EFFECTS OF DIETARY NATURAL ZEOLITE LEVELS ON AMMONIA EXCRETION RATES IN YOUNG RAINBOW TROUTS (*Oncorhychus mykiss*)

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ABSTRACT

The possible effects of dietary zeolite levels on the ammonia excretion rate of young rainbow trouts (*Oncorhychus mykiss*) were investigated. Triplicate groups of rainbow trout were fed diets incorporated with three different levels of zeolite (0.5%, 1% and 2.5%), and a control diet without zeolite at a constant water temperature of 17 ºC. A decline in ammonia excretion level has been recorded with increasing dietary zeolite level. Cumulative ammonia excretion rates for a period of 6 hours, resulted in 6.09 mg N/ 100g fish for the control group (0% zeolite), while an excretion rate of 4.64 mg N/100 g fish has been recorded in fish fed the diet with the highest zeolite level of 2.5%. Based on the findings in the present study, the inclusion of 2.5% natural zeolite in the diet reduced the ammonia excretion rate of rainbow trouts by 24% compared to the control group. This finding could be useful for environmental impact assessment and trout culture management.

KEYWORDS: zeolite, ammonia excretion, feed, rainbow trout (*Oncorhychus mykiss*)

INTRODUCTION

In recent years, there are many discussions in the media concerning the environmental impacts of fish farms and their effluents to the surrounding water, and these discussions have become even more important nowadays. In traditionally operated trout farms in inland flow-through systems, the water effluents are usually not treated for reuse. The availability and quality of the water is a limiting factor for the production capacity of flow-through operated fish farms. The growth of this industry led to new legislations and measures in many European countries, some states of the USA, and Canada, in order to reduce environmental impacts of aquaculture by limiting the concentration and/or mass of specific dissolved/suspended inorganic or organic materials and nutrients contained within the farm effluents, and it also required the accomplishing of environmental monitoring programs [1]. In order to meet the regulations, necessary measures are to be taken by fish farms. A well balanced fish diet is an important factor that can minimize the nitrogenous end products by fish, i.e. a lower ammonia excretion by fish is coupled with a higher digestion of nutrients retained in the fish body. Besides the use of highly digestible ingredients, several feed additives, such as zeolite, are also used in fish diets to reduce ammonia excretion levels.

There are several applications of natural zeolites, such as adsorbents, ion exchangers, and catalysts in agriculture, veterinary medicine and environmental protection. Biological applications include the removal of ammonia from wastewater and animal manure [2]. Furthermore, numerous studies are available on the use of zeolite in diets for terrestrial animals, such as poultry and ruminants. However, to our knowledge so far, only few studies are available on the use of natural zeolite (clinoptilolite) in fish diets, namely sea bass [3] and rainbow trout [4].

The present study was undertaken to investigate the effects of dietary natural zeolite on ammonia excretion rates in rainbow trout, in order to figure out the possible improvement of effluent discharges in fish farms.

MATERIALS AND METHODS

380 rainbow trouts, *Onchornhychus mykiss*, obtained from a local commercial trout farm in Canakkale-Turkey were transported to the research unit of the Fisheries Faculty of the Canakkale Onsekiz Mart University. Prior to the start of the feeding trial, fish were fed a commercial diet (45% protein, 12% fat) for a week in order to adjust them to the experimental conditions. After the conditioning period, three replicate groups of 20 fish, which are initially averaging 70-80 g, were stocked in cylindrical tanks of 300 L, each filled with dechlorinated tap-water.

The experiment was carried out with 380 fish divided into 18 tanks (six groups and triplicate tanks). The water inflow was 3 L/min, and all tanks were aerated by air stones. The recirculating system used in the experiment consisted of 18 300_L tanks, a 300-L sump, 200-L sand filter, and a 300-L biofilter containing bioballs. The total volume of the system was 6200 L. One thousand L of makeup water was exchanged daily corresponding to about 15% of the total volume.

The day before the ammonia-N trial, half of the water in the entire system has been changed, and all experimental tanks were cleaned. Fish body weight and ration size of individual fish were measured and recorded, respectively.

Since the fish consumed 2% of their weight during the adaptation period, this rate was used in the subsequent ammonia excretion trial. Feeding was monitored carefully to avoid any excessive feeding and to ensure an even distribution of feed among all the fish in the tank. After a one-week acclimation period, all fish were weighed individually, and food was withheld one day prior to experiment. On the next day (the day of the trial), experimental feeds (Table 1) were given to the treatment groups, about 2% of body weight at 09:45. After feeding was completed, water inflow was stopped and water samples were taken for a 6-h period. Total ammonia (NH_4^+ and NH_3) concentrations were analyzed by the salicylate method with a Hach DR890 portable spectrophotometer (Hach Co., Colarado-USA). Ammonia-N excretion rate has been calculated by determining the ammonia excreted in each tank after each sampling, using the following formula for a static system: A = [(N2-N1) x V2]/B/T2-1, where A = ammonia excretion rate (µg total NH_3-N/g wet weight/h); N1 = ammonia concentration at time 1 (µg total NH_3-N/ml); N2 = ammonia concentration at time 2 (µg total NH_3-N/ml); V2 = volume of the medium at time 2 (ml); B = wet weight of the fish (g) and T2-1 = time interval between samplings 1 and 2 (h).

TABLE 1 - Proximate composition of the experimental diets.

Feed ingredients	Groups			
	z00	z05	z10	z25
Fish meal	50	50	50	50
Wheat meal	14.0	13.5	13.0	11.5
Soybean meal	24.5	24.5	24.5	24.5
Fish oil	10	10	10	10
Zeolite, clinoptilolite	0.0	0.5	1.0	2.5
Vitamin & Mineral mixture	1.5	1.5	1.5	1.5
Proximate analyses				
Crude Protein	44.96	44.90	44.84	44.86
Crude Lipid	14.06	14.05	14.04	14.01
Crude Ash	8.1	8.6	9.0	10.5

Dissolved oxygen level and water temperature were monitored with a portable oxygen-meter (WTW model, OXI 196), and recorded as 7.0 mg/L and 17.0 °C, respectively. Light was supplied by overhead fluorescent bulbs and also natural light from the windows of the laboratory.

The fish in the z00 group (control) have been fed with pellets without zeolite (clinoptilolite), and experimental fish in the groups z05, z10 and z25 with pellets including zeolite at levels of 0.5%, 1.0% and 2.5% (powdered clinoptilolite). The chemical composition of the original clinoptilolite sample is given in Table 2. The experimental diets were formulated to be isonitrogenic and isocaloric. The pre-weighed dry ingredients were carefully mixed using a laboratory food mixer, with separate addition of the oil and vitamin/mineral premix. The mixtures were primed with water to yield a suitable mash. Moist diets were made into pellets of 1 mm diameter and dried at 40 °C in a fan-assisted drying cabinet. Experimental diet and feedstuffs were analyzed in triplicate for proximate composition according to AOAC [5]. Dry matter was determined after drying at 105 °C until constant weight. Ash content was measured by incineration in a muffle furnace at 525 °C for 12 h. Crude protein was analyzed by the Kjeldahl method after acid digestion using Gerhardt system. Lipid extractions were carried out with petroleum ether in a Soxhlet extraction system.

TABLE 2 - Chemical analysis* of Western Anatolia clinoptilolite used in the present study.

Component	% (wt/wt)
SiO_2	71.0
CaO	3.4
Fe_2O_3	1.7
Al_2O_3	11.8
K_2O	2.4
MgO	1.4
Na_2O	0.4
TiO_2	0.1

*This *Mineralogic-Petrographic* report is related to the sample of *Rota Mining Co.* from Kalabak Damları district, Gordes-Manisa, Turkey, and has been prepared by Istanbul Technical University Mining Faculty on 11.06. 2005.

Statistical analyses were conducted using SPSS 10.0 for Windows. Mean ammonia-N excretion rates between the treatment groups have been compared using one-way ANOVA, and differences were considered to be significant at $p<0.05$ level. Significant ANOVA findings were followed by a post-hoc multiple comparison test (Duncan's new multiple range test; General Linear Model – Univariate procedure).

RESULTS AND DISCUSSION

During a 6-h period, cumulative ammonia excretion rates decreased from 6.09±1.03 mg N/100 g fish in the control group (0% zeolite) to excretion rates of 5.48±0.92, 5.57±0.35 and 4.64±0.73 mg N/100 g fish in the groups fed the diet with 0.5, 1.0 and 2.5% zeolite, respectively. The lowest ammonia-N excretion rate has been recorded in the z25 group, where dietary zeolite inclusion was 2.5%. The

pattern of ammonia-N excretion rates, practically important, in terms of a successful water management, were similar between fish fed diets with 0%, 0.5% and 1.0%. Although there was no significance ($p>0.05$) in ammonia-N excretion levels between these groups and the z25 group, cumulative ammonia-N excretion rate in fish fed the diet with 2.5% zeolite was about 24% lower than that of the control group (0% zeolite).

It has been reported that marine teleosts have basal nitrogen excretion rates of 1.4-34.9 mg N/kg fish/h, and feeding may increase the nitrogen excretion rate to 600 mg N/kg fish/h in some fish species [6].

An increasing trend of excretion rates was recorded during the 6-h period in the present study (Fig. 1). There are several studies on the peak times of postprandial ammonia excretion in fish. It is reported that the peak time may be affected by the moisture content of the diet, namely, a decrease in moisture content showed a delay in the excretion peaks from 2 h in fresh feeds to 6-8 h in dry pellets, respectively [7-9]. However, in interpreting these results, it should be considered that some other factors, such as energy level or amino acid balance, as well as the growth rate, fish size or physiological state, feed intake and some various environmental and cultural conditions, might also affect the postprandial ammonia excretion in fish [8].

As ammonia represents more than 60% of the total nitrogenous end-products in fish [10, 11], lowering the amount of excretion level is an important issue for sustainable and environment-friendly aquacultures. Ammonia excretion rate is one of the most important factors affecting stocking density in intensive fish culture facilities, especially in closed re-circulating systems, due to the accumulation of nitrogenous waste products, which are toxic to fish and can cause deterioration of environmental water quality and threaten fish growth [4, 10]. Diet quality and feed ingredients are also important factors affecting ammonia excretion, i.e. a better diet utilization may result in a lower ammonia excretion by fish. Furthermore, natural compounds, such as zeolite, are used in removing ammonia from wastewater of culture systems and one of the best zeolites in ammonia removal is reported to be clinoptilolite [12]. However, Emadi et al. [12] used zeolite in the water ambiance and not in the diet, and compared it with activated carbon. To our knowledge so far, there are only few studies available on the use of natural zeolite (clinoptilolite) in fish diets, i.e. sea bass [3] and rainbow trout [4]. Obradović et al. [4] reported a positive effect of dietary zeolite (minazel) on fish growth performance, daily feed consumption and utilization. Dias et al. [3] tested incorporation of bulk agents, such as silica, cellulose and a natural zeolite at a 10 and 20 % level, and reported that using the bulk agents did not affect protein digestibility or growth performance in sea bass, and, in comparison to the control treatment, bulk incorporation at 10 and 20 % level did not affect protein retention values in fish. Furthermore, the authors reported that 20% bulk agent incorporation changed the evacuation profile of faeces, and increased faecal egestion time, when compared with the control diet.

Ammonia excretion may result in localized eutrophication and stimulate algal blooms, which can lead to fish mortality [14, 15]. Hence, lowering the amount of excretion level in fish farms is an important issue for sustainable and environment-friendly aquaculture. In the present study, 2.5% zeolite in the diet reduced ammonia excretion by fish to about 24%, with regard to the control group.

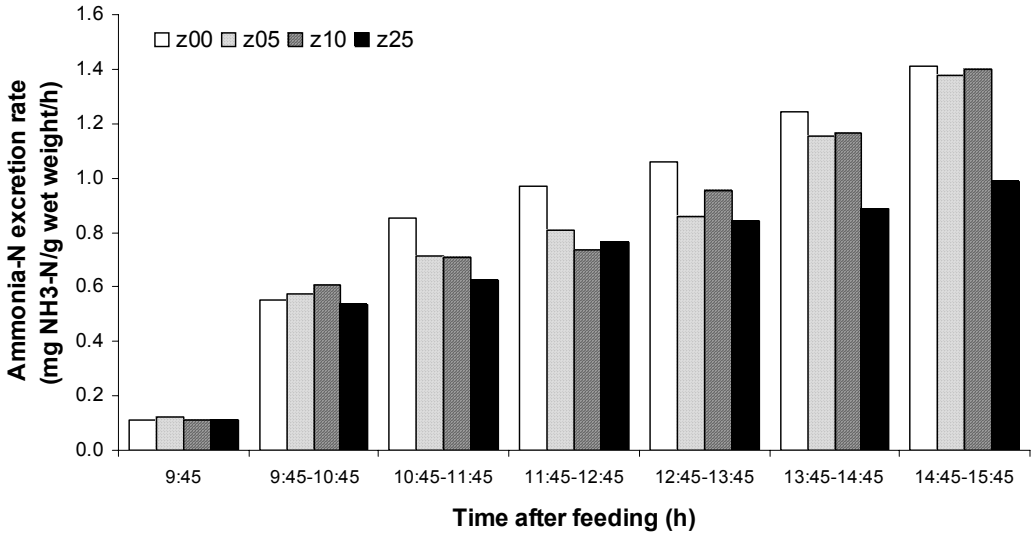

FIGURE 1 - Hourly ammonia-N excretion rate for six hours after feeding rainbow trout with diets including different levels of zeolite (clinoptilolite).

As a conclusion, a certain level of zeolite incorporation to the diet seems to be promising in terms of reducing ammonia excretion, which is an important criterion for a sustainable environment and aquaculture. Additionally, further studies are necessary to assess the possible effects of dietary zeolite on growth performance and feed utilization in rainbow trout.

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