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Effects of Dietary Cure Containing AFIR (Antioxidant Far-Infrared Energy Radiating) Ceramics on Living Body of Hatchery Sea Bream (*Pagrus Major*)

Kikuji Yamashita [1], Shine-Od Dalkhsuren[2], Dolgorsuren Aldartsogt [2],Kaori Sumida[1], Tsuyoshi Morita [1], Otto Baba[1]

[1] *Departments of Oral and Maxillofacial Anatomy, Graduate School of Biomedical Science, Tokushima University, Tokushima, Japan*

[2] *Departments of Gross Anatomy, School of Bio-Medicine, Health Sciences University of Mongolia, Ulaanbaatar, Mongolia*

Abstract: ***Objective;*** *We investigated the effects of oral administration of AFIR (antioxidant far-infrared energy radiating) ceramics mixing food expected flow acceleration on the living body of hatchery sea bream.* ***Design and methods;*** *Ten thousands sea breams were separated to FIR group with the AFIR ceramics powder mixing food and control group with the usual food. After one month, skin, scale, muscle and liver were histologically analyzed and the metabolic enzyme activities of muscle and liver were measured.* ***Results and Discussions;*** *Various changes as promotion of appetite, disappear of blood congestions, well contracting muscle, actively absorbed scales and hypertrophic skin were caused by oral administration of AFIR ceramics mixing food. The lipid, protein, energy and sugar metabolisms of fish meat were all activated by eating the AFIR food. Still more, expanded blood vessels were observed and the energy, lipid and protein metabolisms in liver were intensely activated by eating the AFIR food. From the above results, the blood flow was promoted and the calcium spouted from scales stimulated muscle and internal organ to accelerate the metabolic function in whole bodies.* ***Conclusion;*** *FIR energy radiation by oral administration of AFIR ceramics mixing food activated the blood circulation to accelerate intensely the calcium metabolic from scales to muscle and the energy, lipid and protein metabolism in muscle and liver of hatchery sea bream.*

Keywords: *antioxidant far infrared ray, metabolic enzyme activity, pagrus major*

1. INTRODUCTION

It is an obvious truth which the blood circulation is indispensable for maintaining life of an animal. If any difficulty runs into living body, the blood circulation frequently fails to flow perfectly. Actually, it was reported that a high-fat diet [1], pain [2] and neuro-degeneration [3] cause each abnormal conditions of blood circulation. On thinking from revers side, it was suggested that the advancing blood circulation activated the skin, muscle, bone, internal organ, brain and other organs to facilitate rapidly the condition of health. It was experimentally demonstrated that the up-regulating blood circulation accelerated the differentiation of nephron progenitor in kidney [4]. Then, it was reported that the placentas of hypertensive rats had a higher number of blood vessels to maintain the normal function of placenta after an exercise-training program [5]. Still more, it was shown that exposure of an aged animals to young blood could counteract aging and reverse pre-existing effects of brain aging at the molecular, structural, functional and cognitive level [6]. In this way, it was become to understand how important was the up-regulated blood circulation to accelerate the function of life.

As the hint of means for the keeping up-regulation in blood circulation, pulsed electromagnetic field therapy showed to produce a significant reduction in prostatic volume, testosterone levels or libido with improvement of blood supply on benign prostatic hyperplasia in dogs [7]. Then, we focused on far infrared (FIR) ray as one of the electromagnetic wave for the means of up-regulation in blood circulation. At ordinary temperature, most materials radiated the energy of FIR ray at 5～50 μ m. But, in order to aim biomaterials and medical developments using the fine powder to radiate efficiently and stably FIR energy, natural ore was thought to be the most suitable considering cost, productive capacity and various conditions. Still more, as some natural ore with antioxidant effects being beneficial for living body could be easy obtained, the rhyolite was selected as the AFIR ceramics in the present study. In our previous studies, as FIR energy

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activated the motility of water by stimulating the stretching and bending vibration, it was clarified that AFIR ceramics activated water molecules to accelerate fluidity, volatility and solubility of water [8, 9]. Then, it became evident which the blood circulation was hastened by the activated water and protein molecules in peripheral blood around skin of body surface by radiation of FIR energy. Actually, it was experimentally confirmed that fiber and cream kneaded with AFIR ceramics and touching with human hands as good radiator of FIR accelerated blood circulation [8, 9].

On the other hands, it was clarified that the oral administration of AFIR ceramics with food did not accelerate vertical growth of bone by endochodral ossification, but lateral growth by intramembranous ossification form thick bone [8, 9]. It was thought by these results that oral administration of AFIR ceramics with food strongly affected the FIR reaction to accelerate bone growth by reason why digesting food fever in alimentary canal. To sum up, oral administration of AFIR ceramics with food could be the excellent means strongly affecting FIR power to living body. Then, in the present study, in order to clarify which AFIR ceramics can utilize to activate the culture industry of fish or not, we analyzed effects of oral administration of AFIR ceramics with food on hatchery sea bream as the representation of culture fish.

2. MATERIALS AND METHODS

2.1 Preparation of AFIR (Rhyolite) stone and powder, and handling method for snails and killifish

Two water tanks filled up with ultrapure water of 2.4Lwere separated to control and FIR groups. The AFIR stone (Rhyolite, Matera Inc. Toonn, Ehime) of 2.4g (0.1 %) were spreader at the base of water tank of FIR group. Control group was without any ceramics. The 22 mud snails were each kept in the water tank of both groups at 27℃ and the survival rate was investigated.

Sixty Japanese killifishes were purchased and separated to three groups, AFIR stone group with three stones (average10.92g, 0.36 %), AFIR powder with the peak of 5μm in 1~10μm particle diameter (50g of average particle diameter 10μm, 1 %) and control group without any FIR ceramics with 9L of ultrapure water.

2.2 Analysis of soluble components in rhyolite as AFIR ceramics

500g of Rhyolite in 1 litter pure water were kept staring for 18 hours at room temperature. Supernatant liquid of 600 ml was retrieved by pass through the syringe filter of 0.45μm (close filter, Sartorius, Tokyo) after centrifugation on 3000 rpm for ten min. The supernatant liquid was dried with dry heat sterilizer (SG600, Yamato Science, Tokyo) on 200 ℃ for 3 days. White powder of 13.4 g was obtained after drying and analyzed the components by the x ray diffraction (EDAX, Ametek, Tokyo).

2.3 Preparation of food containing AFIR powder, handling and feeding method for hatchery sea bream

The AFIR powder (Matera Inc. Toonn, Ehime) was mixed at 0.06 % with the usual food for cultivating sea bream. Ten thousands sea breams were separated to FIR and control groups. FIR and control group were feuded with AFIR powder containing food and the usual food for cultivating sea bream respectively. These sea breams of both groups were handled for 1 months and applied for this research then.

2.4 Observation of fish skin and scale with scanning electron microscope (SEM)

The five skin samples of different position from each two sea breams of both groups were observed by the naked eye. Still more, ten scales of different position from each two sea breams of both groups were fixed with 1.5 % glutaraldehyde and paraformaldehyde in 0.1M phosphate buffer (pH7.4). Then, the samples were post-fixed with 2 % osmium oxide in 0.1M phosphate buffer (pH7.4). After fixation, the samples were dehydrated with alcohol and dried by the critical point dryer (HCP-2,Hitachi, Tokyo). After the gold evaporation (Eiko engineering, Tokyo), the samples were observed with SEM (JSM-6335F, Jeol, Tokyo).

2.5 Histological analysis of muscle and liver of sea bream

The muscle and liver samples from five different position of each two sea breams in both groups were fixed with 10 % formalin and embedded paraffin through the generally methods. Thin section of 5 μm was cut from paraffin block to be observed by the light microscope (BX51, Olympus, Tokyo, Japan).

2.6 Analysis of metabolic enzyme activity in muscle and liver of sea bream

In order to estimate the metabolic activity of muscle and liver of sea bream, the activity of enzyme related with energy, lipid, protein, sugar metabolism were

measured using by the enzyme activity measurement kit for research (Apizym, Biomerieux industry, Tokyo Japan). Each five samples of muscle and liver in each two sea breams in both groups were homogenized for sample solution. The sample solution of 65µl was poured into the reaction well composed with 19 enzymes activity measurement wells and control well to incubate for 4hours on the hot plate at 37℃. After incubation, ZYM A solution of 30 μ l and ZYM B solution of 50 μ l were added and the absorbance of all solution at the optimum wave length, 551nm for each enzyme activity of alkaline phosphatase, esterase, esteraselipase, lipase, β-galactosidase, α-fucosidase, 497nm for leucine and valine arylamidases, 403nm for cysteine arylamidase, trypsine, α-chymotrypsine, α-galactosidase, α-mannosidase, 540nm for acid phosphatase, 547nm for naphtol-AS-BI-phosphohydrase, 586nm for β-glucuronidase, 556nm for α-glucosidase, 515nm for β-glucosidase, 416nm for N-acetyl-β-glucosaminidase after keeping 30 min.

2.7 Statistical analysis

The significance of differences between two groups was calculated with the unpaired Student's t test. The results are expressed as the mean ± SD for each group. Values of $p<0.05$ and $p<0.01$ were considered statistically significant

3. RESULTS

3.1 Effects of AFIR stones on mud snails and gold-colored breed of the Japanese rice fishes (*Oryzias latipes)*

In FIR group, the survival rates were 100 % at 9, 16 and 21 day and 72.7 % at 35 day after handling. On the other hand, the survival rates of control group decreased up to 63.6 %at 9days, 40.9 % at 16 day, 24.0 % at 21day and 9.1 % at 35 day after handling. In control group, it was made clear that the presentation of AFIR stone enhanced the survival rates of mud snails. (Fig-1A) The survival rate of Japanese rice fishes increased up to 1.2 fold at 5 day, 1.4 fold at 12 day and maintained high survival rate at almost days from 3 to 24 days without 7 days in FIR powder group compared with control group. The increasing survival rate of FIR stone group was a a little low (Figure 1b). These results suggested that the higher concentration of soluble components was in water, the higher survival rate was

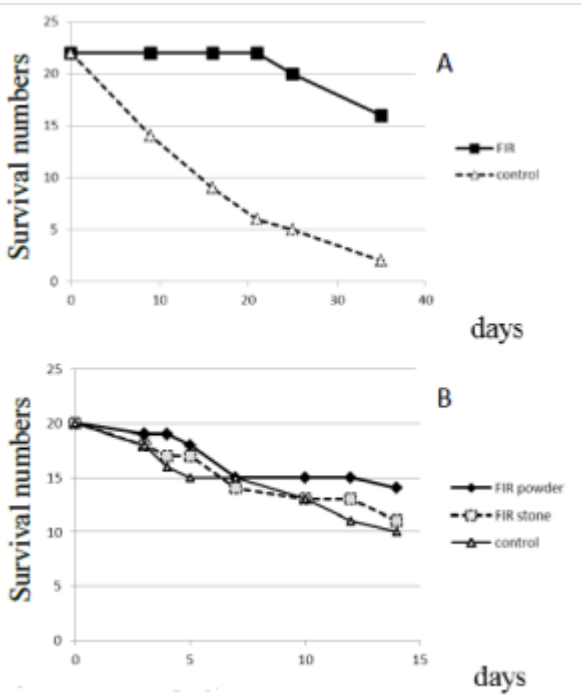

Fig 1: *The effects of AFIR stones on survival rates of mud snails and gold-colored breed of the Japanese rice fishes. The survival rates of mud snails increased up to 1.6 fold at 9 day, 4.2 fold at 21 day and 8.0 fold at 35 days in presentation of AFIR stone (A). The Japanese rice fishes maintained high survival rate at almost days from 3 to 24 days without 7 days in FIR powder group compared with control group. But, the increasing survival rate of FIR stone group showed a little low (B). Data presented are from two independent experiments.*

a little low (Fig-1B). These results suggested that the higher concentration of soluble components was in water, the higher survival rate was maintained.

3.2 Analysis of soluble components in rhyolite

White powder of 13.4 g was obtained after dissolving and drying from 500 g rhyolite powder in 600 ml water. The soluble components in rhyolite as AFIR ceramics contained 43.7% Ca, 15.9% Si, 12.2% Na, 8.9% K, 7.7% Ni, 6.3% Cl, 3.8% S and 1.5% Al respectively (Table 1).

Table 1: *X-ray diffraction analysis of the soluble components in rhyolite*

Element	Count per second	Weight % of Element
Na	55.697	12.227
AL	11.83	1.453
SI	153.084	15.921
S	29.526	3.815
CL	46.289	6.277
K	62.373	8.891
CA	270.502	43.672
NI	18.886	7.746

3.3 Effects of AFIR powder containing food on the handling condition of hatchery sea breams

The feeding was carried out once in two days, namely fifteen times in one month. Total foods of one time were 525kg and 420~450kg respectively in FIR and control group. The sea breams eating AFIR powder containing food became active to jump violently and repeatedly on the surface of sea as if they demanded the additional food when someone accessed the fish tank. On the other hand, the sea breams of control group were quiet even if someone accessed the fish tank. Only feeding time, the sea breams actively move around and jump about.

3.4. Effects of AFIR powder containing food on the skin surface and scale of sea bream

The feeding was carried out once in two days, namely fifteen fold in one month. Total foods of one time were 525 kg and 420~450 kg respectively in FIR and control group. The sea breams eating AFIR powder containing food became active to jump violently and repeatedly on the surface of sea as if they demanded the additional food when someone accessed the fish tank. On the other hand, the sea breams of control group were quiet even if someone accessed the fish tank. Only feeding time, the sea breams actively move around and jump about.

3.5. Effects of AFIR powder containing food on the skin surface and scale of sea bream

The eyes of sea breams eating AFIR powder containing food were pure white color at the area of white of eyes. But, that of control group had some wide red areas like the blood congestion. This result showed that the bleeding treatment was entirely completed at killing the fishes in FIR group (Fig-2A). The skin of the sea breams eating AFIR powder containing food were clear silver with thin scale compared with control group (Fig-2C).

The scales of the sea bream eating AFIR powder containing food easily curve and have many small holes at the surface of scales (Fig-3A). It was the results of proceeding decalcification and activating calcium metabolism that the processes of scales became short at piled up area and the surface of scales (Fig-3B).

The scales at the piled up area of sea bream eating AFIR powder containing food became smooth and have some small holes at the calcified parts of outer and inner surface (Fig-3C).

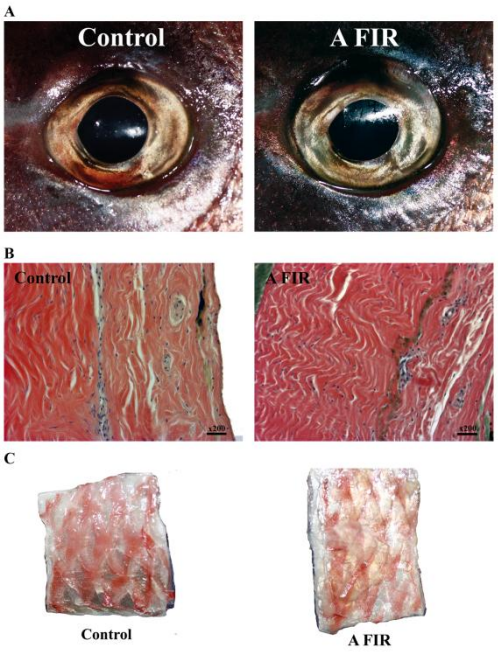

Fig 2: *The effects of AFIR powder containing food on the handling condition of hatchery sea breams.The eyes of sea breams eating AFIR powder containing food were pure white color at the area of white of eyes. Control group had some wide red areas like the blood congestion (A). The histological appearance of muscle fibers had big curve for tightening and strong shrinking by eating AFIR food shown in (B).The skin of the sea breams eating AFIR powder containing food were clear silver with thin scale compared with control group (C). Bars indicate 200μm.*

Scales of sea bream in FIR group became to have smooth phase at outer surface in contact with water and lessor calcified parts and fibrous calcification at inner surface (Fig-3D). A series of change at the surface of processes and folds showed that the calcified area of scales was absorbed to precede the decalcification of scales.

3.6. Change of muscles in sea bream eating AFIR powder containing food

It was considered that the silver-colored skin beneath the scales became thick and the muscle fibers had big curve for tightening and strong shrinking by eating AFIR food (Fig-2b).

The lipid, protein, energy and sugar metabolisms of fish meat were all activated by eating the AFIR food (Table 2). On the lipid metabolism, esterase, esterase-lipase, lipase and average activity increased 8.42, 4.36, 3.55 and 5.44 folds each in lipid metabolism by eating the AFIR food.

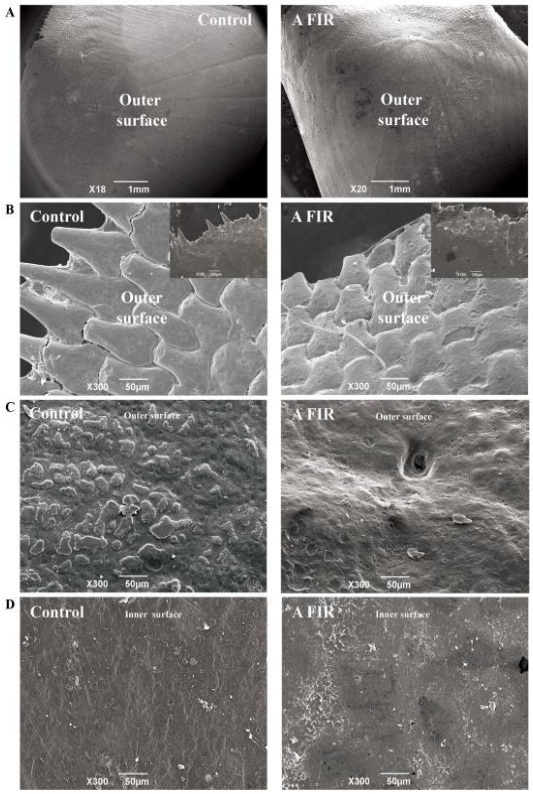

Fig 3: *SEM images of outer and inner structures of AFIR effects powder containing food on the skin surface and scale of sea breams.The scales of the sea bream eating AFIR powder containing food easily curve and have many small holes at the surface of scale (A). The results of proceeding decalcification and activating calcium metabolism that the process of scales became short at piled up area and the surface of scales (B). The scales at the piled up area of sea bream eating AFIR powder containing food became smooth and have some small holes at the calcified parts of outer and inner surface (C). Scales of sea bream in FIR group became to have smooth folds at outer surface in contact with water and lessor calcified parts and fibrous calcification at inner surface (D).*

The results suggested that the neutral fat stored in the muscle was actively decomposed. On the protein metabolism, leucine arylamidase, valine arylamidase, cysteine arylamidase also AFIR containing food increased 2.3, 5.9 and 2.11 folds each. The average of five protein metabolic enzymes increased 2.64 fold.

The alkaline phosphatase, acid phosphatase, naphtol-AS-BI-phosphohydrase and average in energy metabolism increased 4.65, 3.02, 11.3 and 2.93 folds respectively. β-glucuronidase, α-glucosidase, β-glucosidase, β-glucosaminidase, α-mannosidase and α-fucosidase in sugar metabolism increased 10.2, 17.8, 3.21, 2.8 6, 5, 23.7 folds. The average activity of 8 sugar metabolic enzymes increased 6.32 fold.

Table 2: *Effects of AFIR on the enzyme activities of meat metabolism in hatchery sea bream*

	Enzyme name	CTR	AFIR	Rate (M/C)
Lipid metabolism	Esterase	0.019	0.16	8.42
	Esterase lipase	0.025	0.109	4.36
	Lipase	0.027	0.096	3.55
				Average 5.44
Protein metabolism	Leucine arylamidase	0.052	0.120	2.30
	Valine arylamidase	0.031	0.183	5.90
	Cysteine amino peptidase	0.118	0.249	2.11
	Trypsin	0.127	0.214	1.69
	Chymotrypsin	0.167	0.184	1.10
				Average 2.62
Energy metabolism	Alkaline phosphatase	0.023	0.107	4.65
	Acid phosphatase	0.034	0.104	3.02
	Naphtol-AS-BI-phosphohydrase	0.010	0.113	1.13
				Average 2.93
Sugar metabolism	α galactosidase	0.064	0.126	1.97
	β-galactosidase	0.048	0.089	1.85
	β-glucuronidase	0.006	0.062	10.2
	α-glucosidase	0.004	0.071	1.78
	β-glucosidase	0.019	0.061	3.21
	β-glucosaminidase	0.059	0.169	2.86
	α-mannosidase	0.043	0.215	5.00
	α-fucosidase	0.003	0.071	23.7
				Average 6.32

3.6. Change of liver in sea bream eating AFIR powder containing food

The color of liver became white and the sections of blood vessels and bile ducts became round by eating the AFIR food. It was considered that the changes were caused by the blood exhaustion in the liver through the activated circulation at killing the fish (Fig-4a). Still more, the hepatocyte became big and its cytoplasm became abundant. It was regarded that these results showed the elevation of cell metabolism (Fig-4b, 4c).

The section of the bile ducts in the liver became round and its wall became thick by eating the AFIR food (Fig-4a). These results were suggested that the activity of bile secretion became raised. The lipid and protein metabolisms were potently activated by eating the AFIR food. The energy metabolism was definitely activated, but the sugar metabolism except of α-fucosidase inhibited by eating the AFIR food (Table 3). On the lipid metabolism, esterase and esterase-lipase activity increased 4.9 and 17.5 folds by eating the AFIR food. The leucine arylamidase, valine arylamidase, trypsin and chymotrypsin in the protein metabolism,

also increased 4.11, 3.65, 2.80 and 2.27 folds. The activity of each enzyme was definitely activated showed that the protein metabolism became high. The alkaline phosphatase and acid phosphatase in energy metabolism increased 3.48 and 3.91 folds. Though only α-fucosidase in sugar metabolism increased 4.28 fold by eating the AFIR most enzyme in sugar metabolism decreased the activity of enzyme.

Table 3: *Effects of AFIR on the enzyme activities of liver metabolism in Hatchery Sea bream liver metabolism* in hatchery sea bream

	Enzyme name	CTR Liver	AFIR Liver	Rate (M/C)
Lipid metabolism	Esterase	0.020	0.098	4.90
	Esterase lipase	0.008	0.140	17.5
	Lipase	0.119	0.076	0.64
				Average 7.68
Protein metabolism	Leucine arylamidase	0.036	0.148	4.11
	Valine arylamidase	0.047	0.172	3.65
	Cysteine amino peptidase	0.149	0.167	1.12
	Trypsin	0.148	0.415	2.80
	Chymotrypsin	0.162	0.368	2.27
				Average 2.79
Energy metabolism	Alkaline phosphatase	0.035	0.122	3.48
	Acid phosphatase	0.023	0.09	3.91
	Naphtol-AS-BI-phosphohydrase	0.074	0.097	1.31
				Average 2.9
Sugar metabolism	α galactosidase	0.239	0.276	1.15
	β-galactosidase	0.029	0.053	1.82
	β-glucuronidase	0.044	0.054	1.23
	α-glucosidase	0.072	0.056	0.78
	β-glucosidase	0.083	0.094	1.14
	β-glucosaminidase	0.259	0.222	0.86
	α-mannosidase	0.285	0.249	0.87
	α-fucosidase	0.018	0.077	4.28
				Average 1.52

3.7. Change of pancreases in sea bream eating AFIR powder containing food

Still more, the pancreases cells in liver became big and increased the granules. It was regarded that the metabolic activity in pancreases cells swelled (Fig-4D, 4E).

4. DISCUSSION

When fine powder of AFIR ceramics were only applied in water keeping mud snails and gold-colored breed of the Japanese rice fishes, the survival rates increased. As Rhyolite stone as AFIR ceramics was easily broken suitable for grindstone, the mud snails and the Japanese rice fishes might eat fine powder and soluble components of AFIR in water to get high survival rates.

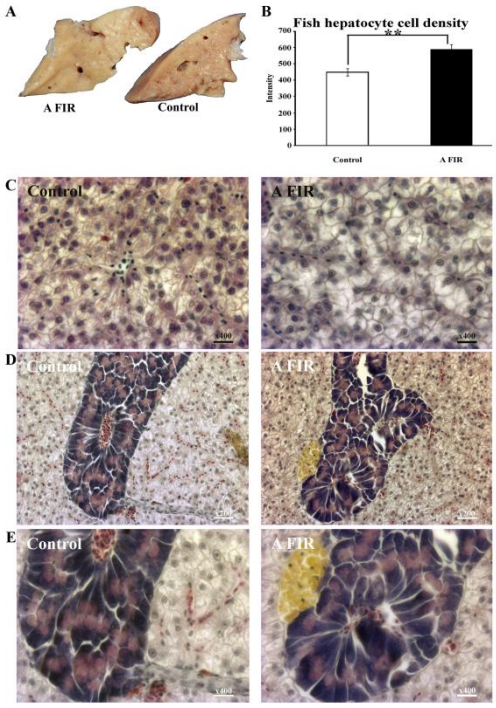

Fig 4: *Change of liver in sea bream eating AFIR powder containing food. The representative picture of liver color became white and the sections of blood vessels and bile ducts became round by eating the AFIR food (A). The histological observation of hepatocyte became big and cytoplasm became abundant (B). The hepatocyte cell density was accelerated in AFIR group compared with control group (C). The pancreas cells in liver became big and increased the granules. It was regarded that the metabolic activity in pancreas cells increased (D) low magnification, (E) Each bar represents the mean ± S.E. **p<0.01. (G). Bars indicate 200 μm and 400μm.*

In the practical manner, 13.4 mg/1000ml (13.4 ppm) of soluble components were obtained by stirring 500 g Rhyolite powder of particle diameter of 5 μm in water for 18 hours. Though that was very few amounts, it was very likely that the mud snails and the Japanese rice fishes assimilated that from water. Then, when the powder of AFIR ceramics were applied to hatchery sea bream with food for increasing the effects, the strong effects to living body were shown more than expected. I should discuss the reason of effects of AFIR ceramics.

Oral administration of AFIR ceramics seemed to affect blood circulation of hatchery sea bream. In the first place, some blood congestions were observed at various areas around eye ball and skeletal muscles not

in FIR group but in control group. The results showed that blood circulation of the whole body was activated and the perfect bleeding was executed, when a live hatchery sea bream was killed. If blood remained in muscle, fish lost its freshness soon and had a smell by its putrefaction through time. It is acceptable that the slices of low fish from hatchery sea bream eating AFIR ceramics with foods can keep freshness for long time without smells in the present study. These results were guided by acceleration of blood circulation with FIR.

So, why can FIR radiation accelerate blood circulation in body? It became to be known that FIR radiated to surface of body with FIR emitter as FIR therapy. It was reported that "Waon therapy" with repeated low-temperature FIR sauna improved chronic total occlusion related myocardial ischemia. The improvement was caused by increasing pre-existing collateral flow and eNOS activity containing up-regulating eNOS expression [10]. Then, FIR therapy was accepted to cause collateral the flow recovery and the new vessels formation in STZ-induced diabetic mice [11]. Still more, it was reported that access blood flow and unassisted patency of the arteriovenous fistula were improved with some skin temperature increasing, when the FIR energy radiated the surface of it in hemodialysis patients for 40 min with hemodialysis [12]. Then, Lai et al. reported that far infrared radiation after percutaneous trans-luminal angioplasties (PTA) up-regulated the micro-circulation of skin to improve hemodialysis access patency for 1 year [13]. These results suggested that FIR therapy caused blood flow promoting action not by some temperature increasing but by direct vasodilation. The mechanism of vasodilation were reported that FIR radiation increased the phosphorylation of endothelial nitric oxide synthase (eNOS) at serine 1179 (eNOS-Ser1179) in time-dependent manner (up to 40 min of FIR radiation) in bovine aortic endothelial cells (BAEC) without alteration in eNOS expression. Thereby, it was almost made clear that NO production and intracellular Ca^{2+} levels in BAEC increased to expand blood vessels [10, 14]. In the present studies, it was speculated that oral administration of the mixing AFIR ceramics and food or water expanded capillary vessels of surrounding tissues around the heat-upped digestive tract by the similar mechanism. From the results of our previous studies, it was made clear that FIR radiation activated vibration of water molecules to promote fluidity and volatility of water [8, 9]. The activated water molecules might stimulate cell membrane of vascular endothelial cells to expand blood vessels through NO production. Still more, it was approved that FIR radiation directly activated vibration of protein structure to accelerate various enzyme activities [9]. Especially, it was suggested that eNOS activity in vascular endothelial cells was directly activated to expand blood vessels by FIR radiation. We advance the further study on the mechanism in the future.

Big difference in temperature was indispensable for strong FIR energy transmission. Then, big difference in temperature between inside and outside of digestive tract is necessary for strong affects by oral administration of AFIR ceramics. It was known that animals containing humans generally increase body temperature after meals [15, 16]. The various causes of increasing temperature were thought to be exothermic reaction of digestive enzymes or electron transport chain of nourishment absorption cells or surrounding tissue. These complex reactions might lead to the high deep body temperature inside or outside of digestive tract. Ventura et al. reported that food was practically wasted by the alternating mesophilic and thermophilic two-stage anaerobic digestion [17]. As the temperature in digestive tract was hard to be directly measured, it was not definitely proved that the temperature in digestive tract increase after meals. But, as the increasing core temperature was directly measured through the gastrointestinal system after exercising what was called the construction of skeletal muscle, it was suggested that the temperature in digestive tract considerably increase by digestion what was called the construction of smooth muscle [18]. Because the average particle diameter of AFIR ceramics was considerably small as 50μm at the best and the difference in temperature between inside of digestive tract and surrounding tissue was big, it was thought that the strong FIR radiated from the eaten AFIR ceramics with food. Consequently, it was discussed that effects of AFIR ceramics intensely emerged by oral administration with food. Still more, it was regarded that the eliminated AFIR ceramics accumulated on the bottom of the sea and contributed to purify the water quality.

Then, scales of hatchery sea bream were being actively absorbed at whole area of overlapped area, outside contacted with water and inside contacted with skin by oral administration of AFIR ceramics. Because of this, the skin became thick. It was known that bone and scale of the fresh water fish body were absorbed with activating digestive tract to supply calcium, once

calcium was required for living [19]. Goldfish and killifish preliminary supply calcium from scales using secondary bone [20]. Still more, scale of Rainbow trout has high-affinity, low-capacity estradiol-17β (E2) binding different from that of liver E2 [21]. In brief, scale of saltwater fish is also controlled by estradiol to repeat the remodeling by osteoblast and osteoclast. Then, it was likely that calcium was supplied from scales according to the change of surround condition. Behavior observation of well swimming, histological observation of well contracting muscle and analysis of promoting metabolic enzyme activities in hatchery sea bream absolutely showed that muscle demanded calcium for swimming. The changes of metabolic enzyme activities suggested following changes of muscle characteristics.

Alkaline phosphatase and Acid phosphatase play roles to decomposed ATP to ADP and mono phosphate, and Naphtol-AS-BI-phosphohydrase remove phosphate from organophosphate related with ATP. An ATP in cytoplasm of muscle fiber was first directly used for skeletal muscles construction. When the ATP was spent perfectly, ATP from keratin phosphate was used for the further moving. When the further movement was continued, a lot of ATPs was formed by sugar metabolism and electron transport chain of mitochondria with oxygen with aerobic exercise. Therefore, it was suggested that the three enzymes might be connected with active movement of muscles.

The sugar and protein metabolisms were indispensable to supply energy for muscle. The activating effects of lipid, protein, energy and sugar metabolisms by eating the AFIR food were indicated to induce the muscles regeneration and the active movement of skeletal muscles in the whole body. It was thought that these changes of environmental condition stimulated osteoclast of scales to increase calcium contents in blood and supply calcium to muscle for swimming. Consequently, it was thought that skin became thick as mechanical reinforcement for scales becoming thin [19, 20].

These active movements and accelerating metabolic activity of whole body muscles were thought to affect whole body to activate internal organic and hepatic function. Actually, intake amount of food and metabolic activity of liver increased by oral administration of AFIR ceramics. In liver, alkaline phosphatase, acid phosphatase and naphtol-AS-BI-phosphohydrase related with ATP metabolism and esterase and esterase lipase for lipid metabolism were activated like that of muscle. These data showed that energy metabolism was also accelerated to exacerbate decomposition of lipid and protein stored in liver by activated blood circulation. As livers (hepatopancreas) of stress loaded cardinal fishes (Apogonidae and Teleostei) from polluted waters demonstrated very strong hyperlipogeny, the exacerbating decomposition of lipid in liver suggested good health condition of the fish [22]. As lipid metabolism in the whole body containing liver and muscle was activated by AFIR effects, vigorous sea breams with a few fats were bred. But, sugar metabolism of liver decreased. As liver plays a role of sugar accumulation as glycogen, it is suggested that sugar metabolism in liver keeps hard up decreasing for glycogen accumulation. Vieira VARO et al reported that muscle glycogen decreased and liver glycogen increased in acidic pH and acute aluminum exposure [23]. These results agreed our data showing differences in muscle and liver. From the above results, FIR energy radiation by oral administration of AFIR ceramics activated the metabolic function in muscle and liver to make whole body of hatchery sea bream more active and healthy. The mechanism of accelerated blood circulation in hatchery sea bream affected by oral administration of AFIR ceramics must be clarified in future.

5. CONCLUSIONS

The blood flow was accelerated by eating the AFIR powder containing food in the sea bream. The activated blood flow guided the strong shrinking of muscles to swim lively with the calcium metabolism of whole body and the absorption of scale in the high label of tissue metabolism. Still more, it was considered that the abdominal viscera such as liver worked strongly to support the activation of motor function by eating the AFIR food.

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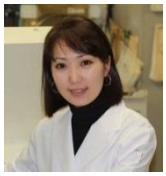
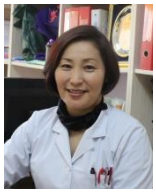
AUTHORS' BIOGRAPHY

Dr. K Yamashita, M. Pharmacy 1985 and Ph. D 1992 is an anatomist and studies the preventive medicine and the application for society with far infrared ray radiation.

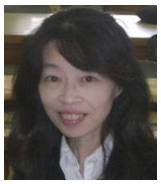


O Dalkhsuren graduated the Health Sciences University of Mongolia by medical doctor in 2007 and completed my master course morphological study in 2009, and studied at the PhD course of the University of Tokushimain Japan. I specialized in anatomy and working now as a senior lecturer at the department of Anatomy of Mongolian National University of Medical Sciences.



A Dolgorsuren have been working in Department of Anatomy, Mongolian National University of Medical Science. She was graduated from Medical University in 1998 as a Medical doctor. A. Dolgorsuren has got master degree in 2009, philosophical doctor degree in 2015 Institute of Health Biosciences, Tokushima University Graduate School Tokushima, Japan

There are 3 textbooks, 31 research articles and over 16 presentations were discussed at the symposiums.



K Sumida, D.D.S., Ph.D. My specialty is Gross Anatomy.

T Morita is a graduate student of Tokushima University and studies the tooth development.

Dr. O Baba, DDS 1990 and Ph. D 1993 is an anatomist and studies on the formation of mineralized tissues.