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Feeding Stimulatory Effects of Nucleotides and Related Compounds on Juvenile Cobia (*Rachycentron canadum*)

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ABSTRACT

The feeding stimulative effects of nucleotides and their related compounds on juvenile cobia (*Rachycentron canadum*) were investigated. Among these nucleotides only inosine-5'-monophosphate (IMP), guanosine-5'-monophosphate (GMP), uridine-5'-monophosphate (UMP), and adenosine-5'-diphosphate (ADP) showed high feeding stimulative responses. In contrast, nucleosides, nitrogen bases, and ribose were not effective. The optimal concentrations of IMP, GMP, UMP, and ADP for juvenile cobia were 2.9, 8.1, 2.8, and 6.6 mmol/kg dry pellets, respectively. These effects of the nucleotides were synergistic when supplemented in pairs. The synergic effects disappeared when the 4 nucleotides were supplemented together.

Key words: cobia, *Rachycentron canadum*, feeding stimulant, nucleotide.

INTRODUCTION

Recently, cobia (*Rachycentron canadum*) has become one of the most important cage-cultured fish in Taiwan. Cobia grows rapidly; its body weight can reach 6~8 kg within a year. Like most commercial marine fish, the protein requirement in the diet for cobia is as high as 44%. For the purpose of reducing the costs of its diet, it is necessary to substitute fishmeal with other inexpensive plant proteins. The use of soybean meal as a replacement for fishmeal has been practiced for many years; however, soybean meal as a dietary component is not as palatable to many fish as fishmeal (Wu *et al.*, 2003). Feed intake of largemouth bass was depressed by an increasing dietary concentration of soybean meal (Kubitza *et al.*, 1997), and a diet containing 70% soybean meal was utilized very poorly by shrimp (Lim and Dominy, 1990).

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It has been reported by many workers that nucleotides are effective in inducing active feeding behaviors in various species of fish (Hidaka *et al.*, 1977; Hidaka *et al.*, 1985; Ishida and Hidaka, 1987). In order to utilize nucleotides as a feeding stimulant for the exploitation of soybean meal-based artificial diets for cobia, the feeding stimulatory effects of nucleotides were examined in 4~11 g juvenile cobia.

MATERIALS AND METHODS

Juvenile cobia were obtained from a local fish farm (Pingtung, Taiwan). They weighed 4 g on average at the beginning and 11 g at the end of the experiment. The experimental fish were kept in three reinforced fiberglass tanks (140 x 60 x 50 cm, with a water depth of 20 cm) containing sea water (30 ppt) at 27~29 °C. Water was vigorously aerated throughout the experiment. A surface light intensity of 250 lux was maintained by fluorescent tubes from 08:30 to 17:30 each day.

The feeding test was carried out once a day at 15:00. Each stimulant was tested in triplicate. A

Table 1 Nucleotides and their related compounds tested in the feeding stimulatory experiments on juvenile cobia

	Pentose	Nitrogen base	Nucleosides	Nucleotides
	Ribose			
Purines		Adenine	Adenosine	AMP (adenosine-5′-monophosphate)
				ADP (adenosine-5′-diphosphate)
				ATP (adenosine-5′-triphosphate)
		Guanine	Guanosine	GMP (guanosine-5′-monophosphate)
		Hypoxanthine	Inosine	IMP (inosine-5′-monophosphate)
Pyrimidines		Cytosine	Cytidine	CMP (cytidine-5′-monophosphate)
		Thymine	Thymidine	TMP (thymidine-5′-monophosphate)
		Uracil	Uridine	UMP (uridine-5′-monophosphate)

group of 20 individuals in each tank was used for the experiment, and the total body weights of the fish were determined at least 5 h before the feeding test. When the fish were removed for weighing, the tanks were thoroughly cleaned, and then the water was replaced. Each test was carried out for 60 min, and the total amount of the test pellet consumed was determined. The feeding stimulatory activity of the test pellet was expressed as the ratio of the test pellet eaten to body weight, and as the intake rate calculated from the following formula:

$$\text{Intake rate (\%)} = (\text{Test pellet eaten} \div \text{Total body weight}) \times 100$$

Fish were fed at 1.5% of their body weight once daily after testing with a commercial eel feed manufactured by Tungli (Pingtung, Taiwan).

In order to estimate the optimum concentration of nucleotides in the test pellets, a least-squares method was employed which examined the relationship between the intake rate and nucleotide concentration levels. The intersection of the ascending line with the line parallel to the y-axis was considered the best estimate of the optimum concentration (Griminger *et al.*, 1955).

For the additive or synergistic effect between the effective nucleotides, the activities of paired combinations of inosine-5'-monophosphate (IMP), uridine-5'-monophosphate (UMP), guanosine-5'-monophosphate (GMP), and adenosine-5'-diphosphate (ADP) at an overall concentration of 1 mmol/kg dry pellet were also assayed.

All nucleotides and their related compounds (Table 1) were obtained from Sigma Chemical (St. Louis, MO), and glycine betaine was a product of Merck Chemical (Darmstadt, Germany). To avoid degradation of the chemicals, test solutions containing single chemicals were prepared at 100 mM immediately before the test by dissolving it in distilled water. Glycine betaine, an amino acid-related compound, was also tested at the same concentration. A sample of Super Seasoning (a product of Weichuan Foods, Taichung, Taiwan) was tested by adding it to the test pellet at a concentration of 40 g/kg dry pellet. For the optimum concentration test, test solutions were serially diluted with distilled water to the required experimental concentrations. For the additive or synergistic effect test, test solutions were prepared from either single compounds at a concentration of 1 mM, by mixing 0.5 mM of each of two compounds, or by mixing 0.25 mM of each of four compounds. Then 1000 ml of the test solution was well mixed with 200 g of binder and 800 g of cellulose powder to make a test pellet.

RESULTS

When nucleotides and their related compounds were tested at a concentration of 100 mmol/kg dry pellet, it was found that only IMP, GMP, UMP, and ADP had high feeding stimulatory activities in juvenile cobias. The effectiveness of thymidine-

5'-monophosphate (TMP) was low, while the others were showed very low or no stimulation (Fig. 1). Glycine betaine and Super Seasoning also showed high feeding stimulatory activity in this experiment (Fig. 1).

The optimum concentrations of nucleotides were determined using the least-squares method. The results suggested that the optimum concentrations of IMP, UMP, GMP, and ADP were 2.9, 2.8, 8.1, and 6.6 mmol/kg dry pellet, respectively (Figs. 2~5).

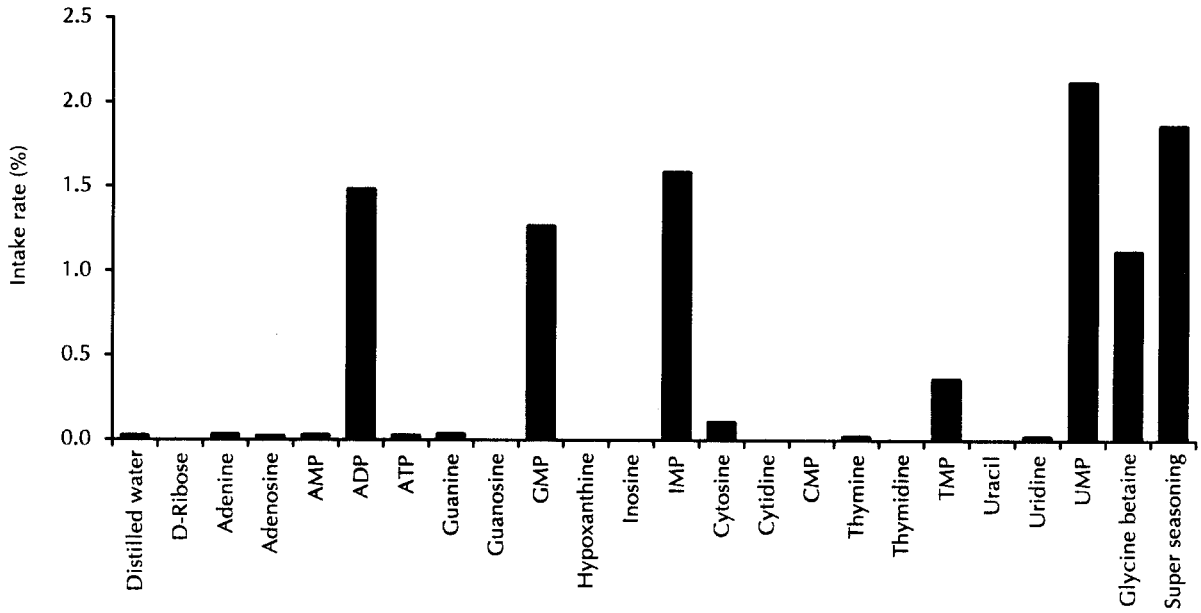

Fig. 1 Feeding stimulatory responses to nucleotides and related compounds and glycine betaine and Super Seasoning. All substances were tested at a concentration of 100 mmol/kg dry pellet, except for Super Seasoning which was added at a concentration of 40 g/kg dry pellet.

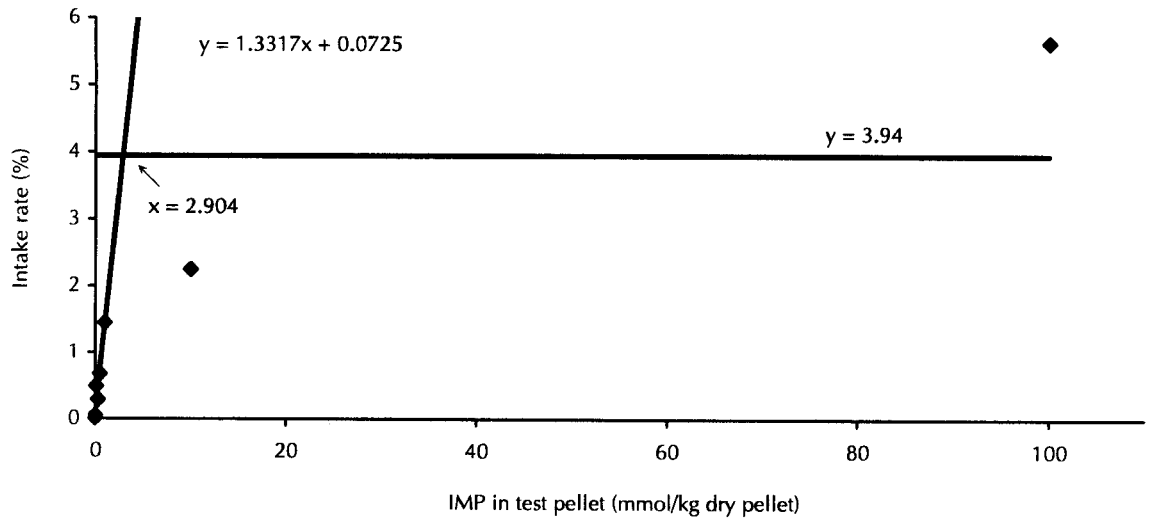

Fig. 2 Stimulatory effect of IMP on the feed intake rate of juvenile cobia. Each point represents the mean of 3 replicates of fish, with 20 fish per replicate. The optimum concentration derived from the least squares method on feed intake was 2.904 mmol/kg dry pellet of IMP.

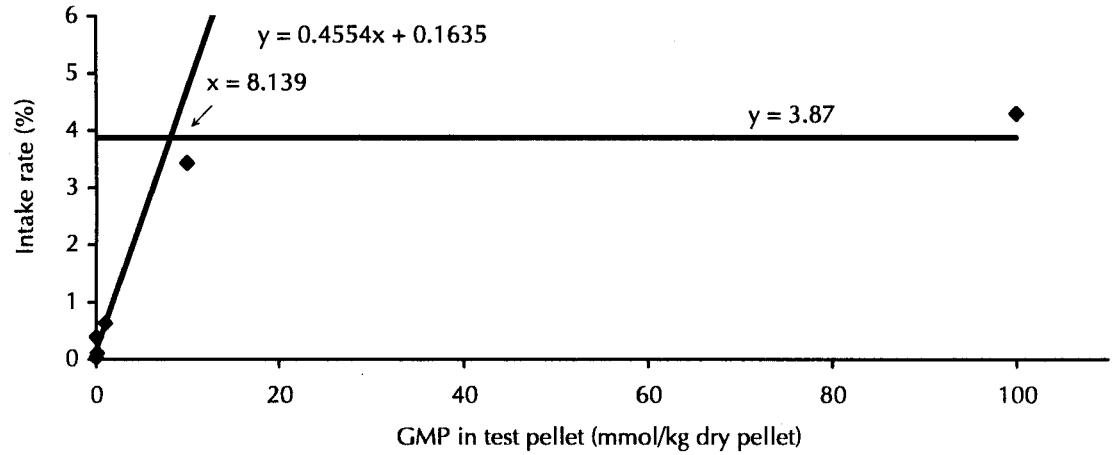

Fig. 3 Stimulatory effect of GMP on the feed intake rate of juvenile cobia. Each point represents the mean of 3 replicates of fish, with 20 fish per replicate. The optimum concentration derived from the least squares method on feed intake was 8.139 mmol/kg dry pellet of GMP.

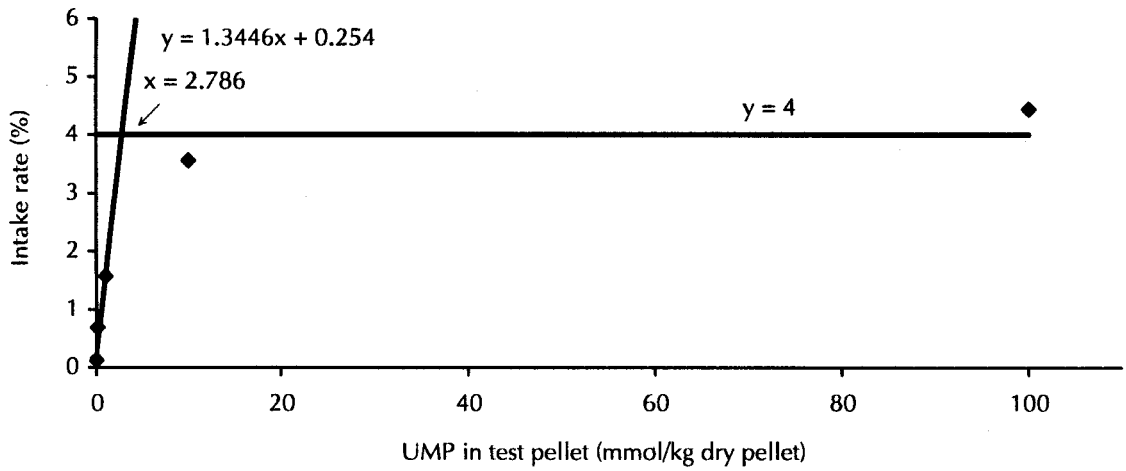

Fig. 4 Stimulatory effect of UMP on the feed intake rate of juvenile cobia. Each point represents the mean of 3 replicates of fish, with 20 fish per replicate. The optimum concentration derived from the least squares method on feed intake was 2.786 mmol/kg dry pellet of UMP.

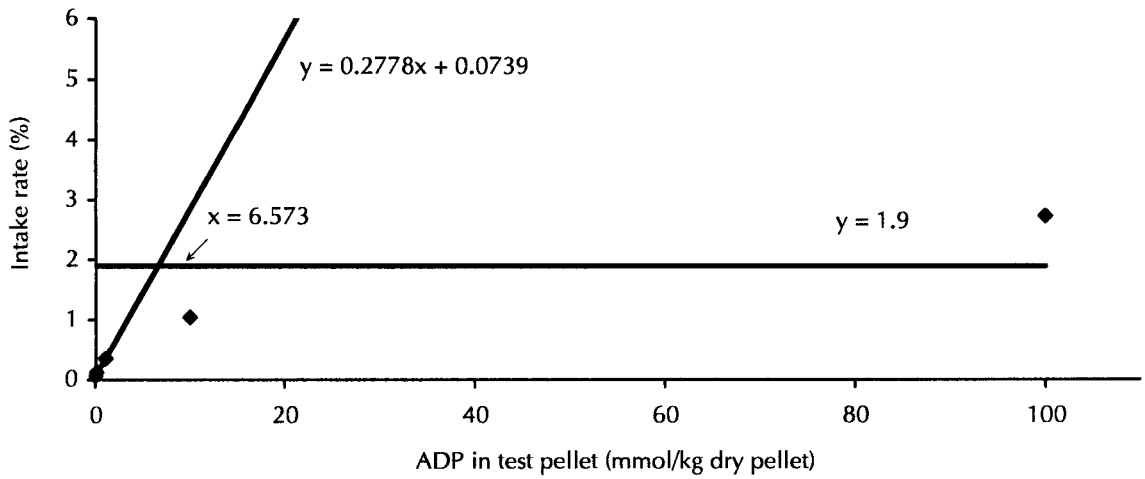

Fig. 5 Stimulatory effect of ADP on the feed intake rate of juvenile cobia. Each point represents the mean of 3 replicates of fish, with 20 fish per replicate. The optimum concentration derived from the least squares method on

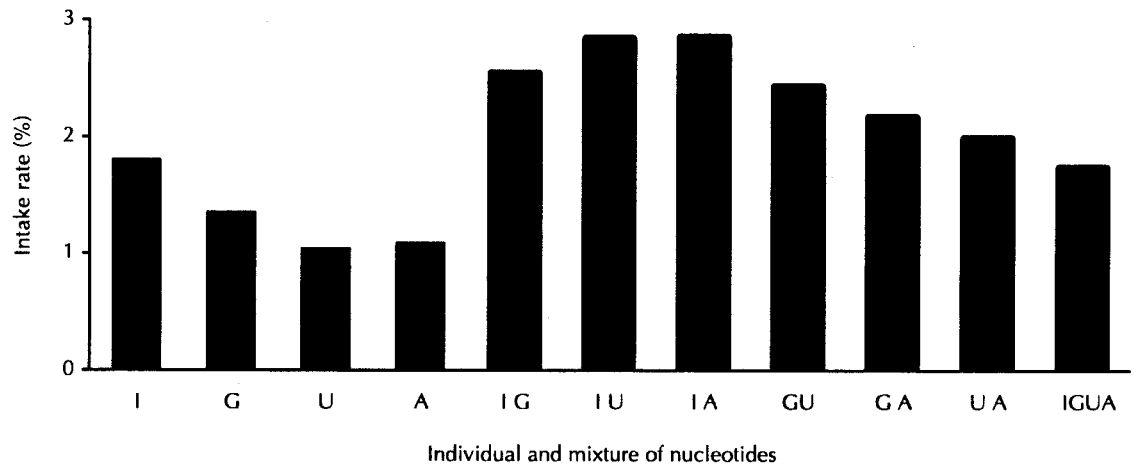

Fig. 6 Feeding stimulatory responses of juvenile cobia to individual or combinations of nucleotides tested at an overall concentration of 1 mmol/kg dry pellet. I, IMP; G, GMP; U, UMP; A, ADP; IG, IMP+GMP; IU, IMP+UMP; IA, IMP+ADP; GU, GMP+UMP; GA, GMP+ADP; UA, UMP+ADP; IGUA, IMP+GMP+UMP+ADP.

The additive or synergistic effects among IMP, UMP, GMP, and ADP are shown in Fig. 6. Combinations of two nucleotides at the same overall concentration of 1 mmol/kg dry pellet were more effective than individual nucleotides. Among them, IMP plus UMP and IMP plus ADP had the strongest feeding stimulatory activities. A mixture of all 4 nucleotides did not produce stimulatory responses surpassing IMP alone.

DISCUSSION

IMP, UMP, and ADP, which were effective in the present study, were also previously found to be effective in the yellowtail and puffer. Hidaka *et al.* (1977) investigated the activities of nucleotides on the gustatory response of puffer by recording electrical responses from the facial nerve innervating the upper lip, and reported that adenosine-5'- monophosphate (AMP), ADP, adenosine-5'-triphosphate (ATP), IMP, and UMP had a marked stimulatory effect at a concentration of 10^{-3} M. The yellowtail taste receptors also responded well to AMP, ADP, ATP, IMP, and UMP (Hidaka *et al.*, 1985). UMP appeared to be the most effective in the present study when tested at a concentration of 100 mmol/kg dry pellet. A similar result was also obtained in several marine fish by comparing the neural responses at 10^{-4} M (Ishida and Hidaka, 1987). IMP showed the strongest activity in this experiment when tested at the low concentration of 1 mmol/kg dry pellet (Fig. 6), and a similar result was observed in jack mackerel (Ikeda *et al.*, 1988).

The minimum concentration of IMP which showed the maximum effect for young yellowtail was estimated to be 8 mmol/kg dry diet (Ikeda *et al.*, 1991). The optimum concentrations of IMP and UMP for juvenile cobia measured in the present study only required 2.9 and 2.8 m mol/kg dry pellet, respectively. This suggests that IMP and UMP can be used as good feeding stimulants for juvenile cobia.

Synergistic interactions between effective nucleotides were evident for juvenile cobia when tested at an overall concentration of 1 mmol/kg dry pellet. Of the nucleotides examined in jack mackerel, only IMP was highly stimulatory, but the presence of ADP interfered with its activity (Ikeda *et al.* 1988). The reason for this discrepancy between cobia and jack mackerel is unknown, but it may be attributed to the different fish species.

Glycine betaine is widely found in tissues of mollusks and crustaceans (Konosu and Hayashi, 1975) and also in a marine worm that is highly recommended by anglers as bait (Konosu *et al.*, 1966). Glycine betaine showed strong activities in this study, and a similar result was also obtained in a previous investigation dealing with the stimulatory effect of amino acids on cobia larvae (Liou and Chen, 2002). Fuke *et al.* (1981) reported that glycine betaine was inert to sea bream either alone or jointly with other components. Super Seasoning, a commercially available cooking flavor enhancer (contained IMP, GMP, and monosodium glutamate), was found to be highly effective in juvenile cobia (Fig. 1). Further investigations are necessary to ascertain the synergistic interactions between nucleotides and amino acids. More work is also needed to determine possible synergetic effects between glycine betaine and Super Seasoning.

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核苷酸及其相關物質對海鱸仔魚的攝餌促進作用

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摘要

核苷酸及其相關物質對海鱸仔魚的攝餌促進作用，經檢測得知僅肉苷單磷酸 (IMP)、鳥苷單磷酸 (GMP)、尿苷單磷酸 (UMP) 和腺苷二磷酸 (ADP) 具有很高的攝餌促進活性。核苷、氮鹼及核糖均無作用。IMP、GMP、UMP 和 ADP 在試料中的最適濃度各為 2.9、8.1、2.8 和 6.6 mmol/kg dry pellet。這四種具有攝餌促進活性的核苷酸混合時，相互間均具協同相加的作用。

關鍵詞：海鱸、攝餌促進劑、核苷酸。

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