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DETERMINATION OF THE PRESENCE OF NUCLEOBINDIN-2/NESFATIN IN Cyprinus carpio (Linnaeus, 1758) AND Cyprinion macrostomus (Heckel,1843)

ABSTRACT

Nucleobindin-2 protein was also termed as nesfatin (for NEFA/NUCB2-encoded satiety and fat-influencing proteins). The aim of this study is to determine to find out whether NUCB2/nesfatin in the blood serum of Cyprinus carpio and Cyprinion macrostomus by enzyme-linked immuno assay (ELISA). Nesfatin level was analysed using Fish Nesfatin ELISA kit (Cat. No: MBS013992). Results were compared between two species and between sexes of each species. In addition, nesfatin levels were also compared with the body weight and length of both C. carpio and C. macrostomus. There has been no study the Nucleobindin-2/Nesfatin described in C. macrostomus, C. carpio in the literature.

Keywords: NUCB2/Nesfatin, Cyprinus carpio, Cyprinion macrostomus, Blood Serum, ELISA

1. INTRODUCTION

Nucleobindin-2/Nesfatin is a 50-kDa protein hormone. It is consist of 396 amino acids, with very high amino acid sequence homology among rat, mouse, and human species [1]. It is suggested that nucleobindin-2 is divided by prohormone convertases into various active peptides, nesfatin-1 (1-82 aminoacid), nesfatin-2 (85–163 amino acid), and nesfatin-3 (166–396 amino acid) [2 and 3]. Of these three peptides, only nesfatin-1 has been shown to have biological functions. The main functions of nesfatin-1 is involved in the regulation of miscellaneous physiological metabolism, including feeding by reducing feed intake [3, 4, 5, 6, 7, and 8], locomotion, reproduction, stress modulation termogenesis [9, 10, and 11] and energy homeostasis [4, 5, 12, 13, 14, 15, 16, and 17]. In a number of animals and humans are present NUCB2 and the nesfatin-1 region displays a high identity among reported NUCB2 sequences [18]. The predicted nesfatin-1 and 2 in Carassius auratus are highly conserved and 82 and 81 amino acids long respectively. Nesfatin-3 is longer in Carassius auratus (309 amino acids) than in rat (231 amino acids) [6]. In fish, two isoforms of NUCB2 (NUCB2A and NUCB2B) exist and nesfatin-1 has been identified Schizothorax prenanti [19], Danino rerio [8 and 20], Carassius auratus [2, 6, and 8], female Oncorhynchus mykiss [21] and Alburnus tarichi [22] most abundantly found in liver but least found in gut brain and tissues. The Both NUCB2A and NUCB2 mRNAs in the Danino rerio were most abundant in the liver but less expression was found in other tissues including the gut and brain. Additionally NUCB2 mRNA expression and protein content has been demonstrated several tissues including the

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hypothalamus, brain, hepatopancreas, liver, intestine and ovary in the Schizothorax prenanti [19] and Carassius auratus [6].

Nesfatin -1 like IR cells in the Carassius auratus were found in the feeding regulatory nucleus of the hypothalamus and gastrointestinal tract [6]. NUCB2/nesfatin-1 like immunoreactivity was detected in the mocosal layer cells of anterior gastrointestinal tract of Danino rerio [20]. Nesfatin-1 is present in the hypothalamo-pituitary-ovarian (HPO) of Carassius auratus and Danino rerio, also primarily determined the relationship between nesfatin-1 and ghrelin, CCK and orexin in Carassius auratus [2 and 8]. The richest and most important family of fish are Cyprinid and Its members are distributed world-wide. This family is representing by approximately 1500 species in the world, about 30 genus and 70 species in Türkiye [23]. The One of the most widely cultured species in the world is Cyprinus carpio [24 and 25]. Cyprinion macrostomus also known as the kangalfish. The genus Cyprinion (type species:Cyprinion macrostomus Heckel, 1843) is a western Asian genus of minnow, distributed from western Syria and the southern Arabian Peninsula to the western tributaries Pakistan and 5 species have been observed in Iran [26]. C. macrostomus is used as an aquarium fish. It is called the "doctor fish", because it plays a therapeutic role in medical treatment [27], and it is also known as "stone fish" due to its feeding activities. There are present studies related peptide as leptin [28 and 29], apelin and ghrelin in the Cyprinus carpio, HSP 70 in the C. macrostomus [30, 31, and 32]. In the both fish has not been observed in studies on nesfatin.

2. RESEARCH SIGNIFICANCE

In this study, we aimed to determine NUCB2/Nesfatin blood/serum level in C. carpio and C. macrostomus. NUCB2/Nesfatin in fishes were compared with between two species and between sexes of each species. In addition to, body weight and length were determined. The for identify nesfatin was used to ELİSA. These results provide evidence supporting the potential metabolic roles of NUCB2 in fish.

Highlights:

- Detection novel information on NUCB2 in fish
- Provide molecular, anatomical and functional evidence to metabolic roles for NUCB2 in fish
- Indicate NUCB2/Nesfatin blood/serum level in C. carpio and C. macrostomus

3. EXPERIMENTAL METHOD-PROCESS

3.1. Materials

In this study, 23 adult C. macrostomus and 13 adult C. carpio obtained from Murat Lake (Elazığ-Bingöl, Türkiye) were used.

3.2. Methods

Fish were anaesthetized with MS-222. After length, weight and sex discrimination of fish was made. Then blood samples taken from the caudal vena were put into the tube with the aprotinin to prevent desaturation of proteins. The samples were separated by centrifugation (4500rpm for 5min at 4°C) and stored at -20°C and analysed immediately at the end of study (Hettich, Zentrifugen Universal 32 R, Germany). Serum NUCB2/nesfatin level was analysed using available enzyme-linkedimmunoassay (ELISA) kit (Fish Nesfatin ELISA kit, Cat. No: MBS013992). The detection range of this kit is 0.25ng/ml-8ng/ml. Sensitivity for the assay is reported to be 0.1 ng/ml. Controls were included in all assays. The plate was then read at 450nm with a SpectraMax Plus 384 plate reader (Molecular Devices LLC, Sunnyvale,

CA). Values are expressed as means ±SEM. Unpaired t-test used to assess between-group data. The Kolmogorov– Smirnov Z test showed that the data were normally distributed. The comparisons were made of both between two species and between sexes of each species, and $p<0.05$ was considered significant. NUCB2/Nesfatin levels, length and weight of each species were compared using Pearson correlation coefficient.

4. FINDINGS

Mean values of the length and weight of C. carpio (26.96±1.17cm/317.69±39.68g) and C. macrostomus (16.6±1.4cm/60.32±3.14g) used in this study were determined.

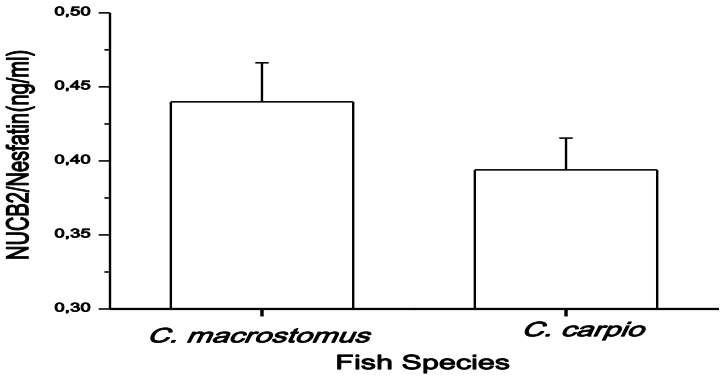

Figure 1. NUCB2/Nesfatin levels in the blood serum of C. carpio (n=13) and C. macrostomus (n=23) (mean±SEM, $p>0.05$)

Sex discrimination of fish were found as 15 female and 8 male for C. macrostomus and 9 female and 4 male for C. carpio. NUCB2/nesfatin level (0.43±0.02ng/ml) in the blood serum of C. macrostomus was found to be no significantly lower ($p>0.05$) than those (0.47±0.01ng/ml) of the C. carpio (Figure 1).

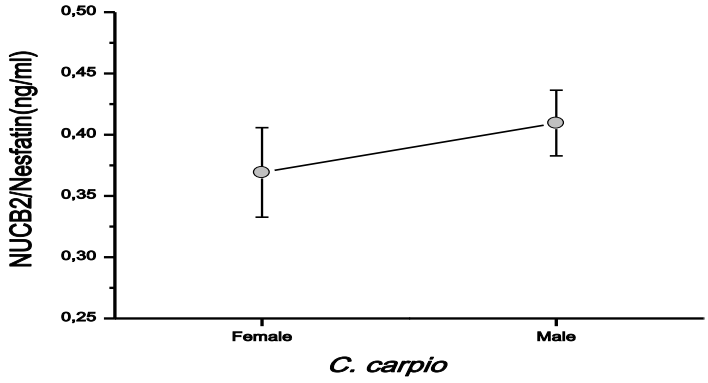

Figure 2. NUCB2/Nesfatin levels levels in the blood serum of male (n=9) and female (n=4) of C. carpio (mean±SEM, $p>0.05$)

It was found to be significant difference ($p>0.05$) that compared to the NUCB2/nesfatin levels of male (0.4±0.02ng/ml) with female (0.36±0.03ng/ml) of C. carpio (Figure 2).

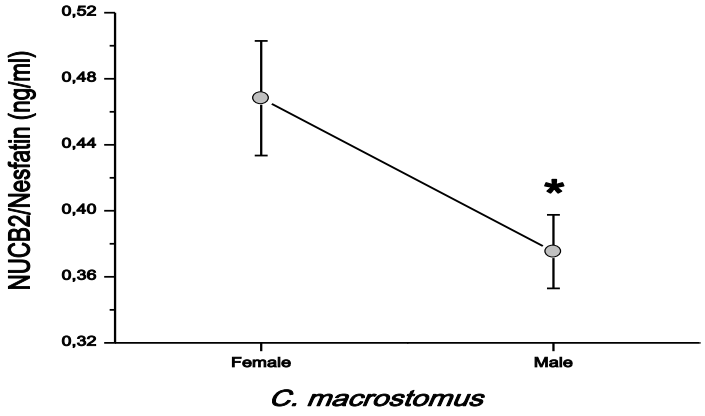

Figure 3. NUCB2/Nesfatin levels in the blood serum of male (n=8) female (n=15) of C. macrostomus (mean±SEM, p>0.05)

It was found to be significant difference (p>0.05) that compared to the NUCB2/nesfatin of male (0.37±0.02ng/ml) with female (0.46±0.03ng/ml) of C. macrostomus (Figure 3).

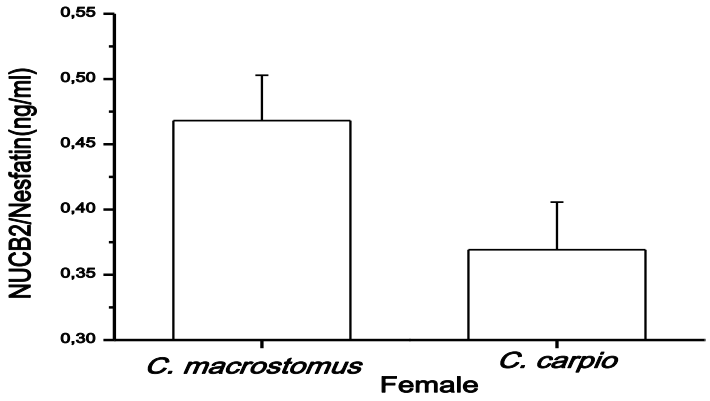

Figure 4. NUCB2/Nesfatin levels in the blood serum of female C.carpio (n=4) and C.macrostomus (n=15) (mean±SEM, p>0.05)

When the NUCB2/nesfatin levels of the female of C. macrostomus (0.46±0.03ng/ml) and female of C. carpio (0.36±0.03ng/ml) were compared It was not found to be significantly higher (p>0.05) (Figure 4).

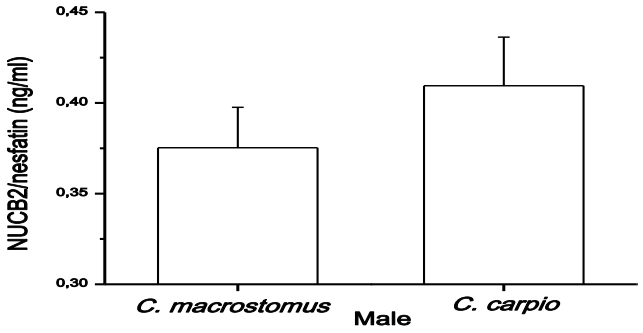

Figure 5. NUCB2/Nesfatin levels in the blood serum of male C. carpio (n=9) and C. macrostomus (n=8) (mean±SEM, p>0.05)

In addition, it has been shown that not significantly correlated when examined correlations between the NUCB2/nesfatin level in the blood serum and body weight (r=0.114, p=0.711) or length (r=0.054, p=0.862) of C. carpio. Similarly, the correlations between NUCB2/nesfatin level in the blood serum and body weight (r=0.023, p=0.916) or length (r=0.150, p=0.494) of C. macrostomus were also found not significant. When the nesfatin levels of male samples were compared, unlike females, C.carpio males (0.4±0.02ng/ml) were found to be higher than C. macrostomum males (0.37±0.02ng/ml). However, it was found to be no significantly lower (p>0.05) (Figure 5).

5. DISCUSSIONS

NUCB2/nesfatin is composed of 396 amino acids, preceded by a 24-amino acid signal peptide with very high amino acid sequence homology among human, Mouse and rat species [3, 5, 14, 15, and 33]. NUCB2/nesfatin-1 is a metabolic hormone that has been widely identified in various hypothalamic nuclei and brain areas with confirmed roles in energy homeostasis of rodents, mammals [7, 11, and 34] and fish [2 and 6]. NUCB2/nesfatin-1 is also expressed in peripheral tissues with relevant metabolic functions, such as the pancreas, stomach, gut, gastric mucosa and the adipose [1, 5, and 6]. Nesfatin-1 is a novel anorexigen in fish [6 and 8]. In Carassius auratus is reported the presence of two paralogous NUCB2 genes (NUCB2A and NUCB2B). It has been indicated that nesfatin-1 involved in the regulation of feeding and metabolism [3, 8, and 9]. In this study, NUCB2/Nesfatin is determined presence in blood/serum of C. carpio (0.47±0.01ng/ml) and C. macrostomus (0.43±0.02ng/ml). In fish, Nucleobindin-2 (NUCB2)/nesfatin-1-like immunoreactive (IR) cells are present in the hypothalamus, nucleus lateralis tuberis, pituitary and anteriör intestine, gonads, gastrointestinal tract [2, 6, 8, 19, and 20]. Furthermore, in the enteroendocrine cells of the goldfish anterior intestine has been found co-localisation of nesfatin-1-like and ghrelin-like immunoreactivity [2]. Nesfatin-1 also found in the follicular cells, but not the oocytes, in both zebrafish and goldfish ovaries [8]. In this study, NUCB2/Nesfatin is determined presence in blood/serum of C. carpio (0.47±0.01ng/ml) and C. macrostomus (0.43±0.02ng/ml).

In the research, NUCB2/Nesfatin determined in blood/serum level of C. carpio and C. macrostomus were compared with between two species and between sexes of each species. NUCB2/Nesfatin levels were found to be no significantly higher (p>0.05) C. macrostomus female (0.46±0.03ng/ml) than C. carpio female (0.36±0.03ng/ml). In the same way It was found to be no significantly lower (p>0.05) NUCB2/nesfatin levels of the C. macrostomus male (0.37±0.02ng/ml) than C. carpio male (0.4±0.02ng/ml). In addition to nesfatin-1 was detected in the circulation of female Oncorhynchus mykiss [21]. Food-deprived C. auratus had significantly less nesfatin-1 in serum than regularly fed controls with 2.52±0.26 and 6.45±3.31ng/ml, respectively (P<0.05). Besides, serum nesfatin-1 levels stayed significantly elevated in circulation (5.97±2.19ng/ml) up to 1 hr after feeding (P<0.05) [6]. Moreover, the correlations between NUCB2/nesfatin level in the blood serum with body weight (r=0.023, p=0.916)/length (r=0.150, p=0.494) of C. macrostomus and body weight (r=0.114, p=0.711)/length (r=0.054, p=0.862) of C. carpio were found not significant. NUCB2 mRNA expression was detected in several tissues including the brain, hepatopancreas, adipoz tissue, intestine, ovary, liver muscle and gill of Carassius auratus [6] and Schizothorax prenanti [19]. NUCB2/Nesfatin immunoreactivity was detected in several tissue using immunohistochemical techniques [1 and 2], Western blot [6], RNA

electrophoresis and DNA purification [19]. In the present study, as in other research, used an ELISA kit measuring the NUCB2/Nesfatin [6]. NUCB2/Nesfatin-1-like in the Carassius auratus and Danino rerio used a fluorescence immunohistochemical tecniques [8]. Using real-time quantitative polymerase chain reaction NUCB2 mRNA expression was detected in the various tissues of Danino rerio, and Carassius auratus [6 and 20].

6. CONCLUSION AND RECOMMENDATIONS

In conclusion, we have shown that NUCB2/Nesfatin levels both between two fish species and between sexes of each species were not significant difference. In addition, we determined that no correlation between NUCB2/Nesfatin levels with both weight and length of C. macrostomus a and C. carpio. Results of this study indicate that NUCB2/Nesfatin blood serum levels are independent of fish species, sexes, body weight and length. The present data demonstrate that more comprehensive studies are needed for conclusive data interpretation. These results provide evidence supporting the potential metabolic roles of NUCB2 in fish.

CONFLICT OF INTEREST

The authors have no conflicts of interest to be disclosed.

FINANCIAL DISCLOSURE

The authors declare that this study has received no financial support.

DECLARATION OF ETHICAL STANDARDS

The authors of this article declare that the materials and methods used in this study require an ethical committee. The Animal Experiments Local Ethics Committee Directive (Protocol No: 2015/02-01/02) of Bingöl University, Republic of Turkey.

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