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EVALUATION OF THE NUTRITIONAL QUALITY OF FOODS WITH THE RED FLOUR BEETLE *(Tribolium castaneum)*

II. Evaluation of corn, wheat and a processed soybean:corn preparation[1]

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ABSTRACT

To assess the nutritional quality of foods with the Red Flour Beetle. *Tribolium castaneum*, two test criteria were utilized: body weight gain in growing larvae in a 4-day period, and the developmental progress of the organism on test. Results were correlated with chemical values and with rat tests utilizing PER (Protein Efficiency Ratio) and a multiple point assay procedure. It was shown that: a) *Tribolium* and rat body weight gain as well as rat PER values showed a response approximately twice as good when fed an Opaque-2 corn in contrast to two other corn types with low lysine content; b) a 70:30 corn:soybean mixture prepared in two treatments with pre-cooked and raw soybeans and passed through a drum dryer was effectively and similarly classified in the two treatments by *Tribolium* larval weight gain, *Tribolium* development, and rat PER values; c) applying a multiple point assay evaluation procedure on three wheat varieties both with *Tribolium* and rat demonstrated another effective procedure as to how *Tribolium* can

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be utilized as test organism. The *Tribolium* assessment methodology that has been proposed is fast and inexpensive, requiring a small amount of sample that can be of particular value as a screening tool for nutritional quality in plant breeding programs.

INTRODUCTION

In an earlier communication (1) the methodology for utilizing the Red Flour Beetle *(Tribolium castaneum)* as a small scale, fast and inexpensive bioassay organism to assess the nutritional quality of foods was proposed. The method has two approaches, one consisting in measuring the weight gain of *Tribolium* larvae (quantitative approach) through a 4-day growth period when placed on a test diet standarized to a particular level of protein and completely balanced in required vitamins, minerals, fatty acids and sterols; and two, measuring the length of time to achieve the pupal and adult developmental stages (qualitative approach) of the insect in the test diet. The methodology, particularly the quantitative approach, is based on the same principle as mammalian test methods (2) where the amount and quality of protein in the diet can result in a gain or loss of body weight.

Considerable information in the literature of the biology of *Tribolium* has been compiled by A. Sokoloff in the treatise *The Biology of Tribolium,* where in Volume 2 (Oxford University Press, 1974) the chapter on "Feeding and Nutrition" is a valuable contribution to the development of feeding trials utilizing this insect. Also, earlier investigations (3-9) where different and longer methods of assessment than the present one were utilized, have consistently ascertained the advantages and potential of *Tribolium* as a test organism in feeding trials.

The insect is a promising tool to be utilized as a test criterion in plant breeding schemes where the number of samples to be analyzed in the initial generations is very large, with generally a small amount of sample available. Presently, selection programs to improve the nutritional quality of grains have to be circumscribed to utilizing chemical indicators due to the impracticality of applying a bioassay criterion with animals like the rat. Therefore, the need to

have an inexpensive, fast and small scale bioassay system like *Tribolium* that could reflect the nutritional potential and physiological performance of the protein or test material in question is a pressing need.

In the present investigation, two common bioassay techniques utilized with rats have been correlated with the *Tribolium* assay: PER, which was tested in three corn varieties and a high protein processed food, and a multiple point assay procedure tested on three wheat varieties. Some of the results of the present investigation have been reported earlier (10).

MATERIALS AND METHODS

The study was conducted with the same *Tribolium castaneum* population maintained following the same procedure and under the same conditions as described in an earlier report (1).

Tribolium test diets (1) consisted of a constant level of protein provided by the test material, cornstarch as a filler, 2.0% mineral premix (3), 0.63% vitamin premix (3), 1.0% cholesterol and 3.0% soybean oil. The trials were performed measuring larval weight gain from 10 to 14 days of age in 5 replicate samples for each diet, each replicate consisting of approximately 1 g of diet and 10 larvae, placed in a 2 x 5 cm Shell vial. Subsequently, at ages 18, 20 or 22 days, the developmental stage of the organisms in each replicate was recorded by counting the number of larvae, pupae and adults present.

PER (Protein Efficiency Ratio) tests were performed with eight 21-day old Wistar rats from INCAP's animal colony per diet. The test diets consisted of a 10% protein level from the test material, cornstarch as a filler, 5% soybean oil, 4% mineral mixture (11), 1% cod liver oil and 5 ml of vitamin premix (12) per 100 g of diet. The animals were kept in individual metal cages for 28 days, offered *ad libitum* food and water, and weekly recordings of body weight and food consumption were taken.

The second comparative method utilized with the rat to correlate with the *Tribolium* methodology was a multiple point assay

procedure recognized as nitrogen growth index (13, 14). Diets were prepared at 0, 3, 5 and 8% protein level for each test material, using the same basal diet ingredients and animal handling procedures as indicated above. Four 21-day old rats were allocated to each protein level, body weight and feed consumption were recorded weekly for a 2-week test period in all the animals. Regression lines were calculated for the trials relating body weight gain as a function of protein intake, and significant differences among regression coefficients calculated according to Steel & Torrie (15).

The above procedures were applied to three different types of material: corn, a high protein processed food and wheat flours, identified as experiments 1, 2 and 3, respectively.

Experiment 1 was conducted on three different Guatemalan grown corn types: a white high lysine Opaque-2 (9.1% protein), a flint yellow (9.7% protein) referred to as Azotea Yellow, and a flint reddish "Tiquisate Dorado" (10.2% protein) referred to as a Tiquisate Red. The *Tribolium* test was performed at a 6% protein level in the diets, and after the quantitative phase of the test the cultures were checked at 18 and 22 days of age to assess the developmental progress of the insects. PER was used as a comparative method with rats. Due to the protein content of the maize types, the test was performed at a 9.0% protein level. Total and available lysine in the corn was quantified by a paper electrophoresis technique (16).

Experiment 2 was carried out on a high protein processed food consisting of a 70:30 corn:soybean mixture prepared in two treatments: a) Precooked, in which the soybean grains were cooked together with corn in an autoclave for 20 min, at 15 psi and 121°C, and b) Raw, where raw dehulled soybean grains were soaked for 24 hours and then mixed with cooked corn. Both treatments were then separately ground in a disk mill, dehydrated and cooked in a drum dryer (VF dryer flakes, laboratory Model 215, General Food Package, Equipment Corporation) at drum speeds of 3, 5 and 7 rpm. In all cases roller pressure was 60 lbs at 143°C. The 6 samples from the precooked and raw treatments at 3, 5 and 7 rpm drum dryer speeds had an average protein content of 19.07 $\pm$ 0.53. They were finely ground and tested with *Tribolium* at a 10% protein level in the diet. PER was used as a comparative method with rats, and was performed at a 10% protein level. The data for experiments 1

and 2 were evaluated by analysis of variance and differences among means were determined by L.S.D. tests.[2]

Experiment 3 evaluated the quality of three varieties of wheat: Nap-Hal (A73-3), Atlas 66 (A73-1) and Commercial (73-5916)[3] in the forms of whole wheat flour and white wheat flour. For the tests, a multiple point evaluation procedure was used both for *Tribolium* and rats. The *Tribolium* assay was carried out measuring the weight gain of the larvae from 10 to 14 days of age including flour levels of protein for each test material. For the whole wheat material, levels of 0, 2, 6 and 8% protein in the diet were used, whereas for the white wheat flour the latter level was increased to 10%. Regression lines were calculated for the trials relating larval weight gain as a function of protein level in the diet, and significant differences among regression coefficients calculated according to Steel and Torrie (15).

RESULTS AND DISCUSSION

Experiment 1:

The performance of *Tribolium* larval weight gain (quantitative analvsis) and rat performance measured as PER and weight gain fed three different corn types is presented in Table I along with the lysine content of the corns.

Tribolium growth response with the Opaque-2 corn was significantly ($P < 0.05$) better and very close to twice as good as with the Azotea Yellow and Tiquisate Red corns, with no significant differences among the last two. This response is paralleled by the

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2 The high protein product was prepared by Ms. Delia Navarrete (Division of Agricultural and Food Sciences, INCAP), who kindly provided the rat test data information.

3 The wheat varieties were kindly supplied by Dr. Paul J. Mattern, Agronomy Department, University of Nebraska, Lincoln, Nebraska 68508, U.S.A.

TABLE I

Performance of *Tribolium* (quantitative analysis) and rats fed 3 different corn types and lysine content of the corns

Corn types	*Tribolium*	Rat		Lysine (g/16g N)	
	Wt. gain (mg)	Wt. gain (g)	PER	Total	Free
Opaque-2	1.21[a]	52.4[a]	2.05[a]	4.07	0.57
Azotea Yellow	0.62[b]	28.0[b]	1.44[b]	3.00	0.13
Tiquisate Red	0.69[b]	26.9[b]	1.34[b]	2.81	0.18

Means with no common superscript differ significantly ($P < 0.05$).

performance in rat weight gain and PER in which the Opaque-2 corn was also significantly ($P < 0.05$) superior to the other two corns. This type of response was expected due to the improved amino acid balance observed in the Opaque-2 corn in relation to other common corns (17), and as shown in Table I, the total and free lysine contents of this corn were markedly higher than in the other two types; a fact which in itself significantly contributes to improve rat performance when fed a common corn diet.

This is a simple example clearly demonstrating *Tribolium*'s ability to separate cereal types of different nutritional quality as accurately as the rat. It should be pointed out that the *Tribolium* tests, as proposed, can be expected to be more accurate and reproducible between laboratories in the assessment of a diet than trials conducted with other species using only 4 to 8 inbred animals per test. This is due to the fact that with *Tribolium:* 1) a large number of organisms are included in each test, and 2) these organisms come from a large random mating population which has greater stability than animals obtained from very distinct inbred strains. In some traits, inbred animals are more phenotypically variable in response on account of their greatly increased environmental variation (18). Also, the ability of the *Tribolium* tests to detect differences with a higher degree of precision can easily be improved by increasing the number of replicates, without having a major effect on the cost of the test.

Table II shows the results of recording the subsequent developmental forms of *Tribolium* present at 18 and 22 days of age in the same three corn diets as above. Following the earlier result at both ages assessed, the Opaque-2 corn was again ranked superior to the other two corns. At 18 days of age *Tribolium* cultures fed the Opaque-2 corn had 2.6 to 3 times as many pupae present as the other two corns. At 22 days of age the same favorable nutritional environment of the Opaque-2 corn produced 2.6 to 4 times as many adult forms. The mortality of the organisms in the test was much higher than has previously been observed when feeding the organism diets containing torula yeast (1). This was possibly due to unknown fractions in yeast that may play an essential role in pupation (19) and failed to be effectively supplied by the mineral mixture utilized in the diets. However, this mortality was really of no consequence to the effectiveness of the test in achieving the goal of separating the corn types according to their nutritional quality.

Experiment 2:

Table III shows the weight gain response of *Tribolium* larva and rat PER values when fed diets containing a mixture of 70% corn with 30% raw soybeans. As has been outlined, this mixture of corn and soybean was passed through a drum dryer at drum speeds of 3, 5 and 7 rpm. These speeds determine the exposure time of the product to the temperature in the rollers. It was the objective of the study to find an optimum roller speed, since a very long exposure time may decrease essential amino acids availability in the protein, rendering it of lower nutritional quality, and a very short exposure time may not entirely eliminate growth inhibitors present in legume grains. Consequently, as shown in Table III, the *Tribolium* larval weight gain and PER values classified the soybean treatments of 5 rpm in both the precooked and raw treatments of better nutritional quality for fulfilling the above conditions.

The rat PER values indicated that the precooked soybean treatment was on the average 23% superior in quality to the raw treatment, indicating that possibly some growth inhibitors were still present in the raw soybean treatment diets. Likewise, *Tribolium* performance on the precooked soybean was on the average 17% better than the raw soybean. The sensitivity of *Tribolium* to separate these two treatments in very similar groupings as the rat is of

TABLE II

Tribolium development for three corn types (qualitative analysis)[1]

Corn types	Age in days							
	18				22			
	D[2]	L	P	A	D	L	P	A
Opaque-2	24	50	26	0	42	20	22	16
Azotea Yellow	34	58	8	0	44	32	20	4
Tiquisate Red	24	66	10	0	40	36	18	6

1 Results represent percentage of adults (A), pupa (P), larva (L) and dead (D) organisms found in five replicate samples of ten individuals each.

2 No. of dead calculated as: 50 – No. of live forms.

Groups enclosed by a continuous line are not significantly different ($P < 0.01$).

TABLE III

Performance of *Tribolium* and rats fed diets containing a mixture of 70%/o corn and 30%/o soybean subjected to different temperature exposure times in a drum dryer

Soybean treatments	Drum dryer rpm[1]	*Tribolium* Wt. gain (mg)	Rat PER
Precooked	3	1.23[a]	2.61[a c]
	5	1.27[a]	2.74[a]
	7	1.16[a c]	2.63[a c]
Raw	3	0.99[b]	2.21[b]
	5	1.08[b c]	2.42[b c]
	7	1.04[b c]	2.42[b c]

1 rpm, revolutions per minute.

Means with no common superscript differ significantly ($P < 0.05$).

particular interest since it further indicates the potential of the insect for testing legume-based foods since *Tribolium*, as well as vertebrates, has been shown to be sensitive to growth inhibitors in raw soybean (4).

Experiment 3

Figure 1 shows an example of the resulting regression lines in applying a multiple point assay evaluation procedure to white wheat flour of three varieties. For the success and appropriate interpretation when this methodology is applied, it is important that the test be conducted along the protein levels in the diet where a linear growth response is observed. It has been shown (1) that when *Tribolium* larvae are fed an "optimum diet" of a 90:10 mixture of unbleached white wheat flour and yeast as a source of protein, the linear weight gain response from 10 to 14 days of age is achieved between the levels of 0 to 6%/o protein in the diet. For the purpose

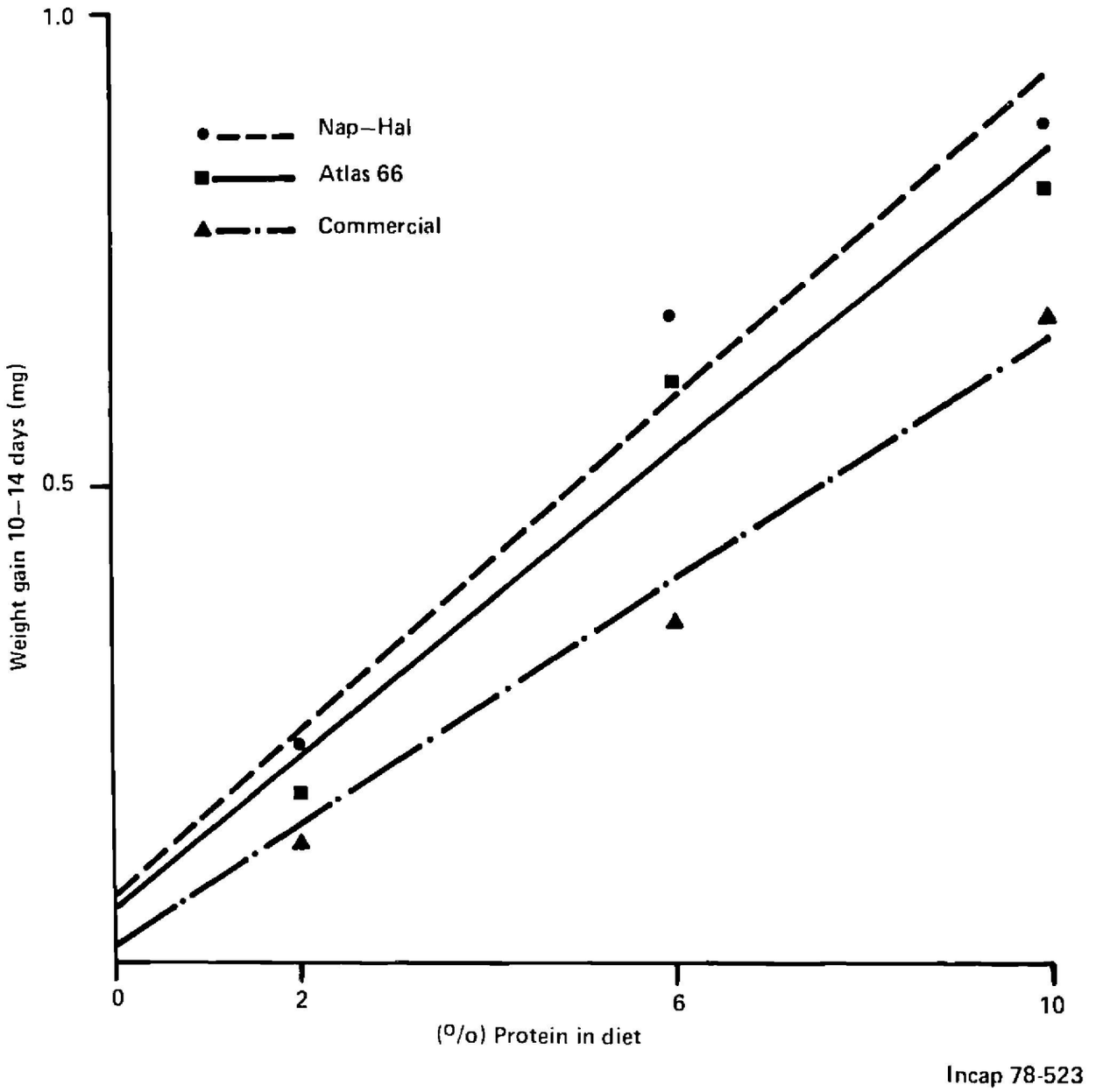

Fig. 1 Multiple point assay evaluation with *Tribolium* of white wheat flour from three varieties.

of the test, an 8% protein level as the terminal point in the analysis can be included with little difference in the results (1). Therefore, in the present study, for the whole wheat flour –which can be considered a good protein source for *Tribolium*– a range of 0 to 8% protein level in the diet was used in the analysis, whereas for the white wheat flour –a lower protein quality source– a range up to 10% protein in the diet was utilized.

The above observation is important when applying a multiple point evaluation procedure with *Tribolium*. Depending on the protein content and general quality of the test product, one can extend

TABLE IV

Multiple point assay evaluation with *Tribolium* and rats of wheat flour from three varieties

Flour Type	Variety	Protein[1] g%/o	*Tribolium*[2]			Rat		
			b	n	r^2%/o	b	n	r^2%/o
White Wheat	Nap-Hal	16.5	.088	20	86	2.27	16	87
	Atlas 66	17.2	.081	20	88	1.43	15	83
	Commercial	12.2	.065	20	92	2.01	16	79
Whole Wheat	Nap-Hal	18.7	.126	20	82	2.43	16	93
	Atlas 66	17.5	.124	20	71	2.20	15	90
	Commercial	13.3	.120	20	89	2.24	16	92

1 Wheat protein calculated on dry weight basis.

2 The letter b refers to regression coefficient, n to the number of points included to calculate the regression values, and r^2%/o to the coefficient of determination r^2 x 100.

beyond 6%/o the range of protein levels to test a diet. This improves the accuracy of the test by working on a wider range where more and more separate points can be included to calculate the regression line.

The regression coefficients calculated for the three varieties of wheat with the *Tribolium* and rat multiple point evaluation procedure are shown in Table IV. The regression values shown for *Tribolium* white wheat flour correspond to the lines drawn in Figure 1. In terms of the magnitude of the regression coefficients, *Tribolium* ranked of better nutritional quality the higher protein content varieties Nap-Hal and Atlas 66 above the Commercial variety in both the white and whole wheat flours. The rat test also consistently ranked the Nap-Hal variety superior to the other two, but in contrast to *Tribolium*, particularly for the white wheat flour, the Commercial variety was ranked above Atlas 66. These rankings are based only on the magnitude of the regression coefficients, but statistically, although all the regressions calculared were significant ($P < 0.01$), the regression coefficients between varieties in the white and whole wheat flour grouping were not significantly different for either rat or *Tribolium* tests. Thus, the study demonstrated that *Tribolium* can be utilized in multiple point evaluation procedures to assess nutritional quality and that it provides a valuable tool that should be further tested and applied.

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