Rate of Ingestion and Proteolytic Activity in Digestive System of Juvenile White Shrimp, *Penaeus vannamei,* During Continual Feeding

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ABSTRACT. Juvenile white shrimp, *Penaeus vannamei,* were studied using tray feeders in relation to food ingestion and enzymatic activity, with data taken every two hours for five days. The feed ingestion was calculated by subtracting the uningested and the leached material from the food provided. Significant differences ($P < 0.05$) in the ingestion rate were found during the experiment. The greatest ingestion occurred between 2000 and 2200 hours. The largest feed ingestion coincided with the nocturnal activity of shrimp. Time-series analysis showed a cycle of increased ingestion before midnight and lower ingestion during daylight. Total protease, trypsin, and chymotrypsin activity were measured during the last 24 hours of the study and included both fed and starved organisms. The enzymatic pattern was similar for both groups. There was a decrease of the protease and trypsin activities before and after 1800 hours, just before the ingestion increased. The study of digestive enzymes provided evidence of a close association between behavior and regulation of the digestion system physiology. The results

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give some clues toward better feeding regimens and the use of feed management for continual monitoring of ingestion to get reliable evaluations for food conversion index. Feed management with tray feeders will reduce pollution in extensive ponds by decreasing the uningested food.

KEYWORDS. Ingestion, feedings, white shrimp, *Penaeus vannamei*

INTRODUCTION

Feed management is an important factor in aquaculture. Uningested food generates several problems in aquaculture systems, because high concentrations of dissolved nutrients induce eutrophication, growth of pathogenic microorganisms, and reduces productivity (Wang 1990). Organic material in the pond water reduces survival and growth rates of the cultivated organisms (Clifford 1992). Feeds used in aquaculture are stable in water for only a limited time. The aquaculturist is also concerned about nutrient loss through leaching. Because of the feeding habits of shrimp, food is not rapidly consumed. Ingestion rates quantify the amount of food ingested by the cultivated animals. Maximum consumption of feed can result in better food conversion factors and therefore better growth (Villalon 1991).

Feeding frequency should depend on the feeding habits and metabolism of the organism. Continual monitoring is needed to avoid over- or underestimation of ingestion. Different approaches have been used to study feeding frequency. Some researchers have used stomach content, feed consumption, or activity to determine the best feeding regimen (Wassenberg and Hill 1993). Alternatively, tray feeders are also used to reduce food loss and as a consumption indicator to allow adjustment of the daily amount of food provided (Jory 1995). Digestive enzymes have been used as an indicator of molt stages (Sellos and Van Wormhoudt 1992), dietary protein quality (Maugle et al. 1982; Lee et al. 1984; Ezquerra et al. 1997), and circadian rhythm (Van Wormhoudt 1973). Trypsin and chymotrypsin are ubiquitous enzymes in aquatic organisms (Garcia-Carreño et al. 1994; Hernandez-Cortes et al. 1997), and the variation in protease activity could yield information about synthesis, secretion, and regulation of the digestive system.

Both biochemistry and behavioral studies will help plan the best feeding strategies to improve the feed conversion ratio and reduce to some extent the deterioration of water quality caused by uningested feed.

The aim of this work was to evaluate feeding habits and biochemical changes in the digestive system. The rate of ingestion of juvenile white shrimp, *Penaeus vannamei*, using tray feeders was estimated during continual feeding over a 24-hour cycle. Proteolytic activity was also evaluated with starved and fed organisms during the daily cycle.

MATERIALS AND METHODS

Experimental Conditions

Juvenile white shrimp, *P. vannamei,* averaging 4.4 ± 0.1 g were obtained from the Centro de Investigaciones Biologicas del Noroeste farm facilities. Outdoor 2,000-L polyethylene tanks (1.5 m long, 1.4 m wide, 0.5 m depth) with sand-layer bottom and with 80 organisms in each were used as the experimental units. Water was filtered by sand, silica, and 5-μm filters and changed 90% each day. The temperature was maintained at $28 \pm 3°C$ with a 250-W heater controlled with a thermostat and monitored every 4 hours. Aeration was provided continually with an air diffuser. Water quality variables such as salinity, dissolved ammonium, and pH were monitored twice per day. Organisms were weighed and randomly distributed into the experimental units. The feeding trial was made over four days, recording the weight gain for both fed and starved organisms. On the fifth day, organisms were taken every two hours for the study of digestive enzymes.

Feeding

Forty-cm diameter trays were used in each tank to provide food. The trays were made of 600-μm mesh fabric attached to a buoyant device to facilitate recovery of the uningested food for analytical purposes. For the fed organisms, food was continually supplied every two hours during the five days. Equal amounts of food were dispensed on the trays through a 5-cm-diameter PVC tube. After two hours of feeding, the remaining pellets were removed and dried for 24 hours at 120°C. The loss of dry food was adjusted according to method of

Lovell (1975). Shrimp feeds were obtained from a commercial source (PIASA Particulado B, Mexico). The chemical composition of the feed was 40% crude protein, 7% crude fat, 11% ash, 3% fiber, 12% humidity, and 27% nitrogen-free extract.

Ingestion Rate

Food ingested was defined as the ingestion rate of dry food per hour (g/hour) according to Sick et al. (1973). Twelve data points of food ingested per hour were obtained from each tank for four days. The ingestion rate, uningested food, and leached material was determined as follows:

$$I = M_i - M_f - M_p/T$$

where:

I = Ingestion rate of food per hour
M_i = Initial dry weight of pellets supplied
M_f = Dry weight of the uningested pellets
M_p = Dry weight of pellets dissolved in the water
T = Elapsed time of pellets underwater

Enzyme Analysis

Three shrimp were collected every two hours during the fifth day of the experiment. Shrimp were de-headed, the hepatopancreas were removed by dissection, stored individually in Eppendorf tubes, and frozen at $-70°C$ until used. The hepatopancreas were individually weighed; homogenized in 50 mM TRIS · HCl, pH 8.0, containing 20 mM $CaCl_2$ (1:2 w/v); and centrifuged for 20 minutes at 11,000 $\times$ *g* to eliminate lipids and tissue debris. The protein content of the enzymatic extract was determined according to Bradford (1976). Total protease activity was assayed with azocasein, trypsin with benzoyl-Arg-p-nitroanilide (BAPNA), and chymotrypsin with succinyl $(Ala)_2$-Pro-Phe-p-nitroanilide (SAAPFNA) substrates according to Garcia-Carreño and Haard (1993) and Hernandez-Cortes et al. (1997). Total protease, trypsin, and chymoptrypsin activity were expressed as units of activity/min/mg protein. Each determination was done in triplicate.

Statistics

The differences in amount of food ingested were analyzed by one-way analysis of variance (ANOVA) and the average of each tested group by the Duncan method using Statgraphics 5.0 software. Time-series analysis was used to identify the feeding periodicity. The method used was the seasonal decomposition (Census method I). Protease activities were also examined by ANOVA analysis. Differences between the means were analyzed by planned comparison (LDS test). The data were analyzed using the program STATISTIC for PCS (Statsoft Inc., Tulsa, Oklahoma, USA).

RESULTS AND DISCUSSION

Water quality conditions were those recommended for optimum growth of white shrimp. Temperature averaged $28.0 \pm 3.0°C$ daily, with the highest value at noon and the lowest temperature at midnight. Oxygen was 7.7 ± 0.3 mg/L, ammonia 0.001 ± 0.0003 mg/L, pH 7.7 ± 0.5, and salinity 37.9 ± 0.5 ppm. The weight increase in each experimental unit was 7.6, 13.6, and 9.7% from the first through fourth day of the experiment. Survival was 98%. Percent of lost material by leaching of food at one, two, and three hours underwater was 1.4%, 3.8%, and 4.5%, respectively. This information was used to correct values of food ingestion.

The rate of food ingestion, grams of food ingested per hour, was calculated each two hours for four days starting at 0200 hours. The fifth day was not included in this analysis because at that time, three organisms were taken two hours apart for digestive enzyme evaluation. An increase in the rate of food ingestion was detected daily during the experiment. An average of 16.5, 19.4, 20.8, and 20.6 g of food was ingested for the first through fourth days of the experiment. The variance analysis of the ingested feed indicates significant differences among sampling periods ($P < 0.05$). Rates of ingestion were characterized by three periods: maximum between 2000 to 2200 hours, medium between 2400 and 0400 hours, and low between 0600 to 1800 hours (Table 1). The percentage of ingested feed between 2000 and 2200 hours was 30% of the total daily amount. Shrimp consumed approximately 6% of their body weight in 24 hours (0.3 g).

TABLE 1. Average separation test (Duncan) and homogeneous groups related to food intake of shrimp (g/hour) at different times. Means followed by a different letter are significantly different ($P < 0.05$).

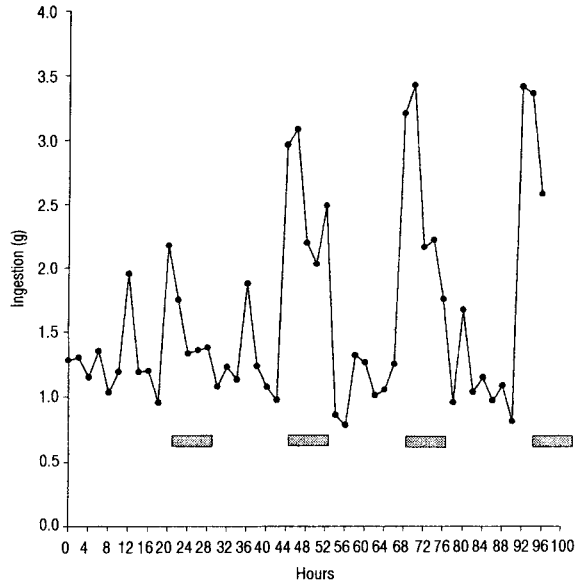
Feeding time	Ingestion (g/hour)	Percentage of daily consumption
0200	2.01 ± 0.13b	9.0
0400	2.02 ± 0.06b	9.0
0600	1.26 ± 0.47a	5.6
0800	1.40 ± 0.11a	6.3
1000	1.38 ± 0.38a	6.2
1200	1.62 ± 0.43ab	7.3
1400	1.30 ± 0.22a	5.8
1600	1.29 ± 0.59a	5.8
1800	1.20 ± 0.51a	5.4
2000	3.44 ± 0.78c	15.4
2200	3.35 ± 0.54c	15.0
2400	2.06 ± 0.49b	9.2

Therefore, the food supplied could be planned in terms of shrimp average weight, number of organisms, and rate of ingestion.

The sequence of the measurements over the four-day experiment was plotted. Several peaks of ingestion were detected during the 96-hour study (Figure 1). There was an increase of ingestion at 1800, a decrease after midnight, and the lowest ingestion occurred during daytime (0400-1800). The peak of the first night of the experiment was low and matched with the amount of food ingested in that day.

The feed ingestion peak between 2000 to 2200 hours can be explained by the nocturnal habits of the shrimp. This behavior is controlled by several factors, including the light cycle. According to our observations, organisms were buried in the bottom sand layer during daytime and became active after dusk. In the kuruma shrimp, *P. japonicus*, food ingestion peaks occur at night, when shrimp become active (Sacayan and Hirata 1986). Cuzon et al. (1982) found a link between behavior and metabolism, supported by a correlation between the period when organisms are active and when digestive enzyme secretion occurs. They also found a better performance when the feeding

FIGURE 1. Average amount of feed ingested by white shrimp.

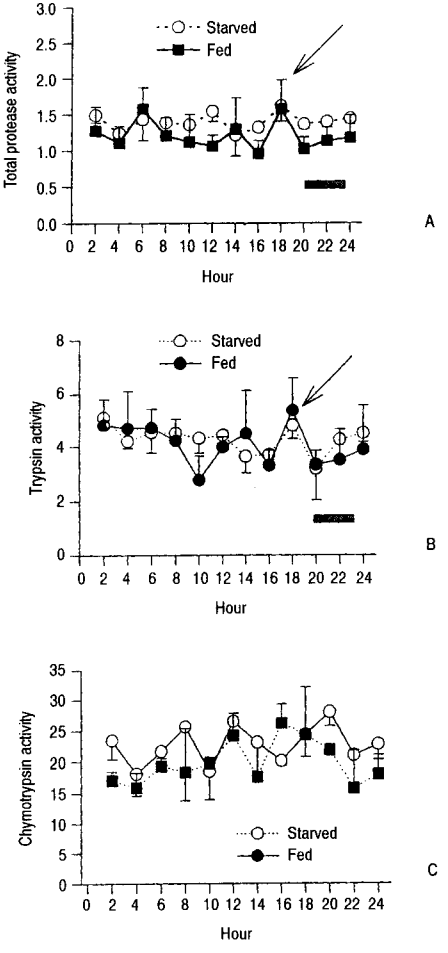


regimen coincided with the period of activity. McTigue and Feller (1989) proposed that inactive organisms in daytime used limited stored energy, and therefore food is transformed into biomass. The diet ingested from 2400 to 0600 hours, before shrimp buried themselves, amounted to 27% of the daily total, which seems to be related to a response directed to storing energy. The food ingested in this period by white shrimp was 34.5%, supported the previous observation. Robertson et al. (1993) found better performance in the same species by diurnal feeding. However, the authors did not give information about food consumption as a function of the feeding regimen. Scura (1995) found that consumption at dawn was 10% lower than the total consumption, and growth of organisms fed after dusk was 60% higher than organisms fed during daytime. Several peneids have feeding habits and increased activity periods coinciding at night (Reymond and Lagardere 1990; Wassenberg and Hill 1993: Seiffert 1997). Even though multiple feedings improved growth and food conversion (Hill and Wassenberg 1987), feeding frequency was limited by pellet stability and the labor involved. A continual monitoring of food ingestion

and biochemical variables will help plan feeding management in cultivated species.

To measure the daily activity of the digestive system and the effect of food ingestion on digestive enzyme activity, total protease (Figure 2a), trypsin (Figure 2b), and chymotrypsin (Figure 2c) activities were eval-

FIGURE 2. Proteolytic activity during 24-hour cycles of white shrimp. Fed and starved organisms were assayed: A. Total protease activity using azocasein as substrate; B. Trypsin activity using BAPNA as substrate; C. Chymotrypsin activity using SAAPFNA as substrate. Units are expressed as specific activity (U/mg protein).



uated in fed and starved organisms. Enzyme activities were plotted starting at 0200 hours of the fifth day of the study. Protease, trypsin, and chymotrypsin activities were detected throughout the day. Because diet protein quality (Ezquerra et al. 1997) and feeding regime (Diaz-Granda 1997) affect the extent of proteinolytic activity, a starved organism group was included in the study as a control. No significant difference ($P < 0.05$) was observed when comparing starved and fed organisms for enzymatic activities. The maximum protease and trypsin activities were found at 1800 hours (1.57 and 5.39 units). The differences between the preceding sample (1600) and before the increase of ingestion (2000) were significant. The reduction of these trypsin activities showed that it is possible that trypsin regulation occurred before the highest food ingestion. An increase in proteinolytic activity was also found in the Caribbean shrimp, *P. schmitti,* at the same period (Diaz-Granda 1997). This shrimp had several protease activity peaks during the day. The increase in enzyme activity, just before the increase in ingested feed in white shrimp and other peneids, regardless of the feeding regimen, suggests an internal regulation of the digestive system. An increase in available protease activities before the peak of feed ingestion indicates a close correlation between feeding behavior and regulation of the digestive system.

Ezquerra et al. (1997) also found an effect on trypsin activity, but not on chymotrypsin, when different diets were used to feed shrimp. Accordingly, trypsin activity seems to be a good biochemical marker of digestive system status. Because proteases are synthesized as inactive precursors or zymogens (Sellos and Van Wormhoudt 1992), it is important to determine when the enzymes are synthesized and when and how they are activated. The processes involved in their expression and control, from the gene to the midgut tract, remain unresolved. Further studies in the expression of digestive enzymes and their inhibitors will help understand how digestion in shrimp is regulated.

The maximum ingestion of white shrimp during the night (2000-2400) and before dawn time (0200-0400) should aim aquaculturists towards adapting their feeding regimens. The proteolytic data, besides the correlation with biochemistry and behavior, gave important clues for a better food conversion. Experimental design on shrimp nutrition must consider the feeding preferences and activity of proteases in these organisms.

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