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RESEARCH ARTICLE

Endocrine Cells in the Gastrointestinal Tract Mucosa of Zander (*Stizostedion lucioperca*)

Mehmet Rüştü ÖZEN * Kenan ÇINAR ** Nurgül ŞENOL **

* Department of Aquaculture, Faculty of Fisheries, Süleyman Demirel University, 32500 Isparta - TÜRKİYE
** Department of Biology, Faculty of Sciences, Süleyman Demirel University, 32260 Isparta - TÜRKİYE

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Summary

The distribution and relative frequency of endocrine cells in the gastrointestinal tract of the zander *(Stizostedion lucioperca)* were studied immunohistochemically using the peroxidase anti-peroxidase complex method. Our aim was to determine the distribution of specific immunoreactivities in some regions of digestive tract of the zander against the antisera Calcitonin gene related peptide (CGRP), Bombesin, Somatostatin-14, Secretin, Trk-A, Trk-B, Trk-C, Histamine, Neurotensin, Neuropeptide Y (NPY). The samples were taken from the stomach, intestine and pyloric caeca. Data were subjected to analysis of variance SPSS 10 statistical software programme and analysis of variance results were significant in the region of average density immunreactive cell type were compared with ANOVA test. The immunoreactivities against all antisera were observed as intensively in the cells of the gland and less in the cells of Lamina epithelialis. The most intensive region of immunoreactive cells were determined as the fundus. Immunoreactivity was not observed in the nerves beloging to the myenteric plexus and muscle tissue and also ganglion cells.

Keywords: *Zander, Stizostedion lucioperca, Endocrine cells, Gastrointestinal tract*

Sudak *(Stizostedion lucioperca)* Gastrointestinal Kanal Mukozasındaki Endokrin Hücreler

Özet

Sudak balığı *(Stizostedion lucioperca)* gastrointestinal kanal endokrin hücrelerinin dağılım ve yoğunluğu peroksidaz anti peroksidaz immunohistokimyasal yöntemi kullanılarak çalışıldı. Spesifik antiserumların Calcitonin gene related peptide (CGRP), Bombesin, Somatostatin-14, Sekretin, Trk-A, Trk-B, Trk-C, Histamin, Neurotensin, Neuropeptide Y (NPY)'nin sudak balığı sindirim kanalının bazı bölgelerinde dağılımlarının belirlemesi amaçlandı. Örnekler mide, bağırsak ve pilorik sekalardan alındı. Veriler SPSS 10 istatiksel yazılım programında varyans analizine tabi tutuldu ve varyans analizi sonucu önemli bulunan bölgelerdeki immunreaktif hücre tipi ortalama yoğunlukları ANOVA testi ile karşılaştırıldı. İmmunoreaktivite gösteren hücrelerin bezlerdeki hücrelerde Lamina epitelyalisdeki hücrelere kıyasla daha yoğun olduğu gözlendi. En yoğun immunoreaktif hücrelerin midede yer aldığı belirlendi. İmmunorektivite miyenterik sinir ağındaki hücrelerde, kas dokusunda ve gangliyon hücrelerinde gözlenmedi.

Anahtar sözcükler: *Sudak, Stizostedion lucioperca, Endokrin hücre, Gastrointestinal kanal*

INTRODUCTION

A large number of endocrine-type secretions are produced in the gastrointestinal tract mucosa of the fish. These secretions, which are called peptide and/or amine, are detected in lamina epithelialis, glands, several connective tissue cells, mucosal nerve ganglions [1,2], and intermuscular nerve plexus [3]. These secretions assure the mobility as well as the proper functioning of the nervous system, regulation of the secretion through the cellular interaction [4], cellular proliferation [5,6], regulation of the intestinal epithelium [7], and the contraction of the smooth muscles [8]. Some researchers suggest that a relationship exists between the immune and endocrine systems [9] and that some secretions which belong to the endocrine cells may be effective in developing immunity

İletişim (Correspondence)
☎ +90 246 3814280
✉ nsenol@fef.sdu.edu.tr

against parasital diseases [10]. Also, it is argued that some peptides (GLP-2) may be used in gastrointestinal region for related injuries [6].

In the studies which were conducted on mammals and fish [3,11,12], endocrine cells in the digestive tract were observed to interact with each other as well as with the neurons, and thus leading to a complex system. In recent years, endocrine cells, which are identified in fish and which secrete peptide and/or amine hormones like gastrin, secretin, CCK (cholecystokinin), and serotonin containing pancreatic polipeptide, such as enteroglucagone, somatostatin, substance P, vasoactive intestinal peptide (VIP), bombesin, gastric inhibitory polypeptide (GIB), motilin have been reported to be found in a scattered manner in pancreas apart from the gastrointestinal mucosa [1,3,7,11,13]. In addition to all these traditional ones, such peptides as Trk-like proteins (TrkA-like, TrkB-like, TrkC-like), neuropeptide Y, calcitonin gene-releated peptid (CGRP), neurotensin, metenkephalin are reported to be found in fish, as well. These peptides regulate the autonome nervous system as well as the activities of the digestive system in many respects [4,7,14-18].

In this study, our aim was to determine the distribution and mucosal location of the cells, which contain CGRP, bombesin, somatostatin-14, sekretin, Trk-A, Trk-B, Trk-C, Histamine, NPY, neurotensin peptides, within the different sections of the gastrointestinal tract (cardia, fundus, pylorus, pyloric caeca, anterior and posterior intestine) of zander *(Stizostedion lucioperca)*.

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MATERIAL and METHODS

Stomach, pyloric caece and intestine of 30 zander *(Stizostedion lucioperca)* were used for this study. The samples which were taken from the esophagus, adjacent parts of the stomach with the intestine (cardia and pylorus), fundus region, pyloric caeca, anterior and posterior intestine were fixed in Bouin's solution for 12 h. The samples which were excised and processed with routine histologic tissue methods were blocked in paraffin. Peroxidase-antiperoxidase (PAP) complex method modified by Sternberger 19 was applied to the 6-7 micron-thick sections. In accordance with this method, the sections exposed to xylol and alcohol were washed and then waited in a hydrogene peroxide solution (0.3 % H_2O_2). The sections which were kept in normal goat serum (1:100) for 1 h were kept in antisera, the name and the dilutions of which are separately given in *Table 1*, at +4°C' for 12-18 h. After the cleaning process with PBS (Phosphate buffered saline 0.01 M, pH 7.4), the sections were kept in anti-rabbit IgG solution (1:200) under room temperature for 1 h. In subsequent stages, they were washed with PBS and waited in PAP complex (1:400). The sections which were exposed to 3,3-diaminobenzidine tetrahydrochloride (DAB) stain were treated with alcohols together with xylol, and then the slides were mounted entellan.

RESULTS

CGRP, Secretin, Trk-A immunoreactive (IR) cells in the lamina epithelialis of cardia were found to be distributed at medium density. On the other hand, Som-14, Bombesin Trk-B and Trk-C IR cells were observed to be less in number in proportion to other peptides. The cells which contain Histamine, NPY and Neurotensin were not detected in this region. Besides, Trk-A and CGRP IR cells found in the glands of cardia region were determined to

Table 1. *Primary antisera used in this study*
Tablo 1. *Bu çalışmadaki primer antiserumlar*

Antiserum	Item No	Dilution	Source
CGRP	sc-28920	1: 200	Santa Cruz Biotec. INC.
Bombesin	NCL-BOMp	1: 200	Nova Castra Lab. UK
Histamine	H 7403	1: 200	Sigma USA
Somatostatin-14	CL-SOMATOp	1: 200	Nova Castra Lab. UK
Sekretin	sc-20938	1: 200	Santa Cruz Biotec. INC.
Neurotensin	sc-20806	1: 200	Santa Cruz Biotec. INC.
NPY	sc-28943	1: 200	Santa Cruz Biotec. INC.
Trk-A	sc-118	1: 200	Santa Cruz Biotec. INC.
Trk-B	sc-12	1: 200	Santa Cruz Biotec. INC.
Trk-C	sc-117	1: 200	Santa Cruz Biotec. INC.

be densely populated. These were followed by Trk-C and Bombesin immunoreactive cells in number, respectively. However, Som-14, Secretin, Trk-B, Histamine NPY and Neurotensin immunoreactive cells were not detected.

With respect to the relative frequency in the lamina epithelialis of fundus region, NPY IR cells were observed to rank first and followed by Trk-C and Histamine IR cells in number, respectively. Except for the small number of Trk-B immunoreactive cells, the other peptides were determined to be at medium intensity. In fundus glands, we observed that no Trk-B IR cell was found but a small number of Trk-C and Neurotensin IR cells. The cells which proved immunreactivity against the other antisera were at medium intensity.

Bombesin IR *(Fig. 1)* cells were observed to be highly dense in the lamina epithelialis of pylorus region, and this was followed by Trk-B IR cells. Apart from Trk-A and NPY IR cells which were found to be small in number in the pylorus region, the other peptides *(Fig. 2)* were at medium density, and there was no Neurotensin immunoreactive cell. In pylorus glands, however, Som-14 immunoreactive cells were determined to be dense, CGRP IR ones at medium density, while Bombesin, Secretin, Trk-A and Histamine cells were in small number *(Fig. 2)*. We could not detect Trk-B, Trk-C, NPY, and Neurotensin IR cells.

Som-14 and Bombesin IR cells were found to be at medium density in the lamina epithelialis of the pyloric caeca region, while there was no Trk-B, Histamine and Neurotensin IR cell detected. Also, other IR cells were determined to be small in number. As for the density in the glands of pyloric caeca region, CGRP and Som-14 IR cells were determined to rank first *(Fig. 3)*. Bombesin, and Histamine immunoreactive cells were found to be at medium density, while the cells which contain the other peptides couldn't be detected.

In the lamina epithalialis of the anterior intestine, CGRP, Bombesin, Som-14, Sekretin, and Trk-A IR cells were found to be distributed at medium density. No immunoreactivity of the other peptides was detected. As for the case in the glands, the density of CGRP, Bombesin, Som-14, Sekretin, Trk-A, and Trk-C IR cells were found to be low, whereas no Trk-B, Histamine, NPY, and Neurotensin IR cell proved to exist.

In the lamina epithalialis of the posterior intestine, Sekretin IR cells were found at medium intensity, while CGRP, Bombesin, Som-14 *(Fig. 3)*. Trk-A, Trk-C, and NPY immunoreactive cells were comparatively less denser.

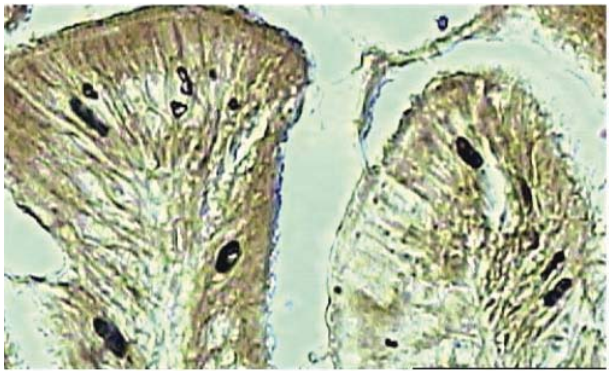

Fig 1. Bombesin IR cell Pylorus x400
Şekil 1. Bombesin IR hücre Pilorus x400

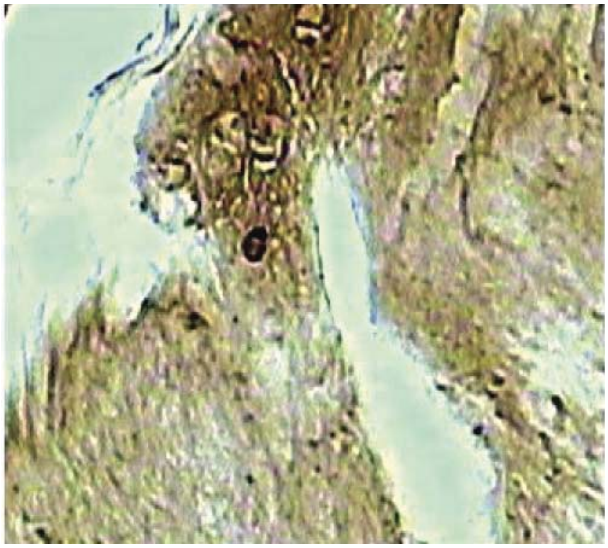

Fig 2. Somatostatin-14 IR cell Pylorus x400
Şekil 2. Somatostatin-14 IR hücre Pilorus. x400

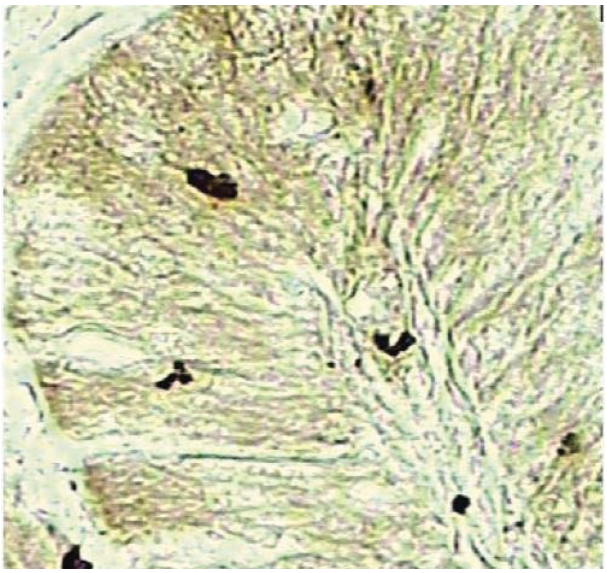

Fig 3. Somatostatin-14 IR cell Post. intes. x400
Şekil 3. Somatostatin-14 IR hücre Post. bağ. x400

Trk-B, Histamine, and Neurotensin immunoreactivities were not detected. With respect to the relative frequency in the glands, the first place was reserved by Sekretin IR cells, and these cells were followed by Som-14, CGRP and Bombesin IR cells in number. Trk-A, Trk-C, and NPY immunoreactive cells, on the other hand, were not observed in the glands.

In this study, a comparison between the cells, which contain 10 different peptides and which are found in 6 regions from which samples were taken, was made using the statistical analysis (ANOVA) *(Table 2)*. It was observed that there was no difference when P=0.000 or P<0.0000 and that the peptides proved to be different in density when P>0.000. Accordingly, significant differences in density were observed to exist between the peptides in the glands and lamina epithelialis of the cardia and fundus regions as well as in the glands and the lamina epithelialis of the posterior intestine. Since P=0.000 in other regions, no remarkable difference was noted. As a result of the immunoreactive staining (PAP), the resulting reaction towards all the antisera was determined to prove weak.

DISCUSSION

As it is the case in fish, the endocrine cells in the gastrointestinal tract of the vertebrates were found to have very different types in related studies. At the same time, these cells were observed to vary in density in the different regions of the gastrointestinal tract [20-22].

In this study, CGRP immunoreactive cells *Anguilla anguilla* [17] and *Salmo trutta* [10] whose regional distribution and localization are to be determined, were reported to be densely distributed in the gastrointestinal tract. Besides, CGRP immunoreactivity was reported to exist in intestinal myenteric plexus nerve fibres and ganglion cells [10,17,23]. In this study, however, no immunoreactivity has been detected in the mentioned regions. On the other hand, CGRP IR cells were found to have been localized in the epithalialis and glands of the stomach and intestine.

Bombesin is a peptide which is produced by ECs cells and found in mammals, amphibians, and gastrointestinal tract of fish [11]. Endocrine function of bombesin regulates the secretion of gastric acid and its motility [16]. In a study conducted on *Amia calva* [16] Bombesin immunoreactive cells were found to be densely distributed in the fundus region, which is in accordance with the findings of this study. While bombesin immunoreactive cells which are found in the fundus region of this species 16 were reported to have a strong immunostaining characteristic in the former study, these cells were observed, in this study, to have a weaker immunostaining characteristic as it was the case with the other peptides.

Although Bombesin IR cells in the pyloric caeca and intestine of *Salmo trutta* [11] were found to be small in number, these cells proved to have a mean density in both epithel and glands of the mentioned regions..

Somatostatin is a polypeptide of 14 amino acids and has been originally isolated from the sheep hypothalamus [24]. As it is the case in this study, somatostatin-14 immunoreactive cells were reported to be localized in the gastrointestinal tract of the species *Oncorhynchus mykiss* [8], *Osteoglossomorpha* [25], *Pelteobagrus fuluidraca, Monopterus albus, Siniperca chuatsi* [3], *Zacco platypus* [24], *Coreoperca herzi* [22]. These cells were also reported to be found in endocrine pancreas of some fish species [3,13,25]. In this study, while these cells were observed to have a mean density in the epithelium and glands of the anterior intestine, they were found high in number in 7 different teleost fish species without stomach [3]. Pan et al.[3], Domeneghini et al.[17]; Maake et al.[23] states that they observed somatostatin immunoreactivity in nerve plexus and fibres as well, whereas we could not detect this immunoreactivity in the mentioned regions.

Secretin is a polypeptide of 27 amino acids and a member of VIP/glucagon family [26]. In this study, secretin IR cells were found in intestines and pyloric caeca in small number, which is in accordance with the findings of Dezfuli et al.[11] and Bosi et al.[12]. On the other hand, these cells were found to exist neither in the stomach nor within the other mentioned regions of *Zacco platypus* [24] and *Coreoperca herzi* [22] species.

Trk-like (A-B-C) proteins which are secreted by the cells making up the sub-population of the endocrine cells carry out the neurotrophin synthesis, amine and/or peptide storage 4 as well as the regulation of the blood circulation of the gastrointestinal tract [23]. The fact that Trk-like proteins (TrkA, TrkB, TrkC) are distributed throughout the intestines of the teleost fish was reported by Lucini et al.[27]. Also, the same researchers stated that these immunoreactivities were found in the nerve fibres of the intestine myenteric plexus and within the ganglion cells. The existence of the cells which show this immunoreactivity in the stomach and intestine of the species Pagrus major and Dentex dentex has been proved [28]. In a study conducted on different telost species [27], Trk-A IR cells were found only in the intestines, Trk-B IR cells only in the stomach, and Trk-C IR cells both in the stomach and intestines. In this study, Trk-A IR cells were observed in all the studied regions, whereas Trk-B ones were detected in cardia, pylorus, and pyloric caeca. Trk-C immunoreactivity

Table 2. *Anova test and the mean values of the immunreactif cells in the lamina epithelialis and the glands of the regions studied*
Tablo 2. *Anova testi ile Lamina epiteliyalis ve bezlerdeki immunoreaktif hücre yoğunluğu ortalama değerleri*

Region	CGRP	Bombesin	Som-14	Sekretin	Trk-A	Trk-B	Trk-C	Histamine	NPY	Neurotensin	Anova (F)	P
Cardia Lam epit.	1.33±0.577	0.67±0.577	1.00±0.000	1.33±0.577	1.33±0.577	0.67±1.155	0.33±0.577	0.00±0.000	0.00±0.000	0.00±0.000	3.210	0.014
Cardia Gland	2.00±1.000	0.33±0.577	0.00±0.000	0.00±0.000	2.33±0.577	0.00±0.000	0.66±0.577	0.00±0.000	0.00±0.000	0.00±0.000	11.926	0.000
Fundus Lam epit.	1.33±1.528	0.67±0.577	1.33±0.577	1.67±0.577	1.33±1.528	0.67±1.155	2.00±1.000	2.00±1.000	4.00±1.000	1.00±1.000	3.111	0.017
Fundus Gland	2.33±1.527	2.00±1.000	2.66±0.577	2.33±0.577	2.00±1.000	0.00±0.000	1.00±1.000	2.00±1.000	2.00±1.000	1.00±1.000	2.206	0.068
Pylorus Lam epit.	1.33±0.577	4.00±1.000	1.33±0.577	1.33±0.577	0.33±0.577	2.00±1.000	1.00±0.000	1.00±0.000	0.67±0.577	0.00±0.000	9.990	0.000
Pylorus Gland	1.00±1.000	0.66±0.577	5.33±0.577	0.66±0.577	0.33±0.577	0.00±0.000	0.00±0.000	0.33±0.577	0.00±0.000	0.00±0.000	29.514	0.000
Ant. Int. Lam epit.	1.00±0.000	1.67±0.577	0.67±0.577	1.00±1.000	1.67±0.577	0.00±0.000	0.00±0.000	0.00±0.000	0.00±0.000	0.00±0.000	7.333	0.000
Ant. Int. Gland	1.33±0.577	2.66±1.527	2.66±0.577	1.00±0.000	2.00±1.000	0.00±0.000	1.00±0.00	0.00±0.000	0.00±0.000	0.00±0.000	8.852	0.000
Post. Int. Lam epit.	0.67±0.577	1.00±1.000	1.33±0.577	2.00±1.000	0.33±0.577	0.00±0.000	1.00±1.000	0.00±0.000	1.00±1.000	0.00±0.000	2.637	0.034
Post. Int Gland	1.66±1.154	1.33±1.527	2.33±1.154	2.66±0.577	0.33±0.577	0.00±0.000	0.33±0.577	0.00±0.000	0.33±0.577	0.00±0.000	4.918	0.001
Pyl. Cae. Lam epit.	1.67±1.155	2.67±0.577	3.00±0.000	1.00±0.000	2.00±1.000	0.00±0.000	0.33±0.577	0.00±0.000	1.33±0.577	0.00±0.000	11.378	0.000
Pyl. Cae. Gland	3.00±1.000	2.00±1.000	3.00±1.000	0.00±0.000	2.66±0.577	0.66±0.577	0.00±0.000	1.33±0.577	0.00±0.000	0.00±0.000	12.741	0.000

was detected in the regions except pylorus gland, anterior intestine, lamina epithelialis, and pyloric caeca.

Histamine is a peptide which assures the smooth muscle contraction of the gastrointestinal tract and stimulates the stomach acid secretion [29]. In accordance with the findings obtained from the studies on the species *Oncorhynchus mykiss* [30]. Histamine IR was found to exist in the fundus and pylorus regions of the stomach. As far as the species *Oncorhynchus mykiss* that Fairgrieve et al.[30] fed with diets containing protein at different doses are concerned, they noted that the diets containing high doses of protein had increased the Histamine immunoreactivity in the samples. However, in this study, Histamine immunoreactive cells which have not been observed in the intestines were found to exist in the intestines of the species *Oncorhynchus mykiss* [31], *Salmo salar* [32], *Dicentrarchus labrax* [33]. Histamine IR was determined to be much higher in density in the samples of the species *Oncorhynchus myxiss* [31], *Salmo salar* [32], and *Dicentrarchus labrax* [33], which carry parasites. In the parasite-carrying samples of the species *Salmo salar*, mast cells in the connective tissues of the intestine were noted to secrete a high amount of Histamine [32]. Once *Oncorhynchus mykiss* was given toxic substances, the Histamine immunoreactivity in the intestine was determined to increase [31].

Neuropeptide Y (NPY) is a 36 amino acid peptide which is abundant and widespread in the central nervous system of all the vertebrates [34]. In this study, these cells were observed in the epithel and glands of the fundus and posterior intestine. Similar findings were reported in previous studies, as well [12,17,25] . Similar to the findings obtained from the studies on *Salmo trutta* [10], a small amount of NPY IR cells were detected in the intestines in this study, as well. Rombout & Reinecke [14] stated that the neurotensin immunoreactivity was found to exist densely in the intestines, myenteric plexus nerve fibres and ganglion cells of the species *Barbus conchonius*. In this study, however, these cells were not observed in the intestines but only in the fundus region. This immunoreactivity was not observed in Plexus myenteric nerve fibres and ganglion cells, either.

Certain difficulties were reported to appear in identifying the mucosal peptides of the digestive tract of fish. Rajjo et al.[16] suggest that these peptides are long-structured in proportion to the mammals and that the determination of the peptide is difficult even if specific antiserum is applied. Besides, Larsson et al.[35] emphasize that apart from the the dilution, heterogeneity, and specifity of the antiserum used, the species should also be taken into consideration. Elbal and Agulleiro [15] state that the difference induced by the terminal ends of the antisera might be species-specific, whereas Holmgren and Nilsson [36] assert that this can be attributable to immunoreactive material difference in neuron and mucosal cells. This resulting interaction was defined as "Quasi Immunoreactivity" by Holmgren and Jönsson [5]. However, some researchers have argued that the positive results obtained in fish-related studies show a structural relationship with the mammals [15] and that although they vary, amino acid arrangements of peptides may also show similarities [35]. Similarly, Rajjo et al.[16] reported that the anticipated innervations happened when antiserum-specific peptide was administered to the fish. Moreover, though the antisera used were obtained from the mammals, it was concluded that the peptides in the digestive tract of the mammals and fish have a similar nature due to the immunoreactivities detected.

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