

# The effect of A-Max and Celmanax yeast commercial productions on energy losing based on ammonia and urea excretion in Common Carp (*Cyprinus carpio*) in comparison with probiotic bacilli

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## Abstract

A-Max yeast of *Saccharomyces cerevisiae* concentrate, Celmanax liquid commercial probiotic products from Arm & Hammer Animal Nutrition Co. (USA) and probiotic bacilli from Protexin Co. (UK) on ammonia and urea-nitrogen excretion in juvenile Common Carp (*Cyprinus carpio*) farmed in a rearing culture system. The experimental diets (A-Max, Celmanax and Probiotic bacilli) were mixed with A-Max suspension (A-Max meal in 200 ml of water were mixed with 1 kg of diet), Celmanax (1 ml in 200 ml of water and concentration of  $1 \times 10^{10}$  spores of 5 species of endospore probiotic bacilli, *B. licheniformis*, *B. subtilis*, *B. polymyxa*, *B. laterosporus* and *B. circulans*, were supplemented respectively in 1 kg of diet and dried at 40°C in incubator for 5 h. This experiment was conducted in a completely randomized design with three experimental treatments (A-Max, Celmanax and Probiotic bacilli) and control group with three replicates for treatment and control. Carp juveniles were fed based on the 5-7% of their body weight by experimental diets with experimental diets and nonsupplemented diet in control group respectively. At the termination of experiment (90 days) fifty fish were sampled and measured the ammonia, urea excretion and calculated the losing energy in testing plastic tanks.

The results showed that the A-Max yeast of *Saccharomyces cerevisiae* concentrate and Celmanax liquid commercial probiotic had beneficial effects amount of ammonia and urea excretion of Common Carp (*Cyprinus carpio*) juveniles and the losing rate of energy decreasing in comparison with control. The best results in were obtained from supplementation diet of Celmanax liquid. The minimum energy losses by ammonia (2.94 KJ/Kg fish/day) and urea (0.68 KJ/Kg fish/day) were obtained in experimental treatment of Celmanax liquid where the Common Carp juveniles fed with supplemented diet with 1 ml of Celmanax liquid per kg of diet.

Keywords: A-Max, Celmanax liquid, experimental diets, Common Carp, urea excretion, energy losses.

## 1- Introduction

Ammonia and urea excretion can be used as indicators of fish nitrogen balance and to determine the effects of environmental and nutritional factors on protein metabolism. The main end-product of protein metabolism in teleosts is ammonia and a significant proportion of nitrogenous waste is also excreted as urea [1]. 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 [2]. Therefore, quantification of ammonia and 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 [3].

The rate of ammonia excretion increases rapidly in response to feed intake [4] and the majority of the nitrogen excreted is derived from deamination of amino acids from dietary proteins [5]. Excretion peaks some hours after feed intake and is mainly dependent upon nitrogen intake; temperature and fish species [6] 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.

Ammonia excretion is known to be affected by factors such as species, body weight, water temperature, feeding and ration size [7,8].

There is not any reports using prebiotic and probiotic for decreasing of losing energy via excretion of ammonia and urea in cultivable fishes. More of studies about the excretion of ammonia and urea were on the relationship between dietary crude protein, temperature, water salinity and ammonia- and urea nitrogen excretion.

The effects of dietary protein to energy ratios, feeding frequency and ration size on nitrogenous excretion by several eel species have been reported [9,10].

Probiotics which compete with bacterial pathogens for nutrients and/ or inhibit the growth of pathogens can be a valid alternative to the prophylactic application of antibiotics and biocides. Fuller (1989) defined a probiotic as 'a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance [11]. A modified and more appropriate definition was proposed by Verschuere et al. (2000a) 'a live microbial adjunct which has a beneficial effect on the host by modifying the host-associated or ambient microbial community, by ensuring improved use of the feed or enhancing its nutritional value, by enhancing the host response towards disease, or by improving the quality of its ambient environment.' [12].

However, there is little information available on using commercial prebiotic and probiotic or their products on ammonia- and urea-nitrogen excretion. Therefore, quantification of nitrogenous excretion in relation to supplementation of these biological agents is of importance in optimising feeding regimes, decreasing the ammonia- and urea-nitrogen excretion and increasing the density of fish in culturing systems and provides an opportunity to losing energy by the common carp and promotion of energy efficiency in rearing system.

Lashkarboloki et al. (2011a) reported that the feeding of Persian sturgeon (*Acipenser persicus*) larvae with bioencapsulated *Daphnia magna* with A-Max yeast of *Saccharomyces cerevisiae* concentrate had a good result on decreasing of ammonia and urea

excretion[13]. In another study using probiotic bacillus with bioenriched *Daphnia sp.* had a good effect on decreasing of excretion of ammonia and urea nitrogen and energy losses in Persian Sturgeon [14].

This study aimed at demonstrating the effect of supplementing dietary with the A-Max yeast of *Saccharomyces cerevisiae* concentrate, Celmanax liquid commercial probiotic products and probiotic bacilli on ammonia and urea-nitrogen excretion in juvenile Common Carp (*Cyprinus carpio*) farmed in a rearing culture system.

## 2- Materials and Methods

The A-Max yeast of *Saccharomyces cerevisiae* concentrate, Celmanax liquid commercial probiotic products from Arm & Hammer Animal Nutrition Co. (USA) and probiotic bacilli from Protexin Co. (UK) on ammonia and urea-nitrogen excretion in juvenile Common Carp (*Cyprinus carpio*) farmed in a rearing culture system.

Three experimental diets with isoprotein, lipid and energy (50%, 10% and 4500 cal/g respectively) were supplemented with 1 g of A-Max per kg of diet, Celmanax of 1 ml of liquid per kg of diet and concentration of  $1 \times 10^{10}$  CFU/kg of diet respectively.

The experimental diets (A-Max, Celmanax and Probiotic bacilli) were mixed with A-Max suspension (A-Max meal in 200 ml of water were mixed with 1 kg of diet), Celmanax (1 ml of Celmanax in 200 ml of water were mixed with 1 kg of diet) and concentration of  $1 \times 10^{10}$  spores of 5 species of endospore probiotic bacilli, *B. licheniformis*, *B. subtilis*, *B. polymyxa*, *B. laterosporus* and *B. circulans*, were supplemented in 1 kg of diet and dried at 40°C in incubator for 5 h.

In experimental treatments of A-Max, Celmanax and Probiotic bacilli the juveniles of Common Carp (*Cyprinus carpio*) were fed by supplemented diets with A-Max, Celmanax and Probiotic bacilli respectively. In control group the Common Carp juveniles were fed on nonsupplemented diet with prebiotics and probiotic bacilli.

Eight-month old Common Carp juveniles with initial weight of  $30.50 \pm 1.50$  g were obtained from Hatchery of Chamran warm water cultivable fish center, Golestan province, Iran. This experiment was conducted in a completely randomized design with three experimental treatments (A-Max, Celmanax and Probiotic bacilli) and control group with three replicates for treatment and control. Common Carp juveniles were fed based on the 5-7% of their body weight by experimental diets for three times a day at 6.00, 14.00 and 22.00 with experimental diets in experimental treatments and nonsupplemented diet in control group respectively.

Twelve circular fiberglass tanks (capacity of 1000 liters) with three replicates for experimental and control treatments, were used in this trial and 50 Common Carp juveniles were introduced to each of fiberglass tanks. Each rearing tank was supplied with running fresh water. Water quality parameters from every tank were monitored each week throughout the experiment. The water temperature was  $24.10 \pm 2.15$ °C, pH was 7.6-8.3 and water oxygen level was maintained above 7.5 mg l<sup>-1</sup> during the experiment by setting electrical air pump.

Fifty fish larvae from each tank were sampled at the termination of the feeding experiment, and the total weight and length of body were measured.

At the termination of experiment (90 days) fifty fish were sampled from each replicates and immediately placed into 12 plastic tanks with capacity of 28 liters for 24h. Aeration and water flow were stopped during the experiment. The water of testing tanks was aerated with electrical pumps for 24 h. before introducing the carp juvenile to these plastic tanks. Water temperature

was 22°C and the experiment was conducted at the natural photoperiod conditions with similar light intensity for all tanks. After 24 h., the water of each tanks were sampled and Ammonia-N and urea-N excretions in each tank were measured by the method of Elliott (1976) and converted into energy by multiplying the coefficient of 24.83 kJ g<sup>-1</sup> N for ammonia and 23.03 kJ g<sup>-1</sup> N for urea [15].

### 3- Results

The amount of ammonia and urea excretion of Common Carp (*Cyprinus carpio*) juveniles is presented in table 1.

**Table 1: The amount of ammonia and urea excretion of Common Carp (*Cyprinus carpio*) juveniles**

Parameter \ Treatment	Control	A-Max	Celmanax	Probiotic bacilli
Ammonia-nitrogen (mg NH <sub>3</sub> -N/kg fish/day)	130.93±21.10 <sup>a</sup>	123.86±21.09 <sup>b</sup>	118.38±26.71 <sup>b</sup>	155.09±19.92 <sup>a</sup>
Urea-nitrogen (mg NH <sub>3</sub> -N/kg fish/day)	32.73±5.27 <sup>a</sup>	30.97±5.27 <sup>b</sup>	29.59±6.67 <sup>b</sup>	38.77±4.97 <sup>a</sup>
Total nitrogen (mg NH <sub>3</sub> -N/kg fish/day)	163.66±26.37 <sup>a</sup>	154.82±26.36 <sup>b</sup>	147.97±58.38 <sup>b</sup>	193.82±24.89 <sup>a</sup>
Energy losses based on ammonia excretion(kJ/kg fish/day)	3.25± 0.52 <sup>a</sup>	3.07± 0.52 <sup>b</sup>	2.94± 0.15 <sup>b</sup>	3.85± 0.49 <sup>a</sup>
Energy losses based on urea excretion(kJ/kg fish/day)	0.75±0.12 <sup>a</sup>	0.71±0.12 <sup>b</sup>	0.68±0.16 <sup>b</sup>	0.89±0.11 <sup>a</sup>
Total energy losses based on urea excretion(kJ/kg fish/day)	4.00±0.64 <sup>a</sup>	3.78±0.64 <sup>b</sup>	3.62±0.42 <sup>b</sup>	4.74±0.60 <sup>a</sup>

The results clearly showed that the A-Max yeast of *Saccharomyces cerevisiae* concentrate and Celmanax liquid commercial probiotic had beneficial effects amount of ammonia and urea excretion of Common Carp (*Cyprinus carpio*) juveniles and the losing rate of energy decreasing in comparison with control.

Effects of Celmanax liquid commercial probiotic on amount of ammonia and urea excretion of Common Carp juveniles were better than A-Max and probiotic bacilli treatments. The treatment of A-Max and Celmanax had significant difference with control group ( $p < 0.05$ ).

The rate of resulted urea excretion showed that control Carp juveniles (32.73 mg/kg fish/day) secreted significantly higher urea in comparison with Common Carp juveniles in experimental treatments of A-Max and Celmanax ( $P < 0.05$ ) while in treatment of probiotic bacilli the ammonia and urea excretion increased but had not significantly difference with control group ( $p > 0.05$ ).

Energy losses rates via Urea and ammonia-nitrogen excretion did not differ significantly between the A-Max yeast of *Saccharomyces cerevisiae* concentrate and Celmanax liquid commercial probiotic ( $P > 0.05$ ).

The maximum of energy losses via excretion of ammonia (3.85 KJ/Kg fish/day) and urea (0.89 KJ/Kg fish/day) were in treatment of probiotic bacilli. But the minimum energy losses by ammonia (2.94 KJ/Kg fish/day) and urea (0.68 KJ/Kg fish/day) were obtained in experimental treatment of Celmanax liquid where the Common Carp juveniles fed with supplemented diet

with 1 ml of Celmanax liquid per kg of diet. The A-Max and Celmanax liquid treatments had significantly difference with control group ( $p > 0.05$ ).

The total energy losses by metabolic wastage (Ammonia and urea) were decreased in experimental treatments of A-Max and Celmanax in comparison with the control. But total energy losses of Common Carp juveniles were significantly increased in probiotic bacilli treatment (4.74 KJ/Kg fish/day) in comparison with the A-Max (3.78 KJ/Kg fish/day) and Celmanax (3.62 KJ/Kg fish/day) treatments ( $P < 0.05$ ).

#### 4- Discussion

All of experimental prebiotic treatments had good results in decreasing of energy losses by excretion of ammonia and urea nitrogen. There are a little researches about the effecting of prebiotic on ammonia and urea excretion rate in cultivable fishes.

Total energy losses based on ammonia and urea excretion rates are directly related to supplement the probiotics of A-Max and Celmanax in diets of Common Carp juveniles.

Many of researches reported that the ammonia and urea excretion rates effected by salinity, levels of crude protein and temperature of water in rearing system.

Handy et al. (1993) reported that total ammonia nitrogen excretion rates are directly related to dietary nitrogen and protein intake in fish [16] and another research showed a relationship between feed offered and ammonia excretion in different species of cultivable fish [6].

In agreement of our results in this study, Lashkarboloki et al. (2011b) reported that the bioencapsulation of *Daphnia magna* with meal of A-Max yeast of *Saccharomyces cerevisiae* concentrate and feeding of Persian sturgeon (*Acipenser persicus*) larvae with its had a good result on decreasing of ammonia and urea excretion [17]. They showed among of 50, 100 and 150 mg of A-Max meal in bioencapsulated suspension broth of *Daphnia magna*, the best result was obtained with 150 mg of A-Max. They highlighted that the level of ammonia excretion in control (0.109 mg/kg fish/day) was decreased by feeding of bioencapsulated *Daphnia magna* with 150 mg per liter of suspension broth and decreased to 0.093 mg/kg fish/day and urea excretion decreasing from 0.079 to 0.030 mg/kg fish/day respectively.

The decrease in  $\text{NH}_3\text{-N}$  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 [18].

In contradiction with our results, Faramarzi et al. (2012) showed that the blend of probiotic bacilli in bioencapsulation broth of *Daphnia magna* and feeding by Persian sturgeon (*Acipenser persicus*) larvae decreased the excretion of ammonia and urea nitrogen 0.11 to 0.094 and 0.080 to 0.045 mg/kg fish/day respectively [14].

Similar results were reported by Faramarzi et al. (2012) on energy losses by excretion of ammonia and urea nitrogen in Persian Sturgeon using probiotic bacillus with bioenriched *Daphnia sp* [14].

The positive effects of *Saccharomyces cerevisiae* concentrate of A-MAX and Celmanax liquid on energy losses by decreasing of ammonia and urea nitrogen excretion in Common Carp juveniles indicated that these prebiotic yeasts can employ as an excellent material for saving energy and enhancement of energy efficiency in Common Carp. Suggesting that the addition of yeast in diet of cultivable fish can promote the feeding and energy performance and reduce the culture cost of fishes in cultivation systems.

The results of this study clearly demonstrate that the *Saccharomyces cerevisiae* concentrate of A-MAX and Celmanax liquid had the best effects on energy losses and increasing of retained energy in Common Carp juveniles.

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