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Reçu le 18 juillet 1969.

PROTEIN NUTRITION OF «TENEBRIO MOLITOR» L. IX. REPLACEMENT CASEINS FOR THE REFERENCE DIET AND A COMPARISON OF THE NUTRITIONAL VALUES OF VARIOUS LACTALBUMINS AND LACTALBUMIN HYDROLYSATES

BY

G. R. F. DAVIS (1) and Jean LECLERCQ

(Research Station, Canada Department of Agriculture, Saskatoon, Canada and Zoologie Générale, Faculté des Sciences Agronomiques de l'Etat, Gembloux)

Because carnitine (LECLERCQ, 1957) and zinc (FRAENKEL, 1958) are necessary for optimal development of larvae of *Tenebrio molitor* L., and because these substances occurred in trace quantities in various caseins, comparisons of the nutritional values of several caseins for these larvae have previously been reported (FRAENKEL and LECLERCQ, 1965; FRAENKEL, 1958). As a result, Labco casein (The Borden Co., New York) or Hoffmann-Laroche casein (Hoffmann Laroche, Basle, Switzerland) were subsequently employed in the reference diet, together with appropriate quantities of carnitine and zinc. These caseins are no longer available. Recent work (LECLERCQ, 1965) indicated again differences in nutritional value of two caseins and consequently suggested that further comparisons of various caseins were necessary to determine those suitable for inclusion in the reference diet.

Eight previous papers in this series have dealt with various aspects of protein nutrition in larvae of *T. molitor* (HUOT and LECLERCQ, 1958*a*, 1958*b*; LECLERCQ and HUOT, 1958*a*, 1958*b*; LECLERCQ and LOPEZ-FRANCOS, 1964*a*, 1964*b*, 1966, 1967). The present paper describes the growth of larvae of this Insect on artificial diets, the protein portion of which consisted of one of four "vitamin free,, caseins, one of three lactalbumins, or one of four lactalbumin hydrolysates.

(1) Recipient of post-doctoral transfer of work from *Research Station, Canada Department of Agriculture*, Saskatoon, Canada, 1968-69.

Material and methods

Diets used in this investigation were formulated according to the composition of an adequate medium for larvae of *T. molitor* (Leclercq and De Bast, 1965). The protein concentration was, however, decreased to 3 g per 100 g of diet, a level previously shown to be optimal for this Insect (Leclercq and Lopez-Francos, 1964*a*). The concentration of glucose was maintained at 94 g per 100 g of diet.

Four caseins were tested for suitability in the reference diet : 1) Vitamin Free Casein (Difco Laboratories, Detroit, Michigan); 2) Vitamin Free Test Casein (General Biochemicals, Inc., Chagrin Falls, Ohio); 3) Vitamin Free Casein (Nutritional Biochemicals Corp., Cleveland, Ohio); and 4) Vitamin-Free Casein N°. 5408 (Dajac Laboratories, Borden, Inc., Philadelphia, Pennsylvania). All are referred to in the text as "casein,,, preceded by the name of the manufacturer.

Difco casein was used in the reference diet, when the nutritive values of lactalbumin and lactalbumin hydrolysate were investigated. Three lactalbumins were compared with it : 1) Lactalbumin (Nutritional Biochemicals Corp., Cleveland, Ohio); 2) Lactalbumin (Fluka A. G. Chemische Fabrik, Buchs, Switzerland; and 3) Lactalbumin (Edible) (Mann Research Laboratories, Inc., New York). Four lactalbumin hydrolysates were also compared with it : 1) Lactalbumin Hydrolysate (Enzymatic) (Nutritional Biochemicals Corp., Cleveland, Ohio); 2) Lactalbumin-hydrolysat enzymat. pulv. (Fluka A. G. Chemische Fabrik, Buchs, Switzerland 3) Lactalbumin Hydrolysate (Enzymatic), Tissue Culture Grade (Mann Research Laboratories, Inc., New York); and 4) Lactalbumin Hydrolysate Enzymatically Hydrolyzed (Sigma Chemicals Co., St. Louis, Missouri). These products are also referred to in the test as "lactalbumin,, or "lactalbumin hydrolysate,,, preceded by the name of the manufacturer.

Larvae of *T. molitor*, race F, weighing 8.5 to 12.4 mg were selected from stock-rearing in whole-wheat flour and 10 % Brewer's yeast and were maintained without food for 48 hours at 27° C and 65 % relative humidity. After starvation, they were weighed individually to the nearest 0.1 mg and were placed in groups of 10 larvae each in plastic egg cups containing 10 g of diet. Each group had an average weight as close as possible to 10 mg. To avoid possible bias from the order in which larvae were chosen, each group of larvae was placed on a particular diet in an order previously determined at random. Four groups of 10 larvae each were used to tests each diet. Although cannibalism is not usually encountered, it occurred in one group fed a diet containing Difco casein and in another containing General Biochemicals casein. In these two cases, interpretation of the results was, therefore, based on three groups of 10 larvae each.

The larvae were reared in the experimental diets for four weeks at 27° C and 65 % relative humidity. They were then weighed individually to the nearest 0.1 mg and the average weight of each group was calculated. Differences between final and initial weights, divided by the initial weight $[(P_4 - P_0)/P_0]$, were determined for each group and an average value was obtained for each diet.

Data from each set of experiments were submitted to an analysis of variance and differences at the 5 % level of confidence were considered significant. Where required, the least significant difference at the 5 % level of confidence ($LSD_{.05}$) was calculated.

Results

Rates of growth of larvae of *T. molitor*, fed artificial diets containing one of four caseins, are given in Table I. Larvae reared on these diets all gained the same amount of weight ($P > 0.05$).

Rates of growth of larvae reared on the reference diet and on diets containing lactalbumin or lactalbumin hydrolysate are outlined in Table II. The larvae gained equal amounts of weight on diets containing lactalbumin, but less weight than on the reference diet containing casein ($P < 0.05$). Similarly, they gained as much weight with any one of the lactalbumin hydrolysates, but less than with lactalbumin or casein ($P < 0.05$).

Discussion

The present results indicate that the four caseins are of equal nutritional quality for larvae of *T. molitor*. They also demonstrate that casein is superior to the two other sources of protein and that

TABLE I. *Nutritional values of four caseins, obtained from four different suppliers, as indicated by gains in weight of larvae of* Tenebrio molitor *L., reared for four weeks on artificial diets, containing 3 % of casein as the only source of protein, at 27° C and 65 % relative humidity.*

Casein	Weight in mg		Gain in weight (mg/mg initial)	
	Initial (P_0)	Final (P_4)	$[(P_4 - P_0)/P_0]$	Average
Difco	10.0 ± 0.7 [a]	44.7 ± 6.9[a]	3.38	3.23
	11.4 ± 0.8	45.7 ± 3.7	3.01	
	10.6 ± 1.1	45.7 ± 3.0	3.31	
General Biolchemicals	10.9 ± 0.9	49.0 ± 7.0	3.50	3.26
	10.9 ± 0.9	45.3 ± 5.9	3.16	
	10.9 ± 0.8	44.9 ± 7.7	3.12	
Nutritional Biochemicals	10.2 ± 0.7	41.4 ± 5.1	3.06	3.21
	10.2 ± 0.7	43.8 ± 4.2	3.29	
	10.5 ± 1.0	43.0 ± 2.4	3.10	
	10.6 ± 1.0	46.7 ± 4.2	3.41	
Dajac	10.6 ± 1.0	47.7 ± 9.4	3.50	3.23
	11.1 ± 0.9	45.8 ± 8.1	3.13	
	10.8 ± 0.9	42.0 ± 7.6	2.89	
	10.6 ± 1.2	46.7 ± 7.9	3.41	

[a] Average weight of 10 larvae ± standard deviation.

lactalbumin hydrolysate is the most inefficient of the three for this Insect.

Because of possible physiological differences among larvae of *T. molitor* of the same size, from one generation to another, direct comparison of these results with those of previous works is difficult. Nevertheless, the values obtained with the casein in the present work resemble closely (Table II) or are slightly lower (Table I) than those previously reported for this Insect (LECLERCQ, 1965; LECLERCQ *et al.*, 1967). For this reason and because of the relationships between results with these caseins and with the lactalbumins (Table II), any or the four caseins can probably be substituted in the reference diet, without adversely affecting the rate of growth of larvae of *T. molitor*.

The results obtained with lactalbumins and lactalbumin hydrolysates are comparable, as far as is possible, with those obtained previously (LECLERCQ, 1965). Contrary to the situation prevailing among caseins supplied by different manufacturers (LECLERCQ, 1965)

TABLE II. *Nutritional values of lactalbumins and lactalbumin hydrolysates, as indicated by gains in weight of larvae of* Tenebrio molitor *L., reared for four weeks on artificial diets containing 3 % of these products as the only source of protein, at 27° C and 65 % relative humidity.*

Protein Source	Weight in mg		Gain in weight (mg/mg initial)	
	Initial (P_0)	Final (P_4)	$[(P_4 - P_0)/P_0]$	Average
Difco casein (control)	11.6 ± 0.9 [a]	61.5 ± 3.9 [a]	4.30	4.35
	9.7 ± 1.5	56.6 ± 10.0	4.84	
	11.0 ± 1.2	59.5 ± 13.1	4.41	
	11.4 ± 1.3	55.3 ± 12.2	3.85	
Nutritional Biochemicals lactalbumin	10.3 ± 1.1	40.9 ± 8.2	2.97	2.93
	10.9 ± 1.9	41.7 ± 6.7	2.83	
	10.9 ± 0.8	42.4 ± 7.4	2.89	
	10.5 ± 1.2	42.3 ± 9.6	3.03	
Fluka lactalbumin	10.9 ± 1.4	43.2 ± 8.0	2.96	3.19
	10.6 ± 1.3	41.5 ± 6.4	2.92	
	11.0 ± 1.9	46.8 ± 9.6	3.25	
	10.3 ± 1.0	47.6 ± 8.2	3.62	
Mann lactalbumin	10.9 ± 1.2	43.5 ± 7.7	2.99	3.20
	9.5 ± 1.1	41.1 ± 5.8	3.33	
	10.7 ± 1.2	44.1 ± 8.7	3.12	
	10.6 ± 1.4	46.1 ± 4.4	3.35	
Nutritional Biochemicals lactalbumin hydrolysate	10.3 ± 1.0	17.4 ± 3.5	0.69	0.53
	10.4 ± 1.2	14.7 ± 1.3	0.41	
	10.0 ± 1.3	15.2 ± 2.1	0.52	
	10.5 ± 1.2	15.8 ± 2.2	0.50	
Fluka lactalbumin hydrolysate	10.6 ± 1.1	16.2 ± 2.3	0.53	0.59
	10.6 ± 1.5	15.7 ± 2.2	0.48	
	10.7 ± 1.5	16.5 ± 2.2	0.54	
	10.1 ± 1.2	18.2 ± 2.4	0.80	
Mann lactalbumin hydrolysate	11.0 ± 1.2	17.7 ± 1.8	0.61	0.58
	10.0 ± 1.2	14.1 ± 2.1	0.41	
	10.9 ± 1.2	18.7 ± 1.6	0.72	
	10.7 ± 1.2	17.1 ± 2.4	0.60	
Sigma lactalbumin hydrolysate	11.0 ± 1.5	16.7 ± 1.5	0.52	0.58
	10.6 ± 1.8	16.4 ± 2.3	0.55	
	10.6 ± 1.4	16.6 ± 2.9	0.57	
	10.2 ± 1.1	17.3 ± 2.0	0.70	
$LSD_{.05}$				0.33

[a] Average weight of 10 larvae ± standard deviation.

the nutritional quality of these products apparently does not vary according to the source of supply. All four hydrolysates tested were the result of enzymatic digestion, so that destruction of constituent amino acids should not have occurred. This reason, then, cannot be used to explain the differences observed between whole protein and hydrolysate.

In Vertebrate nutrition, lactalbumin is always considered superior to casein as a dietary protein (Dr. D. M. HEGSTED, Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts, personal communication) and such is certainly the case for the growing rat (MITCHEL, 1959; HEGSTED and CHANG, 1965*a* and *b*). Therefore, the significant contribution of the present investigation is the fact that larvae of *T. molitor*, contrary to other animals, consistently class lactalbumin as a dietary protein of lower nutritional value than casein. Consequently, some factor in lactalbumin must be detrimental to this Insect.

Work is continuing in this laboratory to determine the causative factors involved in the classification of these proteins in this manner by larvae of *T. molitor*.

CONCLUSIONS

Tenebrio molitor L. differs from other organisms by classifying casein as a dietary protein superior to lactalbumin. It grows as well on diets containing any one of the four vitamin free caseins tested as with the reference diet previously used, which contained a casein no longer available. On diets containing enzymatic hydrolysates of lactalbumin, this Insect grew less well than on diets containing unhydrolyzed lactalbumin.

RÉSUMÉ

Quatre caséines différentes ont été employées dans des régimes artificiels pour larves de *Tenebrio molitor* de race F. Chacune s'est avérée adéquate pour remplacer la caséine utilisée habituellement comme protéine unique de référence dans les recherches sur la nutrition de cet Insecte.

Les trois lactalbumines testées dans les mêmes conditions furent

nettement moins efficaces que les caséines. Quatre hydrolysats de lactalbumine ont aussi été testés; ils se sont avérés considérablement moins efficaces que les lactalbumines. En principe, la préparation de ces hydrolysats par voie enzymatique ne doit pas avoir affecté les teneurs et équilibres en acides aminés, ni entraîné d'autres modifications de proportions dans les constituants de la lactalbumine.

La contribution majeure de ce travail demeure le fait que cet Insecte, contrairement au rat classe la lactalbumine comme protéine de valeur nutritive inférieure à celle de la caséine.

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