Current Trends in Natural Sciences
Vol. 8, Issue 16, pp. 212-219, 2019
Current Trends in Natural Sciences (on-line)
ISSN: 2284-953X
ISSN-L: 2284-9521
Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-9521
ISSN-L: 2284-9521

THE EFFECTS OF CLINOPTILOLITE FROM FEED UPON FISH REARING AND WATER QUALITY

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Current Trends in
Natural Sciences

Abstract
Maintaining and improving the health status of fish as well as increasing production performance of fish ponds are essential for aquaculture systems. The use of natural zeolites as additives in fish feed has become an important concern of researchers over the past two decades. The paper aims to review the global research on the use of clinoptilolite as a feed additive in feeding of various fish species. Clinoptilolite captures heavy toxic metals (Cd, Pb) and also cations, such as ammonium, from fish-ponds water, which leads to optimum media conditions and increased productivity. According to the specific literature, the clinoptilolite zeolite has to be considered as a material with high potential of applicability in the aquaculture. Worldwide, research is ongoing on how to use clinoptilolite more efficiently, as well as finding new opportunities for applying this zeolite to aquaculture.

Keywords: aquaculture, aquatic environment, feed, fish, water purification, zeolites.

1. INTRODUCTION

According to FAO statistics, in 2016 global aquaculture production was over 50% of total production used for human food, respectively 80.0 million tons of fish food products, plus 30.1 million tons of aquatic plants and 37.900 tons of non-food products (FAO, 2018).

Until 2030, freshwater species aquaculture production is expected to increase to 62% as a result of drastic decline of wild fish captures (FAO, 2018).

Expansion of aquaculture is based on the growing need for animal protein and in order to ensure productivity growth in this sector, technologies used must be more efficient.

In the last decades, researchers have been attracted by the possibility of using environmentally friendly materials who can increase the productivity in the aquaculture sector either by increasing the biomass obtained or by optimizing the aquatic conditions for fish rearing (Filep et al., 2016).

The natural zeolite is one of ecologic adsorbents that can be used in aquaculture. Currently, more than 60 types of natural zeolites are known in worldwide and more than other 150 types are synthesized (Ghasemi et al., 2016). Among natural zeolites, clinoptilolite is successfully used for cationic exchange in water and as a feed additive in fish feed.

In animal husbandry, the use of natural zeolites as a feed supplement leads to better production but also to the animal health maintenance. Zeolites are an important mineral source for animals, increase daily average gain and reduce specific consumption, stimulate the immune system, block

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mycotoxins in feed, substantially reduce morbidity and mortality rates, especially for young animals, and have a favourable effect on the conversion of feed.
Technologies that use natural zeolites for water purification are based on ion exchange, especially cation exchange properties of zeolites, whereby dissolved ions can be removed from water by cation exchange from the zeolite composition (Sprynskyy et al., 2005).
Heavy metals from waste water can contaminate natural water sources used in fish farming. These heavy metals, which are not biodegradable, tend to accumulate in the fish body and then in the human body.
Zeolites, particularly clinoptilolite, exhibit a high selectivity for cations of heavy metals such as lead, cadmium and nickel.

2. MATERIALS AND METHODS

The paper presents a review of the most relevant literature on the use of natural zeolites as feed additives in fish diet. The effect of the clinoptilolite natural zeolite on cations of toxic heavy metals in the waste waters in fish farms was also investigated. The study represents an analysis of clinoptilolite influence on fish and water quality from aquaculture.

3. RESULTS AND DISCUSSIONS

Zeolite use as feed additive

Clinoptilolite has been classified as safe regarding healthy issues and, according to EU legislation; it can be used in feed of birds and animals.
Regarding the use of zeolite as a feed additive, a series of research has been carried out demonstrating that its use determined the increasing of biomass, improving media conditions, fish health, and a higher survival rate.
Clinoptilolite used in tilapia (*Coptodon zillii*) feed provided an increase of final fish weight of 11.49% when using 1% zeolite and 19.25% when using 2% zeolite (Yildirim et al., 2009) (Table 1).
At the same time, total ammonia values in feeding tanks for fish were lower when feed the fish with zeolite additive compared to the control pool, respectively 0.34 ± 0.01 mg L^{-1} (1% zeolite), and 0.36 ± 0.01 mg L^{-1} (2% zeolite) versus 0.39 ± 0.01 mg L^{-1}.

Table 1. Nutritional parameters of tilapia (Coptodon zillii) fish feed with zeolite

Nutritional parameters	Control	1% Zeolite	2% Zeolite
Initial weight (g)	1.08±0.01	1.08±0.01	1.08±0.01
Final weight (g)	2.65±0.11	2.95±0.09	3.16±0.35
Specific growth rate (%)	1.99±1.12	2.23±1.08	2.36±1.02
Feed conversion rate (%)	4.03±0.9	3.26±0.33	2.84±0.41
Protein efficiency rate (%)	0.79±0.05	0.91±0.02	0.99±0.12

Similar results were reported by El-Gendy et al., in 2015, when they introduced 2% zeolite into feed for feeding Nile tilapia (*Oreochromis niloticus*).
The experiment was carried out in 6 ponds, with the dimensions: 100m x 42m x 1m and which were populated with brood. The fish had an average initial weight of 9.13g, 8.22g and 8.96g, and the feed rate was 2%, 2.5% and 3% of the body weight.
At the end of the experiment, which lasted 20 weeks, it was found that the addition of zeolite at a feed rate of 3% of body weight had the largest increase in final weight of 239.54 g (Table 2).

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Table 2. Effect of zeolite on growth parameters

Nutritional parameters	No. of fish	Treatment 1	Treatment 2	Treatment 3
Initial weight (g)	60	9.13±1.16	8.22±1.16	8.96±1.16
Final weight (g)	60	211.00±2.03	237.89±2.03	239.54±3.24
Initial length (cm)	60	7.61±0.26	7.11±0.26	7.13±0.26
Final length (cm)	60	27.15±1.24	28.23±1.24	29.13±1.24
Daily increase (g/fish)	60	1.31±0.13	1.49±0.13	1.49±0.13
Growth specific rate (%/day)	60	2.04±0.15	2.19±0.15	2.14±0.15

Kanyilmaz et al., in 2015, looked at the effects of the introduction of clinoptilolite into the gilthead sea bream juveniles (*Sparus aurata*) feed. The initial weight of the fish was 9.06 ± 0.04 g, and the experiment lasted for 10 weeks. The amounts of zeolite used were 10, 20, 30 and 40 g / kg of feed. Fish were fed 2 times a day with 4% body weight in the first 6 weeks, 3% over the next two weeks and 2.5% in the last two weeks.

It has been shown that using clinoptilolite, feed was better used, which led to improved growth performance (Table 3).

Table 3. Growth parameters recorded after zeolite administration in feed

Nutritional parameters	Z 0	Z 10	Z 20	Z 30	Z 40
Initial weight (g/fish)	9.05	9.10	9.08	9.03	9.05
Final weight (g/fish)	50.7	52.6	53.8	54.5	52.0
Growth specific rate (%/day)	2.73	2.78	2.82	2.85	2.78
Feed consumption (g/fish)	50.2	50.1	53.0	49.7	49.7
Daily feed consumption (g/kg medium body weight/day)	22.8	22.2	23.3	22.1	22.1
Feed conversion efficiency	0.83	0.87	0.85	0.88	0.87
Protein efficiency rate	1.72	1.85	1.80	1.84	1.80

The best weight gain was obtained by feeding with 30 g zeolite / kg, of 54.5 g, with 3.8 g more than the control group.

In order to determine the effect of zeolite and perlite introduced in the fish diet, Khodanazary et al., in 2013, conducted a study on juvenile common carp (*Cyprinus carpio*). The experiment was conducted for 8 weeks, during which the fishes were fed 5 days a week, 2 times a day, with 3% of body weight. The average conditions recorded during the experiment were: 6-7 mg L-1 dissolved oxygen, water temperature 25 ± 0.3^{o}C, and pH value 7.5.

The feed administered as a control diet contained 29.35% crude protein, 7.68% crude wheat fats, corn, soybean meal, 26.5% fish meal, 7.5% vitamin-mineral premix. The amounts of zeolites and perlite added were 2.5% and 5% (Table 4).

Table 4. Effects of zeolite and perlite on carp growth performance

Parameter	Control	Z 2,5	Z 5	P 2,5	P 5
Initial weight (g)	30.25±2.76	29.62±2.56	30.35±3.02	28.95±2.49	30.14±2.48
Final weight (g)	58.01±4.16	61.62±8.16	66.41±10.89	59.48±10.08	64.51±9.54
Weight gain (g)	27.52±2.10	32.00±4.60	36.06±4.29	34.53±3.83	34.37±4.24
Feed conversion rate	1.84±0.13	2.03±0.33	1.77±0.19	2.52±0.31	2.24±0.27
Specific growth rate (%/day)	1.14±0.09	2.03±0.33	1.40±0.09	1.28±0.13	1.35±0.05

In terms of serum and glucose levels, they increased in the zeolite and perlite fed lots and the cholesterol level decreased in these groups compared to the control group.

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It was found that by adding 5% zeolite or perlite to the common carp diet, positive effects on apparent protein digestibility coefficients and carp growth performance were obtained.
The natural zeolite effects on ammonia excretion rates (Ergun et al., 2008) were investigated by the addition in feed on the young rainbow trout (*Oncorhychus mykiss*).
A total of 380 fishes were divided into groups of 20, each group being parked in circular tanks of 300 l. Trout juveniles weighed between 70 and 80 g and was fed with a feed of 2% of body weight (Table 5).
The fishes were not fed 24 hours prior to the start of the experiment. On the day of the study, feeds were administered to experimental groups at 9:45. Upon completion of feeding, the water supply was switched off and water samples were taken for 6 hours from hour to hour.

Table 5. Composition of experimental feeds administered

Ingredients	Type of feed			
	Z 00	Z 05	Z 10	Z 25
Fish flour	50	50	50	50
Wheat flour	14	13.5	13	11.5
Soybean flour	24.5	24.5	24.5	24.5
Fish oil	10	10	10	10
Clinoptilolite	0	0.5	1	2.5
Vitamins + minerals	1.5	1.5	1.5	1.5

The results are consistent with those obtained by Nicolae et al. (2017) and revealed that after 6 hours, the ammonia excretion rates decreased from 6.09 ± 1.03 mg N / 100 g of fish, in control group at 5.48 ± 0.92, 5.57 ± 0.35 and 4.46 ± 0.73 mg N / 100 g fish, in the feed fed groups with zeolite. The lowest ammonia excretion rate was recorded in the feed-fed group supplemented with 2.5% zeolite.
Research on the morphometric parameters and water quality in the rainbow trout (*Oncorhynchus mykiss*) tanks was carried out in 2006 by Obradovic et al. Zeolite used as a feed additive in the proportion of 1% is known under the trade name "Minazel", and zeolite "Ambizel V" has been used as a possible corrector of aquatic environmental conditions.
The initial average weight of the fish used in the experiment was 87.93 g and the total body length was 19.80 cm. The group of 9816 fishes was divided into two equal groups - the control group and the experimental group. The duration of the experiment was 150 days. The results at the end of the study show that the use of zeolite had a stimulating effect on fish growth (Table 6).

Table 6. Effects of zeolite "Minazel" on the growth performance of trout

Parameter	Control group	Experimental group
Initial body weight	87.93	87.93
Final body weight	238.41	265.63
Initial total length	19.80	19.80
Final total length	27.23	2.17
Average weight gain (g)	150.48	177.70

Obradovic mentions that the "Ambizel-V" zeolite, used as a corrector of environmental conditions in the experimental pond water, had a positive influence on the decrease in water hardness, ammonia and nitrite. It has been observed, based on the results obtained by Ibrahim et al., in 2016, that feeding of Nile tilapia (*Oreochromis niloticus*) with feed containing 25% crude protein and 2%

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zeolite leads to improved breeding performance and fish health. This variant has proven to be the best in terms of economic efficiency as well, compared to other treatments.

Protective effect of zeolite on heavy metal cations

Natural water sources can be contaminated by heavy metal pollutants, which are not biodegradable and tend to accumulate in the body. Clinoptilolite, the most common zeolite, has a high selectivity for cations of heavy metals such as lead, cadmium and nickel. Research has been carried out to determine the quantities and mode of use of zeolite to prevent the accumulation of metals in the fish body.

The protective effect of clinoptilolite on common carp (*Cyprinus carpio*) exposed to lead toxicity was investigated in the study by Tepe et al., in 2004. Four groups with 20 fishes each were placed in aquariums of 100 l.

Four different treatments were performed: 60 mg L^{-1} $Pb(NO_3)_2$, 60 mg L^{-1} clinoptilolite, 60 mg L^{-1} $Pb(NO_3)_2$ + 50 mg L^{-1} clinoptilolite and control without lead nitrate or zeolite.

The experiment was conducted for 35 days, after which samples were collected and analyzed. It has been observed that clinoptilolite has led to a significant decrease in total ammonia nitrogen concentrations and that it can protect the carp from the adverse effects of lead nitrate. Zeolite, through its high cationic exchange capacity, has reduced the amount of lead nitrate available to fish. The body weight of the fish was measured at the end of the experiment and the results showed no significant differences (Table 7).

Table 7. The effects of lead nitrate on different biochemical components of common carp (Cyprinus carpio)

Parameter	Control	Lead	Lead + clinoptilolite	Clinoptilolite
Cholesterol	104.5 ± 1.5	239.0 ± 3.0	171.5 ± 2.5	164.5 ± 0.5
Glycogen	136.0 ± 2.0	83.5 ± 1.5	123.0 ± 2.0	194.0 ± 1.0
Triglycerides	80.0 ± 1.0	87.5 ± 1.5	105.5 ± 1.5	92.5 ± 1.5
LDH	807 ± 2.0	1751.5 ± 11.5	1475.0 ± 3.0	1387.0 ± 23.0

Similar research was carried out by Mutlu in 2016, which aimed to observing the evolution of metabolic markers in common carp (*Cyprinus carpio*) treated with lead nitrate and clinoptilolite for 30, 60 and 90 days. The treatments were: 60 mg L^{-1} $Pb(NO_3)_2$, 60 mg L^{-1} clinoptilolite, 60 mg L^{-1} $Pb(NO_3)_2$ + 50 mg L^{-1} clinoptilolite and control without lead nitrate or zeolite.

Mutlu considers that zeolite, used for a longer period, by absorbing minerals from water, leads to weight loss of fish. The initial mean weight of the fish was 101.8 ± 0.19 g, and the values reached by it at the end of the experiment are shown in Table 8.

Table 8. Average weight at the end of the trial period

Treatment	Weight (g)		
	30 days	60 days	90 days
Control	148,3 ± 0,21	233,4 ± 0,22	361,7 ± 0,23
$Pb(NO_3)_2$ + Zeolite	141,2 ± 0,20	222,1 ± 0,21	344,6 ± 0,22
Zeolite	137,8 ± 0,20	214,2 ± 0,20	331,4 ± 0,22
$Pb(NO_3)_2$	134,4 ± 0,21	206,4 ± 0,21	319,3 ± 0,21

The results of the metabolic marker measurements performed at 30 days, 60 days and 90 days respectively showed that adding clinoptilolite zeolite to water can protect common carps against the toxicity of lead.

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Research has been carried out on the clinoptilolite zeolite property to reduce the level of cadmium bioaccumulation in Prussian carp (*Carassius gibelio*) bodies exposed to cadmium acetate-induced intoxication (Nicula et al., 2010). The cadmium dose used was 10 ppm and the zeolite content of 0.5 g, 2 g and 4 g respectively.
The data obtained showed that the zeolite forms a complex with Cd that reduces its bioaccumulation in the fish body and at the same time diminishes the Cd antagonistic effect on certain essential minerals such as Fe^{2+}, Zn^{2+}, Cu^{2+}, Ca^{2+} or Mg^{2+}, the intensity of the zeolite action being dose-dependent (Table 9).

Table 9. Cadmium level in fish organs (mg/kg)

Tissue	Cd	Cd + 0,5 g Z	Cd + 2 g Z	Cd + 4 g Z
Gills	24.71	21.42	20.69	11.38
Bowel	47.01	41.60	33.01	29.39
Liver	11.03	10.00	9.07	7.82
Kidney	51.52	4.76	45.20	42.00
Muscle	0.15	0.11	0.05	0.04
Skin	2.01	1.99	1.97	1.65
Brain	1.10	0.90	0.62	0.46
Ovary	1.34	0.90	0.54	0.25
Heart	5.34	4.27	2.53	1.68

In a similar experiment, Ghiasi et al. (2011), used a total of 45 common carps (*Cyprinus carpio*) divided into three groups. The first group was the control group, the second group was exposed to 30 ppb (30 μg L^{-1}) cadmium and the third group was exposed to 30 ppb (30 μg L^{-1}) + 10 g L^{-1} clinoptilolite, for 30 days.
Measurements were conducted after 15 and 30 days after exposure by harvesting kidney, liver, gill and spleen samples. The results obtained showed that the introduction of 10 g L^{-1} of zeolite into cadmium polluted waters reduces the accumulation of heavy metal in the carp organism (Table 10).

Table 10. Cadmium content (ppb) in different organs and water after 15 and 30 days of exposure

Exposure	Kidney	Liver	Gills	Spleen	Water
15 days					
Cadmium	80.455±14.1656	38.405±17.1433	57.654±10.7160	30.950±4.3575	17.9
Cadmium + zeolite	98.420±26.7875	73.916±23.0656	43.708±20.0236	66.037±1.72	5.2
30 days					
Cadmium	121.950±24.684	68.298±9.4479	129.454±15.989	66.073±1.7280	12.8
Cadmium + zeolite	80.340±16.6143	49.670±6.8411	63.825±20.0236	20.618±2.9422	3

Ghiasi et al. published a similar study in 2015 that highlights the pathological effects of cadmium and the effectiveness of clinoptilolite zeolite in reducing its toxicity to the common carp (*Cyprinus carpio*). The fishes were subjected to a concentration of 30 ppb of cadmium, and the lesions were manifested in the kidneys and spleen. Researchers used 10 g of L-1 zeolite to reduce the exposure to cadmium.
The carps, with an average weight of 700 g, were randomly assigned to four groups and parked in 1000 l glass fibber tanks. The first group was the control group, zeolite was introduced into the tank of the second group, cadmium was introduced in the tank of the third group, and in the tank of the group four was introduced both zeolite and cadmium.
The results showed that adding clinoptilolite zeolite to cadmium-contaminated water reduces the toxic effects of cadmium. Decreasing the cadmium level in water and consequently in target tissues

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may be the cause of diminishing the histopathological changes of fish exposed to cadmium + zeolite compared to fish in the cadmium-only group. There is currently insufficient information on the optimal dose of zeolite required to reduce cadmium toxicity and new studies are needed.

Mutlu et al., in 2016, investigated the effects of copper sulphate and zeolite on biochemical parameters of common carp (*Cyprinus carpio*) blood, also confirmed by Uncumusaoğlu, in 2018.

The fishes were parked in four 100-liter aquariums, 10 fishes in each aquarium, underwent a 90-day experiment. The four aquariums included: the control group, one lot treated with 2 mg L^{-1} $CuSO_4$, one lot treated with 60 mg L^{-1} zeolite, and the fourth group treated with 2 mg L^{-1} $CuSO_4$ + 60 mg L^{-1} zeolite. The results shown that when zeolite was administered together with copper sulphate, an increasing of 46.24% in fish body weight was observed. Blood samples were taken to determine hematologic values (glucose, urea nitrogen, total protein, albumin, globulin, creatinine, total bilirubin, uric acid and direct bilirubin) at 30, 60, 90 days.

It has been observed that the use of zeolite in water has had the effect of preventing the toxicity of copper sulphate and therefore a safer environment for fish can be achieved as well as an increase in productivity.

4. CONCLUSIONS

The paper aimed to present the worldwide research on the use of clinoptilolite natural zeolite in aquaculture.

Zeolites improve technological parameters, water quality, and do not cause side effects in freshwater fish ponds.

Research on the use of zeolites as feed additives has highlighted the positive effects on improved growth performance, health status and, implicitly, survival rates.

Clinoptilolite has a high affinity for copper, lead and cadmium ions. With the use of zeolites, new water or waste water treatment technologies can be developed in the field of aquaculture.

Among the advantages of using natural zeolites are low costs for both their exploitation and the purification and obtaining of the desired particle size, they are spread in many areas of the world and, above all, are environmentally friendly materials.

5. ACKNOWLEDGEMENTS

This research work is a part of PhD thesis elaboration ”Research on the clinoptilolite use in carp growth with implications in water quality” and was carried out with the support of Faculty of Animal Production Engineering and Management, University of Agronomic Sciences and Veterinary Medicine of Bucharest.

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