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Effect of live food enriched with the probiotic Spomune©, on the survival and growth of *Cambarellus montezumae* (Saussure, 1857) under controlled conditions

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Abstract

For the present investigation, the effect of a live food enriched with a probiotic was evaluated in 120 juvenile dregs of *Cambarellus montezumae* species with an average size and weight of 21 ± 2 (mm) and 0.490 ± 0.130 (g) respectively. Throughout the investigation, the organisms were kept in six translucent squared aquariums with 40 L of fresh water, at a temperature of 24 ± 1 °C, with a natural photoperiod and with 20 bowls each. The specimens were fed for 24 weeks with *Tubifex tubifex* slimeworm inoculated with 1×10^7 CFU/g with the probiotic Spomune© (*Bacillus subtilis* and *Clostridium butyricum*). To verify the effect of the probiotic on the organisms, survival, growth (height and weight) as well as their degree of well-being were determined. The results show that the specimens fed with the enriched diet have a 95% survival compared to those fed with the unenriched diet was 48%. On the other hand, the growth showed significant differences between the two treatments ($p < 0.003$) since the weight at the end of the experiment was 3.263 and 1.519 g with and without the incorporation of the probiotic in the food, respectively. In addition to this, the organisms fed without the probiotic presented a weight gain of only 0.774 g; while those fed the probiotic was 2.773 g. This knowledge generated allows to propose strategies for the conservation of the species, in the same way with this research the biological bases for a better cultivation in extensive, semi-intensive and intensive systems, under controlled conditions can be laid. This would reduce the pressure on this resource in its natural habitat, giving way to the recovery of natural populations and managing to meet the demand that this organism has at the local level and for aquarium purposes as live food.

Keywords: *C. montezumae*, Spomune© probiotic, survival, growth, aquarium hobby

1. Introduction

Cambarellus montezumae (acocil) is a native and endemic species of the central plateau of Mexico, encompassing Lake Chapala (Jalisco), the crater Lakes of Puebla, Lake Xochimilco (Mexico City), the Guadalupe Victoria dam (State de México), Lake Guadalupe and remnants of the old Lakes of Texcoco and Chapultepec [1-5]. This organism has a great ecological, cultural and commercial value, because it is a food rich in protein, nutritious and even low cost [6-9], therefore it has a demand in the local market, due to its appreciation in Mexican gastronomy (it should be noted that this species has been consumed from pre-Hispanic times to the present by rural ethnic populations); In Europe and the United States, it is consumed as a luxury dish since it is received with great acceptance by high-income populations, on the other hand, acocil is part of the diet of other aquatic organisms in the region that is used as live food in the activity of the aquarium hobby [10]. Unfortunately, in recent decades it has been observed that, due to various factors such as overexploitation, the decrease in the volume and extraction of water for different uses, together with pollution by sewage, pesticides and finally the introduction of exotic species (*Oreochromis niloticus*, *O. aureus*, *Cyprinus carpio* and *Micropterus salmoides*), in their natural habitat their natural populations have decreased [3, 5]. Over the years it has been seen that aquaculture has been the most viable alternative to carry out its cultivation under controlled conditions and thus allow the recovery of wild populations. Currently there are various studies about their morphometric aspects, growth aspects, density, distribution, morphology, predator-prey relationship, eating habits in their natural habitat, as

well as the formulation of plant-based diets. However, the knowledge of their eating habits of these species have diminished in recent years [1, 10-15], in addition to the lack of a commercial balanced food formulated specifically for this specimen that meets their requirements, complicates the development of its cultivation in captivity, so it is very important to look for new alternatives. Research on the nutritional requirements of crustaceans is relatively recent, and no definitive solutions have yet been reached that can solve the problems posed by commercial exploitation [9]. It is frequently observed that the foods used do not contain the nutrients that each of the species require for their optimal growth, mainly in their first stages of life, which is the most critical in all species, since it is where the increased mortality [16]. Regarding the feeding in captivity of these specimens, some researchers have used different types of food for reproduction such as *Artemia franciscana*, fresh charal, carrot and lettuce, regarding the growth of the juveniles they implemented plant detritus, *Tubifex tubifex* and *Daphnia* sp. obtaining good results, due to the high content of proteins, lipids and the carbon-nitrogen ratio necessary for reproduction and shedding of the acocil exoskeleton [17-19]. Despite this, the results have not been satisfactory to carry out its exploitation in semi-intensive crops. In recent years worldwide, biotechnical development cultures have been implemented in live and inert foods enriched with prebiotics, probiotics and symbiotics (extracted from the digestive tract of different aquatic organisms or for commercial use), since it has been shown to be favorable in aquaculture activity, stimulating organisms to have longer survivals, accelerating their growth, strengthening their immune system against some pathogens and improving their reproduction [20, 21, 22, 23]. Therefore, the objective of the present investigation was to evaluate the effect of live food enriched with a probiotic on the survival and growth, height and weight of *C. montezumae*.

2. Materials and Methods

2.1 Obtaining of organisms

For the present investigation, 120 juvenile dregs of the *Cambarellus montezumae* species were obtained with an average size and weight of 21 ± 2 (mm) and 0.490 ± 0.130 (g) respectively, through a sampling carried out in the channel of Cuemanco del Lago de Xochimilco, in Mexico City. The organisms were captured with a triangular spoon net with a 1 mm mesh opening, the specimens were placed in 10 L capacity vats, with 8 L of water of the same medium, at the end of the capture, these were protected and transported to the lab. Upon arrival at the facilities, the specimens were fed and acclimatized for seven days in order to avoid handling stress.

2.2 Experimental design

In the laboratory, six translucent square aquariums of 60 L capacity were prepared with 40 L of fresh water, at a temperature of 24 ± 1 °C, with a natural photoperiod and with 20 bowls each. The specimens were fed daily for 24 weeks with the *Tubifex tubifex* slimeworm inoculated with 1×10^7 CFU/g with the probiotic Spomune© (*Bacillus subtilis* and *Clostridium butyricum*). The treatments were distributed as follows: vat 1 was assigned as a control, in which the organisms were fed unenriched *T. tubifex*; vat 2 was provided with *T. tubifex* enriched with 0.5 g of probiotic Spomune©. It is worth mentioning that each treatment had its three corresponding replicas. In all cases, the feed rate supplied daily represented 5% of the total biomass of the treatment.

For the protection of the couplings, 15 PVC pipes of 2.5 cm diameter and 5 cm long were placed. Food remains and feces were removed from the tubs on a daily basis, likewise 50% water changes were made every week and a total change every 15 days, in order to maintain water quality. On the other hand, the physicochemical parameters (temperature, pH, nitrite and ammonium) were monitored using the API water quality tests.

2.3 Record of morphometric data and survival

With the aim of evaluating the effect of the probiotic and minimizing stress in the couplings, the recording of the sizes and weights of the organisms that make up each treatment was averaged every 4 weeks from the beginning of the investigation to the end and with it adjusted the proportion of food that was offered daily. The morphometric parameters taken from each specimen were the total length (L_T), with a modified plastic ichthyometer of $30 \text{ cm} \pm 0.01$ precision and the weight in (g) using an Ohaus brand digital analytical balance with a precision of ± 0.001 , eliminating the extra weight of water by gently drying the body with a clean cloth. Survival was recorded daily for later analysis.

2.3 Data Analysis

For each treatment, the specific growth rate was calculated using the formula proposed by Ricker (1979) [24].

$$TEC (\%/day) = \frac{(\ln Y2 - \ln Y1)x^2}{T} x 100$$

Where:

TEC= Specific rate of growth

ln Y1= Natural logarithm of weight and total length at the beginning of the experimental period

ln Y2= Natural logarithm of weight and total length at the end of the experimental period

T= Time in days of duration of the experiment

To evaluate the effect of the probiotic on the growth of the acociles, the multiple condition factor was calculated, that is, their degree of well-being, with the formula proposed by (Pokniak *et al.*, 2004) [25].

$$FC = \left(\frac{P}{LT^3} \right) x 100$$

Where:

FC= Multiple condition factor

P= weight and total length at the beginning of the experimental period

LT= total length

Weight gain was determined with the formula used by (Amisah *et al.*, 2009; Vinchira *et al.*, 2014) [26, 27].

$$GP = \left(\frac{Pf - Pi}{T} \right)$$

Where:

GP= Weight gain

Pf= Weight in grams at the end of the period

Pi= Weight in grams at the beginning of the period

T= Time in days of the period

Finally, survival was determined for each of the treatments using the formula proposed by (Uribe and Luna, 2003) [28].

$$\% \text{ Survival} = \frac{\text{Number of organisms at the end of the experiment}}{\text{Number of organisms at the start of the experiment}} \times 100$$

2.4 Statistical Analysis

To determine the average value and standard deviation of the data generated in the different variables considered, a database was made with Microsoft Excel 2020 Software (Microsoft Corp., Washington, USA).

To determine significant differences between the two study treatments, a two-way analysis of variance (ANOVA) was performed. When finding significant differences ($p < 0.05$), the multiple means comparison technique was applied by the Tukey method. For the analysis of variance test, as well as the Tukey technique, the statistical program Systat 12 (Systat Software Inc., California, USA) was used.

3. Results and Discussion

3.1 Survival

The survival of *C. montezumae* in the two treatments studied is presented in Fig. 1, which shows that in the first 4 weeks in both treatments it was maintained with 100%, however, as the study time passed with the non-enriched food the organisms had a lower survival, having at the end 48% in contrast with the enriched food which was 95% in a period of 24 weeks. These results are very similar compared to other studies, where researchers designed and provided an artificial diet based on plants and incorporating a lactobacillus extracted from the digestive tract of angelfish (*Pterophyllum scalare*), obtaining a survival of 90% with the enriched diet, while the organisms fed the unenriched diet were 60% [20]. Similarly, other research shows that by combining a live food without

further enriching an inert food, survival with the same species can be maintained above 75% [29]. These variations could be due to the fact that *Bacillus* sp. used as probiotics, they adhere adequately to the digestive tracts of fish and crustaceans and these cause the organisms to have lower mortality rates [30]. Regarding the use of lactobacilli and bacilli in aquaculture activity, it allows the survival of different species of aquatic organisms to remain above 90% [31]. Various authors [32-34] mention that survival is the precise recording or estimation of the levels of living organisms in cultivation systems, which may be feasible for calculating the daily ration, estimating biomass, production and the state of cultivation in terms of profitability, so providing a live food enriched with a probiotic increases the survival of the organisms and thus the profitability of the crop. Some authors mention that bacteria used as probiotics actually suffer a competitive exclusion in the digestive tract of the host, either by food or by space, so the inclusion of beneficial bacteria in diets for aquatic animals can have very varied effects [34, 35]. The application and incorporation of probiotics is suggested as a preventive measure for diseases of aquatic organisms [23] and can increase the growth rate, either in size or weight, a greater increase in the immune response and helps to improve survival when a disease occurs [36, 37]. With the aforementioned, it can be corroborated in this research that the best survivals with the acociles were with the food enriched with the probiotic Spomune©. Regarding the statistical analysis, ANDEVA pointed out that there are significant differences between the food enriched with the probiotic and the food without enrichment ($p < 0.003$).

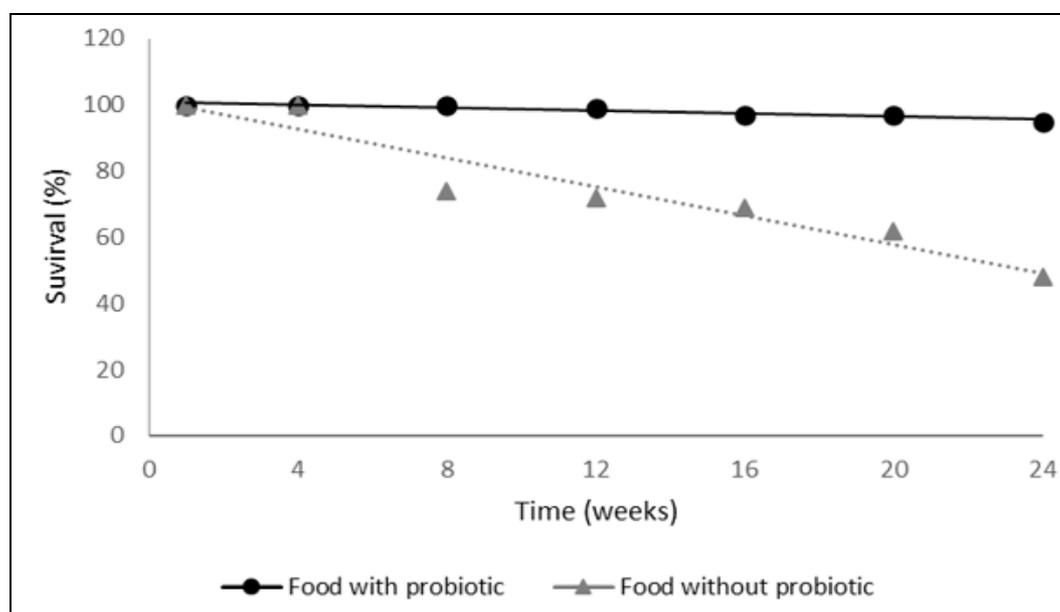


Fig 1: Survival of *Cambarellus montezumae* with respect to time (weeks) with the food enriched with the probiotic and without enriching the food.

Growth (weight and height)

The average values (\pm SD) of the weight and height of *C. montezumae* during the 24 weeks of study are found in Table 1. In general, it is observed that in the food enriched with the probiotic its weight was higher compared to the unenriched food, since at the end of the investigation these organisms obtained a weight of 3.263 and 1.519 (g) respectively. In all forms of animal cultivation, it is sought to provide a sufficient amount of nutrients to cover the growth requirements of the

organisms and whether to reach an efficient point of production and therefore maximize the economic benefit generated by this activity. In terms of the ingestion mechanism of the food, it depends on the way it is supplied. In the case of crustaceans, which generally handle food with their cheipeds, it is necessary that the food be of an appropriate size for the size of the organisms in question [38]. In agreement with other authors [29, 39] they report that by providing *Daphnia pulex* plus a balanced food with 35%

protein, their weight of the couches is 0.858 g in 6 months of experiment, these values are below what was obtained in this studio. Some researchers [40, 41] mention that in the fattening stage the recommended protein values range between 20 and 45%, although there are studies that record a lower efficiency in the assimilation of the protein if it is added in amounts greater than 36%. However, this did not occur in the present investigation since the greater the amount of protein, the greater the growth, and the more if a probiotic was incorporated, it should be noted that the protein percentage of *T. tubifex* is 63.45% [42]. In addition to this, it is important to mention that in the juvenile state, the acociles prefer to consume animal protein and thus present a greater growth in weight without leaving aside the positive effect that the probiotic has on aquatic organisms [4]. On the other hand [20],

when designing an inert plant-based diet and incorporating a lactobacillus, its weight in 180 days was 3.167 g, results similar to the present study. This may be due to the fact that the eating habits that *C. montezumae* presents in its different life stages are detritivores, herbivores, opportunists and omnivores without forgetting that its behavior is cannibalism [43, 44, 45, 46]. The organisms fed without the probiotic presented a weight gain of only 0.774 g; while those fed with the probiotic was 2.773 g (Fig. 2). Due to this, the preparation and quality of this diet was adequate to observe the growth in weight. Regarding the statistical analysis, ANDEVA indicated that there are significant differences between the weight of the organisms fed with the diet enriched with the probiotic and the unenriched food ($p < 0.003$).

Table 1: Average values of length and weight in (mm) and (g) respectively of *Cambarellus montezumae* with respect to time (weeks) with the food enriched with the probiotic and without enriching the food. Similar letters in rows do not show significant differences ($P < 0.05$).

Week	Size		Weight	
	Food with probiotic	Food without probiotic	Food with probiotic	Food without probiotic
0	21 ^a	21 ^a	0.490 ^a	0.490 ^a
D.S.	± 2	± 2	± 0.13	± 0.11
4	27 ^a	23 ^b	0.682 ^a	0.546 ^b
D.S.	± 3	± 1	± 0.132	± 0.11
8	30 ^a	25 ^b	1.203 ^a	0.740 ^b
D.S.	± 2	± 2	± 0.147	± 0.121
12	32 ^a	27 ^b	1.843 ^a	0.939 ^b
D.S.	± 1	± 1	± 0.158	± 0.135
16	36 ^a	28 ^b	2.342 ^a	1.031 ^b
D.S.	± 2	± 1	± 0.148	± 0.124
20	39 ^a	30 ^b	2.809 ^a	1.297 ^b
D.S.	± 2	± 1	± 0.142	± 0.131
24	42 ^a	32 ^b	3.263 ^a	1.519 ^b
D.S.	± 3	± 1	± 0.158	± 0.141

Regarding the growth in size of the acociles, the highest values were obtained with the food enriched with the probiotic, measuring 42 mm in contrast to the specimens fed the diet without enriching 32 mm at the end of the investigation; However, other studies have found greater growth in height when they are fed inert shrimp-based diets, since this type of food is rich in protein and thus stimulates them to have greater growth [2, 29, 47]. Some authors [2, 48] have recorded a maximum size of Xochimilco's *C. montezumae* of 45 mm and an average growth rate of 0.25 cm/month; In the present study, the size is below this value, probably due to the fact that in its natural habitat, the acocil has a greater availability and variability of live food available in contrast when the specimens are kept in captivity and therefore not provide the necessary nutritional requirements. On the other hand, we must not forget that one of the most important resources in aquaculture is food and nutrition, since aquatic organisms obtain all the necessary energy from the transformation of food to maintain their functions and increase of biomass [49]. When the amount of food ingested meets the needs required for maintenance, there is an increase in the dimensions of the organisms (length, volume); what is called growth [50]. One of the food that has been easily studied and adapted for these organisms is the balanced one based on shrimp and which has allowed the acociles to maintain a good state of health, growth, survival and adequate reproduction [29]. However, the results obtained by various authors indicate that food quality is also a limiting factor for optimal growth of *C. montezumae*. Although the consumption of plant material is common, the piglets prefer animal protein, especially in the

early stages, as already mentioned [3, 20, 29]. Although it has been found that under controlled conditions these organisms accept any type of food and present a high digestibility towards a wide variety of materials, it has been reported that there is an 88% to 96% acceptance for balanced diets [29, 51]. During the growth stage, proteins are used by organisms to increase muscle mass, so they are used metabolically in structural and enzymatic functions, in the formation of hormones and in the repair of muscle tissue [52]. This occurs especially in omnivorous and carnivorous organisms, as is the case of acociles, because they obtain the greatest amount of their energy from amino acids in ingested food [53]. Some studies indicate that the preference and acceptance of the type of food, living or inert, by organisms and its relationship with growth and survival largely depends on their stage of development and eating habits [54, 55]. This is also confirmed in the present work where the greatest growth in both height and weight and survival was with the diet enriched with the probiotic. Due to this, the preparation and quality of this diet was adequate to observe growth, but the most important thing is to verify that adding different types of probiotics in the diet of different aquatic organisms accelerates the growth rate. According to the above, the inclusion of beneficial bacteria in diets for aquatic animals can have very varied effects with the last consequence of an improvement in the health and/or growth of the animals, although the latter may be in part a consequence of the first [56, 57]. Regarding the statistical analysis, ANDEVA indicated that there are significant differences between the size of the organisms ($p < 0.002$).

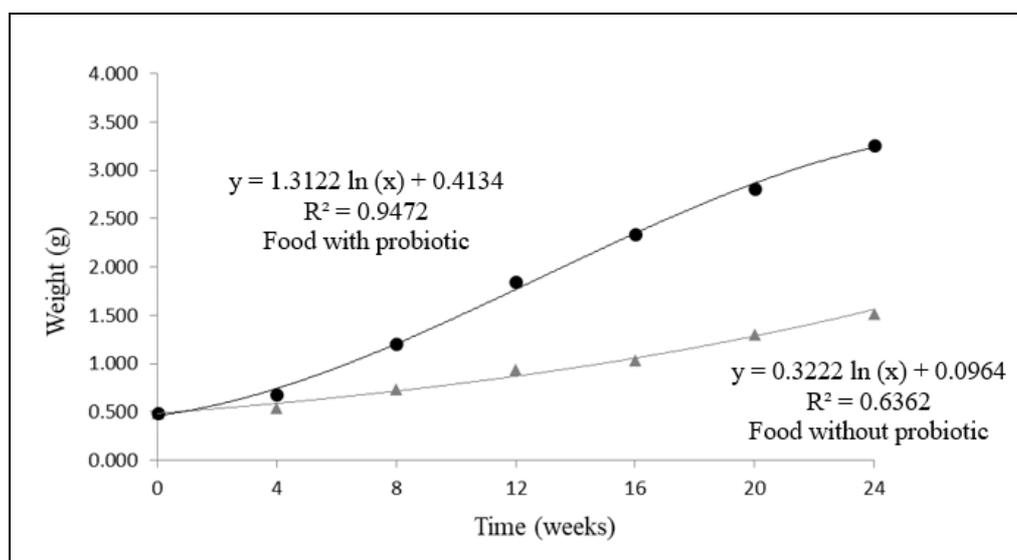


Fig 2: Values of the growth curve with respect to the weight of *Cambarellus montezumae* fed with the diets under study.

Regarding the condition factor (KM) and the absolute growth rate of the acociles with respect to height and weight, they indicate that the organisms fed with the diet enriched with the probiotic Spomune© was superior, contrary to what happened with the unenriched food since its (KM) remained below the

optimum, indicating that the diet does not supply the necessary requirements so that the height correspond to the weight of the animals and with this smaller and low-weight organisms are obtained ^[58] Fig. 3.

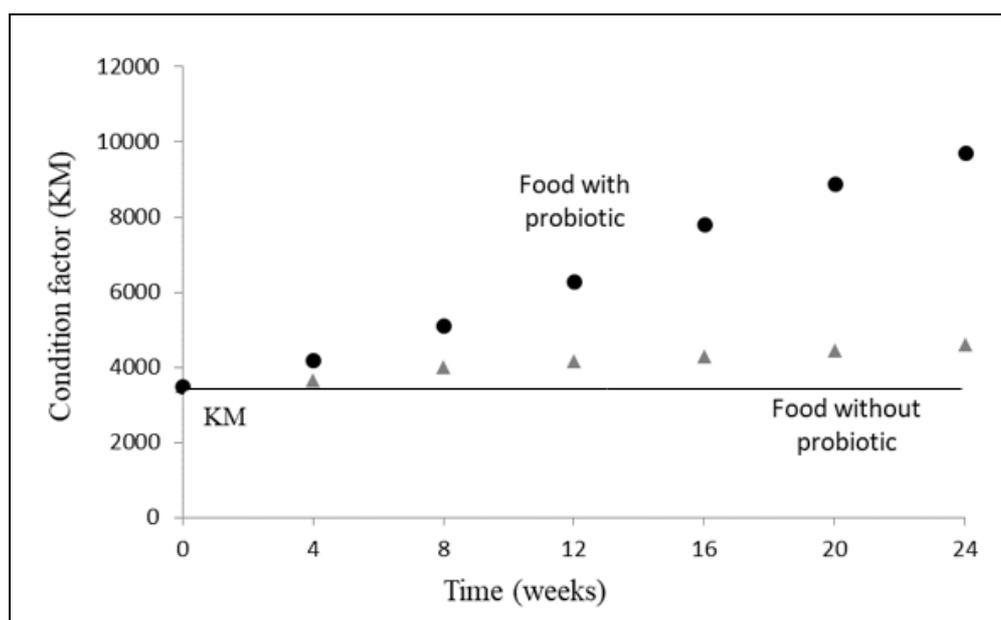


Fig 3: Values of the condition factor (KM) of *Cambarellus montezumae* fed with the diets under study.

4. Conclusion

For everything described in this research, it can be ensured that incorporating a prebiotic, probiotic or symbiotic in the feeding of aquatic organisms plays a very important role since it will have a positive effect, improving and increasing survival, growth (size and weight), reproduction and their immune response. As could be observed in the present study, where survival was 95% with organisms fed the probiotic in contrast to organisms that did not have a probiotic in their diet, it was 48% in a time of 24 weeks. Together, their growth was 42 and 32 (mm) with the organisms with the enriched and unenriched diet, respectively. This knowledge generated allows to propose strategies for the conservation of the species, in the same way with this research the biological bases for a better cultivation in extensive, semi-intensive and

intensive systems, under controlled conditions can be laid. This would reduce the pressure on this resource in its natural habitat, giving way to the recovery of natural populations and managing to meet the demand that this organism has at the local level and for aquarium purposes as live food.

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