour plus soya flour giving biological values almost equal to casein.

PROTEIN SUPPLEMENTATION

The review of supplementation deals only with nutritional implications and covers supplementation by synthetic amino acids, cereal protein concentrates, egg and milk proteins, legume and oilseed proteins, microbial ("single cell") proteins and proteins derived from fish. The technological consequences of adding various protein supplements have recently been reviewed elsewhere (Hulse, in press).

Amino Acids Since lysine is demonstrably, for humans, the first limiting amino acid in wheat, rye and triticale flours, most of the literature relating to synthetic amino acids focusses upon lysine, usually administered as L-lysine hydrochloride. Weight gain in rats is roughly proportional to the L-lysine added to wheat flour up to a level of between 0.2 and 0.25 g lysine per 100 g wheat flour at which point lysine ceases to be the first limiting amino acid. Several papers report the significant loss of added lysine during baking, probably attributable to reactions with reducing sugars present. The unavailability of added lysine in general increased with increase in baking time. In infants fed wheat semolina some benefit of adding lysine and potassium together was demonstrated.

When lysine and threonine, the second limiting amino acid, were added to wheat flour to the point of maximum nutritional efficiency, the resultant wheat bread protein was rated "first class", that is, essentially nutritionally complete. Added lysine improves the nutritional value of most wheat products including bread of various western and oriental kinds, alimentary pastes (macaroni, etc.), chapatis, and biscuits (cookies). The beneficial effect of added lysine appears most marked in the rate of weight gain in rats but improved nitrogen retention with added lysine is also demonstrable. It is also reported, at least over short periods of time, that added methionine improved an all-cereal diet fed to adults. Additions of methionine plus lysine were reported to be beneficial in certain wheat diets.

The need for further research into the role of the "non-essential" amino acids present in cereals is stressed by several authors. Some results indicate that added lysine gives rise to a higher food intake by rats. The reliability of results based upon weight gain in young rats fed amino acid supplemented cereal diets has been questioned by one recognized authority.

Cereal Proteins Supplementation with cereal proteins has, until recently, focussed largely upon added gluten, produced by washing out the starch from a wheat flour dough. While added gluten increases total protein, it does not significantly improve nutritionally the balance of amino acids in any of the cereals under discussion. The nutritional benefit of adding supplements derived from wheat germ and wheat bran is reported by several authors.

The methods and benefits of producing and using wheat protein concentrates are described. The most recent concentration processes entail the fine grinding and screening of certain selected mill feed fractions which are demonstrably higher in both total protein nitrogen and lysine content than the ground endosperm. The benefit to rate of

weight gain and nitrogen retention of such wheat protein concentrates is reported by several authors.

Milk Proteins Apart from the increase in total protein nitrogen, the beneficial effect of adding egg or milk proteins appears to be proportional to the resultant increase in lysine. It is claimed that, as judged by PER, the addition of 6% nonfat dry milk (skim milk powder) is roughly equivalent to the addition of 0.17% lysine. One author reports that 18% of skim milk powder is necessary to achieve a biological value equivalent to the addition of L-lysine and L-threonine added to the point at which each cease to be limiting. Significant losses in PER were reported when wheat flour supplemented with milk was baked into bread. Skim milk powder significantly improved the nutritional value of alimentary pastes, though there is evidence that some leaching of soluble proteins may take place during cooking in hot water.

Legume Proteins For the less developed world, the food legume and oilseed proteins offer the most attractive source of supplementation. Since most of the literature reported originated in North America, soya flour is dealt with at greater length than any other legume protein source. It is worth emphasizing that "soya flour" is by no means a universally homogeneous material and that its biological value is significantly influenced by processing conditions. Some evidence indicates that within limits the biological value of soya protein improves with heat treatment, possibly as a result of the destruction of trypsin inhibitors and other antinutrient factors. Soybean isolates produced by alkaline extraction followed by acid or heat precipitation are of significantly lower biological value than defatted heat-treated soya flours.

Though the evidence is much less weighty there are clear indications that other oilseed and legume proteins can serve as useful supplements to cereal products. Defatted cottonseed flour and proteins derived from chick-pea, pigeon pea and other tropical legume flours have been shown to be nutritionally beneficial. In most instances the benefit, apart from the increase in total protein nitrogen, appears to be roughly proportional to the lysine added.

Microbial Proteins Yeast and other microorganisms offer potentially useful sources of protein supplementation. The level to which they can be usefully added appears to be limited, not so much by their amino acid content but by the concentration of nucleic acids present, excessive ingestion of which by monogastric animals leads to undesirable physiological effects, including kidney stones. The need for more research into the effects of high intakes of nucleic acids from microbial protein has been recommended by several authorities (United Nations 1968). While there are methods available for removing nucleic acids the resultant increase in cost might prove prohibitive for developing countries.

Fish Proteins A good deal of attention has been given to proteins derived from fish, particularly fish protein concentrate produced by extraction with isopropanol or other organic solvents. Unfortunately, all of the literature does not clearly differentiate among the various levels of refinement possible in producing fish protein concentrates. In general, fish protein is a good source of lysine and any significant additions to cereal products improves the biological value in proportion to the amount of lysine added when the diets are judged isonitrogenously. The economic feasibility of producing fish protein concentrates which are sufficiently refined to be acceptable in odour and flavour when blended with cereal products has yet to be demonstrated.

Benefits and Cost of Supplementation In some of the literature reviewed it appears to be assumed that any and all forms of protein supplementation are desirable. It must be emphasized that an ill-informed approach to protein supplementation, particularly with synthetic amino acids, may give rise to undesirable protein nitrogen imbalances, particularly if the supplemented cereal provides most of the protein calories consumed. The problems of amino acid imbalance have been discussed by Bender (1965), Carpenter and de Muelenaere (1965), Harper (1969), Harper and Rogers (1965), Kies and Fox (1972), Sanahuja (1971), Muramatsu et al. (1972). However, where protein malnutrition is apparent or likely to occur, appropriate supplementation of a predominantly cereal diet with an inexpensive protein source is to be recommended, particularly for young children.

In general, as judged by PER and other indices based upon body weight gain, the nutritional benefit derived from supplementing processed wheat, rye or triticale with other protein sources is proportional to the amount of lysine added until the point at which lysine ceases to be the first limiting amino acid. McLaughlan and Morrison (1960) report that in rat diets in which

half the protein was supplied by bread and the other half by each in turn of (*a*) oatmeal, (*b*) whole dried eggs, (*c*) dry beans, (*d*) casein, (*e*) cheese and (*f*) fish flour, there appeared an almost perfect correlation (r = 0.99) between PER and total lysine content, the PERs increasing progressively from (*a*) to (*f*), unsupplemented bread being the lowest, bread plus fish flour the highest.

It might be concluded therefore that the sole purpose of supplementation should be to add lysine until it ceases to be limiting and that this might be best achieved by adding L-lysine hydrochloride. It must be remembered, however, that many of the comparisons reviewed in Chapter 5 are based upon PERs determined in isonitrogenous diets. In practice, however, different protein supplements which contribute the same quantity of lysine will add significantly different amounts of total protein nitrogen to the diets. Altschul and Rosenfield (1970) state that 0.25% is the level at which lysine ceases to be the first limiting amino acid in wheat flour. Table 4 gives (*a*) the approximate quantities of various protein supplements in grams per 100 grams of flour necessary to provide 0.25 grams of lysine and (*b*) the approximate percent increase in protein which would result if such additions were made to a cereal flour of 12% protein. It is evident that all supplements other than lysine will significantly increase the total protein content.

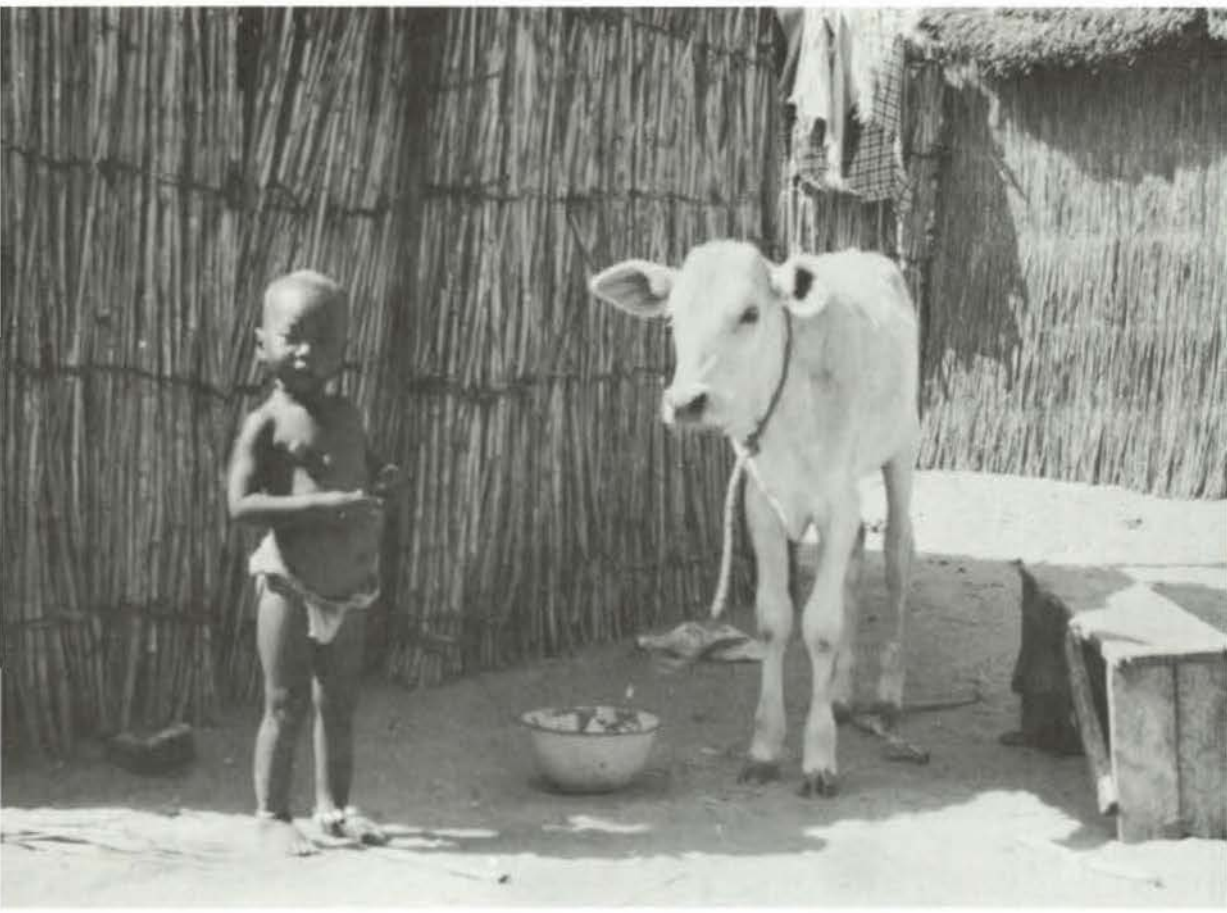
Furthermore, the cost of supplementation to the same level of lysine will vary greatly among protein sources. Hulse (in press) estimated in 1971 that the ingredient cost of adding 0.25 lb of lysine to 100 lb wheat flour in Canada varied from nearly $7.00 for dried egg through $2.30 for skim milk powder, $0.56 for defatted soya to $0.25 for L-lysine hydrocholoride. Obviously these costs will differ among countries and will change from year to year. At the moment of writing (October 1973) all of the relevant prices are markedly higher than in 1971.

Though L-lysine hydrochloride appears least expensive it is available from only one country, Japan. Continued use by another nation would therefore call for a continued expenditure of foreign currency. Many developing nations would doubtless encounter technical and logistical problems in uniformly distributing and dispersing lysine throughout all of their home-produced cereal flours. Nor does added lysine increase, as do the other supplements, the total quantity of protein, food calories and other nutrients. Furthermore if, for example, the cereal flour costs 10 cents per kilo and an added legume flour costs 10 cents per kilo there will be no increase in cost per kilo of the final supplemented mixture. Unless the cost per unit weight of the supplement is equal to, or lower than, the cost of the cereal flour, any degree of supplementation will inevitably raise the cost of the final product.

TABLE 4. Effect on protein content of various supplements to wheat flour.

	(*a*) Approximate quantity necessary to add 0.25 g lysine (*g*)	(*b*) Approximate increase in protein of adding (*a*) to 100 g wheat flour of 12% protein content (%)
Egg (whole dried)	9.0	19
Milk (nonfat dried)	9.5	17
Soya (defatted)	8.0	23
Cottonseed (defatted)	10.0	36
Peanut (defatted)	15.0	40
Chick-pea flour	17.0	12
Fish protein concentrate	3.0	18
Yeast (dried)	6.0	18
Wheat protein concentrate	24.0	17
Lysine	0.25	0

It is the authors' opinion that where necessary, and as far as circumstances permit, the poor nations would be best advised to study seriously the means of supplementing cereals with flours derived from indigenous or locally adapted legumes which generally are much cheaper than most other protein supplements. It is the authors' intention, in the near future, to prepare a review of the nutritional value of certain important tropical food legumes and the products derived from them. The authors also propose to prepare a review similar in scope to the present publication in which the biological values of the sorghums and millets will be considered.



". . . triticales will soon add to the world's food production potential" (Norman Borlaug)

Chapter 2

Methodology for Evaluation of Plant Proteins for Human Use

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Many workers, including a recent expert group (United Nations 1971), have stressed the need for increased intake of good quality protein, particularly by certain segments of the population in developing countries. Probably the simplest way to meet this need is to develop new high protein varieties of crops presently grown in the country or region in question, so that problems of use and acceptability are minimized. Plant breeders have accepted the challenge and have attempted to improve not only the yield, but also the protein quality and protein content of staple cereal and legume crops for human use (Protein Advisory Group 1971).

In any program of crop improvement, consideration must, of course, be given to the possible impact of the program on the nutritional status of the population of the country or area. Thus, it may not constitute good nutritional policy to replace legumes with cereals simply because high yielding cereals can be produced. (Protein Advisory Group 1972a). Such a course of action may reduce, rather than increase, the intakes of some essential amino acids. A very clear assessment must therefore be made first of the nutritional needs of the particular area or country involved and of the possible impact of any plant breeding program on the nutritional status of the population. The program should then be set up to meet these needs.

Protein quality may be defined as the capacity of a protein or a mixture of proteins to satisfy the requirements of the consuming animal for essential amino acids. If the food under study is for human use, the requirements of the test animal should bear a close relationship to those of humans (Campbell and McLaughlan 1970; Bressani et al. 1973).

Because of the fact that the protein value of a food depends primarily on the content of the limiting amino acid, which may vary from food to food, the problem of protein evaluation has been a difficult and controversial one. Thus, many methods have been recommended and used and their relative merits and limitations discussed at length (Campbell 1963; National Academy of Sciences-National Research Council 1963; McLaughlan and Campbell 1969; Porter and Rolls 1973).

In the development of new cereal and legume varieties, protein evaluation revolves largely around a consideration of the lysine, methionine, threonine and tryptophan content of proteins, but may, however, be subject to particular difficulties. Some of the problems involved have been reviewed in recent documents of the Protein Advisory Group (1971, 1972b, 1973). However, in view of the specific problems related to the needs of plant breeders, it was considered necessary in a review such as this to include a section on protein evaluation. It is therefore the purpose of this chapter to present a critique of existing methodology and to suggest appropriate procedures for the evaluation of protein in cereal grains and legumes for human use. Other recent reviews are available (McLaughlan and Campbell 1969; Porter and Rolls 1973).

Need for Methods

The need for nutritional and food quality guidelines, including methods for protein evaluation,

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has been clearly pointed out (Protein Advisory Group 1971). The plant breeder is faced with the fact that he must test small quantities of newly developed lines at the earliest possible opportunity for a variety of qualities including yield, agronomic characteristics and nutritive value for the intended end use be it human food or animal feed. Ideally, methods are therefore needed which will be applicable to individual seeds to evaluate their possible nutritive value and the presence of toxic or harmful substances. While this may not yet be practicable, there is an urgent need for the development of methods applicable to very small samples – at least as screening methods.

In the development and use of such screening methods, it is essential that their meaning and limitations be known and recognized and that the assays be properly standardized. Until appropriate methodology can be developed for small samples, recognition must be given to the fact that larger samples may have to be used for the assessment of protein value and that such testing may have to be conducted at a later stage of the breeding program. It is also obvious that careful consideration must be given to the priorities involved in testing. Thus, if one of the prime purposes is to increase protein value of a crop, protein testing should be initiated at an early stage in testing new lines. On the other hand, if yield is most important, nutritional testing may be given lower priority. It must be recognized, however, that if the new lines are to be used for human food, they must be subjected to critical nutritional tests, including appropriate bioassays. These tests should establish not only the nutritional value of the cultivar but also its freedom from toxic substances.

Total Protein Analysis

Total or crude protein content is normally determined by the Kjeldahl method as specified by the Association of Official Analytical Chemists (1970). This procedure involves the oxidation of the organic matter in the sample, and the reduction of organic nitrogen to ammonium sulphate followed by the quantitative determination of ammonia. For cereals and legume foods the digestion mixture of preference is mercuric oxide, dipotassium sulphate and sulphuric acid (Association of Official Analytical Chemists 1970). Methods of protein analysis have been discussed in considerable detail by an International Conference in 1963 (National Academy of Sciences-National Research Council 1963).

TABLE 5. Factors used for converting nitrogen to protein (World Health Organization 1973).

Foodstuff	Conversion factor
CEREALS	
Wheat	
Whole meal or flour or bulgur	5.83
Flour, medium or low extraction	5.70
Macaroni, spaghetti, wheat pastes	5.70
Bran	6.31
Rice	
Husked or brown (only hulls removed)	5.95
Home-pounded, undermilled, parboiled	5.95
Milled, white	5.95
Rye	
Whole meal, dark flour	5.83
Flour, medium extraction	5.83
Flour, light, low extraction	5.83
Barley	5.83
Whole seed, except hulls and groats	5.83
Pearled, light or dark	5.83
Oats	5.83
Oatmeal, rolled oats	5.83
PULSES AND SEEDS	
Groundnuts	5.46
Soya bean, seeds, flour or products	5.71
Sesame, safflower, sunflower	5.30
OTHER FOODS	6.25

The nitrogen content of any plant material is a mixture of both protein and non-protein nitrogen. To arrive at the protein content of a cereal, the nitrogen content as determined is then multiplied by a conversion factor to give the percentage protein. The conversion factor used is based on the nitrogen which the particular protein contains. The conversion factor employed is not the same for all cereals nor is it always stated in the literature. A recent compilation (World Health Organization 1973) gave the recommended conversion factors listed in Table 5. Tkachuk (1969) recommended a conversion factor of $N \times 5.76$ for triticale. For samples containing appreciable amounts of non-protein nitrogen the protein content may be more accurately estimated by using the summation of the total weight of amino

acids determined by ion exchange chromatography (see next section).

It may be concluded that the first step in any evaluation of protein is the determination of total or crude protein in the sample by the Kjeldahl method. For screening samples of limited size, semi-automatic micromethods are available.

Amino Acid Analysis

Protein Hydrolysis

In order to determine the amino acid content the protein must first be hydrolyzed. There are no official methods for either hydrolyzing proteins or for determining their concentration in the hydrolysate. Blackburn (1968) has selected what he considers the most appropriate procedures for amino acid analysis. Generally, a weighed, finely ground sample is hydrolyzed in 6N HCl for 20–24 h at 110–115°C under nitrogen in sealed glass test tubes or in Teflon bottles with screw caps. The hydrolysate is made up to a given volume, filtered, and an aliquot is evaporated to dryness in a rotary evaporator. The residue is dissolved in citrate buffer (pH 2.2) and diluted for subsequent analysis either by microbiological assay or automated amino acid analyzer.

The effects of acid hydrolysis on different amino acids in various plant foods must be recognized. It is important to obtain full release of amino acids while keeping their degradation to a minimum (Tkachuk and Irvine 1969). It must be realized of course that acid hydrolysis yields total amino acid content and gives no indication of nutritional availability of the amino acid. Since tryptophan is completely destroyed during acid hydrolysis, other methods of hydrolysis must be employed when assaying for this amino acid. The newer procedures use the enzyme Pronase (Calbiochem, Los Angeles, U.S.A.) (Spies 1967; Wall et al. 1971).

Ion Exchange Chromatography

The most satisfactory method for determining amino acid composition is ion exchange chromatography. Most laboratories conducting amino acid analysis use modern automated amino acid analyzers employing the ion exchange principles originally developed by Moore and Stein (1951). Since analyses may not include tryptophan, proline, hydroxyproline and cystine, 100% recovery of amino acid nitrogen cannot be expected. Because the apparent recovery of protein (sum of individual amino acids divided by nitrogen × appropriate factor) varies considerably even among different pure proteins, Knipfel et al. (1971) suggested adjusting the total recovery to a constant value of 90%. However for protein sources containing a high proportion of non-protein nitrogen much lower recoveries are possible.

Microbiological Assays

Microbiological assays are less satisfactory than ion exchange chromatography for complete amino acid analyses. They are, however, convenient for estimating certain individual limiting amino acids such as lysine, methionine, threonine and tryptophan in large numbers of samples. The authors have obtained satisfactory assays for the above four amino acids using a commercially prepared amino acid assay media (Difco), and a 72-h incubation period followed by titration of lactic acid. Microbiological assays have the advantage that they require relatively inexpensive equipment which includes an autoclave, an incubator (or water bath), and general laboratory glassware. Although these assays are relatively simple, the results are affected by many factors including age and number of cells in the inoculum, variation in temperature during incubation, location within the incubator, and contamination with other bacterial species. A useful reference book is by Barton-Wright (1952). Other references relating to microbiological assays include Schiaffino (1959) and Horn et al. (1953).

Amino Acid Units

The amino acid content of protein is expressed in various ways. Older tables of amino acid content (Block and Weiss 1956) gave amino acid values in grams amino acid per 16 g nitrogen, i.e., essentially in terms of percent of protein. This assumes, incorrectly, a constant conversion factor of 6.25 whereby to convert "nitrogen" to protein ($16 \times 6.25 = 100$). Other ways include micromoles per unit of nitrogen or of protein. The convention used by the Food and Agriculture Organization (World Health Organization 1973) is milligrams amino acid content per gram total nitrogen or protein.

Available Amino Acids

It may be concluded that hydrolysis of proteins is probably the most critical analytical step in the

estimation of amino acid content. The methodology appropriate will depend on the number of amino acids being investigated and equipment available. It must also be recognized that in some foods, particularly those which have been heat processed, all the amino acids present may not be fully available. Obviously, there is need for universal testing and adoption of methods, particularly for protein hydrolysis. Until a universally acceptable methodology is agreed upon some contradictions and confusion must be expected among different determinations of the amino acid contents of cereals and other foods.

Chemical Score

The quality of protein in a food is dependent largely on the amount of the limiting amino acid present in the protein. "Limiting amino acid" has been defined (National Academy of Sciences-National Research Council 1963) as the essential amino acid of a protein which shows the greatest percentage deficit in comparison with a standard. Various ways of expressing this deficit have been suggested. The chemical score method (CS) was originally proposed by Mitchell and Block (1946) for predicting the quality of proteins from the amino acid profile. Because amino acid requirements were not established at the time of Mitchell and Block's proposal, egg protein was chosen as a reference standard since it had a known, high biological value. It was assigned a value of 100 and the assumption was made that the amino acid profile of egg contained the same proportions of essential amino acids as the requirement pattern of the animal. Since egg apparently has too much methionine and cystine (McLaughlan et al. 1959; Rao et al. 1964) in relation to the ideal amino acid requirement for the growing rat, its use as the reference standard exaggerates any deficiency of these amino acids in proteins under test. Other problems in the use of chemical score have been reviewed recently by Bender (1973).

At present a variety of amino acid reference patterns (egg, human milk, and estimated human amino acid requirement pattern) are employed in arriving at a chemical score. Although human amino acid requirements would seem to be the logical standard, this pattern is based on exceedingly variable data (Irwin and Hegsted 1971). In 1965 a joint FAO/WHO expert group (World Health Organization 1965a) recommended egg or human milk amino acid patterns as the standards. However, a recent joint FAO/WHO Expert Committee (World Health Organization 1973) has proposed a provisional amino acid scoring pattern which contains considerably reduced amounts of the sulphur-containing amino acids.

The calculation of CS by this method is shown in Table 6 (World Health Organization 1973).

TABLE 6. Calculation of chemical score for cereals, soybean and a mixture of maize and soybean: amino acid content calculated from data in Food and Agriculture Organization (1970b).

Amino acid	World Health Organization (1973) Requirement pattern (*mg/g protein*)	Wheat		Maize		Soybean		Maize–soybean (1:1 protein mixture)	
		Amino acid content (*mg/g protein*)	Chemical score	Amino acid content (*mg/g protein*)	Chemical score	Amino acid content (*mg/g protein*)	Chemical score	Amino acid content (*mg/g protein*)	Chemical score
Lysine	55	31	56	27	49	70	127	48	88
Threonine	40	31	77	36	90	42	105	39	97
Methionine and cystine	35	43	123	35	100	28	80	31	88
Leucine	70	72	103	125	180	85	121	105	150
Isoleucine	40	35	88	37	93	50	125	44	109
Valine	50	47	94	48	96	53	106	50	100
Phenylalanine and tyrosine	60	81	135	87	145	89	148	88	147
Tryptophan	10	–	–	7	70	14	140	10	100

Using the definition given above, the CS for lysine in wheat is $(31 \div 55) \times 100 = 56$. The lowest score indicates both the limiting amino acid and the extent of the amino acid deficiency. Lysine is shown to be the limiting amino acid in each of the cereals but threonine is the second limiting in wheat whereas tryptophan is the second limiting in maize. The information on triticale available at the present time is insufficient to compute a reliable CS along lines comparable to those quoted for other cereals. Based upon the limited analytical data available, triticale would appear to have a CS for lysine of about 64. It is customary to combine the sulphur-containing amino acids in the one score; these are seen to be limiting in soybean protein. It is interesting to note the supplementary relationship when maize and soybean protein are combined. The CS of 88 for lysine and for methionine plus cystine is better than the score of either protein source alone. It should be pointed out that this pattern places less emphasis on sulphur amino acids than do any of the previously recommended reference patterns.

Despite the variety of amino acid reference patterns which have been suggested, many studies have confirmed the general validity and usefulness of CS for expressing protein quality. It is strongly recommended that the most recent reference pattern in Table 6 should be used. It must be remembered however that amino acids in some foods, particularly heat processed ones, may not be fully available, in which event the CS which is calculated from total amino acids, not total available amino acids, will reflect a higher biological value than is in fact the case.

Amino Acid Availability

There are several causes of reduced amino acid availability but probably the most common is simply poor digestibility of the food. Nutrients in plants may not be completely digested because of interference by the cellulosic cell walls. The anti-trypsin factor in soybeans is well known (Liener 1969) but this factor may also be present to some extent in certain cereals (Couch and Hooper 1972). Moderate heating inactivates the trypsin inhibitor, thereby increasing the digestibility of the protein. Excessive heat treatment, however, renders the protein resistant to digestive enzymes, and even in roller dried milks there is frequently a reduction in the availability of lysine (Mauron 1961).

TABLE 7. Available lysine in heated casein–glucose mixtures (Rao and McLaughlan 1967).

Autoclaving time (*min*)	Available lysine		
	DNFB (*chemical*)	Lysine bioassay (*rat*)	Gross Protein value (*rat*)
0	6.10	7.09	7.53±0.26
5	5.62	6.98	6.22±0.36
10	3.50	3.71	4.26±0.55
20	2.11	–[a]	1.47±0.58
40	1.94	–[a]	0.48±0.29

[a]Growth was too erratic to estimate lysine availability.

When a protein source is heated with other foods, reactions occur between free amino groups in the protein with the sugars, aldehydes and fatty acids, which may limit the availability of the amino acids. The rapidity of the reaction between lysine and glucose during heating is illustrated in Table 7 (Rao and McLaughlan 1967). A mixture of casein and glucose in water was autoclaved for varying periods. Available lysine was estimated using rats by a direct lysine bioassay and by Gross Protein Value. The dinitrofluorobenzene (DNFB) procedure of Carpenter and Ellinger (1955) for estimating available lysine was also conducted. Each of the three methods indicated a marked fall in lysine availability with as little as 10 min heating time. Because of its rapidity, the DNFB method is valuable in determining available lysine.

DNFB Method

The DNFB method is based on the reaction between DNFB and the free epsilon amino group of lysine in proteins. Unavailable lysine, i.e. when linked with sugars or other compounds, is not free to react with DNFB. On acid hydrolysis, the product of the reaction between lysine and DNFB, dinitrophenyl-lysine is released and can be measured spectrophotometrically. The original method is not satisfactory, however, for high carbohydrate foods such as cereals (Conkerton and Frampton 1959) and modifications have been proposed. Rao et al. (1963) separated DNP-lysine quantitatively from interfering components by ion exchange chromatography; this modification has been used successfully on plant products (Wall et al. 1971).

Enzymatic Hydrolysis

Since amino acid availability may be related to reduced susceptibility to enzymatic activity, several methods, based on the hydrolysis of proteins by enzymes have been suggested, e.g. Mauron (1961). The basis of these methods is to simulate the sequence of proteolytic enzymes that operate in vivo. The sequence followed is usually digestion with pepsin at pH 2.0 followed by trypsin and erepsin at pH 8.0. Hydrolysis of protein is usually not complete even though it is continued for 2–3 days. A variety of methods (Mauron 1961) have been employed to assess the extent of amino acid release, but none of these methods has achieved widespread acceptance. Enzymatic digestion combined with a microbiological assay for a specific amino acid such as methionine was recommended by Boyne et al. (1967) to assess amino acid availability in high protein supplements for the animal feed industry.

Microbiological Methods

Ford (1962) reported that the proteolytic bacterium *Streptococcus zymogenes* NCDO 592 was suitable for estimating the availability of methionine, leucine, isoleucine, valine, tryptophan, arginine and histidine. The bioassay system measured the amount of each specific amino acid released enzymatically by a preliminary treatment of sample with papain and by the exocellular enzymes secreted by the test organism. The method was subjected to a collaborative assay and it appeared suitable for measuring amino acid availability (Boyne et al. 1967). Unfortunately the assay organism does not require lysine and is therefore unsuitable for estimating available lysine; Ford (1964) recommended *Streptococcus durans* for this purpose. These methods are basically sound but some difference of views exist regarding the preliminary enzymatic treatment (Szmelcman and Guggenheim 1967).

Shorrock and Ford (1973) reported experience with the protozoon *Tetrahymena pyriformis* for the assay of available lysine and methionine. Using the method of Stott and Smith (1966) they concluded that while the *Tetrahymena* assay can predict accurately the results of growth tests with rats, it would be premature to recommend its general use to replace the bioassay.

Effect of Heat Processing

It may be concluded that the availability of amino acids is much more of a problem in heat-processed foods containing sugar than in cereal products that are subjected to relatively mild heating. Nevertheless, amino acid availability is one of the chief criticisms of CS which assumes complete availability. Thus, the determination of available amino acid content of protein is an essential test for the analysis of processed foods.

Since factors such as availability may influence the apparent value of protein in foods, it is important that there be a critical evaluation of protein by rat bioassays. Several methods have been suggested and their advantages and disadvantages critically discussed (National Academy of Sciences-National Research Council 1963: McLaughlan and Campbell 1969; Pellett 1973).

Biological Quality

Protein Efficiency Ratio

One of the most widely accepted procedures for determining protein quality is the Protein Efficiency Ratio (PER) method in which rats are fed under specified controlled conditions for a prescribed time. The PER is defined as the ratio of body weight gain (in grams) to the weight (grams) of protein consumed. Since the results obtained are influenced by factors such as the age of rat, length of assay period, level of protein and sex of rat, these conditions must be standardized. Such standardized procedures are set out in official methods in the United States of America (Association of Official Analytical Chemists 1970) and Canada (Food and Drug Laboratories 1966). In both these assay procedures, the results are computed in comparison with a standardized casein sample to which is assigned a PER value of 2.5. The standardized procedure prescribes the age of the male rats to be used, a 4-week assay period on an ad libitum diet containing 9 or 10% protein on a dry weight basis and adequate in other essential nutrients.

In view of the influence of the factors noted above, the term PER should be applied only to results obtained from standardized assays. In reporting data, any variations from the official methods should be clearly recorded. If animals other than rats as prescribed are used for protein evaluation, the term PER should not be used.

Although the PER method has achieved widespread acceptance, it may be criticized from several aspects. Thus, it has been pointed out that (a) there is not a proportional relationship between

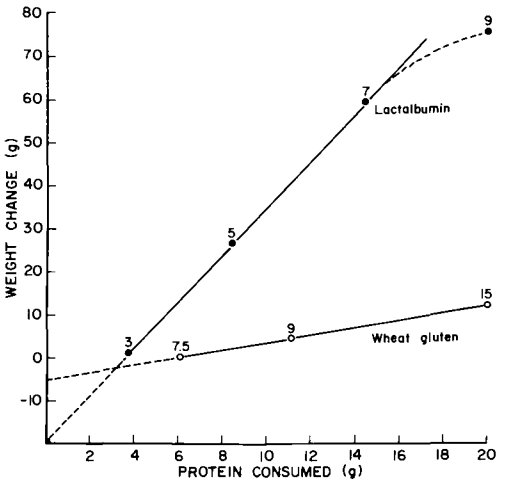


Fig. 1. Weight change of rats fed three levels of lactalbumin and wheat gluten.

one PER value and another, i.e. a PER of 2.0 is not twice as good as a PER value of 1.0, (b) the results may be influenced by level of food intake, (c) maximum PER values are not obtained at the same dietary protein level for different proteins, (d) the method makes no allowance for body tissue maintenance but assumes that all protein consumed is used for growth. In spite of these shortcomings, the PER method, until recently, has been considered the simplest and most generally applicable method (McLaughlan and Campbell 1969). Its apparent simplicity may, however, have encouraged persons lacking a full appreciation of its inherent complexities to report results obtained without due regard to the standardization necessary. Because of its widespread acceptance a description of the method recommended for use in Canada is given in Recommended Methods at the end of this Chapter.

Some of the factors which influence PER assays are shown in Fig. 1 in which body weight change is plotted against protein consumed at three levels for lactalbumin and wheat gluten. Since PER is defined as the ratio of grams of weight gain divided by grams of protein consumed, the PER at 9% protein level for lactalbumin is 75.0 ÷ 20.0 = 3.75. PER values at 3, 5 and 7% protein levels were 0.25, 2.95 and 3.93% respectively. PER values for wheat gluten at 7.5, 9 and 15% protein levels were 0, 0.41 and 0.60, respectively. Although PER is arbitrarily measured at the 9-10% level of protein, this level does not give the maximum value for either lactalbumin or wheat gluten but usually does for casein. It is clear that the highest dietary protein level gives the largest PER provided the point falls on the linear portion of the growth response curve. Over this portion of the curve the protein required for body tissue maintenance is of lesser importance than that required for growth.

Net Protein Retention

To make allowance for body tissue replacement and maintenance, the Net Protein Ratio (NPR) assay was proposed by Bender and Doell (1957). The NPR is equal to the mean weight gain of the test group plus the mean weight loss of a group of rats fed a non-protein diet divided by the average protein consumed by the test group. Thus, for lactalbumin (Fig. 1) fed at a 9% protein level the NPR = (75 + 20) ÷ 20 = 4.74. For wheat gluten the NPR at the 9% protein level is 2.21 and at 7.5% protein the value is 3.54. Thus NPR is also influenced to some extent by the percentage protein level. Details of the procedure are given in Recommended Methods at the end of this chapter.

Net Protein Utilization

The Net Protein Utilization (NPU) assay described by Miller and Bender (1955) is similar to NPR except that body nitrogen rather than body weight is the parameter measured. Bender and Doell (1957) found that NPU and NPR methods correlated closely (r = 0.99) among a variety of 35 foods. Because of the carcass nitrogen analyses required, the NPU assay is not as amenable as the NPR assay to routine testing of a large number of samples and there is evidence that it may be more variable (Chapman et al. 1959).

Slope Ratio Assay

Hegsted and Chang (1965a) proposed the slope ratio (SR) assay in which determinations are made at several dietary protein levels. The slope of the growth response line (i.e. the straight portion from zero protein intake up to the point where the line begins to curve towards the horizontal) of the test protein is expressed as a percent of the corresponding slope for a lactalbumin reference protein. This method has the advantage that the assay defines the slope of the response curve and furnishes an estimate of the error of the assay and its validity.

In Fig. 1 the slope of the line up to the 7% protein (lactalbumin) intake is $(59 + 20) \div 14.5 = 5.45$. Evidently, the slope does not change with different protein intakes up to the point at which the growth response curve begins to flatten. The growth response curve for lactalbumin could be extended to cut the Y axis at -20 g (i.e. the weight loss of the group of rats fed the non-protein diet). The growth response curve for wheat gluten cannot be extended to cut the Y axis at -20 g and consequently the SR assay for wheat gluten is invalid. Similar difficulties have been experienced with other lysine-limiting cereal proteins (Said and Hegsted 1969; Yanez and McLaughlan 1970). This problem seriously limits the usefulness of the SR assay.

It was found from an analysis of available data (McLaughlan and Campbell 1972) that the slope ratio assay could be improved by omitting the group of rats fed the non-protein diet. This finding was also made independently by Hegsted (1972) from the results of a collaborative assay. This modified slope ratio assay which we have called the "growth slope" (GS) assay is essentially the same as the Nitrogen Growth Index of Allison (1964).

In the GS assay the true slope of the response line is used. The slopes (Fig. 1) for lactalbumin and wheat gluten would be 5.45 and 0.86 respectively giving a GS assay value for wheat gluten of $0.86 \div 5.45 \times 100 = 16$. There is at least one problem with the GS method; the response curve for peanut protein tends to have the same slope, and thus the same GS value, as casein yet it is inferior to casein for maintenance purposes. This peculiarity is believed to result from a relatively high requirement of the rat for threonine (and possibly for sulphur-containing amino acids) for maintenance purposes. This aspect of the GS method requires further study. In view of the need to standardize and recognize protein methodology officially, it is urged that this method be so evaluated at an early date.

It is also evident from Fig. 1 that wheat gluten appears to be equivalent to lactalbumin at low protein intakes. The reason is that the relative lysine requirement for maintenance is low compared to its requirement for growth of new tissue. Consequently some proteins, particularly cereal protein, may be more valuable for body maintenance purposes than for growth (Campbell and McLaughlan 1970). The opposite may be true for threonine in peanut protein. Thus, the results of bioassays depend partly on the level of protein in the diet as well as upon which amino acid is limiting in the protein. Particular care needs to be taken therefore in assessing quality of cereal proteins. Despite the shortcomings of rat bioassays, results on a series of protein sources including poor, moderate and good proteins showed close correlation between results of bioassays using rats and humans (Campbell and McLaughlan 1970; Bressani et al. 1973).

Bioassays—Conclusions

It is concluded that in spite of the inherent difficulties of rat bioassays, they must be considered an essential part of any comprehensive testing program. While the PER method is simple and reproducible, there appear to be significant advantages to the use of the GS assay in terms of validity and reliability. Protein values remain constant at different levels of protein intake. Although further investigation is needed, the GS assay is recommended at this time as the most reliable method for the assay of the protein quality of cereal grains and legumes. Where the quantity of cereal or legume to be assayed is small, a single level NPR assay using five animals in a 10-day assay, is a valuable preliminary screening test. Whatever method is selected, it must be standardized and carried out according to recognized procedures. Details of GS assay, NPR and PER methods as recommended by the authors are given in the Recommended Methods at the end of this Chapter. Variations in these procedures should not be used unless carefully tested and specified.

Rapid Screening Methods

Microbiological Methods

Because of the need to test large numbers of small samples the plant breeder has a particular interest in rapid screening methods. From time to time various methods utilizing the growth of protozoa, bacteria or insects, primarily because of their apparent simplicity, have been suggested as being useful for distinguishing between various levels of protein quality for specific foods. Since the protozoon *Tetrahymena pyriformis* requires

the ten amino acids essential for the rat and is capable of digesting intact proteins, its use seemed to offer particular possibilities. The collaborative assay reported by Boyne et al. (1967) made it clear, however, that this organism requires further study before it can be recommended as a rapid screening assay for protein quality. Haenel and Kharatyan (1973) have stated that results from *Tetrahymena* and from other microbiological methods for determining relative nutritive value should not be considered an absolute measure of protein quality without additional confirmation.

Halevy and Grossowicz (1953) and Teeri et al. (1956) among others, have used *S. faecalis* for similar purposes. The problems inherent with such assays have been clearly pointed out by Rogers et al. (1959) who indicated that these methods were valid only when the standard and sample were limiting in the same amino acid. Since this is often not known or needs evaluation, the use of the *S. faecalis* method for screening purposes is not recommended.

Shorrock and Ford (1973) investigated the use of both *Tetrahymena pyriformis* and *S. Zymogenes* and reported that while both could be used for determining available methionine, the *zymogenes* assay seemed simpler. They also concluded that *Tetrahymena* was useful for determining available lysine under controlled conditions. It is suggested that neither bacteria nor protozoa are useful as non-specific screening methods. They can be much more effectively directed toward the estimation of individual amino acid content or available amino acid content using appropriate media.

Dye-Binding Methods

Dye-binding procedures have been used for some time for the estimation of protein and the evaluation of its nutritional quality. Fraenkel-Conrat and Cooper (1944) described how dyestuffs can be used to determine the number of basic groups present in proteins. The precise nature of the dye-protein interaction is obscure but the dominant mechanism is believed to be an electrovalent association between the dye anions and electro-positively charged sites in the proteins. These charges occur in the terminal amino groups and in the basic groups associated with the histidine, arginine and lysine residues present in the protein molecule. Consequently a highly positive correlation exists between the amount of azo dye bound and the number of basic groups associated with the protein in the test sample. Dye-binding methods have been used for several purposes including the determination of total protein content of foods (Cole 1969) and have also been used to detect changes in availability of lysine. Since the methods are rapid and inexpensive and may be automated, they have found favour in testing new lines of crops. They have been officially recognized for the analysis of protein in milk and milk products (Association of Official Analytical Chemists 1970). The UDY dye method for crude protein in wheat and flour has been approved by the American Association of Cereal Chemists (1968).

Lakin (1973a) has recently reviewed the subject in detail and cited examples of the use of dye-binding for the selection of high lysine strains of cereals and legumes (Munck et al. 1969, Kaul et al. 1970) and for studying the effects of factors affecting harvesting and storage of grain (Mossberg 1970). Lakin (1973b) has also indicated that although dye-binding procedures may be used as routine methods to determine protein content of a wide range of foods, they are empirical procedures and a reference method must be used for calibration purposes. It has been found that the calibration will hold as long as the standardization of the dye-binding technique is maintained and the character of the samples remains unchanged.

Lakin (1973b) considered CI Acid Orange 10 to be the best dye for the direct evaluation of protein quality and it may be used, in a modified procedure, for the determination of available lysine.

It must be emphasized that dye-binding is an empirical method and since the results are proportional to the basic amino acids (e.g. lysine, histidine, arginine) present, variations in the percentages of these basic acids among samples of a given cereal may cause discrepancies when dye-binding is used to determine total protein. Furthermore, one cannot use dye-binding procedure to determine total protein and again to determine lysine as percent of protein in the same sample. Obviously, much more work needs to be done on these methods before they can be used reliably to predict the protein value of new cultivars.

Biological Methods

The need for a rapid, simple biological test has been widely recognized. It has been suggested by several workers (Elliot 1963; Shenk et al. 1970) that the meadow vole (*Microtus pennsylvanicus*) has unique advantages for the biological evaluation of certain plant materials. The vole has also been suggested as a means of screening new lines of triticale in that (1) very small samples can be tested quickly, and (2) the vole gives simultaneously an estimate of protein value and an indication of the presence of toxic factors.

In fact, the vole assay suffers from several very serious defects (B. E. McDonald and E. N. Larter 1972 unpublished data). Data provided by McDonald and Larter and cited in Table 8, show that the range of variation within groups of voles is very wide, rendering the vole ineffective as a test animal. It is also evident that voles cannot differentiate between the quality of protein in foods as divergent as casein and cereals. Little is published about the physiology of the vole and practically nothing appears to be known of its amino acid requirements. Thus, the meaning of the vole assay as described is in considerable doubt in terms of human needs. There are no data to describe the reaction of the vole to any "toxic" substance which may be present in new lines of triticale. The vole does not appear to thrive under normal animal laboratory conditions but requires a very moist atmosphere which is difficult to maintain. In view of these limitations, it seems clear that the meadow vole cannot be recommended for the biological testing of new lines of triticale or other cereals intended for human use.

The laboratory mouse was also investigated as a test animal by McDonald and Larter (unpublished data) and while the results exhibited greater uniformity than those derived from voles, a considerable amount of work needs to be done before the mouse assay can be considered adequately standardized and reliable.

TABLE 8. Results of growth assays using voles on four samples (McDonald and Larter 1972 unpublished).

Sample	Vole preliminary Index (*7-day assay*)	SD
Casein	0.69	0.92
Wheat	1.16	0.79
Triticale	1.07	1.09
Rye	1.62	0.69

Since rye may contain certain toxic or undesirable substances, e.g. ergot and resorcinols, the possibility exists that such substances may be present in triticale (*see* Chapter 4). The vole has been suggested as being useful in their detection. It should be emphasized, however, that there is no single level animal test that has any meaning in this regard. One approach that has been found useful (Campbell 1963) is to feed protein supplements or concentrates at various levels up to 15–18% protein in a rat growth assay. If there is evident growth retardation at the high levels, it may be suspected that the food or protein has some toxic or undesirable properties. Unfortunately, this method is not too useful for cereals which contain relatively low levels of protein. Other suggestions are given by the Protein Advisory Group (1972b).

Rapid Screening—Conclusions

In general, it may be concluded that there is no relatively simple, reliable, screening method for evaluating protein quality. There is an urgent need for such a method and research must be directed at once towards this matter if programs for the development of new plant cultivars are to be effective. Non-specific, non-standardized assays of any type whether biological, chemical or microbiological, no matter how simple and easy to apply, should not be used until their meaning, usefulness and limitations have been clearly established, and the techniques for using them carefully standardized.

Bioassays with Humans

The classical method of determining biological value as a measure of protein quality was that of Thomas (1909) and Mitchell (1923–24). However, because this method requires measurements of nitrogen intake and both faecal nitrogen and urinary nitrogen while on both a test diet and a non-protein diet, it is not suitable as a routine test for assessing protein quality. When carried out with human subjects under closely controlled conditions, however, this method still remains the ultimate standard for evaluating protein quality.

Another approach is based on the growth and development of young children consuming the

new food as almost the sole source of protein (Graham et al. 1966). Unfortunately, the growth test can seldom be used because of its long duration.

The most widely used method is nitrogen balance in which the nitrogen intake and nitrogen losses in urine and faeces are measured. The procedure is particularly useful when conducted with young, healthy children. Scrimshaw et al. (1958), Bressani and Viteri (1970) and Bressani et al. (1973) have used nitrogen balance to measure the nutritive value of various proteins. The technique is particularly valuable when applied as the nitrogen balance index in which the slope of the response line is determined. The latter workers concluded that this approach is particularly useful to evaluate protein quality with human subjects, and that comparative tests with rats appear to correlate well with human data. Kies et al. (1965) have applied the method widely in studies with cereals. Graham et al. (1966) have used changes in plasma albumin in conjunction with either growth or nitrogen balance in assessing protein quality in infants.

Although many studies, e.g. Young and Scrimshaw (1972) have explored the possibility of using changes in levels of free amino acids in blood as a basis for estimating protein quality, no practical method has evolved. The level of lysine in the blood may indicate whether or not lysine is the limiting amino acid in a cereal diet but it gives no quantitative measure of the extent of the deficiency. Blood urea levels have also been suggested as a measure of protein quality but as Eggum (1973) has pointed out, the experimental conditions need much further standardization.

It is concluded that the nitrogen balance index method for the testing of proteins with humans is cumbersome to use routinely. The main value of the procedure appears to be in the validation of rat bioassays and the final assessment of new cultivars. A more suitable method for human evaluation of protein quality is urgently needed and particular attention should be paid to the analysis of body fluids.

Suggested Testing Procedures

In any program for the improvement of the nutritional properties of new lines of cereals and legumes, it is essential that the objective of the program be clearly established and understood. In determining the need for new cultivars consideration must be given to the impact that an effective program may have on the overall nutrition of the particular area or country. Since the prime purpose of any program aimed at foods for human use is to improve the quality and quantity of the foods, in this case the quality and quantity of protein, it is most important that there be close collaboration between plant breeders, nutritionists and analysts to ensure that the program is nutritionally sound and that appropriate methodology for evaluation is employed. The following scheme is suggested for the evaluation of protein in new cereal lines.

Procedure 1—For very small samples:

(1) Determine total protein by Kjeldahl method using Association of Official Analytical Chemists (1970) procedure.
(2) Determine amino acid content using ion exchange chromatography (Blackburn 1968), or autoanalyzer.
(3) Determine limiting amino acid content using FAO/WHO reference pattern (World Health Organization 1973).
(4) If sufficient sample is available conduct an abbreviated rat assay, e.g. five animals per group in 10-day NPR assay (see Recommended Methods at the end of this chapter).

Procedure 2—For adequate sample size:

Use Multilevel rat assay, i.e. Growth Slope Assay (see Recommended Methods at the end of this chapter).

Obviously any food for human use must not only have appropriate nutritional value, it must also be free of toxic or harmful substances (Protein Advisory Group 1972b). No test for protein value will give an evaluation of the presence of such substances. If digestible protein is present in sufficient concentration, a multilevel rat growth test should demonstrate the presence of toxic factors.

Summary

1 Any plant improvement program must be designed to meet the nutritional needs of the particular country or area for which the crop is being designed.

2 The first step in the evaluation of protein is the determination of crude or total protein by approved methods.

3 The second step is the estimation of amino acids, particularly the limiting ones, preferably by ion exchange chromatography. The use of Chemical Score is helpful in rating proteins, and determining limiting amino acids.

4 Tests of availability are necessary to estimate the actual amount of amino acids that may be utilized by the human. Availability may be reduced, particularly in proteins processed by heat in the presence of sugars.

5 Biological testing is an indispensable part of any protein evaluation program. Where test samples are adequate the Growth Slope Assay appears to be the best available procedure. (Details of the assay are described in Recommended Methods.)

6 There is no simple, specific, reliable screening method for evaluating protein quality. Non-specific chemical, microbiological or biological screening methods are not recommended unless they have been very carefully standardized and their significance has been established and understood.

7 There is no simple screening method for estimating toxic substances in foods but where applicable multilevel rat growth tests are useful.

8 There is urgent need for the development and standardization of rapid, preferably non-destructive, tests applicable to small samples of grain which will reliably predict protein value.

9 Because of the difficulties inherent in evaluating protein in foods, there is need for close collaboration among plant scientists, nutritionists and analytical chemists.

10 In view of the multiplicity of methods available for chemical, microbiological and biological testing of protein value, there is an urgent need for the comparative standardization, selection and official recognition of demonstrably reliable methods.

The need for standardization of methodology for evaluation of plant proteins for human use is a subject the authors would refer as a matter of urgency to the Protein Advisory Group of the United Nations and to the International Unions of Pure and Applied Chemistry, Nutrition Sciences, and Food Science and Technology.

Recommended Methods

PER Assay

Animals Use male weanling rats of a single strain, 21–23 days of age. Place on a commercial rat chow diet for 2 days. Use groups of 10 rats for each diet with a weight range not greater than 15 g. Distribute rats to various groups so that mean starting weights of all groups are similar.

Diets Use a basal diet of the following composition on an air-dried basis: maize starch 81, maize oil 10, non-nutritive cellulose 5, salts (Bernhart and Tomarelli, 1966) 3, vitamin mixture (Beare-Rogers and Nera 1972) 1. Incorporate the test protein in the diet at the expense of corn starch to 10% protein (nitrogen × 6.25 or other appropriate factor). The protein content of the final diet should be within the range 9.7–10.3% determined by Kjeldahl analysis.

Assay period 4 weeks.

Cages Use individual screen bottom cages provided with feeders which will reduce food spillage to a minimum.

Parameters measured Weigh animals weekly at the same time each day. Estimate food consumption, taking into account wasted food.

Casein control Include a reference standard group of rats given casein (ANRC high nitrogen casein, Sheffield Chemical Co., Norwich, N.Y.) in place of the test protein.

Calculation PER = Weight gain (grams)/Protein consumed (grams). Assume that casein has a PER of 2.5 and correct the PER of the test food to a value of 2.5 for casein.

NPR Assay

Use animals, diets, and cages as for the PER assay. In addition to the test groups include a control group of rats fed the unmodified ration (i.e. the non-protein diet). If the sample size is limited, use only 5–6 rats per test protein. The initial weights of rats in the two groups should be matched to within 1 g. After 10 days on the diets calculate for each pair of rats, the NPR for the test protein.

$$NPR = (WG + WL)/P$$

where: WG = weight gain of test animal (*g*)
WL = weight loss of control animal (*g*)
P = protein consumed by test animal (*g*).

GS Assay

Animals As specified for PER.

Diets As specified for PER except for protein content of diets. Test each protein at 3 levels added at the expense of corn starch.

Reference Protein Use lactalbumin at protein levels of 2.5, 5.0 and 7.5%. Test higher quality proteins at 3, 6 and 9%. Test proteins of lesser quality at 4, 8 and 12, or 5, 10 and 15% of the diet to obtain linear dose–response relation.

Assay period 2 weeks.

Parameters measured Weight changes and food consumption (as for PER).

Calculation of GS Assay Prepare a table containing weight changes, food intake and calculated protein (or nitrogen) intake for each animal. Plot mean group data for weight change against protein or nitrogen consumed for both standard and test protein. Calculate slope from linear portion of each line, and express the slope of the test protein as a percent of the slope of the reference standard (lactalbumin). Appropriate computer programs may be used for calculating results, testing for linearity and giving fiducial limits of the assay.

The plant breeder's treatment of the seedling from the first cross with colchicine has opened the door to the development of improved lines

Chapter 3

Genetic, Varietal, Environmental and Agronomic Factors

Introduction

This chapter contains a review of the literature in which (*a*) genetic and hereditary factors, (*b*) varietal differences and (*c*) environmental influences and agronomic practices are related to the nutritional value of the proteins of triticale and its parents wheat and rye. Every plant is the result of complex interactions among these factors and possibly other, as yet unidentified, influences. Consequently, though a serious effort has been made to separate each of the above defined influences in the text which follows, a clear-cut separation and distinction among them is not easily achieved and, therefore, a considerable degree of overlap will be evident. The literature reviewed is presented under the headings of the cereals with which the text is concerned and the effect of the above influences, (*a*), (*b*) and (*c*), upon the total protein content, the amino acid composition, and the biological value of these cereals.

The nutritional quality of any cereal protein is dependent upon its biochemical composition which in turn is influenced by its genetic background, the environment in which it is grown, the agronomic practices by which it is cultivated and its freedom from infection and infestation.

No earlier reviews covering the literature in precisely this form have been found. Coons (1968) published a bibliography of selected references on cereal grains in protein nutrition, listing over 500 references to papers on wheat and over 50 references to papers on rye, available in English, and published between 1910 and 1966. No references to triticale were included. The Food and Agriculture Organization (1970b), in its compilation of data on the amino acid content of foods and biological data on proteins, and using more selective criteria than Coons, listed 65 references to wheat and wheat products, ten references to rye, and one reference to triticale.

The International Wheat and Maize Improvement Centre (CIMMYT 1971) has published a three-volume bibliography of wheat. Deodikar (1963) prepared a review on rye which included over 1,000 references and in his preface acknowledges his considerable reliance on the publication *Plant Breeding Abstracts*. Pomeranz (1971a) also includes data on the nutritional value of wheat protein and Aykroyd and Doughty (1970) reviewed the role of wheat in human nutrition on behalf of the Food and Agriculture Organization of the United Nations.

The cultivation of triticale has been discussed by Briggle (1969) and Zillinsky and Borlaug (1971a, b). The cultivation of wheat has been reviewed and discussed by Schlehuber and Tucker (1967) and the cultivation of rye by Shands (1969).

Moran (1959) reviewing the nutritional significance of the then recent work on wheat, flour and bread, stated that the differences in the composition of wheat were determined largely by the environment in which the wheat was grown and only to a small extent by heredity. The main object of the breeding and intercrossing of wheat varieties was not to improve the biochemical composition; when such improvement occurred, it was only incidental to improvement in yield capability and resistance to disease.

Genetic and Varietal Factors

Plant breeding consists essentially of (*a*) the establishment of plant populations within which the probability of desired character combinations

occurring is high and (*b*) the identification and selection from within these populations of those parent plants possessed of the characteristics desired. While it may not be excessively difficult to assemble in a single genotype those characteristics which are inherited in a simple manner, a problem of considerable complexity arises when the objective is to combine several characteristics each of which is controlled by a number of genetic factors. Such genetically complex characteristics include yield, resistance to pests and disease, environmental adaptability, and biochemical composition. To bring about the simultaneous combination of as few as 20 desirable genetic factors within a single genotype by conventional intercrossing would require the planting of a population covering an immense area of land and of a size impossible to screen. Consequently, in selecting the parent population for a prospective new variety, the individual entries chosen usually differ in relatively few genetic factors. Thus the deliberate combination of many predetermined genetic factors within a single plant requires a very prolonged breeding process.

Even using the techniques of population breeding, in which composites of freely intercrossing individuals are planted and the best of their progeny selected to form the next composite, a simultaneous combination of complex characteristics is difficult, each major characteristic having to be selected from one or more composite populations.

Largely for economic reasons, the plant breeder's primary concern, traditionally, and until comparatively recently almost exclusively, has been to select high yielding, healthy, disease resistant and environmentally adaptable cereal grains. Some attention has also been given to the technological properties demanded by processors and consumers but among many cereal consuming communities processing technologies have developed which best suit the properties of the cereal grains locally available. To demand of the breeder, in addition to other essential characteristics, a significant change in the biochemistry, particularly in the amino acid composition, of a cereal grain, inevitably adds to the complexity and cost of the breeding program and may, at least for the short or intermediate term, require a compromise with other established characteristics. Nevertheless, particularly for the benefit of people in the developing countries, many of whom rely upon cereal grains to provide more than 80% of their dietary calorie and protein sources, efforts to discover varieties of higher biological value without detriment to yield, disease resistance and adaptability, must be intensified.

HIGH LYSINE MAIZE

The discovery of high lysine mutants in maize gives hope that the biological value of the proteins of triticale, wheat and other cereals may prove susceptible to improvement by genetic manipulation. During the early 1960's the amino acid analysis of a large number of maize varieties led to the discovery of two important mutants, opaque-2 (Mertz et al. 1964) and floury-2 (Nelson et al. 1965). Both mutants contained substantially larger proportions of lysine and tryptophan which, for maize, are the limiting amino acids. Subsequently maize breeders have successfully transferred both opaque-2 and floury-2 genes into "high lysine" hybrid lines and into varietal populations. This discovery has excited widespread interest among plant scientists in the possibility of improving the amino acid balance in other cereals by genetic manipulation. The opaque-2 and floury-2 mutant genes appear to suppress partially the synthesis of the nutritionally unsatisfactory alcohol-soluble prolamin fraction and to stimulate the synthesis of nutritionally more important proteins in the water-soluble albumin and salt-soluble globulin fractions. As would be expected, the compositional differences occur in the storage proteins of the endosperm; no difference is apparent between the germ protein compositions of the traditional varieties and the high lysine mutants. Since in most known varieties the prolamin fraction represents about 45% of the protein of wheat (*Triticum vulgare*) and about 40% of the protein of rye (*Secale cereale*), a genetic mechanism, similar to that in maize, by which to improve the nutritional quality of wheat and rye proteins would represent an important discovery (Nelson 1969). Single identifiable genes with a "high-lysine" effect similar to the opaque-2 and floury-2 genes in maize have not, at the time of writing, been identified in either wheat or rye (Mertz 1971; Johnson and Mattern 1972).

GENETIC CONTROL OF COMPOSITION

The improvement of plant protein quality by genetic manipulation produces an intrinsic and

integral improvement in that it raises the plant's potential to produce protein of higher quality in response to favourable environment and agronomic management. There seems little doubt that if its protein were qualitatively and quantitatively satisfactory, a cereal diet could provide for most human needs.

The fundamental aspects of genetic control of protein composition in cereal grains has been elegantly discussed by Nelson (1969). The composition of plant protein depends upon a genetic information system consisting of a unique sequence of trinucleotide codons (DNA) which is translated through a complementary sequence of trinucleotide codons of "messenger" RNA into the predetermined amino acid sequence. Each codon specifies a particular sequential amino acid, the amino acid sequence in any given protein being invariate. Each protein is believed to be synthesized amino acid by amino acid, the process starting from the N-terminal and finishing at the C-terminal end. Since even a single change in amino acid sequence can prove lethal or at least injurious to the organism, the established amino acid sequence tends strongly to be conserved in healthy plants. This is particularly true of the essential proteins present in the embryo and those which control the photosynthetic and other vital processes in the leaves. Consequently any hope of improving the amino acid pattern of a plant seed protein must be focussed upon the storage protein of the endosperm, the composition of which is less critical to the plant's survival.

In every organism each cellular component is provided with its own unique spectrum of proteins. While the amino acid sequence of each protein remains constant, the proportion of the different proteins within the spectrum may vary. By what control mechanism the ratio in which the various proteins are laid down is by no means clearly understood.

CEREAL PROTEIN CLASSIFICATION

Osborne (1924) provided the classification of seed proteins which, to all intents and purposes, still holds good today. Seed proteins contain albumins (water-soluble), globulins (soluble in saline solution), prolamins (soluble in alcohol), and glutelins (soluble in dilute alkali). Only among the seeds of the *Gramineae*, which include wheat and rye, are prolamins and glutelins found in any quantity. The prolamin fractions of all cereals are rich in the amino acids proline and glutamine but comparatively poor in lysine. It is the prolamin fraction which largely contributes to wheat its unique gluten-forming and traditionally desirable bread-making properties. Technologies developed recently permit bread to be made from flours much poorer in gluten-forming proteins and richer in "high lysine" proteins than was formerly considered possible (Axford et al. 1963). Therefore, the apparent conflict between technological properties as contributed by high prolamin components, and nutritional value from higher lysine components, may no longer be of serious consequence.

PROLAMIN SYNTHESIS

Since the synthesis of prolamin in maize endosperm can be genetically suppressed there is hope that comparable mutant genes may be found whereby to suppress prolamin synthesis in wheat, rye and other cereal endosperm proteins. The presence of a semi-dominant mutation such as occurs in the floury-2 maize in one of the wheat or rye genomes could cause a significant depression of prolamin synthesis and should be identifiable. As already mentioned, no such mutation has been reported. It is possible that a search for such mutations among rye varieties might well prove more fruitful.

It is recorded by Favret et al. (1970) that when prolamins are specific they are regulated by Mendelian genes which act directly in the endosperm tissue and are not influenced by the mother genotype, in sharp contrast to the potential for total protein production in the grain which is strongly influenced by the mother genotype.

As already stated, prolamin is comparatively rich in proline whereas lysine is one of the group of basic amino acids which are present in low proportion in prolamin. The ratio of basic amino acids to proline in the endosperm varies among species and with the age of the plant, the ratio falling as seed endosperm fills out since prolamin production takes place largely after the other storage proteins have been laid down in the endosperm.

In selecting for wheat and rye genotypes of improved biological value, it might be useful to determine the basic amino acid:proline ratio. Geneticists might also explore the activity of prolamin-regulating genes by examining the electrophoretic patterns for the appearance and

disappearance of specific desirable and undesirable proteins.

IMPROVEMENT IN SEED PROTEIN

In selecting for high protein genotypes it would appear to be more meaningful to select on the basis of protein content per seed rather than protein as percent dry matter. The latter is strongly influenced by seed weight and kernel characteristics which in turn are known to be influenced by maturity, environment and agronomic practices.

Other means by which the protein quality of a cereal grain might be improved would be to increase the size of the embryo relative to the size of the endosperm, or to increase the depth of aleurone layer (Nelson 1969). Both the embryo and its surrounding scutellum, and the aleurone layer contain proportionally more protein nitrogen than the endosperm and, in both instances. the protein is of superior biological composition. In many countries, except for a few specialty breads, the embryo is normally excluded from flours used for bread and alimentary pastes. Modern roller and abrasive milling processes can however be adjusted to include in the flour substantial quantities of the aleurone layer, the layer which immediately surrounds the endosperm.

Another possibility suggested by Nelson (1969) is to increase the production of a particular amino acid above the level required for protein synthesis by a mechanism studied by Vogel and Vogel (1967) in microorganisms. Enzyme systems present in mutants of certain microorganisms appear to behave abnormally giving rise to an unusually high production of a particular amino acid in free form. No mutation demonstrating a similar effect has as yet been found in cereals (Nelson 1969).

Environmental and Agronomic Factors

Every plant is the outcome of the interactions which take place between its genotype and the environment in which it is grown. Manipulation of this environment by means of the agronomic practices adopted can influence the productivity, the health and the biochemical composition of the plant.

It is now well established that the quantity of protein in a cereal is genetically controlled (Haunold et al. 1962a,b; Stuber et al. 1962; Johnson et al. 1963, 1967, 1968a,b, 1970; Lonnquist 1969). However the fertility of the soil in which it is grown is an important influence upon whether or not the plant achieves its full genetic potential for protein production. There exists an immense literature which relates to the influence of agronomic variables upon yield, composition and other characteristics of wheat and, to a lesser extent, of rye. Since much of the data recorded is specific for the location, or closely similar locations, in which the experiment was carried out, no attempt has been made to review such literature comprehensively in this publication.

In addition to the influence of environment, fertilizer and irrigation regimes. and the control of pests and diseases, other factors, including the date of planting, can materially influence protein content. It has been reported, for example, that triticale lines planted during the spring contained significantly higher protein contents than the same lines planted in the autumn.

Exceptional progress has been made during the past year in the CIMMYT-Manitoba project in improving the fertility, the seed density, kernel characteristics and the overall yield capability of triticale. At CIMMYT triticale yields in excess of 8300 kg/ha (var. 312) have been reported. Such yields significantly exceed yields of the highest yielding bread wheats (7245 kg/ha) grown under closely similar conditions.

Trials are now in progress at CIMMYT to study the response of promising triticale genotypes to nitrogen fertilizer under a variety of soil and environmental conditions. The first results, which will include grain and straw yields, kernel characteristics and protein content, will be available in the near future.

From India come reports of triticale yielding well at high altitudes and on acid soils, and in Ethiopia five triticale varieties are reported to have performed better than eight durum wheats at five locations. Triticale nurseries are established in 52 countries covering a wide range of soil and environmental conditions under both rain fed and irrigated conditions, the rain fed areas embracing almost every known level of precipitation.

In addition, triticale appears consistently to display protein contents comparable to the best bread wheats together with a biologically superior amino acid composition.

Consequently, though much more research is needed, one can feel extremely optimistic about the future of triticale as a valuable new source of calories and protein for many people.

Triticale

Genetic and Varietal Effects

TOTAL PROTEIN

Chen and Bushuk (1970c), from a comparison of the disc electrophoretic patterns for the albumins, globulins, gliadins, and glutenins of one hexaploid triticale line (6A190) and of its durum wheat (cv. Stewart 63) and rye (cv. Prolific) parents showed that all the protein components of the triticale are present in the parents and conclude that the proteins of hexaploid triticale are simply inherited from its parents. Chen and Bushuk (1970c) cite Yong and Unrau (1964) who, using the same line of triticale but a slightly different extraction procedure, found new proteins in the triticale.

Ellis (1971) using an electrophoretic system, examined the gliadin fraction of a series of hexaploid triticales (PM 1514, 1/1, 2/1, 3/1, AFP1/184) and cultivars of *T. aestivum*, *T. durum* and *S. cereale* obtained from the National Institute of Agricultural Botany and the Plant Breeding Institute, Cambridge, England. He found that the electrophoretic patterns were more variable in triticale than in wheat and about as variable as in rye. Some triticale varieties resembled wheat more closely than rye (e.g. AFP1/184) and some resembled rye more closely than wheat (e.g. 3/1).

Hristova and Baeva (1972) subjected two octaploid triticales to electrophoretic analysis. The triticales were AD-901, of which the parents were the wheat variety Norin-69 and rye Lozen-14, and AD-COC-3-2, of which the parents were the wheat Bezostaia 1 and the rye C_2. They found that certain of the protein components in the spectra of the parents were missing from the triticales and further observed new components in the triticales. They did not find any simple form of inheritance of the protein components of the parents and therefore consider protein spectra of the triticales cannot be reported as a simple addition of the protein spectra of the parent grains.

Although protein quantity and quality are not reported, it may be of interest to refer here to the studies carried out at the Plant Breeding Research Substation, Bajaura, India on the genetic variability, interrelationships and selection index of 31 strains of triticale obtained from overseas and grown during the winter season of 1969-70 (Sethi and Singh 1972). The strains differed significantly for all the characters studied. The estimates of correlation coefficients indicated that the number of spikes per plant was the only effective yield-contributing character. Regression analysis indicated that spikes per plant, 100-grain weight, spike length, days to 70% heading, days to maturity and plant height are important characters, contributing about 78% of the total variability for grain yield, if the selection is based only on these characters.

The protein contents (N × 6.25) percent of four varieties of triticale grown at Fargo, North Dakota, USA in 1970 were: Rosner 18.2; 6TA203 (FasGro 203) 17.9; 6TA204 (FasGro 204) 18.6; 6TA209, 19.7. In 1971, the protein contents were: Rosner 15.3, 6TA203, 15.2; 6TA204, 15.3; 6TA209, 15.1 and of Trailblazer (a reselection of 6TA209) 15.8 (Busch and Wilkins, 1972).

Sisodia (1973), reporting on research in India, states that considerable variability in protein content (N × 5.7), 9.8 to 16.3%, was observed among different head progenies of a triticale variety. Normally in a routine breeding program, no selection for protein content is practised and protein estimations are usually made at a later stage of development of a variety. Thus a variety otherwise homogeneous, is expected to be heterogeneous for characters which have not been selected. Accordingly, protein content in a variety may represent an average estimate of high and low protein genotypes. Selection for high protein content in an otherwise high yielding variety, therefore, is expected to be effective.

AMINO ACID COMPOSITION

Chen and Bushuk (1970a) reported on the solubility characteristics and amino acid composition of one line of triticale (6A190) and of its durum wheat (cv. Stewart 63) and rye (cv. Prolific) grown together in 1966 at the University of Manitoba with the hard red spring wheat, Manitou. The samples were milled into flour on the Buhler experimental mill after tempering overnight to 16.5% moisture. Standard analytical methods of the American Association of Cereal Chemists (1962) were employed to characterize the flours (*see* Table 9). The amino acid composition (by autoanalyzer) is given in Table 10. In general the amino acid composition of triticale

TABLE 9. Characteristics of triticale, rye, durum wheat and hard red spring wheat (data from Chen and Bushuk 1970a).

	Triticale 6A190	Spring rye Prolific	Durum wheat Stewart 63	Hard red spring wheat Manitou
Grain				
1000-kernel weight, *g*	43.4	28.6	44.9	30.6
Moisture content, %	14.0	13.0	13.7	13.5
Flour				
Yield, % *(14% moisture basis)*	58.9	59.6	67.7	71.7
Ash content, % *(14% moisture basis)*	0.42	0.68	0.66	0.48
Moisture content, %	14.0	13.0	13.7	14.8
Protein content, % *(14% moisture basis)*	9.8	9.9	12.1	13.6

was intermediate between its parent species, durum wheat and rye.

Riley and Ewart (1970) reported on studies of the amino acid composition of the *T. aestivum* (bread wheat) variety Holdfast and the rye variety *S. cereale* King II. Chromosome addition lines were constructed by simple backcross procedures using Holdfast as the recurrent parent. In the addition lines the genotype of Holdfast was reconstituted and to it was added, separately and in turn, each of the seven pairs of chromosomes of the rye variety King II. Roman numerals were assigned to the addition lines. Amino acid composition (by autoanalyzer) is shown in Table 11.

TABLE 10. Amino acid composition of flours from rye, triticale, durum wheat and hard red spring wheat (*micromoles amino acid per milligram nitrogen in sample*) (Chen and Bushuk 1970a).

	Rye	Triticale	Durum	HRS wheat
Aspartic acid	2.41	2.23	1.89	1.47
Threonine	1.29	1.34	1.29	1.20
Serine	1.84	2.17	2.26	2.21
Glutamic acid	9.77	11.7	13.2	15.7
Proline	6.26	6.44	6.56	6.21
Glycine	2.40	2.70	2.54	2.73
Alanine	2.15	2.08	1.99	1.84
Valine	2.10	2.20	2.21	2.11
Cystine	0.52	0.70	0.60	0.59
Methionine[a]	0.68	0.79	0.78	0.65
Isoleucine	1.51	1.74	1.87	1.71
Tyrosine	0.56	0.81	0.80	0.78
Phenylalanine	1.47	1.58	1.80	2.10
Ammonia	9.41	10.0	12.5	13.6
Lysine	1.12	1.00	0.85	0.82
Histidine	0.65	0.79	0.83	0.85
Arginine	1.23	1.43	1.13	1.20
Tryptophan		not determined		
N recovery, %	76.2	84.5	87.8	91.7

[a]Including methionine oxide (approximate value).

The use of whole seeds instead of ground material, necessary because of the small quantity of material available, resulted in significant losses in both wheat and triticale of tyrosine, arginine, cystine and methionine. The effects on the amino acid content of grains of the addition to wheat of the entire chromosome complement of rye and some of the effects of each pair of rye chromosomes in turn is shown in Table 11. Riley and Ewart point out that rye chromosome I increased cystine and lysine by 10.7 and 8.7% respectively. Chromosome VII reduced the content of threonine by 10.4%.

Comparison of the values in Table 12 indicates that the contents in the triticale of threonine, serine, glutamic acid, alanine, proline and tyrosine may be influenced by interaction among chromosomes. For the remaining amino acids there is no strong evidence that the contents of the triticale grain cannot be accounted for by simple additivity.

Rye chromosome I is in homoeologous group 5 (Sears 1968), and Tsunewaki (1968) has reported that the winter/spring habit difference in wheat is primarily determined by the chromosomes of group 5. Since Johnson et al. (1968a) found that eight of the ten varieties of wheat with the highest lysine content also had winter habit, group 5 chromosomes may be of particular significance in the control of lysine content in the grain. Confirmation of this would lead to a more rational approach to breeding for higher lysine content.

TABLE 11. Amino acid contents of the grains of wheat, rye, triticale and the seven addition lines with single pairs of rye chromosomes added to wheat (*grams/100 g of recovered anhydro acids*) (Riley and Ewart 1970).

		Addition lines								
Amino acid	Wheat	I	II	III	IV	V	VI	VII	Triti-cale	Rye
Aspartic acid	6.06	6.13	5.74	5.54	6.18	5.83	6.47	6.11	5.81	7.45
Threonine	3.66	3.59	3.34	3.56	3.58	3.83	3.40	3.28	3.62	4.06
Serine	5.66	5.51	5.40	5.54	5.54	5.55	5.54	5.53	5.46	5.50
Glutamic acid	37.15	35.47	36.94	38.90	34.93	37.94	36.94	37.53	36.97	32.63
Proline	12.94	13.62	14.12	13.04	13.31	13.00	14.47	13.14	14.03	13.65
Glycine	4.89	4.86	4.60	4.58	5.11	5.19	4.70	4.90	4.71	4.97
Alanine	4.27	4.19	3.97	4.04	4.28	4.05	4.27	4.05	4.11	4.87
Valine	5.29	5.31	5.14	5.12	5.35	5.06	5.22	5.15	5.11	5.73
Cystine	1.78	1.97	1.80	1.84	1.81	1.94	1.89	1.80	1.80	1.83
Methionine[a]	1.71	1.82	1.66	1.82	1.71	1.67	1.74	1.60	1.68	1.85
Isoleucine	4.43	4.44	4.50	4.32	4.43	4.30	4.30	4.39	4.32	4.44
Leucine	8.30	8.44	8.17	8.00	8.33	8.01	8.13	8.36	7.99	7.75
Tyrosine	2.55	2.67	2.98	2.70	3.00	2.58	2.56	2.72	2.49	2.06
Phenylalanine	6.09	5.97	6.44	5.85	6.19	5.79	5.82	6.11	6.12	6.27
Lysine	3.23	3.51	3.17	3.17	3.42	3.41	3.17	3.21	3.22	4.24
Histidine	2.83	3.03	2.80	2.83	3.05	2.71	2.66	2.83	2.88	2.70
Arginine	5.37	5.69	5.34	5.32	6.00	5.76	4.94	5.43	5.80	6.30
Tryptophan					not determined					
Total	116.21	116.22	116.11	116.17	116.22	116.17	116.22	116.14	116.12	116.30
Protein (N × 5.7), %	15.4	16.7	19.9	16.4	19.7	15.5	18.2	19.0	17.3	18.2
Recovery, %	87.2	84.1	82.9	91.4	82.3	89.2	84.8	81.5	83.6	86.3

[a]Including methionine sulphoxide.

Larter et al. (1968) reported on the protein content and amino acid composition of hexaploid triticale tested in 1967 at the University of Manitoba. The parentage of the lines tested was: (*T. durum* (var. Ghiza) × *S. cereale*) × (*T. durum* (var. Carleton) × *S. cereale*) × (*T. persicum* × *S. cereale*) × (a *Triticum* × *Secale* introduction). The overall mean yield of the best triticale was about equal to the best bread wheat (*T. aestivum* var. Manitou). The percentage protein and amino acid composition (except tryptophan) are given in Table 13. The analytical methods are not stated.

The amino acid balance of triticale compares favourably with that of wheat. The gradual decline in protein level which the Manitoba triticale lines have shown over the years is probably the result of a gradual improvement in kernel characteristics. The original, wide crosses of durum wheat and rye and of bread wheat and rye, produced a high proportion of badly shrivelled grains which, not surprisingly, tended to be high in protein expressed as percent dry matter (Zillinsky and Borlaug 1971a).

Larter et al. (1968) do not discuss the improvement in protein content and quality that may result from selective breeding but point out that the basic weakness in triticale rests in its reproductive system; an improvement in its genetic stability will lead to increased fertility and yield. Their experience in improving kernel type has shown that in transmitting genes with favourable characteristics, some undesirable characteristics may accompany the favourable ones. For example, *T. timopheevi* and *Secale montanum* transmit genes for desirable kernel characteristics but *T. timopheevi* also transmits male sterility and *S. montanum* transmits extreme lateness and chromosome anomalies. Since the Larter et al. paper was published considerable improvement in fertility and kernel characteristics has taken place (CIMMYT 1970-71, Zillinsky 1973).

Tkachuk and Irvine (1969) determined the amino acid composition by autoanalyzer of several cereals, including triticale grown in 1966 by the Rosner Research Group, University of Manitoba, the protein content of which, on an

TABLE 12. Effects on the amino acid content of the grains of the addition to wheat of the entire chromosome complement of rye and the sum of the effects of each pair of rye chromosomes in turn (Riley and Ewart 1970).

Amino acid	Entire rye genome (*Triticale–wheat*)	Sum of effects of individual rye chromosomes (*Addn. line–wheat*)
Aspartic acid	−0.25	−0.42
Threonine	−0.04	−1.04
Serine	−0.20	−1.01
Glutamic acid	−0.18	−1.85
Proline	1.09	4.12
Glycine	−0.18	−0.29
Alanine	−0.16	−1.04
Valine	−0.18	−0.68
Cystine	0.02	0.59
Methionine	−0.03	0.05
Isoleucine	−0.11	−0.33
Leucine	−0.31	−0.66
Tyrosine	−0.06	1.36
Phenylalanine	0.03	−0.46
Lysine	−0.01	0.45
Histidine	0.05	0.10
Arginine	0.43	0.89

TABLE 13. Percentage protein, 1000-grain weight and amino acid composition of triticale in comparison with bread wheat, 1967 (Larter et al. 1968).

	T. aestivum (*var. Manitou*)	Triticale (*mean 6 strains*)
Protein (*dry basis*), %	12.2	12.9
1000-g rain weight, *g*	28.1	37.3
Amino acids, %		
Aspartic acid	4.3	5.7
Threonine	2.4	3.2
Serine	3.5	4.0
Glutamic acid	32.5	27.2
Proline	10.6	9.5
Glycine	3.9	4.4
Alanine	3.1	4.2
Valine	4.1	4.8
Cystine	1.9	2.0
Methionine	1.8	1.2
Isoleucine	3.4	3.7
Leucine	6.5	6.8
Tyrosine	2.1	2.4
Phenylalanine	4.6	4.8
NH_3	6.0	6.0
Lysine	2.6	2.9
Histidine	2.4	2.4
Arginine	4.5	5.2
Tryptophan	not determined	

as-is basis, was 15.6% (N × 5.7), moisture 13.1%. The amino acid composition is presented in Table 14 in grams amino acid per 100 g sample nitrogen. The results for Manitou wheat (protein 15.4%, moisture 8.1%), Selkirk wheat (protein 15.0%, moisture 8.1%) composites, grown in Western Canada in 1966, and for a dark rye flour obtained commercially (protein 13.5%, moisture 8.8%) are also given.

Kurelec et al. (1968) presented data (*see* Table 15) on the protein (N × 6.25) and amino acid composition of three strains of triticale designated 30-66, 57-66 and 64-66 compared with Bankuti (Hungarian), San Pastore (Italian), Bezostaia 4 (Russian), Etoile (French) feed wheats and rye grown under the same conditions in Hungary. Among the wheats the highest content of lysine as percent in the protein, was found in Bezostaia (3.6%). The rye contained 4.3% lysine and the triticales averaged 2.7%.

Gundel et al. (1970) reporting from the same institute as Kurelec et al. (1968) gave details of the average chemical composition of strains of hexaploid triticales raised in Hungary in the years 1966–69. These are given in Table 16, in comparison with the Hungarian Official Standards for barley, wheat and rye. Table 17 gives crude protein (N × 6.25) and the lysine, methionine and cystine content (by autoanalyzer) as percent of grain of selected strains of these triticales. Protein contents ranged from 11.3 to 18.5% with an average of 13.2% at moisture levels ranging from 10.0 to 13.3% with an average of 12%. Among the selected strains, protein varied from 11.06 to 18.0% (13% moisture basis) and lysine, as percent of the grain, ranged from 0.20 to 0.67.

Villegas et al. (1970) determined, by autoanalyzer, the lysine content of the protein of a considerable number of samples of hard red spring wheat, durum wheat, and other wheat species; of rye, grown under differing conditions; and of 25 varieties of triticale developed at the University of Manitoba, and grown at Sonora, Mexico in 1965–66.

Nitrogen was determined by micro-Kjeldahl, the conversion factors used being 5.7 for wheat and triticale and 6.25 for rye. Values for protein are reported on a 14% moisture basis and those

TABLE 14. Amino acid composition of triticale, Manitou and Selkirk wheat, and dark rye flour (*in grams amino acid per 100 g sample N*) (data from Tkachuk and Irvine 1969).

	Triticale 1966	Selkirk wheat composite 1966	Manitou wheat composite 1966	Dark rye flour
Tryptophan	9.90	9.55	9.20	7.57
Lysine	19.00	14.50	14.80	18.10
Histidine	15.50	13.80	14.30	13.10
Ammonia	18.60	22.30	22.10	18.20
Arginine	30.50	24.90	24.40	26.20
Aspartic acid	36.90	29.20	30.90	40.40
Threonine	19.60	17.30	17.20	20.90
Serine	28.50	31.20	29.70	26.80
Glutamic acid	193.00	207.00	207.00	172.00
Proline	66.90	69.60	67.50	65.00
Glycine	24.70	23.50	23.90	22.70
Alanine	22.70	20.40	21.20	23.20
Cystine + Cysteine	17.40	16.20	15.10	14.30
Valine	31.30	27.90	27.80	30.60
Methionine	12.00	10.50	10.60	07.31
Isoleucine	25.90	23.90	21.80	22.80
Leucine	42.00	42.00	41.30	37.40
Tyrosine	14.50	16.70	17.40	11.80
Phenylalanine	29.70	29.70	30.70	28.00
N recovery, %	95.30	96.40	95.80	89.60

TABLE 15. Protein ($N \times 6.25$, *14% moisture basis*) and amino acid content (*as percent protein*) of Hungarian grown triticales, wheats and rye (data from Kurelec et al. 1968).

	Triticale			Wheat				
	30-66	57-66	64-66	Bankuti Hungarian	San Pastore Italian	Bezostaia 4 Russian	Etoile French	Rye
Protein ($N \times 6.25$), %	13.8	12.7	14.2	16.6	13.7	14.0	12.4	9.4
Amino acids, % *protein*								
Leucine	8.0	8.0	7.2	7.7	–	–	8.6	7.3
Isoleucine	3.5	3.4	2.9	5.4	–	–	4.8	3.8
Phenylanine	4.8	3.8	4.4	6.0	3.3	3.3	5.0	4.5
Methionine	1.5	1.3	1.1	1.4	–	–	1.4	1.1
Valine	5.5	5.4	4.8	4.9	–	–	6.5	4.6
Threonine	4.9	4.4	3.9	3.7	2.7	3.0	4.5	3.8
Glycine	7.6	7.0	8.1	7.7	5.3	4.9	4.3	6.6
Glutamic acid	27.2	28.2	34.5	23.9	27.3	26.6	20.5	15.1
Arginine	3.3	3.0	5.1	3.6	4.0	4.4	4.3	5.7
Lysine	2.7	2.6	2.8	2.5	2.5	3.6	2.7	4.3
Histidine	1.8	1.9	2.1	2.4	2.5	2.8	2.2	2.4
Cysteine	1.4	1.5	1.6	1.0	–	–	1.0	–

TABLE 16. Average percent composition (ranges in parentheses) of Hungarian triticales analyzed in 1966–69, compared with Hungarian standards for barley, wheat and rye (data from Gundel et al. 1970).

		MZ6830-66 (Hungarian Standard)		
	Triticale	Barley	Wheat	Rye
Dry matter	87.0 (86.7–90.0)	87.0	87.0	87.0
Moisture	12.0 (10.0–13.3)	13.0	13.0	13.0
Crude protein ($N \times 6.25$)	13.2 (11.3–18.5)	10.2	12.2	11.6
Crude fat	1.8 (1.3–2.7)	1.8	1.9	1.7
Crude fibre	2.4 (1.4–3.8)	3.7	1.9	1.9
N-free extracts	67.8 (65.0–80.0)	68.8	69.3	69.8
Crude ash	1.8 (1.4–2.4)	2.5	1.7	2.0
Starch	50.7 (46.5–54.4)			

TABLE 17. Protein, lysine, methionine and cystine content of various strains of Hungarian triticale (*13% moisture basis*) (data from Gundel et al. 1970).

Sample Number	Protein ($N \times 6.25$) (%)	Lysine	Methionine *percent of grain*	Cystine
20/68	17.57	0.36	0.11	0.19
30/68	18.00	0.67	0.14	0.43
57/68	11.06	0.27	0.10	0.16
57/68	11.79	0.31	0.11	0.20
57/69	14.81	0.37	0.11	0.12
64/St	11.59	0.41	0.09	0.16
64/68	12.91	0.30	0.14	0.15
64/68	11.17	0.20	0.10	0.17
64/69	16.47	0.33	0.11	0.32

for lysine are reported on a dry weight basis. The results for triticale are given in Table 18. The lysine content in the protein was significantly inversely correlated with the protein content of triticale ($r = -0.50$).

The hard red spring wheat varieties, Selkirk, Justin, Chris, Manitou and Crim were grown at two locations in North Dakota, and Selkirk, Justin, Chris and Crim with the soft wheat varieties Lerma Rojo, Narino and 8156, were grown in Mexico. The protein content of the wheats grown in North Dakota ranged from 15.0% (Selkirk at Fargo) to 18.8% (Justin at Minot). In Mexico, the protein content ranged from 10.6% (Crim) to 16.7% (Justin). Lysine in the protein ranged from 2.15 g/16 g N (Justin in Mexico) to 2.77 g/16 g N (Lerma Rojo in Mexico). There was a low negative correlation ($r = -0.4$) for lysine in the protein vs. protein content for the higher protein content wheats grown in North Dakota and a significant high negative correlation ($r = -0.68$) for the lower protein content wheats grown in Mexico. The overall correlation was -0.49 and was significant.

TABLE 18. Lysine content of triticales (data from Villegas et al. 1970) (Villegas 1972–73 data added in proof).

Variety or Cross[a]	Average protein (*N* × 5.7 *14% moisture basis*) (%)	Average lysine		Villegas 1972–73		
		In protein (*g/16 g N dry weight basis*)	In sample (%)	Lab No.	Protein %	Lysine % protein
1593A × 1620	14.7	3.08	0.577	5731	13.0	4.35
1593B	15.1	3.08	0.594	5734	13.2	4.27
1593C	16.4	3.06	0.641	5735	13.9	3.72
1593D	14.3	3.26	0.589	5742	13.0	4.17
1594A	16.2	2.98	0.615	5744	13.0	3.72
1594A × 1601	15.8	3.11	0.627	5746	12.5	4.28
1594A × 1613	16.6	3.35	0.711	5792	12.4	3.81
1594A × 1628A	14.5	3.02	0.559	5793	12.3	4.04
1605	14.6	2.89	0.539	5796	12.2	4.05
1609B × 1636	16.6	2.92	0.618	5800	12.4	3.78
1636A	13.9	3.25	0.578	5801	12.3	3.83
1636A × 1614	15.0	3.08	0.590	5804	12.6	4.05
1636C	14.3	3.02	0.556	5805	13.3	3.87
1636C × 1642	14.5	3.04	0.563	5807	12.4	4.05
1637C	16.0	2.92	0.597	5819	13.4	3.78
1641A	12.8	3.12	0.507	5821	12.2	4.13
1641B	13.1	3.04	0.509	11452	16.3	2.98
1641D	13.8	3.21	0.565	11453	11.2	3.56
6A250 × 6A190[b]	16.1	2.73	0.557			
6A250 × 6A191[c]	13.3	2.94	0.496			
My 64 × Triticale	17.0	2.75	0.595			
Triticale S-112	13.8	2.72	0.479			
Triticale 6913	18.5	2.71	0.639			
Triticale 100-C-132-1	14.7	2.74	0.563			
6A250(6A66.12 × 6A20)	15.0	2.82	0.540			

[a]Average lysine and protein content of selections from each variety or cross.
[b]One selection contained the low of 2.32 g lysine per 16 g N in the protein.
[c]One selection contained the high of 3.42 g lysine per 16 g N in the protein.

Five varieties of durum wheat, Mindum, Wells, Lakota, Stewart 63 and Leeds were grown at three locations in North Dakota, and 23 lines were grown in Mexico. At North Dakota, protein varied from 13.8% (Mindum at Fargo) to 19.9% (Leeds at Edgeley). Lysine in grams/16 g N in the protein varied from 1.84 g (Leeds at Edgeley) to 2.45 g (Mindum at Edgeley) and the negative correlation between lysine in the protein and protein content was highly significant ($r = -0.77$). In Mexico, protein varied from 9.5% (RD176-7A) to 17.1% (RD101-2A). Lysine in the protein varied from 2.17 g (RD182-11B) to 3.10 g (RD176-7A) but the correlation with protein was not significant ($r = -0.33$). However, the overall correlation coefficient for durum wheat was −0.65 and significant. The overall mean protein content was 15.3%. The mean of 2.39 g lysine/16 g N in the protein was lower than the mean of 2.71 g lysine/16 g N in the protein reported by Lawrence et al. (1958) for six durum wheat varieties with an average protein content of 13%.

Altogether 125 varieties or species of rye were analyzed by Villegas et al. (1970); the value for 20 of these are presented in Table 19. Lysine content in the protein correlated significantly with the protein content ($r = -0.69$). It will be observed from Table 19 however that the varieties Explorer, USDA PI 168178, Rye Gator, Detenicke and *Secale montanum*-23-282 were relatively high in both protein and lysine.

Since the lysine varies with the protein content, the regression equation for lysine in protein vs. protein content was used first to estimate the overall lysine content at 13.5 and 17.2% protein and secondly, the variability of lysine content for

TABLE 19. Lysine content of rye species and varieties (Villegas et al. 1970).

Variety and/or Species	Protein (%)	Lysine	
		In protein (*g/16 g N*)	In sample (%)
Antelope	11.4	3.33	0.443
Carsten	9.2	3.65	0.392
Secale dalmaticum CPI 22755	16.5	3.23	0.621
Explorer[a]	14.1	3.30	0.542
USDA PI 168178[a]	14.6	3.22	0.549
USDA PI 227870	10.5	3.71	0.453
Rye Gator[a]	17.3	3.21	0.649
Prolific Spring	14.9	3.25	0.563
Balbo	11.2	3.63	0.474
Detenicke[a]	16.0	3.37	0.628
Secale cereale 5-SC-18	12.8	3.22	0.482
Rye Korean 1	15.3	3.05	0.544
Dominant	11.0	3.80	0.489
Volyanko	11.9	2.91	0.400
Afganistan Winter Rye	10.4	3.35	0.405
Canadian Spring Rye	9.2	3.71	0.397
Secale montanum[a] 23-282	16.9	3.10	0.611
Secale segetale 23709	17.2	3.02	0.604
Abruzzi A	9.9	2.55	0.292
Maia barroso (5053)	10.6	4.26	0.524

[a]Ryes with relatively high lysine and protein.

each cereal examined. The results are given in Tables 20 and 21.

Munck (1972) reported on the amino acid analyses (by ion exchange) of different varieties of wheat, rye and octapoloid triticales grown at two locations in Sweden. From the results presented in Table 22, it will be seen that the mean protein content of the triticale is higher than the wheat and rye, but the lysine and threonine contents, expressed as grams amino acid/16 g N are lower in triticale than in wheat and rye.

Ruckman et al. (1973) compared the hard red spring wheat INIA 66, the white spring wheat Siete Cerros 66, and the amber durum spring wheat Oviachic 65, all short varieties, with the triticales 6TA204 (Jenkins Foundation for Research, Salinas, California); Rosner (University Manitoba) and T-1324 (CIMMYT), all tall varieties, in field experiments at El Centro, Five Points, Davis and Tulelake, California, using

TABLE 20. Estimated lysine content of wheat, rye, and triticale at 13.5 and 17.2% protein (*dry weight basis*) (Villegas et al. 1970).

Cereal	Lysine per 16 g N in protein	
	13.5% protein (*g*)	17.2% protein (*g*)
Spring wheat	2.49	2.36
Durum wheat	2.51	2.26
Rye	3.30(3.39)[a]	3.01(3.11)[a]
Triticale	3.06	2.81

[a]Estimated value when a N-to-protein factor of 5.73 is used [5.73 is average reported by Jones (1931) and Tkachuk (1969b)].

TABLE 21. Variability of the estimated lysine content (*at 15% protein level*) in wheat, rye and triticale (Villegas et al. 1970).

Cereal	Standard deviation (*as lysine per 16 g N in protein*)
Spring wheat	0.13
Durum wheat	0.17
Rye	0.24
Triticale	0.21

TABLE 22. Protein ($N \times 5.7$) and content of nine amino acids in rye, octaploid triticale and wheat grown at two locations in Sweden in 1964 *as grams amino acid/16 g. N* (ranges in parentheses) (data from Munck 1972).

	Winter rye (Svalof)	Winter triticale (Lund)	Winter wheat (Lund)	Winter wheat (Svalof)	Spring wheat (Svalof)
Protein, %	10.8 (9.6–12.4)	14.8 (13.5–16.1)	11.7 (11.1–17.2)	13.1	12.9 (10.5–15.3)
Amino acids					
Lysine	3.72 (3.3–4.0)	2.78 (2.6–3.2)	2.95 (2.5–3.0)	2.85	2.87 (2.6–3.2)
Arginine	5.03 (4.6–5.4)	4.30 (4.0–4.7)	4.74 (4.4–5.1)	4.67	4.66 (4.4–5.1)
Histidine	2.30 (2.1–2.4)	2.11 (1.9–2.4)	2.30 (2.2–2.4)	2.28	2.24 (2.1–2.4)
Methionine (without oxidation)	1.57 (1.4–1.7)	1.44 (1.2–1.6)	1.52 (1.5–1.7)	1.57	1.49 (1.3–1.7)
Threonine	3.58 (3.4–3.7)	3.09 (2.9–3.3)	3.11 (3.0–3.2)	3.10	3.15 (2.8–3.3)
Valine	4.88 (4.6–5.1)	4.42 (4.2–4.7)	4.46 (4.4–4.7)	4.53	4.42 (4.0–4.7)
Isoleucine	3.56 (3.5–3.8)	3.46 (3.2–3.6)	3.48 (3.4–3.9)	3.61	3.55 (3.6–3.7)
Leucine	6.47 (6.1–6.9)	6.57 (6.3–6.9)	6.83 (6.7–7.3)	6.90	6.85 (6.4–7.1)
Phenylalanine	4.82 (4.6–4.9)	4.64 (4.4–4.9)	4.41 (4.4–5.0)	4.61	4.60 (4.4–4.8)

chemical fertilization described as that employed for optimum production at each location. On average, the triticales had higher whole-grain protein content (Kjeldahl $N \times 5.7$) than the wheats (16.3 vs. 14.3% on a dry weight basis), higher lysine (by gas chromatography) in the grain (0.428 vs. 0.307%) and in the protein (2.63 vs. 2.14%). Mean yields of the triticales were lower at all locations; about 71% of the wheats. The best yield of triticale was from 6TA204 at Tulelake which has longer days and cooler conditions than the other locations; the worst was from Rosner, which does not perform well in California, at El Centro.

Grain protein contents did not vary significantly among locations. Protein yields of the triticales, on a dry weight basis, were significantly lower than the wheats at all locations (540 lb vs. 675 lb/acre; 605 vs. 756 kg/ha), but lysine yields were comparable (14.2 vs. 14.5 lb/acre; 15.9 vs. 16.2 kg/ha).

Production of grain showed significant variety × location interaction and this was reflected in the protein and lysine yields per hectare. Wricke's (1962) method was used to determine varietal stability in relation to environment. Among the triticales, T-1324 was unstable for grain, protein and lysine yields and Rosner for lysine yield; 6TA204 showed good stability. Among the wheats, INIA 66 was unstable for grain, protein and lysine yields, Siete Cerros 66 was unstable for grain and protein yields; Oviachic 65 was extremely stable.

BIOLOGICAL QUALITY

Studies on Human Adults Rosner triticale grown in Manitoba, Canada and wheat (a composite of high-protein Atlas 66 × Comanche lines grown in Nebraska, USA) were compared, at two levels of protein intake, in nitrogen balance studies with young human adults by Kies and Fox (1970c).

The subjects were five males and four females and the period of the study was 33 days, consisting of an introductory 3-day nitrogen-depletion period, two 5-day adjustment periods and four experimental periods of 5 days each. The experimental periods were arranged at random for each subject. During the adjustment and experimental periods nitrogen (N) intake was maintained at 4.8 or 6.8 g N per day respectively, 4.0 and 6.0 g N being provided by wheat or triticale, 0.8 g N from

the basal diet. The wheat and triticale grains were whole ground in a hammer mill and were fed in the form of baked, yeast raised rolls taken in equal amounts at each of the three daily meals. The caloric intake for each subject was kept constant during the experimental periods at the level required for weight maintenance.

In nitrogen balance studies, a decrease in body nitrogen loss is generally interpreted as indicative of improved protein utilization. The results showed that at the lower level of protein intake, 4.0 g N/day, the average nitrogen balance was −0.62 g N/day for wheat and −0.44 g N/day for triticale, a difference of 0.18 g N/day in favour of triticale, significant at the 5% level. At the higher level of protein intake, 6.0 g N/day, subjects on both the wheat and triticale diets showed a significantly better nitrogen retention than when receiving the 4 g N level diets. The average nitrogen balance was −0.16 g N/day, for wheat and + 0.01 g N/day for triticale, a difference of 0.17 g N/day in favour of triticale, significant at the 1% level.

Two subjects did not conform to the response pattern. Differences in individual response are not, however, unusual in nitrogen balance studies. Female subjects tended to retain more nitrogen than males possibly because the latter engaged in a higher level of strenuous exercise during the experimental period which may have affected protein as well as caloric need.

Kies and Fox (1970b) also conducted two studies with human volunteers to determine the first limiting amino acid in ground whole triticale grain (Rosner grown in Manitoba, Canada) and in whole ground wheat (a composite of high-protein Atlas 66 × Comanche grown in Nebraska, USA).

Nineteen young adults took part in two studies each 33 days in length. In the wheat study seven males and three females took part and in the triticale study six males and three females took part. Each study had a 3-day nitrogen-depletion period, a 5-day nitrogen adjustment period and five experimental periods of five days each. The experimental periods were arranged at random for each subject. After the nitrogen-depletion period, nitrogen intake was maintained at 5.0 g N per subject per day, 4.0 g N from wheat or triticale plus 0.68 g N from the basal diet and systematically variable amounts of nitrogen from the amino acid supplements. Urea was used to maintain the diets isonitrogenous at 5.0 g N intake level. Caloric intake was kept constant for each subject at the level required for weight maintenance. The wheat and triticale flours were fed as baked yeast raised rolls. The amino acid supplements were L-lysine (0.960 g per day wheat study; 0.891 g per day triticale study), L-methionine (1.152 g per day wheat study, 1.092 g per day triticale study) L-tryptophan (0.212 g per day wheat study, 0.236 g per day triticale study). During one experimental period no amino acid supplements were given (negative control) and in another a combination of all three was given (positive control).

Protein was evaluated primarily by a nitrogen-balance technique and by other laboratory tests, as described by Kies and Fox (1970c). The lysine and methionine composition of the wheat and triticale were determined by autoanalyzer; tryptophan was estimated from reference tables.

Under the experimental conditions of both studies, lysine was shown to be the first limiting amino acid. The average increment in nitrogen retention between lysine-supplemented and unsupplemented wheat diets was 0.73 g N per day, and in the triticale diets 0.54 g N per day, both significant at the 1% level of probability.

Rat, Mouse, and Vole Assays Knipfel (1969) compared a sample of Rosner triticale with wheat and rye samples purchased locally, in diets fed to male weanling rats. The diets contained 10% protein from casein, triticale, wheat, rye or equal-part mixtures of casein plus wheat, casein plus triticale or casein plus rye. The protein contents were: casein 86.3% (N × 6.25); wheat 13.5% (N × 5.7); triticale 16.1% (N × 5.7); rye 12.6% (N × 5.7). Food and drink were provided ad libitum. The amino acid composition (by autoanalyzer) of the diets, except for cystine and tryptophan, is shown in Table 23. The Protein Efficiency Ratios (PER), adjusted to a value of 2.50 for casein, are given in Table 24. The PER of Rosner triticale was equal to the PER of rye, with the PER of wheat significantly lower.

Blood samples from each dietary group were pooled and analyzed by microbiological assay for free lysine, methionine and threonine. The results indicate that lysine was markedly limiting in the triticale and the wheat, but less so in the rye. Subsequent limiting amino acids were not determined. Knipfel (1969) considers that the superiority of triticale over wheat may be attributed to its higher content of lysine and sulphur amino acids.

In isonitrogenous diets fed to weanling rats, Shimada and Cline (1972) compared two unidentified varieties of triticale, one grown in Indiana and

TABLE 23. Amino acid composition of test diets calculated from amino acid analyses of individual protein sources (Knipfel 1969).

	Diets						
Amino Acid	(A) 10% Casein	(B) 10% Triticale	(C) 5% Triticale; 5% Casein	(D) 10% Wheat	(E) 5% Casein; 5% Wheat	(F) 10% Rye	(G) 5% Casein; 5% Rye
	percent of diet						
Aspartic acid	0.68	0.51	0.59	0.49	0.59	0.74	0.71
Threonine	0.37	0.29	0.33	0.32	0.35	0.33	0.35
Serine	0.46	0.40	0.43	0.41	0.44	0.37	0.42
Glutamic acid	2.30	3.19	2.75	3.71	3.00	3.12	2.71
Proline	1.11	1.21	1.16	0.91	1.01	0.90	1.01
Glycine	0.19	0.42	0.30	0.39	0.29	0.43	0.31
Alanine	0.30	0.39	0.35	0.36	0.33	0.44	0.37
Valine	0.63	0.47	0.55	0.48	0.55	0.45	0.54
Methionine	0.26	0.15	0.21	0.10	0.18	0.13	0.20
Isoleucine	0.54	0.41	0.48	0.40	0.47	0.37	0.46
Leucine	0.92	0.69	0.80	0.65	0.79	0.56	0.74
Tyrosine	0.50	0.32	0.41	0.36	0.43	0.27	0.39
Phenylalanine	0.49	0.49	0.49	0.48	0.49	0.42	0.45
Lysine	0.71	0.33	0.52	0.29	0.50	0.47	0.59
Histidine	0.27	0.24	0.26	0.25	0.26	0.26	0.27
Arginine	0.33	0.49	0.41	0.51	0.42	0.52	0.42

TABLE 24. Performance data of rats fed casein, triticale, wheat and rye diets (Knipfel 1969).

	Diets*						
Criteria	(A) 10% Casein	(B) 10% Triticale	(C) 5% Triticale; + 5% Casein	(D) 10% Wheat	(E) 5% Casein; + 5% Wheat	(F) 10% Rye	(G) 5% Casein; + 5% Rye
Weight gain, g	63.7a	31.8bc	67.2a	21.5c	62.6a	37.8b	73.1a
Feed intake, g	220.6a	177.3b	234.0a	180.3b	240.3a	203.0b	239.6a
PER	2.50a	1.55c	2.49ab	1.03d	2.25b	1.61c	2.64a

*Means with same letter(s) not significantly ($P < 0.05$) different.

the other in Texas, with maize and maize plus soya. The triticale varieties were also compared with maize alone on an equal weight in diet basis. As judged by weight gain and feed : gain ratio, both triticales were equal to maize on an isonitrogenous basis and superior to maize on an equal weight basis. When the triticales provided the sole source of protein, at levels of 10% or 15%, and were supplemented with amino acids, lysine was found to be the first, and threonine the second, limiting amino acid. Methionine appeared to be third limiting. The Texas grown triticale was

TABLE 25. Biological evaluation of triticale samples from CIMMYT with mice (*mean values of five mice*) (data from P. J. Mattern 1972 personal communication).

Triticale Sample No.	Protein N × 5.7 (*dry weight basis*) %	Weight gain (*g*)	Feed consumption (*g*)	Feed efficiency ratio
A71-5403	16.1	11.54	112.88	9.78
A71-5404	17.0	9.96	107.30	10.77

TABLE 26. Performance of growing rats on diets of Rosner triticale, Neepawa wheat and Cougar rye, fed to provide 10% and 7% protein in the diet, over 28 days (unpublished data from McDonald and Larter 1972).

	Average daily gain 0–28 days (*g*)	Average daily feed intake 0–28 days (*g*)	Protein index (*g wt. gain/ g protein intake*)
10% protein level			
Casein	3.64	13.95	2.51
Triticale	1.71	13.23	1.29
Wheat	1.11	9.90	1.13
Rye	1.52	12.51	1.29
7% protein level			
Casein	2.60	16.18	2.20
Triticale	1.61	15.02	1.47
Wheat	1.14	12.31	1.26
Rye	0.93	13.70	1.01

judged to be of higher protein quality than the one grown in Indiana. Weber and Reid (1972) have, however, reported briefly that in feeding trials with young mice, the first limiting amino acid for nineteen Mexican dwarf wheat varieties and for three (unidentified) triticale varieties was methionine. The second limiting amino acid was lysine in the wheat and threonine in the triticale. The gains in body weight were about the same for all the wheat and triticale samples studied in Weber and Reid's experiments but the liver GOT (glutamic oxaloacetic transaminase) activity and carcass retention of nitrogen differed though in what manner is not stated.

Octaploid triticale, protein (N × 6.25) 14.8%, winter rye, protein 10.8%, and winter wheat, protein 13%, were included in feeding trials with mice in a 20-day experiment (Munck 1972). The dietary protein varied with the protein content of the cereals. The triticale diet gave good results, comparable to that of the wheat. The rye diet gave relatively poor results which could not be completely explained by differences in protein quantity and amino acid composition.

The College of Agriculture at the University of Nebraska, Lincoln, Nebraska have also conducted mice feeding trials on samples of triticale from CIMMYT. P. J. Mattern (1972 personal communication) reported the results shown in Table 25 and also found substantial differences in feeding trials with mice on wheat samples of the same protein content and consumption.

McDonald and Larter (1972 unpublished data) compared Rosner triticale, Neepawa wheat and Cougar rye in feeding trials using rat, mouse and meadow vole (*Microtus pennsylvanicus*). The cereal (or casein in the control diet) was the sole source of nitrogen and was fed to give a level of 10% protein (N × 6.25) in one series and 7% protein in a second series. In the first series, 10% protein level, diets containing soybean meal, wheat gluten, horsebeans (*Vicia faba*) and oats

TABLE 27. Performance of mice on diets of Rosner triticale, Neepawa wheat and Cougar rye fed to provide 10% and 7% protein in the diet, over 21 days (unpublished data from McDonald and Larter 1972).

	Average daily gain, 0–21 days (*g*)	Average daily feed intake 0–21 days (*g*)	Protein index (*g wt. gain/ g protein intake*)
10% protein level			
Casein	0.62	4.71	1.27
Triticale	0.40	3.91	1.04
Wheat	0.40	3.82	1.06
Rye	0.36	3.70	1.03
7% protein level			
Casein	0.33	4.58	1.10
Triticale	0.41	4.76	1.16
Wheat	0.40	4.95	1.12
Rye	0.34	4.40	1.16

TABLE 28. Performance of voles on diets of Rosner triticale, Neepawa wheat and Cougar rye fed to provide 10% and 7% protein in the diet, over 21 days (unpublished data from McDonald and Larter).

	Average daily gain, 0–21 days (*g*)	Average daily feed intake 0–21 days (*g*)	Protein index (*g wt. gain/ g protein intake*
10% protein level			
Casein	0.30	3.30	0.89
Triticale	0.38	3.20	1.26
Wheat	0.24	3.02	0.75
Rye	0.30	3.18	1.04
7% protein level			
Casein	0.16	3.68	0.60
Triticale	0.35	4.21	1.19
Wheat	0.30	4.12	0.98
Rye	0.29	4.10	1.08

were also compared. In the second series, 7% protein basis, only diets containing casein, wheat, rye and triticale were included.

When fed for 21 days on the twelve different protein sources, the voles showed very little fluctuation in body weight gain (grams per day) the results varying between 0.16 g and 0.38 g, the lowest value being on casein and the highest on triticale. In comparable experiments with rats, body weight gain ranged from 0.22 g per day on wheat gluten to 3.64 g per day on casein. Triticale showed an average gain of 1.71 g per day and soybean meal 3.10 g per day. The important result was that the standard errors in the vole protein indices were of the order of 50% of the mean values, whereas those for rats were of the order of 20% of the means. The measurement of body weight and food consumption for casein, triticale, wheat and rye are presented in Table 26 for rats, in Table 27 for mice and in Table 28 for voles. Further tests of ten days duration were carried out with rats, mice and voles using two samples of wheat and four samples of triticale provided by CIMMYT. The chemical analysis of the wheat and triticale samples, including their resorcinol content, is given in Table 29 and the results of the feeding trials on these samples in Table 30. The laboratory rats and mice rated the biological

TABLE 29. Chemical analyses on triticale and wheat samples from CIMMYT (unpublished data from McDonald and Larter 1972).

Sample	Protein (*air-dry basis*) (*g/100g*)	Lysine (*g/16g N*)	Fibre (*g/100g*)	Resorcinol (*g/100 g*)
Wheat				
4859	11.8	2.40	2.8	0.067
4860	12.4	2.38	2.4	0.083
Triticale				
4861	15.9	2.72	2.7	0.091
4862	15.6	2.80	2.4	0.099
4863	17.1	2.78	2.5	0.089

quality of the cereals in an order predictable from their chemical composition and/or previous experience, but the variability among the voles made it impossible to classify the cereal varieties according to their biological quality. Several of the voles consistently rated cereal grains higher than casein. Consequently the results from the vole assays cannot be regarded as a reliable indication of comparative nutritional values.

Gundel et al. (1970) compared three hexaploid triticales, designated 20/68, 57/68 and 64/68 with Bezostaia wheat, B.40 barley and Kecskemeti rye all grown in Hungary, in nitrogen metabolism trials with rats. The protein (N × 6.25) and moisture content of the grains with their biological value, apparent digestibility of the protein and NPU are shown in Table 31. The authors consider that triticale would be useful as animal feed in Hungary.

Chick Feeding Trials A hexaploid triticale of protein 18.42% (N × 6.25) was investigated by Sell et al. (1962) as a component of rations for male chicks. The amino acid content of the triticale, determined chromatographically, is compared with the average composition of a hard red spring wheat in Table 32.

TABLE 30. Biological evaluation of triticale and wheat samples from CIMMYT with rats, mice and voles (unpublished data from McDonald and Larter 1972).

	Diet					
	(A) Casein	(B) Wheat 4859	(C) Wheat 4860	(D) Triticale 4861	(E) Triticale 4862	(F) Triticale 4863
Rats (means of 8)						
Average daily gain, *g*	4.36 ±0.80	2.23 ±0.52	2.41 ±0.45	3.06 ±0.48	3.24 ±0.64	3.01 ±0.40
Average daily feed, *g*	17.38 ±1.67	17.80 ±1.45	16.24 ±1.37	21.07 ±4.82	17.30 ±1.62	16.94 ±1.41
Protein index (*g wt gain/g protein intake*)	2.28 ±0.31	1.32 ±0.26	1.36 ±0.26	1.62 ±0.17	1.71 ±0.22	1.66 ±0.20
Mice (means of 10)						
Average daily gain, *g*	0.89 ±0.14	0.70 ±0.00	0.73 ±0.00	0.71 ±0.10	0.78 ±0.10	0.85 ±0.10
Average daily feed, *g*	5.30 ±1.07	5.34 ±0.52	5.61 ±0.46	5.43 ±0.64	5.80 ±0.92	5.56 ±0.52
Protein index (*g wt gain/g protein intake*)	1.54 ±0.28	1.26 ±0.14	1.19 ±0.14	1.22 ±0.17	1.25 ±0.24	1.40 ±0.14
Voles (means of 8)						
Average daily gain, *g*	0.39 ±0.14	0.57 ±0.19	0.59 ±0.23	0.58 ±0.16	0.57 ±0.12	0.42 ±0.14
Average daily feed, *g*	2.67 ±0.25	2.99 ±0.60	3.28 ±0.71	3.08 ±0.33	3.23 ±0.53	2.49 ±0.27
Protein index (*g wt gain/g protein intake*)	1.37 ±0.56	1.82 ±0.41	1.64 ±0.49	1.76 ±0.46	1.59 ±0.16	1.53 ±0.38

TABLE 31. Protein and moisture contents of Hungarian grown triticales, Bezostaia wheat, B.40 barley and Kecskemeti rye and their biological value, apparent digestibility and NPU for rats (data from Gundel et al. 1970).

Grain	Protein N × 6.25 (%)	Moisture (%)	Biological value (%)	Apparent digestibility of the protein (%)	NPU (%)
20/68 Triticale	15.30	11.04	71.49±1.09	74.15	61.29
57/68 Triticale	12.00	10.34	72.83±0.91	74.55	65.95
64/68 Triticale	9.45	10.75	72.85±1.80	72.85	64.82
Bezostaia wheat	10.60	8.70	70.77±2.40	72.17	59.83
B.40 barley	11.10	10.09	74.21±0.88	69.12	62.69
Kecskemeti rye	10.80	8.77	74.82±0.81	65.66	60.76

TABLE 32. Amino acid composition of the protein of triticale compared to that of an average hard spring wheat (data from Sell et al. 1962).

Amino acid	Triticale	Wheat
	(percent of protein based on N × 6.25)	
Arginine	4.74	6.15
Glycine	3.78	6.92
Histidine	2.18	2.31
Isoleucine	3.73	4.62
Leucine	6.23	7.69
Lysine	6.07	3.84
Methionine	1.60	1.54
Phenylalanine	4.47	5.38
Threonine	2.50	3.08
Tryptophan	1.17	1.54
Valine	4.26	4.62
Protein, %	18.42	14.18

In the first trial, the basal ration contained 67.5% hard spring wheat (14.18% protein) and 23% soybean meal (44% protein). Triticale was substituted for corresponding quantities of wheat at 30, 45, 60 and 67% of the ration. A further ration which was isonitrogenous to the wheat basal ration but contained 81% triticale was included.

The rations containing from 30 to 67% triticale supported weight gains equivalent to the wheat basal ration. The 81% isonitrogenous triticale ration gave significantly lower weight gains. The efficiency of feed utilization and feed:gain ratio (F/G) by chicks receiving 30 and 45% triticale was significantly better than those receiving the wheat, 60 and 67% triticale rations. The ration metabolizable energy (ME) (Hill and Anderson 1958) values of the 45, 60 and 67% triticale and wheat rations were similar; that of the 30% triticale significantly higher. The 81% triticale ration, which was isonitrogenous with the wheat basal, gave a significantly lower F/G than the wheat basal. The method by which the ration was made isonitrogenous is not stated but Sell et al. suggest that the poorer results from the 81% triticale ration may have been caused by reducing the soybean protein in the isonitrogenous ration to a point where deficiency in lysine resulted.

In the second trial, the basal ration contained 58.5% barley (12.27% protein) and 30% soybean meal (44% protein). Hard spring wheat and triticale were substituted for the barley on a weight for weight basis. Weight gains and F/G were not significantly different but the data suggested that triticale was equal to wheat and superior to barley on a weight for weight basis.

In the third trial, the basal ration contained 90% triticale and 1.5% soybean meal (44% protein), a total of 17.5% protein. The basal ration was supplemented with 0.15% glycine, 0.05% DL-methionine and 0.10% L-lysine HCl in a complete factorial arrangement. L-lysine HCl significantly improved weight gain and F/G. There was no response to glycine or DL-methionine.

In the fourth trial, the basal ration contained 76% triticale and 13.5% soybean meal (44% protein), a total of 21% protein. The test rations were: (A) basal + 0.1% methionine, (B) basal + 0.1% lysine, (C) basal + 0.1% methionine + 0.1% lysine, (D) basal + 0.2% additional protein, and (E) an isonitrogenous ration based on hard spring wheat. Chick growth improved significantly over the basal ration only when both 0.1% methionine and 0.1% lysine were added.

TABLE 33. Comparative amino acid composition of triticale, maize, wheat and rye, calculated as amino acid/16 g N (data from Bixler et al. 1968).

Amino acid	Triticale	Maize	Wheat	Rye
Ammonia	3.2	2.1	3.3	2.7
Arginine	5.5	3.7	4.2	4.9
Histidine	2.2	2.5	2.3	2.3
Lysine	3.1	2.6	2.5	3.8
Tyrosine	1.5	2.8	1.4	1.5
Tryptophan	1.7	2.2	2.0	2.2
Phenylalanine	3.9	4.6	4.0	4.0
Cystine	1.8	1.7	1.9	1.7
Methionine	4.5	1.4	4.8	2.9
Serine	3.9	3.2	4.1	4.2
Threonine	3.1	2.9	3.0	3.7
Leucine	6.0	12.2	6.2	5.9
Isoleucine	3.2	3.4	3.2	3.3
Valine	2.9	4.6	2.8	3.3
Glutamic acid	27.3	17.9	30.6	23.7
Aspartic acid	7.1	7.0	5.1	7.2
Glycine	3.9	3.2	4.0	4.5
Alanine	3.8	7.9	3.4	4.4
Proline	8.9	8.3	9.7	8.9

In summary, on a weight for weight basis, the triticale was approximately equal to hard spring wheat in nutritional value for chicks as judged by weight gain, efficiency of feed utilization and ration metabolizable energy. Lysine appeared as the first limiting amino acid for chicks, with methionine being also limiting.

Bixler et al. (1968) reported on their findings in feeding trials using male chicks at Michigan Agricultural Experimental Station. The triticale, grown in Sonora, Mexico was compared with wheat and rye, also grown in Sonora and with a maize (de Kalb variety grown in USA) diet previously used at the Experimental Station. The amino acid composition of the grain (by auto-analyzer) is given in Table 33. The percentage protein (Association of Official Agricultural Chemists 1965), expressed on an as-received basis was triticale 16.75, wheat 12.94, rye 9.18. The diets were isonitrogenously balanced to give approximately 23% protein. The cereal portions of the four starter diets are given in Table 34. The feeding results are summarized in Table 35.

The maize and wheat diets were significantly better than the triticale but no statistical difference at 5% level of probability was found between the rye and triticale diets. This result is not surprising in view of the relatively low level of soybean protein (16.15%) in the triticale diet. At that level, lysine would probably still be limiting in the triticale diet.

TABLE 34. Percentage composition of all-mash chick starter diets (data from Bixler et al. 1968).

	Triticale	Maize	Wheat	Rye
Titricale, ground	72.50			
Maize, ground		62.21		
Wheat, ground			67.30	
Rye, ground				59.50
Soybean meal (50% protein)	16.15	27.44	21.35	29.50
Other (similar in all diets)	11.35	11.35	11.35	11.35

Bragg and Sharby (1970) reported on three feeding trials to evaluate triticale, produced during 1967, as a source of energy and protein for broiler chicks, and to determine the utilization of amino acids in triticale protein compared to wheat. Each dietary treatment was arranged in a random block design and feed and water supplied ad libitum. Weight gain, F/G ratio and mortality were calculated at 7-day intervals. The cereal portions of the basal, nutritionally balanced, experimental rations, which were nearly isonitrogenous and isocaloric, are given in Table 36.

In trial 1, the three levels of triticale replacement of wheat in the basal ration (A) (zero), (B) 50% and (C) 100%, were fed with three levels of dietary animal tallow, 0, 2.5 and 5% at the expense of dextrose in the basal ration.

Although chick weight gain was slightly less from diets (B) and (C), the differences from the wheat diet (A) were not statistically significant. The addition of 2.5% animal tallow to the diets

TABLE 35. Nutritional value of triticale, maize, wheat and rye in isonitrogenous diets for chicks (data from Bixler et al. 1968).

Grain source in the diet	Weight gain, 2 weeks (g)	Feed consumption/ chicks, 2 weeks (g)	Feed conversion (*feed consumed/ unit of grain*)
Triticale	184.2	281.2	1.52
Maize	208.3	279.3	1.34
Wheat	208.0	296.8	1.43
Rye	174.8	269.4	1.54

TABLE 36. Percentage composition of broiler chick diets (data from Bragg and Sharby 1970).

	Diets		
	(A)	(B)	(C)
Wheat (protein (N × 6.25) 14.4%)	65.5	33.0	—
Triticale (protein (N × 6.25) 14.9%)	-	33.0	66.0
Soybean meal (protein N × 6.25) 44.0%)	20.0	19.5	19.5
Other (similar in all diets)	14.5	14.5	14.5

(B) and (C), improved weight gain compared to diet (A) but there was no significant difference between any of the three diets containing 2.5% fat.

The addition of 5% fat to diet (B) significantly improved the growth compared to diet (B) and diet (C) without fat but was not significantly better than any diet containing 2.5% fat. The 5% fat addition to diet (C) produced a severe growth depression due to a decrease of feed intake which was approximately 80% of the other rations. The F/G ratio was affected more by the addition of fat to the ration than by the substitution of triticale for wheat. There was no difference in metabolizable energy (Hill et al. 1960), of the three basal diets.

In trial 2, four supplemental levels of DL-methionine (0, 0.05, 0.10 and 0.15%) were added to the basal rations. Lysine was maintained at a constant level of 1.1%. The addition of 0.05% methionine to the triticale ration improved growth significantly over the triticale control and the wheat control with no further improvement from the higher levels of addition. No significant improvement in F/G resulted from supplementation.

In trial 3, four supplemental levels of DL-methionine (0, 0.05, 0.10 and 0.20%) and four supplemental levels of L-lysine (0, 0.05, 0.10 and 0.20%) were added to the triticale basal diet (C), with and without methionine; a wheat control was included. Weight gain and F/G ratio were not affected, and the significant improvement noted in Trial 2 with 0.05% methionine was not repeated. These differences are ascribed to the fact that two different strains of commercial chicks were used in the Trials 2 and 3.

Sell et al. (1962) had found that chicks fed triticale rations responded positively to methionine plus lysine treatment. Bragg and Sharby (1970) state that the triticale used by Sell et al. (1962) had a protein content of 18.42% while in their own trials, the protein content of 14.9% (N × 6.25) and the quality of the triticale, was very similar to that of hard wheat (protein 14.4%), which reduced the influence of the higher protein triticale, previously used, on the dietary amino acid pattern.

The amino acid composition (by autoanalyzer; excluding tryptophan) of triticale and wheat and the availability to chicks of the amino acids in the cereals (Bragg et al. 1969) are shown in Table 37. Triticale and wheat protein were efficiently utilized with an average availability of 93.6% and 92.1 respectively. The methionine level of triticale was higher and utilized 10% better than that of wheat. The utilization of cystine was greater than that of glycine in triticale but no significant difference was observed in utilization among the other amino acids. Glycine utilization in wheat was significantly less than the other amino acids. Methionine in wheat was utilized significantly better than glycine but was less available than the other amino acids.

Five triticale selections, grown at CIANO, identified as no. 24, 59, 106, 119 and 132, were fed in chick feeding trials (McGinnis 1972). The triticales provided 8% of the protein in a 14% protein diet and were compared with a similar diet in which 8% of the protein was provided by soybean meal. There were no significant differences between the triticales in chick PERs at two weeks. No. 59, 119 and 132 were not significantly inferior to the soybean meal diet though the PERs from No. 24 and 106 were significantly lower.

The same triticale selections, fed in a practical broiler diet in comparison with maize, gave similar results to maize both when penicillin was, and was not, added. In both the maize and triticale diets, the addition of penicillin resulted in significantly improved growth.

In another trial reported by McGinnis (1972) thirteen selections of triticales from CIANO were compared, in chick diets, with soybean meal, Gaines wheat, "high protein" wheat (protein content not stated), and rye. The triticale selections were identified as no. 27, 37, 38, 39, 44, 48, 52, 53, 54, 61, 64, 65 and 67. The chicks on the soybean meal diet grew at a rate significantly higher than the chicks on any of the other diets. The triticale diets all gave higher rates of growth than those obtained from the rye or from the high protein wheat, or from the Gaines wheat, with the exception of no. 61, which was lower. Penicillin supplementation improved significantly the growth

TABLE 37. Amino acid content and availability of triticale and wheat to growing chicks (data from Bragg and Sharby 1970).

Amino acid	Triticale		Wheat	
	Content (% *total grain*)	Availability* (%)	Content (% *total grain*)	Availability* (%)
Lysine	0.439	93.4ab	0.362	94.3c
Histidine	0.296	95.9ab	0.287	95.5c
Arginine	0.710	92.0ab	0.604	92.0bc
Aspartic Acid	0.879	92.4ab	0.687	91.9bc
Threonine	0.421	91.7ab	0.391	92.7bc
Serine	0.621	94.7ab	0.609	94.5c
Glutamic acid	4.851	97.3ab	5.241	97.5c
Proline	1.196	97.2ab	1.394	96.6c
Glycine	0.673	85.2a	0.627	70.8a
Alanine	0.465	90.3ab	0.394	89.9bc
Cystine	0.163	98.3b	0.136	96.1c
Valine	0.578	92.3ab	0.543	92.2bc
Methionine	0.204	90.0ab	0.180	81.8b
Isoleucine	0.461	93.0ab	0.436	94.2c
Leucine	0.910	94.7ab	0.894	95.2c
Tyrosine	0.417	94.6a	0.384	94.3c
Phenylalanine	0.633	95.8ab	0.645	95.8c
Tryptophan	not determined			
Availability (means of 17 amino acids)	-	93.6±4.66	-	92.1±1.90
Protein (N × 6.25)	14.9	-	14.4	-

*Means with the same letters are not significantly different ($P < 0.01$) within grain source.

response to the rye diet but failed to produce statistically significant growth improvement from any of the other diets. The PERs and the feed efficiency paralleled the results for body weight.

McGinnis (1972) has also reported on chick feeding trials in which 8% of the protein in a 14% protein diet was provided by soybean meal or by one of 27 triticale selections provided by Washington State University, the remaining 6% protein being provided by a premix. A control diet, in which all the protein was provided by the premix, was also included. The soybean meal diet gave better growth than any of the triticale diets. The triticales together with their protein contents (Kjeldahl N × 6.25) are listed in Table 38. The selections which were most effective in supporting chick growth are indicated by an asterisk.

Trailblazer triticale of protein 18.6% (N × 6.25) was also included in the trials. When supplemented with amino acids to a level equal to the soybean meal diet, the triticale-sustained chick growth was not significantly lower than growth on the soybean diet. When Trailblazer was used at levels between 39% and 43% of a practical broiler diet, lysine appeared to be the first and threonine the second, limiting amino acid.

Trailblazer triticale was also used to provide 73% of the diet in broiler diets. Growth response was improved when the diet was supplemented with lysine and when supplemented with penicillin. A significantly greater response was achieved when both were used together, the diet being then equal to the control diet containing maize supplemented with penicillin.

Turkey Poults and Laying Hens Feeding Trials

Sell and Johnson (1969) examined triticales from two suppliers in North Dakota as feed for turkey poults and laying hens. Triticale A had been cleaned and was known to be relatively free of ergot; triticale B was in crushed form and contained a considerable quantity of weed seed; possible contamination with ergot could not be determined.

In the turkey poult trials, both triticales were compared with a durum wheat on a weight for weight basis in nutritionally balanced turkey

TABLE 38. Protein contents of triticale selections from Washington State University (data from McGinnis 1972).

Triticale selection	Protein (N × 6.25) (%)
RS45	15.8
LNTC-1	19.0
RS46	15.7
6TA487	16.6
*RS47	14.4
*6TA484	15.2
*RS48	13.2
*6TA476	16.2
*RS49	14.5
6TA190	16.2
6TA199	17.8
6TA494	17.4
6TA385	17.5
6TA327	18.3
CTC-4	17.1
*6TA518	17.3
*6TA131	18.8
*6TA480B	16.8
*6TA492	17.0
6TA491	16.8
*6TA480A	16.2
*6TA204	16.7
6TA418	16.7
6TA488	16.5
*6TA479	16.6
6TA205	16.3
*6TA486	16.3

*Most effective in supporting chick growth.

starter rations, the grains representing 42% of the test diet. The weight gain of poults on all three rations from one day to three weeks of age was approximately equal indicating little, if any, ergot contamination of the triticale. Efficiency of feed utilization (F/G) was similar for poults fed durum wheat and triticale A but was significantly less efficient with triticale B. The metabolizable energy (ME) contents of the wheat and triticale A were similar but that of triticale B was relatively low; more feed being required per unit of weight gain.

The trial was continued with poults from three to six weeks of age but only durum wheat and triticale B could be compared as there was insufficient triticale A. Again weight gains were essentially similar but the F/G ratio was less efficient from triticale B.

The same grains were included at a level of 53% in rations for laying hens. The ME of durum wheat and triticale A were similar, that of triticale B was significantly lower.

Sell and Johnson conclude that clean triticale, relatively free of ergot, compares favourably with wheat in nutritional value for poultry.

Guenthner and Carlson (1970), compared triticale, maize, wheat and sorghum laying diets (the exact composition is not given) when fed to single comb White Leghorn hens. Two diets were formulated from each grain to contain 12.0% and 15.4% crude protein (N × 6.25). All the protein in the 12% wheat and triticale, essentially isocaloric, diets was provided by the cereals, the other diets being balanced with soybean meal. Methionine and lysine were added to provide a minimum of 0.52% methionine plus cystine and 0.50% lysine. Egg production performance was judged on the averages of three weeks preceding the nine week test period and the final three weeks. Initial average egg production for all groups at 34 weeks of age was 75%. In the 15.4% protein series egg production increased 4% with wheat and maize, 5% with sorghum and decreased 4% with triticale. In the 12% protein series, production decreased 13% with triticale, 10% with wheat, 7% with sorghum and increased 4% with maize. Hen performance on both maize diets was equal; the 12% protein triticale, wheat and sorghum diets did not support egg production as well as did maize. Hens on both the triticale diets and on the 12% wheat diet lost weight, indicating amino acid deficiencies other than lysine and methionine plus cystine. The effect of the superior amino acid composition of the protein in the soybean meal used to balance the maize diet possibly influenced this result.

Weber and Reid (1971) compared two unspecified types of triticale and four varieties of wheat with sorghum in laying hen diets consisting of 17% crude protein (N × 6.25) and metabolizable energy values of 2780 kcal/kg. Differences in egg production and egg weight, but not in shell thickness, were found but no further details are given.

Two triticales, no. 204 and a composite of no. 418 and 419, were compared with the wheat varieties Sonora 64, Siete Cerros and INIA 66, and two sorghums over 252 days in feeding trials with laying hens at the University of Arizona (Weber et al. 1972). Siete Cerros and the triticale composite gave results in egg production and feed conversion similar to sorghum; Sonora 64, INIA 66, and Triticale 204 all lowered egg production

significantly. All the wheat and triticale samples produced lower egg weights than the sorghums.

When, in a second experiment, INIA 66, Siete Cerros, Triticale 203, Triticale 204 and a commercial sorghum were compared, egg production, feed conversion and egg weights were similar. Metabolizable energy values averaged 3130 kcal/kg for the triticales and 3450 kcal/kg for the wheats.

A diet for laying hens in which triticale of unstated origin provided 82% of the protein with the remainder of the protein coming from fishmeal and alfalfa gave results equivalent to those from a conventional layer diet. However, when fishmeal was not included and the protein was supplied by triticale, alfalfa and wheat, and supplemented with lysine and methionine at levels calculated to be adequate, egg production was poorer than from the control diet (Fernandez et al. 1972).

Swine Feeding Trials Stothers and Shebeski (1965) conducted trials with hexaploid triticale grown in the crop years of 1961, 1962 and 1964 as a feed for growing swine.

The protein content (analytical method not stated) of the 1961 crop was 19% and three starter rations on an isonitrogenous basis (approximately 19% protein) were formulated in which (A) barley, (B) barley 50%, triticale 50% and (C) all-triticale, made up the cereal grain portion. Gilts and barrows of the Managra strain were used; in the first part of the test initial weights were approximately 35 lb (16 kg), and in the second part, initial weights were approximately 86 lb (39 kg). Water and feed were given ad libitum.

The results indicated that the triticale was used satisfactorily by pigs started at the heavier weights but not by those started at the lighter weights, when fed over a 4-week period.

In early 1962 grower tests were conducted with Managra barrows and gilts fed from initial weights of 68 lb (30.8 kg) to final weights of 125 lb (56.8 kg). The grower rations again contained (A) barley, (B) barley 50%, triticale 50% and (C) all-triticale as the cereal grain portion. A finisher test was conducted comparing all-triticale with all-barley finisher rations using barrows initially weighing 132 lb (60 kg). Insufficient supplies of triticale limited this particular test.

The growing and finishing tests for 1961 crop showed no significant differences in average daily gain among rations. No carcass abnormalities were apparent in the animals fed triticale.

Triticale, of approximately 15% protein, of the 1962 and 1964 crop years was fed to Managra gilts and barrows and five Yorkshire barrows in finishing rations. The amino acid content of all the triticale used was, except for glycine, similar to that of hard spring wheat but there was in the 1964 triticale crop a 30% decrease in methionine compared to the 1961 crop.

Stothers and Shebeski report a marked palatability problem with the 1962 triticale. When 1962 crop triticale formed 50% of the cereal portion of the finishing ration, average daily feed consumption for both gilts and barrows was approximately 5 lb (2.2 kg) per pig per day. Feed consumption for the first nine days of the finisher test was approximately half this level. When the feeding level of the barley ration was restricted to 5 lb (2.2 kg) per pig per day, average daily gain, feed efficiences and carcass data approximated to those of the pigs fed the triticale ration, suggesting the presence of an appetite depressant within the triticale ration.

When gilts were fed triticale as a sole finishing grain, average daily feed consumption dropped to 0.8 lb (0.3 kg) for the first nine days. Reduction of the triticale level to 20% for eight days improved feed consumption to 4.7 lb (2.1 kg), an increase to 50% level for 21 days further improved feed consumption to 5.6 lb (2.5 kg) but a subsequent increase to 100% triticale reduced feed consumption to 4.4 lb (2.0 kg). Barrows were fed rations containing gradually increased levels of triticale, 20% for two days, 50% for eight days and 100% for an average of 54 days. Average daily feed consumption was 2.5, 4.5 and 4.5 lb (1.1, 2.0, 2.0 kg) respectively. The final shift to 100% triticale resulted in both barrows and gilts showing marked lack of appetite which did not recover to full feeding level.

The 1964 crop gave essentially similar results. Two lots of barrows and gilts were fed either a barley finisher ration or a triticale ration, the lots being subsequently reversed. With both barrows and gilts, feed consumption dropped to almost nil when triticale was the sole cereal grain but a ration which contained 30% triticale and 70% barley was satisfactory. Pelleting the triticale had no apparent effect.

Shimada et al. (1971) compared hexaploid triticale and sorghum in rations for growing swine in Mexico. The triticale used was a mixture of several varieties produced in 1970 with a crude protein content of 16.1% dry basis equivalent to 14.1%

TABLE 39. Essential amino acids of triticale (*as percent of whole dry grain*) and of a typical sorghum used in feeding trials with growing swine (data from Shimada et al 1971).

Amino acid	Triticale	Sorghum (Data from National Research Council 1969)
Arginine	0.97	0.38
Histidine	0.38	0.26
Isoleucine	0.62	0.49
Leucine	1.21	1.60
Lysine	0.57	0.25
Methionine	0.09	0.15
Phenylalanine	0.80	0.60
Threonine	0.55	0.37
Tryptophan	0.16	0.10
Valine	0.82	0.60

protein as fed at a moisture content of 12.5%. The sorghum had a crude protein content of 10.9% dry basis, 9.6% as fed at 12.0% moisture. The amino acid content (by autoanalyzer) of the triticale and of a typical sorghum is given in Table 39.

In the first trial, 16 Duroc gilts with an average initial weight of 14.6 kg received a diet in which triticale was progressively substituted for the grain in a 16% protein sorghum-soybean meal diet. The diets were made isonitrogenous by adjusting the content of soybean meal. Substitution of triticale at 30, 60 and 90% for the sorghum did not significantly affect the performance. Shimada et al. (1971) cite Bowland (1968) as reporting a similar result when triticale replaced 50 and 100% of the wheat and barley constituents in diets for growing pigs.

In the second trial, 12 Duroc pigs, six gilts and six boars, average weight 12.2 kg received a diet in which L-lysine HCl (0, 0.08 and 0.16% of L-lysine) was added to a 96% triticale basal diet. There was a significant linear improvement in weight gain with the addition of lysine. The feed: gain (F/G) ratio also improved but the relation was not statistically significant. The total calculated lysine content of the diet with the highest addition of L-lysine HCl was 0.65% which is still less than the optimum 0.7% suggested (Germann et al. 1958) as being required for 11.4 kg pigs fed 13.4% protein diets.

In the third trial, 16 Hampshire pigs, eight gilts and eight boars, average initial weight 12.3 kg received the same basal diet as in the second trial with and without 0.16% added L-lysine and 0.10% DL-methionine in a 2 × 2 factorial arrangement of treatment in four completely randomized replications. The addition of lysine resulted in a highly significant improvement in average daily gains and F/G. Addition of methionine alone produced a slight depression in weight gain and F/G which was not significant. When both amino acids were added weight gain increased but the combined effect was not statistically significantly better than the addition of lysine alone.

Different results were obtained for the common treatment in the two trials. The triticale used and the initial body weight of the pigs were the same but the breed of pigs, Duroc in the second trial and Hampshire in the third trial, and the time of year, fall vs. winter, were different.

In summary, triticale was substituted successfully for sorghum and for part of the soybean meal in diets for growing pigs.

Harrold et al. (1971) evaluated hexaploid triticale from two crop years as a feed for growing-finishing swine. The basal rations included soybean meal 10.9% and (A) barley 86.9%, (B) barley 43.5%, triticale 43.5%, and (C) triticale 86.9%. All the rations were ground and pelleted. The crude protein content of the rations was (A) 15.5%, (B) 16.3% and (C) 16.7%.

In the first trial, the three basal rations were fed to six pigs having an average initial weight of about 56 lb (25.4 kg). The triticale, grown in 1968, had not been cleaned and contained ergot. The experiment ended after 91 days. Inclusion of the triticale produced a marked reduction in feed intake and average daily gain, the most severe effects resulting when the triticale was the sole grain source.

In the second trial, the cereal portion of the rations were (D) barley, (E) barley 50% and triticale 50% grown in 1969, (F) triticale grown in 1969, uncleaned, (G) triticale grown in 1968, cleaned. Rations were fed to two lots of six pigs with an average initial weight of about 66 lb (30.0 kg). The experiment ended after 45 days. The 1969 triticale produced results comparable to those obtained from the barley ration. Cleaned triticale from the 1968 crop was inferior to barley and triticale from the 1969 crop as the sole grain for growing pigs. The F/G ratios were not markedly different.

In the third trial, the cereal portion of the rations were as in the second trial except that the triticale

TABLE 40. True nitrogen digestibility of soybean, triticale, wheat and barley for 10-kg and 30-kg pigs (data from Sauer 1972).

	True nitrogen digestibility, %	
	10-kg pigs	30-kg pigs
Soybean	94.7	91.6
Triticale	92.0	89.6
Wheat	90.9	88.1
Barley	85.8	88.7

was uncleaned from the 1969 crop. Each ration was fed to three pens of six pigs with an average initial weight of about 86 lb (39.0 kg). It ended after 77 days when the average weight of the heaviest group exceeded 200 lb (90.0 kg). Average daily gain and feed intake was again highest for the pigs fed the barley ration and lowest for those fed the ration with all triticale as the cereal source.

In summary, Harrold et al. recommended that triticale be limited to a maximum of 25% of the ration for growing pigs (50 to 130 lb; 22.8 to 59 kg) or 50% of the grain mixture for finishing pigs (130 lb (59 kg) to market weight). It is not recommended for breeding stock or young pigs. In general triticale appeared to be somewhat unpalatable to growing-finishing swine.

Gundel et al. (1970) compared two hexaploid triticales, designated 57/69 and 64/69 grown in Hungary, in diets for pigs of weights varying from 40 to 60 kg and concluded that triticale could be used successfully in their feed.

Sauer (1972) compared biological availability (de Muelenaere et al. 1967) to Managra barrows, weighing 10 kg or 30 kg at the start of the trials, of 16 amino acids (tryptophan and cystine not included) in triticale, wheat, barley and soybean, all of unstated origin. The faecal analysis method of Kuiken and Lyman (1948) was employed; amino acid analysis by the method of Bragg et al. (1966).

The true nitrogen digestibilities are shown in Table 40 and the mean true essential amino acid availabilities in Table 41. Among the amino acids, lysine was the least available in the cereals. Among the cereals, the essential amino acids were most available from triticale, followed by wheat, followed by barley.

Cornejo et al. (1972) at Davis, California, compared a triticale of 15.3% crude protein (N × 6.25) with commercial wheat, barley and maize in feeding growing-finishing swine. The

TABLE 41. Mean true essential amino acid availabilities (excluding cystine and tryptophan) of soybean, triticale, wheat and barley for 10-kg and 30-kg pigs (data from Sauer 1972).

	Mean true availability, %							
	Soybean		Triticale		Wheat		Barley	
	10-kg pigs	30-kg pigs	10-kg pigs	30-kg pigs	10-kg pigs	30-kg pigs	10-kg pigs	30-kg pigs
Least available in cereals								
Lysine	94.9	91.2	86.3	77.5	80.8	67.3	77.1	65.0
Intermediate availability in cereals								
Isoleucine	95.2	92.1	91.5	87.7	89.2	86.1	84.7	83.1
Methionine	94.5	86.3	90.3	86.8	89.8	85.7	87.3	81.5
Threonine	95.4	91.0	90.9	87.7	87.3	85.4	87.5	82.5
Valine	94.5	90.9	92.4	86.8	89.0	85.9	87.6	84.6
Tyrosine	95.0	93.1	92.9	89.7	90.3	87.5	87.6	85.0
Leucine	95.7	93.2	93.7	91.3	91.2	89.8	88.7	87.6
Most available in cereals								
Arginine	98.4	96.0	94.5	94.1	94.1	93.1	91.4	90.1
Histidine	97.9	94.6	93.8	93.3	94.7	92.7	93.2	89.5
Phenylalanine	95.8	94.2	94.5	93.3	92.4	91.6	90.1	89.5

TABLE 42. Digestible energy (DE), metabolizable energy (ME), corrected ME (ME_n), and nitrogen (N) retention values of triticale, wheat, barley and maize for growing-finishing swine (data from Cornejo et al. 1972).

	DE (*kcal/kg dry matter*)	ME	ME_n	N retention	
				% ingested N	% absorbed N
Triticale	3,603	3,522	3,225	34.4	40.2
Wheat	3,709	3,625	3,335	33.2	37.9
Barley	3,375	3,332	3,052	39.9	51.9
Maize	3,796	3,745	3,560	33.6	41.5

diets were composed of 95% cereal. The experiment was conducted to determine digestible energy (DE), metabolizable energy (ME) and corrected ME value (ME_n) as well as the ability of triticale to promote nitrogen retention. The values obtained, presented in Table 42, indicate that triticale is comparable to wheat and maize and superior to barley as an energy source, and able to support a high positive nitrogen balance.

Chubb (1972 unpublished data) compared triticale of nitrogen content 2.06 with ground barley, and naked or hull-less barley, variety Nackta, in isonitrogenous diets for pigs. The basal diet included, in percent, barley meal 71.0, soybean meal extract 7.5, white fishmeal 2.0, glucose monohydrate 16.0, minerals, etc., 3.5. In the experimental diets, the test cereals were substituted for 25% of the basal diet. The apparent digestibility of dry matter and nitrogen and the gross energy of the triticale and Nackta diets were greater than those of the barley diets.

Allee and Hines (1972a) fed triticale of 18.41% protein (conversion factor not stated) and 0.625% lysine, both expressed on a moisture free basis, in finisher rations to pigs weighing 49 kg. The diets, all in pellet form, were: (A) triticale alone, (B) triticale + 0.1% lysine, (C) triticale and soybean meal, (D) sorghum and soybean meal, (E) wheat and soybean meal. Diets (A), (D) and (E) contained the same level of lysine and all diets received the same mineral, vitamin and antibiotic additions. There were no significant differences in daily gain, feed intake or F/G ratio among the pigs fed diets (B), (C), (D) and (E) but pigs fed diet (A), triticale alone, gained weight significantly more slowly than pigs on the other diets. Carcass analysis showed no significant differences among diets. The results indicate that lysine is the first limiting amino acid in triticale for finishing swine, and also suggest that the lysine in triticale may not be fully available to the pig.

Allee and Hines (1972b) also compared triticale of 15.96% protein (conversion factor not stated) and 0.625% lysine on a moisture-free basis, in meal and pellet form in diets for growing pigs weighing 26 kg. The diets were: (A) (control), sorghum and soybean meal, 16.5% protein, (B) triticale replacing 20% of the sorghum, (C) 40% replacement, (D) 60% replacement, (E) 80% replacement, (F) 100% replacement, all in meal form, (G) 100% replacement in pellet form, (H) triticale and soybean meal, isonitrogenous with diet (A), (I) triticale + 0.2% L-lysine.

The level of triticale and the physical form, meal or pellet, had no significant effect on feed intake, daily gain or F/G ratio but pigs fed diet (H) (isonitrogenous with diet A) and diet (I) gained significantly more slowly than those on the other diets. The results indicate that triticale may be substituted for sorghum on a weight for weight basis, but not on an isonitrogenous basis, in diets for growing pigs.

These findings appear to disagree with those of Shimada et al. (1971).

Cattle and Sheep Feeding Ingalls et al. (1970) compared the feed intake and growth rate of young dairy calves receiving two levels of triticale (27.5 and 55% of the complete ration) with calves receiving barley diets supplemented with urea or soybean meal. The triticale was from the 1967 crop and the ergot content of the 55% triticale diets ranged from 0.03 to 0.11% and was proportionately lower in the 27.5% triticale diet. The highest level of 0.11% just reached the level which might be considered dangerous. The grain

was fed dry rolled but a further ration with 55% pelleted triticale was also fed.

The rations were not isonitrogenous, the percent crude protein contents on a dry matter basis being (A) barley-urea 16.8%, (B) barley-soy 16.7%, (C) triticale (27.5% level) 18%, (D) triticale (55% level dry rolled) 18.5%, (E) triticale (55% level pelleted) 18.9%.

Ten dairy calves, five bulls and five heifers were assigned to each of the experimental diets which were fed once daily in amounts such that feed was available at all times from one week of age. Water was supplied ad libitum. The calves were weighed at 2-weekly intervals and removed from the experiment when they weighed more than 137 kg.

Daily dry matter intake from six weeks of age to final weight was 9 to 11% greater for diets (A) and (B) than for diets (C) and (D) for which intake reduction was similar. Pelleting the 55% triticale ration, diet (E), resulted in a further 12.6% reduction.

Daily weight gains for the same period were 12 to 16% less for calves on diets (D) and (E) than for those on diets (A) and (B). There was no difference in weight gain between calves receiving diets (C) and (D). Pelleting the triticale, diet (E), did not affect weight gain, and feed efficiency was therefore greater for the pelleted triticale than for the dry rolled, and was not significantly different from diets (A) and (B). Differences among diets for apparent dry matter and protein digestibilities were not significant. Bull calves demonstrated greater gains, feed intake and efficiency than did heifer calves.

In summary, complete calf rations containing 27.5 or 55% dry rolled triticale resulted in a small reduction in feed intake and weight gain compared with barley-urea and barley-soy rations but feed efficiency was similar. Pelleting the 55% triticale ration resulted in lower intake, equal weight gain and increased feed efficiency compared to the dry rolled triticale. Low levels of ergot contamination of the triticale may have affected feed consumption.

McCloy et al. (1971) compared a mixture of hexaploid triticales, crude protein 17.8% dry matter basis, grown under irrigation in California and Texas in 1969, with commercial sorghum, crude protein 10.9% dry matter basis (N × 6.25). The triticale was free from detectable ergot.

In a trial to compare performance, feed consumption, efficiency of feed utilization, and carcass traits of finishing steers, 40 Hereford steers, average weight 285 kg, were randomly allotted into two main treatment groups with five replications of four steers in each treatment. The rations were isonitrogenous at 15.1% crude protein as fed, and contained 7% roughage. Dry rolled triticale comprised 92% of that ration. The steers were self fed, were weighed at 28-day intervals and slaughtered on completion of the 146-day feeding period. The average daily gains were significantly higher among steers fed the sorghum ration than among those fed triticale and there was a lower consumption of triticale. Feed conversion of the triticale ration was more efficient. Standard carcass traits were similar, but more condemnations because of liver abscesses occurred among steers fed triticale.

In a trial to compare digestibility, 12 steers of average weight 272 kg and 14 crossbred wethers averaging 43 kg were randomly allotted six and seven per treatment respectively to rations containing dry rolled triticale (79%) or sorghum (71.6%) and roughage. A 7-day adjustment period was followed by 7-day preliminary and collection periods. All animals were initially fed the experimental rations on a limited daily intake basis (1% of body weight) in two feedings and intake gradually increased during the adjustment period. Maximum consumption was limited at 6.35 and 1.20 kg per head daily for the steers and wethers respectively. Feed intake was held constant at the level established for each animal during the remainder of the trial. Water was provided ad libitum. Both apparent and true digestibility of crude protein were significantly higher for the triticale ration.

It had been observed early in the finishing trial that consumption was lower with the triticale ration, and it had also been found that intake levels in the digestibility trial were more difficult to establish with triticale, especially with sheep. Therefore three 28-day acceptability trials, with four treatments in each trial were carried out using 16 Hereford heifers initially averaging 227 kg. There was a 3-week rest period between trials and no heifer received rations with identical triticale levels during any two successive trials. The heifers were fed individually in all three trials. Average daily consumption expressed as kilograms per mean metabolic size, determined from an average of initial and final weights, was used to evaluate comparative acceptability of the rations in each

trial. In the first acceptability trial the rations contained 0, 30, 60 and 90% dry rolled triticale replacing equal parts by weight of sorghum and other concentrates. All rations were isonitrogenous and contained 9% roughage. Consumption decreased at each higher level of triticale, with a significant decrease between the 30 and 60% levels.

In the second acceptability trial rations were similar except that molasses replaced 5% by weight of the grain; in rations containing both grains, each grain was reduced in proportion to its level in the ration. There was also a significant decrease in consumption when the rations contained 5% molasses.

In the third acceptability trial dry rolled triticale and sorghum were compared in rations with 10% roughage and constant grain : roughage ratios, with and without molasses. Consumption was significantly lower with the triticale rations. The addition of molasses did not influence consumption within grain treatments.

McCloy et al. (1971) cite Lofgreen (1969) who found that feed intake and gains were higher and feed utilization more efficient with triticale as compared with sorghum, when both were steam rolled and fed in rations containing 68% grain and 7% molasses. The triticale was from the same source as that used by McCloy et al. (1971).

McElroy (1968), also cited by McCloy et al. (1971), found that steers fed triticale displayed higher and more efficient gains than did steers fed barley, and that consumption of steam rolled triticale rations was higher than dry rolled triticale. He also reported a higher incidence of liver abscesses when triticale made up more than 50% of the grain portion in finishing rations, and considerable damage to ruminal epithelium in steers fed triticale at high levels.

Sherrod (1972) compared two winter triticales designated 131 and 385, two spring triticales, 204 and 208, and two hard red winter wheats, Sturdy and Tascosa, in 50% grain rations, fed at 800 g daily, dry rolled with chopped forage sorghum hay, to sheep in digestibility studies. Crude protein levels, dry matter basis, were comparable among the triticales at 22% and between the wheats at 18%. All rations were eaten readily. Digestibility of the major non-fibre energy components was not significantly different among rations; crude fibre digestibility was comparable among the triticales with Sturdy wheat significantly lower and Tascosa wheat significantly higher, than the triticales. Crude protein digestibility was lower with Sturdy wheat than with the other diets. Nitrogen retained as percentage of intake tended to be lower with the Winter triticales than with the Spring triticales and the wheats, but overall the results indicated that Spring and Winter triticales had comparable nutritive value for sheep with Sturdy wheat lower and Tascosa wheat higher than the triticale.

Triticale grown in the Texas Panhandle area during 1970 was compared with barley grown in Arizona on an equal weight basis in feeding trials with lactating (60 to 120 days post partum) Holstein cows (Moody 1973). The rations were fed individually, and consisted of cubed alfalfa at 1.8% of body weight daily, with the balance of the net energy requirements being provided by concentrates. The concentrates consisted of 92% grain, 7% molasses and 1% salt. The protein values (conversion factors not stated) of the rations as eaten were: (A) steam rolled triticale 18%, (B) steam rolled barley 15.5%, (C) equal amounts of triticale and barley 16.9%. All rations were equally well accepted and no significant differences were found, attributable to the grain source, in milk yield and composition, ruminal volatile fatty acids, or digestibility.

Rosner triticale silage was evaluated against maize silage in the diet of lactating Holstein cows (Fisher 1972). On the triticale silage diet, a marked depression in milk protein content, together with lower milk production and loss in body weight was reported. The apparent digestibility of dry matter for rations containing triticale silage was 64% and for maize silage 67%.

Gundel et al. (1970) compared two hexaploid triticales, designated 20/68 and 57/68 grown in Hungary, in diets for wethers and concluded that triticale could be used successfully in sheep diets.

Environmental and Agronomic Effects

The influence of environment and fertilizer treatment on the protein quality of triticale is evidenced by data provided by Dr. Eva Villegas in June 1970, and presented by Zillinsky and Borlaug (1971a). The protein and lysine content of identical strains from 11 crosses grown at CIANO in 1968-69 and at Navojoa in 1969-70 are compared in Table 43. The average protein content was higher and the percent lysine in the protein lower in the seed from the CIANO crop. Test weight and

TABLE 43. Protein and lysine content of triticale crosses grown at two locations under different levels of nitrogen fertilizer (data from Zillinsky and Borlaug 1971a).

Cross Number	No. of strains	Average % protein		Average % lysine in protein		Lysine in sample	
		Y68-69[a]	N69-70[b]	Y68-69[a]	N69-70[b]	Y68-69[a]	N69-70[b]
× 136	3	16.85	14.84	3.00	3.70	0.505	0.549
× 160	1	16.30	15.39	3.25	3.12	0.529	0.480
× 195	5	16.34	15.65	3.16	3.18	0.516	0.497
× 224	13	17.78	16.03	2.67	3.22	0.475	0.516
× 281	3	16.59	16.62	2.72	3.03	0.451	0.504
× 284	6	16.92	14.81	2.56	3.34	0.433	0.495
× 298	6	16.33	15.27	2.71	3.11	0.442	0.475
× 308	57	15.63	14.35	2.94	3.20	0.459	0.459
× 313	18	16.30	14.17	3.28	3.35	0.534	0.475
× 653	37	16.44	15.19	2.77	3.29	0.455	0.499
× 674	2	16.58	15.62	2.80	3.30	0.464	0.515
Average all samples	151	16.26	14.96	2.83	3.25	0.460	0.486

[a]CIANO winter nursery 1968-69; 120 kg/ha/N application.
[b]Navojoa winter nursery 1969-70; 60 kg/ha/N application.

seed grains were similar for the two seasons but lodging was more serious on the CIANO crop.

All the varieties of triticale used by Villegas et al. (1970) (*see* Table 18), were grown at one location, and the variations should therefore reflect varietal differences.

Szabo (1972) reported that hexaploid triticale had been successfully grown in Hungary, especially in sandy areas. From experiments with five strains of Hungarian grown triticale to ascertain germination requirements, he reported that triticale grains are more sensitive to higher concentrations (2 to 3%) of salt solutions than simultaneously germinated rye grains. The salt tolerance of triticale is roughly equivalent to that of wheat and oats. The optimum germination temperature of the varieties examined was 20°C, the maximum being 35°C (40°C in the case of one variety) and the minimum 5°C.

Tennenhouse and Lacroix (1972) have reported on the effect of treatment with (2-chloroethyl) trimethylammonium chloride (CCC) at 3.0 kg/ha and 6.0 kg/ha with and without nitrogen at 90 kg/ha on Rosner triticale. Both levels reduced the height of the mature plant, the higher level producing greater reduction, but the reduction was less when nitrogen was added. There was no lodging. The 6.0 kg/ha level of CCC on the unfertilized plot increased the yield significantly over the control. The protein content of about 20% dry weight (conversion factor not stated) and 1000-kernel weights, were unchanged by all treatments.

The first section of this chapter has dealt with triticale and has included papers in which triticale was compared with wheat and/or rye.

The subsequent sections of the chapter deal with (*a*) wheat alone, (*b*) comparisons of wheat and rye and (*c*) rye alone.

Wheat

Genetic Effects

TOTAL PROTEIN

The influence of nitrogen metabolism and translocation of nitrogen in the wheat plant upon seed protein content has been reported upon by Johnson et al. (1967), and Johnson and Mattern (1972). Nitrogen metabolism and the nitrate reductase system has been discussed by Croy and Hageman (1970) and Duffield et al. (1972).

Field experiments to estimate the broad-sense heritabilities in wheat, on an individual plant

basis, conducted at Oklahoma State University, showed that nitrate reductase activity was significantly correlated with grain protein, water-soluble protein, and nitrate content. NB 65679, a selection from a cross between Atlas 66 and Comanche, obtained from the Nebraska Agricultural Experiment Station, was a high protein wheat used in these trials.

The results indicated that higher nitrate reduction and more efficient nitrogen translocation were involved in producing higher protein in the grain. The reductase system appeared sensitive to both environmental and physiological influences (Duffield et al. 1972).

Rao and Croy (1972) studied the protease and nitrate reductase patterns in three "high protein" wheats, each having Atlas 66 germ plasm in their parentage, in comparison with Triumph 64, a hard red winter "low protein" wheat widely grown in Oklahoma. There were no significant differences in nitrate reductase and protease activity levels among the three high grain protein wheats but the high grain protein wheat variety NB 65317 (selected as representative) showed higher nitrate reductase activity prior to heading, and higher protease activity after heading, than did the lower grain protein wheat Triumph 64.

Baker et al. (1971) analyzed quality data for hard red spring wheat cultivars grown in Canada in Western Cooperative Wheat Tests in the five years 1965 through 1969. In each year from 23 to 25 cultivars were grown at 15 to 16 locations. Among the quality traits examined for heritability were grain protein and flour protein (American Association of Cereal Chemists 1962). The heritability was estimated from analyses of variance of data from (A) the 13 cultivars that were common to 1965 and 1966, (B) the 12 cultivars common to 1966 and 1967, (C) the 11 cultivars common to 1967 and 1968, (D) the 14 cultivars common to 1968 and 1969. Grain was milled, straight grade, in an Allis-Chalmers mill. Heritability was estimated as the ratio $G/(G + E)$, where G was the component of variance due to average (genetic) differences among cultivars and E was the component due to deviations from average performance (measurement errors and genotype × year interaction). Means, standard deviations and heritability estimates are presented in Table 44. The standard deviations reported include the error of the laboratory analysis and the effect of genotype by location interaction peculiar to each year. The estimates indicate that protein content is highly heritable but Baker et al. state that the heritability estimates are biased upward due to their inability to account for genotype × location interactions. There was a high positive correlation (0.96) between flour and grain protein indicating that milling had essentially no differential effect on flour protein in the cultivars studied.

TABLE 44. Mean, standard deviation and heritability of grain protein, flour yield and flour protein (data from Baker et al. 1971).

	Wheat grain protein (%)	Flour yield (%)	Flour protein (%)
Mean, average of five estimates	14.56	75.6	13.80
Standard deviation, average of four estimates	0.20	0.56	0.18
Heritability (estimates significantly greater than zero ($P = 0.05$) if they exceed 0.46, 0.48, 0.50 and 0.44 in each of the four sets of data, respectively)			
1965-66	0.86	0.81	0.92
1966-67	0.84	0.58	0.87
1967-68	0.61	0.46	0.76
1968-69	0.89	0.80	0.95
Average	0.80	0.66	0.88

AMINO ACID COMPOSITION

In the view of Mertz (1971), the chances of increasing the quantity of protein in wheat and triticale appear to be greater than the chances of increasing the biological quality of that protein. However, encouraging results are emerging from the research being conducted at the University of Nebraska into the nutritive quality of wheat through increased protein content combined with improved amino acid balance. Since lysine is the essential amino acid in shortest supply in cereals, most attention is being given to increasing the proportion of lysine while maintaining a high grain protein content.

The studies at the University of Nebraska have been conducted over many years, and are reported in several publications: Haunold et al. (1962a, b);

Johnson et al. (1967, 1968a,b, 1969, 1970, 1972); Mattern et al. (1968, 1970); Johnson and Mattern (1972).

Among the objectives of the Nebraska research are (1) to analyze systematically the wheats, both *T. vulgare* and *T. durum*, in the world wheat collection for protein and lysine contents, and (2) to incorporate genes for high protein and high lysine into wheats potentially suitable for cultivation in developing countries. It is a further purpose to distribute potentially superior germ plasm to interested developing countries for adaptability and breeding research.

The results of the systematic screening of the world wheat collection were reported as they became available (some 47 publications have been issued between 1966 and 1972) and have been summarized by Johnson and Mattern (1972). More than 12,600 common wheats (*T. vulgare*) and 3,400 durum wheats (*T. durum*) have been analyzed for protein content and amino acid composition with special attention to lysine. Because of the large number of samples of relatively small size to be examined, special analytical techniques have had to be developed. For protein, micro-Kjeldahl and a dye-binding technique are used. Lysine is determined mainly by autoanalyzer following acid hydrolysis; a modification of the Mossberg-Munck dye-binding procedure for basic amino acids has also been used. Tryptophan is determined by a modified ion exchange procedure.

Large variations have been found among the protein contents of wheats so far analyzed, levels ranging from 6 to 23% with a mean of 12.9%. Lysine per unit of protein ranged from 2.2 to 4.2% with a mean of 3.2%.

Low protein wheats (mean of 9.5% protein) and high protein wheats (mean of 18.8% protein) display different amino acid profiles. Lysine per unit of protein is negatively correlated with protein in low protein wheats but no similar correlation is found in high protein wheats. Lysine was positively correlated with threonine, leucine and tyrosine, and negatively correlated with valine; genetically influenced increases in lysine should not therefore be associated with a decrease in the threonine.

When 39 wheats with the highest lysine values were compared with 16 wheats with the highest protein content to find which group provided the largest quantities of the essential amino acids per unit dry weight of the grain, the high protein group provided more of every essential amino acid than the high lysine group.

Potentially useful genetic sources of high protein and/or high lysine are shown in Table 45. Several of the protein sources may carry the same genes for protein. No gene for high lysine comparable to the opaque-2 mutation in maize has yet been identified in wheat but the sources given in Table 45 have been consistently superior in lysine to other varieties examined. In greenhouse tests, Nap Hal (PI 176217) has proved superior in both protein and lysine contents (Johnson and Mattern 1972).

Varietal Influence

TOTAL PROTEIN

An inverse ratio between grain yield and protein content was noted by Williams (1966) who reported that in a series of 24 trials from 1961 to 1963, throughout the Australian wheat belt, the wheat variety Festival was consistently high in protein and low in yield while Heron was high in yield and low in protein.

Feillet (1965) in his thesis on the influence of genetic, agronomic and technological factors on the proteins of wheat described techniques for fractionating wheat protein. By growing different varieties under the same conditions, and the same varieties under differing conditions, he concluded that the albumin fraction is "specific" and may be used to differentiate *T. vulgare* from *T. durum*. The gliadin fraction is, he believes, "varietal" and characterizes each variety. The content of protein in flour is to a large extent influenced by environmental and agronomic factors, though the content of soluble protein appears to be heritable.

The influence of variety, soil and fertilizer treatment on wheat yield, on protein and on other nutrients was studied by El Gindy et al. (1957). Pawnee (hard red winter), Seneca (soft red winter) and Cornell 595 (white) wheat were grown on low-fertility acid soil in 1951 and on well-limed fertile soil in 1956. Apart from soil characteristics, conditions during the two years were much alike.

Protein in the wheat was affected by variety, soil and fertilizer treatment. The influence of each of these factors and the variety × soil interaction were highly significant but variety displayed much

TABLE 45. Useful or potentially useful genetic sources of genes for high protein and/or high lysine in wheat (data from Johnson and Mattern 1972).

	Selection P.I. or C.I. no.	Source	Useful trait	
			High protein	High lysine
Atlas 50	12534	USA	X	
Atlas 66	12561	USA	X	
Atlas × Comanche crosses		USA	X	
Frondoso	12078	Brazil	X	
Frondoso derivative	Purdue 28-2-1	USA	X	
Aniversario	12578	Argentina	X	
Aniversario-derived line	NB66565	USA	X	
Nebraska Male Fertility Restorer	NB542437	USA	X	
Nap Hal	P.I. 176217	India	X	X
Hume[2] × Nb[4]-Agrus-Tc[7]	SD69103	USA	X	
April Bearded	7337	England	X	X
Hybrid English	6225	England	X	X
Pearl	3285	Sweden		X (probable)
Fultz × Hungarian	11849	USA		X (probable)
Fultz Sel.-Hungarian × Minturki-Fultz Sel.	12756	USA		X (probable)
Norin 10-Brevor, Sel. 14 × 27-15-Rio-Rex, Sel. 53	13447	USA		X (possible)
Norin 10-Brevor, Sel. 14 × Brevor Sib, 50-3	13449	USA		X (possible)
22A	5484	USSR		X (probable)

TABLE 46. Kjeldahl nitrogen content and arginine, lysine and valine content as percent of total nitrogen (data from Price 1950).

Wheat	Nitrogen (%)	Arginine	Lysine	Valine
		(*Nitrogen % total nitrogen*)		
White English	1.58	10.2	4.12	3.56
New South Wales Australian	1.81	9.08	3.81	3.44
Plate	2.27	8.94	3.46	3.32
No. 2 Manitoban	2.43	8.75	3.39	3.15

the largest variance and appeared to be the dominant factor.

AMINO ACID COMPOSITION

Price (1950) determined microbiologically the essential amino acid and tyrosine content of four samples of wheat: (1) White English, (2) New South Wales, Australian, (3) Plate and (4) a No. 2 Manitoban. He found that amino acid content tended to be higher in samples of lower Kjeldahl nitrogen content; this was particularly the case with arginine, lysine and valine (*see* Table 46). No similar trend was noted with phenylalanine, histidine, tryptophan or tyrosine though none of these amino acids showed a reverse tendency.

TABLE 47. Protein and lysine in six varieties of *T. durum*, *(14% moisture basis)* (data from Lawrence et al. 1958).

Variety	Protein N × 5.7 (%)	Lysine in protein (%)
Stewart	13.4	3.30
Mindum	12.6	3.04
Langdon	12.4	3.02
Ramsey	13.0	2.93
Sentry	13.8	2.85
Pentad (red durum)	12.6	2.70

Bendicenti et al. (1957) determined the nitrogen, by micro-Kjeldahl, in the Italian soft wheat varieties San Pastore (1.73%), Funo (1.95%), Damiano (1.98%) and Mara (2.01%) and the hard varieties, Cappello (1.99%) and Rossello (2.00%). They also compared the soft varieties Tevere (2.60%) and Mara (2.71%), the hard variety Garigliano (2.62%) and two American varieties Cheyenne (2.78%) and Pawnee (2.84%).

The nitrogen content of the first group ranged from 1.73 to 2.01% and of the second from 2.60 to 2.84%. The amino acid contents (determined chromatographically; cystine by the method of Schram et al. (1954) and tryptophan microbiologically) showed a consistently higher percentage of lysine and lower percentage of glutamic acid in the low nitrogen wheats compared to the higher nitrogen wheats. There were no appreciable differences in the amino acid composition of soft and hard wheat varieties of the same nitrogen content.

Lawrence et al. (1958) found that lysine content (determined microbiologically) varied from 2.46% in the protein (N × 5.7) to 3.84% in some 230 wheat varieties selected to be representative of commercial varieties. The overall mean lysine content on a percent protein basis was 2.89% for all wheats of about 13.5% or more protein; below this level there was a highly significant inverse relationship between protein content and lysine content as percent of protein.

Three winter wheats, Rio, Brevor and Elmar, and three spring wheats, Marfed, Idaed, and Baart grown at Pullman and Lind, Washington, and Moro, Oregon, in 1952, 1953 and 1954 showed no significant difference in the lysine content of the protein.

The protein content and percent lysine in the protein of the durum wheats Stewart, Mindum, Langdon, Ramsey, Sentry and Pentad (red durum) are shown in Table 47. Lysine (% protein) varied from 2.7% for Pentad to 3.3% for Stewart.

The mean values for percent lysine in the protein were: for 18 samples of other *Triticum* species, 3.0; for 13 samples of genera related to wheat, 3.3; for 15 hybrid selections of crosses of *Agropyron elongatum* with wheat, 3.1.

McDermott and Pace (1960) determined the amino acid composition (ion exchange chromatography) of flours milled from a Manitoba wheat and from Hybrid 46 wheat of, respectively, Kjeldahl nitrogen 2.31 and 1.68%, dry matter basis. The amino acid composition of the two flours was generally similar except for lysine and arginine which were both higher in the low protein Hybrid 46. The same inverse relationship between content of nitrogen (N) and content of lysine was also found in the flours milled from Svenno (2.47% N), Bersee (1.85% N), Holdfast (1.44% N) and Arletta (1.18% N).

Sihlbom (1962) reported on the Kjeldahl nitrogen content and essential amino acid composition (Moore et al. 1958) of the winter wheats, Banco and Odin and the spring wheat Svenno, grown in different locations in Sweden. The results are given in Table 48. An inverse relationship between lysine content and total nitrogen is evidenced by these results. Only one of the wheats examined, Svenno from Hasslarp, had a protein content above the 13.5% level at which, according to Lawrence et al. (1958), the inverse relationship is no longer demonstrable.

Simmonds (1962) reported on the variability in amino acid composition (determined by ion exchange chromatography) of six Australian wheats and the flours milled from them. The varieties, origins, and Kjeldahl nitrogen as percent on dry weight were: Crossbred (Tichborne, NSW) 2.81%; Gabo (NSW) 3.14%; Charter (Allsopps NSW) 2.84%; Charter (Boggabri, NSW) 2.74%; Broughton (Querindi, NSW) 1.67%; Sabre (Querindi, NSW) 1.57%.

The wheat samples, although differing in nitrogen content and type, resembled one another in amino acid composition, and the results confirm those of Price (1950), Gunthardt and McGinnis (1957), Lawrence et al. (1958) and McDermott and Pace (1960) all of whom observed an inverse relation between total nitrogen and lysine content,

TABLE 48. Nitrogen and essential amino acid content (excluding tryptophan) (*in grams amino acid/16 g N*) of Swedish winter wheats and the spring wheat Svenno (data from Sihlbom 1962).

	Winter wheat				Spring wheat Svenno			
	Banco, Vaster-gard, Skane	Banco Paarp, Skane	Banco, Gistad, Ostergot-land	Odin, Tierp, Uppland	Tierp, Uppland	Gistad, Ostergot-land	Hasslarp, Skane	Hjortshoj, Skane
Nitrogen % dry matter	1.99	1.46	1.83	1.84	2.41	2.32	2.91	2.34
Amino acids								
Threonine	2.66	3.29	2.89	2.84	2.77	2.76	2.66	2.59
Cysteine	2.20	2.17	2.23	2.16	2.25	2.09	2.03	1.78
Valine	4.32	4.36	4.36	4.65	4.13	4.40	4.24	4.15
Methionine	1.51	1.62	1.61	1.63	1.56	1.44	1.10	1.41
Isoleucine	3.49	3.55	3.05	3.38	3.65	3.62	3.64	3.89
Leucine	6.55	6.96	6.69	6.17	6.62	6.47	6.31	6.81
Tyrosine	3.13	3.32	2.92	3.08	2.97	3.03	3.12	3.08
Phenylalanine	4.56	4.65	4.23	4.34	4.62	4.46	4.60	4.56
Lysine	2.69	3.57	3.14	2.88	2.77	2.50	2.60	2.56

expressed as percent nitrogen, in wheat. The lysine contents found by Simmonds (1962) are higher than those found by McDermott and Pace, and the amide, glutamic acid, phenylalanine, proline and serine contents are 10 to 15% lower. Simmonds (1962) suggests that this may be due to the different methods of hydrolysis employed.

Hepburn and Bradley (1965) compared the nitrogen (Kjeldahl-Gunning procedure) and the amino acid composition (microbiologically or by ion exchange chromatography) of eight varieties of hard red winter wheat, four varieties of hard red spring wheat, one club wheat and one white wheat grown in different parts of the United States in the crop year 1960. The same variety varied in nitrogen content according to location (*see* Table 49). Where the nitrogen content was the same, the proportions of the amino acids were nearly constant for all varieties, though cystine and methionine were somewhat higher in the spring wheats.

Histidine, isoleucine and tyrosine varied least with nitrogen level. In the higher protein wheats glutamic acid, phenylalanine and proline tended to be higher and the remaining amino acids lower. Hepburn and Bradley suggest the total amino acid contribution of any given sample of wheat is determined primarily by the total amount of protein it contains.

Shoup et al. (1966) determined (by auto-analyzer) the amino acid composition (excluding tryptophan), of eight hard red winter wheat varieties and five hard red spring wheat varieties composited from the 1963 crop grown in different areas of the United States. Single samples of Seneca, a soft red winter wheat, Wells, a durum wheat, and Omar, a soft white club wheat, were included.

The histidine, arginine, threonine, glycine and methionine concentrations in the protein were negatively correlated, and the glutamic acid and proline contents positively correlated, with the total protein content of the wheats. As the protein in the wheat increased, the contents of the amino acids increased except for cystine and methionine. The proteins of the hard red spring wheats contained less lysine, arginine and methionine and more cystine than did the hard red winter wheats.

Robinson and Sageman (1968) reported on the amino acid composition (by autoanalyzer) and nitrogen content (by two described methods which agreed exactly) of three South African wheats, Gondveld, Penkop and Wit Spitkop and three Australian wheats, Gabo, Gamenya and Gala sown on four sowing dates in April, May, July and August under irrigation in the Ord River Valley in North Western Australia. The total nitrogen (percent dry matter) varied from

TABLE 49. Nitrogen content of commercial wheats grown in various locations in the USA in the crop year 1960 (data from Hepburn and Bradley 1965).

Type	Variety	Location	Nitrogen (*14% moisture basis*) (%)
Club	Elmar	California	1.41
White	Baart	Washington	1.93
Hard Red Winter	Bison	Akron, Colorado	3.04
		Alliance, Nebraska	2.37
		N. Platte, Nebraska	1.98
	Comanche	Akron, Colorado	2.98
		Alliance, Nebraska	2.37
		Garden City, Kansas	1.92
	Concho	Akron, Colorado	2.97
		Garden City, Kansas	2.37
		N. Platte, Nebraska	1.76
	Pawnee	Akron, Colorado	3.04
		Alliance, Nebraska	2.40
		N. Platte, Nebraska	1.97
	Kaw	Garden City, Kansas	2.37
	Omaha	Fort Collins, Colorado	2.42
	Ottawa	Fort Collins, Colorado	2.40
	Tascosa	Cherokee, Oklahoma	2.34
Hard Red Spring	Thatcher	Williston, N. Dakota	3.34
		Fargo, N. Dakota	2.35
	Canthatch	Williston, N. Dakota	3.13
		Fargo, N. Dakota	2.26
	Selkirk	Langdon, N. Dakota	2.42
		Minot, N. Dakota	2.21
	Conley	Fargo, N. Dakota	2.43
	Lee	Minot, N. Dakota	2.44

3.86 (Gabo) to 3.38 (Gala) but no significant difference in amino acid composition was demonstrated.

Deosthale et al. (1969) found that the protein content (Kjeldahl N × 5.7) of 50 lines of wheat grown under identical conditions at the Indian Agricultural Research Institute, New Delhi, varied from 8.6 to 16.9%. The lysine content (microbiological assay) varied from 2.14 to 2.97 percent of total protein. An inverse correlation was observed between total protein and lysine as percent protein.

Gupta (1970) reported on the lysine and methionine content (microbiological assay) in 17 Indian and nine dwarf Mexican wheat varieties grown at the Indian Agricultural Research Institute, New Delhi. Lysine and methionine as percent of grain were found to be positively and significantly correlated with grain protein. A negative, but not statistically significant, relation was found between lysine percent in the protein and protein content.

Mehdi (1972) reported on the amino acid composition of five wheat varieties, Sharbati Sonora, CA-82, WL-2, Kalyan Sona and D491-5, and 11 strains, grown in 1967-68 at the Indian Agricultural Research Institute, New Delhi. The method of determining the amino acids is not stated nor is the method of determining protein. The highest lysine content was provided by Sharbati Sonora, 2.86 g/100 g protein. Of the strains, HD(M)-1637, HD(M)-1653, HD(M)-1953, HD(M)-1633, HD(M)-1638, HD(M)-1659, HD(M)-1592, HD-1622, HD-1728, HD(M)-1668

and HD-1632, the highest lysine contents were provided (g/100 g protein) by HD(M)-1668, 2.80, and HD(M)-1593, 2.73. HD(M)-1633 and HD-1728 were deficient in lysine, HD-1728 was also deficient in methionine and cystine. Kalyan Sona, HD(M)-1633 and DA 491-5 were highest in methionine which is the first limiting amino acid in diets of mixed cereals and pulses.

Johnson and Mattern (1972) analyzed 12,681 common wheats from the World Collection for protein (mostly by Kjeldahl) and lysine (mostly by ion exchange). Proteins are quoted on a dry weight basis (dwb) or at 14% moisture content (14 H_2O), and lysine as percent protein (% P) or as percent total dry matter (% dm). Protein (dwb) ranged from 6 to 23%, with a mean of 12.9%; lysine (% P) ranged from 2.2 to 4.2% with a mean of 3.2%. An overall negative relation between protein and lysine (% P) was established. In the 8 to 14% protein range a strongly negative relation between protein and lysine (% P) was evident. This relation was less pronounced above 14% protein and virtually disappeared above 16% protein.

Lysine (% dm) displayed a strongly positive linear relation when plotted against protein. As protein increased from 8 to 21% (dwb), lysine (% dm) increases from 0.30 to 0.60%.

Johnson and Mattern (1972) list:

(A) common wheats in the World Collection having highest protein (dwb) contents which range from 22.0 to 18.9%; lysine (% P) ranged from 2.42 to 3.14 and the lysine (% dm) from 0.44 to 0.66,

(B) common wheats having highest lysine (% P) contents which range from 4.26 to 3.83; protein contents (% dwb) range from 6.9 to 10.3,

(C) durum wheats, from 3400 entries in the World Collection, having highest protein (% dwb) which range from 21.3 to 19.4, lysine (% P) ranging from 2.57 to 3.09, and lysine (% dm) from 0.50 to 0.62,

(D) durum wheats with highest lysine (% P) which range from 4.29 to 3.75, protein (% dwb) ranging from 7.3 to 12.1.

They also list common and durum wheats with the highest adjusted lysine values, the negative linear regression of lysine (% P) on protein (% dwb) being used to adjust lysine values to a common protein level of 13.5% (dwb). The fact that fewer wheats appeared with high adjusted than with high unadjusted lysine values reflects the effect of protein level upon lysine content (% P).

Since these results, demonstrating wide variations in protein and lysine contents among the wheats of the World Collection, were all obtained from grain produced at one location, Mesa, Arizona, USA, Johnson and Mattern consider it reasonable to assume that a significant portion of the protein and lysine variation is genetic in origin. However, only extensive trials over a period of years at several locations can firmly establish the genetic potential for high protein. Nevertheless the fact that lysine (% dm) is positively correlated with protein content provides strong evidence that the nutritional value of wheat can be increased by increasing its protein content.

Johnson and Mattern (1972) report that neither protein nor lysine content is influenced by kernel size provided the kernels are plump and healthy. On the other hand, shrivelled kernels were higher in protein and lysine than plump kernels.

BIOLOGICAL QUALITY

Bannerjee and Das (1964) compared 12 varieties of Indian wheat grown at the Indian Agricultural Research Station, New Delhi, in feeding tests with weanling albino rats, five in a group, over four weeks, fed ad libitum. The protein content (method of determination and conversion factor not stated) of the wheats varied from 13.45% (NP 809) to 10% (NP 797). Protein level in the diets varied from 6.3% for NP 797 to 8.61% (NP 823). The ratio weight gain:protein intake varied from 1.63 to 1.96. There was no correlation among any of the statistics quoted.

Triumph 64 winter wheat, protein 15.7% (Kjeldahl N × 5.7, dry basis) and a commercial wheat, protein 10.8%, were fed, on an equal weight of grain basis, in trials with mice, five per group, over 28 days; a 10% casein diet was included for comparison. The higher protein Triumph 64 produced better weight gains and feed efficiency ratios (feed consumption:weight gain) than did the lower protein commercial wheat (Johnson and Mattern 1972).

Environmental Influences

TOTAL PROTEIN

Pereira (1963) reported on the variations in protein content (method of determination not

TABLE 50. Grain yield and protein content (N × 5.7 dry matter basis) of seven varieties at high-yielding and low-yielding nursery sites of the International Winter Wheat Performance Nursery grown in 1969 (taken from data presented by V. A. Johnson at 3rd FAO/Rockefeller Foundation Wheat Seminar, April 29–May 13, 1970, Ankara, Turkey) (Johnson and Mattern 1972).

Variety	Kabul, Afghanistan		Kermanshah, Iran	
	Yield (*q/ha*)[a]	Protein (%)	Yield (*q/ha*)[a]	Protein (%)
Nursery mean	52	13.8	16	14.8
Yorkstar	66	10.4	13	13.5
Heine VII	61	15.9	18	15.4
Bankuti 1201	60	14.1	16	14.7
NB67730	53	17.1	14	15.9
Riley 67	50	14.5	13	14.7
Gaines	46	10.8	11	14.0
Atlas 66	44	16.5	13	18.5

[a]q/ha = quintal (100 kg)/ha.

stated) in the grain of 13 varieties of wheat grown in 10 localities in Portugal. Protein content varied more within the same variety grown in different locations than between different varieties grown in the same locality. For example, Verdeal Rijo varied from 15.9% (dry matter basis) at Braga to 9.8% at Elvas, a difference of 6.1%. At Elvas, the largest difference was 4.5% between Magueija, 13.5% and Quaderna 9.0%, and at Braga between Verdeal Rijo 15.9% and Quaderna 13.1%. Pereira also found that lysine in the protein varied inversely with protein content.

A survey of improved Indian and Mexican dwarf wheats for protein content (Kjeldahl N × 5.7) was reported by Austin et al. (1970). Some 103 varieties were involved and 29 locations in the five wheat zones of India in three crop years but not all the varieties were grown in all the locations. At least nine observations were made on any one variety.

The varieties were divided into:

(i) low (less than 10% protein), 7 varieties;
(ii) medium (10 to 12% protein), 63 varieties;
(iii) above 12% protein (ranging from 12.1 to 14.2%), 33 varieties

The mean protein values for the locations varied from 10.5% (range 9.2 to 11.6%) at Jaunpur to 16.4% (range 14.5 to 17.7%) at Bardoli. There were marked effects with location in protein content of the grain. The highest levels were given by the Mexican dwarf wheats, e.g. Sonora 64, 14.2%, Lerma Rojo, 13.8%, Sonora 63, 13.7%.

Upreti and Abrol (1971) reported on the protein content (Kjeldahl N × 5.7) of 21 strains of triple gene dwarf wheats grown at Delhi, India during the crop season 1968-69. Protein contents ranged from 10.4 to 15.5%, 18 of the wheats having protein contents above 12.0%.

The effect of location on the protein content of different varieties is illustrated by Table 50 which shows the grain yield and protein content of seven varieties at the high-yielding site of the International Winter Wheat Performance Nursery at Kabul, Afghanistan and at the low-yielding site at Kermanshah, Iran (Johnson and Mattern 1972).

AMINO ACID COMPOSITION

McElroy et al. (1949) determined microbiologically the content of nine essential amino acids in Marquis wheat grown in nine different soil zones in Alberta, Canada. The soils were (*a*) gray-wooded, (*b*) gray transitional, (*c*) black, (*d*) black-transitional, (*e*) dark brown, (*f*) light brown. Kjeldahl nitrogen of the wheat ranged from 1.94 to 4.03%, with a mean of 3.02%. Lysine content varied from 2.1 to 2.5% with a mean of 2.2% of the protein (Kjeldahl N × 6.25). An inverse relationship between total nitrogen and lysine as percent of total nitrogen was observed.

Swaminathan et al. (1969) reported on the effect on protein and lysine content of growing six varieties of wheat at four locations in India. The results are presented in Table 51. The lowest protein, 11.72% (Kjeldahl N × 5.7) was recorded for S.308 (Sonalika) at Bijnoor, a low rainfall area with a sandy soil. The highest value was 18.29% for C.273, a local tall wheat grown at Bhind on a clay soil. The lysine (microbiological assay) was also influenced by location.

Eggum (1970) also compared the amino acid content in grams per 16 g nitrogen, and nitrogen content, and the protein value, expressed as true digestibility, Biological Value, NPU, and utilizable nitrogen (NPU × %N/100) of certain higher protein wheats. The comparisons are given in Table 52. Sharbati-Sonora ranks highest as measured by Biological Value and NPU; Super X as measured by true digestibility, and Jarral as measured by utilizable nitrogen.

TABLE 51. Effect of location on protein and lysine content in six varieties of wheat grown at four locations in India (data from Swaminathan et al. 1969).

	Location							
	Delhi		Bijnoor		Bhind		Sri-Ganga Nagar	
Variety	Protein (%)	Lysine (% *protein*)	Protein (%)	Lysine (% *protein*)	Protein (%)	Lysine (% *protein*)	Protein (%)	Lysine (% *protein*)
NP.871	14.60	2.70	13.12	3.09	18.00	2.25	13.40	2.73
C.273	15.10	2.06	14.27	2.69	18.29	2.28	13.26	3.01
S.227	13.00	2.48	12.69	2.63	16.40	2.56	13.70	2.84
S.308	13.70	2.51	11.72	3.32	16.84	2.58	12.68	2.76
Lerma Rojo	14.40	2.24	11.92	2.80	16.93	3.41	12.60	2.86
PV.18	12.10	2.11	12.13	2.43	16.40	2.50	11.96	3.58

TABLE 52. Amino acid content (*grams/16 g N*), nitrogen content and protein value (%) of high protein wheats (all wheats grown in Denmark except Sharbati-Sonora which was grown in India) (data from Eggum 1970).

Species Nationality	Sonora 64 Mexican	Super X Mexican	Inia Mexican	Jarral Mexican	Colibri German	Sharbati-Sonora Indian	Average Danish
Lysine	2.47	2.45	2.30	2.39	2.54	2.39	2.55
Methionine	1.66	1.54	1.49	1.47	1.49	1.52	1.82
Cystine	2.21	2.05	2.05	2.07	2.11	2.30	1.81
Aspartic acid	4.48	4.37	4.51	4.43	4.92	4.95	5.26
Threonine	2.72	2.64	2.59	2.72	2.81	2.84	3.02
Serine	4.45	4.48	4.21	4.69	4.58	4.44	4.73
Glutamic acid	33.26	34.30	34.25	36.25	33.79	30.57	35.77
Glycine	3.78	3.87	3.80	4.15	3.99	3.98	4.19
Alanine	3.17	3.13	3.04	3.22	3.40	3.36	3.77
Valine	4.01	4.07	4.05	4.17	4.31	4.13	4.58
Isoleucine	3.54	3.48	3.48	3.53	3.57	3.39	3.38
Leucine	6.47	6.53	6.28	6.67	6.67	6.33	6.79
Tyrosine	2.44	3.60	3.47	3.81	3.49	3.11	3.14
Phenylalanine	4.74	4.83	4.87	4.88	4.74	4.40	4.41
Histidine	2.27	2.29	2.30	2.23	2.38	2.09	2.29
Arginine	4.54	4.55	4.25	4.36	4.81	4.13	4.65
Tryptophan	1.37	1.11	0.95	1.08	1.00	1.47	1.12
N as % of dry matter	3.02	3.03	3.12	3.64	2.93	2.40	2.01
Protein value expressed as:							
True digestibility[a]	93.4	94.4	90.8	93.2	91.5	89.1	89.6
Biological value[b]	52.0	52.0	49.4	49.4	55.0	60.5	59.0
Net protein utilization (NPU)[b]	48.4	49.1	44.3	45.8	50.3	53.9	52.9
Utilization nitrogen (NPU × % N/100)	1.46	1.49	1.38	1.67	1.47	1.29	1.06

[a]True digestibility is not defined in the paper but is generally regarded as the proportion of food nitrogen that is absorbed (National Academy of Sciences—National Research Council 1963).

[b]Thomas-Mitchell method.

TABLE 53. Average grain yield, protein content, and lysine content for varieties grown in the International Winter Wheat Performance Nursery in 1969 and 1970 (data from Johnson and Mattern 1972).

Variety	2-year average grain yield		2-year average protein content		2-year average lysine content	
	(*q/ha*)	(*Rank*)	(%)	(*Rank*)	(%)	(*Rank*)
Winter varieties						
Bezostaia	42.4	1	13.8	25	2.9	8
Blueboy	36.4	4	14.1	21	3.0	3
San Pastore	33.9	14	14.1	21	2.9	8
Sturdy	36.4	6	14.8	11	2.8	21
Timwin	37.7	2	14.7	15	3.0	3
Parker	36.4	5	14.8	11	2.9	8
Fertodi 293	35.9	8	15.1	8	2.8	21
Benhur	34.2	12	15.1	8	2.8	21
Scout 66	36.6	3	14.5	18	2.9	8
Yung Kwang	34.2	13	14.8	11	2.9	8
Arthur	36.1	7	14.7	15	2.9	8
Gage	34.6	10	14.8	11	2.9	8
Stadler	34.4	11	13.7	26	3.0	3
Heine VII	33.9	15	14.7	15	2.9	8
Lancer	34.7	9	14.2	20	2.9	8
Shawnee	33.4	17	14.1	21	2.9	8
Riley 67	33.9	16	14.5	18	3.0	3
Yorkstar	33.4	18	12.8	28	3.1	1
Bankuti 1201	32.0	20	15.5	5	2.8	21
Triumph 64	32.8	19	15.2	7	2.9	8
NB67730	31.5	21	16.9	3	2.8	21
Atlas 66	30.8	22	17.9	1	2.7	28
Purdue 28-2-1	30.0	24	17.5	2	2.8	21
Winalta	30.2	23	14.1	21	2.9	8
Cappelle Desprez	29.0	25	16.0	4	2.8	21
Gaines	28.7	26	13.4	27	3.1	1
Felix	27.0	27	15.1	8	2.9	8
Odin	24.6	28	15.5	5	3.0	3
Spring varieties						
Lerma Rojo 64	32.4		14.5 (1969 only)		2.8 (1969 only)	
INIA 66	25.5		14.5 (1969 only)		2.8 (1969 only)	

Twenty-eight winter wheat varieties and two spring wheat varieties were grown at the International Winter Wheat Performance Nursery, which has sites throughout the world, in 1969 and 1970. The 2-year average grain yield, protein content, and lysine content of these varieties, with their respective rankings, are summarized in Table 53 (Johnson and Mattern 1972).

The protein, and lysine content (by autoanalyzer), mean and range, for NB 67730, Triumph 64, Bezostaia and Sturdy grown at 15 locations are given in Table 54. A modification of the UDY dye-binding procedure and/or the Kjeldahl method were used for determining the protein. The Russian wheat Bezostaia with a UDY protein mean of 13.2% and a lysine mean of 2.8% compared well with the other varieties and ranked first in yield (*see* Table 53). (Johnson and Mattern 1972).

A selection of wheat varieties with high lysine values when grown at Mesa, Arizona, were regrown at other sites in the United States. The results indicated that in terms of lysine most of the varieties were not genetically different. However, the spring varieties PI 7337 and PI 5484 showed promise, and the winter varieties Atlas 66, PI 135067, PI 135070, PI 166913, PI 94526, PI

TABLE 54. Protein and lysine contents (range in parentheses) for four wheat varieties from 15 locations in the First International Winter Wheat Performance Nursery (data from Johnson and Mattern 1972).

	UDY protein (% *sample*)	Kjeldahl protein (% *sample*)	Lysine	
			In UDY protein (%)	In Kjeldahl protein (%)
NB 67730	16.0 (11.4–21.9)	–	2.7 (2.3–3.1)	–
Triumph 64	14.7 (11.3–18.6)	–	2.7 (2.5–3.1)	–
Bezostaia	13.2 (9.8–15.2)	–	2.8 (2.6–3.2)	–
Sturdy	14.0 (not available)	14.6 (10.7–18.3)	2.8 (not available)	2.7 (2.4–3.9)

173438, PI 11680, CI 13449 and PI 117018 had higher protein and lysine contents than Triumph 64 (Johnson and Mattern 1972).

Agronomic Influences

TOTAL PROTEIN

In general, chemical fertilizers have not been widely used in the past in the major wheat growing and wheat exporting areas of the world but only where intensive cultivation is practised, and with the object of increasing yield (Moran 1959).

The influence of chemical fertilization has received some attention in India where the effect of moisture regimes and fertilization practices on wheat yield and protein in the grain has been studied by Singh and Prasad (1966), Reddy et al. (1968), Panwar et al. (1971), Sharma et al. (1972), Singh and Dastane (1971).

In the United Arab Republic, Barakat et al. (1970) have reported on the interaction between soil salinity and nitrogen fertilizer on the yield and protein content of a 145 Giza wheat variety.

Beech and Norman (1966), Williams (1966) and Taylor and Gilmour (1971) have reported on the effects of climatic factors and agronomic practices on the yield and protein content of wheats grown in Australia.

Dubetz (1972) has reported on the effects of various levels of nitrogen fertilizers on the yield and protein content of a bread wheat, Manitou, and a semi-dwarf high yielding wheat, Pitic 62, grown under irrigation in 1969 and 1970 at Vauxhall and Burdett in Southern Alberta, Canada.

Hutcheon and Paul (1966) reported on growth chamber experiments to control the protein content of Thatcher wheat by nitrogen fertilization and moisture stress and found that it was possible to increase both yield and protein content at the same time in the protein range of 11 to 16%. When protein contents above 16% were obtained, it was at the expense of yield.

The nitrogen nutrition and yield relations of Nugaines, a semi-dwarf high-yielding soft white winter wheat, was studied by Laopirojana et al. (1972) at Oregon Agricultural Experimental Station, Corvallis. High nitrogen fertilization increased grain protein percentage.

Grain and plant nitrogen relations were studied in eight spring wheat crosses seeded at three locations in Montana, USA. Grain nitrogen content was found to be negatively correlated to grain yield and to the grain:straw ratio (McNeal et al. 1972).

Six Indian wheats and 14 wheats from overseas were grown at Durgapura, Jaipur, India under similar conditions and with three levels of nitrogen fertilization (67.2 kg, 134.5 kg and 201.7 kg/ha). The wheats were EG 953, EG 954 and EG 1440 from Egypt; Charter and Gabo from Australia; 184.P.2.A.1.E. and E.220 from Kenya; Cometo Semiduro from Brazil; Trigo Centeira from South America; S.2414, S.54723, S.55362 and S.54887 from Mexico; C.286 and C.591 from the Punjab; Hy 65 from Madhya Pradesh; NP 790 and NP 825

from the Indian Agricultural Research Institute; and R.S. 31-1 from Rajasthan.

Protein (Kjeldahl N × 5.7), was determined and crude gluten content (method of Austin and Miri 1961) and kernel hardness (by a grain-hardness testing apparatus) were assessed. In all the varieties the percentage of crude gluten varied directly with the percentage of crude protein. Kernel hardness was inversely related to gluten and seed weight. Varietal differences were highly significant. In general, higher doses of nitrogen increased the protein and gluten content in the grains and decreased the hardness of the kernel but the varieties did not all respond in the same way to fertilizer. Some varieties showed a decrease in protein content when fertilizer level was raised above 134.5 kg, e.g. Trigo Centeira, others showed an increase only at the highest fertilizer level. The mean protein ranged from 12.0% for the lowest to 12.4% for the medium to 13.1% for the highest level of fertilization (Gandhi and Nathawat 1968).

As the level of nitrogen fertilizer applied to six unnamed varieties of wheat increased (0, 40, 80, 120, 160 and 200 kg/ha) the protein content (Kjeldahl N × 5.7) also increased from 13.7% (no fertilizer) to 16.9% (200 kg/ha) (Swaminathan et al. 1969).

Foliar sprays of urea and parazate, individually and in combination, increased the protein content in NP 4, NP 52, NP 165, NP 718, and NP 825 wheat varieties, but the varieties differed in their response (Swaminathan et al. 1969).

Ermolaev (1970) studied the effects of the rate of sowing, the time of sowing and the level of fertilization on the properties of Bezostaia 1 wheat grown over four years near Russe in Bulgaria. The amount of crude protein (N × 6.25) and dry gluten in the flour was most affected by the level of fertilization. The biggest effects were obtained with 140 kg/ha N active substance, 100 kg/ha P_2O_5 and 60 kg/ha K_2O, at which level the grain yield doubled. Total crude protein increased by 19% and dry gluten by 44.3% compared to the unfertilized control.

Mironovskaya 264 winter wheat grown in the Ukraine in 1964 and 1965, as part of a crop rotation system, contained higher total nitrogen levels when following peas than when following lupins grown for forage (Vlasyuk and Kurinnaya 1971). The best results were obtained using applications of P60, K90 at the ploughing stage, P15, K15 during pre-sowing cultivation and N20 during early spring feeding. Under these conditions total nitrogen, dry matter basis, was 3.12% following peas and 2.70% following lupins in 1964, and 2.25% following peas and 2.13% following lupins in 1965. All nitrogen levels were higher in 1964 than in 1965. Fertilization increased the nitrogen content of the albumins, globulins, gliadins and glutenins in the grain over the control.

Using pot trials in climatically controlled growth chambers, Partridge and Shaykewich (1972) studied the effects of nitrogen, temperature and moisture regime on the yield and protein (Kjeldahl N × 5.7) of the hard red spring bread wheat Neepawa. Photoperiod was 16 hours light and 8 hours dark at all times.

At rates up to 100 ppm of nitrogen, grain yield increased with succeeding increments of fertilizer nitrogen. There was a variable response to the final increment of 200 ppm, probably as a result of phosphorus deficiency.

Percent protein was strongly influenced by nitrogen and temperature treatments through their effect on yield. There was a significant negative correlation between percent protein and grain yield. Significant increases in percent protein over zero nitrogen treatment, resulted from 200 ppm nitrogen. Increased temperatures significantly decreased grain yield in most instances and, indirectly, increased percent protein. Moisture regime had no significant influence on grain yield nor a significant direct effect on percent protein.

A high protein wheat, CI 14016 (protein Kjeldahl N × 5.7 dry weight basis, 12.5%) and an ordinary variety, Lancer (protein 10.8%) were grown over three years at 10 sites in Nebraska and with different levels of soil fertility. Protein content increased in linear relation to nitrogen fertilizer application. The high protein wheat maintained its advantage over Lancer throughout all rates of nitrogen application from 0 to 120 lb/acre (0 to 134 kg/ha) demonstrating that the difference in protein content between varieties is genetically based. Wheat of the high grain protein (14 to 17%) can therefore be obtained from high protein strains grown under conditions of high fertility (Johnson and Mattern 1972).

AMINO ACID COMPOSITION

Gunthardt and McGinnis (1957) compared the amino acid composition of four low protein

(10.3%) and four high protein (16.5%) samples of Idaed wheat. The high protein samples were produced by a combination of nitrogen (120 lb. ammonium nitrate/acre; 134 kg/ha) and water applications at the boot, flowering or milk stages of growth; the low protein samples received from 0 to 40 lb nitrogen (0 to 45 kg) at seeding time only. The high protein samples contained significantly less lysine (microbiological assay) expressed as lysine percent crude protein than did the low protein samples. The lower lysine arose when nitrogen applications were as late as the boot, flowering or milk stages of growth. The lysine content appeared to vary with the year, or yield, or both. Other changes in amino acid composition were not statistically significant. Large differences in protein content were produced by nitrogen fertilization but only slight differences in proportional amino acid content of total crude protein.

Sosulski et al. (1963) found that reduced water supply, nitrogen fertilization, and higher air temperatures increased the protein content of Thatcher wheat grown under controlled environmental conditions in growth chambers. At higher temperatures the soil moisture conditions exerted the greater effect on protein content while the largest responses to nitrogen fertilization were obtained at the medium moisture level. The amino acid distribution was determined by the method of Moore and Stein (1954a, b); cystine and tryptophan were not determined.

Protein content ranged from 10.2 to 23.0% (American Association of Cereal Chemists 1957) expressed on a 14% moisture basis, for the samples grown at three moisture and five fertility levels. Regression and correlation coefficients were calculated between grain protein content and concentration of each amino acid. Glutamic acid and proline were positively correlated with increasing protein content; alanine, arginine, aspartic acid, lysine, serine, threonine, and valine were negatively correlated with protein content. Glutamic acid and arginine were the principal amino acids affected by variations in protein content.

Lindner (1964) examined the amino acid composition (Lindner 1956) of two Russian wheats, Bezostaia and Skorospelka, two Italian, San Pastore and Autonomia, and one Hungarian variety, Bankuti 1201, all grown at the Agricultural Research Institute at Martonvasar, Hungary, all given similar fertilization treatment and harvested in 1961. The protein (N × 5.8) and lysine as percent protein are shown in Table 55.

TABLE 55. Protein content and lysine as percent protein in five wheat varieties grown in Hungary in 1961 (data from Lindner 1964).

	Protein ($N \times 5.8$) (%)	Lysine (% *protein*)
Hungarian		
Bankuti 1201	11.54	3.2
Russian		
Bezostaia	12.99	3.2
Skorospelka	13.74	2.7
Italian		
San Pastore	12.24	2.7
Autonomia	12.47	2.7

Of the varieties studied, Bezostaia had the highest nutritional value as measured by the EAA index of Oser (1951); Bezostaia and Bankuti 1201 rated equally by the chemical score method of Mitchell and Block (1946).

Larsen and Nielsen (1966) determined nitrogen (micro-Kjeldahl) and amino acid content (auto-analyzer) of Koga wheat grown in pots in a mixture of one part clay soil and two parts sand, with a basal dressing and nitrogen as ammonium sulphate added at levels ranging from 0.025 g N/pot to 5.0 g N/pot.

Their results were in agreement with those of other investigators. Increasing applications of nitrogen to wheat caused an increase, in grams amino acid nitrogen per 100 g total nitrogen, in glutamic acid, from 15.6 to 17.4 and proline, from 6.4 to 7.8 in the grain and a decrease in the lysine, from 3.6 to 2.6, and arginine, from 11.25 to 9.0. They report small increases in ammonia and phenylalanine, and small decreases in aspartic acid, threonine, glycine, alanine and valine.

Ewald and Wenzel (1967) examined the effect on protein composition and amino acid content (by column chromatography) on Opal summer wheat, sown on loamy soil, of nitrogen fertilization. As nitrogen fertilization increased, yield increased, first at a higher rate then at a lower rate; grain nitrogen increased, first slightly then, at the higher levels of nitrogen fertilization, from 1.68 to 1.86%, i.e. about 11%. Amino acid content as percent total protein changed little with nitrogen fertilization, the major increase being in glutamic acid;

lysine showed no change. The results of pig feeding trials conducted at another institute, and more fully reported by Brune and Thier (1968a,b), showed that at a fixed nitrogen intake the biological value for pigs was reduced as the nitrogen content increased with fertilization.

Abrol et al. (1971) have reported on the effect of soil fertilizer levels on the protein (Association of Official Agricultural Chemists 1960) and amino acid composition (autoanalyzer; tryptophan colorimetrically) of two dwarf wheats HD(M) 1592 and HD(M) 1620A grown in India. The fertilizer levels were (*a*) high, N134, P68 and K45 kg/ha and (*b*) low, N34, P20 and K0 kg/ha. Protein was fractionated by the method of Nagy et al. (1941).

At high fertilizer levels the percentage of protein increased but the increase in HD(M) 1620A was 38% while the increase in HD(M) 1592 was 14%. In HD(M) 1620A the prolamin and glutelin fractions increased by 51% and 26% respectively, in HD(M) 1592, the prolamin and glutelin fractions increased by 34% and 21% respectively. The higher fertilizer level had little effect upon the albumin and globulin fractions; consistent with other reported results, the increase in total protein was predominantly in the prolamin and glutelin fractions.

In HD(M) 1592 the high fertilizer level increased the glutamic acid, proline, phenylalanine and leucine contents and decreased the lysine, valine and threonine contents calculated as percent protein. In HD(M) 1620A the high fertilizer level increased the glutamic acid, leucine and proline, and decreased the threonine, cystine, isoleucine and tyrosine; lysine was scarcely reduced.

The effect of fertilization and crop rotation on the Kjeldahl nitrogen and essential amino acid composition (determined chromatographically) of wheat and maize grown on reddish-brown forest soil from Saftica in Roumania was reported by Dinca et al. (1971). In a single wheat cropping system, high chemical fertilizer rates reduced the content of the essential amino acids. Without fertilizers, better results were obtained with short rotations of two to four years. The best results in terms of essential amino acid content were obtained with fertilizer N48, P32 and a 2-year rotation. The essential amino acid content (grams amino acid/16 g N) was then 54.6 (lysine 3.3) compared to 31.1 (lysine 2.7) when the same fertilizer was used and single crop cultivation was followed. The 2-year rotation, without fertilizer, produced a total essential amino acid content of 34.3 (lysine 3.4) and the single crop, without fertilizer, 32.6 (lysine 3.0).

Srivastava et al. (1971) studied the effect of nitrogen applied as urea at levels of 0, 20, 40, 60 and 80 kg N/ha on the protein (Kjeldahl N × 5.7) and lysine and tryptophan in the protein, of S-227 wheat grown under rain-fed conditions at the Indian Agricultural Research Institute, New Delhi. In soil treatments the whole dose was applied before seeding; in foliar treatments, half was applied to the soil before seeding and the rest sprayed as a 3% urea solution. In each spray 10 kg N/ha was applied, the first spray at the post-tillering stage, and then, according to the treatment, spraying was repeated at 7-day intervals. Table 56 shows the effect of soil and foliar applications of urea on yield, protein, lysine as percent of protein (enzymatic decarboxylase method, Naik 1968) and tryptophan as percent of protein (colorimetric method of Spies and Chambers 1949). The highest lysine content, 3.22% of the protein, was obtained as 40 kgN/ha applied to the soil; the highest tryptophan, 1.45% of protein, was obtained with soil plus foliar treatment at 80 kg N/ha.

The amino acid composition (by autoanalyzer) of a sample of S-227 wheat showing the highest protein percentage, 10.43%, obtained from soil plus foliar treatment at 80 kg N/ha was compared with one showing the lowest, 7.23% protein from 0 kg N/ha; lysine percent in the protein was inversely correlated with total protein in the wheat.

Mamchenkov and Platonov (1971) found that as the protein content (N × 5.7) of Mironovskaya 808 winter wheat increased through the use of nitrogen, phosphorus and potassium fertilizers combined with crop rotation, the percent amino acid composition in the protein (determined by paper chromatography) altered as follows: lysine, histidine, threonine and glycine decreased; cystine, aspartic acid, glutamic acid, serine, tyrosine, phenylalanine, and leucine increased; alanine, valine and methionine did not change.

Elonen et al. (1972) studied the effects on spring wheats of irrigation and nitrogen fertilization, separately and together, in five field trials in Finland in the years 1967 through 1970. The varieties were Svenno in trials 1 and 2, Norrona in trial 3 and Ruso in trials 4 and 5. The soil was a silty clay with an average pH of 5.6, containing

TABLE 56. Effect of soil and foliar application of urea on yield, protein ($N \times 5.7$), lysine and tryptophan content of 'S 227' wheat (data from Srivastava et al. 1971).

Nitrogen (*kg/ha*)	Method of application	Yield of grain (*kg/ha*)	Protein (*g/100g grain*)	Lysine (*g/100g protein*)	Tryptophan (*g/100g protein*)
0	Control	1,812	8.00	2.50	1.03
20	Soil	2,373	8.09	2.73	1.10
40	Soil	3,146	8.04	3.22	1.17
60	Soil	3,681	8.72	2.96	1.14
80	Soil	3,736	9.10	3.10	1.24
20	Soil + foliar	3,022	8.14	2.23	1.12
40	Soil + foliar	3,295	8.86	2.73	1.09
60	Soil + foliar	3,582	9.35	2.29	1.24
80	Soil + foliar	3,765	9.64	2.38	1.45
SE rates			±0.122	±0.154	±0.0565
SE methods			±0.061	±0.109	±0.04

about 4% of organic carbon in the top soil; the subsoils were heavy clay. The basal fertilizer dressing on the N_1 plots contained on average, 68 kg N, 53 kg P and 81 kg K per hectare and was placed in rows at a depth of 8 cm. Additional nitrogen on the N_2 plots averaged 76 kg/ha and was applied as a surface dressing for the shoots as calcium nitrate in trials 1 and 2, as urea in trial 3, or at a depth of 8 cm in connection with sowing either as urea or ammonium nitrate limestone in trials 4 and 5.

The grains were milled in a Quadrumat Junior to a 60 to 65% extraction flour. Irrigation increased average yields by 1200 kg/ha. Protein (Kjeldahl $N \times 5.7$) decreased with irrigation but this decrease could be offset by additional nitrogen fertilization. Irrigation significantly increased the proportions of lysine and isoleucine (by autoanalyzer) as percent of protein but the decrease in total protein content gave rise to a net decrease in lysine content. Glutamic acid increased while lysine and arginine declined with nitrogen fertilization but as total protein was increased a net increase, on average $8.3 \pm 5.1\%$, was manifested. Table 57 summarizes the position.

By increasing fertilizer nitrogen from 68 to 144 kg/ha and by irrigating twice in June, grain yields increased by 65% without any noticeable changes in flour protein or lysine contents.

There were no significant differences between the amino acid composition of wheat fertilized by urea or by ammonium nitrate limestone but because of the relatively small quantity of material available, the confidence limits were wide.

Khera et al. (1972) examined the relation between the phosphorus level in the soil and the protein quality of the Kalyan Sona wheat grown on it as measured by the lysine, methionine and tryptophan contents of the grain. Kalyan Sona was grown in pots each containing non-saline soil, of pH 7.0 to 8.4, sandy loam to clay loam in texture and low in organic carbon. To each 4.5 kg soil a basal dose of 89 ppm N, 37 ppm K and adequate amounts of trace elements were added. The method of Cate and Nelson (1965) was used to correlate the soil test analyses with the plant response data.

Under these conditions it was found that protein quality, as measured by protein (Kjeldahl $N \times 5.7$) percent in the grain, and lysine (by microbiological assay), methionine and tryptophan (by colorimetric methods) in the nitrogen of the grain was inferior when the available phos-

TABLE 57. Effect of additional nitrogen fertilization (N_1 and N_2) and irrigation on grain yield, protein ($N \times 5.7$) lysine in flour and in protein of flour, from Finnish wheats (data from Elonen et al. 1972).

	Without irrigation		With irrigation	
	N_1	N_2	N_1	N_2
Grain yield, kg/ha	2850	3210	3820	4690
Protein in grain, %	14.31	16.56	12.20	14.43
Protein in flour, %	13.50	15.93	11.25	13.54
Lysine in flour, mg/100 g	292	315	260	283

phorus levels were below 12.5 ppm. Below this level phosphatic fertilizer is required if grain weight and protein quality are not to suffer.

At Pahlavi University, Shiraz, Iran, the effect of potassium and nitrogen applied to the soil, on the lysine, methionine and total protein of Roushan bread wheat was studied by Hojjati and Maleki (1972). The soil was calcareous silty clay loam, initially high in potassium which was added at the rates of 0, 25, 50 and 100 kg K/ha in the form of potassium sulphate under four levels of nitrogen, 0, 50, 100 and 200 kg N/ha as urea. All the potassium and half the nitrogen was applied at seeding in November 1967 and the rest of the nitrogen in April 1968. The field was irrigated before seeding and during the growing season.

Yield of grain and total dry matter production were not affected by potassium applications. Yield was not affected by nitrogen up to 100 kg N/ha but decreased at the 200 kg level. The ratio of grain to total dry matter decreased consistently with increasing application of nitrogen showing that it stimulated vegetative growth more than it did seed production.

Nitrogen fertilizer consistently increased the protein content (micro-Kjeldahl N × 5.83) of the grain, from about 10% to about 14%; an approximate increase of 1% was recorded with each additional 50 kg N/ha. Lysine and methionine (by microbiological assay) increased as percent grain; lysine as percent protein was negatively correlated with the protein content and with the N applied, but positively correlated with K. Methionine as percent protein did not change with varying rates of N or K.

Pahwa et al. (1972) found that the inclusion of cowpeas (*Vigna sinensis* Savi ex Hassk) and berseem (*Trifolium alexandrinum* Juslen) in the crop rotation significantly improved the yield from about 31 to 35 q/ha, of Sonora 64 bread wheat harvested at New Delhi in 1968. The addition of maize (*Zea mays*) stover in the soil before the wheat crops decreased yield but increased calcium in the wheat flour. Crude protein content in the grain (Association of Official Agricultural Chemists 1965) increased following cowpeas and berseem and decreased when maize stover was added but these differences were not significant. Lysine (Naik 1968), methionine and cysteine (autoanalyzer) as grams per 100 g protein were not significantly different; tryptophan (Spies and Chambers 1949) as percent protein was significantly decreased by inclusion of stover and following cowpeas but was unchanged following berseem. Rotation produced no significant differences in the albumin, globulin, prolamin and glutenin fractions (modified Mendel-Osborn method), albumins and globulins accounting for about 40% of total protein. A complete amino acid analysis (autoanalyzer) as grams per 100 g protein, showed that aspartic acid, glutamic acid and proline were slightly higher, and lysine, histidine and arginine slightly lower, in the highest protein sample (16.27%) than in the lowest protein sample (14.18%).

Chlorophenoxy herbicides, particularly 2,4-dichloro-phenoxyacetic acid (2,4-D) are used to control broad-leaved weeds in wheat and one of the effects which may occur is depletion of the starch and sugars and a higher percentage of protein in the grain (Klingman 1953 and Shaw et al. 1955 cited by Pellett and Saghir 1971). Samples of Najah wheat grown in the Lebanon and treated at 2, 4 and 8 kg/ha at the jointing stage of growth with the butoxyethyl ester of 2,4-D were compared with others from unweeded and hand-weeded plots. No significant differences were found in amino acid composition (by ion exchange chromatography) between the grain from the unweeded and the hand-weeded plots. A slight decrease in threonine and arginine and an increase in the proline content of the protein were observed in grain from the treated plot. High doses of 2,4-D may increase protein percentage but have only a minor effect on amino acid composition of wheat (Pellett and Saghir 1971).

BIOLOGICAL QUALITY

Greer and Grindley (1954) reported feeding trials in which weanling rats of both sexes were fed ad libitum on diets in which whole ground winter wheat grown in the United Kingdom provided all of the protein. The mean grain nitrogen contents were: (1) 1.81% N_2 dry basis for grain which had received no spring dressing, (2) 1.92% N_2 dry basis for grain which had received late April dressing (2 cwt nitro chalk per acre), (3) 2.18% N_2 dry basis for grain which had received the late April dressing plus an equivalent dressing in late May. Three feeding trials were conducted, with variations in the oil and vitamin supplementation

to the diets. In two trials 10 pairs of rats were used and in the third, eight pairs. The mean values for nitrogen intake, dry basis, were: (*a*) no spring dressing 0.121 g N_2 per day; (*b*) spring dressing 0.150 g N_2 per day. In all three experiments the growth of the rats receiving the spring dressed wheat exceeded that of the rats consuming the wheat which had received no spring dressing. Growth increment, grams per grams nitrogen intake, was: no spring dressing 9.09; spring dressing 8.67 or a PER (protein N × 5.7) of: no spring dressing 1.58, spring dressing 1.52. Greer and Grindley conclude that the improvement in grain nitrogen brought about by spring dressing represented a protein increase of normal nutritive value.

Bains (1953) studied the effect of nitrogen (60 lb N_2/acre; 67 kg/ha) and phosphorus (25 lb P_2O_5/acre; 28 kg/ha) on the nutritive value of C409 wheat at Punjab Agricultural College and Research Institute, Lyallpur. The fertilizer treatment consisted of (1) green manure (*Cajanus indicus*) 5 tons/acre (12,544 kg/ha), (2) potassium nitrate, (3) potassium nitrate with green manure, (4) superphosphate, (5) superphosphate with green manure, (6) superphosphate with ammonium sulphate, (7) superphosphate with ammonium sulphate and green manure. All the fertilizer treatments improved yield per acre. Treatments (1), (2), (3), (6) and (7) increased, and treatments (4) and (5) decreased protein (N × 5.7) in the grain. However, all treatments improved output of protein per acre, the most productive being treatment (7) with almost double the unfertilized control, followed, in descending order, by (5), (2), (6), (4), (3), and (1).

The biological value of the grain was examined in rat feeding trials using modifications of the "balance sheet" method of Mitchell (1925). The diets, fed to provide 5% protein from the wheat were fed to adult male albino rats, six per group, for a 7-day period following seven days on a nitrogen-free diet. The nitrogen-free diet was also fed at the end of the trial. Under these conditions, that is when the test samples were fed at the same protein level of 5%, there was no noticeable effect of the variations in the protein content of the samples resulting from fertilization.

Brune and Thier (1968a, b) examined samples of the winter wheat Heine VII grown in 1961 at the Institut fur Pflanzenernahrungslehre und Bodenbiologie, Stuttgart/Hohenheim and the summer wheat Opal grown in 1964 at the same institute. The Opal wheat is that referred to by Ewald and Wenzel (1967). The fertilization conditions for Heine VII were 40 kg/ha P_2O_5 and 80 kg/ha K_2O and nitrogen varied to give 0, 30, 60 and 90 kg/ha. The conditions under which the two wheats were grown are not therefore comparable.

The method of amino acid analysis is not stated but since the Heine VII and the Opal were grown at the same institute, although in different years, the amino acid analysis method of the Heine VII may have been the same as that used for Opal by Ewald and Wenzel (1967). A comparison of the amino acid content as percent protein showed no or very slight differences in lysine in response to fertilization. Leucine, isoleucine and phenylalanine were lower in the winter wheat Heine VII with nitrogen fertilization but were higher in summer wheat. Arginine was higher in winter wheat and lower in summer wheat with fertilization. Threonine increased in summer wheat with fertilization but remained the same in winter wheat. There were no significant changes in amino acid composition of either wheat with fertilization.

No correlation was evident between the amino acid scores of the grain (Mitchell and Block 1946; Oser 1951) and the results of feeding trials with pigs and rats. The winter wheat and the summer wheat could not be compared with one another because of the different conditions under which they had been grown but both wheats grown under high levels of nitrogen fertilizer produced statistically significantly reduced "productive protein values" (apparent NPU: nitrogen balance as percentage of nitrogen intake) in pigs compared to the wheat grown without nitrogen fertilization. A modified Thomas-Mitchell method for nitrogen metabolism in pigs was used. The difference was smaller but still significant with young rats (initial weight 40 g, 10 per group) but was not seen in rats weighing 60 g (Brune et al. 1968).

Ferrel et al. (1970) report experiments in which wheat, fortified with widely different levels of lysine, was stored under several conditions. Subsequently PERs were determined at a constant lysine level. It is difficult to draw clear conclusions from the reported results through Ferrel et al. suggest that the lysine naturally present in the wheat suffered greater change than the added lysine.

Wheat and Rye Compared

Genetic and Varietal Effects

Jones et al. (1948) reviewing the comparative growth-promoting (rate of weight gain) values for rats, of the proteins of cereal grains, commented that compared with the proteins of most cereal grains, rye had received comparatively little attention. In their own experimental work they compared whole rye, protein content 10.96% (N × 5.83) with other cereals including hard spring wheat, protein 14.24% (N × 5.83) and soft winter wheat, protein 11.02% (N × 5.83) as the sole source of the protein in rat diets. In two sets of experiments wheat and rye diets were compared at levels of 4.5%, 7.5% and 9.5% protein. The results are shown in Table 58.

The superiority of rye protein over wheat protein found in these experiments confirmed the findings of earlier studies cited by the authors (Mitchell and Hamilton 1929; Kon and Markuse 1931; Johnson and Palmer 1934).

Sure (1954) compared the nutritive values of the proteins (nitrogen conversion factors are not stated) of whole hard wheat flour, protein 14.7%, whole rye (pumpernickel) flour, protein 9.44%, and Arkansas (USA) grown whole rye flour of 10.1% protein in feeding experiments with 12 male and 12 female albino rats per group, over 10 weeks. The cereals provided the only source of protein in the diets, the protein being fed at 9%, 8% and 5% levels. At all levels of protein intake, rye protein was superior to wheat protein in terms of weight gain and ratio of weight gain: protein intake.

A paired feeding experiment was also conducted over 35 days with the protein at the 8% level. On the same amount of food and of protein daily, the rye-fed animals gained 70.4% more in body weight than those on the wheat flour.

de Vuyst et al. (1958) determined the amino acid composition of various grains grown in different locations in Belgium and intended for animal feed. The protein contents (conversion factors not stated) of Alba wheat grown in three locations ranged from 8.16% to 11.10%; Zanda wheat at one location contained 9.08%. Petkuzer rye at two locations contained 8.07% and 8.36% and Court des Flandres rye at two locations 7.82% and 8.80%. The mean lysine and threonine contents (Stein and Moore 1951) expressed as percent total nitrogen were, in the wheats 2.22 and 2.30 respectively and in the ryes 3.90 and 2.34.

Janicki and Kowalczyk (1965) examined the protein content and amino acid composition (by autoanalyzer, tryptophan, Horn and Jones 1945), of five varieties of wheat and three varieties of rye grown in the same areas of Poland and in the same year. The results are presented in Table 59.

Applying the method of Block and Mitchell (1946) and comparing the biological value of egg protein with that of wheat, the authors found the limiting amino acids of all the wheat varieties to be methionine and lysine. The limiting amino acids of rye were methionine and isoleucine. Rye protein has a higher biological value than wheat protein mainly because it contains more lysine and methionine, but wheat has a higher protein content than rye. Janicki and Kowalczyk cite Trzebska-Jeska and Morkowska-Gluzinska (1963) as having found in their work that tryptophan and methionine were the limiting amino acids in rye. The latter however did not examine isoleucine and Janicki and Kowalczyk found higher values for tryptophan than did Trzebska-Jeska and Morkowska-Gluzinska (1963).

TABLE 58. Growth promoting values of wheat and rye fed as sole source of protein in the diet for rats (data from Jones et al. 1948).

	Average weight gains (g)	Average gain/g protein consumed (g)	Average food consumed (g)
	4.5% protein in diet		
Rye	35±2.40	2.26	337
Wheat, hard	18±0.94	1.72	234
Wheat, soft	20±1.32	1.74	216
	7.5% protein in diet		
Rye	60±2.29	2.16	381
Wheat, hard	43±2.11	1.55	362
Wheat, soft	28±1.68	1.21	326
	9.5% protein in diet		
Rye	74±2.44	1.83	434
Wheat, hard	68±2.65	1.60	444
Wheat, soft	66±2.35	1.68	414

TABLE 59. Amino acid hydrolysates of Polish wheat and rye varieties (*in grams/16 g N*) (data from Janicki and Kowalczyk 1965).

	Wheat					Rye		
	Lagiewnicka 52	Odin	Nagradowicka	Zdzislawka	Przodownica	Dankowskie selection	Wloszanowskie	Rogolinskie
Protein N × 5.7 %	11.62	12.45	13.49	14.0	14.33	8.46	9.57	9.79
Alanine	3.83	3.71	3.49	3.36	3.21	4.80	4.35	4.25
Arginine	4.66	4.89	4.90	4.52	4.17	5.59	–	5.55
Aspartic acid	5.41	5.39	5.14	5.20	5.24	7.13	6.82	7.46
Cystine (Cysteine and cysteic acid)	1.43	1.46	–	1.53	1.55	–	–	–
Phenylalanine	3.71	3.74	4.12	3.99	4.10	2.71	3.68	3.34
Glycine	4.02	4.00	3.95	3.85	3.77	4.76	4.36	4.25
Glutamic acid	27.0	27.7	28.0	28.2	28.0	20.9	22.3	21.8
Histidine	–	2.29	2.39	2.33	–	2.54	2.25	2.46
Isoleucine	3.55	3.36	2.98	4.09	3.23	3.34	3.58	3.24
Leucine	6.73	6.51	6.30	6.13	6.35	6.09	6.40	5.85
Lysine	3.02	2.87	2.80	2.79	2.66	4.37	3.94	4.09
Methionine (and methionine sulphoxide)	1.46	1.61	1.56	1.44	1.41	1.79	1.76	1.73
Proline	8.66	9.55	8.58	10.3	10.1	7.96	9.17	8.66
Serine	3.94	4.00	4.02	3.93	3.71	4.45	3.68	4.01
Threonine	2.84	2.67	2.56	2.60	2.46	3.60	2.99	3.07
Tyrosine	1.43	1.77	1.84	2.00	2.07	–	1.41	1.43
Tryptophan	1.11	1.13	1.14	1.04	1.06	–	1.00	0.95
Valine	4.19	4.37	4.00	4.09	4.05	4.50	4.53	4.39

Kalmykov (1968) used column exchange chromatography (Moore et al. 1958) to compare the amino acid composition of Vyatka-2 rye and Bezostaia-1 wheat. The results of these determinations are given in Table 60. The biological value (BV) was determined using the dog as experimental animal. Under the conditions of the experiment, the BV of the rye was 89.5% and of the wheat 76%. In Kalmykov's view the biological tests were in accord with the results of the chemical determinations of amino acid content.

TABLE 60. Amino acid composition of Vyatka-2 rye and Bezostaia-1 wheat in grams/100 g protein (data from Kalmykov 1968).

	Rye	Wheat
Threonine	3.03	3.10
Valine	4.64	3.90
Cystine	2.8	2.7
Methionine	1.8	1.8
Isoleucine	3.34	3.72
Leucine	5.33	6.35
Tyrosine	2.2	2.3
Phenylalanine	4.6	4.7
Lysine	4.54	2.9
Tryptophan	0.87	1.03

Eggum (1970) examined the true digestibility of a number of feedstuffs, including wheat and rye, and of amino acids contained in them, for baby pigs and rats. The results are presented in Table 61 and indicate that amino acids from the same protein source can be digested differently, so making it difficult to estimate the availability of any particular amino acid from the digestibility of the total nitrogen.

Environmental and Agronomic Influences

Pessi et al. (1971) studied the effect of fertilization technique on the protein content of cereals grown in Finland in 1969-70. The fertilizer used

TABLE 61. True digestibility (TD)[a] for pigs and rats of total nitrogen (N) and the single amino acid for casein, wheat and rye (data from Eggum 1970).

	Casein	Wheat	Rye
TD[a] for total N (pigs)	99.4	91.8	80.9
TD[a] for total N (rats)	101.1	89.6	77.0
TD[a] for:			
Cystine	100.1	93.9	86.3
Aspartic acid	98.9	87.1	76.9
Methionine	100.3	88.6	73.9
Threonine	98.8	88.4	74.4
Serine	100.9	96.5	88.1
Glutamic acid	99.6	97.5	92.2
Glycine	97.7	89.7	78.2
Alanine	97.9	86.5	73.3
Valine	99.1	90.8	78.0
Isoleucine	99.1	90.3	73.7
Leucine	99.3	93.2	81.2
Tyrosine	98.7	92.5	79.1
Phenylalanine	98.8	92.0	79.8
Lysine	99.7	84.1	72.2
Histidine	99.7	95.9	88.8
Arginine	99.2	95.0	88.5

[a]True digestibility is not defined in the paper but is generally regarded as the proportion of food nitrogen that is absorbed (National Academy of Sciences—National Research Council 1963).

for winter wheat and rye was calcium ammonium nitrate (26% N) and for spring wheat NPK fertilizer. The winter wheat varieties were Elo, Jyva, Linna, Nisu and Vakha and the rye varieties were Ensi, Pekka, Toivo, Visa and Voima. Differences in yield were found between varieties and with and without chlorcholine chloride (CCC) treatment, but no statistically significant differences in grain crop protein were found which could be attributed to variety, fertilization or CCC treatment.

The six varieties of spring wheats are not named. As the level of fertilization increased, grain protein increased but CCC treatment had no effect nor were there statistically significant differences between varieties.

The grain of one variety of spring wheat, JO-04558 grown in the two years 1968–70 was also analyzed for amino acid composition. (Methods of determining protein and amino acid composition not stated.) The percentage of proline and glutamic acid increased and the percentage of lysine decreased with increased fertilization.

Calcium ammonium nitrate was found to be more effective than urea in improving grain and protein yield of Svenno and Apu spring wheats.

Placement fertilization was more effective than broadcasting fertilization in increasing grain yield of spring wheat and total protein yield even though the former also resulted in slightly lower protein content in the grain.

Fertilization of winter wheat and rye with nitrogen in the spring improved protein content without impairing yield, when compared to fertilization in early winter. Additional nitrogen fertilization at the heading stage increased the protein content.

Janicki et al. (1972) reported on the total Kjeldahl nitrogen and amino acid composition (by autoanalyzer, excluding tryptophan) of five Polish wheat flours, four of which were named varieties, and one rye flour designated only by a code number. On the basis of these, and unpublished results, the authors suggest that a more realistic nitrogen to protein conversion factor for wheat flour is 5.61 instead of 5.7 and for rye flour 5.67 instead of 6.25 as is given in the Polish Official Standard. The protein contents of the flours using both factors are given with the lysine, methionine and threonine content in Table 62.

Rye

Genetic and Varietal Effects

Trzebska-Jeske and Morkowska-Gluzinska (1963) conducted a survey of 33 samples of winter rye harvested in 15 provinces of Poland in 1959. Of the 11 varieties grown, Ludowe and Wloszanowskie were grown in nine provinces, Pulawskie Wczesne and Dankowskie-Selekcyjne in four provinces, and Zeelandskie, Universalne, Rogalinskie, Wierzbienskie, Mikulickie, Kazimierskie and Wielkopolskie in one province each.

No significant differences were found in the mean Kjeldahl nitrogen values for the different varieties but considerable variations in nitrogen content and in weight of 1000 grains were observed within the same variety. For example, the mean nitrogen contents of the widely grown varieties were: Ludowe 1.44% ranging from 1.30 to 1.67% and Wloszanowskie 1.39% ranging from 1.17 to 1.58%. Lysine, leucine, tryptophan, arginine and methionine (microbiological assay) as percent total nitrogen also varied more within varieties

TABLE 62. Protein. ash. lysine. methionine and threonine contents of five Polish wheat flours and one rye flour (data from Janicki et al. 1972).

	Wheat flours					Rye flour
	Luksusowa Code No. 750	Wroclawska Code No. 500	Code No. 950	Krupczatka Code No. 500	Tortowa Code No. 450	Code No. 800
Moisture. %	10.5	11.3	10.7	10.1	10.4	9.0
Ash	0.841	0.553	0.926	0.482	0.485	0.705
Kjeldahl Nitrogen. %	1.97	1.79	2.16	1.48	1.64	1.27
Protein N × 5.7. %	11.23	10.20	12.06	8.46	9.35	7.96
Protein N × 5.61. % (wheat)	11.05	10.05	11.87	8.33	9.20	–
N × 5.67. % (rye)	–	–	–	–	–	7.22
Amino acids as percent of total amino acids						
Lysine	2.60	2.49	2.68	2.40	2.57	3.54
Methionine	1.46	1.55	1.31	1.60	1.29	1.44
Threonine	2.95	2.83	2.98	2.81	2.75	3.77

TABLE 63. Origin. moisture. and (Kjeldahl) nitrogen. and nitrogen efficiency ratio of four rye flours fed to rats (data Kihlberg and Ericson 1964).

	A Russian	B 50% Russian 50% Swedish	C 20% Russian 80% Swedish	D 70% Russian 30% Argentinian
Moisture. %	12.8	13.9	13.2	12.0
Nitrogen. % of fresh weight of flour	2.03	1.75	1.47	1.79
Rat feeding trials				
Average weight gain g/day	1.63±0.10	1.7±0.07	2.09±0.10	1.55±0.08
Nitrogen efficiency ratio g weight gain/g nitrogen eaten	12.25±0.29	13.32±0.31	14.66±0.47	11.51±0.38

than did the mean values. Tryptophan, methionine and lysine were inversely correlated with total nitrogen, though total content of the amino acids increased with the increase in protein content.

Kihlberg and Ericson (1964) compared four rye flours designated (A) Russian, (B) 50% Russian, 50% Swedish, (C) 20% Russian, 80% Swedish, (D) 70% Russian, 30% Argentinian, for amino acid composition (ion exchange chromatography, except for tryptophan and cystine, assayed microbiologically), and for biological quality in rat feeding trials. The moisture content and Kjeldahl nitrogen are shown in Table 63. The amino acid composition of the four rye flour mixtures were essentially similar except for lysine which was, in grams/16 g N, for (A) 4.13, (B) 3.41, (C) 3.16 and (D) 2.88. The rye flours, 91% extraction rate, supplied all the protein and carbohydrate in the diets for young male albino rats, eight per group, fed for 28 days, the diets providing 1.34% nitrogen. The average weight gain and nitrogen efficiency ratios are also given in Table 63. Lysine was found to be the first and threonine the second, most limiting amino acid. No single amino acid or pair of acids increased the protein value of rye flour supplemented with lysine and threonine.

Zhebrak and Kolchin (1968) found little difference in the amino acid content (by paper chromatography), expressed as percent rye protein, in the grain of diploid and tetraploid rye of

TABLE 64. Moisture, nitrogen, protein, 1000-grain weight, lysine and threonine contents of spring and winter rye grain (data from Boronoeva and Kazakov 1969).

	Spring rye			Winter rye	
	1	2	3	4	5
Moisture content, %	8.6	9.0	8.4	8.6	10.4
Weight of 1000 grains, g	33.9	29.7	24.9	16.1	16.2
Nitrogen % dry weight	2.69	2.30	2.65	1.90	2.36
Protein (N × 5.7), %	15.30	13.11	15.10	10.80	13.45
Amino acids as g amino acid per 100 g protein					
Lysine	2.56	2.47	2.65	2.38	2.38
Threonine	4.80	4.35	4.05	3.68	4.20

the Petkusskaya variety. The 1000-grain weights were, respectively, 26.66 g and 57.30 g.

Boronoeva and Kazakov (1969) determined by autoanalyzer the amino acid composition of (1) Onokhoisk spring rye harvested in 1964 at the Buryat Agricultural Research Station, (2) Onokhoisk spring rye harvested in 1965 at the same station, (3) Onokhoisk spring rye harvested in 1965 in the Barguz region of the Buryat ASSR, (4) Udinsk winter rye harvested in 1964 at the Buryat Agricultural Research Station, (5) Winter rye harvested in 1966 in the Orenburg region. Table 64 shows the nitrogen, moisture content, protein, 1000-grain weight, lysine and threonine contents of the spring and winter ryes. There were no substantial differences in the amino acid composition between winter rye and spring rye.

Minja (1969) reported briefly that when the rye varieties Antelope, Dakold, Prolific 6201 and 6203 were, (1) without treatment, (2) autoclaved, (3) solvent extracted, each fed to mice, autoclaving had a detrimental effect; solvent extraction had a beneficial effect on 6201 and 6203. Rations containing Dakold and 6204 were equivalent in apparent biological value to the casein control diet containing 12% crude protein but the treatment, if any, that the rye received is not stated.

Environmental and Agronomic Influences

Somin (1970) determined the amino acid composition (ion exchange chromatography) of 15 varieties of rye grown in the USSR under a variety of environments. The varieties were divided into three groups:

(1) Vyatka 2, grown in five different areas,

(2) Six different varieties grown in the Minsk district,

(3) Four varieties grown in three places.

Total protein content (N × 6.25) ranged from 8.6% to 14.4%. The means were: Group 1, 12.3% (range 10.6 to 13.6%), Group 2, 11.5% (range 9.9 to 13.3%), and Group 3, 12.1% (range 8.6 to 14.4%). There was relatively little difference in the amino acid content as percent rye protein in individual rye varieties, e.g. lysine ranged from 3.1 to 4.2; the means were: for Group 1, 3.9 (range 3.6 to 4.1), for Group 2, 3.9 (range 3.1 to 4.2), Group 3, 3.6 (range 3.4 to 3.8).

Schneider and Lantzsch (1971) reported on the apparent digestibility of three winter ryes and two summer ryes in feeding trials with sheep, four per group and pigs, three per group. The ryes differed in their crude protein content as is shown below:

	Crude protein (N × 6.25) (% *dry matter*)
Karlshuder winter rye	13.62
Karlshuder summer rye	14.44
Petkuser winter rye, short straw	9.29
Petkuser winter rye, normal straw	9.14
Petkuser summer rye	10.17

In spite of these differences in crude protein content, the differences in the apparent digestibility of the ryes were not significant with either sheep or pigs.

Golenkov and Gilzin (1971) determined (by autoanalyzer) the amino acid content of 22 samples of basic varieties of rye from the 1967 Russian harvest and 19 from the 1968 harvest.

About one-third of the amino acid content as percent protein was in the form of glutamic acid (from 21.74 to 35.15%) and proline (8.45 to

12.99%). In all samples examined there was a high content of lysine (3.00 to 5.30%), of threonine (2.82 to 3.91%) and of valine (3.72 to 7.25%). Statistical analysis suggests the variations within amino acids are real and significant and not entirely attributable to analytical errors.

The extent of these variations, however, differs considerably from one year to another. In almost every case, the variations were smaller in 1967 than in 1968, which may be explained by the fact that the environmental conditions were less variable in 1967 than in 1968. Results quoted also show that different varieties grown in the same place, and one variety, Kharkovskaya 60, grown in different regions, were significantly different in their amino acid contents.

The content of lysine in the total protein of the varieties Kharkovskaya 55, 60 and 194 grown at the Kharkov Genetic Institute in 1967 varied only slightly, but in 1968 the variations exceeded the error of determination by three times. A similar picture emerged for the lysine content of Saratovskaya, Krupnozernaya and Volzhanka grown in the Saratovskii region. Lysine (percent grain) from the varieties Kharkovskaya 60, Hybrid 2 and Vyatka Moskovskaya grown in Stupino (Moscow district) was dependent neither upon harvest season nor variety.

The amino acid content of Vyatka rye did not appear to be significantly affected when grown following: (1) fallow, (2) peas, (3) maize, (4) vetch and oats, (5) barley, e.g. lysine content was, respectively, (1) 4.04, (2) 3.92, (3) 3.65, (4) 4.09, (5) 3.99 and threonine (1) 3.24, (2) 3.45, (3) 3.43, (4) 3.45, (5) 3.24.

Fernandez et al. (1972) has reported that when laying hens were fed a diet containing a high level (80%) of rye (of unstated origin), there was a sharp drop in the rate of egg production in the first three weeks followed by an increase in egg production to near normal level. The drop was not prevented by supplementation with penicillin.

Chlorcholine chloride (CCC) treatment, which has been shown to reduce the height of rye (Primost et al. 1967) was applied, with nitrogen fertilization, to Schlagher alt and Kefermarkter varieties of rye at Linz/Donau, Austria. Both these varieties had responded to treatment with CCC (Bayzer and Mayr 1967).

CCC-treated samples of the same (Kjeldahl) nitrogen content as untreated samples had approximately the same amino acid composition (method of Moore et al. 1958; tryptophan by microbiological assay) and there was therefore no increase or decrease in amino acid composition as a direct result of CCC-treatment. Primost et al. state that the higher the nitrogen content in rye, the more glutamic acid and proline and the less lysine, arginine and aspartic acid. Differences in the variety, location and fertilization of rye affect the amino acid composition only in so far as they affect the nitrogen level in the grain. The same effect was produced by CCC-treatment during the vegetative period.

Kernels of triticale infected with ergot (Photo: E. N. Larter)

Chapter 4

Nutritional Inhibitors and Toxic Factors

Introduction

The nutritional value of a cereal may be impaired by the presence of naturally-occurring factors which interfere with the digestibility, and/or availability to human and/or other animals of one or more of its nutrient components. The distinction between a nutritional inhibitor and a toxic substance is indeed fine. The word "toxic" is defined as "poisoned or imbued with poison" and is derived from the Greek name for the poison used for smearing the tips of arrows ($\tau o \xi \iota \kappa o \sigma$, equals or pertaining to the bow). A poisonous (or toxic) substance is one which when introduced or absorbed by a living organism destroys life or injures health. Consequently a nutritional inhibitor, if its inhibiting effect is sufficiently potent to cause serious malnutrition, might properly be described as "toxic".

The nutritional inhibitors reported in triticale, wheat or rye, fall into two broad categories:

(A) Intrinsic substances which occur naturally in the plant. These include resorcinols, phytates, certain enzyme inhibitors, and others as yet unidentified. The presence of nutritional inhibitors is detected by a lower than anticipated rate of weight increase in the animal consuming the food in relation to the quantity and quality of the nutrients ingested.

(B) Substances produced by infection with microorganisms. Many of these, including ergot, aflatoxins and other mycotoxins are, as their name implies, highly toxic.

Naturally-Occurring Inhibitors

Resorcinols

The observed effect of rye, when fed in large amounts, in reducing the food intake and rate of weight increase of cattle, sheep, pigs, poultry, horses and rabbits, has been recognized for many years and was investigated by Wieringa (1967) in the Netherlands.

In experiments using Petkuser winter rye, harvested in 1961, fed to weanling male white Wistar rats, he identified the growth-inhibiting substances as a mixture of 5-n-alkyl resorcinols with odd numbered side-chains of 15–23C atoms, together with smaller amounts of 5-alkenyl resorcinols.

The same group of compounds had been found in wheat by Wenckert et al. (1964). No difference could be found by Wieringa (1967) in growth-inhibiting effect between rye and wheat resorcinols, in experiments in which the rye used contained about twice the level of resorcinol as the wheat. He found great variation in alkyl resorcinol content within a single sample of rye. He also found that the percentage of crude protein correlated positively with 1000-kernel weight, and that the heavier kernels contained relatively less resorcinols than the lighter kernels. It is possible, at least in part, that variation in resorcinol content was attributable to variations in grain composition, the lighter grains probably displaying a higher bran to endosperm ratio than the heavier grains. Chromatographic examination of the rye kernels established that the resorcinols are localized in the pericarp and do not occur in the endosperm or germ. Consequently scientists who encounter apparent varietal differences in inhibitor content should ensure that the differences are truly genetic in origin and not in fact attributable to differences in endosperm size, varying degrees of shrivelling or grain malformation.

Wieringa also established that older rats, with a body weight of about 100 g became accustomed to a diet including the growth-inhibiting factor

whereas weanling rats of approximately 40 g body weight did not.

In experiments with 20 farrows of 'VNL' piglets basic rations were fed to which were added (A) 50% barley meal (control), (B) 50% rye meal, variety D 1964, (C) 50% rye meal, Petkuser, 1964, (D) 50% rye meal, Petkuser, 1965, and (E) 49.15% barley meal with 0.85% rye oil. The average body weight of each group of 10 animals, half male and half female, was 16.2 kg. During the experiment diarrhoea occurred and six animals died, two from the control group. No symptoms of over-feeding with rye were observed but the growth results for all groups were lower than expected.

Taking groups (B), (C), (D), (E) together, the average growth was 11 to 12% lower than that of the control group. This difference was highly significant ($P = 0.01$) though differences between each test group separately and the control were not significant ($P = 0.10$).

Munck (1969) reported that at Svalof, in Sweden, a preliminary screening of 5-alkyl resorcinols in rye showed great variation with genetic and environmental conditions, ranging from 0.12 to 0.22% content of the seed (moisture content about 10%). In winter rye of low protein content 5-alkyl resorcinols were present in higher concentrations than in winter rye of higher protein content. Munck also reported that spring varieties of rye showed no such correlation but gave no further details. In wheat, lower amounts of the same group of compounds were found.

A later report (Munck 1972) on the alkyl resorcinols in rye, wheat and octaploid triticale grown in Sweden gives the findings presented in Table 65. The octaploid triticales resemble wheat more closely than rye in their content of alkyl resorcinols, the content of alkyl resorcinols in triticales being lower than in rye but higher than in wheat.

TABLE 65. Alkyl resorcinol "units" (range in parentheses) in rye, wheat and (octaploid) triticale (data from Munck 1972).

Cereal	No. of varieties	Alkyl resorcinols ("units")
Rye	15	161 (129–192)
Wheat	18	69 (56–79)
Triticale (octaploid)	19	97 (78–124)

Munck (1972) also reported on the resorcinol content of seven commercial milling fractions from a Swedish wheat (*see* Table 88 in Chapter 5). There was high positive correlation ($r = 0.98$) between crude fibre and resorcinol content, again indicating the association of resorcinol with certain bran components.

Tests in England, employing weanling rats have confirmed the presence in wheat bran oil of an unidentified substance which depressed both appetite and growth rate (Flour Milling and Baking Research Association 1971). Most, though not all, of the reduction in growth rate was due to reduced food intake. The smallness of the residual inhibitory effect indicates either that the amount of material in bran, or its potency, is small.

Wieringa (1967-68) has further reported that, in the Netherlands, more of the growth-inhibiting substance is derived from the by-products of wheat milling than from rye because a much larger quantity of by-products incorporated into animal feeds is derived from wheat than rye.

The occurrence of phenolic compounds in the young leaves of one hexaploid and three octaploid triticale lines and of their wheat and rye parents was studied by Dedio et al. (1969). Methanol-HCl extracts, analyzed by two-dimensional thin-layer chromatography, showed a compound present in large amounts in rye to be transmitted to the triticale.

Villegas (1972) gives the resorcinol content of 13 strains of triticale as ranging from 0.070 to 0.092% of sample.

Although the growth-inhibiting effect of 5-alkyl resorcinols in some animals has been demonstrated, their significance in human diets has not yet been determined. Munck (1969) found that 30% of the 5-alkyl resorcinols present disappeared during the baking of soft rye bread.

Zillman (1973, unpublished data) evaluated the effect of resorcinol on growth rate of mice by adding, in predetermined amounts, (1) the acetone-extracted resorcinol compound from rye to diets of triticale, rye, and wheat prepared in the form of wafers, and (2) by physically mixing predetermined quantities of bran (which contained the resorcinol fraction) to the flour of the grains.

The results showed that the resorcinol content of grain per se is not detrimental either to growth rate

or feed intake of mice. Contrary to certain reports in the literature, the feeding value of both triticale and rye was superior to wheat and Zillman concludes that in previous studies in which rye and triticale were found to result in poor growth, the effects could most likely be attributed to the presence of ergot.

Phytic Acid

Phytic acid (inositol-hexaphosphoric acid) and its derived salts, the phytates, occur in the seed coats of many cereal grains. In cereals, phytic acid represents 35 to 97% of the total phosphorus (Gontzea and Sutzescu 1968) and is generally regarded as a poor source of phosphorus to monogastric animals. It is not equally distributed, the pericarp and adherent aleurone layer, together with the germ, containing proportionally 10 to 20 times the level in the endosperm. The importance of phytic acid in nutrition depends upon its property of forming insoluble or nearly insoluble compounds with mineral elements, including calcium, iron, magnesium and zinc, the resultant phytates being excreted in the faeces. Consequently dietary calcium, iron, etc. can be rendered unavailable when combined with phytic acid. Diets high in phytic acid and poor in calcium, iron and zinc, produced mineral deficiency symptoms in experimental animals and in children (Gontzea and Sutzescu 1968).

The quantity of phytic acid found in the products of milling will therefore depend to a large extent on the extraction rate. Urie and Hulse (1952) quote data given in Table 66 to illustrate the increase in phytic acid phosphorus with increase in wheat flour extraction rate. They state that the available (soluble) calcium present in 72% extraction flour is of the order of 10 mg calcium per 100 g flour. Because of the increase in phytate phosphorus, barely 1 mg per 100 g available calcium is present in 100% extraction (wholemeal) flour. For all practical purposes, all of the calcium present is rendered unavailable in 100% extraction flour. Consequently in some countries during wartime legal requirements were introduced to demand calcium supplementation of high extraction flours.

Hay (1942) cited by Kent-Jones and Amos (1967) reported that in commercial wheat products, phytic acid content runs parallel to fibre content.

Phytic acid and the phytates may be decomposed by the enzyme phytase to produce inositol and phosphoric acid. Wheat and rye are rich in phytase, which is unequally distributed through the grain in somewhat the same manner as phytic acid. The phytase of rye is more active than that of wheat (Gontzea and Sutzecu 1968). The extent to which the phytic acid content of wheat and rye may be harmful in a diet will depend on the conditions under which the cereal has been processed for consumption.

Kent-Jones and Amos (1967) cite Pringle and Moran (1942) who reported on the effect of yeast quantity and length of fermentation time upon the destruction of phytic acid in bread made from 85% extraction wheat flour. This effect is shown in Table 67. The quantity of yeast had only a small effect but the longer the fermentation, i.e. the longer the period during which the phytase could act on the phytic acid, the greater the reduction in phytic acid. Because of the distribution of phytic acid and phytase, and the effect of baking temperatures, white bread has a lower content than whole wheat bread, and rye bread has a lower content than wheaten bread of equivalent extraction rate. Table 68 indicates the position (Gontzea and Sutzecu 1968).

Gontzea and Sutzecu (1968) also state that cereals prepared by heating in boiling water or

TABLE 66. Increase in phytic acid phosphorus with increase in wheat flour extraction rate (data from Urie and Hulse 1952).

Extraction rate (%)	Phytate phosphorus (*mg/100 g flour*)
72	50
85	130
100	300

TABLE 67. Effect of fermentation time and yeast quantity on destruction of phytic acid during fermentation and baking 85% extraction wheat flour (data from Pringle and Moran 1942).

Length of fermentation (*hours*)	Proportion of yeast (%)	Proportion of phytic acid destroyed (%)
3	1	59
5	1	64
5	$\frac{1}{2}$	61.5
8	1	76

TABLE 68. Phosphorus and phytic phosphorus in wheat and wheat products, and rye and rye products (ranges, where available, in parentheses). (Gontzea and Sutzecu 1968 citing various references which are not specifically identified against the items).

	Total phosphorus (P) (*mg/100 g*)	Phytic phosphorus (*mg/100 g*)	(*% of total P*)
Bread wheat	320–360	170–280	47–86
Rye	342	247	73
Wheat flour 100%	430 (356–567)	307 (239–385)	71
Bread from wheat flour 100%	415 (330–485)	210 (185–260)	51
Rye flour 100%	328 (226–401)	168 (120–276)	64
Rye flour 95%	302 (219–386)	169 (110–236)	56
Bread from rye flour 95%	286 (235–342)	32 (0–68)	11
Wheat flour 70%	218 (169–285)	125 (66–194)	57
Bread from wheat flour 70%	226 (178–276)	46 (10–102)	20
Rye flour 60%	155 (136–175)	62 (45–82)	40
Bread from rye flour 60%	143 (116–156)	0	0
Wheat bran	1210–1609	1170–1439	89–97

milk may be higher in phytic acid than those prepared by baking since the boiling fluid inactivates the phytase before it can decompose the phytic acid. Autoclaving or prolonged boiling will, to some extent, decompose phytic acid even in the absence of phytase, the degree of decomposition being greater under acid conditions.

Ranhotra et al. (1971) determined the phytic acid phosphorus in the ingredients and in the resultant baked bread when progressive increments of wheat protein concentrate (WPC) from a commercial mill replaced equivalent amounts of the flour in the blend. The white flour, from hard red winter wheat, had a proximate analysis of protein (N × 5.7) 11.83%, ether extract 1.28%, fibre 0.33%, ash 0.43% and moisture 11.07%. WPC, of protein 15.35%, ether extract 3.18%, fibre 1.22%, ash 1.97% and moisture 11.52% replaced 15%, 30% and 45% of the flour. Phytic acid was estimated by the method of Anderson (1963). The effect of these substitutions, and of the baking process is shown in Table 69. The results show the increase in phytic acid with increase in

TABLE 69. Phytic acid phosphorus in bread ingredients and bread made from white flour with additions of wheat protein concentrate (WPC) (data from Ranhotra et al. 1971).

	Phytic acid phosphorus, moisture free sample (*mg/100 g*)
Flour + 0% WPC	
Bread ingredients	13.6
Bread	0.0
Flour + 15% WPC	
Bread ingredients	76.8
Bread	34.7
Flour + 30% WPC	
Bread ingredients	130.7
Bread	71.0
Flour + 45% WPC	
Bread ingredients	190.4
Bread	132.2

WPC and the reduction which occurs during baking.

Ranhotra (1972) has further reported that when WPC progressively replaced, up to 100%, hard red winter wheat flour in blends baked into small loaves by a sponge-dough procedure, the percentage of phytic acid hydrolyzed was inversely related to the amount initially present. All the phytic acid phosphorus was hydrolyzed during baking of the 100% wheat flour blend. The amount hydrolyzed increased up to the 30% level of WPC replacement; thereafter there was no increase in hydrolysis and the amount of phytic acid hydrolyzed was also inversely related to the amount present in the unbaked ingredients. The pH of the breads baked from the blends increased progressively (Ranhotra 1973) with WPC replacements from 5.20 (all wheat flour blend) to 5.75 (all WPC bread) above the optimum for phytase activity in wheat of 5.1 to 5.3 (Peers 1953). Ranhotra (1973) suggests that the progressive decrease in hydrolysis of phytic acid may be caused by increased inhibition of phytase activity or by rephosphorylation of partially hydrolyzed phytic acid, or by both.

Reinhold (1971) has reported on the differences in phytic acid present in Iranian bread made (*a*) in the village and (*b*) in the city. Village bread (tanok or lavosh) is made without fermentation. In the city bakeries bazari and sangak bread are made by a 2- to 4-hour fermentation process followed, for bazari, by baking on the roof of a brick oven or, for sangak, on pebbles, for about 2 min. The wholemeal flours used in tanok and sangak contain nearly all the original phytic acid of the wheat, the meal used for bazari contains rather less. Samples of the three breads were obtained over several months and examined for phytic acid concentration by precipitation as ferric phytate in 0.6% HCl. The mean contents of phytate in milligrams per 100 g dry bread were: bazari bread, 36 samples, 301 (SD 96.9); sangak bread, 30 samples, 401 (SD 116.5) and tanok bread, 96 samples, 630 (SD 140.0).

In both the villages and the city, flat bread provides at least half the caloric intake. It is suggested that the higher phytate intake of the village population, who eat tanok bread, may account for the evidence of zinc deficiency in the village population, in spite of seemingly adequate zinc intake, and also for the prevalence of rickets among village children, and the high incidence of iron deficiency anaemia.

Enzyme Inhibitors

Trypsin is one of the proteolytic enzyme systems present in the internal secretions and essential to the efficient digestion of ingested protein. The literature on protease inhibitors was reviewed by Liener and Kakade (1969). Trypsin inhibitors have been identified in wheat and rye but their importance in practical nutritional terms remains obscure.

Laporte and Trémolières (1962) reported an anti-trypsin factor in uncooked wheat and rye flour. In cooked flour (tubes plunged into boiling water for 5 min, giving an effective temperature of 98°C for 4 min) no trypsin inhibition was found in wheat flour but rye flour retained 80% of the inhibitory activity. Learmonth and Wood (1963) also reported that cooking reduced the inhibitory activity of wheat.

Milner and Carpenter (1969) determined a trypsin inhibitor in an American hard winter wheat of approximately 17% protein (N × 6.25), dry weight basis, by two methods, (1) using haemoglobin as substrate, and (2) using benzoyl-arginine *p*-nitroanilide. Measurable trypsin inhibition was recorded in extracts from the control, untreated, wheat by both analytical methods but extracts from a sample of wheat steeped, as in the preparation of bulgur, for 70 min in water at 80°C and then dried at a temperature below 40°C showed no inhibitory activity.

Polanowski (1967) identified a trypsin inhibitor in the aqueous extract of rye. It was located only in the endosperm and not in the germ or seed coats which included the aleurone layer. Heating the extract at 75°C resulted in loss of inhibitory activity, the inhibitor being completely inactivated after heating for 25 min.

Cho C. Tsen (1973, personal communication) writes: "A trypsin inhibitor and a chymotrypsin inhibitor are extracted from triticale, wheat, and rye flours by means of a sodium acetate buffer at pH 3.8. The inhibitor content of triticale is intermediate between wheat and rye, with the rye exhibiting the highest trypsin inhibitor content and the wheat the highest chymotrypsin inhibitor content. The molecular weight of the trypsin inhibitor as determined by molecular exclusion chromatography is 12–14,000. The trypsin inhibitor is thermally stable at 100°C for one hour. The molecular weight of the chymotrypsin inhibitor determined as above is 17–19,000. Thermal inactivation occurs after 10 minutes at 70°C.

Polyacrylamide gel disc electrophoresis of both inhibitor fractions indicates the presence of several proteins."

Growth-Depressing Factors

Experiments in which rye was used to replace other cereals at high levels in chick diets have shown that it has a low nutritional value for chicks of both sexes (MacAuliffe and McGinnis 1971). Rye was treated (1) by adding an equal weight of water to the ground grain, spreading it on trays and oven-drying at 70°C, and (2) by adding an equal weight of water, autoclaving for 15 min at 121°C and then oven-drying at 70°C. Water-treatment of the rye gave rise to a significant improvement in chick growth and feed efficiency. Alternatively the harmful effect of high levels of rye were largely overcome by addition of the antibiotics terramycin or procaine penicillin G but not by zinc bacitracin. MacAuliffe and McGinnis suggest that the low value of rye is related to a component, possibly the high pentose content, which stimulates the growth of adverse microflora in the intestinal tract of the chick.

Acetone extraction of rye, which would be expected to remove any resorcinols present, did not remove the growth-depressing effect of rye in chick diets in experiments described by McGinnis (1972). Furthermore, the acetone extract of rye did not depress chick growth when added to a diet containing wheat.

Water extraction of rye significantly improved its feeding value for chicks to a level almost equal to, and not statistically significantly different from, the improvement obtained by adding penicillin. The addition of penicillin to the water-extracted rye gave a further improvement. However, adding the water extract of rye to a control diet based on maize did not depress chick growth.

Mild acid treatment, followed by autoclaving, improved the nutritional value of rye in chick diets, a further improvement being observed when penicillin was added. Penicillin also improved the untreated rye diet. The difference between the acid-treated rye and the untreated rye diets, when penicillin was added, was not statistically significant. Alkali treatment of rye, followed by autoclaving, did not effect improvement (McGinnis 1972).

Three commercial enzyme products, Rhozyme H39, Rhozyme CL and Lipase B, improved the utilization of rye by chicks comparably to the improvement with penicillin, but the supplements, in combination, were not more effective than penicillin alone. Inactivation of the enzyme products by autoclaving destroyed the property of chick growth improvement (McGinnis 1972).

Friend (1970) reported on experiments with male Wistar rats, averaging 167 g initial body weight, fed ad libitum a basal diet in which 42% of the total diet was provided by (A) ground barley, (B) ground rye, (C) barley flour plus rye bran, (D) rye flour plus barley bran. Rats given diet (C) containing barley flour plus rye had the highest feed intake, gained most weight and retained more nitrogen than did rats fed the other diets. The least successful diet was (B) ground rye. Weight gains on diet (D) rye flour plus barley bran were better than the ground rye diet but less than on the other two diets. The rye was ergot-free and it would seem that it is rye flour, rather than rye bran, which is associated with the lower feed consumption associated with diets containing rye grain.

Attia and Creek (1965) used male chicks in experiments to establish the nature of a growth depressant factor in raw wheat germ. Raw and autoclaved wheat germ were compared and the tests indicated the presence of a haemagglutinin factor in wheat germ which could be destroyed by autoclaving. The work was carried out partly to meet the criticisms of Parrish and Bolt (1963) who did not accept the presence of an anti-proteolytic factor in raw wheat germ reported by Creek and Vasaitis (1962), and considered that the reduced weight gain then found was caused by a lower feed consumption due to a paste forming on the beaks of the birds fed the raw wheat germ.

Cave et al. (1965) found that raw wheat germ meal when fed as the only source of protein to chicks, produced poor growth and net protein utilization.

Olsen (1967) referring to the work described above on chicks, used male weanling rats to try and establish whether or not an antinutritive factor was present in raw wheat germ. The criteria used were (*a*) amino acid composition, (*b*) digestibility of the protein, (*c*) absorption of nitrogen and amino acids, and (*d*) effect of supplementation with amino acids on the utilization of the protein. The samples of wheat germ were (i) raw, (ii) toasted for 45 min at a product temperature of 121°C, (iii) autoclaved, 15 lb. pressure (121°C) for 20, 45 or 90 min. Olsen found no evidence of a nutritional inhibitor for the rat in raw wheat germ

and recommended that any heat treatment applied to wheat germ should be as mild as possible since excessive heating impaired the biological value of the protein.

Toxic Substances

Ergot

Ergot, which results from the infection of grain or grasses by the fungus, *Claviceps purpurea*, has been recognized for a considerable time as a source of damage to crops and of toxicity to livestock fed infected grain (Seaman 1971). The fungus prevents seeds of the host plant from developing and replaces many kernels with hard, seed-like fungus bodies known as sclerotia.

The size and shape of the sclerotia vary with the host plant. The sclerotia in rye are often horn-shaped and several times larger than the kernels. Grains in which some sterility may occur, such as triticale, and cross-pollinated grains such as rye, are more likely to be infected because their florets are open longer than those of fertile, self-pollinated hosts. Among cultivated crops, rye and triticale are more often infected than wheat, and among the wheats *T. durum* is more often affected than *T. aestivum*.

Treatment of the seed with fungicide does not appear to control ergot. Zillinsky and Borlaug (1971b) state, however, that Larter has reported the wheat variety Kenya Farmer as being resistant to ergot. Crosses between Kenya Farmer and triticale varieties have been made at CIANO in 1970 in an attempt to transfer resistance to ergot from Kenya Farmer to triticales. Crosses were also made between Kenya Farmer and several varieties of rye to produce new octaploid triticales.

E. N. Larter (1973, personal communication) informed the authors that similar attempts are being made at the University of Manitoba to build ergot resistance into triticale by crossing with Kenya Farmer and other resistant wheat varieties. The results will not be available until late in 1974.

The problem of ergot infection of triticale is illustrated in the findings of Busch and Wilkins (1972) for five triticales and two wheats grown at Fargo and at Carrington, North Dakota in 1971. The figures are given in Table 70. Ergot infection was, in general, higher in triticale than in wheat, and, again in general, higher at Fargo than at Carrington.

TABLE 70. Ergot infection in wheat and triticale grown in two locations in North Dakota in 1971 (data from Busch and Wilkins 1972).

	Per cent ergot (weight)	
Cereals	Fargo 1971	Carrington 1971
Wheats		
Era	0.01	0.01
Waldron	0.25	0.13
Triticales		
Rosner	0.53	0.06
6TA203 (FasGro 203)	0.39	0.18
6TA204 (FasGro 204)	0.92	0.25
6TA209	0.86	0.47
Trailblazer (a reselection of 6TA209)	0.62	0.22

Wide variations in the tolerance of livestock to the ergot toxin have been recorded. In Canada, limits are imposed on ergot in grain and Seaman (1971) states that feed containing 0.1% ergot should be regarded as dangerous.

Bragg et al. (1970) however, investigating the effect of triticale ergot on the performance and survival of broiler chicks found they could tolerate up to 0.8% ergot in the diet. They recommended, however, that commercial feed should not contain more than 0.4%.

Two feeding trials were carried out simultaneously with 330 commercial broiler chicks distributed at random into 33 experimental pens of 10 birds each at one day of age. In the first trial eight levels of triticale ergot (0, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 and 12.8%) were supplied in a basal ground wheat ration, at the expense of wheat. Dietary treatments were arranged in a randomized block design. In the second trial, three dietary treatments were prepared with a ground triticale basal ration (the triticale at the same weight for weight level as in the wheat ration). Ergot was supplied at two levels (0 and 1.6%) at the expense of the triticale. Average weight gain, feed: gain ratio (F/G) and percent mortality were calculated at the end of 28 days and 56 days of feeding the experimental diets.

In the first trial results at 28 days of age showed no significant difference in weight gain between chicks fed 0.4%, 0.8% ergot or the control diet. Maximum weight gain was obtained with 0.2% ergot which was significantly higher than 0.8%

dietary ergot. A very severe depression in weight gain was shown in chicks fed 1.6% dietary ergot and the depression continued as the dietary level increased until growth practically ceased at 12.8% dietary ergot. The F/G ratio was not adversely affected at ergot levels of 0.2, 0.4, and 0.8%; there was a small but consistent increase with increasing levels, the result of a voluntary restriction of feed intake. Dietary levels of 1.6% and above showed very poor F/G ratios.

Chicks fed ergot diets from 29 to 56 days of age showed the same growth pattern observed during the first 28 days. There was no significant growth depression until the 1.6% level of dietary ergot was reached. The F/G ratio was not severely affected even at the 1.6% level fed to 56 days of age. Ergot levels of 3.2% and above however led to signs of toxicity and by 48 days of age all the chicks in the 3.2% ergot group had died and by 56 days the mortality in the 1.6% group was 23%.

In the second trial weight gain and F/G ratio were equal for chicks fed ergot-free wheat and triticale diets. The addition of 1.6% ergot to either wheat or triticale diets depressed growth. Pelleting the triticale feed increased the growth depression significantly compared to the wheat, triticale, or 1.6% ergot and triticale diets in mash form.

In experiments conducted by Ingalls and Phillips (1971) 50 early-weaned dairy calves were fed five experimental diets at 5 to 7 weeks of age. Ergot taken from triticale was added to a complete barley diet at rates of 0, 0.07, 0.14, and 0.28% weight for weight. In the fifth diet triticale containing 0.07 to 0.1% ergot was used in place of barley. Diets were fed ad libitum over 14 weeks; rumen samples were taken and ration digestibility was determined after 4 and 10 weeks. Diet had no significant effect on total volatile fatty acids (VFA), molar percent of VFA, or rumen ammonia level. Dry matter and energy digestibilities were lower for the 0.07% ergot diet compared with 0% ergot, triticale, and 0.28% ergot diets. The effect on dry matter intake, weight gain and feed efficiency is shown in Table 71. Dry matter intake of the zero ergot diet was significantly greater than the triticale diet, which was greater than the 0.28% ergot diet. The zero ergot diet produced significantly greater weight gains than 0.07% ergot or triticale diets, which produced greater gains than the 0.28% ergot diet. Feed efficiencies were not significantly different among diets.

TABLE 71. Effect on dry matter intake, weight gain and feed efficiency of dairy calves fed ergot-containing diets (data from Ingalls and Phillips 1971).

	Dry matter intake (*kg*)	Weight gain (*kg/day*)	Feed efficiency
Barley	3.39	0.97	3.50
Barley + 0.07% ergot	3.10	0.84	3.71
Triticale + 0.07 – 0.1% ergot	2.89	0.85	3.42
Barley + 0.14% ergot	3.20	0.88	3.68
Barley + 0.28% ergot	2.61	0.72	3.20

McCloy et al. (1971) cite E. W. Stringham (1968) and H. H. Nicholson (in a personal communication) who observed reduced performance in cattle fed triticale, compared to barley and maize, but who also noted relatively high, but unstated, levels of ergot in the triticale used.

Harrold et al. (1971) comparing triticale from two different crop years as a feed for growing-finishing swine used grain which contained ergot for part of the trials but the level of contamination is not stated. The results from cleaned 1968 crop were however inferior to those from uncleaned 1969 crop.

Stothers and Shebeski (1965) encountered palatability problems in feeding triticale of 1962 and 1964 crop years to growing swine. Although the paper states that "ergot has been essentially absent from the triticale used in these tests", the low palatability is now suspected to be at least in part attributable to ergot in the grain (L. H. Shebeski 1972, personal communication).

Triticale, of unstated origin, containing 0.0015% ergot, was fed to lambs in trials of finishing rations. Little is known of the effect of ergot on lambs but the level present did not appear to affect feed intake. Shelled maize was superior to triticale, to triticale with maize, and to barley, as judged by weight gain and feed efficiency (Jordan and Hanke 1972).

Aflatoxin

Toxic substances, known as mycotoxins, are produced by mould fungi which grow upon many grains, grain legumes and oilseeds. The incidence and control of mycotoxins has been recently reviewed by Scott (1973). Of the mycotoxins, the

aflatoxins, formed by *Aspergillus flavus*, are probably the most important as a hazard to human health and are certainly those which have been the most extensively studied.

Kao and Robinson (1972) found marked changes in amino acid composition when hard red spring wheat (var. Selkirk) and hard red winter wheat (var. Triumph) were inoculated with *Aspergillus flavus* spores. The amino acids were analyzed by ion exchange chromatography and aflatoxin by thin-layer chromatography. In the sound wheat, the ratio of amino acid to Kjeldahl protein was about 100%. In mouldy Selkirk the ratio was 78.86% and in mouldy Triumph, 90.63%. Some amino acids appeared to have been metabolized to form other nitrogenous compounds still detectable as Kjeldahl nitrogen. In mouldy Selkirk wheat, cysteine decreased by about 74%, arginine, histidine and glutamic acid also decreased. Lysine decreased, but not to such an extent as in mouldy Triumph wheat. Methionine was the only amino acid to increase, its increase probably being derived from cysteine. In mouldy Triumph wheat, arginine, histidine and lysine decreased, while methionine increased 35%. The amino acid composition of the mould-free wheats were essentially similar.

Aflatoxin was detected only in the hard red spring wheat, Selkirk, and practically all was distributed in the bran.

The incidence of aflatoxin infection and its level in cereal grains appears to be relatively low, compared, for example, with certain oilseeds.

Lafont and Lafont (1970) examined, chromatographically, contamination with the aflatoxin B_1 of samples of mixed animal feed from two factories in France, and the raw materials of which they were composed, taken direct from the storage silos.

Detectable levels (above 0.25 μg/kg) of aflatoxin B_1 were found in 15 of the 32 lots of wheat (47%), 7 of the 25 lots of wheat flour (28%), and 4 of the 21 lots of rye (19%). No sample of rye but two lots of wheat (6%) and two lots of wheat flour (8%), were heavily contaminated (levels above 100 μg/kg). In comparison, 24 of 51 lots of soya cake (47%) contained detectable levels, 7 lots (14%) of which were heavily contaminated.

Tolerance Levels

The reader will note the marked discrepancy among different recommended maximum limits for ergot infection. The problem of specifying limits of tolerance appears with virtually all potentially injurious substances which occur in or contaminate foods. What constitutes a "safe" tolerance limit is influenced by the total quantity of the food consumed, and the creature for which it is intended. Weanlings and young infants are often more sensitive than healthy adults. One can only urge, in the case of ergot and mycotoxins, the development of varieties and the implementation of agronomic, storage, handling, and inspection practices which minimize infection. Where there is doubt the advice of competent public or animal health authorities should be sought. In any event where tolerance limits are recommended or prescribed the detailed method of sampling, inspection and analysis proposed should also be prescribed.

Processing Additives

It is recognized that a wide range of additives may find their way into processed cereal foods. Such additives include flour bleaching and improving agents, dough and alimentary paste conditioners, amylose complexing agents, and others. Some of these additives affect the biological value of the protein but no attempt has been made in this publication to review the literature relating to this subject.

In most countries of the developed world there are regulations governing the additives which may be included in food intended for human consumption, e.g. Food and Agriculture Organization (1959, 1960, 1961a, b, c, 1963a, b, 1969b). The World Health Organization (1957, 1958, 1961, 1962, 1964, 1965b, 1966, 1967a, b, 1970, 1972) has issued monographs on the technology, evaluation and toxicology of several food additives.

The principle of the quern, the earliest form of grain mill, is still followed in many parts of the developing world

Chapter 5

Processing and Supplementation with Other Protein Sources

Introduction

Chapter 3 dealt with how the protein content and composition of triticale, wheat and rye, are influenced by genetic, varietal, agronomic and environmental factors. The biological value of cereal grain proteins can be markedly affected by what happens to them after they are harvested and in particular by how they are processed. Processing comprehends a wide variety of treatments including grinding (milling), mixing with other materials to form doughs, pastes and slurries, drying, cooking and baking. It might be argued that processing should take account of threshing, drying, storage and chemical treatment of the whole grain. While recognizing the importance of these latter components of the total grain production and utilization system, their impact upon the protein value is of little consequence except, in a negative sense, if they are carried out inefficiently and the grain in consequence suffers significant infection and/or infestation. The deleterious effects of infection with ergot and mould-fungi are discussed in Chapter 4. Insect infestation can result not only in a gross loss of grain but also in a selective nutritional loss in that many insects preferentially attack the protein-rich moieties of the grain.

The basic structure of the wheat grain, which is broadly similar to that of triticale and rye, is illustrated in Fig. 2. The diagram shows that the cereal seed consists at one end of the germ, which is the embryonic new plant representing 2 to 3% by weight of the total grain, and that the remainder consists largely of the endosperm which is the initial food store for the new plant and forms between 80 and 85% of the grain. The germ and endosperm are surrounded by the seed and fruit coats which, when removed, are collectively called the "bran" or "husk". As Fig. 2 illustrates, the three other layers, collectively called the pericarp, are the epidermis, the epicarp and the endocarp, and they consist largely of cellulose. The episperm or testa contains the pigments which give the seed its distinctive colour. The aleurone layer is important because of its high protein and high mineral content.

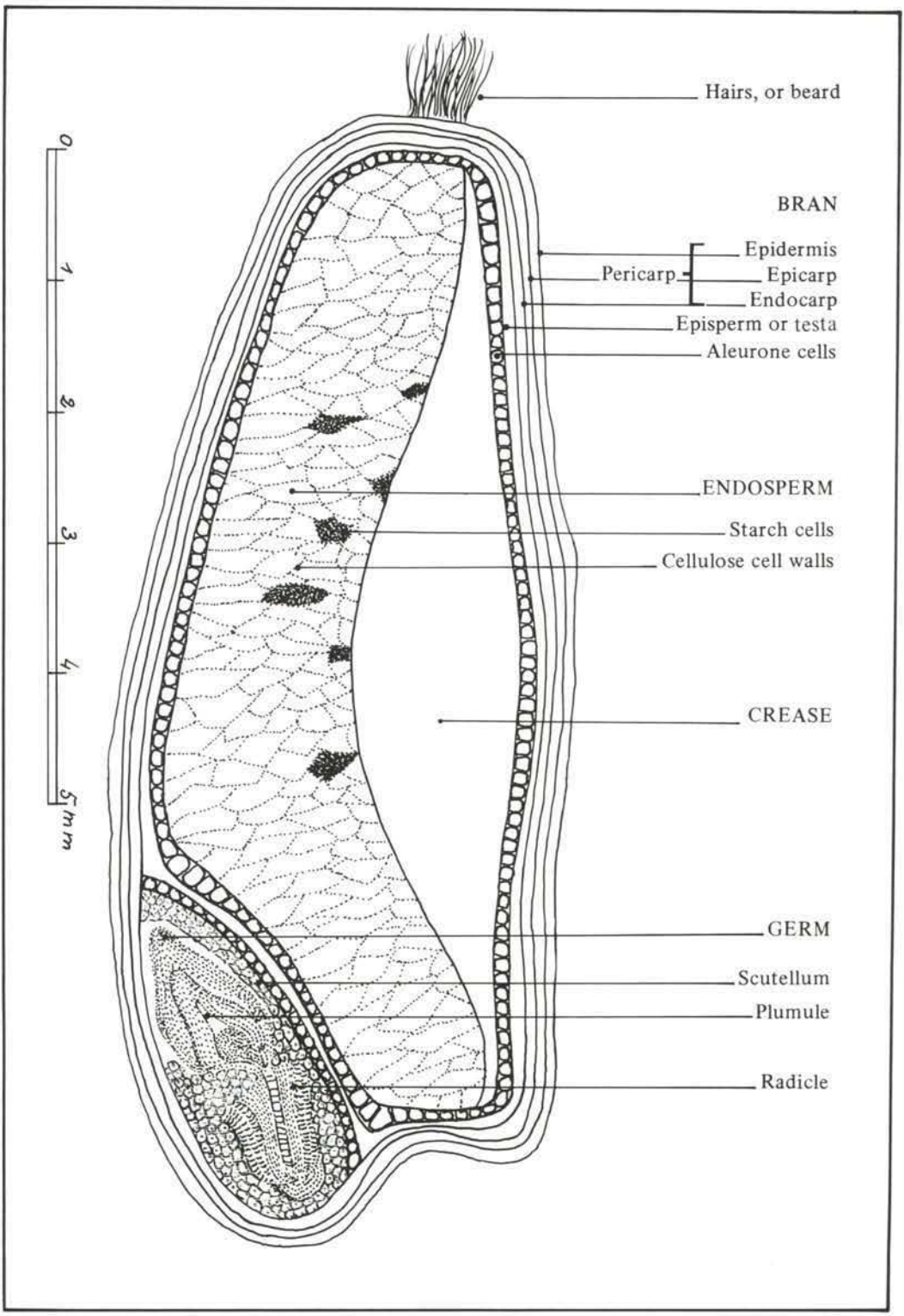
By manual dissection and subsequent analysis, Hinton (1953) has demonstrated the typically wide variation in protein content which occurs among the various fractions of the cereal grain seeds. Hinton's results, which are based upon a variety of English wheat, are given in Table 72. From Hinton's results it can be seen that the highest percentage protein levels are to be found in the germ, the scutellum which surrounds the germ, and the aleurone layer, and that the protein proportion declines from the aleurone towards the centre of the endosperm and from the aleurone towards the outer seed coats.

Hinton's general findings are supported by several other investigators. Grewe and LeClerc (1943) analyzed 19 samples of wheat germ from the USA and demonstrated an average protein content of 28.9%. Fraser and Holmes (1959) analyzed the principal fractions of British wheats and found the following average protein contents: endosperm 9.6%, germ 28.5%, bran 14.4%. Kent-Jones and Amos (1967), from a wide range of samples, quote a spread of wheat germ protein from 22 to 32%.

Milling

The influence of milling on the overall nutritive value of wheat was reviewed by Moran (1959) and earlier by Dawbarn (1949). Kasarda et al. (1971) reviewed the composition of the proteins and amino acids of wheat fractions, bringing up to date an earlier related study by Pence et al. (1964). Lockwood (1960) reported that the protein content of wheat in typical British bread wheat blends varied between 7.5% and 18%.

It is readily evident that the protein content and composition of any milled product will be influenced both by the protein present in the



Drawing: Sabine Clerget-Vaucouleurs

Fig. 2. Cross-section of a typical grain of wheat.

TABLE 72. Protein contributed by wheat fractions (data calculated from Hinton 1953).

Fraction	Fraction/ 100 g whole grain (g)	Protein/ 100 g fraction (g)	Protein/ 100 g grain (g)	Protein/ 100 g protein in whole grain (g)
Pericarp } Testa }	8.0	4.4	0.3	3.5
Aleurone	7.0	19.7	1.4	15.9
Germ	1.0	33.3	0.3	3.5
Scutellum	1.5	26.7	0.4	4.5
Outer endosperm	12.5	13.7	1.7	19.3
Middle endosperm	12.5	8.8	1.1	12.5
Inner endosperm	57.5	6.2	3.5	39.9
Whole grain	100.0	8.8	8.8	100.0

original cereal grain and by the proportion of the various fractions of the grain which the milled product contains.

The milling of cereals into flour is probably man's most ancient food processing industry. Primitive man pounded the cereal seeds between rocks until, about 4000 B.C. the more efficient hand quern appeared. Within the ancient quern are to be seen the first traces of modern milling techniques in that rotary stones, carved with radial grooves, revolve one upon the other thus breaking open the grain and freeing the white starchy endosperm which is then ground and sieved to produce the fine white powder known as "flour" which was from earliest times described as the "flower" (the finest portion) of the grain.

ROLLER MILLING

Lockwood (1960) and Ziegler and Greer (1971) have presented comprehensive reviews of modern roller-milling technologies. Briefly, a roller-mill consists of two series of paired rolls, (*a*) the break rolls which are corrugated and counter-rotate at different angular velocities thereby shearing and breaking the cereal grains and permitting the endosperm to spill out in large chunks, (*b*) the reduction rolls, which are smooth, or very finely corrugated, and crush or reduce the endosperm to fine particles. Interspersed with the flow of broken and crushed wheat particles between the break and reduction rolls one finds a series of sieves, gravity tables and aspirators which separate particles of different size, density and effective mass. The comparative toughness of the bran enables it to withstand the application of crushing and shearing stresses encountered in the roller-mill which consequently cause detachment and partial disintegration of the endosperm particles initially attached to the bran.

The purpose of most roller-milling operations is to separate the endosperm from the bran and germ and to convert it to a fine white flour or, in the case of durum wheats intended for alimentary pastes, into clean, uniform, yellow particles called semolina. The percent proportion by weight of flour obtained from a given quantity of wheat or other cereal grain is known as the extraction rate. Thus a 50% extraction rate would contain 50% by weight of the grain milled and would consist of almost pure endosperm. A 100% extraction rate would consist of the whole ground grain.

EXTRACTION RATE

Ziegler and Greer (1971) present data which illustrates the relation between extraction rate and protein content. Table 73, derived from Ziegler and Greer, indicates the contrast in protein content between low (76% and below) and high (above 80%) extraction rates.

In producing low-extraction flours, the protein-rich aleurone layer is virtually eliminated with the bran. The 85% extraction flour would contain most of the aleurone layer and therefore would retain between 96 and 100% of the protein originally present. The question of what constitutes an optimum percentage extraction rate needs to be carefully considered, particularly in the less developed countries, several of which convert imported wheat to low-extraction rate flours for local consumption and export the separated bran

TABLE 73. Compositions[a] of flours of various extraction rates in series milled from given wheats (data from Ziegler and Greer (1971).

		Extraction rate of flour (%)						
		42–46	65	70	75	80	85	100
Protein (N × 5.7), % (at 14% moisture)	A	11.9	–	12.9	13.2	13.4	13.7	13.8
	B	7.73	–	8.01	8.08	8.31	8.65	9.0
	C	–	10.7	10.9	–	11.6	12.0	12.0
	D	–	–	11.3	–	11.9	12.4	12.3
Protein (N × 5.7), as % of protein in wheat	A	86.6	–	93.6	95.7	97.1	99.5	100.0
	B	85.9	–	89.0	89.8	92.4	96.1	100.0
	C	–	89.2	90.8	–	96.6	100.0	100.0
	D	–	–	92.0	–	96.8	100.0	100.0

[a]Flour A: Manitoba wheat: laboratory milled
Flour B: English wheat: laboratory milled
Flour C: Manitoba 65%, English 35%: laboratory milled
Flour D: Mixed wheats: commercially milled.

and germ to developed countries for use in animal feeds.

AIR CLASSIFICATION

The endosperm of wheat consists of starch granules of various sizes embedded in a matrix of protein. There is a limit to the degree of fineness in wheat flour which can be produced by break and reduction milling. If the reduction rolls are set too close together the particles are simply compressed into flat discs. By employing impact-milling, as exemplified by the pin disc-mill, the endosperm particles can be reduced to flours of extremely fine particle size. During such fine grinding much of the endosperm protein is liberated as free protein in the form of small wedge shaped slivers. Since these fine protein fragments possess the smallest average effective mass, they can be separated from the starch-rich fraction by air classification in which a centrifugal force is counteracted by a centripetal air drag. The relative magnitude of these opposing forces determines the critical particle size or effective mass at which the flour will be fractionated (Jones and Halton 1958; Jones et al. 1959). The finest particles of lowest effective mass will be richest in protein.

Because of the physical nature of their endosperm, soft wheats respond in greater degree to fine grinding and air classification than hard wheats. Starting with Ontario soft wheat flours of roughly 9% average protein content, fractions containing 18 to 20% protein can be obtained by fine grinding and a single air classification. In the authors' experience the most extreme result was obtained from a sample of Gaines wheat in which the original 75% extraction flour contained 8.5% protein. After pin-mill grinding and fractionation in an Alpine air classifier, the fraction below an average diameter of 17 microns was found to contain an average of 24% protein (Hulse, 1966 unpublished data).

ABRASIVE DECORTICATION

An alternative principle of milling, which deserves more attention than it has received, consists of abrasive decortication in which the seed coats (bran) are removed by passing the grain between moving abrasive surfaces. Such a process has been described by deMan et al. (1973).

The theoretical advantages of such a system are that the proportion and thickness of bran coats removed can be varied according to the intensity and duration of the abrasive forces applied. Ideally one could remove the high cellulose epicarp and testa without removing the high protein aleurone layer thus leaving perhaps more than 95% of the original grain seed protein intact. Furthermore, certain of the antimetabolites and microorganisms present in and on the outer seed coats would also be removed. Preliminary evidence from an IDRC project in West Africa suggests that grain processed by abrasive decortication as described by deMan et al. (1973), and subsequently

converted to flour, is more stable in storage, probably because most of the mould-fungi present on the seed coats are removed with the bran. During break milling or roller milling the micro-organisms on the outer seed coats tend to be homogenized with the ground whole grain particles. It would be worthy of experiment to discover if both ergot, where present, and some of the antimetabolites present in the outer seed coats described in Chapter 4, might be removed by abrasive decortication with a consequent improvement in the quality of the grain.

RYE MILLING

The influence of milling upon the protein value of rye grains appears to have received scant attention though some of the information is to be found in Kent (1966) and Kent-Jones and Amos (1967). In Canada rye is normally milled by a break and reduction process to produce two main products, dark rye flour which consists essentially of the whole rye grain, and light rye flour of approximately 70% extraction. One set of commercial data indicates that the protein content of light rye flour is roughly 25% lower than that of dark rye flour milled from the same grain.

Secondary Processing

The secondary processing of cereal grains consists in the main of cooking or baking the milled products with water and other ingredients. The simplest processing consists of cooking in steam or water to produce a gruel, porridge or granular material of which couscous is typical (Ferhi 1970). In the making of alimentary pastes such as macaroni, noodles and spaghetti, the cereal flour is combined with water and sometimes other ingredients, before being dried or sheeted and cut into thin strips or shapes and dried. Alimentary paste production is discussed by Hummel (1966), Irvine (1971) and Winston (1971).

BAKING

Baking is perhaps the oldest and most widely practised method of processing cereal grain flours. The principles and practices of baking bread, cakes and biscuits are the subject of many publications, e.g. Pyler (1952), Calvel (1962), Fance (1968), Fance and Wragg (1968), Kiger and Kiger (1968), Matz (1968, 1972), Sultan (1969), Pomeranz and Shellenberger (1971), Smith (1972), Bennion and Bamford (1973).

The four basic and essential ingredients of leavened bread are flour, salt, yeast and water. Leavened bread comes in a remarkable range of compositions, weights, shapes and sizes.

In the Near East, thin discs of fermented dough are baked for a few minutes at temperatures close to 400°C which causes the dough to rise rapidly almost to the conformation of a hollow sphere (Dalby 1969).

On the Indian sub-continent chapatis are made from unfermented, unleavened discs of dough baked upon a hot plate (Chowdhary 1954, Dharam Jit Singh 1956).

Supplementation with Other Protein Sources

In making bread, wheat or other cereal flour can be supplemented with a very wide range of protein sources and other nutrients. Hulse (in the press) has reviewed the technological and nutritional implications of supplementing bread and other baked cereals with various protein sources.

At the end of this Chapter, two aspects of protein supplementation are considered: (*a*) that which improves the amino acid balance and (*b*) that which increases the total proportion of protein nitrogen. Since, in much of the work reported, the two aspects are virtually inseparable, the section has been organized according to the sources of protein supplementation rather than according to the primary purpose of supplementation. The somewhat lengthy review of supplementation is, the authors believe, relevant particularly to the needs of developing countries. In several of these countries, where the demand for bread is steadily increasing (the annual increase exceeds 8% in Africa) one encounters a growing interest in bread made from composite flours in which wheat flour is supplemented with other cereal flours, including maize, sorghum and millet, with root starches, such as cassava, plus legume proteins (Food and Agriculture Organization 1970c; Hulse (in press)). This is a subject that will doubtless receive increased attention as more countries seek comparative self-sufficiency in cereal grains. The performance and acceptability of composite flours containing triticale is being studied at the University of Manitoba.

This Chapter is concerned primarily with how the biological value of the protein of triticale, wheat and rye may be influenced by primary and secondary processing and by supplementation. Not surprisingly in view of its long history as a

TABLE 74. Generic identification key of wheat, rye and triticale varieties (Unrau and Jenkins 1964).

Ploidy	Designation	Botanical Description
Tetraploid	4B113	*Triticum turgidum* var. *ramoso megalopolitanum*
Tetraploid	4B121	*T. turgidum* var. *plinianum*
Tetraploid	4B249	*T. polonicum* var. *levissimum*
Tetraploid	4B276	*T. persicum* var. *stramineum*
Tetraploid	Ramsey (durum wheat)	*T. durum* var. *hordeiforme*
Tetraploid	Stewart (durum wheat)	*T. durum* var. *hordeiforme*
Tetraploid	Fourex (rye)	*Secale cereale* (induced tetraploid)
Hexaploid	Pembina (bread wheat)	*T. aestivum* var. *lutescens*
Hexaploid	Selkirk (bread wheat)	*T. aestivum* var. *lutescens*
Hexaploid	6A49 (bread wheat)	*T. aestivum* (synthesized from *T. turgidum* var. *nigro-baratum* × *Ae. squarrosa*)
Hexaploid	6A20.1 (Triticale)	*T. durum* "Carleton"—*S. cereale*
Hexaploid	6A66.1 (Triticale)	*T. dicoccoides*—*S. cereale*
Hexaploid	6A67.1 (Triticale)	*T. persicum*—*S. cereale*
Octaploid	8A112 (Triticale)	*T. aestivum*—*S. cereale* (awnless)
Octaploid	8A125 (Triticale)	*T. aestivum*—*S. cereale*

staple of man's diet, a formidable body of literature can be found which relates to bread and other baked products from wheat flour and to a much lesser extent to bread made from rye flour. At the time of writing, comparatively little has been published concerning bread and other cereal foods from processed triticale. Nevertheless, it is believed that much of what is known concerning the influence of processing and supplementation upon the nutritional value of wheat and rye products may be extrapolated to triticale. It is for this reason that the following rather extensive chapter is included in this publication.

Milling

Triticale

Unrau and Jenkins (1964) investigated several triticale varieties, both hexaploid and octaploid, together with species of wheat and rye. A generic description of the varieties used is given in Table 74.

Grain samples were milled in a Buhler laboratory mill. The hexaploid triticales were passed through the mill stream a second time to obtain a higher extraction. Table 75 records the 1000-kernel weight, the crude protein of the seed, on a dry weight basis, the flour yield, and the flour protein also on a dry weight basis. Protein content was determined by Kjeldahl procedure (Association of Official Agricultural Chemists, 1950).

The high crude protein content of the triticale grain was considered to be associated to some extent with the rye genomes, as was, also, the shrivelled kernel characteristic.

Vaisey and Unrau (1964) in a paper published before Unrau and Jenkins (1964) but describing later work, examined the same varieties primarily for their sugar content. The values obtained for protein were in close agreement with those described by Unrau and Jenkins (1964).

Berry et al. (1971) compared a hard red spring (HRS) flour, durum semolina and hexaploid triticale, all grown in North Dakota and milled on a Buhler mill, and a commercial rye flour. They were primarily concerned with the characterization of the triticale starch but the protein contents were determined using the official methods of the American Association of Cereal Chemists (1962). Expressed on a dry matter basis, the percent protein contents were: HRS flour 16.1; durum semolina 17.5; rye flour 11.0; triticale 12.4.

Lorenz and Maga (1972) investigated the compositions of fatty acids, carbonyls and hydrocarbons in eight samples of grain all grown on irrigated sites at Fort Collins and Center, Colorado in 1971. Two winter wheats (Scout and Caprock), two spring wheats (Inia Res and Ciano Sib.), two spring hexaploid triticales (6TA204 and 6TA206) and two winter hexaploid triticales (TR385 and TR386) were tempered and milled on a Brabender Quadrumat Junior mill. Moisture and ash were

determined by the methods of the Association of Official Agricultural Chemists (1960); protein content by the Udy Protein Analyzer method (American Association of Cereal Chemists 1962). The grain protein, flour protein and flour ash of the cultivars examined are given in Table 76.

Madl and Tsen (1973) determined the proteolytic activity by a modified Ayre-Anderson method (McDonald and Chen 1964; Wang and Grant 1969; Bushuk et al. 1971) of six triticales, three ryes and four wheats milled on Brabender Quadrumat and Miag experimental mills. The grain protein (N × 5.7, dry basis), flour yield, flour protein, flour proteolytic activity, bran protein and bran proteolytic activity of the samples are shown in Table 77. The Mexican triticales, Armadillo and Bronco, showed higher protein contents and flour yields than the Kansas grown varieties. The proteolytic activity of triticale was comparable to that of rye which was significantly higher than that of wheat. The proteolytic activities of the brans were considerably higher than those of the flours. No direct correlation between protein content and proteolytic activity was observed. Moisture, protein contents and proteolytic activity of the milling fractions of a Kansas triticale are shown in Table 78. The proteolytic activity of the fractions increased as the protein content of the fractions increased.

Chen and Bushuk (1970a, b, c) reported on the solubility characteristics and amino acid composition of one line of triticale and of its durum wheat and rye parents. Included in the trials was a cultivar of hard red spring wheat, Manitou. The triticale, 6A190, was originally produced at the University of Manitoba by crossing the durum wheat variety Stewart 63 and the rye variety Prolific. With the hard red spring wheat, Manitou, the four species were grown together in 1966 at

TABLE 75. 1000-kernel weight in grams, crude protein of seed, flour yield, and flour protein of varieties examined (data from Unrau and Jenkins 1964).

Variety	1000-kernel weight (g)	Crude protein of seed (%)	Flour yield (%)	Flour protein (%)
Durum wheat				
Ramsey	43.7	16.5	74.1	15.7
Stewart	51.8	16.0	72.3	14.4
Tetraploid wheat				
4B113	44.3	15.0	63.3	14.2
4B121	44.6	15.0	67.1	14.1
4B249	67.2	18.3	71.8	17.1
4B276	29.3	17.9	63.1	15.5
Bread wheat				
Pembina	29.7	17.2	69.6	16.2
Selkirk	36.9	17.1	71.9	16.3
6A49	44.6	18.0	65.6	17.2
Rye				
Fourex	36.9	19.1	59.7	13.6
Hexaploid triticale				
6A20.1	46.3	19.8	63.7	18.8
6A20.2	44.0	19.5	63.0	17.3
6A66.1	45.6	19.3	62.7	17.1
6A67.1	43.6	17.3	60.4	15.1
6A67.2	42.5	17.1	63.4	15.3
6A67.3	41.8	17.3	61.5	16.2
6A67.4	44.1	17.2	64.9	15.3
6A67.5	43.7	16.9	66.7	15.3
6A67.6	42.2	17.3	66.8	15.5
6A67.7	43.1	16.6	66.1	15.2
6A67.8	42.4	16.8	66.8	15.2
6A67.9	41.8	17.1	68.8	15.5
6A67.10	42.0	17.3	66.6	15.1
6A67.11	43.4	16.3	63.4	15.1
6A67.12	44.5	18.1	64.5	16.9
Octaploid triticale				
8A112	26.1	17.9	64.7	16.6
8A125	38.8	19.2	67.5	18.0

TABLE 76. Characteristics of wheat and triticale cultivars (14% moisture basis) (Lorenz and Maga 1972).

	Grain protein (N × 6.25) (%)	Flour protein (N × 5.7) (%)	Flour ash (%)
Spring triticale			
6TA204	14.6	11.3	0.53
6TA206	14.5	11.7	0.54
Winter triticale			
TR385	12.0	8.7	0.47
TR386	10.8	8.6	0.47
Spring wheat			
Inia Res.	12.7	12.4	0.46
Ciano Sib.	13.2	12.5	0.48
Winter wheat			
Scout	12.0	11.6	0.43
Caprock	11.2	11.0	0.41

TABLE 77. Relative proteolytic activities of flours and brans of wheat, rye, and triticale (data from Madl and Tsen 1973).

	Whole Grain		Flour		Bran	
	Protein ($N \times 5.7$ *dry basis*) (%)	Flour yield (%)	Protein ($N \times 5.7$ *dry basis*) (%)	Activity (*mg protein per ml supernatant*)	Protein ($N \times 5.7$ *dry basis*) (%)	Activity (*mg protein per ml supernatant*)
Wheats						
Eagle (Manhattan, 1972)	12.7	70	11.8	1.74	14.3	4.31
Centurk (Manhattan, 1972)	12.7	73	11.4	1.75	15.2	4.87
Scout-1 (Garden City, 1971)	13.6	72	13.1	1.73	16.7	5.37
Scout-M (Manhattan, 1971)	14.6	72	13.9	1.65	17.7	4.80
Ryes						
Minn. 11 (Columbia, Mo. 1972)	10.0	51	7.1	2.61	13.9	6.48
Balbo (Manhattan, 1972)	12.6	45	8.1	2.07	15.7	5.77
Balbo (Manhattan, 1971)	16.3	48	12.7	2.08	19.5	5.75
Triticales						
Triticale-1 (Manhattan, 1971)	13.7	64	12.6	1.98	18.1	6.60
Triticale-F (Garden City, 1971)	15.1	61	14.1	2.01	18.6	6.15
Triticale-385 (Garden City, 1971)	15.4	62	13.6	2.00	20.0	5.70
Triticale-298 (Manhattan, 1971)	17.5	58	15.5	1.93	21.0	5.96
Armadillo (Mexico, 1971)	16.2	67	13.9	2.68	21.3	6.35
Bronco (Mexico, 1971)	19.9	69	17.6	2.53	23.3	6.45

the University of Manitoba. The samples were milled into flour on a Buhler experimental mill after tempering overnight to 16.5% moisture. The flours were characterized using the standard methods of the American Association of Cereal Chemists (1962), unless otherwise specified. Protein contents (N × 5.7) were obtained using a semi-micro Kjeldahl method and amino acids by autoanalyzer. The 1000-kernel weight and moisture content of the grain of the four varieties with their flour yield, ash, moisture and protein content are given in Table 79. The amino acid compositions of the flours from the four varieties are given in Table 80.

In general Chen and Bushuk (1970a) found the amino acid composition of the triticale to be intermediate between its parent species, durum wheat and rye. They comment that the recovery of amino acids from the rye sample was rather low, for reasons which were not investigated.

Anderson et al. (1972) reported on the effect of milling and air classifying a triticale (FasGro 204). The composition of the triticale grain on a moisture free basis is given in Table 81.

Using a Buhler pneumatic laboratory mill a straight grade flour was obtained from this triticale of about 64% extraction, flour protein 12.1% and ash 0.54%, both on a 14% moisture basis. Bran and shorts were separated from the flour without difficulty. A second straight grade flour, 12.7% protein and 0.57% ash was ground more finely by three passes through an Alpine Kolloplex mill (Model 16 oz). The finely ground flour was air classified in a Pillsbury laboratory model classifier. Yields of the air classified fractions and their analyses are given in Table 82.

TABLE 78. Proteolytic activity in various milling fractions of a Kansas triticale (data from Madl and Tsen 1973).

Mill fraction	Moisture (%)	Protein ($N \times 5.7$ *dry basis*) (%)	Proteolytic activity (*mg protein per ml supernatant*)
Straight grade	12.7	11.2	1.78
1st break	14.5	7.5	1.33
2nd break	14.0	9.1	1.43
3rd break	12.6	12.6	2.07
1st middlings	13.0	12.0	1.55
3rd middlings	11.6	15.5	2.80
4th middlings	10.3	16.5	3.31
5th middlings	9.5	18.0	4.16
Reduction shorts	8.2	14.8	6.10
Red dog	9.0	15.6	6.40
Break shorts	11.0	18.4	6.43
Germ	9.6	21.0	5.20
Whole grain	9.7	13.3	–

Anderson et al. (1972) also quote fractionation results for triticale compared with those found by other authors for rye and wheat (*see* Table 83).

It is interesting to note that whereas the yields of recovered fractions from triticale more closely resemble those from rye than from wheat, the total protein shift is significantly higher in the case of triticale than the other two cereals. Total protein shift is defined by Anderson et al. (1972) as the sum of the protein shifted into the high-protein fractions and out of the low-protein fractions, expressed as percentage of the total protein present in the flour, as described by Gracza (1959).

Anderson et al. (1972) also wet-milled triticale grain by a laboratory procedure (Anderson, R. A., 1963). No particular problems were encountered, the triticale responding in a manner similar to soft wheats. Starch and gluten were separated by the Martin, or dough ball process (Knight 1965) but the softer nature of the gluten presented difficulties in the washing process.

Hexaploid triticale, var. Cachirulo, provided by the (Spanish) Instituto Nacional de Investigaciones Agronomicas, was test milled using a Buhler model M.C.K. and a Miag model Vario C ex 2. The authors (de la Plaza et al. 1969) suggest a milling diagram from which six final products may be obtained, two flours, two protein concentrates and two brans. Four of these fractions, two flours and two protein concentrates obtained from a combination of the Buhler and Miag Vario experimental mills were compared with a triticale flour obtained from a normal milling on the Buhler and a similarly Buhler milled control wheat flour obtained from a blend of 10% Florencia Aurora, 10% Impeto, 30% Aragon 03 and 50% Negrillo (Vallejo et al. 1969). The chemical characteristics are given in Table 84. Nitrogen was determined by Kjeldahl, ash by the method of the American Association of Cereal Chemists (1962). The methods for fat and fibre determination were not identified.

Wheat Flour

Table 3 in Chapter 1 shows average differences and ranges in amino acid composition among whole wheat, germ, bran and flour of 70 to 80% extraction rate. The data in this table cannot be directly related to each other since they are

TABLE 79. Grain, 1000-kernel weight and moisture content; flour yield, ash, moisture and protein of triticale, its parental species and a hard red spring wheat (Chen and Bushuk 1970a).

	Triticale 6A190	Spring rye Prolific	Durum wheat Stewart 63	Hard red spring wheat Manitou
Grain				
100-kernel weight, g	43.4	28.6	44.9	30.6
Moisture content, %	14.0	13.0	13.7	13.5
Flour				
Yield, % (14% moisture basis)	58.9	59.6	67.7	71.7
Ash content, % (14% moisture basis)	0.42	0.68	0.66	0.48
Moisture content, %	14.0	13.0	13.7	14.8
Protein content, % (14% moisture basis)	9.8	9.9	12.1	13.6

TABLE 80. Amino acid compositions of flours from rye, triticale, durum wheat and hard red spring wheat (micromoles amino acid per milligram nitrogen in sample) (Chen and Bushuk 1970a).

Amino acid	Rye (Prolific)	Triticale	Durum (Stewart)	HRS wheat (Manitou)
Aspartic acid	2.41	2.23	1.89	1.47
Threonine	1.29	1.34	1.29	1.20
Serine	1.84	2.17	2.26	2.21
Glutamic acid	9.77	11.7	13.2	15.7
Proline	6.26	6.44	6.56	6.21
Glycine	2.40	2.70	2.54	2.73
Alanine	2.15	2.08	1.99	1.84
Valine	2.10	2.20	2.21	2.11
Cystine	0.52	0.70	0.60	0.59
Methionine[a]	0.68	0.79	0.78	0.65
Isoleucine	1.51	1.74	1.87	1.71
Tryosine	0.56	0.81	0.80	0.78
Phenylalanine	1.47	1.58	1.80	2.10
Ammonia	9.41	10.0	12.5	13.6
Lysine	1.12	1.00	0.85	0.82
Histidine	0.65	0.79	0.83	0.85
Arginine	1.23	1.43	1.13	1.20
Tryptophan		not analyzed		
N recovery, %	76.2	84.5	87.8	91.7

[a]Including methionine oxide.

TABLE 81. Composition of triticale (FasGro 204) (*moisture free basis*) (data from Anderson et al. 1972).

Crude protein (N × 5.7), %	17.1 (ca 14.6 at 14% H_2O)
Crude fat, %	1.7
Crude fibre, %	3.1
Ash, %	2.1
Nitrogen-free extract, %	76.0

derived from several sources, but it can be observed from the table that, compared to wheat, the contents of lysine, arginine, alanine, aspartic acid and glycine are lower in flour and higher in bran and germ, while the contents of glutamic acid and proline are higher in flour and lower in bran and germ. This is a reflection of the relatively high amounts of endosperm protein present in com-

TABLE 82. Fine grinding and air classification of triticale flour (*14% moisture basis*) (data from Anderson et al. 1972).

Kind of flour and fraction number	Yield (%)	Protein (%)	Ash (%)	Fat (%)	Maltose values	Mass median diameter μ
Straight flour		12.7	0.57	0.69	161	29
Reground flour (3 passes)		12.0	0.59	0.62	246	20
Fraction 1	10.7	34.3	1.15	1.56	372	11
Fraction 2	8.0	28.4	0.88	1.13	374	12
Fraction 3	6.4	20.7	0.73	0.85	359	15
Fraction 4	22.8	6.7	0.50	0.41	187	18
Fraction 5	14.0	4.8	0.45	0.34	184	21
Fraction 6	16.8	4.4	0.42	0.30	157	23
Fraction 7	11.7	5.6	0.46	0.31	149	25
Fraction 8 (coarse residue)	9.6	13.3	0.50	0.42	204	31
	100.0					

TABLE 83. Comparison of fractionation responses of reground flours from triticale, rye, and wheat (*14% moisture basis*) (Anderson et al. 1972).

Sample	Triticale	Rye[a]		Wheat		
		Balboa	Commercial Mix	Durum Wells[b]	Hard Red Spring Selkirk[b]	Hard Red Winter Wichita[c]
Straight flour, protein, %	12.7	8.2	10.9	15.7	12.8	10.9
Maximum range of protein contents, %	34.3-4.4	18.6-3.4	21.5-6.2	26.2-11.4	23.7-7.6	29.4-5.5
Combined high-protein fractions 1-3						
Protein, %	29.0	15.9	19.8	23.6[d]	19.4	24.4
Yield, %	25.1	26.8	25.8	9.2[d]	18.8	21.0
Combined starchy fractions 4-7						
Protein, %	5.5	4.8	7.4	12.0[e]	8.7	6.5
Yield, %	65.3	60.4	56.8	30.3[e]	49.5	52.9
Coarse residue fraction 8						
Protein, %	13.3	5.2	9.6	16.4	14.2	9.8
Yield, %	9.6	12.8	17.4	42.2	31.7	26.1
Protein shifted, total, %	73.0	50.0	41.0	15.0	29.0	50.0

[a]Unpublished data.
[b]Peplinski et al. (1965).
[c]Stringfellow and Peplinski (1966).
[d]Combined fractions 1 and 2.
[e]Combined fractions 4, 5, and 6.

TABLE 84. Characteristics of triticale milling products and control wheat flour (*dry matter basis*) (data from Vallejo et al. 1969).

	Triticale					Wheat blend
	Buhler milled flour	Flour 1	Flour 2	Concentrate 1	Concentrate 2	Buhler milled flour
Ash, %	0.67	0.59	0.77	1.38	1.78	0.58
Protein (N × 6.25), %	18.2	16.2	19.3	22.2	24.1	10.6
Fat, %	1.5	1.0	1.0	2.1	3.0	1.2
Fibre, %	0.6	0.7	0.8	0.9	1.8	0.4

mercially milled flour, from which bran and germ are customarily excluded.

Kasarda et al. (1971) in their review of the amino acid composition of wheat flour provide tables which compare the amino acid composition of flour fractions with the wheats from which they are milled citing, amongst others, Hepburn et al. (1957, 1960b); Horn et al. (1958); Bruggemann and Erbersdobler (1967); Kohler and Palter (1967); Waggle et al. (1967); Tkachuk and Irvine (1969a). A similar pattern emerges to that shown in Table 3. However, soft wheats, comparatively low in protein, tend to have a higher content of lysine, arginine and other essential amino acids

TABLE 85. Relative nutritive value (RNV) of wheat and wheat products (*as percentage of RNV of lactalbumin ± the standard error*) calculated using rat weight gain and body water as measures of response (data from Miladi et al. 1972).

	Based on weight gain	Based on body water
Wheat germ	80±5	79±3
Wheat protein concentrate	72±4	75±3
Red Dog	60±3	60±3
Shorts	55±3	57±2
Bran	49±3	51±2
Whole wheat	36±3	39±2
Patent flour	23±4	26±3
Clear flour	25±3	27±2

The mean value ± 2 standard errors gives the 95% confidence limits of each value.

than do strong (higher protein) wheats (Kasarda et al. 1971 citing Lawrence et al. 1958; Hepburn and Bradley 1965; and Waggle et al. 1967).

Soft wheats are customarily used more extensively in cakes and biscuits (cookies) than in bread flours. However, recent technological developments, including the Chorleywood Bread Process (Chamberlain et al. 1966) have made it possible to include significantly larger proportions of soft wheat flours in bread than heretofore.

Nunnikhoven and Bigwood (1959) reported on the amino acid composition (ion exchange chromatography) of a sample of Manitoba No. 2 wheat, and of the flour commercially milled from it in Belgium at two extraction rates, 75% and 65%. Tryptophan was not determined. The content of the essential amino acids was not greatly affected by the milling process, except for lysine, which was materially decreased by milling to 75% extraction and showed a further decrease on milling to 65% extraction rate.

Miladi et al. (1972) examined germ, wheat protein concentrate, red dog, bran, shorts, patent flour and clear flour obtained by standard milling procedures from a single sample of hard red spring wheat. The amino acid content was determined by autoanalyzer. Protein availability was determined by an in vitro method (Kohler et al. 1969). The relative nutritive value (RNV) was determined as described by Hegsted and Chang (1965a, b), Hegsted and Worcester (1966), Hegsted et al. (1968), using young male rats weighing approximately 50 g. The RNV is defined as the slope of the dose-response curve of the protein under test divided by the slope of the dose-response curve obtained with the standard protein, lactalbumin. Body-weight gain in the 3-week experimental period and the body-water of the animals at the end of the experiment were used as measures of "response".

The RNV of the various fractions is given in Table 85. Patent flour has the lowest, and germ the highest, RNV, and the RNV correlates highly with the lysine content of the proteins of the fractions analyzed. The essential amino acid contents are shown in Table 86. A chemical score based on the essential amino acid content of egg protein did not predict the RNV. The total protein content and the digestibility of the fractions

TABLE 86. Essential amino acid composition of wheat mill fractions (*in grams amino acid/16 g N*) (data from Miladi et al. 1972).

	Clear Flour	Patent Flour	Whole Wheat	Red Dog	Bran	Shorts	WPC[a]	Wheat Germ
Lysine	1.88	1.95	2.47	3.78	4.10	4.21	4.59	4.92
Tryptophan	1.11	1.10	1.28	1.38	1.92	1.53	1.35	1.28
Threonine	2.62	2.67	2.82	3.24	3.29	3.31	3.49	3.52
Valine	4.53	4.58	4.88	5.16	5.17	5.18	5.40	5.22
Isoleucine	4.08	4.30	4.01	3.83	3.63	3.61	3.83	3.68
Leucine	6.78	6.78	6.54	6.30	5.98	5.96	6.26	6.04
Tyrosine	2.93	3.11	2.89	2.69	2.66	2.66	2.78	2.58
Phenylalanine	4.99	5.08	4.73	4.29	3.91	3.81	4.02	3.78
Cystine	2.76	2.64	2.44	2.36	2.29	2.24	2.21	1.88
Methionine	1.88	1.97	1.89	1.92	1.74	1.81	2.13	1.89

[a]Wheat protein concentrate.

as measured by the in vitro method of Kohler et al. (1969) are given in Table 87.

The effect of varying extraction rates of wheat flour on the nutritional value of the breads made from the flours was described in the classic experiments of Widdowson and McCance (1954) in feeding undernourished European children. Contrary to what had been expected from rat feeding trials, no significant differences were found between the breads, which supplied about 75% of the calories in diets which were otherwise poor. Hughes (1955) estimated the amino acid content of the different diets used and from this it appeared that the lysine content in the diets was adequate, and that this amino acid was therefore no longer limiting. Under these conditions the differences in extraction rate would not be of nutritional significance.

Hutchinson et al. (1956) fed male weanling rats with bread made from wholemeal flour and from 70% extraction flour, laboratory milled from a clean sample of Manitoba No. 2 wheat. When, in the diets, the lysine content of both the wholemeal and white bread was raised to about 1%, lysine was no longer limiting and the rats grew equally well on the lysine supplemented diets. Hutchinson et al. found no evidence of any other amino acid becoming limiting, once the lysine requirement had been satisfied. Weanling rats appear to be extremely sensitive to small changes in the lysine content in diets of which the lysine content is within the range of 0.3 to 0.5%.

Gontzea et al. (1970) reported on rat feeding trials in which wheat wholemeal and flours of 85%, 75%, 50% and 10% extraction rates were compared. Male weanling Wistar rats, in groups of 10, were pair-fed the flours, as such, with only the addition of 2% liver oil, over 30 days. Growth rate, food consumption and protein efficiency ratio were measured. Growth rate was highest on the wholemeal diet and fell as the extraction rate was reduced; the most important reduction, statistically, was between flours of 85% and 75% extraction rates. Flours of 75% and 50% extraction rate were twice, and those of 10% extraction rate, five to eight times, worse than the 85% extraction rate and wholemeal as measured by growth rate.

In nitrogen balance tests with adult rats, pair-fed, the digestibility coefficient of the 50% extraction flour exceeded 90%, of the 85% extraction flour was approximately 85%, and of the wholemeal 78%. However, the nitrogen retained as a percentage of the nitrogen absorbed was approximately 29% for the wholemeal, 25% for the 85% extraction flour and 21% for the 50% extraction flour.

TABLE 87. Total protein content and digestibility of the protein in wheat fractions, in vitro method of Kohler et al. 1960 (data from Miladi et al. 1972).

Wheat fraction	Protein content ($N \times 6.25$) (%)	Digestibility (%)
Wheat germ	25.25	91.6
Wheat protein concentrate	27.40	96.5
Red dog	17.40	94.1
Shorts	19.70	83.9
Bran	17.20	69.4
Whole wheat	16.67	91.0
Patent flour	14.39	99+
Clear flour	19.15	99+

The whole wheatmeal, and seven of the 42 milling fractions obtained from it at a commercial mill in Sweden, were fed to provide 9.4% protein in the diet of rats and mice in trials reported by Munck (1972). The fractions fed included the embryo, P42, two fractions of the inner endosperm, P10 and P12, and four fractions from the outer endosperm, P37, P38, P39 and P41. The composition of the fractions in protein, lysine, fibre, ash and resorcinols is given in Table 88.

The nitrogen retention (NR) in mice and the net protein utilization (NPU) in rats were reasonably well correlated. There was a marked negative relationship between crude fibre content and true digestibility for rats. Because of the high degree of correlation between crude fibre and digestibility, and between resorcinol content and digestibility, it was not possible to differentiate statistically between the effects of these two factors.

The high lysine P37 fraction gave an NPU in rats equal to that of the P42 germ diet; in mice the P37 diet gave a higher NR than did the P42 diet. In the mouse trials, the P37 to 41 diets showed a continuously decreasing gain in protein with decreasing consumption of lysine. The wheat germ diet lies on the upper side, and the low-lysine P10, P12 and the whole wheat (W) diets on the lower side, of the regression line of the P37 to 41 diets. The digestible lysine in the germ was thus utilized less effectively, and the P10, P12 and W diets more effectively, than expected when

TABLE 88. Protein, lysine, fibre, ash and resorcinol contents of commercial milling fractions of a Swedish wheat (data from Munck 1972).

	Crude protein ($N \times 6.25$) (%)	Lysine (*g/16 g N*)	Fibre (%)	Ash (%)	Resorcinol relative units
P 10 } inner	10.0	2.31	0.10	0.24	14
P 12 } fractions	14.2	3.11	0.90	1.80	39
P 37 }	19.3	6.01	4.70	3.52	124
P 38 } outer	14.8	5.88	9.30	3.93	382
P 39 } fractions	14.0	5.66	11.60	5.10	516
P 41 }	12.8	4.86	12.75	6.44	538
P 42 embryo	30.1	7.70	2.40	4.55	40
Whole meal	10.7	3.34	1.95	1.28	90

compared with the results of the P37 to 41 fractions.

The addition of bran to a diet based on white flour improved the growth rate and food consumption of weanling rats partly, it is suggested, because of the supplementary effect of the higher lysine in the bran on the protein of the flour (Hutchinson and Martin 1970). The 71% extraction rate flour was milled from a No. 2 Northern Manitoba wheat, in a Buhler laboratory mill. Diets in which bran replaced 0, 25, 45 and 65% of the white flour were compared. As the proportion of bran increased, the nitrogen level in the diet increased as did (*a*) the growth rate per rat per day, (*b*) the food intake and (*c*) the growth rate per rat per nitrogen intake. However, the growth rates were still poor and well below those attainable if flour, without bran, is supplemented with lysine and threonine as found by Hutchinson et al. (1956, 1964). Fine grinding of the bran did not affect growth rate nor food intake significantly but slightly depressed the apparent digestibility of the nitrogen in the diet.

Girish et al. (1971) reported on the protein content of 450 samples of wheat and wheat products including maida (commercial white flour) and resultant atta (used in chapatis). Not surprisingly, the maida, being lower in bran, contained less protein (Kjeldahl N × 5.7) than resultant atta and whole wheat. In several instances, resultant atta was higher in protein than the whole wheat. No information is given on extraction rates but ash contents in the whole wheat ranged from 1.3 to 1.69%, in the maida from 0.46 to 0.61% and in the resultant atta from 1.26 to 1.54%.

Tara et al. (1971) examined 33 samples of flour from Indian commercial roller mills. A composite grist was prepared by mixing equal amounts of wheat, mainly US red wheat, from the mills, conditioning the mix to 15.5% moisture and milling to give a 69.5% extraction flour. Lysine, threonine and methionine were analyzed microbiologically and the results are presented in Table 89. It was noted that the variations were slightly higher when expressed as percent of flour than as percent of protein. In the commercial flours the correlation coefficients between protein, threonine and methionine were positive and significant. There was a significant positive correlation ($r = 0.695$) between protein and lysine as percent flour but the correlation was negligible between protein and lysine expressed as percent protein. The average overall loss of lysine during milling was 36.3%; losses in methionine and threonine as a consequence of milling appeared to be substantially lower.

Air Classified Wheat Flour Hutchinson et al. (1959) determined the amino acid composition (ion exchange chromatography) of an air classified high-protein flour (Jones et al. 1959) and of its parent flour. The results given in Table 90 are compared with a typical United Kingdom baker's flour. The amino acid composition of the air classified flour differed little from that of the parent flour.

Air classified flours of protein content as high as 26% (Kjeldahl N × 5.7) were used in rat feeding trials in the form of bread by Hutchinson et al. (1959). The bread was made in the laboratory with 15 g yeast per 800 g flour. Neither salt nor milk powder was included in the bread doughs but the

TABLE 89. Protein, lysine, threonine and methionine contents (with range in parentheses) of 33 samples of Indian commercial flours, and of a composite grist wheat and laboratory-milled flour (*14% moisture basis*) (data from Tara et al. 1971).

	Commercial flour	Composite grist wheat	Laboratory-milled flour
Protein (N × 5.7), mean %	9.55 (7.7–12.1)	9.90	9.0
Lysine g/100 g flour	0.176 (0.122–0.256)	0.286	0.189
g/100 g protein	1.84 (1.46–2.45)	2.89	2.10
Threonine g/100 g flour	0.301 (0.252–0.376)	0.333	0.310
g/100 g protein	3.13 (2.71–3.31)	3.37	3.40
Methionine g/100 g flour	0.170 (0.129–0.196)	0.180	0.177
g/100 g protein	1.78 (1.53–1.98)	1.82	1.96

diets as fed over 28 days from weaning contained added salt, a vitamin mixture and other non-protein nutrients. As the level of bread protein in the diet increased from (A) 10.8% to (B) 15.0% to (C) 18.8% to (D) 24.6%, a moderate increase in rat growth was recorded. Throughout lysine remained the limiting amino acid.

Graham et al. (1969) reported that white flour air classified to contain 21% protein provided infant diets similar in their components to control casein diets. Table 91 shows the essential amino acid composition (methods of determination not stated) of the protein of (*a*) human breast milk (HBM) and (*b*) the air classified wheat flour used, expressed as grams per 100 g protein and as milligrams per gram total essential amino acids (EAA). The subjects, six severely undernourished infants in the British American Hospital, Lima, Peru, received the four experimental wheat diets in random sequence, for 15 to 36 days each, with intervening 9-day periods on casein. The four wheat diets, in which wheat provided the only protein source, were fed isonitrogenously and isocalorically per unit of body weight and included: (A) white wheat flour (21% protein); (B) white wheat flour plus L-lysine HCl to bring the lysine and threonine levels to approximately 85% of the levels in HBM (i.e. equivalent to an enrichment level of 0.12% lysine in ordinary white flour); (C) white wheat flour with L-lysine HCl to bring lysine to the same proportion of the essential

TABLE 90. Amino acid composition of air classified flour, its parent flour and a baker's flour (*protein Kjeldahl N × 5.7*) (data from Hutchinson et al 1959).

	Amino acid nitrogen (*g/100 g total N*)		
Amino acid	Parent flour (*11.3% protein*)	Derived high-protein flour (*26.8% protein*)	A typical UK[a] baker's flour (*12.9% protein*)
Alanine	2.70	2.87	2.87
Arginine	–	–	6.72
Aspartic acid	2.80	2.60	2.66
Cystine	1.65	–	1.68
Glutamic acid	18.66	19.69	19.94
Glycine	3.84	–	3.93
Histidine	3.26	3.66	3.59
Isoleucine	2.57	2.56	2.55
Leucine	4.72	4.76	4.81
Lysine	2.30	2.38	2.36
Methionine	0.98	0.98	1.05
Phenylalanine	2.61	2.75	2.80
Proline	8.30	8.75	8.83
Serine	3.90	4.08	3.89
Threonine	1.98	2.07	2.05
Tryptophan	–	–	0.98
Tyrosine	1.41	1.46	1.61
Valine	3.18	3.13	3.28

[a]Values from McDermott and Pace (1957).

TABLE 91. Essential amino acid (EAA) composition of human breast milk (HBM) protein and of air classified wheat flour protein expressed as grams/100 g protein (% *protein*) and as mg/g total essential amino acids (*mg/g EAA* (data from Graham et al. 1969).

	HBM protein		Wheat protein		W/HBM, (%)[a]
	(% *protein*)	(*mg/g EAA*)	(% *protein*)	(*mg/g EAA*)	
Isoleucine	6.4	131	4.22	125	95
Leucine	8.9	183	6.51	193	105
Lysine	6.3	129	2.67	79	61
Phenylalanine + Tyrosine	10.1	207	8.25	244	118
Cystine + Methionine	4.3	88	3.62	107	121
Threonine	4.6	94	2.8	83	88
Tryptophan	1.6	33	1.2	36	109
Valine	6.6	135	4.5	133	98
Total	48.8	1,000	33.77	1,000	

[a]The % EAA for each amino acid in wheat as a percentage of that in HBM.

amino acids, 129 mg lysine per gram EAA, as is found in HBM protein, (*see* Table 91, i.e. equivalent to an enrichment level of 0.2% lysine in ordinary white flour), and (D) wheat flour with L-lysine HCl to bring lysine to the same proportion of the wheat flour protein (6.3%) as is found in HBM protein (*see* Table 91, i.e. equivalent to an enrichment level of 0.4% lysine in ordinary white flour).

All the diets were well accepted and tolerated. Diet (B) increased rates of weight gain and nitrogen retention, stability of serum albumin and elevation of the ratio of plasma lysine. Diets (C) and (D) produced further progressive increases in weight gain, nitrogen retention and plasma lysine. Graham et al. (1969) conclude that enrichment of ordinary white wheat flour with lysine to the 0.12% and possibly the 0.2% level would be beneficial in those areas where wheat provides the main source of protein in the diet, particularly that of infants and children.

The prolonged feeding of wheat flour enriched at the same levels as in the previous study confirmed the apparent superiority of the 0.2% level over the 0.12% level, and in the one case in which it was used, of the 0.4% level over the 0.2% level (Graham 1971).

Toepfer et al. (1969, 1972) have reported on the amino acid composition of selected wheats, the flours milled from them and the products into which they were made. The methods by which the amino acids were analyzed are not given but may be expected to be published in a paper in preparation by Hepburn.

The protein contents of (*a*) a hard red wheat, and (*b*) the 72% extraction flour milled from (*a*), and of (*c*) a soft wheat, and (*d*) patent flour, (*e*) a straight flour and (*f*) a cut-off (air classified) flour milled from (*c*) are given in Table 92. The essential amino acid (EAA) compositions of (*a*) and (*c*) in milligrams per 100 g dry weight are also quoted in Table 92 with the percentage of wheat EAA found in the fractions (*b*), (*d*), (*e*), and (*f*). The highest losses on milling occur in lysine, tryptophan and threonine.

In their review of milling principles Ziegler and Greer (1971) point out that the yields and protein contents of the fractions obtained by air classifying wheat flour vary with different wheats. The yield of the fine fraction from hard wheat flour is much lower than that from soft wheat. The ratio between the protein contents of the fine fraction and that of the parent flour is about 1.5:1 from hard wheat, 2:1 from soft wheat. The yield of medium fraction is lower, and of coarse fraction higher, from hard wheats than from soft wheats; the protein content of the medium fraction of hard wheats is about two-thirds, and of soft wheats less than half, the protein content of the original flour.

Amino Acid Availability The effect of extraction rate on the availability of the amino acids (assayed microbiologically) in wheat was reported by Calhoun et al. (1960) for lysine, and by Hepburn et al. (1966) for methionine, threonine, tryptophan, valine, phenylalanine, leucine and isoleucine. The blend, consisting of a hard red spring wheat and a hard red winter wheat, had a nitrogen content of

TABLE 92. Protein content of (*a*) hard red wheat and (*b*) 72% extraction flour milled from (*a*); (*c*) soft wheat and (*d*) patent flour about 54% extraction, (*e*) straight grade flour about 72% extraction and (*f*) cut-off (air classified) flour milled from (*c*), with essential amino acids (EAA) composition of (*a*) and (*c*) and percentage of wheat EAA found in (*b*), (*d*), (*e*) and (*f*) (data from Toepfer et al. 1972).

	(*a*) Hard red wheat	(*b*) 72% extraction	(*c*) Soft wheat	(*d*) Patent flour	(*e*) Straight grade flour	(*f*) Cut-off flour (air classified)
Protein (N × 5.83 dry basis), %	14.8	13.7	11.7	9.7	10.6	11.3
Essential amino acids	(*dry weight mg/100 g*)	(% *of wheat EAA*)	(*dry weight mg/100 g*)	(% *of wheat EAA*)	(% *of wheat EAA*)	(% *of wheat EAA*)
Lysine	433	69	360	66	74	83
Cystine	310	94	223	96	101	115
Valine	742	90	581	84	89	102
Methionine	248	103	206	89	95	111
Isoleucine	617	98	464	90	96	107
Leucine	1,068	97	812	91	95	107
Tyrosine	461	94	344	90	95	109
Phenylalanine	765	101	567	91	97	110
Tryptophan	266	83	225	68	70	77
Threonine	471	86	393	76	81	93

2.46% (Kjeldahl-Gunning procedure) and the 72% extraction patent flour commercially milled from this blend, a nitrogen content of 2.31%.

The availability of lysine was evaluated by weanling rats (Calhoun et al. 1960), fed two basal diets, of which wheat or flour constituted 70% by weight. Diet (A) contained 20% wheat gluten, Diet (B) an amino acid mixture equivalent to the amount of gluten in Diet (A) but from which lysine was omitted. Performance was rated by the gain in live weight, gain in empty body weight after removal of the intestinal contents, and gain in carcass nitrogen over a 3-week period. The response of the samples was referred to standard curves and the results compared with those obtained by microbiological assay. The closest agreement between the basal diets was found when carcass nitrogen gain was related to total available lysine consumed. On that basis the availability of lysine in wheat was 75% and in the flour 72% with the gluten basal diet, and 78% and 80% respectively with the amino acid basal diet.

For the other amino acids (Hepburn et al. 1966) the wheat and flour were from the same lot as in the lysine study and the methods were essentially similar. As in the lysine study (Calhoun et al. 1960), the best agreement between basal diets and between sample levels was obtained from the relation between increase in carcass nitrogen and the amount of available amino acid consumed. The increase in total carcass nitrogen content was found to vary directly with the amount of available amino acid consumed when that amino acid was limiting in the diet. The availabilities as calculated are shown in Table 93.

Saunders and Kohler (1972) have described an in vitro method for the measurement of protein digestibility using *Streptomyces griseus* protease (Pronase B grade, Calbiochem, Los Angeles), followed by treatment with chick pancreas acetone powder. This method correlated well with an in vivo method using rats, fed isocaloric and isonitrogenous diets equivalent to 10% protein, the comparative standard being casein. Table 94 gives the protein digestibility determined by the two methods on wheat mill feeds from a sample of hard red spring wheat and also quotes the values obtained by the in vitro procedures of Booth and Moran (1946), Chick et al. (1947) and Akeson and Stahmann (1964).

TABLE 93. Availability of seven amino acids in wheat and flour fed at two diet levels (*calculated by increase in rat carcass nitrogen*) (data from Hepburn et al. 1966).

	Wheat		Flour	
	Diet level (%)	Availability (%)	Diet level (%)	Availability (%)
Methionine	22.5	96	25	96
	45	88	50	95
Tryptophan	25	88	25	88
	40	91	40	86
Isoleucine	20	73	20	91
	35	91	35	86
Phenylalanine	15	76	15	90
	25	73	25	79
Valine	25	76	25	78
	40	72	40	82
Leucine	25	94	25	75
	40	91	40	97
Threonine	30	90	30	102
	45	101	45	88

TABLE 95. Extraction rate and protein content of seven milling fractions of a Polish rye (data from Bartnik 1964).

Flour	Extraction rate (%)	Protein content (*N* × 5.83 *dry matter basis*) Total (%)	Digestible[a] (%)
Light rye flour	45.4	6.65	4.96
Rye flour	60.0	6.70	4.97
Rye flour	70.0	7.29	5.26
Rye flour	78.5	8.92	6.20
Rye flour	82.0	8.63	6.00
Army rye flour	87.0	9.56	6.89
Coarse rye flour	98.0	9.79	6.64

[a]Bartnik (1960).

Rye Flour

Bartnik (1964) reported on feeding trials with rats bred at the Institute for Food and Nutrition, Warsaw, on seven flour fractions from a single sample of rye grown in Poland. The fractions were fed, contained in the proportion of 1 : 1, with a basal diet simulating the average composition of the Polish diet. The control diet was the basal diet alone which provided 480 kcal and 11.7 g protein (N × 6.25) per 100 g diet. Of this 11.7 g protein about two-thirds was in the form of animal protein (dried whole milk, pulverized dried beef and dried whole egg).

The growth rate method of Osborne et al. (1919) was used with groups of 12 rats, six male and six female of initial weight about 49 g, over six weeks. The composition of the seven fractions reported by Bartnik (1964) is given in Table 95.

When these seven fractions of rye flour were incorporated on a 1 : 1 basis with the basal diet, the protein content in the diets rose progressively, from 9.74% (N × 6.25) to 11.08%; the proportion of flour protein in the total protein of the diet rose, from 37.1% to 46.1%, but the protein efficiency ratio of the various diets did not differ. The weight increases were approximately 2 g per gram total protein and 2.6 g per gram digestible protein in all

TABLE 94. Protein digestibility in wheat mill feeds by in vivo and in vitro procedures (data from Saunders and Kohler 1972).

	Protein digestibility, (%)				
	Saunders & Kohler (1972)		Booth & Moran (1946)	Chick et al. (1947)	Akeson & Stahmann (1964)
	(*In vivo*)	(*In vitro*)	(*In vitro*)	(*In vitro*)	(*In vitro*)
Casein control	99.3	98.5	–	–	–
Patent flour	93.2	96.7	99.4	99.0	98.9
Red dog	84.6	89.5	95.2	94.4	93.9
Germ	86.7	85.5	91.5	92.8	88.3
Shorts	77.4	79.1	82.7	84.1	80.8
Bran	73.0	71.5	74.5	72.0	69.7

TABLE 96. Amino acid composition of rye grain, rye flour and bran (data from Rukosuev and Silant'eva 1972).

	Rye grain		Sieved flour		Break flour		Bran	
	Total protein (%)	(*g/100 g product*)	Total protein (%)	(*g/100 g product*)	Total protein (%)	(*g/100 g product*)	Total protein (%)	(*g/100 g product*)
Lysine	4.23	0.46	3.30	0.32	3.46	0.37	4.06	0.67
Histidine	2.09	0.22	1.90	0.19	1.82	0.18	2.19	0.35
Arginine	5.62	0.60	4.57	0.44	4.94	0.53	6.31	1.02
Aspartic acid	7.16	0.77	6.12	0.58	7.16	0.80	7.47	1.22
Threonine	3.11	0.34	2.54	0.25	3.29	0.35	3.34	0.57
Serine	4.54	0.48	4.56	0.44	5.05	0.55	4.53	0.73
Glutamic acid	29.91	3.25	34.46	3.22	34.94	4.03	27.93	4.53
Proline	5.20	0.55	5.90	0.56	5.96	0.65	4.93	0.79
Glycine	4.79	0.52	3.73	0.36	4.84	0.52	5.44	0.89
Alanine	5.13	0.55	4.22	0.41	4.78	0.51	5.36	0.88
Cystine	1.19	0.13	1.20	0.13	0.91	0.08	1.90	0.32
Valine	5.56	0.60	4.92	0.48	5.45	0.60	5.32	0.86
Methionine	0.65	0.07	0.21	0.02	1.08	0.10	0.44	0.08
Isoleucine	3.88	0.42	3.10	0.30	4.09	0.44	3.69	0.61
Leucine	7.00	0.75	5.85	0.56	7.33	0.81	6.76	1.09
Tyrosine	2.86	0.31	2.67	0.26	2.90	0.31	2.66	0.43
Phenylalanine	5.25	0.56	4.99	0.48	5.57	0.62	4.56	0.73
Ammonia	2.92	0.30	2.74	0.28	2.16	0.22	2.19	0.37

the diets. The total weight increases over the six weeks varied from 75 g to 96 g but in parallel with the percentage protein of the flour added to the basal diet. The weight increases of the animals on the test diets per gram protein consumed was higher in the test diets than on the basal diet alone. Bartnik (1964) comments that these results could indicate, so far as growth rate is concerned, either that there is no difference between the protein of the different milling fractions of rye flour examined or that the protein in the basal diet balanced the protein in the rye flours. He considers that the probability is that there is no difference between the proteins of the milling fractions. This view is supported by the findings of Szkilladziowa (1961), who used rats from the same Institute strain as Bartnik (1964).

The digestibility coefficients of these seven fractions with three other rye flour fractions and four of bran were reported by Bartnik (1960). Digestibility decreased with increasing extraction rate. However, since the protein content rose with extraction rate, there was a net increase in digestible protein from the flours of higher extraction rates.

Szkilladziowa (1961) compared Polish rye flour of 98% extraction and of 45% extraction with egg in feeding tests with rats using the original growth method of Osborne et al. (1919) and a modification. The diets were fed at 5.5% protein (N × 6.25) level. At this low level of protein, both rye flours gave very similar biological values by both methods, means of 45.8% and 45.9% for the 98% extraction flour and 44.8% and 49.0% for the 45% extraction flour. These values were approximately that of whole egg.

Szkilladziowa (1960a) determined the biological value of rye flour of three extraction rates, (A) 98%, (B) 87% and (C) 45% by the rat growth method of Osborne et al. (1919) and by the chemical method of Block and Mitchell (1946). Wheat flour of (D) 98% and (E) 50% extraction was also included, egg providing the control diet. The average level at which the protein (N × 6.25) was fed varied, 8.3% in Diet (A) (98% extraction rye), 8.5% in Diet (B) (87% extraction rye), 5.3% in Diet (C) (45% extraction rye), 8.7% in Diet (D) (98% extraction wheat), 8.8% in Diet (E) (50% extraction wheat).

The biological values for the rye flours (Osborne et al. 1919) were 54 (Diet A), 52 (Diet B) and 30 (Diet C) respectively, and for the wheat flours 47 (Diet D) and 23 (Diet E). The equivalent values by the chemical method of Block and Mitchell

(1946) were (A) 41, (B) 39 and (C) 37, (D) 40 and (E) 35.

Rye flours of similar extraction rates, (A) 98%, (B) 87% and (C) 45%, and wheat flour of (D) 98% and (E) 50% extraction rates were also examined by Szkilladziowa (1960b) in feeding trials with 290 white rats using the method of Miller and Bender (1955). Various levels of protein (N × 6.25) were fed (between approximately 5% and 9% in the diets) but the mean NPU values were respectively (A) 55.9, (B) 58.8 and (C) 61.0 for the rye flours and (D) 52.9 and (E) 43.6 for the wheat flours.

Kurzepa et al. (1960) reported on the essential amino acid content (assayed microbiologically) of 10 flours of differing extraction rates and four brans from a single sample of experimentally milled Polish rye. As the extraction rate rose from 45.5% to 98%, the content of lysine and arginine in relation to total nitrogen increased and the content of leucine decreased. The content of the other amino acids determined: phenylalanine, tryptophan, histidine, valine, isoleucine, methionine and threonine did not alter substantially.

Rukosuev and Silvant'eva (1972) compared the amino acid composition of the proteins in a sample of whole rye, 1966 harvest, sieved rye flour (63% extraction), break flour (87% extraction) and bran. The amino acids, determined by autoanalyzer, are quoted in Table 96.

Secondary Processing

Triticale

No published work has so far been encountered on the effects of processing for human consumption on the nutritional value of triticale-containing products. The literature covering such effects on the nutritional value of wheat and rye is considerable and is referred to later in this chapter. Papers have, however, appeared on the utilization of triticale in baked and other products, and these are referred to briefly below.

Bread Unrau and Jenkins (1964) conducted baking tests using the hexaploid triticale samples described above under Milling (*see* Table 74 and 75). Baked alone, the triticale did not give good results but when blended with Pembina wheat flour, up to a limit of 40% triticale to 60% Pembina, loaf volumes were comparable to those when Pembina was baked alone.

Vallejo et al. (1969) compared experimentally milled triticale flours with a similarly milled flour from a mixed grist of Spanish grown wheats (*see* Table 84). Baked alone, the triticale flours gave poor bread compared with the wheat flour control but mixtures of 20 parts triticale flour and 80 parts wheat flour produced bread superior in overall baking quality to the wheat flour control, probably because the triticale corrected the deficiency in diastatic activity which is general in Spanish wheat flours. Vallejo et al. suggest that the addition of triticale flour would improve the nutritional quality of bread protein.

The spring triticales 6TA204 and 6TA206 and the winter triticales TR385 and and TR386 were compared with two winter wheats, Scout and Lancer, and two spring wheats Inia Res. and Chris, for the production of bread and rolls (Lorenz 1972). Characteristics of the flours are given in Table 97. The values, where they may be compared, are similar to those given by Lorenz and Maga (1972) (*see* Table 76). The triticale bread had a mild rye flavour. Some changes in procedure were needed to produce the best results.

Lorenz et al. (1972b) discuss more fully the mixing and baking properties of these triticale varieties. The winter triticales produced bread of lower specific volume, more open grain, slightly harsher texture, and slightly darker colour than the winter wheats. The spring triticales produced bread of acceptable quality, 6TA204 had the highest volume of all the breads baked and 6TA206 was only slightly inferior to the spring winter wheat bread.

Bushuk (1972) reported that acceptable bread may be obtained from triticale flour using a mechanical development process.

Tsen et al. (1973) compared the baking quality of three hexaploid triticales of protein (N × 5.7, 14% moisture basis) of (I) 11.1%, (II) 13.6% and (III) 12.8%, and a wheat flour of 12.7%. The amino acid composition (by autoanalyzer) of triticales I and II and the wheat flour are given in Table 98. Acceptable bread could be obtained from the triticales by eliminating bulk fermentation and, for triticales II and III, adding 0.5% sodium stearoyl-2 lactylate, 0.25% sucrose tallowate, or 0.25% ethoxylated monoglyceride. Triticale I required the addition of 20% wheat flour with 0.5% sodium stearoyl-2 lactylate, or 60% wheat flour alone. Triticale bread staled twice as rapidly as wheat bread, as measured by changes in crumb firmness with a Bloom Gelometer.

Cake Thompson (1971) and Thompson and Vaisey (1971) cited by Bushuk (1972) found that

TABLE 97. Characteristics of wheat and triticale cultivars (*14% moisture basis: yield through 100 mesh screen*) (data from Lorenz 1972).

	1970 crop				1971 crop			
	grain protein ($N \times 6.25$) (%)	flour protein ($N \times 5.7$) (%)	flour ash (%)	flour yield (%)	grain protein ($N \times 6.25$) (%)	flour protein ($N \times 5.7$) (%)	flour ash (%)	flour yield (%)
Spring triticale								
6TA204	14.6	11.3	0.53	42.1	15.7	12.7	0.54	61.9
6TA206	14.5	11.7	0.54	42.8	15.2	12.8	0.53	61.5
Winter triticale								
TR385	12.0	8.7	0.47	33.9	13.1	11.6	0.49	56.4
TR386	10.8	8.6	0.47	32.7	13.4	11.5	0.46	58.2
Spring Wheat								
Inia Res.	12.7	12.4	0.46	45.2	–	–	–	–
Chris	–	–	–	–	14.3	14.0	0.46	69.1
Winter wheat								
Scout	12.0	11.6	0.43	57.0	12.5	11.7	0.44	66.8
(No details are given for Lancer)								

cake made from chlorinated triticale flour, even at the highest levels of chlorination examined, was inferior to that obtained from standard cake flour, as measured by volume, subjective crumb scores, textureometer evaluation of hardness, cohesiveness, gumminess and adhesiveness of the cake crumb.

Alimentary Pastes Lorenz et al. (1972a) prepared acceptable noodles from a triticale flour of unspecified origin, of 9.3% moisture and 13.3% protein (N × 5.7).

Bushuk (1972) reported that spaghetti made by laboratory equipment from triticale semolina was darker and inferior overall to spaghetti from durum wheat. On cooking, triticale spaghetti behaved similarly to durum spaghetti and had a rye flavour.

Breakfast Cereals Triticale made into breakfast cereals, flakes, puffs and muffets ("shredded wheat" type product) on a commercial plant were similar to those made from wheat but with a slight rye flavour (Bushuk 1972).

Malting and Brewing Pomeranz et al. (1970) reported on the use of triticale in malting and brewing. The triticales used are characterized in Table 99. Beers from the worts of 6T204 (Texas), Rosner (Winnipeg and North Dakota), 6714 and 6804 (Winnipeg) had both satisfactory clarity-stability and gas stability.

TABLE 98. Amino acid composition of triticale and wheat flours in grams amino acid per 100 g Kjeldahl protein (data from Tsen et al. 1973).

Amino Acids	Triticale I	Triticale II	Wheat
Lysine	2.63	2.85	2.16
Histidine	2.03	2.23	2.24
Ammonia	3.82	3.76	4.33
Arginine	4.13	4.39	3.88
Aspartic acid	5.73	6.45	4.54
Threonine	2.98	3.36	3.06
Serine	4.70	5.25	5.58
Glutamic acid	32.03	37.22	45.85
Proline	10.77	12.51	12.24
Glycine	3.62	3.91	4.09
Alanine	3.40	3.81	3.52
½ cystine[a]	3.27	2.74	3.56
Valine	4.43	4.86	5.17
Methionine[a]	1.60	1.44	1.08
Isoleucine	3.37	3.83	4.26
Leucine	6.76	7.45	8.16
Tyrosine	3.07	3.35	4.01
Phenylalanine	4.77	5.11	5.99

[a]By oxidation.

In a later paper, Pomeranz (1971b) compared the Kjeldahl nitrogen contents and amino acid composition (Robbins and Pomeranz 1971) of the

TABLE 99. Kernel weight, and nitrogen in grain and in malt (*dry matter basis*) of triticales (data from Pomeranz et al. 1970).

Selection	Location	Kernel weight (*mg*)	Kjeldahl nitrogen (% *in grain*)	Nitrogen (% *in malt*)
6T204	Bushland, Texas	27.6	3.20	3.33
6T208	Bushland, Texas	25.6	3.26	3.35
6T209	Bushland, Texas	27.7	3.14	3.23
6450-3-1	Bushland, Texas	20.2	3.10	3.24
Rosner[a]	Winnipeg, Canada	33.3	2.48	2.48
6714[a]	Winnipeg, Canada	30.4	2.84	2.88
6804[a]	Winnipeg, Canada	30.3	2.65	2.76
Rosner	Fargo, N. Dakota	29.6	2.94	2.94
6437-6	Madison, Wisconsin	28.7	2.96	2.98
6450	Madison, Wisconsin	25.9	2.82	2.93

[a]Strains from a double cross in which two varieties of *Triticum durum* Desf., a variety of *T. persicum*, and a fourth *Triticum* species were used.

TABLE 100. Nitrogen contents and amino acid composition (*grams/100 g amino acid recovered*) of rye, wheat and triticale grains grown under comparable conditions (data from Pomeranz 1971b).

	Rye (composite)	Chris Wheat	Rosner Triticale
Kjeldahl Nitrogen, %	2.38	3.38	3.23
Amino acids			
Lysine	3.1	2.4	2.6
Histidine	2.0	2.2	2.0
Ammonia	3.5	3.9	3.4
Arginine	4.9	4.1	5.0
Aspartic Acid	7.5	5.0	6.5
Threonine	3.1	2.6	2.9
Serine	4.2	4.2	4.2
Glutamic acid	27.2	33.2	34.2
Proline	13.1	10.8	10.6
Cystine/2	0.3	1.0	0.5
Glycine	4.1	3.8	3.8
Alanine	4.1	3.3	3.8
Valine	4.5	4.2	4.1
Methionine	2.3	2.1	1.8
Isoleucine	3.4	3.5	3.1
Leucine	5.7	6.4	5.4
Tyrosine	2.2	2.6	2.3
Phenylalanine	4.7	4.7	4.0

grain, malt and sprouts of Rosner triticale, Chris wheat and a composite rye, all grown in Bushland, Texas, USA in 1969. The Kjeldahl nitrogen and amino acid composition of the grain is given in Table 100. When malted under comparable conditions all three cereals showed a decrease in glutamic acid and increases in lysine, arginine, aspartic acid, valine, isoleucine and leucine. The increase in nitrogen and lysine in malts and malt sprouts is shown in Table 101.

TABLE 101. Effect of malting on Kjeldahl nitrogen and lysine content of triticale, wheat and rye (data from Pomeranz 1971b).

	Nitrogen (%)	Lysine (*g/100 g amino acid recovered*)
Rosner triticale		
grain	3.23	2.6
malt	3.35	3.1
sprouts	4.76	4.8
Chris wheat		
grain	3.38	2.4
malt	3.36	2.7
sprouts	5.10	5.7
Rye		
grain	2.38	3.1
malt	2.36	3.9
sprouts	4.50	4.8

W. O. S. Meredith (1969 unpublished report) cited by Bushuk (1972) malted triticale to produce a product of high enzymatic activity which, it was concluded, could be used successfully in brewing.

TABLE 102. Nitrogen and protein content ($N \times 5.7$, *dry matter basis*) of Chilean wheat and wheat products, and their NPU_{op}, percentage of protein calories in each diet (P), NPU_{st} and net protein concentration (NPC = $NPU_{op} \times P/100$) (data from Ballester et al. 1962).

	Total N (%)	Total protein (%)	NPU_{op}	P	NPU_{st}	NPC
Wheat	1.75	9.98	40.2	10.0	40.3	4.0
"Mote" of wheat	1.75	9.98	31.3	10.0	31.3	3.1
Flour (76% extraction rate)	1.50	8.50	38.6	8.4	38.6	3.2
Bread	1.65	9.40	47.9	9.4	49.3	4.5
Roasted flour	1.44	8.21	34.1	8.1	34.1	2.8

Finney et al. (1971) cited by Bushuk (1972), found that triticale malts were superior to barley malts in breadmaking.

Miscellaneous Products Bushuk (1972) cites E. N. Larter (1972 unpublished report) as reporting the test marketing of triticale pancake flour and of a mix based on triticale flour for pizza pastry.

Wheat and Rye

Wheaten Bread Hepburn et al. (1957) assayed, microbiologically, the amino acids in the crust and crumb of bread from wheat flour and compared the results with those from the whole loaf. Only in the case of lysine was the value for the crust significantly less than that for the crumb. The content of lysine in the whole bread sample equalled the value expected from the proportion of crust and crumb in the bread.

McDermott and Pace (1957) determined colorimetrically the amino acid contents of hydrolysates of wheat flour, and of the whole bread and of bread crust, made from the flour. The contents of phenylalanine, tryosine and serine in the bread hydrolysate were lower by between 5 and 7% than in the flour hydrolysate. The contents of phenylalanine, tyrosine and lysine were 15 to 16% lower in the crust than in the flour hydrolysate.

Using microbiological assay methods, Horn et al. (1958) found that no destruction of amino acids occurred during fermentation of bread doughs prior to baking but losses of cystine, lysine and methionine during baking were statistically significant in small experimental loaves in which the ratio of crust to crumb is greater than in larger commercial loaves.

Clegg and Davies (1958) using a chemical method (Bruno and Carpenter 1957) found that the apparent available lysine in the crust of wheat bread was about 15% less than that of the flour from which it was baked.

Burke (1960) determined by autoanalyzer the lysine, histidine and arginine contents of bread made from 90% extraction (South African) flour and of the unbaked dough. A loss of about 10% lysine occurred during baking but histidine and arginine were not noticeably affected.

Ballester et al. (1962) reported on the nutritive value of Chilean cleaned, milled white wheat, the "mote" prepared from it, white flour available commercially in Santiago (76% extraction), pan bread and roasted flour. The "mote" was prepared by boiling (for about 2 hours) and decorticating the wheat in an alkaline lye, grinding and washing with water. The fresh product contained about 65% water. It was dried at temperatures below 60°C and ground. The bread was prepared with flour 100 g, salt 3 g, sugar 3 g, yeast 3 g and cooking fat 3 g and was baked at 220°C for 20 to 22 min. The roasted flour was made from whole, cleaned, white wheat, roasted at 160°C.

The analysis of the products and results of NPUs (Miller and Bender 1955; Miller and Payne 1961) are given in Table 102.

The NPU_{st} of the bread was higher than the NPU_{st} of the wheat and of the flour. The roasted flour did not support growth and by the tenth day the rats on that diet had fallen below their starting weight. The best rate of growth was shown by the rats on the wheat diet, followed by those on the bread, commercial flour and "mote" in that order.

Gotthold and Kennedy (1964) examined the effect of baking and steaming on the ingredients of whole wheat bread. The same bread formula containing whole wheat flour, protein content 15.5% (Kjeldahl $N \times 5.83$) was baked and steam-cooked and the nutritional value of each compared

with that of the unbaked ingredients by the following four criteria derived from rat feeding experiments: (*a*) change in body weight, (*b*) PER (modified method of Osborne et al. 1919), (*c*) BV (N-balance method of Mitchell (1923-24) and Mitchell and Carman (1926) (baked bread only), and (*d*) NPU (Bender and Miller 1953; Miller and Bender 1955).

By all methods, after 28 days, there was little significant difference between the unbaked ingredients and the steamed bread whereas the baked bread showed significantly lower values. The following are percentage differences for each of the method:

	Difference between unbaked ingredients and baked bread (%)
Body weight	53
BV	8.6
PER	11.0
NPU	15.0

The above were all computed after 28 days' feeding tests. After 10 days the results were inconclusive.

While all the 28-day results reveal the same general trend, the difference in magnitude among methods is worthy of note.

Gotthold and Kennedy (1964) cite Adolph and Tsui (1935) who compared the growth-promoting qualities of steamed and baked white bread. They fed the two breads ad libitum to weanling rats for 8 weeks and reported satisfactory growth and little, if any, difference, between the two types. Gotthold and Kennedy (1964) from an analysis of the growth curves given by Adolph and Tsui (1935) concluded that the rats fed baked bread gained 28% less in 4 weeks and 15% less in 8 weeks than did those fed steamed bread. This difference is in agreement with the findings of Gotthold and Kennedy in which the rats fed baked bread gained 27% less weight in 4 weeks than those fed steamed bread.

The Chorleywood Bread Process (CBP) is a method of making bread from wheat flour in which the normal 2 to 4-hour period of bulk fermentation of the dough, customarily used in the United Kingdom, is replaced by a few minutes of intense mechanical agitation. The effect of this process on the nutritional quality of the flour protein was examined by Chamberlain et al. (1966). The NPUs of bread made from (*a*) a strong flour and (*b*) a weak flour by both CBP and a conventional breadmaking method were compared and no significant difference was recorded between methods for either of the two flours.

Rye Bread Kon and Markuse (1931) report feeding commercially baked rye bread from 70% extraction flour as 95% of the diet of female rats. The crust and the crumb of the same bread were similarly fed to male rats. The PER for the whole bread, the crust and the crumb are given below:

	Protein ($N \times 6.25$) (%)	PER
Whole bread (female rats)	6.4	1.25
Crumb (male rats)	6.7	1.26
Crust (male rats)	6.3	0.83

Although the rats in the trials were of different sexes, and the results are therefore not strictly comparable, the findings indicate that, as reported elsewhere, there is a significant loss in protein value in the crust, but the difference between whole bread and bread crumb is not of consequence.

Kofranyi (1957) reported on nitrogen balance tests, conducted on himself, to compare the biological value of milk protein with rye protein. In all, the tests were conducted in stages over a period of 10 months. The rye protein was taken in the form of rye bread of 0.84 to 0.91% N, the only "foreign" protein amounting to 0.074 g N per day. He found that the addition of glutamic acid did not improve the biological value of the rye protein and slightly reduced that of the milk protein. The values found in the nitrogen balance tests were so low that absolute figures for biological value were not obtained. He concluded, however, that proportionally the biological value of rye protein was 22% lower than that of milk protein.

Kofranji and Muller-Wecker (1960) reported on dietary studies with three men and one woman to compare the biological values of the proteins of milk, egg, rye, and wheat. They report that the ratios were respectively 100:100:83:41. When lysine was added to the wheat and rye diet, the biological values improved; the addition of methionine also effected some improvement but the addition of leucine did not.

The minimum protein requirements of the individuals were found to differ considerably but reproducible results were obtained, within individuals and within protein sources, provided the

results of the first 10 days of the experimental period were not included. No agreement was found between the results of the feeding experiments and biological values calculated from amino acid analyses whether by the chemical score method of Mitchell and Block (1946) or with the EAA index of Oser (1951).

Crisp Bread Kihlberg and Ericson (1964) in rat feeding trials found marked differences between a rye flour composed of 70% Russian and 30% Argentinian rye and the corresponding crisp bread. Nitrogen in the diet was 1.63% and the differences are shown below:

	Average weight (*gain g/day*)	Nitrogen efficiency ratio (*g wt. gain/g N eaten*)
Rye flour	1.99±0.11	12.68±0.35
Rye crisp bread	1.15±0.09	7.73±0.47

During baking, crisp bread suffered a much greater decrease in protein value than did soft bread probably because of the higher average temperature reached. As reported elsewhere, lysine loss is much greater in the crust than the crumb of "soft" breads. Wheat crisp bread fed at the same time did not even maintain the starting weight of the young rats.

Munck (1972) described experiments with Swedish whole wheat bread baked in thin flat cakes containing 54.3% water in the dough. For (A) soft bread, the dough was baked at temperatures of 450–340–400°C in sequence over 2.0 min and for (B) hard bread, at 430–340–380°C in sequence over 3.6 min. The moisture content in the cooled bread was for (A) 26.3% and for (B) 11.0%. The composition of lysine, histidine, arginine, threonine and methionine in the soft bread changed little in comparison with the whole wheat meal, but in the hard bread all the amino acids showed a reduction, especially marked in the case of lysine.

In mice feeding trials, the whole wheat meal and the breads were fed at about 14.0% protein (dry matter basis). There was little difference in gain per animal, food consumption and grams weight gain per gram protein consumed between the whole wheat and the soft bread diets (lysine consumed per animal 234 and 277 mg respectively) but there were reductions from the hard bread diet (lysine consumed per animal 188 mg).

Microwave baking Clarke and Kennedy (1962) evaluated the effect of baking, by microwave and in a conventional oven, on the protein quality and lysine availability of whole wheat bread with an addition of milk powder. The protein quality was assessed by gain in body weight, PER, and increase in carcass nitrogen of rats, and apparent lysine availability by faecal excretion. The diets provided approximately 10% protein. The dough was baked in an air-oven in metal pans and by microwave baking in glass containers.

The nutritional value of the air-oven baked bread was significantly lower than that of the unbaked ingredients by all the criteria used, the decreases ranging from 24 to 45%, and between 20 and 32% lower than that of the microwave baked bread. The body weight gains of the animals fed the microwave bread diet were significantly lower than those fed the unbaked ingredients, but since they ate less food, the gain per unit of protein eaten was not significantly different. The apparent availability of lysine was about 20 to 30% less in oven baked bread than in the unbaked ingredients but little difference was found between the ingredients and the microwave baked bread.

Chapatis Milner and Carpenter (1969) prepared chapatis from three parts whole wheat flour and one part water, rolled into thin discs and cooked on a hot plate at 210°C for 2 min each side, without fat. After air drying, they were ground for incorporation in a diet for male and female weanling rats. The chapatis, representing approximately 60 to 64% of the diet, were fed to provide 10% crude protein (N × 6.25).

The PER values were higher for chapatis than for uncooked wheat. Milner and Carpenter (1969) consider that this improvement is due to increased palatability of the cooked product and hence to increased weight gain rather than to any improvement in the nutritional value of the protein resulting from the processing.

Chapatis were also included at a 13% protein level in a diet for chicks. The responses to the chapati diet and to the control, untreated wheat, diet were similar.

Shyamala and Kennedy (1962) compared, in rat feeding tests, (A) uncooked whole wheat flour, 1.67% nitrogen (N), (B) chapatis of whole wheat flour, 1.60% N, (C) chapatis of 90% whole wheat flour and 10% defatted soya flour, 2.35% N, (D) a skim milk diet, 5.93% N. Each diet provided 1.5%

N and the tests continued over 4 weeks. Body weight gains and PERs were determined. The factors used to convert N into protein were 5.83 for the whole wheat flour, 5.79 for the flour plus defatted soya flour and 6.38 for the milk diet.

The chapati diet was significantly better than uncooked flour, which Shyamala and Kennedy attribute to the destruction of trypsin inhibitor(s) but which Bender (1969) suggests is the result of improved palatability. The soya supplemented chapatis produced 51% increase in PER over the chapatis without soya and were not significantly different from the milk diet in body weight gain or PER.

In a second series, puris (similar to chapatis but deep fried in hot vegetable oil) displayed PER values 22% lower than whole wheat chapatis. The food intake from the puri diet was lower than that from the chapati diet.

Baking Powder Biscuits (Scones) The effect of baking on the PER of the protein in baking powder biscuits (scones), muffins and griddlecakes was assessed by Kennedy and Sabiston (1960) in rat diets providing approximately 10% protein fed ad libitum over 4 weeks. Cookies were also fed but assessed over 3 weeks. The PERs for the unbaked ingredients were significantly greater than for the baked products. The reductions in PER of the baked products compared to unbaked ingredients are shown below:

	Baking time (*min*)	Baking temperature (°C)	PER reduction compared to unbaked ingredients (%)
Biscuits (scones)	12	218	12.7
Muffins	23	204	14.5
Griddlecakes	2	204	17.8
Cookies	10	177	15.5

No apparent relation was noted between degree of reduction in PER and the extent of colouration of the crust. It is not mentioned whether the approximate ratios of crust:crumb were determined in the various products.

Clark et al. (1959) assayed microbiologically the loss of lysine in powder biscuits (scones) baked at 232°C for 20 min. Biscuits were prepared for analysis by drying at room temperature and extraction by petroleum ether. The mean concentration of lysine in the standard biscuits was 126.4 ± 9.7 mg/g N; the mean percentage of Kjeldahl N was 2.18. The lysine content of the flour was 139.3 mg/g N. Therefore 9.1% of the lysine in the flour was destroyed or rendered unavailable to the microorganisms as a result of baking. As baking time increased so did the loss of lysine; 18.1% in 25 min, 27.0% in 30 min. When L-lysine HCl was added the proportional losses were comparable to those of the lysine naturally present. Variations in the other ingredients had no effect except that, for any given baking time, higher proportions of sugar increased the loss of lysine. The concentration of lysine was significantly lower in the crust than in the crumb.

Cake An unbaked cake mixture (with a contribution of essential amino acids approximating that of whole egg) was found by Block et al. (1946) to have a protein efficiency between 3.3 and 3.5 when fed to protein-deficient adult rats. When the mixture was baked into a cake, and dried before feeding, the efficiency fell to 2.4, and when slices of the cake were toasted at 100 to 130°C the efficiency fell to 0.7. The reduction was apparently due to the destruction or non-availability of the lysine since the addition of 0.63% of L-lysine to the toasted cake restored the protein efficiency to 3.2.

Alimentary Pastes Cubadda et al. (1970) conducted rat feeding trials to determine the effect of drying at various temperatures on the nutritive value of alimentary pastes. Earlier work by the same authors had shown that using the chemical method of Carpenter (1960) as modified (Roach et al. 1967), and a microbiological method, there was a reduction in available lysine depending on the drying temperature, amounting to a 40% loss at 80°C. In the present experiments, the pasta dried at (A) ca 20°C, (B) 60°C and (C) 80°C contributed 89% of the 10% protein (N × 5.7) diet for male and female albino rats, six per group.

The PER, NPU and FCE (Food Conversion Efficiency) values are given in Table 103. The lysine content of the pasta (determined by autoanalyzer) fell from 1.84% of protein for pasta (A) to 1.75% for pasta (B), to 1.67% for pasta (C). The reductions in nutritional value, and in lysine, with increase in drying temperature are significant.

Toepfer et al. (1969, 1972) compared the amino acid composition of a durum wheat of 15.6% protein (N × 5.83, dry basis), with that of the

TABLE 103. Food consumption, weight gain, FCE (food conversion efficiency), PER and NPU of male and female rats fed pasta dried at various temperatures (Cubadda et al. 1970).

Treatment	Weight gain (g)	Average food consumption per head (g)	FCE	PER	NPU
Males					
Diet (A) (Ambient temperature) ca 20°C	19.41 ± 3.29	163.7	0.116 ± 0.017*	1.07 ± 0.15	52.0
Diet (B) (Temp. 60°C)	18.09 ± 5.02	161.8	0.109 ± 0.014	1.01 ± 0.16	51.6
Diet (C) (Temp. 80°C)	16.25 ± 4.04	161.2	0.104 ± 0.017	0.96 ± 0.18	46.6
Females					
Diet (A) (Ambient temperature) ca 20°C	24.33 ± 5.46	175.4	0.135 ± 0.022	1.25 ± 0.21	51.1
Diet (B) (Temp. 60°C)	19.33 ± 4.03	168.3	0.118 ± 0.017	1.09 ± 0.18	47.8
Diet (C)	16.67* ± 2.29	162.5	0.108 ± 0.010	0.98** ± 0.12	46.2

*$P < 0.01$. **$P < 0.001$.

semolina (15.2% protein) and the macaroni (14.9% protein) prepared from it.

The macaroni was prepared commercially with a dough absorption of 25% and a drying time of 41 hours at 38°C. Lysine (determination method to be published) in the durum wheat was 433 mg/100 g grain, dry weight. Lysine in the semolina was 76% and in the macaroni 77% of that found in the wheat.

Breakfast Cereals Griswold (1951) reviewing the literature on the effect of heat on the nutritive value of proteins concluded that the nutritive value of the protein of cereals was not damaged by cooking with water but was lowered by toasting, as in processing flaked or puffed cereals. Bender (1966) in a later review concluded that heating, rolling and flaking processes do not affect the protein but the process of "explosion" puffing, in which temperatures of about 200°C and pressures of 100 to 200 lb are used, may reduce PER values significantly. For example, wheat balls fell in PER from 1.6 to 0.3 during puffing. Since breakfast cereals are not normally a major component of diet, and since they are usually a vehicle for milk, these losses are not regarded as serious in developed countries. However, high temperature extrusion and explosion puffing should be avoided in the processing of cereal and protein foods for infants and malnourished persons, especially in developing countries.

Bulgur Bulgur or burghul consists of parboiled wheat and is a common food of the Middle East. In its preparation, the whole wheat is steeped in hot water to swell the grain, then boiled and finally dried (Milner and Carpenter 1969). Jamalian and Pellett (1968) determined the amino acid composition (by autoanalyzer, tryptophan by Lombard and DeLange 1965) for two samples of bulgur. The analysis for the essential amino acids is given in Table 104.

TABLE 104. Essential amino acid composition of bulgur (data from Jamalian and Pellett 1968).

	(mg/g N)
Tryptophan	45
Threonine	172
Isoleucine	195
Leucine	390
Lysine	160
Methionine	90
Cystine	123
Phenylalanine	253
Tyrosine	183
Valine	230

The data of Jamalian and Pellett (1968) does not relate the nutritive value of the protein of the bulgur to that of its original wheat. The effect of the processing is however reviewed and discussed by El-Lakany et al. (1969) and by Milner and Carpenter (1969).

El-Lakany et al. (1969) reviewed earlier work on the effect of various treatments on the biological value of the protein of whole wheat. They cite Beaudoin et al. (1951) who found that the biological value improved when the wheat was cooked in boiling water as in the preparation of shredded wheat, and also cite Shammas and Adolph (1954), Yang and Al-Nouri (1962) and Kohler (1964) who reported that the nutritive value of wheat is unaffected by mild cooking as in the preparation of bulgur.

In their own experiments, El-Lakany et al. (1969) compared the metabolizable energy (ME) for chicks of two samples of wheat of protein 15.4% and 12.1% respectively (Kjeldahl N, conversion factor not stated). The wheat was: (A) untreated, ground; (B) autoclaved for 60 min at 15 lb pressure, fan-dried at room temperature and ground; (C) water-soaked overnight, autoclaved for 60 min at 15 lb pressure, fan-dried at room temperature, ground; (D) frozen for 5 days at $-4°C$, thawed and ground.

All the treatments significantly improved the metabolizable energy (ME) content of the wheat for chicks compared to the untreated control wheats. Soaking the wheat in water before autoclaving in treatment (C), did not enhance the effect of autoclaving. The cause of the significant increase in ME values following freezing is not explained.

Wheat of protein content 15.4% was fed, (A) untreated, and after autoclaving for (E) 60 min, (F) 90 min and (G) 120 min at 15 lb pressure, followed by fan-drying at room temperature. Again, the ME value of the wheat autoclaved for 60 min was significantly improved over that of the untreated control but the ME values of treatments (F) and (G) were significantly reduced compared to that of the untreated control.

Milner and Carpenter (1969) compared the PER and NPU values for male and female rats, fed over 10 days, on American hard winter wheat of 17% protein ($N \times 6.25$, dry matter basis) treated as shown below:

(A) Raw wheat; (B) wheat steeped at 80°C for 70 min, cooked at 5 lb pressure for 30 min and oven-dried at a temperature below 40°C (mild treatment); (C) wheat steeped at 80°C for 70 min, cooked at 10 lb pressure for 60 min and oven-dried below 40°C (moderate treatment); (D) wheat steeped at 60°C for 4 hours, cooked at 15 lb pressure for 15 min, oven-dried below 40°C, then steeped at 80°C for 70 min, cooked at 10 lb pressure for 1 hour and oven-dried below 40°C (severe treatment).

The results are given below:

	PER	NPU
(A) Raw wheat	1.12	39.7
	As percent of control (A)	
(B) Mild treatment	110	101
(C) Moderate treatment	89	91
(D) Severe treatment	85	90

The higher PER given by bulgur (B) was probably due to improved palatability; NPU was unaffected.

In further trials, growth response and protein efficiency of chicks was used to compare (A), (B) and (D) with: (E) wheat steeped in a large volume of water at 80°C for 70 min, and then dried; (F) wheat steeped as for (E), boiled for 15 min in excess water, drained and oven-dried at a temperature below 40°C.

Growth response and "PER" were significantly lower for (D) than for (A); there was no positive response to the other products.

When (A), (E) and (F) were fed at 92.7% of the diet to rats, PERs were significantly higher than control, (E), 153% and (F), 145%.

When (A) and (E) were fed in a further experiment with rats, PER and NPR increased significantly but NPU did not: (A), 36.9; (E), 37.8. In this trial, the appetite quotient (rate of food intake in relation to its metabolic size, i.e. body weight) 0.88, was significantly higher for (E) than for (A), that is the steeped wheat was more palatable than the uncooked control. The digestible energy (cal/g dry matter) of (E) was 4% higher than for (A).

Milner and Carpenter (1969) also compared PERs and appetite quotients of rats fed 13% protein diets in which the sole source of protein was (A) untreated wheat; (G) wheat germinated until the grains just began to sprout, dried; (H), (G) treated as (E); (J), (G) treated as (B); (K), (G) treated as (B). The trend in results was as for the other experiments; the best performance being that of (H), the steeped, germinated wheat.

There was no difference in total lysine (Moore et al. 1958 and Carpenter et al. 1963) between (A)

and (D) but FDNB-available lysine (Carpenter 1960) fell by 14% in the production of (D), severely treated bulgur. Total cystine also fell by 14% but none of the other amino acids investigated showed any change. Heat processing had no significant effect on the availability of leucine, tryptophan and methionine determined microbiologically.

In summary, mild heat processing of wheat improved its palatability for rats which grew faster and gave higher PER values than when fed unprocessed wheat. Severe heat treatment resulted in a 10% fall in NPU with some destruction of cystine and reduced availability of lysine.

Pence et al. (1965) reported on the results when seven samples of bulgur prepared commercially in the United States, and the wheats from which they were prepared, were compared for the effect of processing on the nutritive value of the wheat. Processing conditions are not reported in detail but range from continuous pressure cooking to continuous cooking at atmospheric pressure with differing intermediate treatments. The protein (N × 5.7) contents of the wheat and the bulgur were not significantly different nor were the PER values (method not given). Because of the nature of the survey, statistical analysis was not possible. In summary, Pence et al. (1965) consider that, in general, the nutritional values quoted in the literature for wheat may be used for bulgur.

Shepherd et al. (1965) compared bulgur produced from four different wheats: (A) by a hot lye treatment followed by a thorough washing, (WURLD WHEAT); (B) by a conventional method, with (C) the wheats from which they were made. Lye-peeling, which produces a light coloured bulgur even from red wheats, did not affect protein content (N × 5.7). Average PER values were not significantly different between wheat and conventional bulgur (wheat 1.17; bulgur 1.15) but the PERs of the lye-peeled bulgur averaged 1.02. Shepherd et al. (1965) suggest that this reduction may be due to the leaching of some of the water-soluble proteins and/or to partial destruction of lysine.

Steamed Wheat Hutchinson et al. (1964) compared the growth rate (grams per gram nitrogen consumed) of male weanling rats, eight per group, over 21 days, on diets containing 1.53% Kjeldahl nitrogen, and composed of: (A), unheated wheat from a commercial (UK) mill, laboratory-milled to about 65% extraction rate; (B), wheat from the same grist, steamed at the mill, and laboratory-milled as (A). Flour (B) gave a significantly better growth rate of 4.5 compared to 3.3 from flour (A).

In further trials, six rats per group were fed (C), Manitoba wheat, unsteamed; (D), the same as (C), laboratory-steamed, as wholemeal, in diets containing 2.55% Kjeldahl nitrogen over 28 days. Wholemeal (D) gave a significantly higher growth rate, 7.45, than wholemeal (C), 6.55, showing that the effects were not caused by variability in the milling process.

The improvement given by the steamed wheat diets were shown, statistically, to be accounted for, almost entirely, by the increased food intake on the steamed wheat diet. Hutchinson et al. suggest that this increase in food intake could be due to (1) the destruction of the trypsin inhibitor, (2) improved palatability, or (3) the treatment having destroyed the capacity of the wheat to form gluten, thus making the meal or flour more easily and efficiently masticated and digested. The last suggestion was based on the observation that rats fed barley (which does not form gluten), steamed and unsteamed, showed no significant difference in rate of growth, food intake and efficiency of utilization.

Rhizopus oligosporus fermentation Tempeh is an Indonesian product prepared by fermenting soya beans with species of the genus *Rhizopus*. Wang et al. (1968) compared the PER of: (A) casein; (B) wheat, slightly cracked, washed and boiled for 12 min, drained and cooled; (C), as (B), followed by inoculation with a spore suspension of *R. oligosporus* NRRL 2710, and incubated at 31°C for 24 hours; after incubation, steamed for 5 min to destroy the mould, freeze-dried and ground; (D) soybeans, slightly cracked, washed and boiled for 25 min, drained and cooled; (E), (D) inoculated and treated as (C); (F), equal weights of (B) plus (D); (G), equal weights of (B) plus (D), followed by incubation as for (C). The results are given in Table 105.

Fermentation of wheat improved the growth rate and PER significantly but did not have a significant effect on soybean. However, the fermented mixture of soybeans and wheat produced a PER comparable to that of casein.

The amino acid composition (by autoanalyzer; tryptophan, microbiologically) and availability (in vitro by pepsin-pancreatin digestion) was determined on (B), (C), (F) and (G). The essential

TABLE 105. Rat PERs of wheat and soybean fermented by *R. oligosporus* (data from Wang et al. 1968).

	PER
(A) Casein	2.81±0.10
(B) Wheat, boiled	1.28±0.05
(C) Wheat, fermented	1.71±0.05
(D) Soybeans, boiled	2.17±0.03
(E) Soybeans, fermented	2.27±0.05
(F) Soybeans + wheat (1:1), boiled	2.49±0.04
(G) Soybeans + wheat (1:1), fermented	2.79±0.04

amino acid composition of wheat and wheat plus soybean was not significantly altered by fermentation but the available lysine in wheat was increased.

Wheat fermented for 12, 24, 48 and 72 hours was also incorporated into rat feeding diets. On the basis of PER value and growth of the rats, the optimal fermentation time for wheat by *Rhizopus* was 48 hours.

Enzyme Treatment Although wheat bran contains about 17% protein, on a dry basis, monogastric animals are able to utilize only about 60 to 70% of the protein (Saunders et al. 1972). Different cellulolytic enzymes available commercially in the USA were used to process wheat bran and one of these (Pectinol 41P) increased the nutritive value of bran as determined by rat feeding.

Supplementation with Other Protein Sources

Protein supplements and supplementation have been considered by several conferences and working committees (United Nations 1968; Association of Food Scientists and Technologists (India) 1969; Milner 1969). The text which follows has been organized according to the sources of supplementation; synthetic amino acids, cereal proteins, egg and milk proteins, food legume and oilseed protein, protein from microorganisms and fish protein.

Synthetic Amino Acids

Rosenberg and Rohdenburg (1951) compared American bread containing shortening, sugar and dry skim milk with similar breads fortified with 0.5% DL-lysine HCl and 0.25% L-lysine HCl. The loss of lysine in the standard during baking was found by microbiological assay to average 15% varying from 9.5 to 23.8%. The average loss in bread fortified with DL-lysine HCl was 11% (range 2.4 to 21.8%) and with L-lysine HCl the loss averaged 32% (range 25 to 36.8%). The lysine content fell between 5 and 10% during toasting.

Rosenberg and Rohdenburg (1952) demonstrated a significant increase in PER by the addition of 0.3% L-lysine.

Sure (1954) in rat feeding tests reported the nutritive values of whole rye flour to be markedly superior to whole wheat flour when fed at 9, 8 and 5% protein levels over a period of 10 weeks. Additions of L-lysine and L-lysine plus DL-threonine improved the growth response to whole wheat flour at an 8% protein level. Little growth response was noted when lysine and threonine were added to whole rye flour at the 8% protein level.

Sabiston and Kennedy (1957) compared whole wheat bread and its unbaked ingredients containing additions of 0, 3, 6 and 12% nonfat dry milk and 0.166% L-lysine, with white bread containing 6% nonfat dry milk. Rat growth studies indicated that white bread containing nonfat dry milk was superior to whole wheat bread without milk and somewhat inferior to whole wheat bread containing 3% nonfat dry milk. The PER of whole wheat bread containing 0.166% L-lysine (roughly equivalent to the amount of lysine in 6% nonfat dry milk) was similar to that of bread containing 6% nonfat dry milk. The PERs of all baked bread were approximately 20% lower than the PERs of their unbaked ingredients.

Stromnaes and Kennedy (1957) reported the PER of bread to be increased by 9%, and of the unbaked ingredients by 11%, by the addition of up to 6% milk solids. The addition of 0.166% lysine increased the PER of both the bread and unbaked ingredients by roughly 21%.

Jahnke and Schuck (1957) reported that the addition of three parts of nonfat milk solids and 0.25 part of lysine per 100 parts of flour were comparable in protein value to the addition of 12 parts of nonfat milk solids without lysine.

Bender (1957) reported that, when supplemented with 0.2% lysine, 1.4% threonine and 1.1% methionine, British bread gave an NPU value of 80. The NPU values were 46 and 57 respectively for bread alone and bread to which 0.2% lysine had been added.

Hutchinson et al. (1958) demonstrated that lysine is the first limiting and threonine the second limiting amino acid in unsupplemented

wheat bread. Rat body weight gain on unsupplemented bread was about 20% of that on an egg protein diet. When lysine and threonine were added to bread to the point that they were no longer limiting, growth efficiency rose to approximately 85% of the egg protein diet.

Culik and Rosenberg (1958) compared white bread containing 6% nonfat dry milk with bread supplemented with 0.25% L-lysine HCl. The comparison was carried out through five successive rat generations. Lysine supplementation improved reproduction and lactation significantly and uniformly throughout the seven litters of the parent generation and through all successive generations. Breeding performance on the commercial bread diet was inferior to the lysine supplemented diet. Bread supplemented with 0.25 L-lysine HCl as the sole source of dietary protein maintained near normal reproduction and lactation.

Brown et al. (1959) compared a commercial white bread and three types of commercial high protein bread in rat growth tests in which bread provided the sole source of protein. Bread was tested with and without lysine supplementation, the percentage of protein from wheat varying from 89 to 60% and from milk and soy proteins from 11% to 40%. The PERs were found to be a direct function of the lysine content of the mixed protein regardless of whether the lysine was added as L-lysine HCl or as a constituent of milk or soya. PER values above 2.0 were recorded only when L-lysine exceeded 200 mg per gram of nitrogen.

Hutchinson et al. (1960) also confirmed that, when fed white bread as the sole source of protein, the mean rate of growth of weanling rats was directly proportional to the mean intake of lysine. From the results of the experiment, in which the protein content of the diet was between 12 and 13% and the amounts of added lysine ranged from 0 to 0.25%, Hutchinson et al. related the rate of growth y (grams per rat per day) to the intake of lysine x (milligrams per rat per day) by the empirical equation $y = 0.06154x + 0.03$. Substitution in the equation of data from growth rates comparing bread crumb with bread crust indicated a loss of 20% lysine in the crust. The slightly lower rates of growth recorded suggest a reduction in available lysine during processing of wheat germ.

Zentner (1961) reported an increased browning of bread crust baked from wheat flour to which amino acids had been added. Added glycine and lysine produced a decrease in gas production which, Zentner suggests, resulted from the formation of N-substituted glycosylamines by amino acids-reducing sugar interaction.

Yang et al. (1961) studied first the effect in rats of graded additions of lysine to wheat flour and second, whether a single daily dose of lysine administered by stomach tube was nutritionally equivalent to the same amount of lysine included in the diet.

Between 0.2 and 0.25% lysine improved body weight gain though certain adverse effects were noted at the 1% lysine addition. No difference in growth was noted between lysine in the diet and lysine fed by stomach tube 4, 8, 12 or 16 hours after normal feeding.

Barness et al. (1961) compared white wheat flour (commercial "cream of wheat") as the chief source of protein in the diets of 22 malnourished male Latin American infants aged 3 to 17 months in a children's hospital in Texas, in diets (A) with and without milk, (B) with and without lysine supplementation. Following an adjustment period, nitrogen balance studies were carried out for nine days.

Lysine in the "cream of wheat" was determined as 135 mg/g N by an unstated method. L-lysine HCl was added to yield a total of 0.55% of the wheat. The diets also included starch and sugar, and the infants received a multivitamin mixture daily. Caloric intake varied from 75 to 100 to 120 kcalories/kg per day and protein (N × 6.25) from 1.2 g to 4.0 g protein/kg per day. In the wheat-milk mixture 70% of the protein was obtained from the wheat and 30% from the milk on which diet the infants remained in N equilibrium even when total protein was less than 2 g/kg per day and caloric intake 75 kcal/kg per day. Supplementation with lysine and potassium gave no improvement.

A positive effect of supplementation was considered to exist if the N balance during the supplemented period differed by more than 10% of the N intake from the most positive control period; a negative effect if the control period exceeded the supplemented period by more than 10% of the intake, and no effect if the balances were between those limits.

Of the two infants fed wheat alone at 75 kcal/kg per day and protein at approximately 2.0 g/kg per day, one showed increased N reten-

tion and one no effect when lysine was added. When the wheat was supplemented with potassium alone, there was an increased retention in all three periods studied, and when both lysine and potassium were added, there was increased retention in two of the three periods, and no effect in one. In the baby who showed increased N retention from lysine alone, the effect was much greater with the double addition of lysine and potassium.

With five babies fed wheat alone at 100 kcal/kg per day and protein at 2.0 g/kg per day, there was no effect in two periods when lysine alone was added, no effect in the three periods when potassium alone was added, but a significant effect when both lysine and potassium were added.

When wheat alone was fed to four babies at 100 kcal/kg per day and protein levels of respectively, 2.5, 3.5, 4.0, and 4.0 g/kg per day, only the baby receiving the 2.5 g protein level showed a positive effect in N retention with potassium supplementation and an even more marked effect after the addition of lysine and potassium.

Potassium balances determined in 14 babies indicated a relationship between increased potassium retention with increased N retention.

Positive nitrogen balances were obtained when wheat was fed alone at total protein levels of 2 to 4 g/kg per day. The data indicated that wheat supplemented with lysine and potassium, as determined in the present study, is an adequate source of protein for growing infants for the period covered in the study. The lysine and potassium were effective only when mixed with the daily wheat diet.

Pomeranz (1962) did not find any significant correlation between the lysine and protein contents of flours of equivalent extraction rates, but higher extraction flours contained significantly higher lysine contents. Small losses of lysine in the crumb occurred in bread made from commercially milled flours enriched with soy flour, yeast, gluten or wheat germ. Pomeranz suggests that protein-fortified breads should be classified by their protein and lysine contents rather than by a description of the level of fortification.

King et al. (1963) studied the effect of feeding bread, made from white wheat flour enriched to United States standards and the same bread additionally fortified with L-lysine HCl, at the level of 625 mg/100 g flour to children aged 6 to 18 years in two villages in Haiti. The bread contained 4.0 to 4.6 mg L-lysine HCl, which was consistent with an expected loss of 15% during baking. The daily ration of 150 g per child was given in two portions, and was provided on 150 school days in a total period of 261 days. Some 450 children were divided into four age groups for each sex and examined for height and weight before the feeding program began, at a mid point, and at the end. King et al. reported that although the increments in stature, weight and mean corpuscular haemoglobin concentration "are not impressive", they were, in certain cases, statistically significant, indicating some benefit from lysine supplementation. Marked weight gains occurred even in the children fed bread without lysine, compared to those in the control school.

Hutchinson et al. (1963) reported when dried bread crumb provided 85% of the diet and the sole source of protein for rats that the addition of 0.5% L-lysine and 0.2% of L-threonine improved bread protein quality to the rank of "first class". The supplemented bread crumb contained a total of between 0.75 and 0.80% lysine and between 0.5 and 0.6% threonine. Equivalent levels of lysine and threonine required additions to the bread crumb of 6.2% casein, 8% egg albumin, 21% soy flour or 18% skim milk powder. It should be noted that whereas the addition of lysine and threonine did not affect the protein content (12.5%) of the dried bread crumb, the additions of casein, egg albumin, soy flour and skim milk raised the protein to 18.6%, 20%, 18.6% and 17.2% respectively.

Gates and Kennedy (1964) fed to rats unbaked bread ingredients containing 3, 6 and 12 parts of nonfat dry milk per 100 parts of flour with and without 0.25 part of L-lysine HCl, and the corresponding breads. The unbaked ingredients without added lysine showed significantly increasing PER values with increasing milk solids. The addition of lysine resulted in significant increases in PER both with unbaked ingredients and with bread at all levels of added milk, the proportional increase declining as the level of milk solids increased. At all levels of milk, and in samples with and without lysine, increases in PER were significantly inferior in animals fed bread as compared with the unbaked ingredients, the average decrease in PER when the ingredients were baked into bread being of the order of 21%.

Jansen et al. (1964a, b) demonstrated that PERs of both unsupplemented bread and bread supplemented with 0.3 of L-lysine HCl per 100 g of flour were reduced as baking time increased from 0 to 50 min. Of the added lysine, 30% became un-

TABLE 106. Protein content of macaroni, total L-lysine HCl in protein, mean protein intake and PER of plain and enriched macaroni, with and without added lysine HCl, on rat PER (data from Bains et al. 1964).

	Protein content of macaroni (%)	Total L-lysine HCl in protein (%)	Mean protein intake (g)	PER
(A) Plain macaroni	12.0	3.16	19.59	1.10
(B) (A) diet plus L-lysine HCl 0.25 g/100 g	12.0	5.40	26.95	2.11
(C) Enriched macaroni (plus casein and skim milk solids)	17.0	6.80	28.98	2.48
(D) (C) diet plus L-lysine HCl 0.25 g/100 g protein	17.0	9.60	29.08	2.63

available after baking for 30 min at 450°F (232°C). When the baking time was reduced to 20 min no significant nutritional loss was found by rat growth assay though an 18% loss was indicated by ion exchange chromatography. In bread without milk powder 15% of the added lysine became unavailable when the bread was baked for 30 min at 450°F (232°C). The loss of lysine during baking increased significantly in the presence of nonfat dry milk. When additions of between 6 and 14% of nonfat dry milk were made to doughs baked for 20 min at 450°F (232°C) the loss of lysine was about 36%.

Bains et al. (1964) compared rat PERs of: (A) macaroni made from Indian durum wheat semolina, (B) diet (A) fortified with 0.25 g L-lysine HCl per 100 g protein after processing, (C) enriched macaroni made from durum wheat semolina, casein, and skim milk solids in the weight ratios of 90:7:3, (D) diet (C) fortified with 0.25 g L-lysine HCl per 100 g protein, after processing. The macaroni was included in 10% protein rat diets fed ad libitum over 28 days. The results are given in Table 106.

Addition of lysine to plain macaroni in diet (B) significantly improved the quality of the protein but fortification with milk protein and casein in diet (C) effected a still greater improvement. The PER of macaroni in diet (C) was not significantly improved by the addition of lysine. Rats fed diet (B) had significantly lower percentage of fat in their livers than those on diet (A).

Howe et al. (1965a, b) discuss the effect on PER of supplementing a wide range of cereals with protein sources including fish flour, peanut flour, sunflower, cottonseed flour, sesame flour, soybean flour and mixtures with and without added lysine and other amino acids.

Yamazaki (1968) recommended that bread sold in Asia should be fortified by lysine up to 0.25% of the flour weight.

Maleki and Djazayeri (1968) studied the supplementation of Arabic bread with lysine, threonine and methionine. They found the PER (0.57) of non-supplemented flour to decrease to 0.4 during baking. The addition of 0.3% L-lysine raised the PER of flour to 1.4 and of bread to 1.5, and 0.6% threonine added before baking raised the bread PER to 2.3. Methionine produced no significant change.

Hedayat et al. (1968) studied the biological value of Iranian commercially milled whole wheat flour, with and without additions of L-lysine HCl when baked into Iranian village type bread. The flour was sifted by the baker before use to remove about 5% of the bran and coarser particles.

The NPU of unenriched whole wheat flour was found to be 42.0 and of village bread 42.5. The equivalent Biological Values were 46.0 and 46.5. The NPU of the flour with 0.2 g L-lysine HCl per 100 g flour was 50.5 and of the village bread 60.0. The equivalent BV were 55.5 and 67.0.

Food consumption and growth rate were higher in the rats fed bread from enriched flour than in those fed bread from unenriched flour.

Hedayat et al. also found that weanling male Wistar rats showed significantly higher serum albumin, liver fat and liver nitrogen when fed whole wheat flour supplemented with 0.2 g L-lysine HCl/100 g flour than those fed unsupplemented wheat flour. No similar differences were found among female rats.

The effect of the supplementation with L-lysine and DL-threonine, of Indian wheat and of a poor wheat diet, was studied in groups of eight 20-day-old male albino Wistar rats by Daniel et al. (1968b). The lysine and threonine contents of the wheat and of the diets were assayed microbiologically. The protein content of the basal wheat diet was 10.3% and of the basal poor Indian wheat diet (PWD) was 12.3% (N conversion factor not stated). The lysine and threonine contents of the wheat were, respectively, 3.0 and 2.4 g/16 g N and of the PWD 3.3 and 2.8 g/16 g N. L-lysine and DL-threonine were added to raise the levels approximately to those in egg proteins, namely 6.6 g and 4.9 g/16 g N respectively. PER of the wheat increased from 1.62 to 1.99 when L-lysine was added and to 3.02 when L-lysine and DL-threonine were added. The PER of skim milk powder was 3.13. The PER of the PWD diet when also fortified with vitamins and minerals increased significantly from 2.03 to 2.58 on the addition of L-lysine and to 2.70 on the addition of both lysine and threonine. The difference between the PERs on the addition of lysine and of lysine plus threonine was not significant. The PER (1.73) of the PWD diet when it was not also fortified with vitamins and minerals showed no significant improvement on the PER (1.90) with addition of lysine; the addition of lysine and threonine improved the PER to 2.18 which was significantly higher than the basal diet but not significantly higher than the diet supplemented with lysine alone. It was concluded that deficiencies in vitamins and minerals must be corrected in order to gain greatest benefit from supplementary amino acids.

The effect of supplementing a poor wheat diet with L-lysine and DL-threonine was also studied by Daniel et al. (1968a) in feeding trials with seven boys aged 10 to 12 years living in a boarding house in Mysore, India. The basal wheat diet was that customarily eaten in the home and provided daily 44.7 g protein (N × 6.25), of which whole wheat flour contributed 33.3 g, red gram dhal (*Cajanus cajan*) 3.4 g, skim milk powder 1.8 g, and vegetables and condiments 6.2 g. A diet based on skim milk powder in which maize starch and skim milk powder were substituted for the whole wheat flour and the red gram dahl in the wheat diet was used for comparison.

The experiment comprised six periods each of 10 days duration, the first five days being treated as a preliminary period, faeces and urine being collected in the second five days. The order in which the diets were fed was (1) basal wheat, (2) wheat plus 1.5 g L-lysine HCl/child per day, (3) wheat plus 0.86 g DL-threonine/child per day, (4) wheat plus 1.5 g L-lysine HCl and 0.86 g DL-threonine/child per day, (5) skim milk powder diet, (6) low protein diet, similar to that of Parthasarathy et al. (1963). The proteins of the wheat diet contained, in grams/16 g N, lysine 3.1 and threonine 3.8 (wheat protein containing lysine 2.4 and threonine 2.8).

Nitrogen in the diet, urine and faeces was determined by micro-Kjeldahl. The digestibility coefficient and the biological value of the diets were calculated according to Tasker et al. (1962). The net protein utilization operative (NPU_{op}) was calculated according to Platt et al. (1961).

The wheat diet at a level of 1.5 g protein/kg body weight met all the essential amino acid requirements of children as assessed by Nakagawa et al. (1962) except for lysine. Nitrogen retention on the wheat diet was 10.2% of intake. Supplementation with L-lysine raised N retention significantly to 21.5% of intake. Supplementation with DL-threonine raised retention to 14.4% but this was not significant. Supplementation with both L-lysine and DL-threonine increased retention to a highly significant 30.4% of intake, comparable with 31.3% N retention on the milk diet.

The NPU of the wheat diet was 37.7, which increased significantly to 48.7 by supplementation with L-lysine and non-significantly to 42.0 by supplementation with DL-threonine. Supplementation with L-lysine and DL-threonine significantly increased the NPU to 57.3, comparable to that from the milk diet of 59.1.

The protein intake ranged from 1.78 to 1.80 g/kg body weight on the different diets. The net available protein in grams per kilograms body weight was: wheat diet 0.67, wheat plus L-lysine 0.80, wheat plus DL-threonine 0.75, wheat plus L-lysine and DL-threonine 1.04, milk diet 1.11. These were compared with the Food and Agriculture Organization (1965) reference protein requirements of 0.72 g/kg body weight.

The effect of supplementing wheat with L-lysine HCl in the all-vegetable diet of children aged 2 to 5 years in an orphanage in Vellore, South India, was studied over six months by Pereira et al. (1969), the children being divided into two matched groups, (A) experimental with lysine supplementa-

tion and (B) control, without supplementation. The basic orphanage diet provided 2 g vegetable protein and 100 kcal/kg body weight per day. The wheat was fed as wheat flour and broken wheat grains, boiled, roasted or fried, and provided 54% of the daily calories and 85% of the daily protein. The only difference in the diets of the two groups lay in the lysine supplementation of the wheat portion of the experimental group's diet. From analyses (by autoanalyzer) of samples of cooked foods, children in the control group had an intake of 0.54 g lysine per day, those in the experimental group 0.73 g lysine per day. The losses of lysine during cooking were 21% in the control group and 30% in the experimental group.

At the end of the six months, the children in the lysine-supplemented group had grown significantly taller than those in the control group. Statistically significant differences in weight were not observed, both groups losing weight in the last month of the trial which occurred during the hottest part of the year. No statistically significant differences were observed between the groups in haemoglobin, packed cell volume, total serum proteins, serum albumin, retention of absorbed nitrogen, or daily creatinine excretion. Nor were significant differences observed in minor illnesses or general health.

Mitsuda (1969) described how bread fortified with lysine was tested among groups of Japanese school children. The bread was enriched with 0.8 g of L-lysine HCl per school lunch serving to offset the loss of lysine during baking. At this lysine level excessive browning of the bread together with a slightly bitter taste was noted. The loss of lysine was reduced by baking at a lower temperature for a longer time.

Adrian and Frangne (1969) studied biscuits baked from wheat flour plus gluten enriched alternatively with groundnut (peanut) meal, fish meal, skim milk powder or lysine. During baking the PER of groundnut- and fish meal-enriched biscuits fell by 30% and 40% respectively. Greatest losses occurred in the milk-enriched biscuits. Adrian and Frangne state the loss of amino acids from the "digestible" fraction to be less than is accounted for by the drop in PER. It would appear that lysine was rendered unavailable by interaction, possibly with reducing sugars.

Matthews et al. (1969) compared the effect of adding lysine to chapati and bread made from wheat flours of 93% and 85% extraction. They report that 25% of the added lysine was lost in the bread but only 4% in the chapati.

Jansen (1969) reported adding several protein supplements to bread. The protein content (dry weight basis) of the unsupplemented bread was 13% and the net protein value (NPV) 7.3. The addition of 4% nonfat dry milk raised the protein content to 16% and the NPV to 8.5; 4.1% toasted soya increased the protein to 17% and the NPV to 9.0, and 10% nonfat dry milk raised the protein to 17% and the NPV to 10. The addition of 0.3% L-lysine HCl did not affect the protein content but raised the NPV to 10.0, the equivalent of adding 10% nonfat dry milk.

Efremov et al. (1970) described how five middle-aged men employed in the baking industry of the USSR were fed a standardized mixed diet of 3400 Calories per day containing 500 g bread over five successive 10-day periods. Only the composition of the bread in the standard diet was changed for each 10-day period. During the first 10 days they received ordinary wheat bread (composition not recorded); during the subsequent periods the bread contained, per 100 g flour (A) 10 g dried skim milk, (B) 5 g fat-free fish flour, (C) 20 g soybean (composition not stated), (D) 0.5 g lysine HCl. The nature and composition of the non-bread portion of the diet is not given but the tabulated data indicates the bread provided about 40% of the total nitrogen intake. The subjects' body weight did not change and nitrogen balance remained positive throughout. The methodology is not described but Efremov et al. quote the Indices of Net Utilization of Protein (%) as follows: bread (control) 12.6, (A) plus skim milk 10.0, (B) plus fish flour 15.7, (C) plus soya 17.3, (D) plus lysine 15.1.

The quoted skim milk result appears to conflict with the conclusion that the addition of skim milk, fish flour, soya or lysine to bread improved the efficiency of protein utilization.

Clark et al. (1970) determined the requirements of adult human subjects 20 to 25 years old for methionine and cystine using white wheat flour as the dietary base. There were six men in the first experiment and five young men and two women in the second experiment. Amino acids were determined by autoanalyzer.

Part of the amino acids were provided by the flour and the remainder in crystalline form. Total daily nitrogen intake was 6.0 g per person. The requirements varied from 700 mg methionine plus

400 mg cystine to 260 mg methionine plus 280 mg cystine. The sulphur-containing amino acids present in 142 g white wheat flour appeared to be adequate for five of the six men in the first experiment.

Hedayat (1971) and Hedayat et al. (1971) describe a school lunch study conducted among children aged from six to 12 years of both sexes in five Iranian villages over eight months in the school year 1968-69. Children in three villages received bread enriched with lysine, vitamins and minerals. In the fourth village the lunch included bread plus vitamins and minerals but without added lysine. In the fifth village, neither lunch nor bread was provided. The bread was made locally from Iranian flour of 95% extraction enriched with a vitamin-mineral mixture alone, or with the same mixture and lysine HCl to give 2.3 g of lysine per kilogram of flour. Chemical and biological tests on rats conducted earlier (Hedayat et al. 1968) had shown that most of the added lysine was still available after baking into bread.

The bread was served at breakfast (125 to 150 g) and with lunch (150 to 200 g). The lunch meals provided approximately 50% of the recommended dietary allowances (National Academy of Sciences (US) 1964) for protein, calories, calcium and ascorbic acid, 66% of the thiamine, 85% of the riboflavin and niacin and 199% of the iron. The composition of the lunch dishes varied according to the seasonal availability of vegetables. Animal protein and pulses were excluded, the intention being to ensure a limitation in lysine. There was no control of food eaten by the children in their homes but a food consumption survey indicated that dietary protein was limiting in lysine in 97% of the relevant village households surveyed. In total, 203 children received lunch and lysine-supplemented bread, 90 children received lunch with bread without lysine supplement and 117 children received no lunch or bread. The children received a standardized clinical examination, and height, weight, skinfold thickness of the triceps and arm circumference were measured.

The children receiving the school meal showed a marked gain in weight and mid-arm circumference compared with the control group, but no significant difference in weight gain between the lysine and no-lysine groups was noted. The group receiving the school meal without lysine showed significantly higher height gains compared with the lysine and control groups, but the lysine groups showed significantly higher gains in mid-arm circumference than the no-lysine group.

The point is made that the final measurement of weight and/or arm circumference gain was taken in mid-spring when food was most scarce in the villages; this could explain the considerable number of children in the control group who showed loss of weight.

Hedayat et al. (1973) discussed the possible causes of the essentially negative results of the enrichment of bread with lysine and concluded that since the provision of school meals had a very positive result in improving weight and anthropometric measurements in children compared to those who did not receive school meals, school feeding programs might be more beneficial than lysine enrichment.

Ghai and Chaudhuri (1971) studied the effect of supplementing wheat flour with lysine in the diet of malnourished preschool children in India. The 44 children, aged one to six years, though free from chronic and acute diseases, had weights below 60% of the mean weight for age standards. They were divided into two groups of 22, matched for age, sex and weight. The diet provided 120 Calories and 2 g protein per kilogram body weight, all the protein and most of the Calories being provided by the wheat flour. The allowances were adjusted once a week to allow for weight gains, and the observations were carried on for a period of two months.

One group received the diet without supplementation, the other wheat supplemented with 0.1% lysine. The level of 0.1% was chosen because studies at the Nutrition Research Laboratory, Hyderabad (1967-68) had indicated that lysine supplementation at the 0.1% level increased the PER of wheat flour from 1.78 to 2.15 but that no further increase occurred when the level of lysine was raised to 0.2%.

The differences found between the two groups in the rate of weight gain, changes in serum proteins, and ratio between non-essential and essential amino acids in the serum were not significant.

Oiso (1971) reviewing clinical studies of amino acid fortification in Japan refers briefly to studies on adult males on a regimen consisting of 600 g bread and 60 g sucrose. Whether receiving supplements or not, the men remained in negative

N balance though supplementation with L-lysine HCl tended to improve the N balance and diminish fatigue.

Young and Scrimshaw (1971) reviewed clinical studies in the United States on the amino acid fortification of protein foods. They cite Hegsted et al. (1955) who found a positive effect of methionine supplementation at 1.0 g/day in young women fed an all-vegetable protein diet in which wheat provided 48% of the total nitrogen, and rice and other cereal products the remainder. Hegsted et al. (1955) also found that 0.9 g/day supplementary lysine improved nitrogen retention. The unsupplemented diet provided about 1200 mg lysine/day.

Young and Scrimshaw (1971) question whether the positive effect of supplementary methionine would have continued during a longer balance period since the intake of total sulphur-amino acids provided by the unsupplemented diet appeared adequate. They cite Hoffmann and McNeil (1949), who found that the nutritive value of wheat gluten fed to adult men could be significantly enhanced by supplementation with L-lysine. Using the nitrogen balance index (NBI) method developed by Allison (1959, 1964), they found, with nine subjects, that 4% L-lysine added to the protein improved the NBI from 0.62 ± 0.09 without supplementary lysine, to 0.76 ± 0.08, with supplementary lysine. The latter figure compares with a value of about 0.73 for casein (Allison 1959).

Bressani (1971) summarized earlier work (Bressani et al. 1960; Bressani et al. 1963) on the amino acid supplementation of Guatemalan wheat flour, of 70% extraction, in nitrogen balance studies in children. The essential amino acid composition of the flour is given as, in milligrams per grams nitrogen: arginine 229, histidine 155, isoleucine 239, leucine 375, lysine 159, methionine 101, cystine 112, phenylalanine 324, tryosine 191, threonine 183, tryptophan 52, valine 245. The basal diet in percent by weight consisted of: wheat 85, gluten 7, glycine 3, maize starch 5, to give a protein content (N conversion factor not stated) of 18.7 and 385 calories/100 g. In most of the studies the children received each diet for a total of nine days with a 4-day adjustment interval between treatments. Three balances were obtained per dietary treatment.

The weight of amino acids replaced an equal weight of maize starch and the nitrogen replaced an equivalent amount of nitrogen from glycine. Amino acids were added to give 270 mg lysine, 90 mg tryptophan, 270 mg methionine, 270 mg isoleucine, 270 mg valine and 180 mg threonine/g N in the diet. The results suggest that the main improvement in protein quality resulted from the addition of lysine although the addition of tryptophan and methionine further improved nitrogen retention.

Bressani (1971) considers that some of the effects observed with the mixture of amino acids may result from their low biological availability in wheat or to certain imbalances among them. Supplementation may not be beneficial if the amounts of individual amino acids added are greater than the optimum, thus giving rise to an overall imbalance.

To determine the optimum level of lysine to be added to wheat flour, balance tests were carried out at constant caloric intake on three children at two levels of dietary protein. At a dietary protein intake of 2.0 g/kg per day, 61 mg lysine per gram nitrogen was sufficient to increase nitrogen retention. At a protein intake of 3.0 g/kg per day, 107 mg lysine per gram nitrogen were needed. The ratio of lysine need to nitrogen intake was similar for both levels.

Reddy (1971) conducted nitrogen balance studies among Indian children, from two to five years, who were moderately undernourished though not showing signs of severe protein malnutrition. The diet, composed of wheat flour of protein content 10.7% (conversion factor not stated), safflower oil and sugar, was fed to provide 2 g protein and 100 kcal/kg body weight. Vitamin and mineral supplements were also provided. Baking the flour into chapatis did not give rise to a significant loss in available lysine (method of Carpenter, 1960). The lysine content was assayed microbiologically (Steele et al. 1949), from a water extract of the fortified wheat; the free lysine in the water extract was considered to be available. The experiment was divided into three 10-day diet periods: Period I, an unfortified wheat diet; Period II, wheat diet supplemented with 0.1% lysine HCl; Period III, unfortified wheat diet.

The unsupplemented wheat diet provided 56 mg lysine/kg body weight; the supplemented diet 72 mg lysine/kg body weight. All of the children gained weight over a period of four weeks and in all the nitrogen retained during the three periods

was essentially similar. The suggested protein requirement for infants is about 1.7 g/kg and for pre-school children about 1 g/kg (World Health Organization 1965a). Snyderman et al. (1959) reported the lysine requirements for infants to be about 90 mg/kg body weight. Assuming that amino acid requirements run parallel to protein requirements, the calculated lysine requirement for pre-school children would be 53 mg/kg body weight which is close to the 56 mg/kg in the unsupplemented wheat diet. This may explain the absence of any beneficial effect from lysine supplementation in the children discussed above.

Young and Scrimshaw (1971) reviewed clinical studies in the United States on the level and kind of non-specific nitrogen in the human diet and emphasized the need for extensive additional clinical studies into the contribution which the "non-essential" amino acids may take, in extending available protein supplies.

Kofranyi (1971) following long-term nitrogen balance tests on human subjects, also states that the biological value of proteins is not determined by the limiting essential amino acids alone but also by the proportions of the amino acid mixtures including sources of "non-essential" nitrogen.

Kies et al. (1972) reported on nitrogen balance studies in 10 adult men fed diets providing equal, but sub-optimal, amounts of protein (4 g nitrogen per subject per day) from rice, maize, wheat or milk plus, in each case, 0, 4.0 or 8.0 g N/day from non-specific nitrogen (NSN) consisting of an isonitrogenous mixture of glycine and diammonium citrate. The wheat source was enriched wheat flour with a nitrogen content of 0.0184 g N per gram flour, fed as drop biscuits. The experimental plan consisted of three successive 27-day periods. Each period consisted of an introductory 2-day nitrogen depletion phase (2.0 g N per subject per day), a 5-day nitrogen adjustment phase (4.0 g N per subject per day) and four experimental phases of 5 days each. These are considered to be of sufficient length to give data for accurate comparison (Kies et al. 1967; Kies and Fox 1970a). The sequence of the three periods and the order of the experimental phases within each section were randomized for each subject. Caloric intake for each subject was maintained at the level required for approximate weight maintenance by varying maize starch and fat intake. The essential amino acid content provided by 4.0 g N from the wheat was estimated from tables by Orr and Watt (1957). The nitrogen balance was the principal method of evaluation.

The mean nitrogen balances achieved on the wheat diet were (1) with no supplementation, −2.38 g N; (2) with 4.0 g NSN, −0.81 g N; (3) with 8.0 g NSN, −0.12 g N. These results confirm earlier findings that NSN supplementation has a sparing effect on the protein requirements of humans.

The importance of glutamic acid as a source of non-specific nitrogen in diets for rats on an amino acid diet was tested by Hepburn et al. (1960a). Male weanling rats when fed a diet containing an amino acid mixture patterned on that of wheat gluten, and supplemented with histidine, lysine, methionine, threonine and tryptophan showed an unusually high rate of weight gain. When the non-essential amino acids (except cystine and tyrosine) were eliminated from the diet, it was found that the omission of glutamic acid in each instance resulted in a reduced growth rate. The elimination of other non-essential amino acids did not produce the same effect. When increments of glutamic acid were added to glutamic acid-free diets, progressively greater weight gains were obtained. Maximum growth rate was obtained at the 5.66% level of glutamic acid. Food intake was also decreased at low levels of glutamic acid, perhaps as a result of reduced palatability in the absence of glutamic acid, or loss of appetite due to amino acid imbalance.

Taylor et al. (1972) examined the effect of lysine supplementation of wheat gluten in young male students at the Massachusetts Institute of Technology. Two levels of protein intake, 0.27 g and 0.73 g protein/kg per day and two levels of caloric intake were studied in seven subjects and eight subjects respectively. Caloric intake was either adequate for weight maintenance or 80% of the adequate level. Mean nitrogen balance at low protein and adequate caloric intake was, in grams nitrogen per day, −1.29 without, and −1.05 with, lysine supplementation. At low protein and inadequate caloric intake the corresponding balances were −2.02 and −1.70 g N per day. At the higher protein intake, lysine improved nitrogen balance by +0.43 g at both levels of caloric intake.

Tara et al. (1972) used atta (milled cleaned commercial wheat) to prepare chapatis and baked yeast-raised bread, without and with additions of L-lysine HCl. They found a loss of lysine of 12.5%,

as assayed microbiologically, in yeast-raised loaves fortified with 0.2% lysine HCl and a loss of 5.5% in the unfortified loaf. Loss of lysine in the chapatis was negligible.

Biological testing was carried out using 21-day old weanling male albino Wistar rats in groups of 10, over a period of 4 weeks. Each diet contained amounts of baked or unbaked wheat ingredients to provide about 10% protein (N × 5.7) and the animals were fed ad libitum.

Differences between the PER of the chapatis and the unbaked ingredients were statistically non-significant. The PER of the chapatis fortified with 0.15% L-lysine HCl was significantly higher (38%) than that of the unfortified chapatis.

The microbiological finding that the loss of lysine on cooking chapatis was negligible was confirmed by the finding that the PER of chapatis fortified with lysine before cooking (2.04) and the PER of those fortified after cooking (2.13) were little different. The animals fed lysine-fortified diets ate more food than did those on unfortified diets.

Although a loss of 12.5% in lysine content on baking bread was found microbiologically, and the PER of bread fortified with 0.2% lysine HCl before baking was 2.21 compared with a PER of 2.34 for bread fortified after baking, this difference in PER was not statistically significant. The PER of the fortified bread was however about 60% higher than that of the unfortified bread and the amount of food consumed by the rats on the lysine-fortified diets was higher than that eaten by the rats on the unfortified diets.

Hegsted (1971) stated there is abundant evidence that when an amino acid is added to a diet containing a nutritionally inadequate supply of that amino acid, the rate of weight of young rats is improved. He considers that none of the assay techniques so far available are satisfactory for evaluating the relative potency of protein for maintenance. He is doubtful that assays obtained with young rats are valid estimates of the utility of proteins for man, either adult or growing child, because during all stages of postnatal development a much larger proportion of dietary protein is utilized for maintenance than for growth. Hegsted has said: "A minimum conclusion, it seems, would be that amino acid fortification programs based upon rat assay alone would be foolish if not dangerous". ". . . amino acid scores appear to be quite inadequate estimates of the nutritional quality of proteins. This is especially true of cereal and vegetable diets . . ."

Cereal Proteins

Bakers of bread have mixed other cereals with their wheat flours for as long as their art is recorded. The ancient Egyptians and Romans mixed barley, oats or rye with wheat flour and pioneers in North America mixed upwards of 25% rolled oats with wheat flour to make flat breads which they baked on griddles over open wood fires.

The protein content of wheat flour can be concentrated in at least two general ways (1) concentration of the endosperm protein by removing part of the predominant starch; (2) concentration with selected high protein fractions from the bran and heat-stabilized germ.

Wheat Gluten, Bran and Germ Wheat endosperm protein can be concentrated by either wet or dry processes. The "wet" processes depend upon washing out the starch from flour-water doughs leaving a concentrate of gluten which can be added subsequently to bread doughs either in the wet state or after drying. The "dry" concentration processes depend upon controlled grinding and fractionation of the wheat components.

Research on wheat proteins began in the Institute of Anatomy and Chemistry at Bologna, Italy, where Beccari (1745), the professor of medicine, described how he separated gluten from a wheat flour dough by washing out the starch with water. Patents for the production and utilization of wheat gluten in bread, biscuits and other foods were reported in the middle of the 19th Century, e.g. Johnson (1853).

Supplementation of wheat protein with wet or dry gluten increases the total proportion of protein but does not greatly alter the balance of amino acids. The protein content of dried washed crude gluten ranges from 75 to 85% (Kasarda et al. 1971). Hale (1963) describes how to make high protein bread from added wheat germ and gluten flour of 41% protein content. Fance and Wragg (1968) describe how "gluten bread" containing not less than 16% protein and "high protein bread" containing not less than 22% protein on a dry weight basis can be made to British statutory standards.

Results which indicate that the proteins of wheat bran and germ are biologically superior to endosperm proteins were summarized by Mitchell

TABLE 107. Effect of adding wheat germ and gluten on protein and lysine content of bread baked from three flours (data from Pomeranz 1962).

	Patent			Brown			Wholemeal		
Flour supplement	Protein (%)	Lysine (%)	Lysine (% *of protein*)	Protein (%)	Lysine (%)	Lysine (% *of protein*)	Protein (%)	Lysine (%)	Lysine (% *of protein*)
0	13.8	0.46	3.3	15.1	0.52	3.4	14.6	0.50	3.4
Germ 3%	14.4	0.49	3.4	16.6	0.58	3.5	15.7	0.61	3.9
Gluten 3%	15.2	0.49	3.2	17.2	0.63	3.7	16.7	0.58	3.5
Germ 5%	14.7	0.51	3.5	16.4	0.60	3.7	16.3	0.62	3.8
Gluten 5%	17.0	0.51	3.0	18.3	0.63	3.4	18.7	0.60	3.2
Germ 10%	15.9	0.59	3.7	17.1	0.69	4.0	17.0	0.68	4.0
Gluten 10%	20.2	0.55	2.7	21.7	0.67	3.1	21.1	0.64	3.0

(1925) and Boas Fixsen and Jackson (1932). By and large, both in developed and developing countries, most of the bran and germ are consigned to animal feeds.

Westerman et al. (1952) described the biological superiority of defatted wheat germ added at 4 and 6% levels to nonenriched white flour. Rand and Collins (1958) described the nutritional improvement of adding 10 and 15% of solvent-defatted wheat germ to white flour.

Pomeranz (1962) fortified a variety of patent, long extraction (brown) and whole meal flours with gluten and wheat germ. The gluten contained 71.7% protein (N × 5.7) and 1.47% lysine, and the wheat germ 33.1% protein and 2.24% lysine. The results of adding 3%, 5% and 10% of wheat germ and gluten on the bread baked from three flours of different extraction rates are quoted in Table 107.

Pomeranz also reported a high positive correlation ($r = +0.91$) between the protein and the lysine contents of low extraction flours.

Howard and Anderson (1965, 1968) described the treatment of obesity with a commercial dietetic bread, the "Cambridge Formula Loaf", containing 25% protein derived largely from added stabilized wheat germ. Among 108 patients, the mean weight loss was significantly higher over eight weeks in those fed the high protein, wheat germ loaf than those fed brown bread or a commercial wheat germ bread.

Improved milling technologies offer several means of concentrating the natural wheat protein resource in flour and bread.

Wheat Protein Concentrate In the late 1960's a new method of concentrating wheat protein and improving protein value was described by Fellers et al. (1966), Sullivan (1967a, b) and Rozsa (1968). Bradley (1965) has demonstrated that the milling fraction known as "Shorts" contains roughly double the quantities of lysine and threonine found in an isocaloric quantity of human milk.

Fellers et al. (1966), Sullivan (1967a, b), and Rozsa (1968) described how carefully selected mill feeds were ground to fine particle size and subsequently screen-separated to produce wheat protein concentrates. Sullivan (1967a) discussed several such wheat protein concentrates, one of which contained 23% protein, approximately 1.05% lysine and 0.74% threonine. Sullivan (1967b) described how a straight grade hard winter wheat flour was blended with (*a*) 30% of "Shorts" and (*b*) 30% of a wheat concentrate produced by fine grinding and screening "Shorts". These blends were tested in Egyptian flat bread, and in India and Pakistan in chapatis, biscuits and other cereal products. The nutritional values of the blends were reportedly superior to Indian atta which approximates to a whole wheat flour.

Graham et al. (1970) evaluated a mixture of equal parts of unbleached white flour and a protein concentrate from wheat shorts, protein content 18.8% (N × 6), in feeding trials with infants and young children. About two-thirds of the protein was derived from the shorts, the lysine content of which is higher than the flour. The PER of the mixture as obtained was 1.73 ± 0.041 (standardized 1.31 c.f. casein 3.27 ± 0.044) and the NPU 47, these values being superior to those reported for wheat flour.

All the children received daily vitamin-mineral mixtures. The approximate minimum intake of

milk protein and total calories required by each child for continued accelerated growth and normal serum proteins were obtained. Each child received isonitrogenous and isocaloric levels of the cereal mixture. The test periods of 15 or 30 days were preceded and followed by 9-day periods in which casein was the protein source.

As judged by nitrogen absorption and retention, rates of weight gain and serum albumin levels in three infants receiving the wheat flour-wheat concentrate mixture as the sole source of protein, the mixture's protein value was approximately 60% that of casein. The mean of 60% N retention preceding and following casein periods is close to the 63% found in previous studies with wheat flour (Graham et al. 1969). Rates of weight gain for the same periods gave a mean of 74% that of casein, higher than the 67% found in the wheat flour studies (Graham et al. 1969). In the wheat flour studies there was a mean fall of 0.08 and a final level of 4.02 g of albumin/100 ml serum; in the present wheat flour-wheat concentrate studies there was a mean fall of 0.77 and a final level of 3.49 g/100 ml serum.

When L-lysine was added to the mixture at a level of 48 mg/kg body weight per day in trials with two infants the mixture's protein value improved by about 50%. This improvement still left the biological value of the mixture significantly below casein whereas with wheat flour alone, lysine fortification brought the relative biological value very close to that of casein.

The theoretical improvement in lysine content provided by the wheat flour-wheat concentrate mixture seems, at least in infants and young children, to have been offset by inferior digestibility.

The effect of substituting a commercial wheat protein concentrate (WPC) of protein content 15.35% (N × 5.7) for 0, 15, 30 and 45% of the flour in a bread formula on the nutritional value of the ingredients and on the baked bread was studied by Ranhotra et al. (1971). Amino acids (except tryptophan) were determined by autoanalyzer; tryptophan by the method of Tkachuk and Irvine (1969). PER and NPU (Miller and Bender 1955) values of a 10% protein diet were determined with six rats per diet, fed ad libitum for 14 days, using casein as control.

The WPC was lower in protein content, lysine and fibre, than previous samples examined. A loss in all essential amino acids during baking is reported, the proportional loss increasing with increasing proportion of WPC in the blend. The percentage losses in lysine were 2.6, 11.7, 11.9 and 18.8% as the WPC was progressively increased from 0 to 45%. Tryptophan fell 2.0% when no WPC was present but thereafter showed no loss during baking.

The rats fed the diets with the unbaked flour showed a progressive increase in food intake, weight gains and liver weights as the proportion of WPC increased. On average, for each 15% replacement of flour with WPC there was an increase of 0.2 in PER. The NPU also increased progressively.

Rye Flour The effect of supplementing rye flour of 98%, 87% and 45% extraction rates with a range of other foods of animal and vegetable origin was studied by Szkilladziowa (1962) in rat feeding tests using the methods referred to in her previous work (1960a, b, 1961). A mixture of one part rye flour and one part wheat flour, both of 98% extraction gave no improvement over the rye flour alone.

Egg and Milk Protein

Athenaeus, in his book "The Deipnosphistai", written in 230 AD, tells us that early Egyptian bakers made bread from wheat mixed with cow's milk. Cato also describes how Roman bakers added milk and cheese to their bread, in addition to nuts and sesame seed.

The high cost of egg proteins restricts their use to specialty and highly priced breads and they make little major contribution to bread protein supplementation. One exception is Jewish "Challa" bread which may contain as much as 2.5% of additional protein derived from egg yolk. In many developed countries, skim milk, skim milk powder (nonfat dry milk), and in some cases buttermilk, are used to supplement bread protein, and several countries regulate the quantity to be added to such breads (e.g. Government of Canada, Food and Drug Act and Regulations; Government of United States of America, Federal Food, Drug and Cosmetic Act; United Kingdom Government, Bread and Flour Regulations).

Brown et al. (1959) and McLaughlan and Morrison (1960) have reported a highly significant correlation between the PER of bread and the total amount of lysine present irrespective of the source of lysine.

TABLE 108. Effect of additions of nonfat dry milk with and without L-lysine HCl content and PER of bread and the unbaked ingredients (data from Gates and Kennedy 1964).

	Nonfat dry milk (*parts per 100 parts by weight of flour*)	Nitrogen (%)	Adjusted PER (*Casein = 2.50*)
Unbaked ingredients	3	2.67	1.07
	6	2.75	1.44
	12	2.93	1.87
Bread	3	2.74	0.93
	6	2.84	1.05
	12	3.02	1.35
Unbaked ingredients + 0.25 lysine	3	2.73	2.11
	6	2.82	2.11
	12	2.98	2.28
Bread + 0.25 lysine	3	2.76	1.70
	6	2.91	1.78
	12	3.09	1.74

Several research scientists in post-war Germany explored the fortification of bread with milk products and other protein sources. Bruchner (1949) proposed that up to 40% of the total liquid used for the preparation of rye bread doughs could be in the form of fresh whey.

Sabiston and Kennedy (1957) state that white bread and whole wheat bread containing 0.166% L-lysine (roughly equivalent to the amount of lysine contributed by 6% nonfat dry milk) is roughly equivalent in PER to bread containing 6% nonfat dry milk. The PER of the baked bread, whether supplemented by nonfat dry milk or its equivalent in lysine, was approximately 20% lower than the equally supplemented unbaked ingredients.

Jahnke and Schuck (1957) report upon the effect of increasing levels of milk solids added with and without additional lysine to (*a*) bread and (*b*) unbaked ingredients. The results showed a progressive increase in nitrogen efficiency ratio (NER, weight gain in grams per gram nitrogen consumed) with increase in milk solids from 3% to 12% in both bread and unbaked ingredients. The addition of 0.25% lysine further increased the NER at each level of addition of milk solids.

Mauron and Mottu (1962) compared evaporated and sweetened condensed milk in isonitrogenous quantities in a white flour diet for rats. Sweetened condensed milk was found to be superior to evaporated milk as measured by PER. The addition of lysine restored the supplementary value of the evaporated milk.

Hutchinson et al. (1963) studied the protein supplementation of dried bread crumb which provided 85% of the diet and the sole source of protein for the rats under test. They report that an addition of 0.5% L-lysine plus 0.2% L-threonine raised the biological quality of British type bread protein into the range which could be ranked as "first class". They report that 18% of skim milk powder was necessary to achieve an equivalent biological value.

Gates and Kennedy (1964) compared milk solids added at 3%, 6% and 12% levels with and without 0.25% L-lysine HCl in bread and unbaked ingredients. Their results, expressed as adjusted PERs as determined by the method of Osborne et al. (1919) modified by Chapman et al. (1959) are shown in Table 108 and demonstrate the following: (*a*) an increase in PER with increasing milk solids in both unbaked and baked products; (*b*) an average reduction of 21% in PER when the ingredients were baked into bread; (*c*) an overall significant increase in PER with the addition of 0.25% lysine to milk solids but no significant difference between 3%, 6% and 12% milk solids when lysine was present.

Bains et al. (1964) compared rat PERs of: (A) macaroni made from Indian durum wheat semolina, (B) diet (A) fortified with L-lysine HCl, 0.25 g/100 g protein after processing, (C) enriched

TABLE 109. Protein content of macaroni, total L-lysine HCl in protein, mean protein intake and PER of plain and enriched macaroni, with and without added lysine HCl, on rat PER (data from Bains et al. 1964).

	Protein content of macaroni (%)	Total L-lysine HCl in protein (%)	Mean protein intake (g)	PER
(A) Plain macaroni	12.0	3.16	19.59	1.10
(B) (A) + L-lysine HCl 0.25 g/100 g	12.0	5.40	26.95	2.11
(C) Enriched macaroni (plus casein and skim milk solids)	17.0	6.80	28.98	2.48
(D) (C) + L-lysine HCl 0.25 g/100 g protein	17.0	9.60	29.08	2.63

macaroni made from durum wheat semolina 90, casein 7, and skim milk solids 3 parts, (D) diet (C) fortified with L-lysine HCl, 0.25 g/100 g protein, after processing. The macaroni was included in 10% protein rat diets fed ad libitum over 28 days. The results are given in Table 109.

Diet (B) significantly improved the quality of the protein over diet (A), but fortification with milk protein and casein produced a still greater improvement. The PER of macaroni, diet (C), was not significantly improved by the addition of lysine.

It is very well recognized that, from their own resources, developing nations cannot normally seriously consider the supplementation of cereal foods with milk protein. Nevertheless from time to time more affluent nations see fit to present dried milk to those less fortunate. It may be that in some circumstances the most beneficial use of these gifts may be as supplements whereby to enhance the biological value of cereal proteins.

Food Legume and Oilseed Protein

There is much recorded evidence from ancient Egypt, Greece and Rome, and from later societies, to indicate that bean, pea and other legume flours were added to wheat flour either to extend the available wheat supplies in times of emergency or to provide cheap bread for poor people. McCance and Widdowson (1955) presented a comprehensive historical review of the conflicting opinions concerning the relative nutritional merits of brown and white breads. They described the supplementation of wheat flour with other cereals and legume flours in wartime Europe.

Soybean Protein In the past, greater attention has been given to soybean than to any other legume or oilseed protein as a bread protein supplement. The recent international interest in other legumes gives hope that other vegetable proteins might be used to fortify bread in the less developed countries in the future.

In the developed world soya products predominate among legumes used as supplements to cereal proteins. It is probable that more than 22 million kilograms find their way into baked products alone. "Soya flours" even in developed countries vary significantly in nature and composition, a fact which causes difficulties in relating data from one source to that from another.

The chemical and physical properties of soya products vary markedly according to the method of processing. High fat soya products may vary in protein content (N × 6.25) from 38 to 46%, low fat and defatted soya flours from 46 to 53%. Commercial soybean protein concentrates range in protein from 65% to 70% and soybean isolates from 90 to 95%.

The nutritional value also of soya products is influenced by processing conditions. Horan (1967) compared the protein efficiency (the method is not stated) of commercial types of defatted soya flours as illustrated in Table 110.

Data extracted from a variety of sources including Food and Agriculture Organization (1970b) suggests approximate ranges of biological values for various kinds of processed soya products and these are quoted in Table 111.

Mayr (1948) reported on the addition of 55% protein soya meal to bread in Germany at levels between 2.5 and 5%. At the 2.5% level bread protein content on a dry weight basis increased by 1.1% and at the 5% level by 2.2%.

Henry and Kon (1949) reported that the combined effect of 3% skim milk powder and 2.78%

TABLE 110. Effect of heat treatment on protein quality of commercial defatted soya flours (data from Horan 1967).

Heat treatment	Protein dispersible index	Relative Protein Efficiency (dried skim milk = 100%)
Negligible heat	90–95	40–50
Light heat	70–80	50–60
Moderate heat	35–45	75–80
Toasted	8–20	85–90

soya flour was superior in biological value to 6% of dry skim milk when included in bread.

Westerman et al. (1954) found no significant difference between wheat germ and soya flour added to enriched wheat flour on the rate of growth of rats during the first or second generation but soya flour added to non-enriched wheat flour appeared to promote a faster rate of growth in the second generation than did wheat germ. Rats fed enriched flour plus soya flour stored more B vitamins in their livers.

Guggenheim and Friedman (1960) reported on bread baked from flours varying in extraction rate from 74 to 95% and fortified with up to 12% soya flour. Lysine (milligrams per gram nitrogen) increased with increase in extraction rate and with percent soya flour added. Threonine increased and methionine decreased with the percent added soya but neither changed appreciably with extraction rate.

NPR (Bender and Doell 1957) rose with extraction rate and with percent of added soya. Bornstein and Lipstein (1962) using the same materials in feeding trials with chicks, reported a marked improvement in growth rate with soya supplementation, possibly from the increased dietary protein level and the additional lysine. The growth rate was more marked than with increasing rate of extraction. The findings of Guggenheim and Friedman and of Bornstein and Lipstein, are in good agreement.

Later, Guggenheim et al. (1962) reported on feeding trials conducted in Israel over eight months with adolescent boys and girls consuming large amounts of bread and doing heavy physical work in addition to their school studies. There were 88 boys and girls at agricultural school E and 96 at agricultural school K aged $13\frac{1}{2}$ to $18\frac{1}{2}$ years. At school E the flour used was of 87% extraction from imported wheat fortified with 5% of a commercial toasted soya meal. At school K the flour was of 74% extraction and unfortified. Both were supplemented with 2.5 mg riboflavin and 2.5 g calcium carbonate per kg of flour. The 74% bread contained, on average, per 100 g bread, 8.6 g protein (conversion factor not stated), 274 mg lysine and 174 mg methionine. The 81% bread contained on average 10.4 g protein, 348 mg lysine, 186 mg methionine. Other flour products were eaten at both schools, and at school E, the traditional white bread and cakes were eaten on the Sabbath.

TABLE 111. PER values of processed soya products (data from various sources including Food and Agriculture Organization 1970b).

	PER (casein = 2.5)
High fat soya flour	2.0–2.5
Defatted soya flour	2.0–2.5
Soya protein concentrate	2.0–2.5
Soya protein isolate	1.1–2.1

Individual food consumption of the subjects was not recorded but flour products provided over 40% of the calories in both schools, the experimental flours providing 32% at school E and 36% at school K. There were other differences between the diets at the two schools but at the end of the observation period no appreciable differences attributable to the bread were found in increase in weight and height, change of skin fold thickness, general nutritional status and haematological conditions.

Mizrahi et al. (1967) reported on the effect of using isolated soya proteins, produced by an isoelectric wash and calcium coagulation, on the baking characteristics, acceptance and nutritive value of bread. They describe how these additions increased water absorption and how loaf volume decreased proportionately with the level of protein isolate added. The decrease in loaf volume from mixtures containing less than 6% soya protein was counteracted by the addition of 1% of lecithin. Protein additions up to 8% of the flour weight did not significantly affect flavour. PER of the bread increased with increase in the percentage of soya protein and was found to be

in linear relationship with the lysine content of the bread.

Pomeranz and Finney (1973), United States Department of Agriculture, have recently been awarded a US Patent No. 3,679,433 covering a process for the addition of up to 16% soya flour, or other protein-rich, high-lysine concentrate, with 0.25 to 4.0% glycolipids, to wheat flour to provide acceptable bread containing approximately 70% more protein and three times as much lysine.

The volume of literature covering the supplementation of wheat and rye with other oilseed and grain legume proteins is sparse compared with that which treats of soya bean supplements. Some attention has been given to fortification of bread with cottonseed flour though a considerably greater volume of literature describes the nutritional properties of unbaked cereal-cottonseed mixtures.

Cottonseed Protein When fed to young rats at 10% protein levels, bread containing 10 parts of cottonseed flour per 100 parts of wheat flour produced higher rates of gain per gram of nitrogen consumed than bread without the cottonseed flour (Womack et al. 1954). Womack et al. also compared bread containing skim milk powder and bread with skim milk powder and cottonseed flour, but found no significant difference in weight gain when the two breads were fed isonitrogenously. However, when fed at the same percentage by weight, the cottonseed flour bread, having a higher protein content, produced a significantly higher rate of weight gain.

Dalby (1969) described how Egyptian Baladi bread was supplemented first with a mixture of chick-pea flour and inactive dry yeast and later with two samples of low-gossypol cottonseed flour, the first having been made from a glandless cottonseed variety; the second rendered gossypol-free by solvent extraction. Dalby stated that at 7% of the wheat flour, cottonseed flour, containing roughly 60% protein, provided a protein supplementation equivalent to 12% of nonfat milk solids.

Several other authors have reported upon the use of various oilseed and legume proteins in bread but most of the published discussion relates to technological rather than nutritional implications of their experiments.

Other Vegetable Proteins Phansalkar et al. (1957) added various pulses and leafy vegetables to wheat in rat diets. The pulses included bengal gram (chick-pea, *Cicer arietinum*), black gram (*Phaseolus mungo*), green gram (*Phaseolus radiatus*), and red gram (*Cajanus indicus*). The leafy vegetables were agathi (*Sesbania grandiflora*), amaranth (*Amaranthus gangeticus*), murungu (*Moringa oleifera*) and parpukeerai (*Portulaca olearacea*). All materials were locally purchased. The diet, about 10% protein (conversion factor not stated), consisted of 7% wheat and 3% pulse, or 6% wheat plus 3% pulse and 1% amaranth. The rats were male and female weanling albinos, fed ad libitum and weighed once a week for four weeks.

TABLE 112. Effect of additions of pulses with and without amaranth on rat PER (data from Phansalkar et al. 1957).

Diet	Protein (%)	PER	
		Average	S.E.
(A) Skim milk diet	10.48	2.57	0.095
(B) Wheat	10.58	1.77	0.082
(C) (B) + bengal gram	11.11	2.18[a]	0.092
(D) (B) + black gram	10.50	2.15[a]	0.100
(E) (B) + green gram	10.98	2.22[a]	0.108
(F) (B) + red gram	10.24	2.19[a]	0.065
(G) (B) + amaranth	10.94	1.65	0.072
(H) (C) + amaranth	10.94	2.19[a]	0.080
(J) (D) + amaranth	10.32	2.37[a]	0.073
(K) (E) + amaranth	10.85	2.23[a]	0.118
(L) (F) + amaranth	10.54	2.35[a]	0.080

[a]$P < 0.01$, all, with the exception of diet (G), amaranth, significantly different from wheat diet (B).

Of the leafy vegetables, only amaranth is reported upon. Results are given in Table 112. There was no significant difference between the PER, 2.35, of diet (L), (wheat, red gram and amaranth, protein 10.5%) and the skim milk diet (A), (PER 2.57, protein 10.5%).

The rat diets contained vitamin mixtures and since these would not be present in the normal Indian diet, a trial with bajra (sorghum) plus red gram (pigeon pea) plus amaranth was carried out to see if omission of the mixtures would affect the PER. The PER was lowered when the salt plus vitamin mixture was not added but the difference was not statistically significant.

It was observed that PER varied according to the season in which the experiments were carried out though no systematic seasonal differences in food intake were observed.

The effect of supplementing a poor Indian diet based on wheat (PWD) with full fat soya flour, red gram dhal (pigeon pea, *Cajanus cajan*), bengal gram dhal (chick-pea, *Cicer arietinum*), or skim milk powder, was studied using male albino rats, eight rats per group over four weeks, by Daniel et al. (1965). The basic PWD consisted of wheat 78.5 g, red gram dhal 5.0 g, groundnut oil 5.0 g, skim milk powder 0.9 g, common salt 0.3 g, green leafy vegetable (*Amaranthus gangeticus*) 2.1 g, brinjal (egg plant, *Solanum melongena*) with potato 8.2 g. This diet provided 11.26% protein (N determined by micro-Kjeldahl method) and, in g/16 g N, 4.5 g lysine and 3.8 g threonine assayed microbiologically. The basic diet (protein 11.26%) was supplemented with bengal gram or red gram at a 15% level, soya flour at 5.5% or skim milk powder at 9.0% level to provide 2.2% extra protein and corresponding increases in lysine and threonine. All these diets were fed with and without standard mineral and vitamin premixes.

The growth rates of the rats increased significantly as a result of the supplementation with bengal gram or red gram at 15%, or soya flour at 5%, and they also increased significantly when vitamins and minerals were added to the basic diet. With both types of supplementation there was a further significant increase. The PERs of the rats fed supplements of bengal gram, red gram and soya to provide 2.2% extra protein were similar (1.90, 1.92 and 1.93), and when the diets included vitamins and minerals there was a significant improvement (to 2.31, 2.41, 2.39) respectively. There was no significant difference between these PERs and those for diets supplemented with skim milk powder with (2.46) and without (2.46) vitamins and minerals. By the criteria employed supplementation of a poor Indian wheat diet with 15% bengal gram (chick-pea) or red gram (pigeon pea) is roughly equivalent to supplementation with 5% soya flour.

Bolourchi et al. (1968) examined 12 male students aged 19 to 27 years given a diet in which 90 to 95% of the protein came from US commercial enriched wheat flour eaten as bread and rolls made without milk. The remainder of the dietary protein came from fruits and vegetables. The experimental "core" diet provided 3,320 kcal per day, 11.8 g nitrogen, 67.3 protein, 80 g fat and 559 g carbohydrate. All the core diets were supplemented with protein-free calories to maintain the weight of the subjects. For the first 10 days on the wheat diet the subjects were in negative nitrogen balance but for the remainder of the 50-day experimental phase the subjects were in nitrogen equilibrium and displayed good health.

Three blends of hard wheat and bengal gram dahl (*Cicer arietinum*) obtained in Mysore, India, were evalutated in rats by Daniel et al. (1969). The blends were (A) 80 wheat, 20 bengal gram (total protein (N × 6.25) 14.3% dry weight basis), (B) 70 wheat, 30 bengal gram (total protein 16.1%), (C) 40 wheat, 60 bengal gram (total protein 20.7%). The ratio of wheat to bengal gram protein in the blends was (A) 2:1, (B) 1:1 and (C) 1:3. The lysine, methionine, cystine and threonine contents were assayed microbiologically. The blends were fortified with L-lysine, DL-methionine and DL-threonine to the levels of these amino acids in human milk.

The PERs (corrected, Osborne et al. (1919) taking the PER of skim milk powder as 3.0) were (A) 2.43, (B) 2.37 and (C) 2.25. The PER of (A) increased to 2.70 when supplemented with lysine and threonine, and to 2.96 when supplemented with lysine, threonine and methionine. The PER of (B) increased to 2.72 when fortified with lysine and threonine and to 2.93 with lysine, methionine and threonine. The PER of (C) increased to 2.66 when fortified with methionine and to 2.80 with methionine and threonine. The mean weekly gain in body weight of the female rats fed blends (A), (B) and (C), with different levels of protein, fortified with vitamins and minerals were 18.4 g, 15.8 g and 17.0 g respectively over a period of 4 weeks. Supplementation with lysine and threonine increased the mean weekly growth rate on blend (A) to 23.6 g and on blend (B) to 19.6 g, and supplementation with methionine and threonine increased the mean weekly growth rate on blend (C) to 20.0 g.

The results of the PER test and of the growth test are not strictly comparable but Daniel et al. suggest they indicate that a blend of 80 parts of wheat to 20 parts of Bengal gram, of about 14.0% protein on a dry basis and fortified with vitamins and minerals would meet the protein needs of growing children.

Edwards et al. (1971) reviewed earlier work, including that of Bolourchi et al. (1968), on the nutritive value of wheat for adult men. They also investigated the nutritional value of wheat flour

protein in 12 male students, aged 23 to 30 years during a 74-day period. The commercial white flour was fed in the form of bread made without either milk or any other source of animal protein. The wheat diet contained 46 g protein, of which 35 g was provided by the bread and 11 g by such other plant foods as potatoes, turnips, green vegetables and fruit. In other diets 20% of the wheat nitrogen provided was replaced, isonitrogenously, by pinto beans, rice, or peanut butter. The daily calories supplied were: wheat diet 3,036; wheat plus pinto bean 3,006; wheat plus rice 3,043; wheat plus peanut butter 3,043. Nitrogen balance, blood urea nitrogen, urinary urea, plasma lipid concentration, and essential and non-essential amino acids were determined at the beginning and end of four 15-day intervals following the wheat diet regimen.

Nitrogen balance was maintained over the trial period and Edwards et al. conclude that a diet providing 46 g protein per day of which 76% of the nitrogen was supplied by wheat and the remainder by potatoes, other vegetables and fruit is adequate for nitrogen maintenance in adult man. Replacement of 20% of the nitrogen with pinto beans, rice or peanut butter did not significantly improve the utilization of the wheat proteins.

Protein from Microorganisms

Yeast acts in bread doughs as a leavening agent and, until recently, yeast has been studied nutritionally more as a source of B-complex vitamins than of protein. The natural yeast cell contains about 30% dry matter of which more than 50% is crude protein (N × 6.25). Its chemical composition is comparatively well known (Von Loesecke 1946, Inskeep et al. 1951) but some uncertainty is apparent concerning the long term effects of feeding large amounts of yeast to human beings. Those yeasts which over thousands of years have been consumed in bread, beer and wine have been ingested in relatively small daily quantities.

During World War II workers, mostly in Germany, examined the influence of dried yeast in bread. Schwarz et al. (1942) found that additions of 2.5% and 5% of dried brewers' yeast significantly improved the vitamin B-complex and protein content of white bread.

Light and Frey (1943) reported that 5% dry yeast containing approximately 50% protein compared favourably with bread containing 6% nonfat milk solids. These authors found the growth response to be proportional to the lysine added from the dry yeast and dry milk supplements.

McCollum (1945) reported on the nutritional benefit of adding dried brewers' yeast and indicated that palatability was not seriously impaired. Nevertheless, both he and Schwarz et al. (1942) reported a significant decrease in bread quality, assessed technologically, as the amount of added dry yeast was increased.

Sure (1948) attributed a superior weight gain in rats fed wheat flour enriched with dried food yeast to the lysine added by the yeast.

Seeley et al. (1950) found that when non-viable dried yeast was added to bread, at a concentration not exceeding 3%, rats fed on the resulting bread showed an increase in daily weight gain over controls. Dried yeast was found to be, nutritionally, a better supplement than nonfat dry milk but the maximum increase in weight gain occurred in bread containing both dried yeast and nonfat milk solids.

Lang (1950) discussed the fortification of bread with lysine and torula yeast and emphasized that a greater improvement in biological value was obtained than by using whole wheat flour.

Cremer et al. (1953) discussed various means of supplementing bread with protein, including milk powder and dried yeast, and stated that the addition of 3% protein significantly raised the biological value of the bread.

Hundley et al. (1956) described the fortification of bread with two types of algae: (i) *Scenedesmus obliquus* (ii) *Chlorella pyrenoidasa*. The first contributed significant amounts of both lysine and threonine; the second, though an adequate source of threonine, was comparatively deficient in lysine.

Pomeranz (1962) found that the addition of 3%, 5% and 10% of dry yeast to bread raised the initial protein content from 13.8% to 14.4%, 14.7% and 15.9% respectively. By microbiological assay he determined that the control lysine content of 0.46% was raised respectively to 0.49, 0.51 and 0.59%. The protein (N × 6.25) and lysine content of the active dried yeast were 42.4% and 2.97% respectively.

Spicer (1970, personal communication) undertook the screening of a large number of microorganisms found in carbohydrate sources and describes the discovery of a microfungus with a protein content of 45% and NPU in the region of 70, which grows well on a variety of hydrolyzed

TABLE 113. Lysine, threonine, methionine and cystine content with and without petroleum yeast (*in grams/16 g N*), and protein score of rat diets (data from Narayanaswamy et al. 1972).

	Petroleum yeast	Poor wheat diet (PWD)	PWD plus 6% yeast
Lysine	7.4	3.2	3.8
Threonine	5.3	3.1	3.5
Methionine	1.4	1.9	1.8
Cystine	0.5	1.2	1.1
Protein Score (assuming hen's egg as 100)	34	50	52

starches and the sucrose present in cane juice and molasses. The organism is being studied as a protein supplement in bread and other cereal foods. Spicer does not indicate the level of digestibility nor the content of nucleic acids present.

Narayanaswamy et al. (1972) compared the PER for young female rats, eight per group, fed ad libitum over 28 days, of a poor wheat diet supplemented by yeast grown on hydrocarbons. The yeast was composed of, in grams per 100 g, moisture 9.8, protein (N × 6.25) 45, fat 1.1, mineral matter 10.3, calcium 0.16, phosphorous 2.10 (Association of Official Agricultural Chemists 1955).

The experimental diets consisted of a basic mixture of vegetables, peanut oil, skim milk powder and common salt totalling 16.59 to which were added: Diet (A) 78.5 g wheat, 5.0 g bengal gram; diet (B) 75.5 g wheat, 5.0 g bengal gram, 2.0 mineral mix, 1.0 vitamin premix; diet (C) 72.5 g wheat, 5.0 g bengal gram, 6.0 g petroleum yeast; diet (D) 69.5 g wheat, 5.0 g bengal gram, 6.0 g petroleum yeast, 2.0 g mineral mix, 1.08 vitamin premix.

The lysine, threonine, methionine and cystine contents (assayed microbiologically) of the diets is shown in Table 113. The protein content (micro-Kjeldahl N, conversion factor unstated) and the PERs are given in Table 114.

The addition of 6% yeast, providing about 2% extra protein in the diets, plus vitamins and minerals, produced highly significant increases in the rate of rat growth, and significant improvement in PER. The differences in PERs of diets (A) 1.50, (B) 2.02, (C) 1.86 and (D) 2.34, were all significant.

TABLE 114. Effect of addition to poor wheat diets of 6% yeast on protein content of diet and rat PER (data from Narayanaswamy et al. 1972).

Diet	Protein content (*dry basis*, %)	PER
(A) Poor wheat diet	12.46	1.50
(B) (A) + mineral and vitamin premix	12.38	2.02
(C) (A) + 6% yeast	14.48	1.86
(D) (C) + mineral and vitamin premix	14.36	2.34

Yanez et al. (1973) found that the substitution of Chilean flour (70% extraction rate, Florana wheat) by *Candida utilis*, cultivated on sugar beet molasses, at levels of 0, 3, 6 and 10%, improved the crude protein (N × 6.25) content of the resultant bread from 14.4% at the zero level to 21.3% at the 10% level. The rat PER (Chapman et al. 1959) increased significantly from 0.84 at zero level to 1.14 (3% level) to 1.45 (6% level) to 1.74 (10% level) but did not attain the PER of 2.71 of the casein diet. Loaf volume decreased noticeably and colour darkened as fortification levels rose above 1%. Yanez et al. point out that when the nucleic acids of yeast are metabolized by human beings the final product is uric acid, which in excess may lead in susceptible subjects to gout or the formation of stones in the urinary tract. Food and Agriculture Organization (1971) have set the maximum tolerable intake of nucleic acids at 2 g per day, equivalent to 10 to 30 g of unicellular proteins. In Chile bread enriched with *Candida utilis* at the 3, 6 and 10% levels would provide, respectively 400, 800 and 1300 mg nucleic acids daily, assuming an average consumption of 250 g bread daily per child. This level is much lower than the recommended maximum.

Fish Proteins

Before presenting their review of supplementation with fish proteins the authors would offer a word of caution to the reader. The terms "fish flour", "fish protein concentrate", "marine protein concentrate" appear in the literature sometimes to designate closely similar materials, sometimes to describe widely different products. In

TABLE 115. Effect of supplementing wheat flour diet with fish flour on rat PER (data from Metta 1960).

Diet	Percent of diet	Dietary protein (%)	Total food intake (g)	Weight gain (g)	PER
(A) Whole wheat flour	66	12.9	280	71±1.5	1.980±0.038
(B) Whole wheat flour	65	13.6	280	81±1.3[c]	2.127±0.035[a]
fish flour	1				
(C) Whole wheat flour	63	14.8	280	102±1.1[c]	2.470±0.028[c]
fiish flour	3				
(D) Enriched wheat flour	63	9.7	279	32±3.1	1.183±0.096
cellulose	3				
(E) Enriched wheat flour	62				
fish flour	1	10.6	279	56±4.1[b]	1.882±0.134[b]
cellulose	3				
(F) Enriched wheat flour	60				
fish flour	3	12.1	279	79±2.8[c]	2.364±0.077[b]
cellulose	3				

[a] $0.01 < P < 0.02$.
[b] $P < 0.01$.
[c] $P < 0.001$.

some instances the products referred to appear to be dried products of whole fish or fish muscle which retain some or all of the characteristic odour and flavour of the original. Others consist of refined protein concentrates produced by a variety of extraction methods from a variety of raw materials. Consequently comparison of the data and findings among authors may not always prove meaningful.

Metta (1960) examined the effect of odourless, defatted fish flour (Viobin Corporation) at supplementation levels of 1 and 3% in simulated cereal-based Indian diets. The basal diet included 5% mung bean, *Phaseolus aureus*, (protein 23.6%), and 5% yellow split pea (protein 27.2%) and was balanced for vitamins and minerals. The isocaloric experimental diets were: (A) basal diet, plus whole wheat flour providing 63% of the diet; (B), diet (A), plus fish flour replacing 1% of the wheat flour; (C), diet (A), plus fish flour replacing 3% of the wheat flour; (D), basal diet, plus enriched wheat flour providing 63% of the diet with 3% cellulose; (E), basal diet, plus enriched wheat flour providing 62% of the diet, fish flour 1% and cellulose 3%; (F), basal diet, plus enriched wheat flour providing 60% of the diet, fish flour 3% and cellulose 3%. The fish flour contained 83.1% protein (N × 6.25).

Growing male rats six per treatment were trio-fed amounts of the control and the supplemented diets for 28 days (method of Mitchell and Beadles 1930). Weight gain and PER are shown in Table 115.

Though Metta attributes the higher weight gain mainly to the better amino acid balance in the fish protein-supplemented diet, it is difficult to assess how much is the result of the higher protein content, compared with the unsupplemented diet.

Morrison and Campbell (1960) compared the PER of diets containing fish flour obtained from the Fisheries Research Board of Canada. Defatted fish flour was made from codfish muscle and contained 90% protein (N × 6.25), 5.4% moisture and 6.4% ash.

In the first trial, fed at the 10% level, the diets included: (A) whole wheat flour; (B), diet (A) plus fish flour, 2 lb/100 lb wheat flour; (C), diet (A) plus fish flour, 4 lb/100 lb wheat flour; (D), diet (A) plus fish flour, 6 lb/100 lb wheat flour. The results are presented in Table 116. PER increased with increasing addition of fish flour.

In a second trial the diets, also fed at the 10% protein level, included: (E) casein; (F) enriched white bread made with water; (G), as (F) with 10% fish flour replacing an equivalent amount of white flour; (H) enriched white bread including 4.2%

TABLE 116. Effect of supplementation of whole wheat flour with defatted fish flour on rat PER (data from Morrison and Campbell 1960).

	Diets			
	(A)	(B)	(C)	(D)
Fish flour added lb/100 lb wheat flour	0	2	4	6
Weight gain, g	37	67	84	102
Food consumption, g	249	314	337	365
PER	1.49	2.12	2.48	2.80

milk solids; (J), as (H) with 10% fish flour replacing an equivalent amount of white flour. The results on rat PER are shown in Table 117.

The lysine contents of the flour and bread diets, determined microbiologically, are shown in Table 118.

The bread used in diet (G) had a protein content of 16.5% and that used in diet (J) a protein content of 18%, air-dry basis. These "fish-flour" breads showed reduced loaf volume, were darker in colour, and somewhat "rubbery" in texture but the smell and taste were not noticeably affected.

A highly significant correlation of 0.993 was found when the lysine content was plotted against the PER, indicating that the PER values in these diets are a direct function of the percentage lysine in the protein. Morrison and Campbell cite Rogers et al. (1959) and Flodin (1959) as also observing a significant relationship between lysine concentration and PER in foods low in lysine.

Howard et al. (1958) has suggested that an ideally balanced "complete" protein should contain 5.3 g lysine per 16.0 g N. The supplemental value of fish flour, based on its content of "complete" protein, was calculated according to the formula given by Howard et al. (1958) and found to be 185. The addition of 2 lb of fish flour per 100 lb wheat flour, as in the first trial, increased the protein content of the mixture by 10% but increased the "complete" protein content by 42%. Fish flour contains approximately 10.9 g lysine per 16.0 g N (Rogers et al. 1959) so it also contributes "extra" lysine.

TABLE 118. Effect of defatted fish flour on lysine content of bread and flour proteins (data from Morrison and Campbell 1960).

	Lysine content (*g/16 g N*)
Whole wheat flour	3.02
+ 2 parts fish flour	3.63
+ 4 parts fish flour	4.37
+ 6 parts fish flour	4.68
White bread	2.43
+ 10% fish flour	6.32
Milk bread (4.2% milk solids)	3.16
+ 10% fish flour	5.86

The protein rating (Campbell and Chapman 1959) of the white bread (diet F) was 11.1 and of the bread containing fish flour (diet G) was 51.5.

Jansen et al. (1966) studied the effect of alcohol-extracted fish flour at 3% and 6% in combination with L-lysine and L-lysine plus DL-threonine, the additions being made to standard white bread after drying. From rat feeding studies, they concluded that the addition of lysine and threonine doubled the utilizable bread protein, with lysine alone being responsible for approximately 75% of the increase. Bread supplemented with 6% of fish flour displayed a protein retention efficiency of 58; the addition of 0.2% of lysine raised this value to 72; 0.4% lysine plus 0.15% threonine with no fish flour gave a value of 78 compared to 88 for the reference sample of casein. Since these additions were made to dry bread after baking,

TABLE 117. Effect of supplementation of white bread with defatted fish flour on rat PER (data from Morrison and Campbell 1960).

	(E) Casein	(F) Bread	(G) Bread + 10% fish flour	(H) Milk bread	(J) Milk bread + 10% fish flour
Weight gain, g	109	23	145	43	130
Food consumption, g	311	177	376	216	363
PER	3.49	1.30	3.87	1.97	3.59

TABLE 119. Biological quality of bread and of bread with 6% fish flour, 12% skim milk powder and 6% fish flour plus 0.5% L-lysine (data from Yanez et al. 1967).

	NPU_{op}	Protein calories (%)	Net protein concentration	NPU_{st}	Score
Bread	35.4	10.4	3.7	35.4	45
+ 6% fish flour	42.8	14.3	6.1	48.9	72
+ 12% skim milk powder	44.5	13.5	6.0	50.4	71
+ 6% fish flour + 0.5% L-lysine	58.8	14.6	8.6	71.4	70

they do not reflect any degree of loss which might occur during baking.

Sidwell (1967) discussed the nutritional advantages of supplementing wheat flour with fish protein concentrate as developed and produced by the Bureau of Commercial Fisheries in the United States. He stated that a mixture of 10% FPC and 90% wheat flour in cookies showed a PER of 3.0 compared to 3.4 for casein. The FPC was used as an ingredient of bread and cookies. FPC was added to the bread formula at levels of 5, 10, 15, 20 and 25%. At all levels of addition there was a noticeable effect on crumb colour and loaf volume, the former becoming progressively darker and the latter smaller with progressive increases in FPC. The crude protein content of the bread (N × 6.25 moisture free basis) rose from 15.0% in the control to 33.9% at a 25% level of addition. The author's estimates of protein and lysine in a 40 g slice of bread were:

% FPC	0	5	10	15	20	25
Protein, g	3.8	4.6	5.7	6.4	7.1	7.8
Lysine, mg	70	96	136	154	214	259

The crude protein content of cookies increased from 5.4% at zero addition to 9.8% at 15% of FPC. Sidwell states that as the FPC increased the degree of sweetness decreased and the colour changed from bright yellow to dull grey yellow.

Yanez et al. (1967) examined samples of fish flour produced in a pilot plant at Quintero, Chile from *Merluccius gayi*. In the products examined, available lysine (Carpenter 1960) ranged from 8.3 to 9.5 g per 100 g of crude protein (Viobin Corp. product, for comparison, 8.7), and NPU (Miller and Bender 1955) from 63.5 to 70.7 (Viobin 69.2). Fish flour was added to "marraquata" bread at the 10% level and compared with control bread, and bread with 12% skim milk powder. The protein content in grams per 100 g dry matter was: control bread 10.2 (N × 5.7), bread with 6% fish flour 13.9 (N × 5.9), bread with 12% skim milk powder 13.2 (N × 5.9). The biological quality of the breads is shown in Table 119. It is not clear from the text exactly how these results were obtained.

"Tallanines", a pasta product, was made locally, under normal manufacturing conditions, containing 10% of fish flour. The chemical composition and the biological value are shown in Table 120.

TABLE 120. Moisture, protein content and biological quality of commercial pasta, pasta enriched with 10% fish flour, and the unprocessed ingredients of the enriched pasta (data from Yanez et al. 1967).

	Moisture (%)	Protein ($N \times 6.25$) (%)	NPU_{op}	Protein calories (%)	Net protein concentration	NPU_{st}	Score
Commercial pasta	11.5	10.2	38.4	11.9	4.6	40.2	47
Pasta enriched with 10% fish flour	10.6	16.1	47.0	19.1	9.0	64.4	73
Ingredients of enriched pasta	14.1	15.8	51.6	19.2	9.9	71.8	73

TABLE 121. Biological quality of roasted whole wheat meal without and with 10% fish flour (data from Yanez et al. 1967).

	NPU_{op}	Protein calories (%)	Net protein concentration	NPU_{st}	Score
Roasted whole wheat meal	41.2	8.2	3.6	41.2	47
Roasted whole wheat meal with 10% fish flour	70.2	11.6	8.2	81.6	72

The biological quality of roasted whole wheat meal, enriched with 10% fish flour is given in Table 121.

"Ulpo", a preparation of roasted whole wheat meal with water or milk, is widely eaten by young children, especially in rural areas of Chile. A ration of "ulpo" consisting of a mixture of roasted whole wheat meal 70 g, fish flour 10 g and sugar 20 g, mixed with 200 ml of cold water was presented to each of 300 children aged 9 to 14 years and found completely acceptable.

When fish protein concentrate was used as a supplement to wheat in the diet of Peruvian children, acceptance and consumption were excellent during the six years of the project (Baertl 1971). The study was carried out in four villages each of about 1000 inhabitants where evidence of poor growth, a high incidence of malnutrition and mortality existed among pre-school children. Villages 1 and 2 served as controls; in village 3 each family received enough ordinary wheat noodles to provide a daily supplement of 250 kcal and 7 g of wheat protein per person. In village 4, fish protein concentrate replaced 10% of the noodle flour. There were only minor effects on growth but the mortality of infants and pre-school children in villages 3 and 4 dropped in comparison with the preceding 12-year pattern and was not matched in villages 1 and 2. It is worthy of note that during the experimental period, improvements in housing and sanitation took place in villages 1, 2 and 3 but not in village 4.

Stillings et al. (1971) compared the PER and NPU of wheat flour, and of bread made from it, unsupplemented and supplemented with either fish protein concentrate (FPC) or lysine. The samples were commercial (United States) baker's grade wheat flour of 86.9% dry matter, protein 14% (N × 6.25), to which was added L-lysine HCl or FPC of 94.9% dry matter, protein 85%, prepared from red hake (*Urophycis chuss*) by extraction with isopropyl alcohol.

In summary, when unprocessed wheat flour mixtures were fed at 10% protein level, maximum weight gain, PER, NPU and total protein and fat in the carcasses were produced by 15% FPC and 0.2 to 0.4% lysine. Maximum responses from FPC were greater than those from lysine. When bread baked from the wheat flour mixtures was fed at the same protein level, 25% FPC and 0.4% lysine produced maximum weight gain and PER. Protein ratings for bread supplemented with 10 to 25% FPC were 35 to 150% higher than those for bread supplemented with lysine. When bread was included in diets on the basis of 80% by weight, 10% FPC or 0.4% lysine produced the highest weight gains, the weight gain being 36% higher with FPC than with lysine.

A variety of authors including Donoso et al. (1963), Santa Maria (1969), Bacigalupo (1969) describe the addition of dehydrated fish flour (harina de pescado) at various levels up to 12% of the wheat flour but their publications are concerned more with acceptability than with improvement in nutritional value.

Sen et al. (1969) describe several alternative methods of producing fish protein concentrate from Bombay duck (*Harpodonn nehereus*) and the influence of processing conditions upon the nutritional and organoleptic properties. The samples were incorporated into bread, chapatis, puris and other cereal foods.

Conclusion

Though the most widespread benefit to mankind would probably result from the development of nutritionally superior varieties of triticale, the biological value of products derived from triticale, wheat and rye can be improved or impaired by how they are processed. Among malnourished

peoples it is important that as much as possible of the protein-rich aleurone layer be retained in the milled flour. More efficient milling techniques applicable in the developing countries whereby to retain the aleurone layer in the flour should therefore be sought. In addition research is needed wherewith to produce inexpensive acceptable protein supplements derived from legumes and oilseeds grown in the triticale producing areas. Milling devices which can process both cereal grains and legumes within the same facility will, it is believed, make possible the production and use of nutritious cereal-legume mixtures. The International Development Research Centre is encouraging a series of integrated projects with this end in view.

Epilogue

Triticale is well along the way to becoming the first commercially viable cereal grain to result from a scientifically controlled genetic wide-cross. Several triticales produce significantly higher yields of grain than the most productive known bread wheats grown under comparable conditions. Lysine contents equivalent to high lysine maize, and protein contents in the same order as bread wheats are also to be found among advanced triticale lines.

More research is required to determine the overall biological value of advanced triticales in animal feeds, much of the earlier reported work being inconclusive. Greater attention should also be given to exploring fully the potential of the rye parent in the synthesis of triticales of higher biological value.

Much progress is apparent in the improvement of inherent fertility and in kernel characteristics. Grain and endosperm shrivelling, though not yet totally eliminated, is currently of a significantly lower incidence than in the past. Further improvements in genetic, agronomic, technological and biological properties may be confidently expected.

These are the tangible and predictable products of the Triticale Project.

The Triticale Project provides more than these tangible benefits. The Project clearly demonstrates the feasibility of producing viable plants from wide genetic crosses. The Project also serves as a model of what can be achieved by a scientifically integrated approach to plant breeding in which equal concern is afforded to the plant's agronomic properties, its biological quality and its utility.

The Triticale Project demonstrates that, in the words of Radhakrishnan, "We can take a hand in shaping the future of things."

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