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THE USE OF CLINOPTILOLITE ZEOLITE AS A FEED ADDITIVE IN JUVENILE CARP FEED (*Cyprinus carpio*)

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Abstract

The clinoptilolite natural zeolite can be used as a dietary supplement in fish breeding to improve nutritional parameters and maintain their health. In this regard, within the Aquaculture Laboratory of the USAMV Bucharest, an experiment was carried out with juvenile carp species (Cyprinus carpio). The fish, divided into three groups, were fed with 1% and 2% zeolite feed additive, and non-additive feed, respectively, for 10 weeks. The comparative analysis of the results obtained for the morpho-productive characters (live weight, total length and maximum body height) revealed that the group fed with 2% clinoptilolite additive feed, obtained the best performances. Clinoptilolite in feed has contributed at maintaining favorable media conditions for the growth and development of fish from the controlled systems used. Although there were no significant differences in medium performances, it was found that clinoptilolite positively influenced the studied characters.

***Key words**: aquaculture, feed, fish, morphological characters, zeolite.*

INTRODUCTION

Worldwide, aquaculture supplies for human consumption, over 50% of total fish production and due to the decrease of catches in fishing areas, this percentage will increase to 20% by 2032 (FAO, 2014). Global freshwater deficiency, strict regulations regarding quality of wastewater from fish farms and the limited space available are obstacles that need to be overcome for the development of aquaculture. The technologies used in aquaculture must ensure increased production and a minimal negative impact on the environment due to the toxic contaminants in the effluent waters of the recirculating systems.

Clinoptilolite, registered as a food additive - DIN53770, is declared safe for final consumers of meat, milk or eggs from animals that have zeolite in feed (EFSSA, 2007).

Due to their chemical and physical properties, zeolites and especially clinoptilolite, have a wide range of use. In recent decades, a lot of researchers have paid particular attention to the use of zeolites in biochemistry. The use of zeolite as a feed additive leads to growth rate improve and to maintenance of general state of health of animals, implicitly of the fish. In recirculating systems, fish feed, additive with zeolite, contributes to improving fish productivity, but is also a corrector of environmental conditions (Obradovic et al., 2006). Weight gain of biomass is the result of the detoxification effect of zeolite (Ortatatli and Oguz, 2001; Rizzi et al., 2003), and by slower passage of feed through the intestine, a better utilization of the nutrients is achieved (Dias et al., 1998; Eya et al., 2008). One of the important factors that influence the health of fish in recirculating aquaculture systems is the level of ammonia in the technological water (Badiola et al., 2012).

It is assumed that the rate of biomass growth is stimulated by suppressing the formation of ammonia, which is considered toxic in cells, and in the gastrointestinal tract of animals (Papaioannou et al., 2005). By using fish feed additive with zeolite, the amount of oxygen used in the oxidation of ammonia is reduced (Florian et al., 2002). The composition of the feed and the technological parameters of the water are determining factors of the sensory quality of fish meat.

Clinoptilolite has been used as an additive in fish feed at concentrations of 1 to 10% (Edsall and Smith, 1989; Yıldırım et al., 2009; Khodanazary et al., 2013).

In Romania, research on the use of clinoptilolite in animal husbandry has been carried out only since 2000. The results of the research highlighted the favorable effects of zeolite on the feed conversion coefficient (Pogurschi et al., 2017). The quality of milk production, animal health and welfare were improved by using the Romanian volcanic tuff rich in clinoptilolite as a food additive and as a supplement in bedding. The zeolite thus used has led to the provision of optimal technological conditions for the environment (Marin et al., 2018).

Research conducted in 2017 in the Aquaculture Laboratory of USAMV Bucharest showed that the use of clinoptilolite with the granulation of 1-3 mm, in column form, ensures the filtration of water from a controlled system (Sava et al., 2017; Nicolae et al., 2017).

MATERIALS AND METHODS

The experimental research, which was carried out in the Aquaculture Laboratory of the USAMV Bucharest, followed the study of influence of the feed additive with clinoptilolite zeolite on the development of juvenile carp (*Cyprinus carpio*). The duration of the experiment was 70 days. The controlled system used consisted of three aquariums with a capacity of 220 l each, the juvenile carp being distributed in them, in lots of 36 individuals (Figure 1).

The average weight of the fish was 28.28 g/pcs in aquarium 1, 29.28 g/pcs in aquarium 2 and 26.30 g/pcs in aquarium 3. Two of the fish groups were fed with zeolite feed additive in percentages of 1% (aquarium 1) and 2% (aquarium 2), and the third lot was the control group (aquarium 3), fed with non-additive feed with zeolite. The feed dose, administered in 3 rations, was 4% of body weight in the first 42 days and 5% of body weight in the next 28 days.

Clinoptilolite zeolite used as a feed additive is a hydrated crystalline aluminosilicate, with a frame-like structure containing pores occupied by water and alkaline cations that give it a high ion exchange capacity and molecular sieve properties. Table 1 shows the chemical composition of the zeolite used.

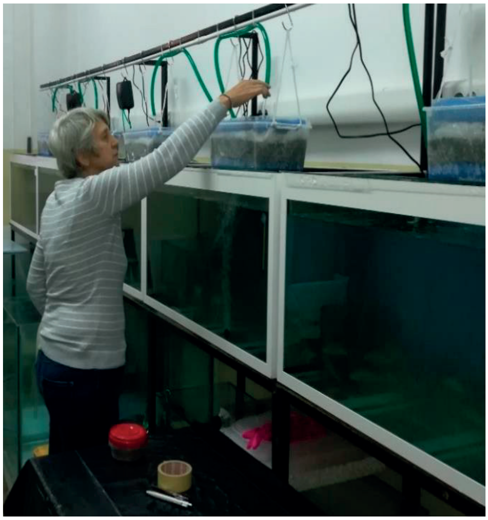

Figure 1. Controlled system for fish growth (original photo)

Table 1. The chemical composition of the clinoptilolite zeolite used

Compound	Percentage (%)
SiO_2	68.75-71.30
Al_2O_3	11.35-13.10
CaO	2.86-5.2
K_2O	3.17-3.40
Fe_2O_3	2.10-1.90
MgO	1.18-1.20
Na_2O	0.82-1.30
P.C.	9.77

When was the recipes made, the chemical composition of the ingredients and their digestibility level were taken into account, so that the level of catabolics released in the environment is as low as possible (Table 2 and Table 3).

It is known that the feeding method used and amount of daily ration are important elements, which condition the degree of acceptance and consumption of feed and efficiency of bioconversion (Misăilă, 2004).

Table 2. Raw materials in the used recipes

Raw materials	Recipe 1 - 1% zeolite	Recipe 2 - 2% zeolite	Recipe 3 - 0% zeolite
Fish meal 65%	25.00%	25.00%	25.00%
Corn	28.67%	26.63%	30.70%
Wheat	10.00%	10.00%	10.00%
Soy bean 46%	31.31%	31.68%	30.95%
Sunflower oil	2.81%	3.50%	2.13%
Zeolite	1.00%	2.00%	0.00%
Lysine HCl	0.13%	0.12%	0.14%
DL-Methionine	0.08%	0.08%	0.08%
Premix 1%	1.00%	1.00%	1.00%
Total	100.00	100.00	100.00

Table 3. The chemical composition of recipes

Nutritional value	Recipe 1 - 1% zeolite	Recipe 2 - 2% zeolite	Recipe 3 - 0% zeolite
Metabolizable energy	3000 kcal	3000 kcal	3000 kcal
Crude fat	7.02	7.64	6.41
Crude ash	6.58	6.58	6.58
Calcium	1.61	1.64	1.58
Total phosphorus	1.15	1.14	1.15
Crude cellulose	2.21	2.16	2.25
Crude protein	35.00	35.00	35.00
Total lysine	2.20	2.20	2.20
Digestible lysine	1.98	1.98	1.98
Total methionine	0.80	0.80	0.80
Digestible methionine	0.73	0.73	0.73
Arginine	2.20	2.20	2.19
Histidine	0.87	0.87	0.86
Isoleucine	1.47	1.47	1.47
Leucine	2.60	2.59	2.61
Phenylalanine	1.53	1.53	1.53
Threonine	1.36	1.36	1.36
Valine	1.68	1.68	1.68
Tryptophan	0.37	0.37	0.37

Also, through the administered food was aimed at maintaining the juvenile carp health as well as the quality of the water in the aquariums. Water filtration was performed using zeolite filters (Sava et al., 2017). Each filter used 4 kg of clinoptilolite, with a granulation of 2-3 mm. Zeolite was regenerated at 48 hours with saline solution (Nicolae et al., 2017).

Each individual was measured for three morphological characters: living body weight, maximum body height and total body length, at 2 weeks intervals. Body weight (W) was determined by weighing with a scale for small weights. Maximum body height (H) and total length (L) were measured using the graded line. Maximum body height was measured in the highest region of the body, at the level of the first radius of the dorsal fin (Nicolae et al., 2013) (Figure 2). The total length was measured on the midline of the body, from the tip of the muzzle to the midline joining the extremities of the two caudal lobes.

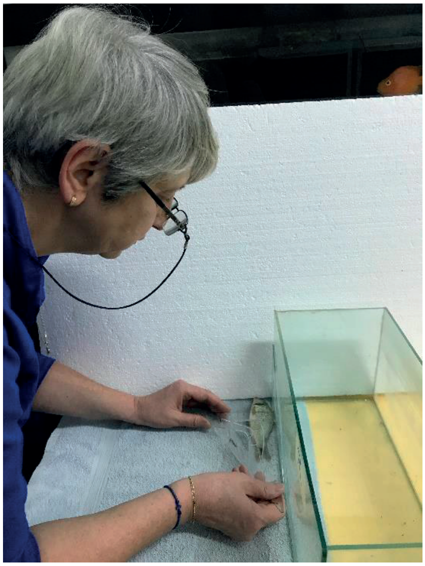

Figure 2. Maximum height measurement (original photo)

Statistical analysis was required to rank the categories of individuals taking into account the analysed characters: living weight, maximum body height and total length. The performance averages and their errors were established based on the following calculation relationships:

$$\overline{X} = \frac{\sum X}{n_x} \quad (1)$$

$$S_{\overline{X}} = \sqrt{\frac{S^2}{n_x}} \quad (2)$$

Where:

$\overline{X}$ = average;

ΣX = values sum;

n_x = values number/ specimen size/lot size;

$S_{\bar{x}}$ = average error;

S^2 = variant.

The Fisher test was used to determine whether or not there are significant differences between the groups of individuals made up of the amount of zeolite administered in the ration,

with respect to average body performance. Fisher value determination was done using variance analysis (ANOVA) with two sources of variation: intergroup and intragroup.
The chemical analyzes of the fish meat were carried out according to Regulation (EC) no. 152/2009 and ISO standards, by the gravimetric method for the dry substance, the Kjeldahl method, using a semi-automatic KJELTEC 2300 auto system - Tecator (Sweden), for crude protein, the organic solvent extraction method for crude fat and the gravimetric method for ash.

RESULTS AND DISCUSSIONS

At two-weeks intervals weighing was performed and the total length and maximum height of the fish body in the three aquariums were measured.
The results obtained after performing the 5 weightings are presented in Table 4.

Table 4. The average performances determined for live weight

Specification	N	Live weight (W), g $\overline{X} \pm s_{\overline{X}}$				
		Interval 1 (2 weeks)	Interval 2 (2 weeks)	Interval 3 (2 weeks)	Interval 4 (2 weeks)	Interval 5 (2 weeks)
Aquarium 1	36	29.33±0.80	31.71±0.98	34.97±1.14	37.20±1.35	40.85±1.75
Aquarium 2	36	29.36±0.87	32.78±0.97	34.83±1.12	39.39±1.37	42.61±1.63
Aquarium 3	36	27.14±0.84	31.42±0.98	33.31±1.17	36.25±1.34	38.42±1.56
Total	108	28.61±0.48	31.97±0.56	34.36±0.65	37.62±0.78	40.63±6.87

Interval 1 (first two weeks of the study)
Comparing the performances recorded by individuals from each aquarium it can be observed that the average body weight of the individuals in the control aquarium deviates the most from the statistical population average, respectively by 1.47 g, this group of individuals achieving the lowest performance.
The averages of the performances achieved by individuals from the aquariums where zeolite was administered were close, the difference between the two aquariums being only 0.03 g.
Interval 2 (weeks 2-4 of the study)
Analyzing the registered performances, it can be observed that between all three aquariums there are very small differences, of maximum 1.36 g, the smallest weight being established for the control group.
Interval 3 (weeks 4-6 of the study)
The performance averages established for each group show that between groups there are very small differences, respectively between the average of the lowest performing group and the statistical population average being 1.05 g.
Interval 4 (weeks 6-8 of the study)
The best weight was determined for the aquarium 2 group, which achieved a performance with 2.19 g more than in the other aquarium where additive feed was administered with 2% clinoptilolite and 3.14 g more than the control group.
Interval 5 (weeks 8-10 of the study)
The evolution of body weight in the last study interval is similar to the situation presented for interval four. Individuals who received 2% clinoptilolite in ration differ from the control group by an additional 4.19 g. The poorest performance was determined for the control group, which achieved with 2.21 g less than the statistical population average.
Simultaneous analysis of the performances recorded in all three aquariums, throughout the experiment showed that the individuals in the aquarium where zeolite was administered 2% in ration achieve the best weights in 4 of the 5 intervals, and the lowest performances were established for the individuals in the control group (Figure 3).

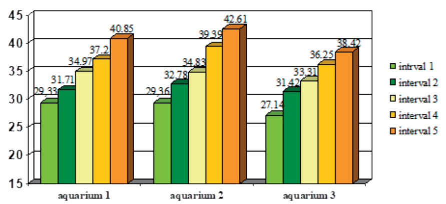

Figure 3. Diagram of live weight recorded performances

It can conclude that the administration of 2% clinoptilolite influences the character of the study, live weight. Regarding the second character that represented the object of this study, respectively the total body length, in the first interval, the best performance is described for the group of individuals in the aquarium 2 (Table 5).

Table 5. The average performances determined for total body length

Specification	N	Total body length (L), mm				
		$\overline{X} \pm s_{\overline{X}}$				
		Interval 1	Interval 2	Interval 3	Interval 4	Interval 5
Aquarium 1	36	11.85±0.12	12.13±0.34	12.76±0.13	13.15±0.15	13.49±0.17
Aquarium 2	36	12.13±0.11	12.47±0.11	12.79±0.12	13.30±0.13	13.62±0.14
Aquarium 3	36	11.81±0.13	12.09±0.13	12.45±0.15	13.01±0.16	13.10±0.18
Total	108	11.83±0.07	12.12±0.13	12.66±0.08	13.15±0.08	13.40±0.09

Individuals in aquarium 1 achieve a total length of only 0.05 mm longer than the control group, for which the lowest performance was established. The situation is similar for next four intervals.

The largest difference was observed in the second interval, respectively in Aquarium 2. The average of total body length was 0.35 mm higher compared to the average calculated for the statistical population (Figure 4).

The averages of performances recorded by all three groups of individuals for the maximum body height character are very close (Figure 5). Between the best and the poorest performance, a difference of only 0.1 mm in the first interval, 0.06 mm in the second, 0.11 mm in the third, 0.16 mm in the fourth and 0.17 mm in the last interval was achieved (Table 6).

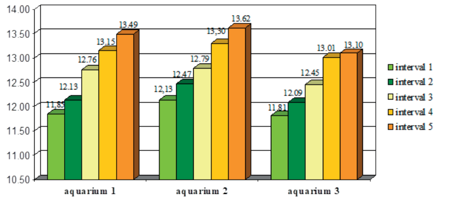

Figure 4. Diagram of total length recorded performances

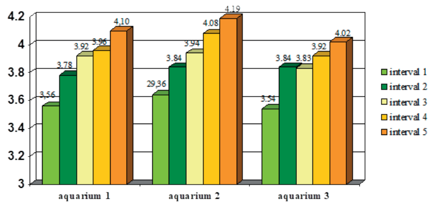

Figure 5. Diagram of maximum body height recorded performances

Table 6. The average performances determined for maximum body height

Specification	N	Maximum body height (H), mm				
		$\overline{X} \pm s_{\overline{X}}$				
		Interval 1	Interval 2	Interval 3	Interval 4	Interval 5
Aquarium 1	36	3.56±0.04	3.78±0.05	3.92±0.05	3.96±0.05	4.10±0.07
Aquarium 2	36	3.64±0.05	3.84±0.06	3.94±0.06	4.08±0.06	4.19±0.07
Aquarium 3	36	3.54±0.04	3.84±0.04	3.83±0.04	3.92±0.05	4.02±0.06
Total	108	3.58±0.03	3.82±0.03	3.90±0.03	3.10±0.03	4.10±0.04

The results of the test of the significance of the differences between the averages of the performances for weight, length and height, determined for the three groups, taking into account the evolution in each interval, are presented in Table 7.

Table 7. Fisher test for studied characters

Character	Period	The average of the squares intergroups	The average of the squares intragroups	Fisher test
Live weight	Interval 1	58.528	25.320	2.311ns
	Interval 2	18.400	34.213	0.538ns
	Interval 3	30.587	46.477	0.658ns
	Interval 4	93.192	66.143	1.409ns
	Interval 5	159.728	97.034	1.646ns
Total length	Interval 1	1.106	0.501	2.207ns
	Interval 2	1.519	0.543	2.797ns
	Interval 3	1.291	0.649	1.989ns
	Interval 4	0.795	0.790	1.006ns
	Interval 5	2.654	0.957	2.773ns
Maximum body height	Interval 1	0.095	0.078	1.218ns
	Interval 2	0.037	0.083	0.446ns
	Interval 3	0.129	0.101	1.277ns
	Interval 4	0.253	0.116	2.181ns
	Interval 5	0.276	0.167	1.652ns

The calculated Fisher values for all three characters are smaller than table values. This shows that between all three groups of individuals there are no significant differences in terms of average performances, in any of all three characters studied. The low values of morpho-productive parameters of juvenile carp from the experience are due to the relatively low water temperature (18-20°C), the experiment being carried out between November 2019 - January 2020.

At the end of the study period, of 10 weeks, the chemical composition of fish meat was analysed, in terms of the proportion of dry matter (DM), crude protein (CP), crude fat (CF) and ash (A). The results obtained are presented in Table 8.

Table 8. Chemical analysis of fish meat

Zeolite (%)	DM 65°C (%)	DM103°C (%)	CP (%)	CF (%)	A (%)
0	27.78	96.26	58.67	28.99	5.78
1	27.03	96.93	60.01	28.25	5.60
2	27.80	96.85	58.52	31.50	5.30

Chemical analysis of juvenile carp meat showed that the highest percentage of crude protein was recorded by the group fed with feed additive with 1% zeolite. The group fed with feed additive with 2% zeolite had the highest percentage of crude fat.

CONCLUSIONS

In the present study the influence of the feed additive with the clinoptilolite zeolite on the development of juvenile carp (*Cyprinus carpio*) was highlighted.

Even if there were no significant differences by comparing the recorded performances, it can be concluded that the administration of clinoptilolite influences productive characters.

The best results regarding live weight, total length and maximum body height characters were achieved by the group fed with 2% zeolite recipe.

It is important to note that no deaths were recorded during the experiment.

Research has shown the benefits of using clinoptilolite as a feed additive in juvenile carp feed.

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