Protein/Energy Requirement of the Red Crawfish

by

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ABSTRACT

A study was conducted to determine the optimum protein to energy ratio for red crawfish (*Procambarus clarkii*). Six experimental diets were formulated which contained 20, 30, or 40% protein and 2500 or 3500 kcal/kg gross energy. Growth decreased as dietary energy increased in crawfish fed 20 and 30% dietary protein. Growth of crawfish fed 40% dietary protein decreased as dietary energy decreased. In general body protein decreased as dietary energy increased. Crawfish fed low energy (low fat) diets exhibited lower body fat. Also, body fat tended to decrease as dietary protein increased. Data indicated that dietary protein/ energy imbalances affected performance. In addition, 30% dietary protein and 2500 kcal/kg dietary energy appeared to be adequate for good growth and protein deposition.

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INTRODUCTION

The red and white crawfish (*Procambarus clarkii* and *Procambarus acutus acutus* respectively) are important commercial food species and their culture is a multimillion-dollar industry in the United States. Crawfish are cultured for food in approximately 30,000ha in Louisiana and 3,000ha in Texas with smaller areas under culture in other states. Traditionally, Louisiana has produced and consumed the bulk of the crawfish available in the U.S. However, market expansion and the generally higher price paid for crawfish in markets that are not affected by natural crawfish crops (as in Louisiana), has increased interest in crawfish production. States such as South Carolina, Mississippi, Missouri, Arkansas, Washington, Oregon and California are becoming involved or have a keen interest in commercial crawfish culture.

Although crawfish are omnivorous, they feed primarily on vegetation in pond culture. Formulated feeds are not normally used in pond culture, rather a detritous-based ecosystem is established to provide food. Vegetation is generally provided, however, a major problem in crawfish farming is depletion of vegetation resulting in stunted (submarketable) crawfish (Avault et al., 1974). Supplemental feeding or feeding a complete feed has been beneficial in preventing stunting (Huner and Barr, 1981). Although early work indicates that feeds formulated for other aquatic animals were beneficial in providing rapid growth, they were not economical (Smitherman et al., 1967; de la Bretonne et al., 1969; Meyers et al., 1970; Clark et al., 1974; Tarshis, 1978). Crawfish now command prices sufficient to make feeding economically feasible, especially if feeds are formulated based on the nutrient requirements of the crawfish. Feeding a nutritionally balanced feed should increase yield and provide a means to produce a crop on a year-round basis.

Although several researchers have investigated the use of supplemental feeds and forages for crawfish and a few diets have been tested, essentially no information exists concerning the nutritional requirements of the crawfish. Based on a study by Huner and Meyers (1977) the protein requirement for the red crawfish is estimated to be below 25% of the diet. In later reports these researchers stated the protein requirement to be in the 20 to 30% range (Huner and Meyers, 1979). However, no study has adequately determined the protein requirement nor have any considered the protein to energy relationship of crawfish.

The optimum dietary protein to energy ratio is important for formulating both experimental and commercial feeds for crawfish. Optimum levels of protein to energy are important both economically and nutritionally. Since protein sources are generally more expensive relative to energy sources, it would be economical to use a source of energy such as carbohydrate and/or fat to provide the energy requirements, thereby sparing dietary protein for tissue deposition. Nutritionally, a balance between dietary protein and energy should allow an animal to satisfy its energy requirement and simultaneously consume adequate protein for growth. The information gained from protein-energy studies will allow an efficient experimental diet to be developed for determination of other nutrient requirements as well as providing data to be used in formulation of commercial feeds. The objective of this study was to determine the optimum dietary protein to energy ratio for red crawfish.

LITERATURE REVIEW

Because of the popularity of crawfish as a food and bait species, numerous researchers have studied it. Early work largely concentrated in taxonomy, distribution, biology and physiology as indicated in a bibliography by Spohrer et al., (1974). More recently, several publications have summarized pertinent information concerning the culture of crawfish (Visoca, 1966; Avault, 1972; Gary, 1975; LaCaze, 1976; Huner and Avault, 1977; Huner and Barr, 1981).

Economics (Roberts, 1980) as well as the legal aspects (Williams et al., 1975) of the crawfish industry have been studied. Other areas of study include crawfish diseases (Johnson, 1977), effects of pesticides on crawfish (Muncy and Oliver, 1963; Cheah et al., 1980), and the ecology and social importance of the crawfish (Huner and Barr, 1981).

In the last two years an aritifical crawfish bait has been formulated and tested (Avault, 1983). The attractants used in the baits may have application in practical feeds for crawfish, serving as a stimulus for consumption.

Supplemental feeds such as alfalfa, cottonseed meal, cracked corn and other grain by-products have been used by crawfish farmers with varying success (Huner and Barr, 1981). Sweet potato vines and leaves, sweet potato trimmings and various types of aquatic vegetation have been used as supplemental feeds (Goyert et al., 1977). Range pellets have also been used as a supplement (Cange et al., 1981). These researchers reported that the addition of range cubes increased total yield but stunting was not prevented. Other agricultural forages and by-products such as bahia grass and rice hays have been shown to be beneficial as supplemental feeds (Rivas et al., 1978; Romarie et al., 1978). The benefit of supplemental feeds appears to depend on the C:N ratio which should be 17:1 or less before crawfish will eat the feed (Huner and Barr, 1981); therefore, their benefits are not immediate.

Although information concerning supplemental feeds and forages for crawfish is available, essentially no nutritional information exists. No studies have been conducted to determine the optimum dietary protein to energy ratio.

Relatively little is known about the protein requirements of crustaceans. The optimum dietary protein level for juvenile blue crabs (Callinectes sapiclus) was reported to be between 23 and 37% (Millikin et al., 1980a). In a review of shrimp and prawn dietary studies New (1976) concluded that a dietary protein level of 27-35% was optimum, but noted that if amino acids were balanced, protein requirements might be reduced. More recently, optimum dietary protein levels ranging from 40-55% have been reported for various species of shrimp and prawn (Deshimaru and Yone, 1978; Millikin et al., 1980b; Kanazawa et al., 1981; Alava and Lim, 1983). However, the wide variation in experimental techniques makes comparisons difficult (New, 1976).

Although the protein/energy requirement of shrimp and prawns has not been well researched, Hysmith et al. (1972) concluded that low protein-high energy or high protein-low energy diets were better than either low protein-low energy or high protein-high energy diets for growth of Panaeus axtecus. Deshimaru and Kuroki (1974) found that adding 6% dextrin to a casein-based diet resulted in increased growth rates for Panaeus japonicus.

The dietary protein requirements of the American lobster (Homarus americanus) have been investigated. Castell and Budson (1974) reported that incremented increases in dietary protein from 0-60% improved the performance of lobsters. As an explanation they proposed that the protein source (casein) may have been limiting in one or more essential amino acids or that the lobsters utilized casein as an energy source.

The protein sparing action of dietary carbohydrate was investigated by Capuzzo and Lancaster (1979). They concluded that dietary carbohydrate had a sparing effect on protein in Homarus americanus. In addition, they showed that protein efficiency and protein utilization efficiency increased as dietary protein/carbohydrate ratios decreased. Callagher et al. (1979) concluded that protein/energy ratios were important considerations when formulating lobster diets. These researchers reported that growth was better in animals fed low protein/energy ratio diets than those fed high protein/energy ratio diets.

Few studies have been reported concerning dietary protein requirements of crawfish. Tarshis (1978) found no differences in growth for *Procambarus acutus acutus* fed diets containing protein levels between 31.7-50.5%. Butler (1971) fed dietary protein levels ranging from 30.0 to 43.2% and noticed no significant differences in growth for *Procambarus clarkii*. Possibly these values were above the optimum protein requirement for crawfish. Huner and Meyers (1977) reported the dietary protein requirement for *P. clarkii* to be below 25%. More recently the protein requirement of *P. clarkii* was reported to be near 20-30% (Huner and Meyers, 1979). Dietary protein to energy ratios have not been studied in crawfish.

METHODS

Diets and experimental design

An 8 week study was conducted to determine the optimum protein to energy ratio for red crawfish. Six experimental diets (Tables 1, 2 and 3) were formulated using casein and gelatin as protein sources. Dietary lipid was maintained at 2 or 10% of the dry diet depending on the energy level and dextrin levels were varied to give the desired dietary energy. Since neither digestible nor metabolizable energy values are available for crawfish, dietary energy values were calculated using standard physiological fuel values of 4 kcal/g for protein or carbohydrate and 9 kcal/g for lipid.

Three protein levels, 20, 30, and 40%, were used and 2 energy levels, 2500 and 3500 kcal/kg at each protein level. Protein to energy ratios ranged from 57.0 to 160.0 mg protein/kcal. Experimental diets were prepared in our laboratory. Dry ingredients were blended in a V-mixer, then mixed with the oil and water (30-40%) to yield a consistency capable of being pressure pelleted. The pelleted diets were frozen until needed. Prior to feeding the diets were thawed and mechanically broken into the appropriate particle size.

A total of 112 crawfish were maintained in 10cm diameter cylindrical pvc containers, one crawfish per container. Four

Table 1. Experimental diet composition.[1]

Ingredient[2,3]	Diet number					
	1	2	3	4	5	6
Casein	18.7	18.7	28.1	28.1	37.4	37.4
Gelatin	3.0	3.0	4.5	4.5	6.0	6.0
Dextrin	35.2	42.2	24.3	31.3	13.5	20.6
Menhaden Fish Oil	2.0	10.0	2.0	10.0	2.0	10.0
Carboxymethyl-cellulose	2.0	2.0	2.0	2.0	2.0	2.0
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin Mix[4]	0.5	0.5	0.5	0.5	0.5	0.5
Mineral Mix[5]	5.6	5.6	5.6	5.6	5.6	5.6
Cellulose	37.5	17.5	37.5	17.5	37.5	17.4

[1]Commercial Trout feed containing 38% crude protein and 8% crude fat fed as control diet.

[2]Expressed as percentage of dry diet.

[3]Ethoxyquin added as an antioxidant.

[4]See Table 2.

[5]See Table 3.

Table 2. Composition of vitamin premix.

Vitamin (form)	Amount/ kg of diet	Activity/ kg of diet
	(mg)	
A (Ester conc.)	42.25	8250.00 IU
D-3 (cholecalciferol)	0.04	1500.00 IU
E (d-a-tocopherol)	73.53	100.00 IU
K (menadione)	15.00	15.00 mg
Niacin (nicotinic acid)	110.00	110.00 mg
Riboflavin	22.00	22.00 mg
Pyridoxine (HCl)	40.00	40.00 mg
Thiamin (mononitrate)	43.54	43.54 mg
Pantothenate (d-calcium)	100.00	100.00 mg
Biotin	0.11	0.11 mg
Folacin (Folic acid)	10.00	10.00 mg
B-12	0.04	40.00 ug
Inositol	110.00	110.00 mg
Choline (bitartrate)	1260.42	605.00 mg
Ascorbate	500.00	500.00 mg

Table 3. Composition of mineral premix.

Mineral (form)	Amount/ kg of diet
	(mg)
Calcium ($CaHPO_4 \cdot 2H_2O$)	20700.0
Calcium ($CaCO_3$)	14800.0
Phosphorus (KH_2PO_4)	10000.0
Potassium (KC1)	1000.0
Sodium (NaC1)	6000.0
Manganese ($MnSO_4 \cdot H_2O$)	350.0
Iron ($FeSO_4 \cdot 7H_2O$)	500.0
Magnesium ($MgSO_4$)	3000.0
Iodine (KIO_3)	10.0
Copper ($CuSO_4 \cdot 5H_2O$)	30.0
Zinc ($AnCO_3$)	150.0
Cobalt ($CoC1_2$)	1.7
Molybdenum ($Na_2MoO_4 \cdot 2H_2O$)	8.3
Selenium (Na_2SeO_3)	0.2

containers were placed in 50x25x25 cm glass aquaria. Four aquaria were used for each treatment (16 crawfish per treatment). A recirculating water system was used in which water flowed through individual aquaria into a common drain which returned it to a settling chamber. Water passed through the settling chamber into a biofilter before being returned to each aquaria by use of a low pressure high volume blower.

Juvenile crawfish obtained from a commercial crawfish farm were transported to the Aquaculture Research Center and acclimated to environmental conditions. Uniform size crawfish were stocked into individual containers and fed a casein-gelatin conditioning diet for a period of 2 weeks prior to the start of the experiment. During this time they readily acclimated to experimental diets and environmental conditions.

At the beginning of the experiment, individual weights and lengths were recorded. During the first week of the experiment mortalities were replaced after which no replacements were made. Crawfish were fed ad libitum twice daily. Aquaria were checked 2-3 times daily for molts, which were removed to prevent consumption. Weight and length measurements were taken biweekly.

Sample Collection and Analysis

Upon termination of the experiment, all crawfish from all aquaria were collected and frozen for body analysis. Due to the small sample size pooled samples of 6-8 crawfish were used for analysis. Samples were prepared by blending each pooled sample (6-8 crawfish/sample) until a homogenous slurry was obtained. Each analysis was conducted in duplicate on 2 pooled samples per treatment.

Dry matter and ash were determined using A.O.A.C. methods (1965). Whole body protein was determined by the macro-kjeldahl method (AOAC, 1965). Whole body lipid was determined by chloroform-methanol extraction (Folch et al., 1957).

Water Quality

Water quality was checked periodically. Dissolved oxygen was checked with a YSI model 51B oxygen meter. Ammonia, nitrate, nitrite, pH and calcium hardness were determined using a Hach DR-EL/2. Calcium hardness was maintained above 50 ppm through the addition of CaCl and $CaCO_3$. Water temperature was checked 3 or 4 times a week.

Statistical Analysis

Data were analyzed with the Statistical Analysis System, SAS-79 (Helwig and Council, 1979) using the General Linear Models Procedure. Duncans multiple range test was used to determine statistical differences (Steele and Torre, 1960). Results were considered significant at the 0.05 level.

RESULTS AND DISCUSSION

Water quality varied somewhat during the experimental period (Table 4). Ammonia ranged from 0.03 to 0.46 ppm and nitrite from 0.07 to 0.83 ppm. At the higher levels growth may have been affected; however, it was assumed that all crawfish were influenced equally. Dissolved oxygen was in a range (6.9 to 8.0 ppm) which was considered to be optimum for aquatic organisms. Except for a two week period in which the temperature dropped to 22°C, the temperature was near 27° C. The drop in temperature appeared to reduce the feed consumption of all crawfish. Hardness was maintained above 50 ppm during the study. De la Bretonne et al. (1969) reported that 50 ppm or above was adequate for normal growth of crawfish. Changes in water quality did affect performance, but it was assumed that the effects were the same for all crawfish in the system. It is possible that fluctuations in water quality contributed to the variation observed during the experiment.

Although the precise dietary protein to energy ratio cannot be determined from the present study, an estimate of the protein/energy requirement can be made. Growth was best in crawfish fed Diet 3 (30% protein and 2500 kcal/kg), although it was not significantly different from growth of crawfish fed Diets 1, 2, or 6 (Table 5). Growth of craw-

Table 4. Water quality data.

Parameter	Range (ppm)
NH_3	0.03-0.46
NO_2	0.07-0.83
pH	8.18-8.51
Dissolved Oxygen	6.9-8.0
Hardness	57-213
Temperature	22-27[1]

[1]Expressed in degrees Celcius.

Table 5. Initial weight, final weight and percent increase for juvenile crawfish fed experimental diets.

Diet	P:E ratio	Initial weight	Final weight	% Increase
1	80	1.1	4.5[bc] [1]	305.0[bc]
2	57	0.9	3.6[bc]	290.0[bc]
3	120	1.0	4.8[b]	369.2[b]
4	86	1.2	4.0[bc]	243.9[c]
5	160	1.1	3.4[c]	222.1[c]
6	114	1.0	4.2[bc]	330.5[bc]
Control	95	1.1	6.7[a]	532.3[a]

[1] Same letters are not significantly different ($\alpha = 0.05$).

fish fed diets containing 20 and 30% protein was reduced as dietary energy levels increased. Dietary energy concentrations influence feed consuption; i.e., animals eat, in part, to satisfy energy needs (NRC, 1983). Assuming this to be true for crawfish, increasing dietary energy levels may have restricted feed consumption resulting in insufficient protein intake, thus a reduction in growth.

Dupree and Sneed (1967) and Prather and Lovell (1973) reported reduced growth in catfish fed high protein diets containing insufficient energy. Based on the magnitude of percent growth increase between crawfish fed the high protein-low energy diet (Diet 5) and those fed the high protein-high energy diet (Diet 6) this would appear to be true for crawfish. However, their growths were not statistically different when crawfish fed the control diet were included in statistical analysis. Their growth were statistically different though when the control group was not considered. Although not conclusive, there were indications that diets containing an imbalance of protein and energy depressed growth in crawfish.

Percent length increases are presented in Table 6. Length increases in crawfish fed Diet 3 were greater than crawfish fed other diets; however, it was not significantly different than length increases for crawfish fed diets 1 and 2. Length increases in crawfish fed the high protein-low energy diet (Diet 5) was significantly reduced compared to those fed the high protein-high

Table 6. Initial length, final length, and percent increase for juvenile crawfish fed experimental diets.

Diet	P:E ratio	Initial length (mm)	Final length (mm)	% Increase
1	80	34.5	53.5[bc][1]	54.8[bc]
2	57	33.0	50.2[bc]	52.0[bc]
3	120	32.7	53.8[b]	64.6[ab]
4	86	36.2	49.8[bc]	37.6[d]
5	160	36.1	48.8[c]	35.2[d]
6	114	34.8	52.3[bc]	50.5[c]
Control	95	34.8	60.3[a]	73.8[a]

[1]Same letters are not significantly different ($\alpha = 0.05$).

energy diet (Diet 6). These results are similar to the growth data and support the theory that a dietary protein/ energy imbalance affects performance.

Body composition data are presented in Tables 7 and 8. Crawfish fed Diet 3 had significantly higher protein deposition (wet weight basis) than crawfish fed other experimental diets. There was a significant increase in protein deposition as dietary energy decreased within each protein level. This suggests that the lower energy diets were adequate in energy and that at higher energy levels protein intake may have been restricted.

Protein deposition (dry weight basis) was similar in crawfish fed the low protein and high protein diets, but not those fed the medium protein diets; i.e., protein deposition was higher at lower energy levels except for crawfish fed the 30% protein diets. Generally this indicates that the lower energy diets were adequate.

A significant decrease in body fat was observed in crawfish fed low energy diets (Tables 7 and 8) which is probably reflective of both low dietary energy and fat. High levels of dietary energy and/or fat have been shown to increase body fat in fish (NRC, 1983). Body fat decreased significantly in crawfish fed diets containing 3500 kcal/kg as dietary protein increased. Fat deposition in crawfish fed diets containing 2500 kcal/kg energy was not significantly different in crawfish fed 20 and 30% dietary protein, but did decrease significantly in those 40% dietary protein.

Table 7. Body composition (wet weight basis) of juvenile crawfish fed experimental diets.

Diet	Crude dietary protein (%)	Dietary lipid (%)	P:E ratio	Whole Body Composition			
				Protein (%)	Lipid (%)	Moisture (%)	Ash (%)
1	20	2	80	10.19^{c}[1]	1.26^{d}	78.65^{abc}	6.64[2]
2	20	10	57	9.68^{d}	2.77^{b}	78.47^{bc}	6.75
3	30	2	120	11.96^{a}	1.35^{d}	76.08^{d}	7.40
4	30	10	86	11.00^{b}	2.28^{c}	77.67^{c}	5.99
5	40	2	160	11.24^{b}	0.81^{e}	79.64^{a}	5.95
6	40	10	114	10.17^{c}	1.27^{d}	79.38^{ab}	6.47
Control	38	8	95	11.88^{a}	3.27^{a}	74.23^{e}	7.52

[1] Same letters are not significantly different ($\alpha = 0.05$).

[2] No significant differences were found.

Table 8. Body composition (dry weight basis) of juvenile crawfish fed experimental diets.

Diet	Crude dietary protein (%)	Dietary lipid (%)	P:E ratio	Whole Body Composition			
				Protein (%)	Lipid (%)	Dry Matter (%)	Ash (%)
1	20	2	80	47.73[c][1]	5.93[c]	21.35[cd]	31.14[a]
2	20	10	57	45.02[d]	12.84[a]	21.53[c]	28.98[b]
3	30	2	120	49.95[b]	5.63[c]	23.92[b]	30.94[a]
4	30	10	86	49.23[bc]	10.22[b]	22.33[c]	26.82[c]
5	40	2	160	55.18[a]	3.97[d]	20.36[d]	28.26[bc]
6	40	10	114	49.33[b]	6.15[c]	20.37[d]	31.37[a]
Control	38	8	95	46.13[d]	12.67[a]	25.77[a]	29.17[b]

[1] Same letters are not significantly different ($\alpha = 0.05$).

These data may reflect a change in energy requirements as dietary protein increased.

Protein deposition was high and fat deposition low (wet weight basis) in crawfish fed Diet 3 (30% protein and 2500 kcal/kg). In addition, crawfish fed Diet 3 demonstrated the best growth of crawfish fed experimental diets. Based on dry weight crawfish fed Diet 5 (40% protein and 2500 kcal/kg) were highest in body protein and lowest in body fat, but growth was poor.

There were no significant differences in whole body ash percentage on a wet weight basis (Table 7). There were some changes in whole body ash when expressed on a dry weight basis (Table 8); however, their importance is not known. Previous researchers did not observe significant differences in ash content due to diet formulation (Phillips and Brockway, 1959; Phillips et al., 1966; Page and Andrews, 1973; Garling and Wilson, 1976).

Condition factors (Table 9) for crawfish were generally the same regardless of dietary treatment. Although there were some statistical differences, all crawfish appeared to be in good condition. Thus, diet did not appear to have a major impact on overall condition.

Crawfish fed the control diet gained more weight than those fed experimental diets (Table 5). The control diet was a commercial trout feed which contained approximately 38% crude protein (primarily from fish meal) and 4000 kcal/kg. Properly processed fish meal is a high quality protein

Table 9. Initial, final and percent change in condition factors and percent survival for juvenile crawfish fed experimental diets.

Diet	P:E ratio	Initial[1] condition factor	Final condition factor	% Increase	% Survival
1	80	2.63^{ab}[2]	2.74^{b}	6.8^{bc}	100
2	57	2.51^{b}	2.78^{b}	9.9^{abc}	100
3	120	2.96^{a}	2.99^{ab}	2.3^{c}	94
4	86	2.42^{b}	3.10^{a}	28.2^{a}	88
5	160	2.25^{b}	2.82^{ab}	25.9^{a}	94
6	114	2.40^{b}	2.88^{ab}	20.7^{abc}	100
Control	95	2.47^{b}	2.96^{ab}	21.4^{ab}	100

[1] Condition factor = wet weight x 10^5/length^3.

[2] Same letters are not significantly different ($\alpha = 0.05$).

source for fish and is often used as an attractant (NRC, 1983). Protein quality (i.e., high levels of essential amino acids) and acceptability (i.e., increased consumption) are primary reasons for the better growth of crawfish fed the control diet. Based on percent increase in length, crawfish fed Diet 3 (30% protein and 2500 kcal/kg) performed as well as those fed the control diet. Based on other parameters, i.e., body composition and condition factors, the performance of crawfish fed the control diet was no better than those fed the experimental diets. Overall (except for weight increase) there was no advantage to the commercial feed. The increased weight gain of crawfish fed the control was probably more a result of increased consumption rather than protein or energy levels.

Variation in the present study may be accounted for, in part, by environmental differences (i.e., changes in water quality), sex or species differences. Relatively high variation has been reported in other studies with crawfish (Huner and Romaire, 1979).

CONCLUSION

Data from the present study, although not conclusive, suggest that 30% dietary protein and 2500 kcal/kg is adequate for normal growth of crawfish reared in a closed system.

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