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# Establishing Relationships of Nutrient Composition and Quality of Wheat and Triticale Grains Using Chicken, Quail, and Flour Beetle Bioassays

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The growth of chickens, quail, and *Tribolium castaneum* larvae fed isonitrogenous diets containing 15 percent protein mostly derived from cereals was correlated with the chemical composition using ridge regression analysis. The following variables were included in the analysis for two varieties of triticale, three varieties of soft and common wheat, two of hard wheat, and three of durum wheat: moisture, crude protein, lipid, ash, amylase inhibitor activity, pearling index, reducing and nonreducing sugars, glucose, soluble and insoluble starch, available carbohydrate, amylopectin/amylose ratio, water-soluble pentosans, cellulose, pectic substances, lignin, hemicellulose, nonavailable carbohydrate, and acid and neutral detergent fiber contents.

The triticales were significantly better than the wheats for the growth of test organisms. Although the constituents of cereals are not nutritionally independent for supporting growth, the results of these studies showed that starches may be a more important nutritional component of cereals than was previously presumed. Both starch and crude protein content were positive nutritional components for weight gain. Grain hardness was negatively correlated with weight gain in all test organisms.

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## Establishing Relationships of Nutrient Composition and Quality of Wheat and Triticale Grains Using Chicken, Quail, and Flour Beetle Bioassays<sup>1</sup>

## **INTRODUCTION**

PLANT BREEDERS ARE CONTINUALLY ALTERING CEREALS through genetic manipulations to develop new varieties for increased yields and pest resistance. The ultimate use of these cereals in the diets of humans and animals is of direct concern to the nutritionist. Components of nutrient quality in cereals include protein content and amino acid profiles, the kinds of carbohydrates, lipids, vitamins and minerals, and the presence of any deleterious antinutrients. Availability of nutrients must be evaluated by feeding trials, experimentally difficult with humans, but practical with mice, rats, or poultry. Insects have also been used successfully to evaluate the nutritional value of cereals. (Vohra et al., 1973; Medrano et al., 1979; Loschiavo, 1980; Shariff, Vohra, and Qualset, 1981).

Usually, the nutritional quality of cereals is studied in relation to an individual nutrient. Much emphasis has been placed on protein content and the amino acid profile of the grain. The role of various carbohydrates in the nutritional quality of cereals or diets has been determined with isolated purified sugars, starches, or unavailable carbohydrates (Vohra et al., 1973). Effects of individual components of cereal grains, especially the components of the carbohydrate complex, on the nutritional value of whole ground cereals have not been evaluated extensively. The purpose of this study was, therefore, to report the detailed chemical composition of selected varieties of triticale and common and durum wheats and to evaluate their nutritional properties as indicated by the growth responses of chickens, quail, and *Tribolium castaneum* larvae to diets based on these cereals.

## MATERIALS AND METHODS

## Grain samples

We selected 47 varieties of wheat and triticale from international performance trials conducted at the University of California Agronomy Research Farm in the 1977–1978 crop season. Preliminary results with flour beetle larvae were then used to select 10 of these varieties for detailed study. The 10 varieties were grown under identical conditions with irrigation at the Agronomy Farm in the 1978–1979 crop season to produce sufficient grain for chemical analyses and bioassays. The varieties chosen and their origins are as follows:

Hexaploid triticale (XTriticosecale Wittmack), selected from the International Triticale Yield Nursery: Mapache and 6TA-204. Mapache was developed by the International Maize and Wheat Improvement Center (CIMMYT) and 6TA-204 was developed by B. C. Jenkins.

Hard common wheat (Triticum aestivum L.), selected from the International Spring

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Wheat Yield Nursery (ISWYN): Nacozari 76 from CIMMYT and Anza from CIMMYT and the University of California, Davis.

Soft common wheat:  $H-RA^2-F_2$ , a spring wheat selected from ISWYN and developed by CIMMYT; Atlas 66 and NapHal/Atlas 66, winter wheats developed in North Carolina and Nebraska, USA, respectively, which were selected from the International Winter Wheat Performance Nursery organized by the U.S. Department of Agriculture and the University of Nebraska. These varieties will be designated hereafter as HRAF, A66, and NHA66, respectively.

Durum wheat (*Triticum turgidum* L.), selected from the International Durum Yield Nursery organized by CIMMYT: Mexicali 75, Mallard 'S', and Rokel 'S', all developed by CIMMYT.

### Chemical analyses

All test samples were finely ground for chemical analysis in a Thomas-Wiley intermediate mill and were passed through a 100-mesh screen. Moisture, ash, lipids, and crude protein were determined in triplicate by the methods given in AOAC (1975). A factor of 5.8 was used to convert Kjeldahl nitrogen to crude protein for wheats and triticales. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined as described by Robertson (1978).

Starch was isolated from test samples as described by Wolf (1964). Amylose content in starch was measured according to the procedure of William, Kuzina, and Hylnka (1970). Amylopectin was estimated by subtracting the value of amylose from that of total starch.

The methods described by Southgate (1969a, 1969b) were used to determine fractions of available and unavailable carbohydrates, including water-soluble and water-insoluble starch, reducing and nonreducing sugars, water-soluble pentosans (pectin), cellulose, hemicellulose, pectic substances, and lignin. Another enzymatic method suggested by Hellendoorn, Noordhoff, and Stagman (1975) was also used in determining total unavailable carbohydrates.

Alpha-amylase inhibitor activity in test varieties was determined by a modified method of Yetter, Saunders, and Boles (1979). A finely ground 500-mg sample was weighed in a centrifuge tube. Each sample was assayed in six replicates. Exactly 30 ml of 1.0 M sodium bicarbonate (pH 8) solution were added to three replicates of each sample and shaken gently for 10 minutes. The soluble extract was separated from insoluble residue by centrifugation for 20 minutes at  $7,700 \times G$ . These tubes were test samples, and the remaining three replicates of each sample were controls. Human saliva (1 ml), 4 ml of 0.02 M phosphate buffer (pH 6.5), and 1 ml of 0.15 M sodium chloride solution were added to each replicate (both test samples and controls), mixed gently, and allowed to incubate for 10 minutes at  $30^{\circ}$ C. The soluble portion was then removed by centrifugation for 10 minutes at  $5,000 \times G$ , and glucose was determined by the oxidase method in the supernatant. Alpha-amylase inhibitor activity was estimated in percent of glucose in the control samples in relation to the amount of glucose in the test samples.

Grain hardness was measured by the pearling index (Beard and Poehlman, 1954).

#### **Bioassay methods**

The procedure for nutritional bioassay using red flour beetle (*Tribolium castaneum*) larvae, Japanese quail (*Coturnix coturnix japonica*), and chickens has been described

	Trit	icale	Soft	wheat	Hard	wheat	D	urum wh	eat
Item	Mapache	6TA-204	HRAF	A66	Nacozar 76	i Anza	Mallard	Rokel	Mexicali 75
Test cereal (g/kg)	862.2	866.4	822.6	910.0	859.7	856.5	848.5	830.6	849.2
Glutamic acid (g/kg)	47.8	43.6	87.4	-	50.3	53.5	61.5	79.4	60.8
Basal mix <sup>+</sup> (g/kg)	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0
Calculated metaboliz	able								
energy (kcal/g)	2.78	2.82	2.80	2.50	2.83	2.80	2.63	2.65	2.79

TABLE 1. COMPOSITION OF TEST CEREAL DIETS\*

\* Diets were planned to contain 15 percent crude protein. Glutamic acid (8.25% N) was used to adjust nitrogen content in diets.

<sup>†</sup> Contained: (in g) DL-methionine, 4.5; CaHPO<sub>4</sub>·2H<sub>2</sub>O, 27.0; CaCO<sub>3</sub>, 10; soybean oil, 20; KCl, 2.97; K<sub>2</sub>HPO<sub>4</sub>, 4.95; MgSO<sub>4</sub>·7H<sub>2</sub>O, 3.97; NaCl, 5.5; MnSO<sub>4</sub>·H<sub>2</sub>O, 0.297; CuSO<sub>4</sub>·5H<sub>2</sub>O, 0.097; ZnO, 0.12; Ca(C<sub>2</sub>H<sub>3</sub>O<sub>2</sub>)<sub>3</sub>·4H<sub>2</sub>O, 0.02; Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O, 0.009; KIO<sub>3</sub>, 0.09; Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O, 0.00066; FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.664; (in mg), menadione bisulfite 1.5; thiamin HCl, 4; riboflavin, 8; niacin, 60; calcium pantothenate, 25; folic acid, 1.2; biotin, 0.2; vitamin B<sub>12</sub>, 0.01, pytidoxine HCl, 8; choline chloride, 1300; BHT, 100; (in IU), vitamin A, 5000; vitamin D<sub>3</sub>, 4500, vitamin E, 88.

earlier (Vohra et al., 1973).

Day-old chicks and Japanese quail were fed the test cereal diets of the composition given in Table 1. A combination of test cereals and D, L-glutamic acid (8.25 percent N) was used to make diets isonitrogenous with 15 percent crude protein. A combination of soybean meal and yellow corn was used in control diets for chickens and quail, also with 15 percent crude protein. The gain in body weight of chickens and Japanese quail was determined over an experimental period of two weeks. *Tribolium castaneum* larvae were also fed the chicken diets (Table 1), and they were weighed at 14 days of age. All diets were finely ground to pass through a 100-mesh sieve. The procedure was essentially the same as described previously by Shariff et al. (1981).

#### Data analysis

The chemical composition parameters of the grains were subjected to analysis of variance, and the means with the standard errors are reported. Similar analyses were done on the growth data in the bioassays. The influence of the test cereal composition on growth of chickens, quail, and *T. castaneum* larvae was studied by multiple regression analysis. The relationship of grain hardness to growth was determined by simple regression analysis. The interrelationships of independent variables were determined by ridge regression analysis and the resulting ridge traces (Williams, Qualset, and Geng, 1979).

## **RESULTS AND DISCUSSION**

#### Chemical composition and grain hardness

Compositions of the noncarbohydrate fraction of tested wheat and triticale samples are given in Table 2. Two of the soft wheats (A66 and NHA66) did not produce wellfilled grains, because they matured late. As a result, they had a significantly higher protein content (Table 2) and lower total available carbohydrate concentrations (Table 3) than the other common wheats. However, no significant differences in crude protein,

					Alpha-amylase	
Test material	Moisture	Crude protein (X1)	Lipid	Ash	inhibition	Pearling index <sup>†</sup>
Triticale	%	%	%	%	%	%
Mapache	$9.1 \pm 0.7a$	$14.2 \pm 0.4a$	$1.6 \pm 0.1a$	$1.4 \pm 0.1a$	31.6±1.7a	$6.0 \pm 1.2a$
6TA-204	$8.6 \pm 0.4a$	$14.4 \pm 0.4a$	$1.6 \pm 0.1a$	$1.3 \pm 0.1a$	$28.8 \pm 1.4a$	$8.0 \pm 1.5a$
Soft wheat						
HRAF	$8.2 \pm 0.6a$	$12.0 \pm 0.5a$	$1.6 \pm 0.1a$	$1.4 \pm 0.1a$	$32.2 \pm 2.0a$	$8.0 \pm 1.1a$
A66	$8.2 \pm 0.5a$	17.5 ± 0.9b	1.7 ± 0.1a	$1.5 \pm 0.1a$	35.0±1.9b	$8.6 \pm 1.0a$
NHA66	$8.1 \pm 0.4a$	$20.3 \pm 0.8b$	$1.7 \pm 0.1a$	$1.5 \pm 0.1a$	39.7 ± 1.3b	8.6±0.9a
Hard wheat						
Nacozari 76	$9.0 \pm 0.3a$	$14.7 \pm 0.7a$	$1.4 \pm 0.1a$	$1.4 \pm 0.1a$	38.3 ± 1.5b	16.6 ± 2.1b
Anza	$8.6 \pm 0.3a$	13.9±0.8a	$1.5 \pm 0.1a$	$1.3 \pm 0.1a$	$37.3 \pm 1.7b$	$19.4 \pm 1.4$ b
Durum wheat						
Mallard	$8.4 \pm 0.5a$	13.5 ± 0.3a	$1.3 \pm 0.1a$	$1.4 \pm 0.2a$	$39.4 \pm 1.1b$	$25.4 \pm 2.3c$
Rokel	$8.0 \pm 0.4a$	$12.5 \pm 0.6a$	$1.4 \pm 0.1a$	$1.5 \pm 0.1a$	$32.9 \pm 1.2a$	$24.6 \pm 2.0c$
Mexicali 75	$8.5 \pm 0.5a$	13.5 ± 0.5a	$1.6 \pm 0.1a$	$1.5 \pm 0.1a$	34.6 ± 1.0ab	23.0±1.7c

TABLE 2.	PERCENTAGE COMPOSITION,	ALPHA-AMYLASE INHIBITION	AND PEARLING INDEX
	OI	F TEST CEREALS*	

\* Values given are means ± standard deviations. Values in a column followed by the same letter are not statistically different by Duncan's multiple range test (P<0.01).</p>

† Percentage of sample left unground.

TABLE 3. COMPOSITION OF DIGESTIBLE CARBOHYDRATE FRACTION OF THE TEST CEREALS\*

Test material	Reducing sugars (A)	Non-reducing sugars (B)	Glucose	Soluble starch (C)	Insoluble starch (X2) (D)	Total available CH2O (A+B+C+D)	Amylose	Amylo- pectin/ amylose ratio
Triticale	%	%	%	%	%	%	%	
Mapach <del>e</del>	$0.41 \pm 0.07a$	$1.19 \pm 0.04a$	$0.11 \pm .002a$	5.7 ± 0.6b	58.4 ± 2.9b	65.76b	$18.1 \pm 1.2a$	4.5a
6TĀ-204	$0.45 \pm 0.08a$	1.30 ± 0.07a	0.07 ± .003a	$5.6 \pm 0.2b$	$58.6 \pm 2.4b$	65.92b	20.0±0.9a	4.0a
Soft wheat								
HRAF	$0.45 \pm 0.08a$	$1.26 \pm 0.10a$	$0.08 \pm .001a$	5.2 ± 0.4b	58.1 ± 1.7b	64.95b	$21.0 \pm 1.1a$	3.8a
A66	$0.52 \pm 0.09$ b	$1.49 \pm 0.08b$	$0.20 \pm .004$ b	3.6±0.7a	$45.4 \pm 1.4a$	50.91a	$19.0 \pm 1.7a$	4.3a
NHA66	$0.62 \pm 0.04$ b	$1.44 \pm 0.07 b$	$0.19 \pm .003 b$	$2.8 \pm 0.5a$	$42.1 \pm 2.4a$	46.95a	$20.5 \pm 1.0a$	3.9a
Hard wheat								
Nacozari 76	$0.37 \pm 0.02a$	$1.29 \pm 0.10a$	$0.10 \pm .004a$	$5.4 \pm 0.8b$	55.8 ± 2.1b	62.87b	$23.1 \pm 1.6a$	3.3a
Anza	$0.47 \pm 0.05a$	$1.23 \pm 0.04a$	0.08 ± .005a	$4.9 \pm 0.9b$	55.9±2.1b	62.47b	23.5±1.8a	3.2a
Durum who	at							
Mallard	$0.47 \pm 0.05a$	$1.33 \pm 0.05a$	0.07 ± .006a	$4.5 \pm 0.6b$	$57.4 \pm 2.4b$	63.62b	$19.5 \pm 0.8a$	4.1a
Rokel	$0.38 \pm 0.02a$	$1.01 \pm 0.11a$	$0.06 \pm .003a$	$4.8 \pm 0.7 b$	$56.1 \pm 3.1b$	62.34b	$22.1 \pm 1.2a$	3.6a
Mexicali 75	$0.48 \pm 0.04a$	$1.17 \pm 0.06a$	$0.09 \pm .006a$	$4.9 \pm 0.3b$	$54.5 \pm 2.5b$	61.00b	$21.0 \pm 1.1a$	3.8a

\* See first footnote (\*), table 2.

carbohydrates, lipid, or ash content of other samples were observed.

Alpha-amylase inhibitor activity was high in two abnormal soft wheats (A66 and NHA66), the hard wheats, and Mallard durum (Table 2). However, Mexicali 75, a durum wheat, was not significantly different from other test varieties. The two triticales, one normal soft wheat, and one of the durum wheats had significantly less alpha-amylase inhibitor activity than the other varieties.

The hardness values or pearling indices for the tested varieties of triticales and soft wheats were not significantly different from each other but were significantly (P<0.01) lower than those of hard wheats and durum wheats (Table 2). The durum wheat

varieties were the hardest of the group examined. The values for hard wheat varieties were significantly (P<0.01) lower than those obtained for durum wheat varieties, but significantly (P<0.01) higher than the triticales and soft wheat varieties.

Results of fractionation of the available carbohydrate complex by the Southgate (1969a) procedure are presented in Table 3. Total available carbohydrate values were calculated as the sum of reducing sugars, nonreducing sugars, and soluble and insoluble starch fractions. A66 and NHA66 had significantly (P<0.01) higher levels of reducing and nonreducing sugars and glucose than the other varieties. However, water-soluble and water-insoluble starch contents were significantly lower in these two varieties with the result that total available carbohydrates were reduced. No significant differences were observed among other varieties of wheats or triticales.

Cerning and Guilbot (1973) stated that, in general, the mono-, di-, and oligosaccharide levels decreased during grain maturation. It seemed as if di-, and oligosaccharides were degraded into monosaccharides by an enzyme system and then used in polysaccharide synthesis. Earlier, Harris (1962) found that starch was synthesized at the expense of reducing and nonreducing sugars. These reports might explain the higher values of reducing and nonreducing sugars and lower values of soluble and insoluble starch in one of the soft wheat varieties. The structural carbohydrates and protein components were present, but the storage carbohydrates were deficient and the grain did not fill properly.

No significant differences in the amylose content of the starches from any of the test cereals were detected. The amount of amylose was about one quarter the amount of amylopectin, as indicated by the amylopectin/amylose ratio.

Results of fractionation of the unavailable carbohydrate complex by the procedure of Southgate (1969b) are given in Table 4. The abnormal soft wheat had a significantly higher level of pectic substances and lignin than the triticales, the normal soft wheat, and durum wheats. Its pectin content was not significantly different from one of the hard wheats and one of the durum wheats. In general, hard wheats were not significantly different from other tested cereals in most of the constituents of nondigestible carbohydrate fraction.

		Sou	thgate metho	d		Total	Hellen-		
Test material	Water-solubl pentosans (X6)	e Cellulose (X4)	Pectic substance (X7)	Lignin (X5)	Hemi- cellulose (X3)	South-	doorn, et al, method enzymatic	Van met NDF	Soest hod ADF
Triticale	%	%	%	%	%	%	%	%	%
Mapache	$0.42 \pm 0.11a$	2.37 ± 0.09a	$0.87 \pm 0.10a$	$1.06 \pm 0.02a$	$3.3 \pm 1.4a$	7.9 ± 1.2a	$10.4 \pm 0.5a$	6.0±0.4a	2.9±0.2
6TA-204	$0.55 \pm 0.42a$	$2.43 \pm 0.06a$	$0.77 \pm 0.11a$	$1.00 \pm 0.01a$	$3.4 \pm 1.1a$	$8.2 \pm 0.8a$	11.0 ± 1.5a	$6.2 \pm 0.3a$	3.2±0.14
Soft wheat									
HRAF	0.69 ± 0.30a	$2.80 \pm 0.11a$	$0.82 \pm 0.09a$	$1.08 \pm 0.01a$	$4.4 \pm 1.1a$	$9.8 \pm 1.0a$	$11.3 \pm 1.0a$	$7.6 \pm 0.4a$	$3.4 \pm 0.12$
<b>A</b> 66	$1.30 \pm 0.42b$	$3.13 \pm 0.08b$	$1.01 \pm 0.02b$	1.54 ± 0.03b	$6.3 \pm 1.4a$	13.2±0.3b	15.7 ± 0.4b	10.1 ± 0.5a	4.2 ± 0.1t
NHA66	$1.41 \pm 0.39b$	$3.01 \pm 0.12$ ab	$1.21 \pm 0.07b$	$1.62 \pm 0.05 b$	6.6±1.2a	$13.9 \pm 0.5b$	16.2 ± 0.9b	10.1±0.2a	4.0±0.2b
Hard wheat									
Nacozari 76	1.07 ± 0.22ab	$2.63 \pm 0.14a$	0.97 ± 0.04ab	1.04 ± .007a	6.7 ± 1.3a	$12.4 \pm 0.1b$	13.6 ± 0.2ab	$9.4 \pm 0.4a$	2.9±0.22
Anza	0.76 ± 0.19a	$2.86 \pm 0.07a$	$0.92 \pm 0.04 ab$	$1.18 \pm 0.04a$	$5.0 \pm 1.4a$	10.7 ± 0.7a	$13.4 \pm 0.8a$	$8.4 \pm 0.2a$	$3.4 \pm 0.12$
Durum whea	ıt								
Mallard	$0.90 \pm 0.29a$	$2.06 \pm 0.05a$	$0.88 \pm 0.05a$	$0.99 \pm 0.02a$	$4.9 \pm 1.5a$	9.7 ± 0.6a	$11.3 \pm 0.1a$	7.9 ± 0.5a	$3.1 \pm 0.1$
Rokel	$0.59 \pm 0.20a$	$2.87 \pm 0.04a$	$0.85 \pm 0.07a$	1.07 ± 0.03a	5.0±1.7a	$10.4 \pm 0.9a$	12.9 ± 1.5a	$8.2 \pm 0.4a$	3.5±0.1a
Mexicali 75	1.00 ± 0.32ab	$2.69 \pm 0.07a$	$0.82 \pm 0.08a$	$1.22 \pm 0.04a$	$6.1 \pm 1.4a$	11.8 ± 1.2ab	13.9±0.5ab	$9.2 \pm 0.3a$	$3.4 \pm 0.12$

 TABLE 4.
 COMPOSITION OF NON-DIGESTIBLE CARBOHYDRATE FRACTION OF THE TEST CEREALS BY DIFFERENT METHODS\*

\* See first footnote (\*), table 2.

Significantly higher values for total unavailable carbohydrate content were found in the two abnormal soft wheats and a hard wheat variety, Nacozari 76, when the method of Southgate (1969b) was used (Table 4). A66 showed significantly higher unavailable carbohydrates as determined by acid detergent method. However, no test samples were significantly different in NDF content. A hard wheat, Nacozari 76, and a durum wheat, Mexicali 75, were not significantly different from A66 when total unavailable carbohydrates were determined by the method of Hellendoorn, Noordhoff, and Stagman (1975).

Values of unavailable carbohydrate or dietary fiber content, as determined by enzymatic method of Hellendoorn, Noordhoff, and Stagman (1975), were higher than those determined by Van Soest's detergent method or by Southgate's method (1969a, b) (Table 4). Acid detergent fiber was always less than neutral detergent fiber, because hemicellulose was not measured in ADF. ADF measures cellulose and lignin only. ADF and NDF were lower than total unavailable carbohydrates determined by the Southgate method. Because pectin and gums are lost in solution in the detergent fiber method, this method is not very suitable for measuring total unavailable carbohydrates of cereals.

#### Nutritional bioassay

Weight gain of chickens and quail fed the test cereal diets showed that the triticales were superior to durum wheats and hard wheats in supporting growth (Table 5). The diet containing A66 was nutritionally as good for quail as those containing triticales. No clear-cut patterns were noticed for other samples. Because of a shortage, A66 and NHA66 were not fed to chickens, and only A66 was available for the quail diets.

The level of crude protein in test diets was kept at 15 percent, which is less than optimal for the growth of chickens and Japanese quail (Table 1). In a preliminary study, differences in quail growth due to differences in chemical composition of cereals were masked when the crude protein level was 30 percent. For the estimation of protein quality of cereals, Fernandez, Lucas, and McGinnis (1974) found it desirable to keep the protein level of chicken diets at 14 percent. Our diets included glutamic acid as a source of nitrogen to bring the crude protein level to about 15 percent, since it is not deleterious for chickens, quail, or the *Tribolium* larvae. The resulting data suggested that the triticales were nutritionally better than the wheat varieties tested in this study and further support our earlier results (Shariff, Vohra, and Qualset, 1981).

To relate the growth responses to the chemical composition of the cereals, we selected seven chemical composition variables for analysis by regression methods: crude protein (X1), starch (X2), nemicellulose (X3), cellulose (X4), lignin (X5), pectin (X6), and pectic substances (X7).

Simple correlations showed the weight gains of the test organisms to be generally correlated positively with starch and, to a lesser extent, with crude protein, but negatively with the other five composition variables (Table 6). The partial regression coefficients for several of the composition variables were highly unstable during stepwise regression analysis. Examination of the simple correlation matrix of the composition variables revealed a close negative association between X2 and X3 (r = -0.87) and high positive association between X3 and X6 (r = 0.90) (Table 6). Consequently, variance inflation factors (VIF) were calculated to determine the degree of multiple correlation (R) among the composition variables, i.e., the degree of multicollinearity or interrelatedness. Any VIF value exceeding 10 (equivalent R = 0.95) is considered likely to cause difficulty in

	We	eight gain	Larv	al weight
Cereal variety in diet		0 1		2
in diet	Chicken	Quail	1	2
	g	g	mg	mg
Control	$107 \pm 11c$	$20 \pm 3c$	$2.98 \pm .09 bc$	2.94 ± .07d
Triticale				
Mapache	$62 \pm 6b$	13 ± 3b	3.05 ± .02c	1.57 ± .09c
6TA-204	$62 \pm 7b$	$14 \pm 4b$	$3.13 \pm .04c$	$1.59 \pm .03c$
Soft wheat				
HRAF	54 ± 4a	11 ± 4ab	2.97 ± .12bc	1.41 ± .03bc
A66	_	$14 \pm 5b$	2.76 ± .05a	
NHA66	_	_	$2.80 \pm .04a$	—
Hard wheat				
Nacozari 76	54 ± 5a	$10 \pm 4a$	2.88 ± .11ab	0.87 ± .02a
Anza	$51 \pm 4a$	7 ± 2a	2.85 ± .07ab	$1.15 \pm .07b$
Durum wheat				
Mallard	53 ± 7a	$7 \pm 2a$	2.99 ± .07bc	$0.54 \pm .03a$
Rokel	$54 \pm 5a$	$8 \pm 2a$	2.90 ± .03b	$0.71 \pm .05a$
Mexicali 75	$51 \pm 4a$	6 ± 1a	$2.79 \pm .06a$	$0.62 \pm .04a$

#### TABLE 5. WEIGHT GAINS OF CHICKENS AND JAPANESE QUAIL AND WEIGHTS OF TRIBOLIUM CASTANEUM LARVAE\*

\*See first footnote (\*), table 2.

Composition variable			Weight g	gain	Composition variable					
		Chick	Quail	Larva	X2	X3	X4	X5	X6	<b>X</b> 7
X1	Crude protein	.38	.25	.36	.06	11	48	22	.03	.23
X2	Starch	.74	.79	.80		87	47	52	71	39
X3	Hemicellulose	74	67	74			.32	.15	.90	.56
X4	Cellulose	36	24	28				.67	.00	.07
X5	Lignin	40	44	42					01	05
X6	Pectin	69	65	72						.47
<b>X</b> 7	Pectic substances	26	31	32						

TABLE 6. SIMPLE CORRELATION COEFFICIENTS BETWEEN WEIGHT GAIN OF CHICKENS, QUAIL, AND *TRIBOLIUM* LARVAE AND SELECTED CHEMICAL COMPOSITION VARIABLES OF CEREALS.

estimating partial regression coefficients because of multicollinearity (Marquardt, 1970).

In the chicken experiment, VIF values calculated for ordinary least squares (OLS) estimates of the regression coefficients exceeded the value 10 for starch, hemicellulose, cellulose, and pectin (Table 7). For quail and *T. castaneum* the VIF values for starch, hemicellulose, lignin, and pectin exceeded 10 (Tables 8 and 9). The use of ridge regression (Williams, Qualset, and Geng, 1979) with K = 0.06 to 0.1 brought all VIF values far below 10. The evidence from VIF calculations confirmed that part of the instability of coefficients perceived before resulted from interrelations among independent variables.

With the detection of collinear relations among the independent variables, the next step was to identify the influential variables from the ridge traces. These plots of standard partial regression coefficients against the bias parameter K over the range from 0 to 1 were prepared for each experiment using the ridge regression computer program developed by Jeffery and McKinney (1975).

Examination of the ridge traces for the chicken experiment showed some instability, since hemicellulose was the largest positive coefficient at K = 0 and then rapidly decreased to slightly below 0 (Figure 1A and Table 7). Cellulose started near -0.2 and rose above 0 and again changed its sign as the K value increased. Similarly, lignin started out near 0 and decreased to about -0.2. However, starch, protein, and pectin remained stable.

In the quail experiment, ridge traces also showed instability in hemicellulose and lignin: both of these variables started out strongly positive, but rapidly changed sign (Fig. 1B and Table 8). The unstable cellulose trace started out negative, rose sharply positive, and then approached 0. Again, starch, protein, and pectin remained quite stable.

TABLE 7. WEIGHT GAIN OF CHICKENS. PARTIAL (b) AND STANDARD PARTIAL REGRESSION COEFFICIENTS ( $\hat{\beta}$  AND  $\hat{\beta}$  [K]) AND VARIANCE INFLATION FACTORS (VIF) BY ORDINARY LEAST SQUARES (OLS) AND RIDGE REGRESSION ESTIMATION

		0	OLS (K = 0) Ridge regre				
Composition variable		b	β	VIF	b	β̂(K)	VIF
X1	Crude protein	2.769	0.437	1.6	2.118	0.335	1.0
X2	Starch	3.261	0.895	32.8	0.817	0.224	1.5
X3	Hemicellulose	6.221	1.380	119.5	- 0.472	- 0.105	0.5
X4	Cellulose	- 4.318	-0.227	11.1	1.738	0.091	1.3
X5	Lignin	- 0.089	-0.001	9.8	- 17.392	- 0.269	1.3
X6	Pectin	- 28.163	- 1.190	36.1	- 8.584	- 0.364	1.3
<b>X</b> 7	Pectic substances	- 22.620	- 0.296	3.0	- 8.122	- 0.106	1.1

#### TABLE 8. WEIGHT GAIN OF QUAIL. PARTIAL (b) AND STANDARD PARTIAL REGRESSION COEFFICIENTS ( $\hat{\beta}$ AND $\hat{\beta}$ [K]) AND VARIANCE INFLATION FACTORS (VIF) BY ORDINARY LEAST SQUARES (OLS) AND RIDGE REGRESSION ESTIMATION

	OLS (K = 0)			Ridge regression (K = 0.06)			
Composition variable		b	β	VIF	b	β̂(K)	VIF
<b>X</b> 1	Crude protein	2.388	0.673	2.1	1.099	0.310	1.0
X2	Starch	5.473	2.680	32.6	1.143	0.560	1.4
X3	Hemicellulose	9.463	3.750	103.7	0.162	0.064	0.5
X4	Cellulose	- 3.319	- 0.311	8.4	3.316	0.310	1.3
X5	Lignin	25.636	0.708	10.4	- 9.971	- 0.275	1.2
X6	Pectin	- 25.031	1.890	29.1	- 3.235	- 0.245	1.3
<b>X</b> 7	Pectic substances	- 26.527	- 0.551	2.8	- 5.184	- 0.108	1.1

#### TABLE 9. WEIGHT OF *TRIBOLIUM* LARVAE. PARTIAL (b) AND STANDARD PARTIAL REGRESSION COEFFICIENTS ( $\hat{\beta}$ AND $\hat{\beta}$ [K]) AND VARIANCE INFLATION FACTORS (VIF) BY ORDINARY LEAST SQUARES (OLS) AND RIDGE REGRESSION ESTIMATION.

		C	OLS $(K = 0)$			Ridge regression (K = 0.1)			
Con	position variable	b	β	VIF	b	β̂(K)	VIF		
X1	Crude protein	0.355	0.701	2.1	0.198	0.390	1.0		
X2	Starch	0.677	2.320	32.6	0.225	0.772	1.4		
X3	Hemicellulose	0.635	1.760	103.7	- 0.056	- 0.156	0.5		
X4	Cellulose	0.558	0.366	8.4	0.740	0.485	1.3		
X5	Lignin	3.939	0.762	10.4	0.530	0.103	1.2		
<b>X</b> 6	Pectin	- 0.982	-0.520	29.1	0.001	0.001	1.3		
<b>X</b> 7	Pectic substances	- 2.080	- 0.302	2.8	- 0.346	- 0.050	1.1		

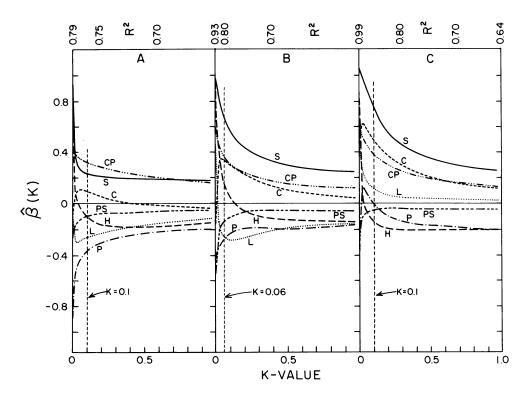


Figure 1. Ridge traces of standard partial regression coefficients  $\hat{\beta}(K)$  for increasing values of K and coefficients of determination R<sup>2</sup> for seven composition variables related to: A, chicken growth; B, quail growth; and C, *T. castaneum* growth (larval weight 2 of Table 5). CP = crude protein; S = starch, H = hemicellulose, C = cellulose, L = lignin, P = pectin, PS = pectic substances.

In the *T. castaneum* experiment where the chicken diet was used (larval weight 2 in Table 5), ridge traces showed marked instability in the variables hemicellulose and lignin as these two variables started out strongly positive and either became negative or became the smallest ones with increasing K-values (Fig. 1C and Table 9). Pectin started out negative then changed sign twice and ended up near -0.2. Crude protein and starch were quite stable as in the chicken and quail experiments.

The data in Tables 7, 8, and 9 for standard partial regression coefficients at K = 0.1 indicate that starch and crude protein were consistently positive nutritional components leading to weight gain. Cellulose was an important positive component for quail and larval weight. Lignin and pectin were negative components for chickens and quail but not for the larvae. Pectic substances did not show an important role in any of the experiments. These findings confirmed that hemicellulose, lignin, and cellulose are not independent of each other. The results further confirmed that the starch content of cereals has the most significant effect on their nutritional value for fowl, followed by crude protein and pectin. Starch and crude protein contents had a positive effect, while pectin had a negative effect. Pectin has been shown to be a growth depressant for chickens, quail, and *T. castaneum* larvae when added at 2 percent in the diet (Vohra et al., 1973).

The influence of alpha-amylase inhibitor activity in cereals on nutritional value was determined by multiple regression. First, three main constituents—starch, crude protein, and alpha-amylase inhibitors—were used, and R<sup>2</sup> was determined for each experi-

ment. Then the variable for the alpha-amylase inhibitor was omitted, and no significant decrease in  $\mathbb{R}^2$  was observed. These results indicate that alpha-amylase inhibitor did not significantly affect growth of chickens, quail, or *T. castaneum* larvae. Macri et al. (1977) reported that native wheat albumin did not show the depressive effect upon chicken growth. They concluded that the chicken's gastric digestion is very effective in inactivating albumin amylase inhibitor. However, Pace et al. (1978) reported that growth of *Tenebro moliter* L. larvae was depressed when purified wheat albumin was added in the diets. It is possible that more purified alpha-amylase inhibitor was more deleterious than that in the cereals, or *T. moliter* was more sensitive to amylase inhibitors than our test organisms.

The hardness of the test cereals negatively influenced the growth of chicken, quail, and T. castaneum larvae as indicated by the results of simple regression analysis. The equations are:

C = 93.32 - 1.39 X	r <sup>2</sup> =	0.58	(P<0.05)
Q = 40.48 - 2.50 X	r <sup>2</sup> =	0.83	(P<0.01)
T = 165.06 - 53.60 X	r <sup>2</sup> =	0.79	(P<0.01)

where C = weight/chicken, Q = weight/quail, T = weight/larva, and X = hardness (%).

## CONCLUSIONS

We conclude that triticales are significantly better for the growth of chicken, quail, and *Tribolium* larvae, and that starch plays a more important role in the nutritional value of cereals than was previously presumed. Also, the constituents of cereals should not be considered nutritionally independent. The physical hardness of the cereals (a positive factor for milling and eventual human food use) seems to decrease significantly the availability of nutrients to the test animals used in these experiments, and is a factor that can be modified relatively easily in plant breeding programs designed to improve nutritional quality of cereal grains.

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