Degradation analysis of microencapsulated diet in pacu (*Piaractus mesopotamicus* Holmberg, 1887) larvae intestine through scanning electron microscopy (SEM)

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ABSTRACT. This research analyzed the microencapsulated diet degradation in pacu (*Piaractus mesopotamicus*) larvae intestine. The pacu larvae received the following feeding treatments: AMD- larvae fed initially *Artemia* nauplii for six days, followed by microencapsulated diet; C_6MD- larvae fed initially *Artemia* for six days, followed by six days of co-feeding and the rest of the experiment (8 days) with microencapsulated diet; C_9MD- larvae fed initially *Artemia* for six days, followed by nine days of co-feeding and the rest of the experiment (5 days) with microencapsulated diet. The pacu digestive tract contents were removed, processed and analyzed under scanning electronic microscopy. Diets from AMD larvae treatment showed few degradation areas, when compared to original dry diets. On the other hand, diets removed during co-feeding period showed the highest degradation areas. The results suggest a positive effect of *Artemia* on diet degradation.

Key words: fish larvae, diet degradation, microencapsulated diet, co-feeding, scanning electron microscopy.

RESUMO. Análise da degradação de dieta microencapsulada por larvas de pacu (*Piaractus mesopotamicus*, Holmberg, 1887) através de microscopia eletrônica de varredura. Este trabalho teve como objetivo analisar o grau de degradabilidade de dietas microencapsuladas por larvas de pacu (*Piaractus mesopotamicus*), através da microscopia eletrônica de varredura. Os seguintes tratamentos alimentares foram testados: AMD - larvas alimentadas por 6 dias, com náuplios de *Artemia*, com transição brusca para dieta microencapsulada; C_6MD - alimentação inicial com *Artemia* por 6 dias, 6 dias de co-alimentação e o restante do tempo (8 dias) somente com dieta microencapsulada; e C_9MD - idêntico ao tratamento anterior, porém, com 9 dias de co-alimentação. O conteúdo presente no trato digestório das larvas foi coletado e processado para análise em microscópio eletrônico de varredura. Os grânulos provenientes das larvas do tratamento de transição brusca (AMD) possuíam poucas áreas de degradação, semelhantes às dietas secas. Já as dietas coletadas das larvas durante o período de co-alimentação possuíam uma maior área degradada. Os resultados sugerem uma influência dos náuplios de *Artemia* sobre a degradação das dietas microencapsuladas.

Palavras-chave: larva de peixe, degradação, dieta microencapsulada, co-alimentação, microscopia eletrônica de varredura.

Introduction

Nutrient digestion, absorption and assimilation studies on fish larvae fed with artificial diets are limited, due to several factors, such as the small larvae's size when they start exogenous feeding, the need of preparing a diet consisting of small particles, and the low acceptance of artificial diets by the larvae. Thus, low and variable ingestion rates are obtained in feeding experiments (Ronnestad *et al*., 2001). Moreover, the lack of knowledge about size, texture, atractivity and nutrients leaching, together with failure in administration methods, due to the limited understanding of feeding physiology, nutritional requirements and especially of larval behavior in the presence of inert food (Fernández-Díaz *et al*., 1994), raises difficulties when adopting artificial diets.

Up to now, no artificial diets that can sustain the same growth and survival rates observed in larvae fed with live prey were developed. According to Dabrowski (1984), the determination of larval and juvenile nutritional requirements by the traditional methods such as standard diets, semi-purified or

even by commercial diets, represents a difficult task, since those preparations generally do not support larval growth. Hence, works conducted with larval fish nutrition have been based on yolk sack components utilization, estimation of the essential fatty acids and some vitamins that can be easily incorporated by live organisms (Watanabe and Kiron, 1994) and the imitation of live organisms' composition (*Artemia* or rotifers).

Artificial diet introduction in larval rearing has been more effective when furnished together with a live prey period (Walford and Lam, 1987; Martinez-Tapia *et al*., 1990) known as co-feeding. Kolkovski *et al*. (1997) suggest that *Artemia* basically influences the ingestion, digestion and assimilation of microencapsulated diet during co-feeding by two manners. The first could be a remote influence through chemical stimuli, when the free amino acids released from live organisms activate larval chemical receptors stimulating appetite and orienting larvae to prey. The second could be a direct influence of *Artemia* biochemical composition in the digestion and assimilation of the nutrients process by larvae.

Experiments dealing with degradation of microdiets by larvae are scarce. Fernández-Díaz and Yúfera (1995) verified that soft microdiets dispersed in gelatin were easily degraded, compared to hard diets dispersed in alcohol. However, there is still a need for information and techniques to determine diets degradation in fish larvae digestive tract. The knowledge about microencapsulated diet degradation capacity by larval fish inside their digestive tract is of great importance for understanding the larval digestion process, producing bases for future development of artificial diets that can replace live prey (Jones *et al*., 1993).

The aim of this work was to study the degradation of microencapsulated diet by pacu larvae intestine, through SEM, which could be used as a food digestion index and to evaluate the possible influence of live organisms in diet degradation.

Material and methods

Pacu (*P. mesopotamicus*) larvae from induced spawning were obtained from the Hydrobiology Station of *Furnas* (state of *Minas Gerais*, Brazil). Before complete yolk exhaustion, 4-days-old larvae were individually counted and distributed into experimental tanks at a density of 10 larvae/L. The experiment was carried out in polyethylene tanks with a 100L volume of well water in a flow through system, continuous aeration and natural photoperiod.

A commercial microencapsulated diet "Hatchfry Encapsulon" from ARGENT Labs, USA (containing 50% protein, 12% lipids, 3% fiber, 6% moisture, 7% mineral matter, and particle size between 150μm and 250μm) and *Artemia* nauplii were used according to the following treatments:

- AMD: *Artemia* for 6 days, weaned to microencapsulated diet;
- C_6MD: *Artemia* for 6 days, 6 days of co-feeding (*Artemia* + microencapsulated diet), and the last 8 days fed exclusively on microencapsulated diet;
- C_9MD: *Artemia* for 6 days, 9 days of co-feeding (*Artemia* + microencapsulated diet), and the last 5 days fed exclusively on microencapsulated diet.

The introduction of the microencapsulated diet in co-feeding (C_6MD, C_9MD) and in the AMD treatments occurred on day 7. In the first treatments, *Artemia* amounts were gradually reduced during co-feeding. In all treatments, the larvae were fed four times a day, and during the co-feeding period, the microencapsulated diet was offered *ad libitum* 10 minutes before the live diet.

Larvae were sampled for microencapsulated diet degradation every three days during the co-feeding period and every four days when they were receiving only artificial diet. The diet degradation analyses were carried out utilizing 10 larvae from each treatment. They were fixed in glutharaldehyde 2,5% (Sigma) in phosphate buffer (pH 7.2). Afterwards, they were dissected under stereoscopic microscope to remove and collect the digestive tract contents. Since the quantity of food collected was low, the contents of ten larvae were joined, following post-fixation in osmium tetroxide (Sigma) for two hours. After this, each sample underwent crescent alcoholic dehydration and drying in critical point dryer (Tousimis model Sandri 80A). After that, the samples were coated with a gold layer using a Jeol model JFC 1100 coating unit and scanned in electron microscope (Jeol JSM model 5410) at 75 Kv, where they were electronmicrographied.

Electronmicrographies of dry and hydrated microencapsulated diet granules were taken in order to compare with those sampled in the pacu larvae digestive tract.

Results and discussion

To our knowledge, few articles have been found in literature showing microencapsulated diet degradation. Fernández-Díaz and Yúfera (1995) observed microdiets expelled from *Sparus aurata* larvae digestive tract and concluded that diets prepared with gelatin were easily degraded by larvae compared to another prepared with alcohol in its

final processing. Chu and Ozkizilcik (1999) observed under the microscope the digestive tract of anesthetized *Morone saxatilis* larvae and reported that microdiets digestion was slow, because a reduction in the food aggregation was observed only after three hours. Due to the low transparency of pacu larvae digestive tract, allied to the difficulty in distinction between *Artemia* and microencapsulated diet (both were dark orange) this method was not used, although it would reduce the analytical costs and allow larvae survival.

The analysis of the digestive tract contents of pacu larvae during co-feeding, revealed a small quantity of microencapsulated diet granules and a large quantity of *Artemia*, which made it impossible to observe the degradation index as proposed by Fernández-Díaz and Yúfera (1995). However, even with low incidence, some observations were conducted.

The microencapsulated diet granules ingested by larvae of AMD treatment showed the same aspect of the dry diet granules (Figure 1A), without major degradation areas, characterized by the loss of the covering pellicle and sizes similar to the manufacturer specifications (150 to 250μm) (Figure 1B). On the other hand, diets collected during the co-feeding period, deriving from C_6MD and C_9MD treatments showed greater degradation areas (Figures 1C and 1D). However, after the suppression of *Artemia* in these treatments, when only microencapsulated diet was furnished, the granules degradation areas diminished (Figures 1E and 1F), when compared to anterior samples during co-feeding period, which could indicate an *Artemia* influence in the digestion breakdown of microencapsulated diet, as suggested by Kolkovski *et al*. (1997). Walford *et al*. (1991) working with *Lates calcarifer* stated that co-fed (microdiets and rotifers) larvae did not possess undigested diets in the digestive tract, while others larvae, fed exclusively with microdiets, showed only intact microcapsules in their intestine, even expelling them intact. Koven *et al*. (2001) pointed out that, although a direct and significant contribution of enzymes by the live food to larval digestion has not been demonstrated, ingested zooplankters may instead be releasing post-prandial factors that stimulate enzyme secretion. Microdiets may contain proteins and others ingredients that are difficult for fish larvae digest as well anti-nutritional factors (Lindner *et al.*, 1995 *apud* Kolkovski, 2001).

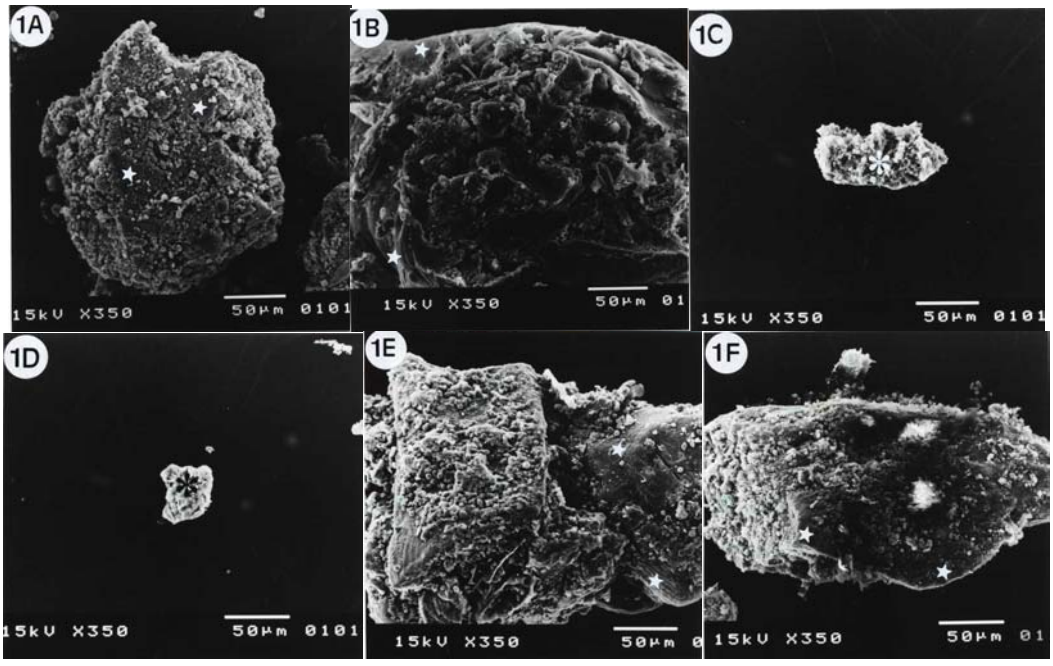

Figure 1A. Original dry microencapsulated diet, with non-degraded areas (ι), 350X. **1B.** Microencapsulated diet derived from AMD larvae treatment, with non-degraded areas (ι), 350X. **1C.** Microencapsulated diet derived from C_6MD larvae treatment, during co-feeding period, with degraded areas (~), 350X. **1D.** Microencapsulated diet derived from C_9MD larvae treatment, during co-feeding period, with degraded areas (~), 350X. **1E.** Microencapsulated diet derived from C_6MD larvae treatment, exclusively fed microencapsulated diet, with non-degraded areas (ι), 350X. **1F.** Microencapsulated diet derived from C_9MD larvae treatment, exclusively fed microencapsulated diet, with non-degraded areas (ι), 350X.

Due to the low amount of inert particles in the pacu larvae digestive tract contents, the observations about diet degradation were impaired. In the Fernández-Díaz and Yúfera (1995) experiment, diets made with protein cross link possessed regular size and shape (spherical), characteristics that contributed in the determination of diets degradation classes. The diets used in this experiment possessed irregular size (150 to 250μm) and shape, factors that increased the difficulties in producing a more precise analysis. However, this work could be used as a base for future investigations about diet administration, ingestion and degradation by native larvae fish.

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