Nutritional Quality of Oilseed Protein Isolates as Determined with Larvae of the Yellow Mealworm, *Tenebrio molitor* L.[1]

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ABSTRACT Previous studies have shown that larvae of the yellow mealworm, *Tenebrio molitor* L., classify the nutritive values of oilseed meals in a manner similar to weanling mice. The present study was designed to determine whether such classification was mediated entirely by the oilseed proteins or was influenced by nonprotein constituents of these meals. Larvae of *Tenebrio molitor* fed a diet of 90% ground wheat + 10% brewer's yeast gained twice as much weight as those fed synthetic diets containing wheat or two brands of casein. The results suggest that the yeast may have supplied essential amino acids or unknown growth factors, absent or deficient in the other protein sources. Autoclaving of the six oilseed proteins, in general, improved the nutritive value by over 50% (range, 7.6% for safflower isolate to 176.4% for soybean isolate). Larvae fed diets containing autoclaved isolates of soybean, sunflower or turnip rape gained weights similar to those fed a diet of wheat + brewer's yeast. The nutritional quality of the autoclaved isolates was classified by larvae of *Tenebrio molitor* as follows: soybean = sunflower = turnip rape > flax = rape = safflower. These results are discussed in relation to the amino acid composition of the larvae and the dietary protein sources. J. Nutr. *104:* 1172–1177, 1974.

INDEXING KEY WORDS oilseed protein · protein quality · evaluation techniques · *Tenebrio molitor* · effects of heating · amino acids · insect nutrition

Recently, larvae of the yellow mealworm, *Tenebrio molitor* L., were shown to classify the nutritive values of oilseed meals in a manner similar to weanling mice (1). Larval growth when autoclaved soybean, sunflower or turnip rape meal was fed for 4 weeks was nearly equivalent to that when the control diet of ground wheat + brewer's yeast was fed. The growth rates of both mealworm larvae and mice when fed safflower and rape meals were intermediate. However, the principal difference between mice and larvae was the apparent more efficient utilization of flax meal proteins by mice, perhaps due to a palatability factor.

The present study was initiated to evaluate the growth of *T. molitor* on oilseed protein isolates. These isolates were relatively free of nonprotein constituents that might depress growth by inhibiting feeding. They were also autoclaved to destroy trypsin inhibitors and other antimetabolites which had depressed the growth of mice fed similar diets (2). Experiments were also conducted to demonstrate the differences in protein quality among ground wheat, wheat + brewer's yeast and two brands of casein.

MATERIALS AND METHODS

Materials. Isolated proteins of seeds of Altona soybean, Commander sunflower, Gila safflower, Echo turnip rape, Target rape and Redwing flax were prepared by alkali extraction of the respective meals and precipitation at the isoelectric point, as described by Sosulski and Sarwar (3). Because of high losses of essential amino acids in the previous study, these isolates were not washed as extensively. Portions of

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TABLE 1

Composition of the diet[1]

Diet composition		Vitamin B solution	
	%		μg/0.1 ml
Protein	10.00	Choline chloride	±500.00
D-Glucose	84.64	Nicotinic acid	50.00
McCollum-Davis Salt Mixture # 185[2]	3.65	Thiamin·HCl	25.00
Cholesterol, U.S.P.	0.96	D-Calcium pantothenate	25.00
Linoleic acid (95+%)	0.75	Riboflavin	12.50
Vitamin-B solution	0.10 ml/g	Pyridoxine·HCl	12.50
		DL-Carnitine·HCl	6.00
		Folic acid	2.50
		D-Biotin	0.25
		Zinc chloride[3]	40.00

[1] All dietary components obtained from ICN Nutritional Biochemicals Corporation, Cleveland, Ohio, except as otherwise noted.
[2] McCollum, E. V. & Davis, M. (1914) Observations on the isolation of the substance in butterfat which exerts a stimulating influence on growth. J. Biol. Chem. *19*, 245. Composition as follows: Calcium lactate·$5H_2O$, 35.19%; calcium biphosphate·H_2O, 14.60%; potassium monophosphate, 25.78%; sodium monophosphate, 9.38%; sodium chloride, 4.67%; magnesium sulfate anhydrous, 7.19%; ferric citrate, 3.19%. [3] Fisher Scientific Co., Fair Lawn, N.J.

the protein isolates were autoclaved at 120° for 15 minutes and other portions were not heat treated.

The ground wheat and wheat + brewer's yeast were taken from the same source as the rations used to rear *Tenebrio* larvae. Casein A [2] and casein B [3] were obtained from commercial sources. These protein sources were not autoclaved.

The amino acid analyses were conducted on duplicate hydrolysates of each sample with an automatic amino acid analyzer.[4] Sixteen amino acids were determined by the two-column procedure after hydrolysis with 6 N HCl under vacuum, in a sealed ampule for 24 hours at 100° (3). Cystine and methionine were measured as cysteic acid and methionine sulfone by performic acid oxidation of the samples, followed by acid hydrolysis. For tryptophan analysis, the samples were hydrolyzed with 14% barium hydroxide under vacuum at 110° for 18 hours.

Experimental diets. Diets were prepared containing the protein sources plus glucose, salt mixture, cholesterol, linoleic acid and a vitamin solution (table 1) according to a formulation used extensively in nutrition studies with *T. molitor* (4, 5). Larvae of *T. molitor* are said not to require vitamins A, B-12, C, D, E and K (4). Each diet contained sufficient protein source to provide 10% of dietary protein. A constant energy value was maintained at 4.1 kcal/g by adjusting glucose and linoleic acid concentrations of the diet.

The wheat + yeast diet consisted of 90 parts of ground whole wheat and 10 parts of brewer's yeast, without added minerals, cholesterol, linoleic acid or vitamins. This diet was the same as that used in routine stock rearing of larvae of *T. molitor* and served as the control diet in the previous investigation (1).

Mealworm experiments. Larvae of *T. molitor*, Gembloux strain, race F, fed from hatching a stock diet of ground wheat and brewer's yeast, were starved for 48 hours to ensure readiness to eat test diets. Thirty larvae were fed each diet for 4 weeks. They were placed in individual vials containing 1 g of diet at 27 ± 0.25° and 65 ± 5% relative humidity. The initial weights of larvae ranged from 8.4 to 12.5 mg and the average group weights for 30 larvae, from 9.8 to 10.1 mg. The standard error of the mean of the initial weight approximated 0.2 mg. After 4 weeks, they were weighed again and the changes in weight during the experimental period were calculated. The larvae were then dried to constant weight in a vacuum oven at 38° and the dry weights were recorded. Fifteen of the dried larvae from the group fed wheat + yeast were selected at random and amino acid analysis was performed on duplicate hydrolysates, as for the protein

[2] Vitamin-free casein, ICN Nutritional Biochemicals Corp., Cleveland, Ohio.
[3] ANRC Reference Protein-137F, ICN Nutritional Biochemicals Corp., Cleveland, Ohio.
[4] Beckman model 120C analyzer, Beckman Instruments, Inc., Fullerton, Calif.

isolates of this study. For comparison purposes, 20 larvae, within the initial weight range of larvae used in this investigation, were obtained from stock rearing, were dried and were analyzed as above.

Data on changes in fresh weight and on final dry weights were analyzed, using analysis of variance and Duncan's multiple range test (6). Differences at the 5% level of confidence were considered significant.

RESULTS AND DISCUSSION

Larvae of *T. molitor*, fed diets containing ground wheat, gained as much weight as those fed diets containing either of the two samples of casein (table 2). Source of casein did not have a significant influence on the final fresh or dry weight of the larvae. Incorporation of 10% brewer's yeast in the ground wheat diets resulted in twice the weight gain obtained with wheat or casein. The yeast may have contributed essential amino acids or unknown growth factors, which were absent or deficient in the other protein sources.

Differences in final weights or in weight gains of larvae fed the unautoclaved protein isolates were not large, except for the very low values obtained with the diet containing soybean isolate (table 2). Sunflower and turnip rape were better sources of dietary protein for larvae than flax. Rape and safflower were intermediate in nutritive value.

The general effect of autoclaving the isolates was to increase larval growth by over 50% (table 2). However, the magnitude of this increase was not identical for all protein isolates, because larvae fed a diet with autoclaved soybean isolate gained 2.8 times as much in dry weight as those fed a diet containing unautoclaved soybean isolate. The improvement in nutritive value of isolates by heating was probably due to the destruction of heat-labile inhibitors, such as the trypsin inhibitor, to improved protein digestibility and to increased palatability of the diet. These effects cannot be readily separated until techniques are developed for quantitating the levels of protein and indigestible indicator in the feces of the test organism. However, soybean is known to contain a potent trypsin inhibitor which adversely affects the growth of *Tenebrio* larvae (7).

Larval growth when fed autoclaved oilseed protein isolates showed that soybean, sunflower and turnip rape were superior to flax, rapeseed and safflower as sources of protein. The highest values for final dry weight and for fresh weight gain, obtained with autoclaved soybean isolate, were only slightly less than those obtained with the wheat + brewer's yeast diet. The larvae classified these autoclaved protein isolates in the same order as the respective autoclaved meals in the previous investigation (1). In earlier studies with mice (1, 2),

TABLE 2

Average weights of larvae of Tenebrio molitor *L. fed for 4 weeks at 27.0±0.25° and 65±5% r.h. meridic diets containing 10% protein*[1]

Protein source in diet	Unautoclaved: Final weight dry basis[2]	Unautoclaved: Weight gain fresh basis[2]	Autoclaved: Final weight dry basis[2]	Autoclaved: Weight gain fresh basis[2]
	mg	*mg*	*mg*	*mg*
Wheat	15.5±0.8[3]*b*	28.0±2.2 *b*	—	—
Wheat + yeast	28.2±1.1 *a*	55.9±2.9 *a*	—	—
Casein A[4]	14.7±0.3 *b*	25.4±2.4 *b*	—	—
Casein B[5]	14.9±1.1 *b*	26.5±2.4 *b*	—	—
Oilseed protein isolates				
Sunflower	13.8±0.9 *b*	26.6±2.2 *b*	21.8±1.2 *b*	46.7±3.0 *a*
Turnip rape	12.8±0.6 *b*	23.0±1.3 *b*	19.0±1.3 *b*	40.9±3.3 *a*
Soybean	8.9±0.6 *d*	12.9±1.5 *d*	24.6±1.5 *a*	52.3±3.7 *a*
Rape	12.4±0.7 *b*	22.5±1.8 *bc*	13.7±0.8 *c*	24.6±2.1 *b*
Flax	9.7±0.6 *cd*	17.0±1.6 *cd*	14.7±0.8 *c*	29.2±1.8 *b*
Safflower	11.8±0.6 *bc*	21.5±1.5 *bc*	12.7±0.7 *c*	22.5±1.5 *b*
Average	11.6±0.7	20.6±1.7	17.7±1.0	36.0±2.1

[1] Values are the mean of 30 larvae per group. [2] Within each type of diet, values followed by the same letter do not differ from one another at $P = 0.05$. [3] Values are mean ±SEM. [4] Vitamin Free Casein, ICN Nutritional Biochemicals Corp., Cleveland, Ohio. [5] ANRC Reference Protein-137F, ICN Nutritional Biochemicals Corp., Cleveland, Ohio.

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TABLE 3

Protein content and amino acid composition of larvae of Tenebrio molitor *fed the wheat + yeast diet for 4 weeks and of dietary sources of protein*[2]

Protein source	*Tenebrio* larvae[1] Initial	Final	Wheat	Wheat + B. yeast	Casein	Soybean	Sunflower	Turnip Rape	Rape	Flax	Safflower
Protein content,[3] %	69.0	47.2	15.0	19.8	93.5	87.3	95.9	81.8	86.5	84.9	92.5
Essential amino acids	%	%	%	%	%	%	%	%	%	%	%
Arginine	5.9	4.6	4.3	4.9	3.0	7.2	10.3	7.0	7.1	10.3	8.9
Histidine	2.9	2.8	2.0	2.1	2.2	2.2	2.4	3.0	2.5	1.8	2.1
Isoleucine	4.5	4.5	2.8	3.8	5.0	4.6	4.3	4.0	4.3	4.1	4.1
Leucine	8.0	7.7	6.1	6.9	9.9	7.9	6.8	7.8	7.7	8.0	6.8
Lysine	6.7	6.5	2.2	3.7	7.6	6.3	3.0	6.0	5.0	3.0	2.4
Methionine	1.6	1.5	1.6	1.4	2.5	1.2	2.4	2.6	2.1	1.8	1.7
Phenylalanine	6.5	6.8	4.3	4.7	4.6	5.1	5.3	4.1	4.1	4.4	4.8
Threonine	4.0	3.9	2.3	3.2	3.8	3.5	3.3	3.7	3.8	3.2	3.1
Tryptophan	0.8	0.7	1.1	1.2	2.6	1.4	1.4	1.6	1.5	1.3	1.2
Valine	6.7	6.5	3.9	4.8	6.2	4.7	5.6	5.1	5.4	5.2	5.2
Total	47.6	45.5	30.6	36.7	47.4	44.1	44.8	44.9	43.5	43.1	40.3
Nonessential amino acids											
Alanine	9.9	8.9	3.1	4.6	2.6	4.1	4.4	4.4	4.4	4.3	4.8
Aspartic acid	7.9	8.7	4.2	6.5	6.8	12.9	10.5	6.1	7.3	10.7	11.6
Cystine	1.9	1.8	2.3	1.6	0.5	1.6	1.8	3.5	4.2	2.3	1.6
Glutamic acid	12.1	10.7	33.2	27.8	20.9	19.5	22.1	21.6	21.7	21.6	24.7
Glycine	5.7	5.6	3.9	4.3	1.4	4.2	5.2	5.2	5.5	5.6	5.6
Proline	4.6	7.5	9.7	9.1	9.8	5.0	4.4	7.3	6.4	4.5	4.1
Serine	4.5	4.2	4.0	4.7	5.4	5.2	3.8	4.1	4.0	4.3	4.2
Tyrosine	5.8	7.0	2.4	3.1	5.0	3.5	3.0	2.9	2.8	2.8	3.1

[1] For determining the protein content and amino acid composition of initial larvae, 20 larvae were obtained from stock rearing in wheat + brewer's yeast, within the weight range of 8.4 to 12.5 mg. They were dried to constant weight at 38° and protein hydrolysates were prepared. For final larvae, 15 dried larvae were selected at random from those fed the diet of wheat + brewer's yeast in this investigation and were prepared as above. Amino acid analyses were performed on duplicate samples of the hydrolysates in both cases. [2] Determination of the protein content and amino acid composition of the dietary components was based on duplicate determinations except for methionine and cystine, which were single determinations. [3] The protein content of the various products was determined as N × 6.25.

the protein efficiency ratio and the net protein utilization values for the autoclaved meals also ranked these oilseed species in the same relative order as the larvae in the present study. Sarwar et al. (2) also fed the oilseed protein isolates to mice and obtained results comparable to those recorded for unautoclaved samples in the present investigation. However, the mice grew poorly when fed the diets containing protein isolates of sunflower and this result was attributed to the low level of lysine in the protein source. For this reason, studies were made on the amino acid composition of the protein sources in the present work and on their relationships to larval performance.

The average final moisture content of the larvae fed the wheat + brewer's yeast diet was 56.6%, but the mean for those fed the oilseed isolates was 61.8%. Initially, larvae of 10 mg in weight contained 69.0% protein, dry weight basis (table 3). In contrast, larvae fed for 4 weeks the control diet of wheat + brewer's yeast having a final weight of 65.9 mg had an average protein level of 47.2%. Despite this large difference in protein concentration, initial and final larvae were very similar in amino acid composition. During growth, a substantial increase in the amount of proline occurred in larval proteins. Slightly increased levels of aspartic acid and tyrosine were associated with possible decreases in arginine, alanine and glutamic acid during growth. However, the changes in amino acid composition were small in relation to the changes in total protein concentration during the maturation of the larvae.

In comparison with the animal protein, casein, the *Tenebrio* larvae were richer in arginine, phenylalanine, alanine, cystine and glycine but lower in methionine, tryptophan, glutamic acid and proline (table 3). Except for lower lysine and tyrosine levels, the wheat + brewer's yeast was closer in amino acid composition to the larvae than was casein.

The soybean, sunflower and turnip rape isolates contained almost the same level of total essential amino acids as the final larvae (table 3). Sunflower protein was comparatively lower in lysine and proline, but soybean was lower in methionine and glycine than *T. molitor*. Turnip rape was deficient in phenylalanine and all three isolates were lower than the larvae in valine, alanine and tyrosine. Rape, flax and safflower isolates tended to be lower in total essential amino acid content and showed greater divergence from the larval composition than soybean, sunflower and turnip rape. Some insects require a dietary source of phenylalanine and tyrosine (8) and the larvae of *T. molitor* contained high levels of these amino acids. Wheat + brewer's yeast, casein, soybean, sunflower and safflower were generally higher in these aromatic amino acids than the rape species and flax. The larvae were relatively low in arginine and tryptophan, but supplementation with these essential amino acids has been recommended for this insect species (8, 9). The present data show that larvae utilize high levels of proline during protein synthesis and the effects of the wide range in proline composition among the present samples are of interest. Although it would be premature to assign the differences in larval growth to specific variations in amino acid composition, flax and safflower proteins tended to be low in many of the above amino acids in comparison with soybeans, sunflower and turnip rape. The results suggest larvae of *T. molitor* may be useful in predicting protein quality among oilseed protein sources.

The yellow mealworm is adapted for survival on grain or moldy grain and was expected to grow more rapidly when fed the wheat or wheat + brewer's yeast than when fed the animal protein, casein. The present and the previous (1) studies indicate that consistent differences in growth rates can be obtained among oilseed meals and protein isolates. Whether these differences in weight gains are due to variations in protein quality or to palatability and rate of consumption can only be determined by quantitative measurements of protein digestibility and responses to amino acid supplementation. Generally, the larvae of *T. molitor* grow poorly when fed synthetic diets of pure amino acids (9), but supplementation of deficient proteins with individual amino acids has not been extensively explored except in relation to animal proteins (8). These topics are currently under investigation.

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