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THE EFFECT OF PROTEIN LEVELS AND SOURCES ON GROWTH OF *Penaeus aztecus*[1]

by

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CONTENTS

THE EFFECT OF PROTEIN LEVELS AND SOURCES ON GROWTH OF *Penaeus aztecus*

Abstract

Extruded feeds based on a soy-flour, rice bran base were fed to laboratory-reared juvenile shrimp, *Penaeus aztecus*. Measured protein levels ranged from approximately 24 to 63 percent in increments of approximately 10 percent. Best growth was obtained with a feed containing 51.5 percent protein, while growth decreased at the highest protein level. Essentially no growth occurred amongst animals fed at the three lowest protein levels.

Simultaneous tests of animals fed three other diets varying in composition from those above suggested that protein level alone was not the factor involved in growth stimulation. Two feeds varying only one percent in protein content differed considerably in growth stimulation, while a third feed with almost 25 percent less protein was about as efficient. Analysis of amino-acid composition of the feeds did not provide an explanation as to the differences in growth response.

Apparently, factors other than protein concentration or quality, which have not yet been identified, are important in the growth of penaeids.

INFLUENCE DES NIVEAUX PROTEIQUES ET DE L'ORIGINE DES PROTEINES SUR LA CROISSANCE DE *Penaeus aztecus*

Résumé

Des aliments extrudés à base de farine de soja et de son de riz ont été administrés à des juvéniles de *Penaeus aztecus* élevés en laboratoire. Les niveaux protéiques mesurés s'échelonnaient de 24 à 63 pour cent environ, par échelons de quelque 10 pour cent. Un aliment contenant 51 pour cent de protéines a donné la meilleure croissance, cette dernière diminuant à un niveau protéique plus élevé. La croissance a été pratiquement nulle parmi les crustacés auxquels on avait administré des aliments aux trois niveaux protéiques inférieurs.

Des tests simultanés portant sur des crustacés auxquels on a administré trois autres régimes alimentaires dont la composition était différente de ceux mentionnés précédemment permettent de penser que le niveau protéique n'est pas le seul facteur qui intervienne dans la stimulation de la croissance. C'est ainsi que deux aliments dont la teneur protéique ne différait que de un pour cent ont provoqué une stimulation de croissance très différente, alors qu'un troisième aliment contenant près de 25 pour cent de protéines en moins a été à peu près aussi efficace. L'analyse de la composition des aliments en acides aminés n'a pas permis d'expliquer à quoi sont imputables ces réactions de croissance.

Apparemment, d'autres facteurs que la concentration ou la qualité protéique, qui n'ont pas encore été identifiés, jouent un rôle important dans la croissance des pénéides.

EFECTOS DEL CONTENIDO DE PROTEINAS DE LOS PIENSOS Y DEL ORIGEN DE LAS MISMAS EN EL CRECIMIENTO DE *Penaeus aztecus*

Extracto

Se alimentó a formas juveniles de camarón, *Penaeus aztecus*, criadas en laboratorio con piensos extruídos a base de harina de soja y salvado de arroz. El contenido de proteínas de los piensos varió entre un 24 y un 63 por ciento, aproximadamente, con diferencias del orden del 10 por ciento. Los mejores crecimientos se obtuvieron con piensos con un 51,5 por ciento de proteínas, mientras el crecimiento disminuyó con el contenido máximo de proteínas. En los animales a los que se suministraron piensos con los tres niveles más bajos de proteínas no se registró esencialmente ningún crecimiento.

Los ensayos realizados simultáneamente con animales alimentados con otras raciones de composición distinta de las indicadas sugieren que el contenido de proteínas no es el único factor que influye en el crecimiento. Dos piensos con una diferencia de contenido de proteínas de sólo 1 por ciento arrojaron resultados considerablemente diversos, mientras un tercer pienso con casi un 25 por ciento menos de proteínas resultó casi igualmente eficaz. El análisis de la composición en aminoácidos de los piensos no permitió explicar las diferencias de crecimiento.

Parece ser, pues, que para el crecimiento de los peneidos son importantes otros factores diversos de la concentración o calidad de las proteínas, pero hasta la fecha no ha sido posible determinarlos.

1. INTRODUCTION

Of major importance in the economic cultivation of any animal is the food requirement for protein. The economically valuable penaeid shrimp species have been studied by several groups to determine requirements for this parameter. Results have, however, been varied. For this reason, we have attempted to evaluate protein requirements in laboratory-reared Penaeus aztecus using a series of diets of similar composition, but varying in protein quantity.

2. METHODS

All Penaeus aztecus used in this study were hatchery-reared, either at the Galveston Laboratory of the National Marine Fisheries Service or at Dow Chemical Company in Freeport, Texas, following procedures and feeding regimens described by Mock and Murphy (1971). Following development to postlarva, animals were maintained on a diet of live nauplial brine shrimp until the young were approximately 45 mm total length and approximately 0.6 g in weight. Ten animals were then randomly assigned to glass aquaria of 60-l capacity with undergravel filters, sterilized substrates, and filtered sea water (Zein-Eldin and Meyers, 1973), and held in constant temperature rooms maintained at 28 ± 1°C. Salinity in all aquaria was 25 ppt. Two randomly assigned groups of shrimp (20 individuals in all) were given each feed. Individual animals were measured to the nearest 0.5 mm (rostrum-telson length), blotted and weighed to the nearest 0.1 mg and identified individually by clipping uropods. Individuals were weighed, measured, and reclipped every two to three weeks. Mean initial weight was 0.705 ± 0.193 g with a range of 0.4389-1.3331 g. Mean final weight (after 106 days) of survivors (all feeds combined) was 5.21 ± 1.33 g with a range of 2.56-9.28 g.

Animals were fed weighed amounts of extruded feeds twice daily with amounts adjusted at each feeding depending upon the presence or absence of feed in the aquarium. Excess feed was removed, but no correction for dry weight of uneaten feed was made in calculation of approximate conversion rates.

Because the design of isocaloric diets requires the modification in amount and kind of several ingredients (notably the addition of fats), the decision was made to vary only two components without regard to the caloric values of the resulting feeds. Experimental feeds in this experiment differ from the control customarily used at the Galveston Laboratory (Zein-Eldin and Meyers, 1973). None of these feeds included components other than those used in the control, and these in different ratios. All used menhaden extractives as attractant and were bound with alginate (Meyers and Zein-Eldin, 1972; Meyers, Butler and Hastings, 1972). The principal protein sources were of vegetable origin: rice bran (defatted) and soy flour. The ingredients of the experimental feeds are listed in Table I as well as the analytical values for protein percentage which ranged from approximately 24 to 63 percent in 10 percent intervals.

3. RESULTS

As is apparent from Fig. 1, there was no significant increase in mean weight among shrimp fed at the three lowest protein levels over a period of 47 days. This contrasts with an approximate tripling of weight at the two higher protein levels during this time. Although moulting did occur in the tanks fed at the lower protein levels, it was less frequent and instances of negative growth (decrease in weight) were recorded.

The protein content of the feed affected not only the final mean size of the shrimp but also the ability of the individuals to withstand the stress of handling during measurement. Only 70 percent of the animals fed diets of 43.4 percent protein or less (diets 1, 2, and 3) survived the 19 days prior to the first measuring period (Table II) and an additional 20 percent of these animals died within 24 hours following the 19-day measurement. The decreased survival among these shrimp is reflected in an absolute decrease in biomass of animals fed at the lower protein levels (Fig. 2). It must also be noted that animals at the lower protein levels consumed only about one fifth the feed eaten by animals at the higher protein levels (Table III).

Table I

Composition of experimental feeds in percent

Ingredient[a]	Control 5-5/70B	1	2	3	4	5
Menhaden meal	8	5	5	5	5	5
Shrimp meal (sundried)	31.5	3	3	3	3	3
Fish solubles	2	2	2	2	2	2
Lecithin	1	1	1	1	1	1
Vitamin mix	2	2	2	2	2	2
Kelgin	2.5	2.5	2.5	2.5	2.5	2.5
Sodium hexametaphosphate	1	1	1	1	1	1
Rice bran (defatted)	49[b]	83.5	64.5	45	25	5.5
α-soy flour	3	0	19	38.5	58.5	78
Protein content (by analysis)	29.6	23.9	33.8	43.4	51.5	62.9

a/ Ingredient sources described in Meyers and Zein-Eldin (1973)

b/ Non-defatted rice bran used in this feed

On the basis of survival, resistance to stress (handling), moulting frequency, recorded increase in individual size and biomass, it would appear that the protein content of a feed satisfactory for P. aztecus must be greater than 43.4 percent. The range of protein content would also appear to be critical, because growth was less with a feed of 62.9 percent protein than with one of 51.5 percent protein (Fig. 2; Table II).

Simultaneously, however, three additional feeds were tested: a modification of our standard feed containing non-defatted rice bran, and two commercial feeds obtained from Japan, K-25 and F-1. Growth with feed K-25[1/] was greater than with any other feed tested, whether expressed as increase in biomass or increase in mean weight of individuals (Fig. 3; Table II). Growth with feed F-1 (which has a protein content only 1-2 percent less than that of K-25) was significantly less and animals fed this diet did not greatly exceed in mean weight those fed the control which had only about 30 percent protein. Detailed amino-acid analyses of these three feeds did not provide a ready explanation of the observed differences in growth rate (Table IV).

4. DISCUSSION

This study emphasizes the need for further experimentation concerning both the requirements for and the utilization of protein by penaeids. Previously published studies have suggested a variety of protein optima. Penaeus setiferus is said to have a protein optimum of 28-32 percent (Andrews, Sick and Baptist, 1972), Penaeus aztecus (Venkataramiah, Lakshmi and Gunter, 1975) and P. monodon (Lee, 1971) optima of about 40 percent, while P. japonicus requires more than 60 percent protein (based upon descriptions of diets tested by Deshimaru and Shigeno, 1972) and at least 50 percent purified protein (Deshimaru and Kuroki, 1975). Although our results are in general agreement with the studies on P. aztecus and P. monodon, they strongly suggest that factors other than protein content, or even protein quality, may be as important as the content of protein in feeds for penaeids, particularly those in intensive culture systems.

1/ Obtained from Kyowa Hakko Kogyo Limited

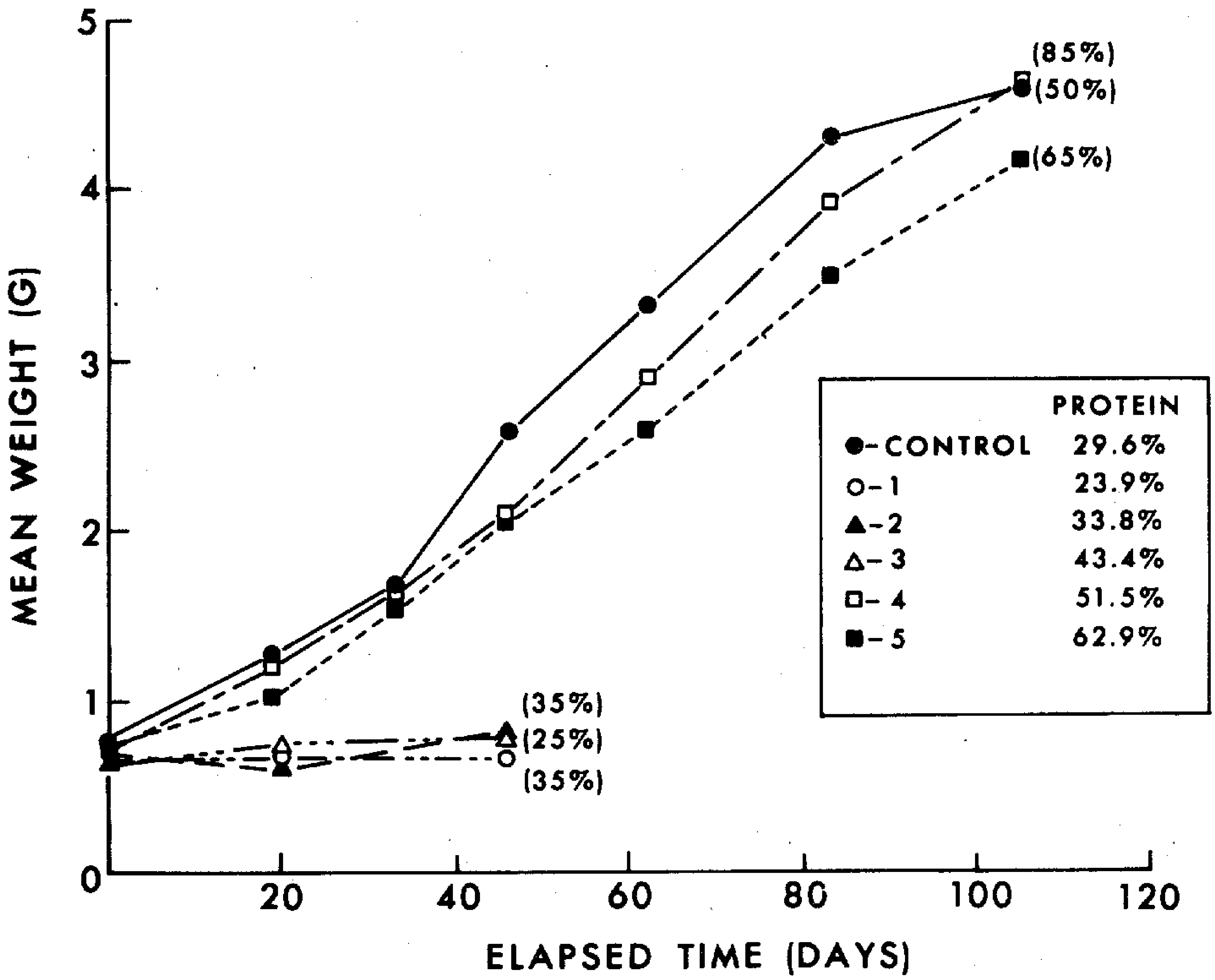

Fig. 1 Mean weight of Penaeus aztecus given diets containing various levels of protein. Feed composition as in Table I. Figures in parenthesis indicate survival

Thus, the presence of 20 percent more protein than in our standard feed did not ensure a consistent increase in growth rate in P. aztecus. Almost equivalent growth rates were obtained with diets varying in total protein between 30 and 53.5 percent protein, while diets differing only 1 percent brought about significantly different increases both in weight and biomass.

Decreased growth at the highest protein level was noted in P. setiferus fed diets of 40 and 52 percent protein (Andrews, Sick and Baptist, 1972) and has also been described in P. aztecus juveniles by Venkataramiah, Lakshmi and Gunter (1975) and by Lee (1971) in P. monodon. This raises a question concerning the high protein feeds described by various Japanese workers culturing P. japonicus: are there such significant differences in protein requirements among penaeid species? If so, culture would be best restricted to those species which can be grown adequately using feeds of lower protein content and of lower cost (e.g., high quantities of cereal or grain protein rather than animal protein).

Table II

Increase in mean weight of <u>Penaeus</u> <u>aztecus</u> juveniles fed rations of varying protein levels. Mean weight in g ± standard deviations. Test aquaria listed separately. Number of animals measured in parenthesis

Feed	Elapsed Time (Days)						
	0	19	33	46	62	83	105
1 (a)	.729 ± .221 (10)	.696 ± .186 (8)		.689 ± .134 (4)			
(b)	.677 ± .173 (10)	.679 ± .127 (10)		.659 ± (3)			
Combined	.700 ± .195 (20)	.687 ± .151 (18)		.676 ± .103 (7)			
2 (a)	.702 ± .259 (10)	.664 ± .250 (9)		.809 ± (3)			
(b)	.707 ± .241 (10)	.611 ± .085 (9)		.906 ± .296 (4)			
Combined	.704 ± .244 (20)	.637 ± .183 (18)		.864 ± .267 (7)			
3 (a)	.693 ± .172 (10)	.720 ± .114 (4)		.813 (2)			
(b)	.656 ± .166 (10)	.758 ± .153 (6)		.814 (3)			
Combined	.674 ± .166 (20)	.742 ± .133 (10)		.814 ± .082 (5)			
4 (a)	.744 ± .062 (10)	1.237 ± .475 (9)	1.708 ± .481 (9)	2.281 ± .488 (9)	2.986 ± .646 (9)	3.947 ± .798 (9)	4.595 ± .975 (9)
(b)	.719 ± .198 (10)	1.178 ± .187 (9)	1.591 ± .250 (9)	2.131 ± .347 (9)	2.820 ± .477 (7)	3.954 ± .642 (8)	4.798 ± .697 (8)
Combined	.732 ± .220 (20)	1.207 ± .352 (18)	1.650 ± .418 (18)	2.206 ± .418 (18)	2.914 ± .566 (16)	3.950 ± .706 (17)	4.691 ± .867 (17)
5 (a)	.730 ± .169 (10)	1.001 ± .263 (9)	1.408 ± .246 (7)	2.050 ± .182 (8)	2.571 ± .344 (7)	3.478 ± .490 (6)	4.129 ± .716 (6)
(b)	.679 ± .178 (10)	1.064 ± .267 (8)	1.700 ± .264 (8)	2.055 ± .262 (8)	2.665 ± .407 (8)	3.550 ± .887 (7)	4.243 ± 1.135 (7)
Combined	.705 ± .171 (20)	1.033 ± .247 (17)	1.562 ± .288 (15)	2.053 ± .218 (16)	2.621 ± .368 (15)	3.516 ± .704 (13)	4.190 ± .928 (13)
5-5/70B (a)	.727 ± .279 (10)	1.282 ± .318 (8)	1.480 ± .290 (6)	2.507 ± .384 (5)	3.480 ± .293 (5)	4.157 ± .552 (5)	4.679 ± .698 (5)
(b)	.694 ± .183 (10)	1.301 ± .296 (10)	1.869 ± .274 (7)	2.651 ± .308 (8)	3.220 ± .475 (8)	4.463 ± .709 (6)	4.614 ± 1.116 (5)
Combined	.710 ± .227 (20)	1.293 ± .297 (18)	1.690 ± .337 (13)	2.596 ± .331 (13)	3.320 ± .421 (13)	4.324 ± .631 (11)	4.668 ± .877 (10)
F-1 (a)	.749 ± .168 (10)	1.297 ± .331 (10)	2.118 ± .551 (9)	2.892 ± .469 (9)	3.516 ± .502 (8)	4.901 ± .558 (8)	5.424 ± .560 (8)
(b)	.682 ± .115 (10)	1.210 ± .243 (10)	1.869 ± .350 (9)	2.753 ± .319 (10)	3.308 ± .340 (10)	4.225 ± .412 (10)	5.022 ± .405 (9)
Combined	.716 ± .145 (20)	1.254 ± .279 (20)	1.994 ± .466 (18)	2.819 ± .392 (19)	3.400 ± .420 (18)	4.525 ± .581 (18)	5.211 ± .512 (17)
K-25 (a)	.690 ± .168 (10)	1.926 ± .170 (8)	2.812 ± .332 (9)	3.751 ± .377 (9)	4.512 ± .449 (7)	5.763 ± .256 (6)	6.802 ± .531 (7)
(b)	.707 ± .220 (10)	1.908 ± .303 (8)	2.717 ± .358 (8)	3.704 ± .407 (8)	4.884 ± .694 (8)	6.062 ± .904 (8)	7.523 ± 1.418 (7)
Combined	.699 ± .191 (20)	1.917 ± .237 (16)	2.767 ± .337 (17)	3.729 ± .400 (17)	4.710 ± .604 (15)	5.934 ± .699 (14)	7.163 ± 1.095 (14)

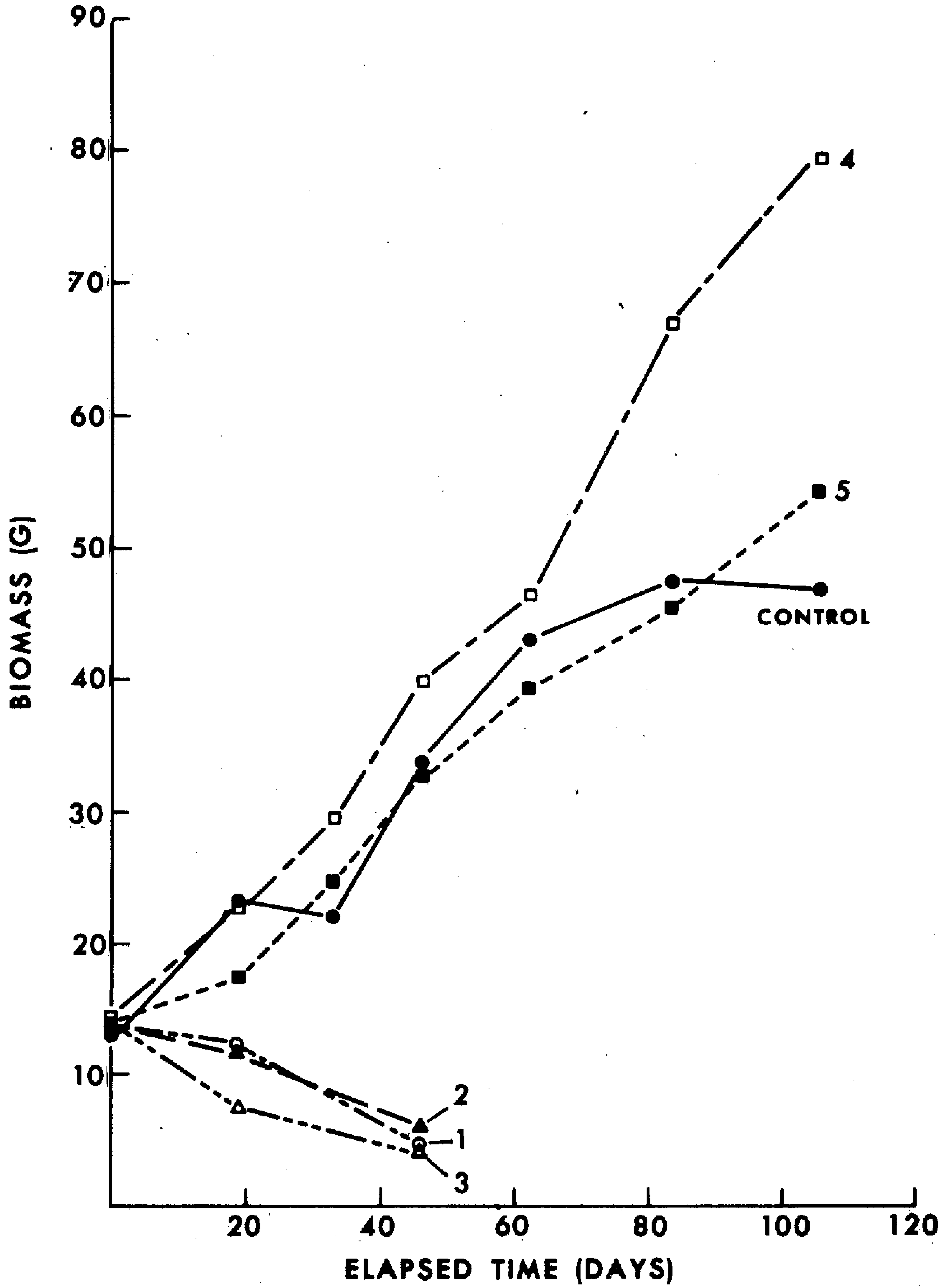

Fig. 2 Combined biomass of Penaeus aztecus fed various levels of protein. Symbols as in Fig. 1

Table III

Conversion ratios of Penaeus azteous juveniles fed diets of varying protein content and composition

Feed	Elapsed time: 46 days (1) Feed supplied (g)	(2) Protein supplied (g)	(3) Biomass change a/ (g)	(4) Conversion ratio b/	(5) Cols. 2 ÷ 3	105 days (1) Feed supplied (g)	(2) Protein supplied (g)	(3) Biomass change a/ (g)	(4) Conversion ratio b/	(5) Cols. 2 ÷ 3
1	30.5	7.3	- 9.3	- 3	- 1	-	-	-	-	-
2	38.4	13.0	- 8.0	- 4	- 2	-	-	-	-	-
3	28.9	12.5	- 9.4	- 3	- 1	-	-	-	-	-
4	161.3	83.0	25.07	6.4	3.3	487.1	250.9	65.11	7.5	3.9
5	85.0	53.5	18.75	4.5	2.9	252.6	159.0	40.37	6.3	3.9
Control	192.7	57.0	19.55	9.9	2.9	430.3	116.0	32.48	13.2	3.6
F-1	162.6	93.5	39.24	4.2	2.4	499.8	287.0	74.30	6.7	3.9
K-25	172.3	100.0	49.41	3.5	2.0	438.5	257.0	86.34	5.7	3.0

a/ Absolute change in biomass, not corrected for mortalities

b/ Conversion ratio = feed supplied/biomass change; not corrected for uneaten feed

Table IV

Amino-acid composition of experimental feeds expressed as percent of total sample weight. Mean of duplicate samples

	5-5/70B	F-1	K-25
Lysine	1.676	3.678	4.240
Histidine	0.726	1.011	1.203
Arginine	2.207	2.932	4.097
Taurine	a/	0.464	0.223
Aspartic acid	3.653	6.442	7.310
Threonine	1.052	2.166	2.576
Serine	1.197	2.379	2.464
Glutamic acid	4.083	8.259	8.613
Proline	1.262	2.693	2.431
Glycine	1.792	3.919	3.035
Alanine	1.612	3.674	3.337
Valine	1.280	2.860	2.779
Methionine	0.518	1.356	1.336
Isoleucine	1.003	2.372	2.545
Leucine	1.895	4.865	4.569
Tyrosine	1.470	1.930	1.978
Phenylalanine	1.208	2.300	2.425
Ammonia	0.728	0.910	0.956
Total protein	29.51	55.58	57.93

a/ Not determined

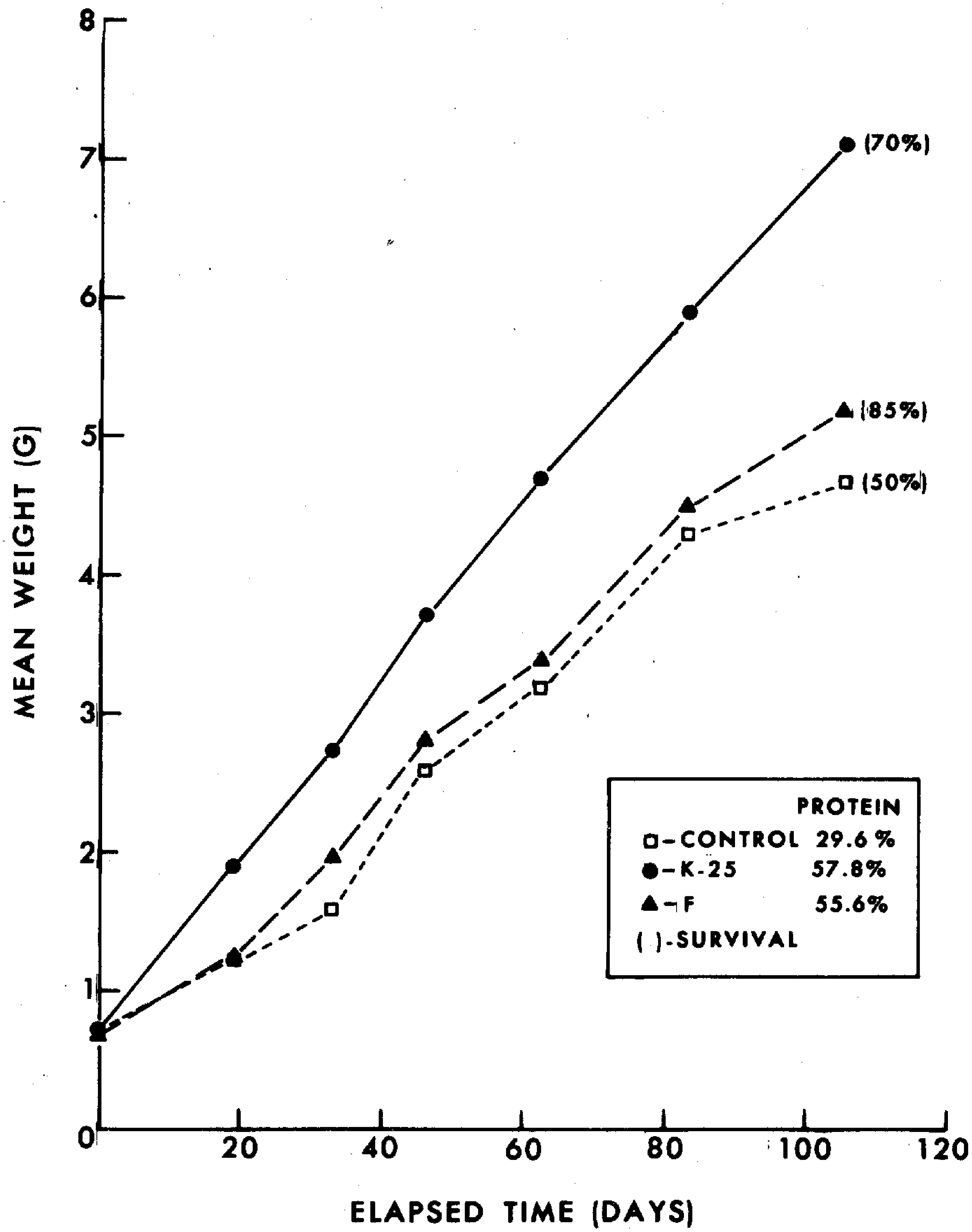

Fig. 3 Mean weight of Penaeus aztecus fed two commercial feeds of greater than 50 percent protein, compared with standard feed (control) 5-5/70B. Figures in parenthesis indicate survival

It must also be mentioned that utilizable protein may be lower than that suggested from analytical results, particularly at the lowest protein levels described, since Lee (1970) has indicated that P. monodon uses rice bran about 10-20 percent less efficiently than other vegetable proteins. Should this be true of P. aztecus, effective protein levels might be considerably less in our control feed (5-5/70B) than indicated by the analytical results.

Conversion ratios were calculated using the absolute biomass change. Although many authors have used a ratio which corrects for mortality (Kitabyashi et al., 1971), we feel that the use of actual biomass change provides a more realistic evaluation of the economic potential of a given feed under actual culture conditions. Animals in these experiments were fed ad libitum, and under some conditions of physiological stress (e.g., immediately prior to or following moult) they ate less than expected. The weight of uneaten feed was not subtracted from the total amount of feed supplied so that conversion rations as given in Table IV may be considered maximal. After either 46 or 105 days, the control feed was least efficient (highest conversion ratio) among feeds yielding a positive change in biomass. If, however, percentage of protein is considered, the control was second in rank in the long-term experiment. Thus, evaluation of the potential of these feeds in economic terms requires knowledge of growth rates, biomass changes (independent of survival) and the cost of feed per protein unit.

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