Current fisheries crisis and the rising demand for animal protein have increased consideration of aquaculture as a replacement for wild fisheries. However, aquaculture production is vulnerable to impacts of disease and environmental conditions that in recent years have affected fish farms globally resulting in partial or sometimes total loss of production.

Anima Sanumparticularly as food supplement ensures: increased **bioavailability**, more efficient **adsorption capacity** and higher **cation exchange capacity**.

Zeolites and Agriculture

There are nearly 50 different types of natural zeolites, and more than hundred synthetic ones with varying physical and chemical properties. They are used in a variety of applications, like petrochemical cracking, water softening and purification, the separation, removal of gases and solvents, in construction, agriculture, animal husbandry and as a humanfeed supplements. Different types of Zeolites are used as feed supplements for humans and animals. Crystal structure and chemical composition account for the primary differences.Zeolites can act as ion-exchangers. Due to high total volume of zeolite cavities (so-called micro-pores), zeolites can play a role as absorbers of various small molecules (absorbents; molecular sieves) whose size is smaller than that of pores. Additionally, zeolites can have various defects in above-mentioned ideal structure forming larger irregular cavities of higher dimensions: mesopores from 5-20 Å; and macropores from 20-100 Å. In this manner, zeolites can act as adsorbents of large molecules and various other particles (e.g. bacteria and viruses). Adsorbates are bounded in these larger cavities as well as various semi-cavities on the surface of the zeolite particles. For example, several scientific studies prove that zeolite, due to its profound absorption and adsorption actions, acts as strong agent for removal of harmful compounds such as mycotoxins, polycyclic aromatic hydrocarbons, nitrosamines, arsenic compounds, harmful amines, dioxins, ROS (particularly hydroxyl radical) and many other types of toxins. Furthermore, natural zeolite **Clinoptilolite** has been used as effective adsorbent of radioactive waste, particularly various radionuclides from water. More important, Clinoptilolite was substance of choice for animal radionuclide poisoning protection, where it clearly demonstrated affective adsorption of radionuclides.

The zeolite group has emerged as having considerable potential in a wide variety of agricultural processes. The unique ion exchange, dehydration-rehydration, and adsorption properties of zeolite materials promise to contribute significantly to many years of agricultural and aquaculture technology[1]
Most of the initial research on the use of zeolites in agriculture took place in the 1960s in Japan, Japanese farmers have used zeolite rock for years to control the moisture content and malodor of animal wastes and to increase the pH of acidic volcanic soils. The addition of small amounts of the zeolites clinoptilolite and mordenite to the normal protein diet of pigs, chickens, and ruminants gave noticeable increases in the body weight and general health of the animals[2]. The use of zeolites in rations also appeared to reduce odor and associated pollution problems and to provide a means of regulating the viscosity and nitrogen retentivity of animal manure.

[1]Mumpton, F. A., and Fishman, P. H., “The Application of Natural Zeolites in Animal Science and Aquiculture, ” J. *Anim. Sci. 45, 1188-1203,1977ž*
[2]Minato, Hideo, “Characteristics and Uses of Natural Zeolites”, Koatsugasu 5, 536-547, 1968.

Effect of Dietary Inclusions of Clinoptilolite in husbandry animals

Clinoptilolite is not the most well known, but is one of the more useful natural zeolites. Clinoptilolite is used in many applications such as a chemical sieve, a gas absorber, a feed additive, a food additive, and an odor control agent and as a water filter for municipal and residential drinking water and aquariums. Clinoptilolite is well suited for these applications due to its large amount of pore space, high resistance to extreme temperatures and chemically neutral basic structure, what might strike many as odd is the food and feed additives. Clinoptilolite has been used for several years now as an additive to feed for cows, pigs, horses and chickens. It absorbs toxins in the feed that are created by molds and microscopic parasites and has enhanced food absorption by these animals. Similar uses in actual people food are being tested. Clinoptilolite can easily absorb ammonia and other toxic gases from air and water and thus can be used in filters, both for health reasons and for odor removal.
Clinoptilolite belongs to the class of huge family of zeolites alumino-silicates. Basic structural motif of zeolites represents SiO_4tetrahedra. Tetrahedra build complex tridimensional silicate skeleton (network) closing relatively huge cavities, accommodating cations. Like in other zeolites, cations in clinoptilolite are exchangeable. For example, sodium or calcium cations can be exchanged by mercury or lead cations. Dietary inclusions of clinoptilolite could be beneficial for animal production. Pigs fed clinoptilolite experience beneficial weight gains and are less subject to disease than pigs fed normal diets. They also show regular digestion, an appetite increase, and the meat-content increases at the expense of the fat. Clinoptilolite, chabazite, mordenite, erionite and phillipsite actively adsorb ammonia, carbon dioxide, hydrogen sulphide and mercaptanes and have a strong deodorizing effect. It is also possible that zeolites remove toxins and create changes in enzymology and immunological responses. All these events have resulted from application of zeolites in the animal production industry.

Piglets aged 27 days were for four weeks fed the natural Clinoptilolite mannelite as 2% of their feed, corrected for nutrient dilution. Mannelite gave a tendency for higher growth and lower feed-to-gain ratio. When the diet was not corrected for nutrient dilution, the piglets showed significantly higher growth and a better feed-to-gain ratio (corrected for differences in nutrient concentration of the diets) over the total experimental period. They were able to compensate for the energy diluting effect of mannelite addition by increasing their feed intake. Authors concluded that dietary dilution of piglet feed with 2% mannelite significantly increased daily gain and decreased feed-to-gain ratio, corrected for nutrient dilution[3].
70% Clinoptilolite, elevated nitrogen excretion in faeces and lowered nitrogen excretion in urine.
Therefore, dietary inclusion of Clinoptilolite for growing pigs changed the excretion in urine without altering protein deposition[4,5].
Single-combed, 16-week-old pullets of 3 strains were fed a diet containing 135 g protein/kg with or without 50 g Clinoptilolite/kg. Sterile river sand replaced Clinoptilolite in the control diet in order to keep the diets isoenergetic. Significant dietary effects of feeding clinoptilolite were observed with improvement in number of eggs laid per hen, shell thickness, efficiency of food utilization, and droppings moisture content. No significant dietary effects between treatments were observed with body weight, age at first egg, egg weight, food intake of hen, and rate of amino acid absorption of radioactive lysine and methionine into the bloodstream[6].

Effect of Clinoptilolite on BroilerChicken Production and Aflatoxicosis

Zeolite as a feed additive in broiler chickens diets increase performance and feed efficiency and decrease ammonia levels in the poultry houses. Often the ammonia levels in poultry houses exceed 100 ppm close to lethal for growthers and birds. High ammonia levels decrease gradable weight gain in broiler chickensoperations and egg production in layers. Adding zeolite in broiler chickens diet lead to decrease primarymortality, rapid growth of chickens, and aflatoxin control in broiler chickens and reduce bedder moisture. Many farms have eliminated most of their odor and realized greater bird health, welfare and production by feedingzeolite between 0.5 to 2 % of the total ration. Feed efficiency was markedly higher at all levels of zeolitesubstitution in broiler chickens.

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[3] A Veldman, PJ Vanderaar.Agribiol Res – Z AgrarbiolAgrikultOkol 50:289-294, 1997
[4] HD Poulsen, N Oksbjerg.Animal Feed Science Technology 53:297-303, 1995.
[5] J Mojzis, NF Kovac, G Mojzisova. Vet Human Toxicol 36:533-535, 1994.
[6] MD Olver. British Poultry Science 38:220-222, 1997

Clinoptilolite incorporated into the diet can reduce the deleterious effects of aflatoxin because it strongly adsorbs aflatoxins and zearalenone [7].Mineral adsorbents based on natural zeolite and bentonite may be used in animal diets in order to prevent poisoning caused by mycotoxins. The addition of clinoptilolite to the aflatoxin diet reduced the adverse effects of aflatoxin and should be helpful in a solution to the Aflatoxicosis problem in poultry[8].

Zeolite and Aquaculture

At this stage, the number of published papers dealing with "zeo aquaculture " is quite small, and hard data are few; however, the potential of these materials in such areas is apparent, and zeolites show promise of contributing directly to increased aquaculture productivity in the years to come. In addition to that, in aquaculture there are many results on the biological benefits of adding zeolites to fish feeds.
Clinoptilolite, one of the types of zeolites or zeolites itself, are used in aquaculture applications to provide pollution control in ponds; to remove N-compounds from water of hatcheries, fish transport and aquariums; to increase oxygen in aquarium and fish transport; and to increase growth parameter values of fish by adding into feed.
Study by American Society of Animal Science [9]showed that ion-exchange and adsorption properties of natural zeolites can be exploited to make more efficient use of feed nitrogen in animal nutrition, to reduce intestinal diseases, to control moisture and ammonia content of animal manure, to purify recirculating hatchery waters in aquaculture, to provide oxygen-enriched air for fish breeding and transportation, and to reduce the nitrogen content of feedlot- and hatchery-runoff waters.

Anima Sanum and Fish Nutrition

Intensive aquaculture continues to expand, which requires high quality protein sources. Fishmeal is major and increasingly expensive component of salmon and trout feeds, since it has high levels of digestible protein and energy, excellent amino acid and fatty acid profiles (Ozogul*et al.*, 2006)[10].
Although there is lack of published papers on zeolites/clinoptilolite in fish feed, the physiology of fish and poultry is remarkably similar, and if the results achieved with chickens can be duplicated with fish, the substitution of small amounts of inexpensive zeolites in normal fish food, with no adverse change in growth and perhaps a small increase in feed efficiency, could result in considerable savings.
The quality of the water in recirculating systems should also be improved by the use of zeolite-supplemented food, as should that of the effluents.
The study conducted in Serbia[11]has researched the influence of zeolite type as a feed supplement for trout. Zeolite has been applied in a concentration of 1%. Moreover, the influence of zeolite type was researched as a corrector of environmental conditions on production of Rainbow trout (*Oncorhynchusmykiss*Walbaum). The results have shown that zeolite as a feed supplement had a positive effect on fish growth, their final weight and final length. Zeolite introduction has resulted in 13.62% lower feed conversion ratio (mass of feed vs. mass of produced fish). Introduction of zeolite as a corrector of environmental conditions led to slight decrease of numerical values of total water hardness; nitrate and ammonia.
Leonard[12] reported preliminary results of experiments where 2 percent Clinoptilolite was added to the normal 48 percent protein food of 100 rainbow trout: after 64 days, a 10 percent improvement in the biomass increasewas noted, with no apparent ill effects on the fish (
Table 1Effect of Clinoptilolite Additions to the Diet of Trout).

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[7]Tomasevic-Canovic, M Dumic, O Vukicevic, M Duricic, S Jovanovic.ActaVeterinaria 46:227-234, 1996
[8]H Oguz, T Kececi, YO Birdane, F Onder, V Kurtogha. Res Vet Sci 69:89-93, 2000.
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[11]The application effects of natural zeolite in feed and water on production results of OncorhynchusMykiss (Walbaum), Obradovic, Adamovic, Vukasinovic, Jovanovic, Levic, Roumanian Biotechnological Letters Vol. 11, No. 6, 2006, pp. 3005-3013, Bucharest University
[12]Leonard, D.W., 1979.The role of natural zeolites in industry. Transactions of the Society of Mining Engineers A.I.M.E. Preprint, 79: 380-401

	Control 100% normal feed[b]	Test 98% normal feed 2% clinoptilolite
Average starting weight (g)	10.2	10.1
Average 64-day weight	48,6	52.3
Average weight gain	38.4	42,2
Mortality ... ~ ~	4	3

a100 rainbow trout.
bStandard 48% protein fish food

Table 1Effect of Clinoptilolite Additions to the Diet of Trout

Smith (Smith, R.R., 1980) reported that the addition of 10% sodium bentonite to a commercial trout feed increased the growth rate of rainbow trout *(Salmogairdneri*) by 14% and reduced the amount of feed required per unit of weight gain by 20%. Also, Reinitz (Reinitz, G., 1984) demonstrated that dietary inclusion of sodium bentonite, at 5, 10 and 15% adversely affected weight gain in rainbow trout. [13]
Results of the study conducted in Department of Biology, West Virginia State University, USA[14]indicated that dietary bentonite and mordenite supplementation improved weight gain, feed efficiency, specific growth rate and body protein deposition in juvenile rainbow trout.
Also scientific study[15]results showed that zeolite was able to protect fish against lead toxicity by decreasing the adverse effects of lead nitrate. Because of the high cation exchange capacity of zeolite, ionic lead may replace other cations on zeolite, thus becoming less available to fish.
Study at Tuncely University[16], Fisheries faculty, Turkey demonstrated that fatty acid compositions are improved by adding Clinoptilolite to fish feed.
The preventive effect of zeolites on the intoxication of organophosphate poisoning has been described[17]. Zeolite tuff containing 61% clinoptilolite has been shown to prevent and eliminate organophosphate poisoning. The low resorption rate from the gastrointestinal tract creates conditions for inclusion of natural zeolites in the arsenal of rational prevention of organophosphate poisoning.

Anima Sanum -Why is particle size important:Milled vs. Micronized Zeolite/Added Value to Fish Nutrition

New physical properties, bioavailability and bio-efficacy of solid substances are often intrinsically related to the primary particle size and proportion of amorphous surface area. The natural zeolite, crystal structure (crystallite) and framework limit the particle size. Too large of a particle size distribution profile limits the bioavailability and detox properties. Too small particles (e.g., in the sub-micron and nano-range) reduces adsorption and cation exchange capacity (CEC), because the crystal structure, will be when the particles are ground or processed to particle sizes smaller than the basic, crystallite. Therefore, to achieve maximum bio-function, bio-effect, it is a trade-off, between finer particle size and effectiveness per mg dose of the zeolite. As the particles decrease in medium size (diameter) from macro particles to micro particles, then smaller to colloidal and the nanoparticle range the surface area (S) decreases as a (A^2) square function and volume (V^3) as cubic function and the S/V Ratio becomes larger.
Thus, many attempts have been conducted to obtain good bioavailability achieved by creating amorphous product. Particle size reduction by top-down processing i.e. milling is one of various strategies for improving solubility and reactive characteristics of poorly water-soluble ingredients. When zeolite rocks are “milled”, they are grinded to powder. This is normally takes place at the mine and it

[13] Effects of Dietary Zeolites (Bentonite and Mordenite) on the Performance Juvenile Rainbow trout *Onchorhynchusmyskis*, Jonathan C. Eya, Andrea Parsons, Iyerusalem Haile and PremalathanJagidi, Australian Journal of Basic and Applied Sciences, 2(4): 961-967, 2008
[14] Effects of Dietary Zeolites (Bentonite and Mordenite) on the Performance Juvenile Rainbow trout *Onchorhynchusmyskis*, Jonathan C. Eya, Andrea Parsons, Iyerusalem Haile and PremalathanJagidi, Australian Journal of Basic and Applied Sciences, 2(4): 961-967, 2008
[15]Protective role of zeolite on short- and long- term lead toxicity in the teleost fish *Heteropneustesfossilis*, Subodh Kumar Jain, Chemosphere, 39(2): 247-251, 1999
[16]Fatty acids Profiles of Rainbow Trout (*Oncorhynchusmykiss*Walbaum, 1792), Fed With Zeolite (Clinoptilolite), D.Danabas, The Journal of Animal and Plant Sciences, 21(3), 561-565, 2011
[17]Mojzis, F Nistiar, G Kovac, G Mojzisova. Vet. Med. 42: 443-449, 1994.

the cheaper way to create powder. The problem with the milling process is that it crushes the zeolite cage structure rendering the zeolite un-absorbable in the body and largely less effective. However, certain particular dynamic micronization has been recognized as one of the most effective way to improve dissolution behavior.

More recently Clinoptilolite nutrientsupplement, nutraceuticals and heavy metals, detox products have been introduced into the market place for humans. However, micronized product has the advantage for metals detox, because of the micronized particle size range, micro-crystal structure and ultrahigh surface area, which translates to: a) more efficient adsorption per gm of mineral; and b) higher CEC (meq/gm) loading cations (e.g., As, Hg, Pb, Cd, Cu, Zn, etc.), nutritional and bio-medical application.

Micronizing of clinoptilolite increases specific, amorphous and mesoporous surface area, enhancing adsorptive and detoxifying properties[18]. It is possible to enhance the NH_4^+retention capacity of natural clinoptilolite just by decreasing particle size without incorporating any further exchangeable cations within the framework of zeolite[19]. Micronized zeolite Clinoptilolite (MZC) particles below 5 microns can be entrapped within intestinal payer's patches via micro fold cells and exhibit lymphocyte response[2021]followed by cascade of superantigens immunization signals (**Figure** 1).

These effects (immune modulation) were confirmed in both animal and human studies.[30,22]In mice, due to immune stimulation, MZC exhibited anti metastatic effect[30]. Finally, MZC exhibited excellent protective and regenerative effect on liver [31,23,24,25]and in patients with burn wound trauma[26]

Although micronized zeolites have not yet been introduced to aquaculture on a larger scale, there are a number of sources on the benefits of introduction in aquaculture. The most important effect in fish farming is the strong ability to remove ammonia. Ammonia is an unavoidable excretion by fish and its accumulation in fish production systems has numerous negative influences. Ammonia is very toxic to fish, in smaller concentrations it can cause reduced growth, poor feed conversion and reduced disease resistance; in higher concentration it can be lethal to the fish. Furthermore, its ability to catch various toxic elements and microorganisms reduces their concentration in fishponds. Micronized zeolites in fish feed have beneficial effects on fish digestion system, it restores intestinal flora and improves the transport of enzymes resulting in decreased body fat in fish meat and improves fatty acids profile.

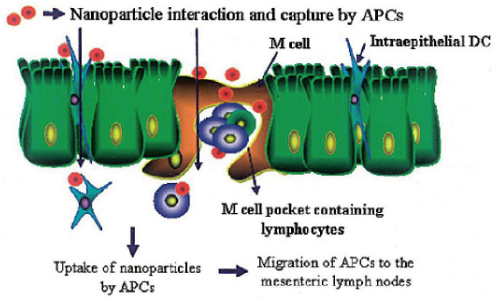

Figure 1 .Zeolite immune stimulation

Veterinary Faculty in Zagreb [27] has conducted a research of the use of Anima Sanumas a supplement to fish feed. Study has shown that AS supplement has

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significant impact on fish health, better immunity and overall better condition. Given in the dosage of 6-8% of total daily feed, zeolite supplement had three major effects on fish:

1. Antitoxic effect,
2. Higher growth rate and
3. Lower mortality rate.

The study has shown **19% higher growth rate** and **improvements in fish meat quality.**
Moreover, micronized zeolite clinoptilolite have **reduced mortality rate by 14%. Total production has increased by 35%**. In addition to high impact on fish production and fish meat quality, zeolite has also beneficial impact on environment, significantly reducing ammonia, toxic elements and microorganisms in fishponds and reducing fresh water needs.
Zeolite adsorption capacity is directly proportional to the specific surface of the micronized zeolite particles. Current technology in grinding zeolite Clinoptilolite demonstrates increase of specific surface of the material, which obviously leads to increased adsorption capacity.

Conclusion:

Studies and field-testing indicates the fact that Anima Sanum as supplement to fish feed has several significant beneficial effects:

- Antitoxic effect: ammonia removal, purification of pond water
- Better growth and FCR: restores intestinal flora and improves the transport of enzymes resulting in decreased body fat in meat and better food assimilation
- Higher survival rate: reduced mortality
- Supports immune system
- Improves fatty acid profile
- Better meat quality
- Protect against heavy metal absorption and elimination of organophosphate poisoning
- Prevent mycotoxins poisonings

Anima Sanum technology provides answer to major challenges in conventional and organic fish farming: to get safe products without heavy metal or organophosphate poison, reduce mycotoxins and use of antibiotics,reduce the dependency on fishmeal and fish oil, better feed conversion ratio and less environmental impact due to decreased need for forage fish in feed production. Anima Sanum also provides solution to feed and feeding strategies in aim to grow a healthy fish fast at the lowest possible cost.