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Selective non digestion of yellow mealworm *Tenebrio molitor* larvae by arowana

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Abstract : This study reveals the unusual case reported for the first time on the selective non digestion of yellow mealworm (*Tenebrio molitor*) larvae by arowana fish. In January 2005, an Asian arowana (*Scleropages formosus*) (red variety), from Daesang Tropical Fish Corporation, Seoul, Korea, mortality was observed due to unknown cause. No putative causal factors were suggested by bacteriological and parasitological examinations. Internal examination of the dissected stomach showed some undigested debris with mandible parts of mealworms attached to the mucosal lining of stomach wall. Feeding experiment of yellow mealworm (*Tenebrio molitor*) was conducted on the silver arowana (*Osteoglossum bicirrhosum*). Result showed that on the test group, fish released vomitous material containing undigested mandibles of mealworm was observed on the 24th day of the feeding experiment. Histopathological examination of the cross section of the stomach wall layers of the test groups, showed detached parts of the mucosal layer and gastric pits around the damaged area with intact mucularis tissues. While the control group fed with mealworm larvae without head part showed intact stomach wall layers consisting of gastric pit, mucosa and mucularis tissues. Fish on both treatments survived until the termination of experiment. The removal of mealworm larvae head before feeding probably help or aid in the fast digestion of these insect larvae.

Key words : non digestion, *Osteoglossum bicirrhosum*, *Scleropages formosus*, *Tenebrio molitor*

Introduction

Arowanas are large, majestic fish with enormous, pearly scales [2]. It is the most valuable and unique group of ornamental fishes and always very much in demand in the ornamental fish trade, commands high price ranging hundreds or thousands of dollars per fish. The breeding and farming of arowana has been carried out in several countries such as Indonesia, Singapore and Malaysia [9, 14]. There are 4 major varieties of species of arowana and are classified as giant Arapaima Pirarucu (*Arapaima gigas*), African arowana (*Heterotis niloticus*), silver arowana (*Osteoglossum bicirrhosum*) and Asian arowana (*Scleropages formosus*) [3]. Among the varieties of arowana, the most common type of species that is being sold in the fish aquarium shop is the silver arowana while the most expensive species is the Asian arowana. Asian arowana is an ancient osteoglossid bonytongue fish species and its natural distribution covers vast areas of Southeast Asia, including Cambodia, Indonesia, Laos, Malaysia, Philippines, Vietnam and possibly Thailand [4, 10], which is thought to be one of the most primitive teleosts, dating back to the Mesozoic age of reptiles about 100 million years ago [6]. The number of individuals in the natural habitat had been reduced rapidly due to over-fishing, low fecundity, long generation interval, oral-brooding habit and open-water spawner makes this species

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vulnerable to overexploitation. Therefore, this species has been listed as one of the most highly endangered fish by Convention on International Trade on Endangered Species of Wild Fauna and Flora (CITES) since 1975 as an Appendix I protected fish [6, 7, 12]. The diet of Asian arowana is wide-ranging, including insects, arachnids, non-woody roots and tubers [13]. Nutrition remains an important and costly component in the farming of arowana. The development of an economical, palatable dry feed with optimized nutrient content is therefore desirable. Use of nutrient in fish depends on the activities of digestive enzymes present in various digestive organs.

Invertebrates such as insects comprise a large portion of the diets of many species of arowanas maintained in captivity especially in broodstock conditioning and in grow-out phase for juveniles. Insects and other invertebrates in general contain high levels of protein, although a portion may be chemically bound within the exoskeleton [1]. One of the common foods feed for arowana is the *Tenebrio molitor* larvae, also known as yellow mealworm. This is common pests that grow to be about 2.5 cm long, weigh 0.2 g and are rich in protein and minerals [1].

In January 2005, an Asian arowana (*Scleropages formosus*) (red variety, about 47 cm in total length) with CITES serial no. 132146345A, from Daesang Tropical Fish Corporation, Seoul, Korea, reported mortality of unknown cause. No putative causal factors were suggested by bacteriological and parasitological examinations. Internal examination of organs especially on the dissected stomach showed some undigested debris attached to the mucosal lining (Fig. 1). Based on these observations, we hypothesize that mandible parts of mealworms that were accumulated and attached to the mucosal lining of the stomach wall were not digested and eliminated and have caused the death of this fish. The present study aims to report the selective non digestion of mealworm larvae that might have caused the death of arowana.

Materials and Methods

Feeding experiment

Due to unavailability and expensive cost of Asian arowana juvenile fish, 2 juvenile fish samples of silver arowana (*Osteoglossum bicirrhosum*) (18 cm average body length) commonly sold in the fish aquarium shop were purchased and used for the feeding experiment. Two 70 *l* fish aquaria were prepared and used for the experiment with 1 fish stocked per aquarium. The aquaria were equipped with flow-through fresh water, aerated and covered with nylon mesh. One fish was used as test group and fed daily with 1-2 larvae of mealworms with complete mandible. While the other fish was used as control group and fed with 1-2 larvae without head part. Daily observation for the feeding behavior and mortality was done for 30 days.

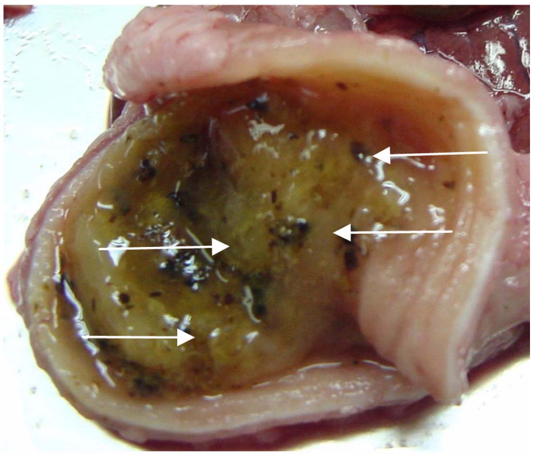

Fig. 1. Macroscopic internal examination of Asian arowana stomach with food debris and mandible (arrow) parts of mealworm larvae attached to the mucosal lining of the fish stomach.

Histopathology

The stomachs of the 2 fish were Sampled and fixed in 10% buffered formalin. After dehydration and clearing, samples were embedded into paraffin using automatic tissue processor. Paraffin blocks were further processed, cut into sections and stained by haematoxylin and eosin staining method and observed under light microscope.

Results

Clinical signs and macroscopic observations

In the test group, fish does not show any clinical signs or unusual behavior from 1st day up to the 19th day of the experiment. However on the 20th day up to the 23rd day of the experiment, fish did showed loss of appetite. On the 24th day, vomitous material (Fig. 2A) containing undigested mandibles (Fig. 2B) of the

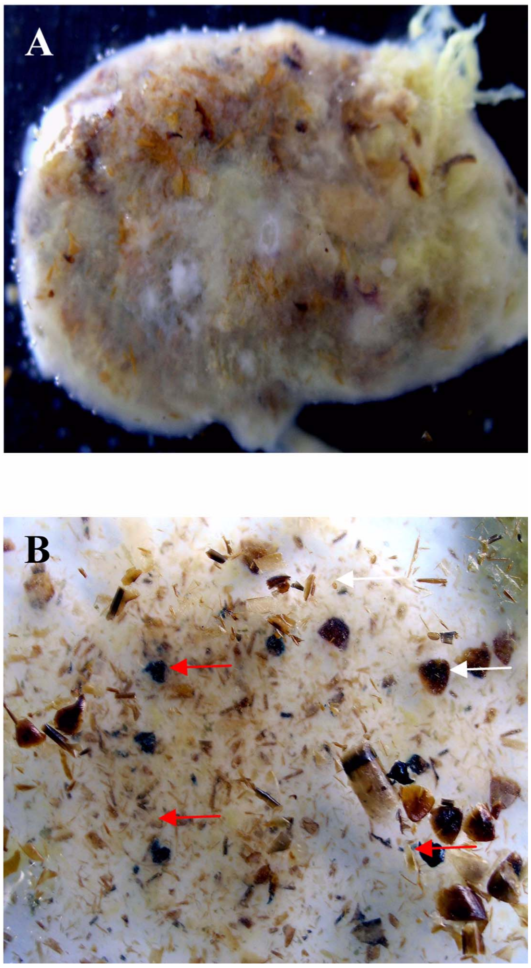

Fig. 2. A. Macroscopic examination of vomitous material found inside the aquarium. B. Macroscopic examination of debris, mandible (red arrow) and skin (white arrow) parts of the mealworm larvae found inside the vomitous material.

mealworm larvae were observed in the water. Also on the 25th day up to the 30th day of experiment, the test group showed reduced feeding on the mealworm larvae. The control group fed with mealworm larvae without head part did not show any unusual signs of abnormality all throughout the feeding experiment. No mortality was observed until the termination of the experiment. The fish were sacrificed and the stomachs were processed for histopathology.

Histopathological findings

Histopathological examination of the cross section of the stomach wall layers of the control group, showed intact stomach wall layers consisting of gastric pits,

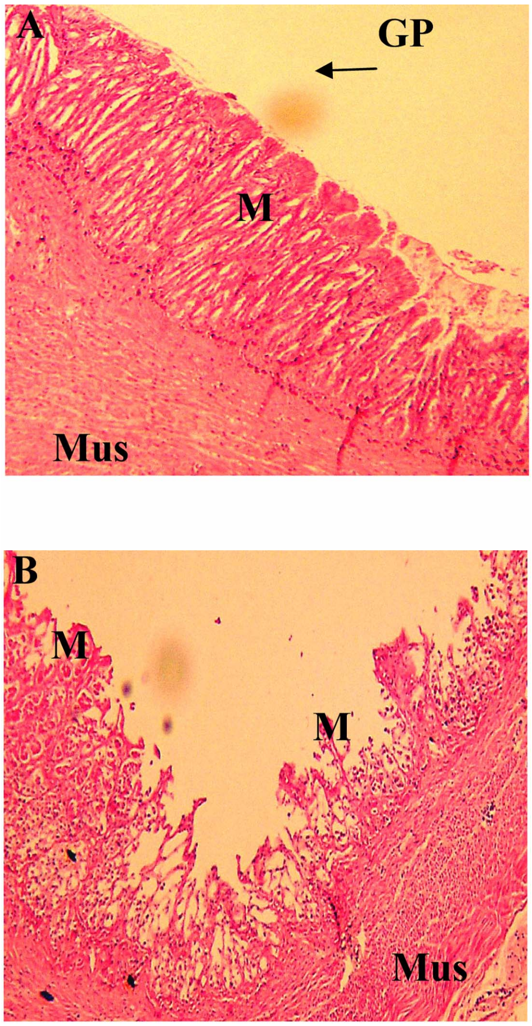

Fig. 3. A. Cross section of the normal stomach of silver arowana feed with mealworm larvae (without mandible), showing intact mucosal layer (M). B. Cross section of the stomach of silver arowana that vomited the non-digested materials, showing damage mucosal layer (M). Mus, muscularis; GP, gastric pits. H & E, ×100.

mucosa and muscularis tissues (Fig. 3A). For the test group, detached parts of the mucosal layer and gastric pits (Fig. 3B) were prominent around the damaged area. The mucularis tissues are still intact.

Discussion

In fish, after ingestion of food, sensory input to local and central control systems elicited by the food itself starts specific patterns of motility, such as swallowing, gastric emptying and peristalsis. When food is swallowed it enters the alimentary canal proper and proceeds via the esophagus to the stomach where digestion takes place, followed by the intestine and the waste products are excreted thru the anus. There are 3 basics digestive functions that are regulated in the fish. These are motility, secretions, and absorption. Regulation of these functions is accomplished by combined actions of nervous system inputs and chemical signals. Gastrointestinal motility includes several different features. Standing contractions mix the food, while peristaltic movements propulse the food in an anterograde (from mouth to anus) or a retrograde (vomiting, regurgitation) direction. Digestion in fish concerns the breakdown of foods by enzymatic and, in many cases, acidic secretions in the gut. The lower gut of many fish (especially carnivorous ones) contains a true stomach as in the case of arowana, characterized by a smooth muscle (muscularis mucosa) layer of tissue.

The dead Asian arowana that was submitted to our laboratory for diagnosis was fed with yellow mealworm larvae with complete head parts as a daily diet. The water parameters in the aquarium are in the optimum level. Fish showed loss of appetite a week before prior to the mortality. Generally, arowana have a great resistance against diseases so long as they are not weakened by bad treatment, such as spoiled food, polluted and contaminated water, lack of oxygen, sudden change in water temperature, or other conditions, the result of human negligence and ignorance that will cause stress and injury to the fish. Due to the predating nature of arowana, broodstock were usually fed with a variety of live feed such as insects (e.g. mealworms), prey fish, shrimps, wild guppy, freshwater prawn (*Macrobrachium lanchestrii*), low grade goldfish and chopped fish meat. These are mainly used for broodstock conditioning and grow-out phase for juveniles. The risk of possible introduction of harmful pathogens through the use of trash fish is also present. Most of the mealworm feed to the arowana, usually contained adequate levels of Cu, Fe, Mg, and Zn to meet dietary requirements, based on domestic animal recommendations [1]. Regarding proximate body composition, larval stages of mealworms contained significantly more fat than adults and in general it contains high levels of protein. The neutral detergent fiber, used as a measure of chitin content comprised about 15% of dry matter in the larvae of most species of mealworms. Most of the mealworms sampled met the dietary vitamin E recommendations for domestic mammalian carnivores [1].

Use of nutrient in fish depends on the activities of digestive enzymes present in various organs. It was reported that the gastric mucosa of the stomachs of carnivorous fish produces a protease (protein breakdown) enzyme (e.g., pepsin) with an optimal activity at a pH of 2 to 4. Hydrochloric acid is also secreted by the gastric mucosa glands in these species, creating the low-pH environment. The groups of Natalia *et al*. [11] have already characterized several types of digestive enzymes such as proteases, amylase and lipase functioning at various major digestive organs of arowana fish and the existence of these enzymes also means that arowana possess the ability to digest a wide range of ingredients in formulated feeds. So far no studies were conducted and reported on the mechanism of how these different digestive enzymes reacted to the different type of foods or diets especially mealworms taken by arowana fish. Arowana will require substantial lipase activity to effectively digest the high dietary fat intake from live insect and smaller prey fish [11]. Knowing and understanding the importance and optimized conditions of digestive enzymes activities and its presence in the digestive organ of this carnivorous fish enable better comprehension of nutrient digestibility and might aid in the process of the digesting mealworm [5, 8].

In the present study, these were similarly observed when silver arowana (test group) fed with yellow mealworm larvae with complete head part as a daily diet had showed loss of appetite for 1 week before vomited the undigested food that contains the mandible parts of the mealworm larvae. While the control group fed with yellow mealworm larvae without head part digested the food properly and eliminated the waste products by feces. We speculated that when the mealworm mandibles with hooks were accumulated, stacked or attached to the mucosal layer of the stomach wall and not eliminated properly together with the waste products, it might cause erosions and ulcerations, and eventually cause internal bleeding and death of the fish. Another reason maybe the anatomical structure of the intestine of the arowana might cause difficulty in the elimination of mealworm with head part. In another experiment conducted to Oscar fish fed with yellow mealworm larvae with complete head part eliminated the mandible parts together with feces. Moreover the anatomical structure of the intestine of this fish is different from that of the arowana (data not shown).

Although in the present feeding experiment, fish survived until the termination of the experiment. It might be that some fish encounter difficulty in the elimination of this mandible part that could lead to the death of fish.

We recommended to those hobbyists and fish farmers to remove the mealworm head part before feeding to the arowana fish. Further studies should also investigate the type of enzymes responsible in the fast digestion of mealworm larvae and also the mechanism behind the non-digestion and elimination of the mandible parts of mealworm larvae.

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