SHORT COMMUNICATION

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# Effects of intracerebroventricular injection of apelin-13 on food intake in broiler chicks

**Razieh Amini Zadeh[1], Hossein Jonaidi[1]*, Saeed Esmaeili Mahani[2], Mahsa Salehi[1], Mojtaba Emam Bakhsh[1]**

*[1] Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran; [2] Department of Biology, Faculty of Basic Sciences, Shahid Bahonar University of Kerman, Kerman, Iran.*

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## Abstract

Apelin is an endogenous peptide ligand for G protein coupled apelin receptors (APJ orphan receptors) which are very similar to angiotensin II receptors. Apelin is expressed in most tissues of the body including hypothalamus that is responsible for regulating water and food intake, the gastrointestinal tract, the circulatory system, adipose and muscle tissues, and the immune system. The physiological actions of apelin, including food intake, has not yet been reported in birds. In this study, the effect of intracerebroventricular injection of different doses of apelin-13 was investigated on food intake in neonatal broilers at the age of five and seven days. The chicks had access to food immediately after injection and cumulative food intake was measured at half, 1, 2, 3, 4, 8 and 21 hr after injection. The 2-way ANOVA analyzed data showed that apelin-13 at dose of 1.00 µg significantly reduced food intake at 21 hr after injection in five-day old chicks. In addition, in dose of 1.50 µg, it could significantly reduce food intake at 2, 3, 4, 8 and 21 hr after injection. In seven-day-old chicks, the doses of 1.00 and 4.00 µg of apelin-13 had no effect on food intake compared to the control group. Apelin-13 at dose of 2.00 µg significantly reduced food intake at 8 and 21 hr after injection. The results of this study showed that apelin-13 had a reducing effect on food consumption in neonatal broiler chicks.



## Introduction

The regulation of appetite is involved with a number of organs, tissues and neural circuits throughout the body with a feedback loop between the brain and peripheral tissues to provide energy for metabolic needs.[1] In the brain, the hypothalamus is the most important center for receiving and processing environmental signals for regulating appetite and energy balance.[2] The apelinergic system which has been considered in recent years is involved in regulating appetite.[3] Apelin, an endogenous peptide, was first isolated from bovine stomach and identified as a G protein receptor ligand (APJ).[3] The apelin gene in chickens encodes a 78 amino acid peptide that shares 42.00% to 73.00% sequence matching with other vertebrate species, including humans, turtles and some aquatic species.[4] Like mammals, in chickens pre-proapelin forms two active fragments including apelin-13 and apelin-36. Two apelin receptor (APLNR) genes have been identified in chickens named APLNR1 and APLNR2.

The APLNR1 encodes a 370 amino acid receptor that shares a 64.00% amino acid sequence matching with the human APLNR and APLNR2 encodes a 365 amino acid receptor in chicken which shares a 43.00% amino acid sequence with the human APLNR. Like humans, both apelin receptors in chickens have seven membrane-spanning domains. APLNR2 has been reported to be activated only by apelin-36 at concentrations much higher than the physiological range.[4] Findings have shown that in chickens apelin-36 is a stronger ligand for APLNR1 than apelin-13.[4]

Apelin and its receptors are present in several hypothalamic nuclei such as paraventricular (PVN) and arcuate (ARC) nuclei which are involved in feeding behaviors and energy homeostasis.[5] Central injection of apelin-13 in mice also inhibits gastric emptying and chymus movement along the small and large intestines,[6,7] indicating a close correlation between food intake and bowel and stomach movements.[8,9] These findings suggest that apelin can play a regulatory role in feeding behaviors and energy homeostasis.[10]

***Correspondence:**

Hossein Jonaidi. DVM, PhD
Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran
**E-mail:** hjonaidi@uk.ac.ir

As far as we know, the physiological actions of apelin including food intake have not yet been reported in birds. In this study, for the first time, the effect of apelin-13 on food intake was investigated in neonatal brioler chicks. For this purpose, the effect of intracerebroventricular (ICV) injection of various doses of apelin on cumulative food intake was investigated.

## Materials and Methods

**Animals.** This study was carried out under the proposal number 48062 at the Faculty of Veterinary Medicine of Shahid Bahonar University of Kerman, Iran. One-day-old broiler chicks (Ross 308) were prepared. The birds were initially kept in mass in an automatic controlled cage with free access to water and food. For the first 48 hr, the cage temperature was 33.00 ˚C and humidity was 55.00 ± 5.00% and then temperature was reduced to 30.00 ˚C and humidity to 50.00 ± 5.00%. The chicks were kept in the continuous lighting.

**Ration.** The birds' diet, a starter diet containing 21.00% crude protein and 3,200 kcal ME $kg^{-1}$, was constant throughout the experiment and they had free access to water and food at all times except during the three hours of fasting.

**Intracerebroventricular (ICV) injection method.** The ICV injection was performed into the right lateral ventricle of chicks using a stereotaxic device based on the method of Davis *et al*.[11] The injection was performed using a 100-µL Hamilton syringe (Hamilton Co., Reno, USA) with a barrier inserted into the needle therefore only 4.00 mm of the needle could enter the bird's brain. The volume of injection was 10.00 µL of 0.85% saline solution (Shahid Ghazi Pharmaceutical Co., Tabriz, Iran) plus 0.10% Evans Blue (Farzaneh Arman Co., Isfahan, Iran) for the control group and the same solution with the same volume containing the corresponding dose of apelin-13 (Phoenix Pharmaceuticals Inc., Créteil, France) for the treatment groups (doses of 1.00 and 1.50 µg for five-day-old chicks and doses of 1.00, 2.00 and 4.00 µg for seven-day-old chicks). Each injection lasted 15 sec to prevent the solution from escaping from the bird's brain. Since the skulls of five and seven-day-old chicks are cartilaginous, the needle was easily entered the skull.

**Study protocol.** In the first stage of the experiment, the chicks were kept for five days. The chicks were transferred to individual cages for adaptation 48 hr before injection. On the day of injection, the chicks were fasted for 3 hr before injection in order to synchronize their food intake. The doses of 1.00 and 1.50 µg were injected in this stage. After the injection, the fresh weighed food was given to the chicks and the food intake was measured at half, 1, 2, 3, 4, 8 and 21 hr post injection. The second experiment was similar to the first experiment except that seven-day-old chicks were used. The doses of 1.00, 2.00 and 4.00 µg were injected in this stage.

**Confirmation of the injection site.** At the end of each experiment, the chicks were anesthetized and decapitated and their brain were removed to check the accuracy of the injection site. Due to the color of Evans Blue in the injected solution, the data from birds not properly injected with the solution were not included in the analysis (Fig. 1).

**Statistical analysis.** In order to analyze data and draw the diagrams, GraphPad Prism Software (version 9.0.0; GraphPad Software Inc., San Diego, USA) was used. Data was analyzed using a two-way analysis of variance (ANOVA) with repeated measure. Significance level was considered $p < 0.05$ using Tukey test.

## Results

**The effect of ICV injection of apelin-13 on food intake in five-day-old chicks.** The effects of ICV injection of apelin-13 at doses of 1.00 and 1.50 µg were compared to the control (vehicle) group. According to the results, the dose of 1.00 µg significantly reduced food intake at 21 hr after injection (Fig. 2). Also, the dose of 1.50 µg of apelin-13 significantly decreased food intake at 2, 3, 4, 8 and 21 hr after injection compared to the control group (Fig. 2).

**The effect of ICV injection of apelin-13 on food intake in seven-day-old chicks.** In this experiment, the effect of ICV injection of apelin-13 at doses of 1.00, 2.00 and 4.00 µg were compared to the control group. The results showed that the doses of 1.00 and 4.00 µg had no effect on food intake in chicks compared to the control group (Fig. 3). The dose of 2.00 µg of apelin-13 significantly reduced cumulative food intake 8 and 21 hr after injection (Fig. 3).


**Fig. 1.** Evans Blue traces in the right lateral ventricle of chickens' brains.

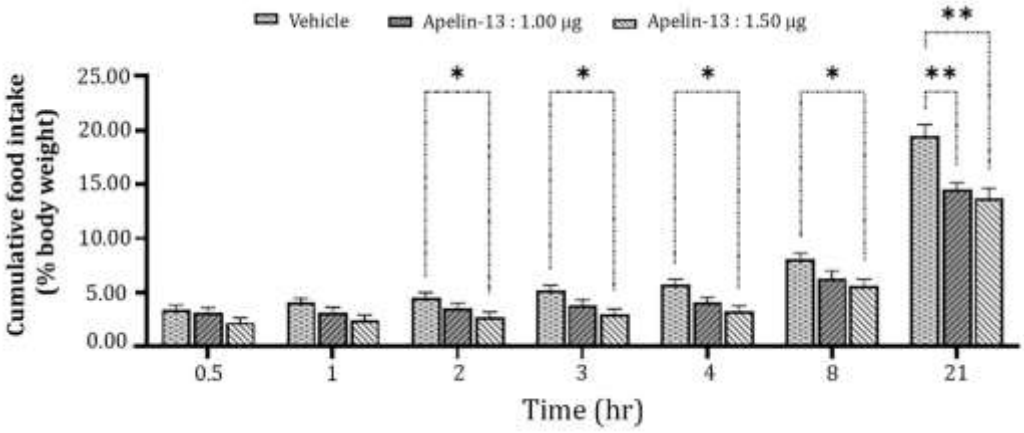


**Fig. 2.** Average cumulative food intake based on body weight percentage. 1.00 µg and 1.50 µg doses of apelin-13 were ICV injected to five-day-old broiler chicks. All data are presented as mean ± SEM (n = 10 per group). * Significant difference $p \leq 0.05$; ** significant difference $p \leq 0.01$.

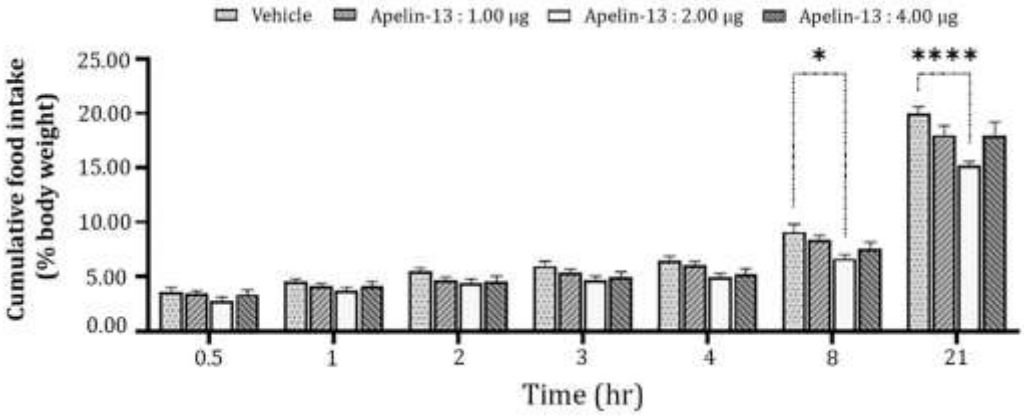


**Fig. 3.** Average cumulative food intake based on body weight percentage. 1.00, 2.00 and 4.00 µg doses of apelin-13 were ICV injected to seven-day-old broiler chicks. All data are presented as mean ± SEM (n = 12 for control group, n = 11 for dose of 1.00 µg group, n = 12 for dose of 2.00 µg group and n = 8 for dose of 4.00 µg group). * Significant difference $p \leq 0.05$; **** significant difference $p \leq 0.0001$.

## Discussion

The present study showed that central injection of apelin-13 reduced food intake in neonatal broiler chicks. Given that the apelin gene and its receptor are expressed in chicken brain,[5] it shows the involvement of apelinergic system in controlling food intake in the central nervous system of chicks.

The result of this study also revealed that apelin-13 decreased food intake in a very narrow range dose of 1.50 µg and 2.00 µg. This reduction was not occurred rapidly, and the effect was slow and chronic. The possible reason for this slow effect, may be related to low number of receptors or low interaction with other systems involved in feeding regulation in the appetite control centers or low penetration into the brain tissues. However, in agreement with our study, Lv *et al*. reported that ICV injection of antagonist apelin-13 (F13A), a selective antagonist of APJ receptors, had no effect on cumulative food intake in mice, however, injection of apelin-13 significantly reduced food intake. The combination of F13A and apelin-13 increased food intake compared to the group that received only apelin-13. This indicates that the anorexia effect of apelin-13 acts via the APJ receptors.[10] It has also been shown that apelin-13 inhibits gastric emptying and chymus movement along the small and large intestines which can be completely blocked by F13A.[6,12] This effect represents the action of apelinergic system on gastrointestinal tract. The apelinergic system has been found in several hypothalamic nuclei such as PVN, and ARC which are involved in feeding behaviors and energy homeostasis.[5] In addition, apelin is present in the circulatory system, peripheral tissues such as gastric mucosa[13] and adipose tissue.[14] As mentioned earlier, adipose tissue and the gastrointestinal tract are involved in regulating appetite by sending signals such as leptin and ghrelin.[15,16] In addition, apelin increases in obesity disorders associated with hyperinsulinemia that alters feeding behavior and energy balance.[14,17]

The mechanisms underlying the action of apelin-13 regarding feeding control in chicks have not been reported.

Anatomical evidence shows the localization of APJ mRNA in several areas of hypothalamus such as PVN and ARC nuclei which play an important role in regulating food intake.[3,5]

Several evidences have shown an inhibitory effect of central corticotrophin releasing factor **(**CRF**)** in food intake in rodents and chicks.[10,18] Injection of CRF into the PVN caused anorexia in rats.[10] The CRF antagonist (α-helical $CRF_{9-41}$) also attenuates the anorexia effects of CRF.[10] These findings were consistent with ICV injection of CRF in neonatal broilers which reduced cumulative food and water intake in chicks.[18] Recent *in vitro* studies have shown that apelin-13 stimulates CRF and Arginine vasopressin (AVP) secretion from the hypothalamus.[19] In addition, the APJ receptor is expressed in neurons in the hypothalamus that secrete CRF.[10] The studies also showed that injection of α-helical $CRF_{9-41}$ into mice did not affect cumulative food intake, although, its combination with apelin-13 reversed the inhibitory effects of apelin-13 on food intake and increased food intake compared to the group receiving only apelin-13. These results indicated that the CRF system was involved in the inhibitory effects of apelin-13 on food intake in small laboratory animals.[10]

In conclusion, in this study we showed for the first time that ICV injection of apelin-13 had a weak decreasing effect on food intake in chicks compared to strong effect of some anorectic peptides like Ghrelin. Although anorectic effect of apelin-13 was not seemed to be as important as other anorectic factors like CRF, POMC and CART in chicks, further investigation is needed to clarify the mechanisms underlying its effect.

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**Conflict of interest**

The authors have no conflict of interests to declare that are relevant to the content of this article.

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