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Influences of Probiotic Bacilli on Ammonia and Urea Excretion in Two Conditions of Starvation and Satiation in Persian Sturgeon (*Acipenser persicus*) Larvae

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Abstract: This study (at 4 weeks) was carried out to evaluate influences of probiotic Bacilli on ammonia and urea excretion in Persian sturgeon (*Acipenser persicus*) larvae. *Bacillus* bacteria (*B. licheniformis, B. subtilis* and *B. circulans*) (three species in a commercial preparation, Protexin Aquatic) were bioencapsulated within *Daphnia magna* at three concentrations by holding the Daphnia in suspensions of 1×10^7, 2×10^7or 3×10^7 bacteria per milliliter for 10 hours (T1, T2 and T3, respectively). The sturgeon larvae in experimental treatments were fed one of the three probiotic treatments at a level of 30 percent body weight 5 times a day but larvae in control treatment (C) fed by nubioencapsulated Daphnia. The ammonia and urea excretion in Persian sturgeon (*Acipenser persicus*) larvae fed by Daphnia enriched with protbiotic were compared to those larvae fed a control treatment of unbioencapsulated *Daphnia magna*. The results showed that ammonia and urea excretion in both two condition, in experimental treatments had significantly decreased in comparison to control treatment ($P<0.05$). Lower amount of ammonia and urea excretion in both two condition observed in treatment T2. Maximum amount of ammonia and urea in both two condition observed in control treatment. Also, there is significant different about ammonia and urea excretion in both two condition between experimental treatment ($P>0.05$). Results indicate that use of probiotics bacilli can reduce amount of ammonia and urea excretion.

Key word: Acipenser Persicus · Probiotic Bacilli · Daphnia Magna · Ammonia and Urea Excretion

INTRODUCTION

The abuse of antimicrobial drugs, pesticides and disinfectants in aquaculture has caused the evolution of resistant strains of bacteria and concern of the society [1,2]. Thus, the use of probiotics in the culture of aquatic organisms is increasing with the demand for more environment-friendly aquaculture practices [3]. Probiotics is the use of microbial supplements to benefit their host [4]. Although application of probiotics in aquaculture seems to be relatively recent [5], the interest in such environment friendly treatments is increasing rapidly. Moriarty [6] proposed to extend the definition of probiotics in aquaculture to microbial "water additives." A growing number of studies have dealt explicitly with probiotics and it is now possible to survey its state of the art, from the empirical use to the scientific approach [7-11]. The potential benefits of probiotics in aquaculture ponds include improvement of water quality with decreasing of ammonia excretion, enhancement of nutrition of host species through the production of supplemental digestive enzymes, lower incidence of diseases and greater survival and improved immune response [12].

The main end-product of protein metabolism in teleosts is ammonia and a significant proportion of nitrogenous waste is also excreted as urea [13]. Consequently, measurements of ammonia and urea excretion have been used as indicators of the effects of various environmental and nutritional factors on protein metabolism and can give an insight into the nitrogen balance of fish [14-17]. Therefore, quantification of

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ammoniaand urea-nitrogen excretion for fish species in relation to nutrition is important for intensive fish culture operations because protein metabolism partly defines the success of a particular nutritional regimen [18,19].

The rate of ammonia excretion increases rapidly in response to feed intake [20-22] and the majority of the nitrogen excreted is derived from deamination of amino acids from dietary proteins [13, 23]. Excretion peaks some hours after feed intake and is mainly dependent upon nitrogen intake, temperature and fish species [24-26]. Used the same diet to show that ammonia excretion patterns were related to nitrogen intake in three species of marine fish and indicated no inter-species difference. Conversely, urea-nitrogen excretion rates were species specific in turbot and gilthead sea bream [27]. Although in early studies, urea-nitrogen excretion was not found to correlate with nitrogen intake in the same way as ammonia-nitrogen excretion [21], several authors have now demonstrated a linear relationship in flatfish [28-31]. The mechanism behind this is not clear but the adaptive significance of urea synthesis in some teleosts appears to be ammonia detoxification during times when ammonia cannot be freely excreted into the environment, such as a high environmental ammonia concentration [32].

There is not information available on ammonia and urea-nitrogen excretion in Persian sturgeon fed by probiotic bacilli. This study aimed to demonstrating the effect of probiotic bacilli on ammonia- and urea-nitrogen excretion in Persian sturgeon larvae.

MATERIALS AND METHODS

The probiotic *Bacillus* was prepared from the commercial product Protexin aquatic (Iran-Nikotak), which is a blend of three *Bacillus* species. The blends of probiotic *Bacillii* (*B. licheniformis, B. subtilis* and *B. circulans*) from suspension of spores with special media were provided. Three concentrations of bacterial suspensions, 1×10^7, 2×10^7 and 3×10^7 bacteries per milliliter (CFU mL^{-1}) were provided by Protexin Co and the colony forming unit (CFU) of probiotic *Bacillii* were tested by microbial culture in Tryptic Soy Agar (TSA).

Daphnia magna were obtained from intensive production ground ponds of the center of sturgeon culture of Marjani (Iran). The *Daphnia magna* at a density of 5 g live *Daphnia* $litter^{-1}$ was held in a broth suspension with *Bacillus circulans*, *Bacillus subtilis* and *Bacillus licheniformis* at densities of 1×10^7, 2×10^7 and 3×10^7 bacteria per milliliter for 10 hours.

This experiment was conducted in a completely randomized design with four treatments (three probiotic levels and a control) and three replicates per treatment for a total of twelve fiberglass tanks (each with a capacity of 40 liters). Larvae of Persian sturgeon (initial weight: 74.9±0.89 mg) were obtained from the center of sturgeon culture of Marjani (Iran). The density of fish larvae in per tank were 71 fish. Persian sturgeon larvae in control and experimental treatments were fed 30 percent of their body weight for 5 times a day (2.00, 7.00, 12.00, 17.00 and 22.00). The control treatment was fed unbioencapsulated *D. magna*. Water quality parameters of input water to rearing system were monitored each week throughout the experimental. The water temperature was 19.46±1.23°C, pH was 7.85±0.26 and water oxygen level was maintained above 7.65±0.55 mg L^{-1} during the experiment an electrical air pump (by a single filtration unit).

Two ration levels were tested in the growth experiment for characterize amount of ammonia and urea excretion: starvation and satiation. This study carried out with three replicates for each treatment (C, T1, T2 and T3) and 10 fish for each replicate. One hundred and twenty *Acipencer percicus* larvae, which had been starved for12h (9:00 to 21:00), were captured, blotted of excess water weighted and then placed into 12 individual experimental tanks at the end of the experimental feeding for 6h. Similar activities were done for satiation. For this purpose, One hundred and twenty fish were captured immediately after feeding and weighted, then placed into 12 tanks for 6h. Aeration was water flow were stoped during the experiment. During this period, water temperature was 19°C and the experiment was conducted at the natural photoperiod conditions with similar light intensity for all tanks. After experiment period, the water of each tanks were sampled and sent to lab for analysis.

One-way ANOVA and Duncan's multiple range tests were used to analyze the significance of the difference among the means of treatments by using the SPSS program.

RESULTS AND DISCUSSION

The amount of ammonia and urea excretion of larvae in both two conditions is presented in Figure 1-4. The results clearly showed that the probiotic bacilli had beneficial effects amount of ammonia and urea excretion of larvae in both two condition. Effects of probiotic treatments on amount of ammonia and urea excretion of Persian sturgeon larvae in both two condition resulted

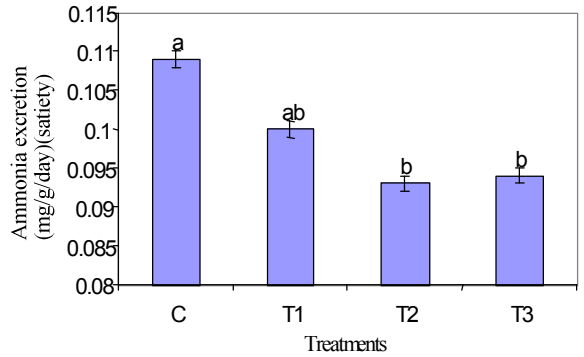

Fig 1: Amount of ammonia excretion in conditions of satiety ($p<0.05$)

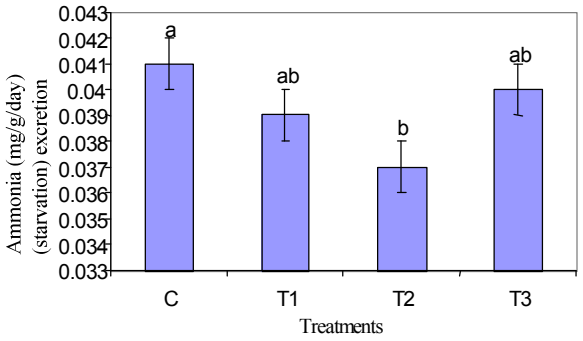

Fig. 3: Amount of ammonia excretion in conditions of starvation ($p<0.05$)

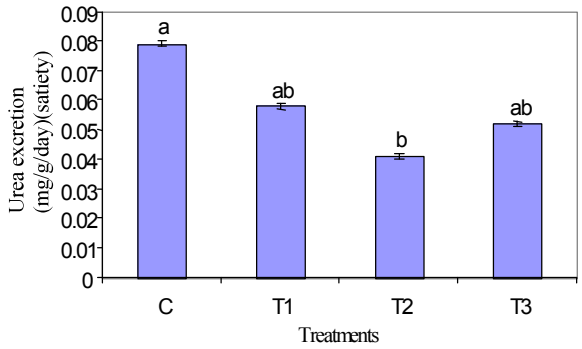

Fig. 2: Amount of urea excretion in conditions of satiety ($p<0.05$)

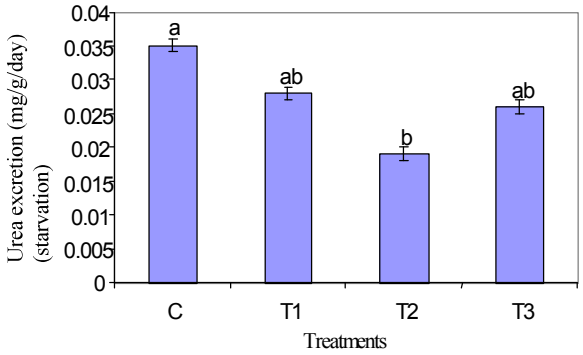

Fig. 4: Amount of urea excretion in conditions of starvation ($p<0.05$)

better than control treatment ($p<0.05$). The rate of urea excretion showed that control larvae secreted higher urea in comparison with experimental treatments ($P<0.05$).

Urea-N (ammonia) excretion decreased in both of condition when larvae were fed by probiotic bacilli. The higher rate of urea and ammonia excretions were observed in control treatment ($P<0.05$). Results shown in figure 1-4 showed that *Acipenser persicus* larvae secreted higher ammonia and urea excretion in satiation condition in comparison with starvation. The best results were observed in T2.

The present study showed that ammonia and urea excretion were decreased experimental treatments by inclusion of the probiotic bacilli in comparison with control treatments. Similar decreases have been reported in previous studies [33].

Ammonia is excreted mainly by gills as NH_4^+ ions [34]. In crustaceans, the possible mechanism of ammonia excretion is passive NH_3 (un-ionized ammonia) NH_4^+ (ionized ammonia) effluxes [35] and exchange of NH_4^+ for Na^+ [36].

Koshio, *et al.* [37] showed in *P. japonicus* after 1 h feeding period, cumulative ammonia excretion over a subsequent 5 h period increased with increasing dietary protein content. Rosas *et al*. [38] Effect of dietary protein on apparent heat increment and postprandial nitrogen excretion of *P. setiferus, P. schmitti, P. duorarum and P. notialis* postlarvae. Rosas *et al.* [38] measured postprandial nitrogen excretion (PPNE) in postlarval (PL 25-30) of *P. setiferus*, *P. schmitti*, *P. duorarum* and *P. notialis*, fed with different levels of protein content. The nitrogen excretion increased with increasing dietary protein.

Increasing the dietary level of non-protein digestable energy increase nitrogen retention by decreasing nitrogen losses [39]. The decrease in NH_3-N concentration in this study appears to be the result of increased incorporation of ammonia into microbial protein and may be the direct result of stimulated microbial activity [40].

Whereas there are no premeditate on ammonia and urea excretion by enrichment activity, this study showed enrichment of daphnia with probiotic bacilli can reduce

ammonia and urea excretion and also cause increase protein retention in *Acipenser percicus* larvae body notably.

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