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***Original Article***

# Interactions of Cholecystokinin and Glutamatergic Systems in Feeding Behavior of Neonatal Chickens

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**Abstract**

This study aimed to assess the possible feeding behavior alterations by central interactions of cholecystokinin (CCK) and glutamatergic systems in neonatal chickens. In experiment 1, chickens received intracerebroventricular (ICV) administration of saline and CCK ($CCK_4$; 0.25, 0.5, and 1 nmol). In experiment 2, birds were ICV injected with saline, $CCK_{8s}$ (0.25, 0.5, and 1 nmol). In experiment 3, chickens received the ICV injection of saline, $CCK_{8s}$ (1 nmol), MK-801 (15 nmol), and co-injection of the $CCk_{8s}$+MK-801. Experiments 4-7 were performed similar to experiment 3, except for chickens that were injected with CNQX (390 nmol), AIDA (2 nmol), LY341495 (150 nmol), and UBP1112 (2 nmol) instead of MK-801. Subsequently, the total amount of the consumed food was determined. According to the results, the ICV administration of $CCK_4$ (0.25, 0.5, and 1 nmol) could not affect the food intake in chickens ($P$>0.05). The ICV injection of the $CCK_{8s}$ (0.25, 0.5, and 1 nmol) led to a dose-dependent hypophagia ($P$<0.05). Moreover, hypophagia induced by $CCK_{8s}$ decreased by the co-injection of the $CCK_{8s}$+MK-801 ($P$<0.05). These results showed that the hypophagic effects of the CCK on food intake can be mediated by NMDA glutamate receptors in layer-type chickens.

**Keywords:** Chickens, Cholecystokinin, Food intake, Glutamatergic

## 1. Introduction

Feeding behavior is considered a complex physiological function in animals that can be affected by external (e.g., environmental and dietary factors) and internal factors (e.g., digestive, hormonal, and brain ones) (1). Food intake can be regulated via interactions between neurotransmitters and neurological pathways in the central nervous system (CNS) (2). Cholecystokinin (CCK) which is best- known as a gastrointestinal hormone is also one of the most abundant neuropeptides in the brain of mammals and chickens (3). The bioactive CCK occurs in multiple forms, including CCK33, CCK (26-33) (sulfated $CCK_8$, $CCK_{8S}$), and CCK (30-33) ($CCK_4$). The best-known form is $CCK_{8S}$. In the gastrointestinal tract, CCK acts on the contracted gallbladder, gut movements, gastric emptying rate, gastric acid, and the secretion of pancreatic enzymes (4). However, it is known as an anorexigenic neurotransmitter in the brain. Feeding behavior in rodents was suppressed after peripheral injection of CCK (5). Food intake was decreased in chicks following intraperitoneal injection of $CCK_{8S}$ (60 and 300 nmol/kg). $CCK_4$ could not affect food intake in chicks (3). Furthermore, the hypophagic effects were observed in chicks following the intracerebroventricular (ICV) administration of $CCK_{8S}$ (0.2 and 1 nmol) (3). Glutamate

is considered a major excitatory neurotransmitter in the CNS and its two classified subtypes, namely the ionotropic and metabotropic receptors. The ionotropic receptors include N-Methyl-D-aspartate receptor (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and Kainate receptors, while the metabotropic receptors include $mGLUR_1$, $mGLUR_2$, and $mGLUR_3$ receptors. Hypophagia was observed after AMPA receptor agonists that were ICV injected into the lateral hypothalamus in mammals (6). In addition, the ICV administration of the DL-AP5 (NMDA receptor antagonist) could enhance the consumption of food in broiler-type cockerels (7).

Based on reports, close connections are observed between central CCK and glutamatergic systems. Interestingly, the atypical vesicular glutamate transporter 3 is expressed by the CCK interneurons, indicating that both glutamate and GABA from their terminals contacting pyramidal cells can be released via these interneurons (8). Systemic administration of a $CCK_1$ receptor antagonist or hindbrain NMDA receptor antagonist could significantly decrease CCK-induced $ERK_{1/2}$ phosphorylation occurring in NTS neurons and vagal afferent fibers. Glutamate release increases by the ICV injection of the CCK in the hippocampal interneurons (9). For instance, it is reported that the hypophagic effects of the CCK on the vagal afferent neurons can be mediated via NMDA receptor phenotypes in male Sprague-Dawley rats (10). Furthermore, both CCK-induced reduction of food intake and hindbrain MAPK signaling can be mediated by NMDA receptors. However, there is no report regarding the effect of the interaction of the CCK with glutamatergic systems on food intake in chickens. Accordingly, this study aimed to assess possible feeding behavior alterations by central interactions of CCK and glutamatergic systems in neonatal chickens.

## 2. Materials and Methods

### 2.1. Drugs

$CCK_4$ (Cholecystokinin fragment 30-33 amide), $CCK_{8s}$ (Cholecystokinin fragment 26-33 amide), MK-801 (NMDA glutamate receptors antagonist), CNQX (AMPA glutamate receptors antagonist), AIDA ($mGLUR_1$ glutamate receptors antagonist), LY341495 ($mGLUR_2$ glutamate receptors antagonist), UBP1112 ($mGLUR_3$ glutamate receptors antagonist), and Evans Blue were obtained from Sigma Co. (USA).

### 2.2. Animal Procedure

A total of 352 one-day-old layer-type chickens (Hy-line) were included in this study. They were bought from a regional incubation (Morghak Co. Iran) and randomly allocated into the experiments, each with four treatment groups (n=44 per group). Birds were held at a temperature range of 30˚C+1˚C and 50%+2% moisture. Moreover, they were maintained as a flock for two days before they were randomly moved to their individual birdhouses (11). As shown in table 1 the chickens were fed with a commercialized meal comprising raw protein (21%) and digestible calories (2850 kcal/kg) (Chineh Co. Iran). Additionally, freshwater was given to all birds daily. Just 3 hours before the ICV injections, chickens were food-deprived (FD3) but they could receive water. In experiment 1, chickens were ICV injected with saline, $CCK_4$ (0.25 nmol), $CCK_4$ (0.5 nmol), and $CCK_{8s}$ (1 nmol). In experiment 3, chickens in the four groups received the ICV injection of saline, $CCK_{8s}$ (1 nmol), MK-801 (15 nmol), as well as the co-injection of the $CCk_{8s}$ and MK-801. In experiment 4, four groups of birds received the ICV injection of saline, $CCK_{8s}$ (1 nmol), CNQX (390 nmol), and the co-injection of the $CCk_{8s}$+CNQX. In experiment 5, birds received the ICV administration of saline, $CCK_{8s}$ (1 nmol), AIDA (2 nmol), and the co-injection of the $CCk_{8s}$+AIDA. In experiment 6, the ICV injection of the saline, $CCK_{8s}$ (1 nmol), LY341495 (150 nmol), and the co-injection of $CCk_{8s}$+LY341495 were performed on all chickens. In experiment 7, chickens in the four groups received the ICV injection of saline, $CCK_{8s}$ (1 nmol), UBP1112 (2 nmol), and co-injection of the $CCk_{8s}$+UBP1112. The dosage of ICV injections was chosen based on previous studies and our pilot study (unpublished data).

**Table 1.** Ingredient and nutrient analysis of the experimental diet

| Ingredient | (%) | Nutrient analysis | |
|---|---|---|---|
| Corn | 52.85 | ME, kcal/g | 2850 |
| Soybean meal, 48% CP | 31.57 | Crude protein (%) | 21 |
| Wheat | 5 | Linoleic acid (%) | 1.69 |
| Gluten meal, 61% CP | 2.50 | Crude fiber (%) | 3.55 |
| Wheat bran | 2.47 | Calcium (%) | 1 |
| Di-calcium phosphate | 1.92 | Available phosphorus (%) | 0. 5 |
| Oyster shell | 1.23 | Sodium (%) | 0.15 |
| Soybean oil | 1.00 | Potassium (%) | 0.96 |
| Mineral premix | 0.25 | Chlorine (%) | 0.17 |
| Vitamin premix | 0.25 | Choline (%) | 1.30 |
| Sodium bicarbonate | 0.21 | Arginine (%) | 1.14 |
| Sodium chloride | 0.20 | Isoleucine (%) | 0.73 |
| Acidifier | 0.15 | Lysine (%) | 1.21 |
| DL-Methionine | 0.10 | Methionine (%) | 0.49 |
| Toxin binder | 0.10 | Methionine + cystine (%) | 0.83 |
| L-Lysine HCl | 0.05 | Threonine (%) | 0.70 |
| Vitamin $D_3$ | 0.1 | Tryptophan (%) | 0.20 |
| Multi enzyme | 0.05 | Valine (%) | 0.78 |

ME: metabolisable energy, CP: crude protein, per kg of diet, the mineral supplement contains 35.2 g manganese from $MnSO_4 \cdot H_2O$; 22 g iron from $FeSO_4 \cdot H_2O$; 35.2 g zinc from ZnO; 4.4 g copper from $CuSO_4 \cdot 5H_2O$; 0.68 g iodine from ethylene diamine dihydroiodide; 0.12 g selenium from $Na_2SeO_3$. The vitamin supplement contains 1.188 g of retinyl acetate, 0.033 g of dl-α-tocopheryl acetate, 8.84 g of tocopherol, 1.32 g of menadione, 0.88 g of thiamine, 2.64 g of riboflavin, 13.2 g of nicotinic acid, 4.4 g of pantothenic acid, 1.76 g of pyridoxine, 0.022 g of biotin, 0.36 g of folic acid, 1500 mg of choline chloride.

### 2.3. ICV Administration

Before each treatment, the chicks were assigned to groups according to the mean body weight to uniform the treatment groups. All chickens at five days of age received the ICV administration using a microsyringe (Hamilton, Switzerland) without anesthesia based on the method previously presented by Davis, Masuoka (12) and Furuse, Matsumoto (13) with an acrylic device (14). This method induces no physiological stress in neonatal chickens (15). The volume of ICV administration (with vehicle or drug solution) was 10 μL in each bird. At the end of the experiments, the correct placement of the injection in the ventricle was confirmed by the presence of Evans Blue, followed by euthanizing the chickens and slicing the frozen brain tissue.

### 2.4. Food Intake

Following the ICV injections, chickens were immediately returned to their individual cages, and they received food (pre-weighed). Afterward, the cumulative food intake of chickens injected with ICV was measured at 30, 60, and 120 min. Food consumption was estimated as a percentage of body weight to minimize the impact of body weight on the amount of food intake. Chickens in each experimental group were used only once.

### 2.5. Statistical Analysis

A two-way repeated-measures ANOVA and t-test were applied to analyze the data. Statistical analysis was performed via SPSS software (version 16.0) (SPSS, Inc., Chicago, IL, USA). The data were presented as mean±SEM (standard error of the mean). The Tukey-Kramer test was also used to compare the means of the groups with the main effect obtained by ANOVA. The means were compared considering $P<0.05$.

## 3. Results

According to the findings, the injection of $CCK_4$ (0.25, 0.5, and 1 nmol) did not show any effects on food intake in four groups of chickens, compared to the controls ($P>0.05$) (Figure 1). A dose-dependent

hypophagia was observed following the ICV injection of the $CCK_{8s}$ (0.25, 0.5, and 1 nmol), compared to the controls ($P$<0.05) (Figure 2). As observed in figure 3, $CCK_{8s}$ (1 nmol) significantly decreased food intake in four groups of chickens, compared to the controls ($P$<0.05). MK-801 (15 nmol) did not show any effects on food intake, compared to the controls ($P$>0.05). Hypophagia induced by $CCK_{8s}$ was decreased following the co-injection of the $CCK_{8s}$+MK-801 ($P$<0.05).

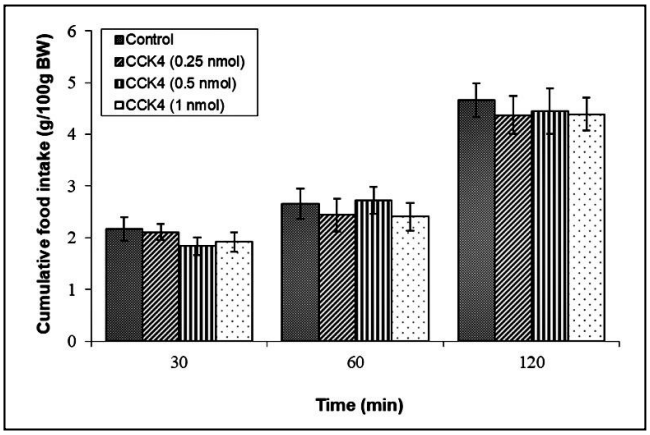


**Figure 1.** Effect of the ICV injection of $CCK_4$ (0.25, 0.5, and 1 nmol) on the cumulative food intake in neonatal layer chicken (n=44). $CCK_4$: Cholecystokinin fragment 30-33 amide. Data are expressed as mean±SEM.

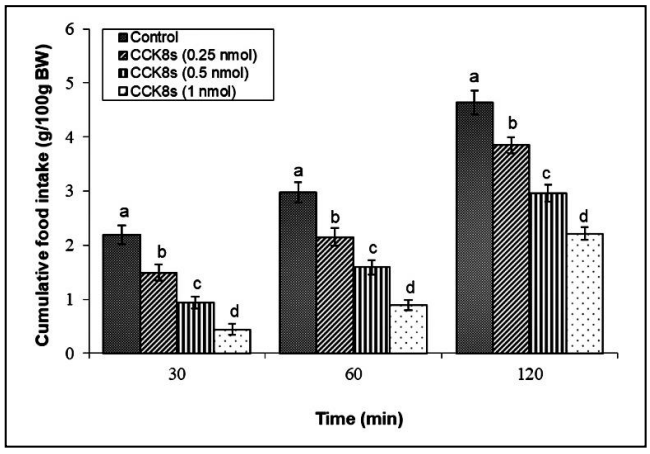


**Figure 2.** Effect of the ICV injection of $CCK_{8s}$ (0.25, 0.5, and 1 nmol) on cumulative food intake in neonatal layer chicken (n=44). $CCK_{8s}$: Cholecystokinin fragment 26-33 amide, sulfated. Data are expressed as mean±SEM. Different letters (a, b, c, and d) indicate significant differences among treatments ($P$<0.05).

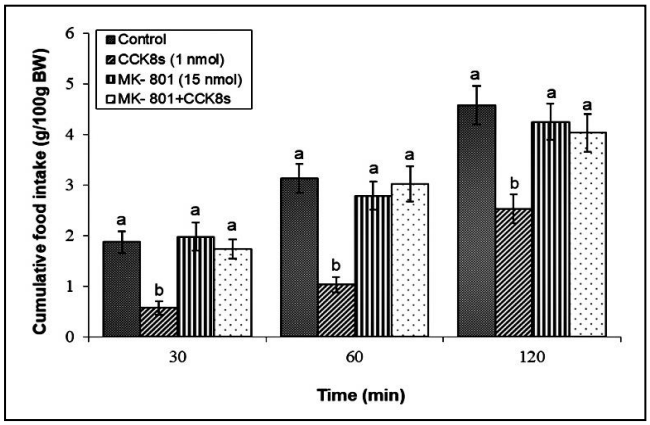


**Figure 3.** Effects of the ICV injection of control solution, $CCk_{8s}$ (Cholecystokinin fragment 26-33 amide, sulfated; 1 nmol), MK-801 (NMDA glutamate receptors antagonist; 15 nmol), and co-injection of the $CCk_{8s}$+MK-801 on cumulative food intake (g/100g BW) in neonatal layer chicken. Data are expressed as mean±SEM. Different letters (a and b) indicate significant differences among treatments at each time ($P$<0.05).

As shown in figure 4, $CCK_{8s}$ (1 nmol) could significantly decrease food intake in four groups of chickens, compared to the controls ($P$<0.05). CNQX (390 nmol) did not show any effects on food intake in all treatment groups, compared to the controls ($P$>0.05). The co-injection of the $CCK_{8s}$+CNQX showed no effects on food intake in the treatment groups, compared to the controls ($P$>0.05). According to figure 5, $CCK_{8s}$ (1 nmol) significantly decreased food intake in the treatment groups, compared to the controls ($P$<0.05). AIDA (2 nmol) did not show any effects on food intake in the treatment groups, compared to the controls ($P$>0.05). The co-injection of the $CCK_{8s}$+AIDA could not affect food intake in the treatment groups, compared to the control group ($P$>0.05).

As shown in figure 6, $CCK_{8s}$ (1 nmol) could significantly decrease food intake in the treatment groups, compared to the controls ($P$<0.05). LY341495 (150 nmol) had no effects on food intake in the treatment groups, compared to the control group ($P$>0.05). The co-injection of the $CCK_{8s}$+LY341495 could not influence food intake in the treatment groups, compared to the control group ($P$>0.05). As shown in figure 7, $CCK_{8s}$ (1 nmol) could significantly decrease food intake in the

treatment groups, compared to the controls ($P$<0.05). UBP1112 (2 nmol) did not show any effects on food intake in the treatment groups, compared to the controls ($P$>0.05). The co-injection of the $CCK_{8s}$+UBP1112 could not affect food intake in the treatment groups, compared to the controls ($P$>0.05).

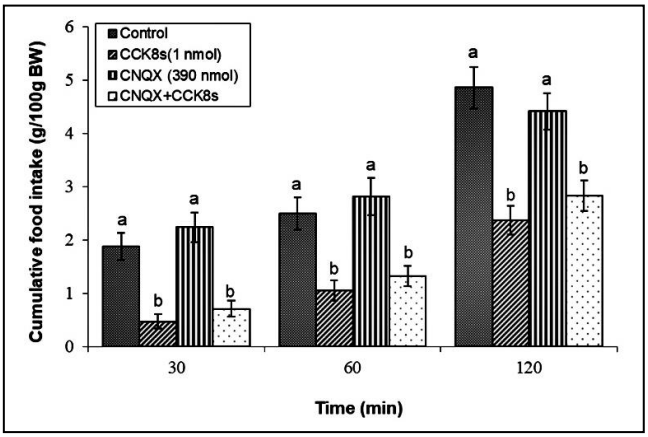


**Figure 4.** Effects of the ICV injection of control solution, $CCk_{8s}$ (Cholecystokinin fragment 26-33 amide, sulfated; 1 nmol), CNQX (AMPA glutamate receptors antagonist; 390 nmol), and co-injection of the $CCk_{8s}$+CNQX on cumulative food intake (g/100g BW) in neonatal layer chicken. Data are expressed as mean±SEM. Different letters (a and b) indicate significant differences among treatments at each time ($P$<0.05).

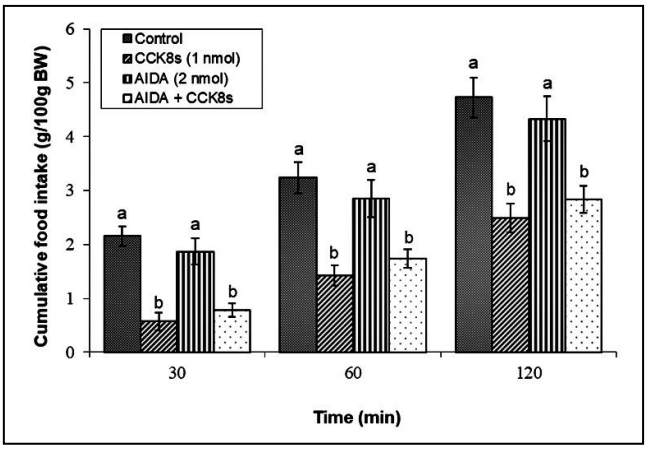


**Figure 5.** Effects of the ICV injection of control solution, $CCk_{8s}$ (Cholecystokinin fragment 26-33 amide, sulfated; 1 nmol), AIDA ($mGLUR_1$ glutamate receptors antagonist; 2 nmol), and co-injection of the $CCk_{8s}$+AIDA on cumulative food intake (g/100g BW) in neonatal layer chicken. Data are expressed as mean±SEM. Different letters (a and b) indicate significant differences among treatments at each time ($P$<0.05).

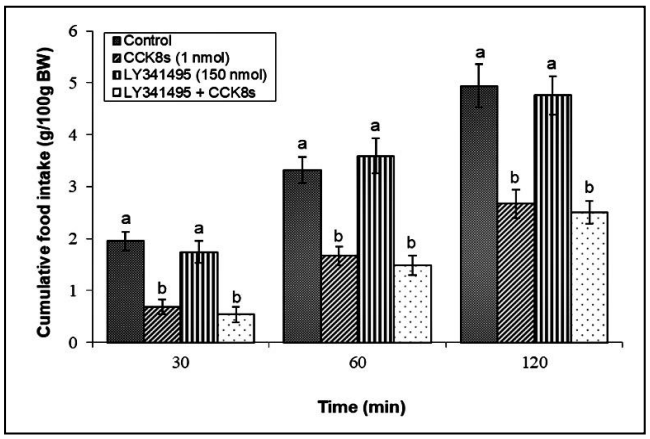


**Figure 6.** Effects of the ICV injection of control solution, $CCk_{8s}$ (Cholecystokinin fragment 26-33 amide, sulfated; 1 nmol), LY341495 ($mGLUR_2$ glutamate receptors antagonist; 150 nmol), and co-injection of the $CCk_{8s}$+LY341495 on cumulative food intake (g/100g BW) in neonatal layer chicken. Data are expressed as mean±SEM. Different letters (a and b) indicate significant differences among treatments at each time ($P$<0.05).

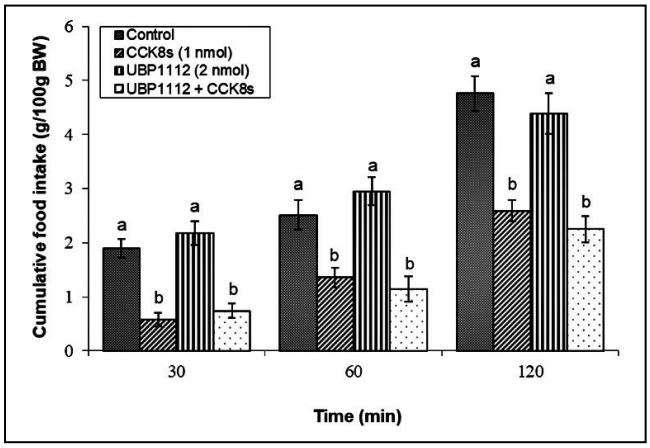


**Figure 7.** Effects of the ICV injection of control solution, $CCk_{8s}$ (Cholecystokinin fragment 26-33 amide, sulfated; 1 nmol), UBP1112 ($mGLUR_3$ glutamate receptors antagonist; 2 nmol), and co-injection of the $CCk_{8s}$+UBP1112 on cumulative food intake (g/100g BW) in neonatal layer chicken. Data are expressed as mean±SEM. Different letters (a and b) indicate significant differences among treatments at each time ($P$<0.05).

## 4. Discussion

To our knowledge, this is the first report regarding the effects of possible interactions of glutamate and CCK systems on food intake in layer-type chickens. The findings showed that the injection of $CCK_4$ (0.25, 0.5, and 1 nmol) could not affect food intake in chickens. A dose-dependent hypophagia was observed following the ICV administration of the $CCK_{8s}$ (0.25, 0.5, and 1 nmol). The co-injection of the $CCK_{8s}$+MK-801 decreased hypophagia induced by $CCK_{8s}$. However, no interaction of $CCK_{8s}$ with AMPA, $mGLUR_1$, $mGLUR_2$, and $mGLUR_3$ receptors on food intake regulation was observed in chickens. The CCK acts on the hypothalamus, leading to the inhibition of feeding behavior (16). Exogenous CCK induces satiation and the full pattern of satiety behavior in rodents, primates, and humans (17). It has been suggested that the anorexigenic properties of CCK is related to metabolic status and the presence or absence of other factors involved in food intake (17). CCK regulates vagal afferent neurons sensitivity to satiation and hunger signals. The gastric load can increase CCK-induced intake reducing the effect (18). The activation of CCK-induced vagal afferent neurons can be regulated by Neuropeptide Y, proopiomelanocortin, and neurons in the nucleus of the solitary tract, including leptin and glutamate (19).

CCK-induced ERK1/2 phosphorylation in NTS neurons and vagal afferent fibers was significantly reduced by the CCK receptor antagonist or hindbrain NMDA receptor (9). The ICV injection of the CCK could increase glutamate levels in the hippocampal interneurons (9). For instance, CCK acts via NMDA receptor phenotypes on vagal afferent neurons obtained from male Sprague-Dawley rats (10). Furthermore, CCK-induced reduction of food intake and hindbrain MAPK signaling can be mediated by NMDA receptors. Afferents from the entorhinal cortex make glutamatergic synapses onto dentate gyrus granule cells, and the highest density of CCK receptors is observed in the region near the granule cell layer (20). The rate of recovery from vesicle depletion could not be affected by the application of the sulfated $CCK_{8s}$ releasable vesicles that release the probability effect. The effects of CCK on glutamate release can be mediated by the inhibition of a sensitive $K^+$ channel requiring the functions of CCK-2 receptors, PLC, intracellular $Ca^{2+}$ release, and PKC (9).

The CCK could not enhance glutamate release by the direct interaction with presynaptic $Ca^{2+}$ channels since the use of the selective P/Q- and N-type $Ca^{2+}$ channel blockers did not block CCK-induced increases in AMPA EPSCs, and the application of CCK failed to change $Ca^{2+}$ channel currents recorded from stellate neurons, the cell body of the pre-frontal pathway (21). CCK also increases GABA release in the hippocampus, and some effects of CCK on food intake may be mediated by this pathway (21). These results suggested that the hypophagic effects of the CCK on food intake can be mediated by NMDA glutamate receptors in neonatal layer-type chickens. Given that there are no reports regarding the chickens, we were not able to compare our results with those found in previous studies.

Regarding the limitations, we were not able to determine molecular signaling pathways in the interconnections between CCK and glutamate receptors. However, more studies are needed to elucidate the underlying cellular and molecular signaling pathways in the interconnections between CCK and glutamate receptors regulating food intake in chickens.

## Authors' Contribution

Study concept and design: M. Z.
Acquisition of data: M. J.
Analysis and interpretation of data: M. Z.
Drafting of the manuscript: M. Z.
Critical revision of the manuscript for important intellectual content: M. Z.

## Ethics

All experiment procedures were approved by the Faculty of Veterinary Medicine, Islamic Azad University, Science and Research Branch, Tehran, Iran.

## Conflict of Interest

The authors declare that they have no conflict of interest.

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