Journal of Research in Biology

FicusPublishers

OPEN ACCESS

Original Research paper

An International Online Open Access Publication group

The effect of feeding with *Saccharomyces cerevisiae* extract(Amax) on ammonia and urea excretion in Persian sturgeon (*Acipenser persicus*) larvae by bioenrichment of *Daphnia magna*

Authors:
[1]Mohamad Lashkarbolouki,
[1]Hojatollah Jafaryan,
[1]Moein Faramarzi,
[2]Ali Zabihi ,
[1]Hosein Adineh.

Institution:
[1] Department of Fishery, Gonbad University of Agricultural Sciences and Natural Resources, Gonbad, Iran

[2] Department of Fishery, Tehran University of Agricultural Sciences and Natural Resources, Karaj, Iran.

Corresponding author:
Lashkarbolouki M.

Email:
3772214@gmail.com

ABSTRACT:

Acipenser percicus larvae (mean weight:80mg) were fed enriched with *Daphnia* and *Saccharomyces cerevisiae* product (Amax) to ammonia and urea excretion in two conditions of starvation and satiation. Persian sturgeon larvae were fed by bioencapsulated *Daphnia* with Amax in three concentrations (50, 100 and 150 mg/l Amax) for four consecutive weeks also control larvae were fed by nonbioencapsulated *Daphnia.* They were acclimatized to the laboratory conditions for 10 days before starting of the experiment. Moreover, a 12 h dark: 12 h light photoperiod was provided. The amount of postprandial Ammonia and urea excreted by *Acipenser percicus* larvae within 24 h was related to dose of Amax in broth. also results showed when larvae were fed with 50,100,150mg/l Amax, has decreased ammonia and urea excretion. Lower amount of ammonia(.093) and urea(.030) excretion in satiation condition and urea(.016) excretion in starvation condition were observed in treatment with 150 mg/l Amax ($P < 0.05$).but there is no significant different about ammonia excretion in starvation between other treatments ($P > 0.05$). Results indicate that use of probiotics *Saccharomyces cerevisiae* product (Amax) can reduce the amount of ammonia and urea excretion interestingly.

Keywords:
Acipenser percicus ,*daphnia*,ammonia,urea,starvation,satiation.

Article Citation:
Mohamad Lashkarbolouki, Hojatollah Jafaryan, Moein Faramarzi, Ali Zabihi, Hosein Adineh.
The effect of feeding with *Saccharomyces cerevisiae* extract(Amax) on ammonia and urea excretion in Persian sturgeon (*Acipenser persicus*) larvae by bioenrichment of *Daphnia magna*
Journal of research in Biology (2011) 2: 110-115

Web Address:
http://jresearchbiology.com/Documents/RA0042.pdf.

Dates:
Received: 04 Jun 2011 /**Accepted:** 09 Jun 2011 /**Published:** 13 Jun 2011

INTRODUCTION

Over 50% of the nitrogen input into a marine fish culture system may be lost to the water as excretions (Gowen and Bradbury, 1987). Fish may excrete nitrogen in the form of ammonia, urea, amines and amino acids(Wood, 1958; Porter et al., 1987). Marine teleosts are predominantly ammoniotelic, with 70 to 90% of nitrogen excretion usually being in the form of ammonia(Brafield, 1985; Dosdat et al., 1996). Ammonia is toxic to fish and is considered to be a major factor limiting fish biomass and stocking density in intensive culture systems (Cai and Summerfelt, 1992). which can lead to fish mortality (Skjoldal and Dundas, 1989).

Rate of endogenous nitrogen excretion in fishes is an indicator of various environmental and nutritional factors influencing the protein metabolism, and thus gives an insight into the nitrogen economy of fishes (Birkett, 1969; Savitz, 1977). While Gerking (1955) measured the endogenous nitrogen excretion by feeding the fishes a non protein diet (glucose), Kaushik (1980) and Roy and Das (1986) could demonstrate that excretion following a short term starvation (total food deprivation) served as endogenous nitrogen excretion. The major proportion of ammonia excretion occurs through the gills, whereas antennal and maxillary glands play a minor role (Binns and Peterson, 1969; Cameron and Batterton, 1978).In general, most teleosts are vulnerable to elevated internal ammonia levels (Person-Le Ruyet et al., 1995). However, under certain circumstances such as high ambient ammonia or aerial exposure, ammonia excretion is inhibited, and toxic ammonia becomes concentrated in blood and body tissues. Fishes are generally known to tolerate relatively higher accumulation of ammonia than mammals (Saha and Ratha 1994). Some fish species have evolved various mechanisms to tolerate or avoid very high environmental and internal ammonia Species such as the Lake Magadi tilapia (*Alcolapia grahami*), toadfish (Batrachoididae) and air breathing mudskipper (*Heteropneustes fossilis*) are capable of converting ammonia to less toxic urea via the ornithine urea cycle, then excreting a part or all of their nitrogenous waste as urea (Saha and Ratha, 1994).

In some fish, urea synthesis by uricolysis has been identified as the ureogenic pathway is used to eliminate excess ammonia (Wright and Land, 1998).Plasma total ammonia ($NH3 + NH4^{+}$) normally remains between 0.05 to 2 mM in most teleost fishes (Campbell and Anderson 1991; Wood 1993). In contrast, blood ammonia levels greater than 0×05 mM can be toxic to the central nervous system of most mammals (Meijer et al 1990).

Ammonia excretion is known to be affected by factors such as species, body weight, water temperature, feeding and ration size (Jobling, 1981; Yager and Summerfelt, 1993) .Whilst postprandial nitrogenous excretion has been studied in a number of temperate fish species (Echevarria et al., 1993; Kikuchi, 1995), few studies have been carried out to investigate the interactions between body weight, water temperature and ration size on ammonia excretion. Recently, investigations of the advantages of microorganisms and fermented products as feed additives have been conducted to evaluate their effect on growth performance, body composition and ammonia production. These investigations were also stimulated by the fact that broiler industries are faced with lower pollutants mainly ammonia.Continuous feeding of microorganisms to animals provides a constant inoculation of the organism in the alimentary tract (Santoso et al., 1999).Fermented products were also found to be beneficial in reducing fat accumulation (Tanaka et al., 1992) and ammonia production (Santoso et al., 1999). One of the fermented products which may have beneficial in its effects on reducing ammonia production is the fermented product from *Saccharomyces cerevisiae*(Amax). Inclusion of microorganisms or fermented products were also proven to reduce ammonia production (Santoso et al., 1999). The mechanism by which the culture and the fermented products reduceding ammonia production was unknown. The aim of this experiment was to determinate the amount of ammonia and urea excretion at two condition of starvation and satiety in *Acipenser percicus* larvae fed bioencapsulated *daphnia* with *Saccharomyces cerevisiae* extract.

MATERIALS AND METHODS

Feeding trial

Ten-day old healthy larvae of Persian sturgeon (*Acipenser persicus*) with initial weight of 80±7 mg and total length of 22±5 mm were obtained from Hatchery of Marjanii sturgeon center, Golestan, Iran. The Baker's yeast (*Saccharomyces cerevisiae*) product under the commercial title of Amax, were used for enriching of *daphnia*. The *Daphnia magna* was cultured in earthly ponds at Marjani sturgeon center. Three concentrations of yeast suspension (50, 100 and 150

mg/l Amax in suspension of broth) were provided. Twelve fiberglass tanks (capacity of 50 liters) with three replicates for experimental treatment and control were used. The density of fish larvae in each tank was 70 fishes.

After 10 h. the bioencapsulated *Daphnia magna* was collected on a 120mm-pore-size sieve, washed with fresh water and was used as a live food and vector to carry Amax to digestive system of *Acipenser persicus* larvae. In experimental treatments of T1, T2 and T3 the Persian sturgeon larvae were fed by bioencapsulated *D. magna* by 50, 100 and 150 mg/l *Amax*. In control the fish larvae were fed on unbioencapsulated *D. magna*. Each treatment was in triplicate. Sturgeon larvae were fed based on the 30% of their body weight for six times a day at 2.00, 7.00, 12.00, 17.00 and 22.00 with bioencapsulated *Daphnia magna* in experimental treatments and unbioencapsulated *Daphnia magna* in control treatment respectively.

Each rearing tank was supplied with running fresh water which had been filtered through the special cotton filter (flow rate: 1 L min^{-1}). Water quality parameters from every tank were monitored each week throughout the experiment. The water temperature was 19.8± 0.6 °C, pH was 7.6-8.3 and water oxygen level was maintained above 7.5 mg l^{-1} during the experiment by setting electrical air pump. .

Experimental treatment

Two ration levels were tested in the growth experiment to characterize the amount of ammonia and urea excretion: starvation, and satiation. This study carried out with three replicates for each treatment(control, 50, 100 and 150 mg/l Amax) 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 activity 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 and water flow were stopped 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 experimental period,the water of each tanks were sampled and sent to lab for analysis.

Statistical analysis

All data were analyzed statistically using descriptive statistic and analysis of variance (ANOVA). Duncan's multiple range test was used to evaluate the mean differences among different treatments at the 0.05 significant levels.

RESULTS

The rates of ammonia excretion of the larvae were relatively stable over time and showed no significant variation with the durations of starvation. But the rate of urea excretion over the starvation showed that control larvae secreted higher urea compared with other treatment ($P < 0.05$). Urea-N excretion decreased in both of condition when larvae were fed by *Saccharomyces cerevisiae* extract*(* Amax). The higher rate of urea excretions were observed in control treatment ($P < 0.05$). Urea-nitrogen excretion accounted for between 30–50% of total ammonia-nitrogen excretion rates at each treatment Table 2. showed that *Acipenser persicus* larvae secreted higher ammonia and urea in condition of satiation comparison with starvation. Generally fish larvae are able to secrete ammonia towards urea increasly.

Table1. Amount of ammonia and urea excretion at two conditions of satiety and starvation

Treatment / Parameter	control	50 mg/l Amax	100mg/l Amax	150mg/l Amax
Ammonia excretion mg/g/day (satiety)	.109± 0015.^{a}	.095± 0001.^{b}	.095± 0041.^{b}	.093± 0044.^{b}
Urea excretion mg/g/day (satiety)	.079± 018.^{a}	.055± 0007.^{ab}	.050± 0022.^{ab}	.030.± 0296^{b}
Ammonia excretion mg/g/day (starvation)	.041 0018.±^{a}	.043 ± 0026.^{a}	.043± 0004.^{a}	.044± 0007.^{a}
Urea excretion mg/g/day (starvation)	.035± 001.^{a}	.030± 0003.^{ab}	.027± 0012.^{ab}	.016± 0160.^{b}

DISCUSSION

The rates of postprandial, starvation ammonia and urea excretion of *Acipenser persicus* larvae were reported in Table 1 . The methodology followed in this research was very similar to that used by Thomas and Piedrahita (1998). As in their work, TAN and urea-N excretion rates measured did not discriminate between the fish and any microorganisms present in the culture tanks and are in fact, "apparent excretion rates". The present study showed a small decrease (8-15%) in ammonia and urea excretion by inclusion of the *Saccharomyces cerevisiae* extract in feeding treatments. Similar decreases have been reported in previous studies (Erasmus et al, 1992). Feeds for some fish species typically have a high protein content that supplies a large proportion of dietary energy and results in high nitrogenous excretion.

Ammonia excretion rates are directly related to dietary nitrogen and protein intake in teleosts (Rychly, 1980; Beamish and Thomas, 1984). Increasing the dietary level of non-protein digestable energy increases nitrogen retention by decreasing nitrogen losses (Kaushik and Oliva-Teles, 1985; Medele et al., 1995). 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 (Harrison et al., 1988). This study showed the use of probiotics *Sacharomyces cerevisiae* extract(Amax) could reduce ammonia and urea execration reasonably. Observed in control treatment at both of satiation and starvation conditions, the decrease in ammonia excretion with increasing Amax in broth was in agreement with previous findings for eels (Gallagher and Matthews, 1987; Degani and Levanon, 1988). Postprandial ammonia and urea excretion rates of larvae decreased with ration size, but were not significantly affected by body weight. A similar conclusion was reached by Cui and Wootton (1988) in their studies on *Phoxinus phoxinus.* Paulson (1980) also demonstrated that nitrogen consumption was the most important factor influencing ammonia excretion of brook trout (*Salvelinus fontinalis*) and rainbow trout (*Oncorhynchus mykiss*) , while effects of body weight was less important. Extrapolating from the results,Yager and Summerfelt(1993) concluded that body weight only accounted for 30% of the variability in ammonia excretion of juvenile walleye (Stizostedion vitreum).

Whereas there are no premeditate on ammonia and urea excretion by enrichment activity,this study showed enrichment of daphnia with *Saccharomyces cerevisiae* extract(Amax) probiotics can reduce ammonia and urea excretion and also cause increase protein retention in *Acipenser percicus* larvae body notably.

REFERENCE

Beamish FWH, Thomas E. 1984. Effects of dietary protein and lipid on nitrogen losses in rainbow trout, *Salmo gairdneri*. Aquaculture., 41:359-371.

Binns R and Peterson AJ. 1969. Nitrogen excretion by the spiny lobster *Jasus edwardsii* (Hutton). The role of the antennal gland. Biol Bull Mar Biol Lab Woods Hole., 136:147-153.

Birkett L. 1969. The nitrogen balance in plaice, sole, and perch. Journal of Experimental Biology 50:375-386.

Brafield, AE. 1985. Laboratory studies of energy budgets. In: Tytler, P., Calow, P. (Eds.), Fish Energetics: New perspectives. Croom Helm, London. 257-282.

Cai YJ and Summerfelt RC. 1992. Effects of temperature and size on oxygen consumption and ammonia excretion in walleye. Aquaculture 104:127-138.

Cameron JN and Batterton CV. 1978. Antennal gland function in the freshwater crab *Callinectes sapidus*: water, electrolyte acid-base and ammonia excretion. J Comp Physiol., 123:143-148.

Campbell JW and Anderson PM. 1991. Evolution of mitochondrial enzyme system in fish: the mitochondrial synthesis of glutamine and citrulline; in Molecular biology of fishes (eds) P W Hochachka and T P Mommsen (Elsevier: Amsterdam) vol. 1:43-76.

Cui Y and Wootton RJ. 1988. The metabolic rate of the minnow, *Phoxinus phoxinus* (L.) (Pisces: *Cyprinidae*), in relation to ration, body size, and temperature. Funct Ecol., 2:157-161.

Degani G and Levanon D. 1988. The relationship between ammonia and oxygen concentration in water and the biomass and level of protein in the

diet of eel, *Anguilla anguilla*. Aquacult. Eng., 7:235-244.

Dosdat A, Servais F, Metailler R, Huelvan C and Desbruyeres E. 1996. Comparison of nitrogenous losses in five teleost fish species. Aquaculture., 141:107-127.

Echevarria G, Zarauz N, Lopez-Ruiz J and Zamora S. 1993. Study of nitrogen excretion in the giltheadseabream Sparus aurata L. : Influence of nutritional state. Comp. Biochem. Physiol., 105A, 17–19.-**,** Effect of yeast culture supplement, rumen fermentation and duodenal digesta flow in dairy cows. J. Dairy Sci. 75:3056.

Erasmus LJ, Botha PM, and Kistner A. 1992. Effect of yeast culture supplement, rumen fermentation and duodenal digesta flow in dairy cows. J. Dairy Sci. 75:3056.

Gallagher ML and Matthews AM. 1987. Oxygen consumption and ammonia excretion of the American eel *Anguilla rostrata* fed diets with varying protein energy ratios and protein levels. J. World Aquacult. Soc., 18:107-112.

Gerking SD. 1955. Influence of rate of feeding on body composition metabolism of bluegill fish. Physiological Zoology 28:267-282.

Gowen RJ and Bradbury NB. 1987. The ecological impact of *salmonid* farming in coastal waters: a review. Oceanogr. Mar. Biol. Ann. Rev., 25:563-575.

Harrison GARW, Hemken KA, Dawon R, Harmon J and Barker KB. 1988. Influence of addition of yeast culture supplement to diets of lactating cows on ruminal fermentation and microbial populations. J. Dairy Sci., 71:2967.

Jobling M. 1981. Some effects of temperature, feeding and body weight on nitrogenous excretion in young plaice *Pleuronectes platessa* L. J. Fish Biol., 18:87-96.

Kaushik SJ. 1980. Influence on the nutrient status on the daily patterns of nitrogen excretion in the carp (*Cyprinus carpio*) and the rainbow trout (*Salmo gairdneri*). Reprod Nutr Dev., 20:1751-1765.

Kaushik SJ and Oliva-Teles A. 1985. Effects of digestible energy on nitrogen and energy balance in rainbow trout. Aquaculture 50:89-111.

Kikuchi K. 1995. Nitrogen excretion rate of Japanese flounder—a criterion for designing close circulating culture systems. Israel J. Aquacult. Bamidgeh., 47:112-118.

Medale F, Brauge C, Vallee F and Kaushik SJ. 1995. Effects of dietary proteinrenergy ratio, ration size dietary energy source and water temperature on nitrogen excretion in rainbow trout. Water Sci., Technol. 31:185-194.

Meijer AJ, Lamers WH and Chamuleau RAFM. 1990. Nitrogen metabolism and ornithine cycle function; Physiol. Rev., 70:701-708.

Paulson LJ. 1980. Models of ammonia excretion for brook trout (Salĺelinus fontinalis)and rainbow trout (*salmo gairdenri*). Can. J. Fish. Aquat. Sci., 37:1421-1425.

Person-Le Ruyet J, Chartois H and Quemener L. 1995. Comparative acute ammonia toxicity in marine fish and plasma ammonia response. Aquaculture 136:181-194.

Porter CB, Krom MD, Robbins MG, Brickell L and Davidson A. 1987. Ammonia excretion and total N budget for gilthead seabream(*Sparus aurata*) and its effect on water quality conditions. Aquaculture 66:287-297.

Rychly J. 1980. Nitrogen balance in trout: II. Nitrogen excretion and retention after feeding diets with varying protein and carbohydrate levels. Aquaculture 20:343-350.

Saha N and Ratha BK. 1994. Induction of ornithine-urea cycle in a freshwater teleost, *Heteropneustes fossilis*, exposed to high concentrations of ammonium chloride. Comp. Biochem. Physiol., B108:315-325.

Santoso U, Ohtani S, Tanaka K and Sakaida M. 1999. Dried Bacillus subtilis culture reduced ammonia gas release in poultry house. AsianAus. J. Anim. Sci.,. 12:806-809.

Savitz J, Albanese E, Evinge MJ. 1977. Effect of

ration level on nitrogen excretion, nitrogen retention and efficiency of nitrogen utilization for growth in largemouth bass (*Micropterus salmoides*). Journal of Fish Biology 11(2):185-192.

Skjoldal HR and Dundas I. 1989. The Chrysochromulina polylepis bloom in the Skagerrak and Kattegat in May–June 1988: environmental conditions, possible causes, and effects. ICES Coop. Res. Rep. No. 175:59.

Tanaka K, Youn BS, Santoso U, Ohtani S and Sakaida M. 1992. Effects of fermented products from chub mackerel extract on growth, and carcass compositison, hepatic lipogenesis and on contents of various lipid fractions in the liver and the thigh muscle of broilers. Anim. Sci. Technol., 63:32-37.

Thomas S and Piedrahita R. 1998. Apparent ammonia-nitrogen production rates of white sturgeon (*Acipenser transmontanus*) in commercial aquaculture systems. Aquacultural Engineering., 17:45-55.

Wright PA and Land MD. 1998. Urea production and transport in teleost fishes. Comp. Biochem. Physiol., A119:47-54.

Wood CM. 1993. Ammonia and urea metabolism and excretion. In: Evans, D.H. (Ed.), The Physiology of Fishes. CRC Press Inc, Boca Raton, Ann Arbor, London, Tokyo. 379-425.

Wood JD. 1958. Nitrogen excretion in some marine teleosts. Can. J. Biochem. Physiol., 36:1237 -1242.

Yager TK and Summerfelt RC. 1993. Effects of fish size and feeding frequency on metabolism of juvenile walleye. Aquacul. Eng., 12:19-36.