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Original Articles

CRF_1/ CRF_2 and MC_3/ MC_4 Receptors Affect Glutamate- Induced Food Intake in Neonatal Meat-Type Chicken

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Glutamate, Melanocortin, Corticotrophin, Food intake, Neonatal chicken.

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ABSTRACT

Central glutamate, melanocortin and corticotropin systems have mediatory role on several physiologic functions in the brain, but their interactions on appetite regulation are not fully elicited. So, the aim of the current study was to determine interaction of the glutamate with melanocortin and corticotropin systems on food intake in 3-h food-deprived (FD_3) neonatal meat-type chicken. In experiment 1, chicken intracerebroventricular (ICV) injected (A) phosphate-buffered saline (PBS), (B) glutamate (75 nmol), (C) glutamate (150 nmol) and (D) glutamate (300 nmol). In experiment 2, (A) PBS, (B) astressin-B (CRF_1/CRF_2 receptors antagonist, 30 µg), (C) glutamate (300 nmol) and (D) astressin-B+glutamate were ICV injected. Experiments 3-5 were similar to experiment 2, except birds were injected with astressin2-B (CRF_2 receptor antagonist, 30 µg), SHU9119 (MC_3/MC_4 receptor antagonist, 0.5 nmol) and MCL0020 (MC_4 receptor antagonist, 0.5 nmol) instead of the astressin-B. In experiment 6, the injections were (A) PBS, (B) MTII (MC_3/MC_4 receptor agonist, 2.5ng), (C) glutamate (75nmol) and (D) MTII+glutamate. Then, cumulative feed intake was recorded at 30, 60 and 120 minutes after injection. According to the results, dose dependent hypophagia observed by ICV injection of the glutamate (75, 150 and 300nmol) compared to control group in neonatal broiler chicken (p<0.05). Co-injection of the astressin-B+glutamate and astressin2-B+glutamate decreased glutamate-induced hypophagia in neonatal broiler chicken (p<0.05). Co-injection of the glutamate+MC_3/MC_4 receptors antagonist decreased hypophagic effect of the glutamate (p<0.05). These results suggested hypophagic effect of the glutamate mediates via CRF_1/CRF_2 and MC_3/MC_4 receptors in chickens.

INTRODUCTION

Feed intake, satiety and energy expenditure regulates via diverse signals from central and peripheral tissues (Hassanpour *et al.*, 2015; Zendehdel *et al.*, 2017). Neurotransmitters interact by a wide distributed neurological network on feed intake regulation in the central nervous system (CNS) (D'Addario *et al.*, 2014). Appetite regulation regulates in several brain areas such as striatum, hypothalamus, amygdala, nucleus tractus solitarius (NTS) and arcuate nucleus (ARC) (D'Addario *et al.*, 2014).

The melanocortin system is one of the central neurotransmitter systems and to date its five subtypes (MC_1R-MC_5R) have been identified (Alvaro *et al.*, 2003). It has prominent role in several physiologic functions e.g. grooming, thermoregulation, learning and energy balance regulation (Schneeberger *et al.*, 2014). In the brain of the avian Melanocortin receptors have also, been identified (Takeuchi *et* *al.*, 1998). Among melanocortin receptors only MC_3R and MC_4R subtypes are responsible for the central feed intake regulation (Schneeberger *et al.*, 2014). The MC_3R and MC_4R mainly found in arcuate nucleus (ARC), ventromedial hypothalamus (VMH) and periventricular nucleus (PVN) regions of the hypothalamus (Liu *et al.*, 2003). It is reported ICV injection of the MC_3/ MC_4 receptors agonists decreased feed intake in rats (Strader *et al.*, 2003).

Corticotrophin-releasing factor (CRF) is a 41 amino acid peptide and has major role in regulating central aspects of the stress response (Silberman and Winder, 2013). Corticotrophin receptors (CRF_1 and CRF_2) are G-protein-coupled receptors and regulators pituitary function in anxiety and stress (Yamada and Bruijnzeel, 2011). Activation of the CRF_1 and CRF_2 receptors decreases feed intake (Richard *et al.* 2002). The ICV injection of the Astressin-B or Astressin2-B decreased feed intake in rats (Stengel *et al.*, 2009). Also, Finelli *et al.*, (2014) reported ICV injection of the Astressin2-B decreased feed intake in rats (Finelli *et al.*, 2014). Recently, Heidarzadeh *et al.*, (2017) reported ICV injection of the Astressin-B affects nesfatin-1 induced hypophagia in FD_3 broiler chicken.

Glutamate is the main excitatory neurotransmitter in the CNS and its two classified subtypes, the ionotropic and metabotropic receptors have been identified. N-methyl-D-aspartate receptor (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and Kainate receptors belongs to the ionotropic while $mGLUR_1$, $mGLUR_2$ and $mGLUR_3$ receptors are metabotropic (Baghbanzadeh and Babapour, 2007). In several reports, effect of both ionotropic and metabotropic receptors on feeding behavior is identified (Zeni *et al.*, 2000; Baghbanzadeh and Babapour, 2007; Zendehdel *et al.*, 2009). Hypophagia reported by ICV injection of AMPA receptors agonist into the lateral hypothalamus in mammals (Ciranna, 2006; Hettes *et al.*, 2010). Also, the ICV injection of the DL-AP5 (NMDA receptor antagonist) increased food consumption in FD_3 broiler cockerels (Taati *et al.*, 2011; Mortezaei *et al.*, 2013). Injection of the AMPA, NMDA and kainate receptors antagonist into the brain increased feeding behavior in pigeon (Da Silva *et al.*, 2003).

Both melanocortin and glutamatergic neurons are identified in the nucleus of the tractus solitarius (NTS), PVN, amygdala, ARN and area postrema of the brain which engaged with feed intake regulation centers in mammalian (Liu *et al.*, 2003). In rats VTA, excitatory glutamatergic transmission is potentiated through CRF_2 modulation of the NMDA transmission (Ungless *et al.*, 2003). Also, CRF increase glutamatergic neurotransmission in the central amygdala (Silberman and Winder, 2013). According to the reports there are differences in central food intake regulation mechanisms between mammals and avian (Richards, 2003). There is no report on the interaction of glutamate with melanocortin and corticotropin systems on appetite regulation in avian. Based on comparative physiology, it is logical to assume the regulatory mechanisms governing these processes in birds (Furuse *et al.*, 2007). Therefore, the main purpose of the current study was to determine interaction of the glutamate with melanocortin and corticotropin systems on feed intake in FD_3 neonatal meat-type chicken.

MATERIAL AND METHOD

Animals

A total of 264 one-day-old male meat- type chickens were purchased from a local hatchery (Mahan Co., Iran). Birds were maintained in stabilizing electrically heated batteries at a temperature of 32 °C ± 1, kept at 40-50 %relative humidity and 23:1 lighting/dark period (Olanrewaju *et al.*, 2006). They were kept for 2 days as flocks and then the birds were randomly allocated and transferred into their individual cages. A commercial starter diet containing 21% crude protein and 2850 kcal/kg metabolizeable energy (Animal Science Research Institute Co. Iran) were provided to the animals (table 1). During the study all birds had *ad libitum* access to diet and fresh water. 3 h prior to the injections, the birds were food deprived (FD_3) but had free access to water. ICV injections were done at 5 days of age. Animal handling and experimental procedures were performed according to the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health, USA (publication No. 85-23, revised 1996) and the current laws of the Iranian government for animal care, and were approved by the Institutional Animal Ethics Committee of Faculty of Veterinary Medicine, University of Tehran.

Experimental drugs

Drugs used include glutamate, astressin-B (CRF_1/ CRF_2 receptors antagonist), astressin2-B (CRF_2 receptor antagonist), SHU9119 (MC_3/MC_4 antagonist), MCL0020 (MC_4 receptor antagonist), MTII (MC_3/MC_4 agonist) and Evans blue. They were purchased from

Table 1 – Ingredient and nutrient analysis of 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 D3	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 MnSO4·H2O; 22 g iron from FeSO4·H2O; 35.2 g zinc from ZnO; 4.4 g copper from CuSO4·5H2O; 0.68 g iodine from ethylene diamine dihydroiodide; 0.12 g seleniumfrom Na2SeO3. 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 pyridoxin, 0.022 g of biotin, 0.36 g *of folic acid, 1500 mg of* choline chloride.

Sigma-Aldrich (USA) and Tocris (UK) Co. To remove possible effect of the PBS as well as clarifying the accuracy of injected site, drugs were dissolved in a fresh PBS (phosphate-buffered saline) containing Evan's blue and the pH was adjusted to 6.5–7.5.

ICV injection protocol

In each experiment, the birds were weighed and based on their body weight allocated into experimental groups so the average weight between treatment groups was as uniform as possible. The chicken was ICV injected once in each experiment using a microsyringe (Hamilton, Switzerland) without anesthesia using the Davis *et al.*, (1979) and Furuse *et al.*, (1997) method. Briefly, the head of the chicken was held with an acrylic device in which the bill holder was 45° and the calvarium was parallel to the surface of the table as explained by Van Tienhoven & Juhasz (1962). An orifice was made in a plate over the skull of the right lateral ventricle. A microsyringe was inserted into the ventricle through the orifice in the plate and the tip of the needle perforated only 4 mm below the skin of the skull (Jonaidi & Noori, 2012). All injections were done in a volume of 10 µL (Furuse *et al.*, 1999). The control group received a control solution (saline containing Evans blue, 10 µL) (Furuse *et al.*, 1999). This technique does not induce any physiological stress in neonatal chicks (Saito *et al.*, 2005). At the end of the experiments, to recognize the accuracy of the injection, the chicks were sacrificed by decapitation. Accuracy of placement of the injection in the ventricle was verified by the presence of Evans blue followed by slicing the frozen brain tissue. In each group, 12 birds received the injection, but just the data of those individuals where dye was present in their lateral ventricle were used for analysis (11 chickens per group). All experimental procedures were done from 8:00 A.M. until 3:30 P.M.

Feeding experiments

In this study, six experiments were designed to determine interaction of the glutamate with CRF_1/ CRF_2 and MC3/MC4 receptors in neonatal meat-type chicken. (Each experiment included 4 groups with 11 replicates in each group; n=44). In experiment 1, chicken ICV injected with (A) phosphate-buffered saline (PBS), (B) glutamate (75 nmol), (C) glutamate (150 nmol) and (D) glutamate (300 nmol). In experiment 2, (A) PBS, (B) astressin-B (30 µg), (C) glutamate (300 nmol) and (D) astressin-B + glutamate were ICV injected. In experiment 3, birds ICV injected with (A) phosphate-buffered saline (PBS), (B) astressin2-B (30 µg), (C) glutamate (300 nmol) and (D) astressin2-B + glutamate. In experiment 4, FD_3 chicks received ICV injection of (A) PBS, (B) SHU9119 (0.5 nmol), (C) glutamate (300 nmol) and (D) SHU9119 + glutamate. In experimet 5, the ICV injection to the birds were (A) PBS, (B) MCL0020 (0.5 nmol), (C) glutamate (300 nmol) and (D) MCL0020 + glutamate. In experiment 6, the injections were (A) PBS, (B) MTII (2.5 ng), (C) glutamate (75 nmol) and (D) MTII + glutamate. The injection procedure in the experimental procedure is presented in table 2 and in the flow chart. Immediately after the injection feed was provided to the birds and cumulative feed intake (g) was measured at 30, 60 and 120 min after the injection. Food consumption was calculated as a gram of body weight (g/100g BW) to minimize the impact of body weight on the amount of feed intake. These doses of drugs were determined according to the pilot and previous studies (Zeni *et al.*, 2000; Baghbanzadeh & Babapour, 2007; Zendehdel *et al.*, 2009; Ahmadi *et al.*, 2017; Heidarzadeh *et al.*, 2017).

Table 2 – Treatments procedure in experiments 1-7

Experiment 1	ICV Injection
Treatment groups	
I	solution(PBS) *
II	glutamate (75,nmol)
III	glutamate (150 nmol)
IV	glutamate (300 nmol)
Experiment 2	ICV Injection
Treatment groups	
I	solution(PBS) *
II	astressin-B (30 µg)
III	glutamate (300 nmol)
IV	glutamate (300 nmol) + astressin-B (30 µg)
Experiment 3	ICV Injection
Treatment groups	
I	solution(PBS) *
II	astressin2-B (30 µg)
III	glutamate (300 nmol)
IV	glutamate (300 nmol) + astressin2-B (30 µg)
Experiment 4	ICV Injection
Treatment groups	
I	solution(PBS) *
II	SHU9119 (0.5 nmol)
III	glutamate (300 nmol)
IV	glutamate (300 nmol) + SHU9119 (0.5 nmol)
Experiment 5	ICV Injection
Treatment groups	
I	solution(PBS) *
II	MCL0020 (0.5 nmol)
III	glutamate (300 nmol)
IV	glutamate (300 nmol) + MCL0020 (0.5 nmol)
Experiment 6	ICV Injection
Treatment groups	
I	solution(PBS) *
II	MTII (2.5 ng)
III	glutamate (75 nmol)
IV	glutamate (75 nmol) + MTII (2.5 ng)

PBS: phosphate-buffered saline, astressin-B: CRF_1/ CRF_2 receptors antagonist, astressin2-B: CRF_2 receptor antagonist, SHU9119: MC_3/MC_4 antagonist, MCL0020: MC_4 receptor antagonist, MTII (MC_3/MC_4 agonist).

Statistical analysis

Cumulative feed intake was analyzed by repeated measure two-way analysis of variance (ANOVA) and is presented as the mean ± SEM. For treatments found to have an effect according to the ANOVA, mean values were compared with Bonferroni test. *P* values <0.05 were considered to indicate significant differences between the treatments.

RESULTS

Effects and interactions of central glutamate with CRF_1, CRF_2, MC_3 and MC_4 receptors on cumulative feed intake in FD_3 neonatal broilers are shown in figures 1-6. In experiment 1, dose dependent hypophagia was observed by ICV injection of the different doses of the glutamate (75, 150 and 300 nmol) compared to the control group in neonatal broiler chicken [treatment effect: F (3, 80) = 385.1, *P*<0.0001; time effect: F (2, 80) = 824.7, *p*<0.0001; treatment and time interaction: F (6, 80) = 73.42; *p*<0.0001; Fig. 1].

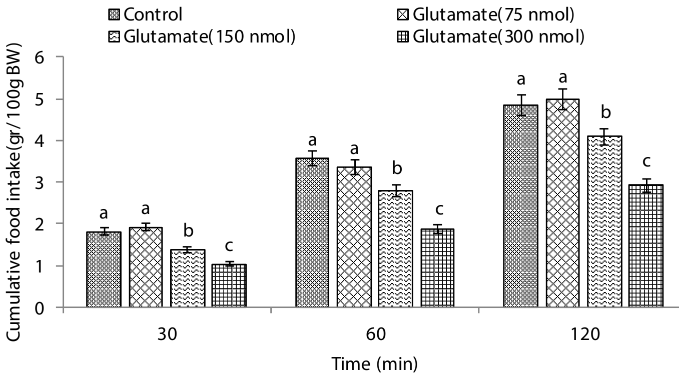


Figure 1 – Effects of intracerebroventricular injection of control solution(PBS) and different doses of the glutamate (75, 150 and 300 nmol) on cumulative food intake (gr/100gr BW) in neonatal meat-type chicks. PBS: phosphate- buffered saline. Data are expressed as mean ± SEM. Different letters (a-c) indicate significant differences between treatments at each time (*p*<0.05).

In experiment 2, ICV injection of the astressin-B (30 µg) had no effect on feed intake (*P* >0.05). The glutamate injection (300 nmol) significantly decreased feed intake compared to the control group (*P*<0.05). Co-injection of the astressin-B + glutamate, decreased glutamate-induced hypophagia in neonatal broiler chicken [treatment effect: F (3, 80) = 241.7, *p*<0.0001; time effect: F (2, 80) = 541.8, *p*<0.0001; treatment and time interaction: F (6, 80) = 35.18; *p*<0.0001; Fig. 2].

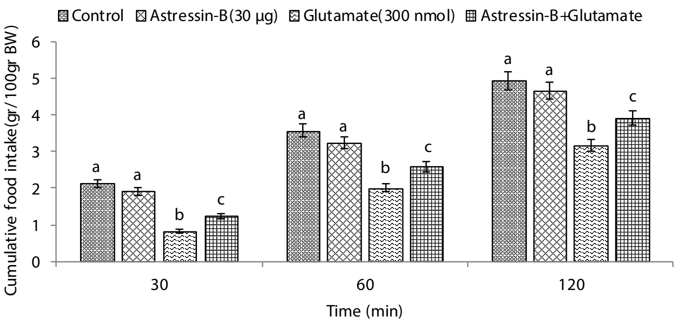


Figure 2 – Effects of intracerebroventricular injection of control solution(PBS), astressin-B (CRF_1/ CRF_2 receptors antagonist; 30 µg), glutamate (300 nmol) and co-injection of the astressin-B + glutamate on cumulative feed intake (gr/100gr BW) in neonatal meat-type chicks. PBS: phosphate- buffered saline. Data are expressed as mean ± SEM. Different letters (a, b and c) indicate significant differences between treatments at each time (*p*<0.05).

In experiment 3, ICV administration of the astressin2-B (30 µg) had no effect on feeding behavior (*P*>0.05) while 300 nmol of the glutamate significantly decreased feed intake compared to the control group

(p<0.05). Co-injection of the astressin2-B + glutamate, decreased glutamate-induced hypophagia in neonatal broiler chicken [treatment effect: F (3, 80) = 230.7, p<0.0001; time effect: F (2, 80) = 235.3, p<0.0001; treatment and time interaction: F (6, 80) = 21.6; p<0.0001; Fig. 3].

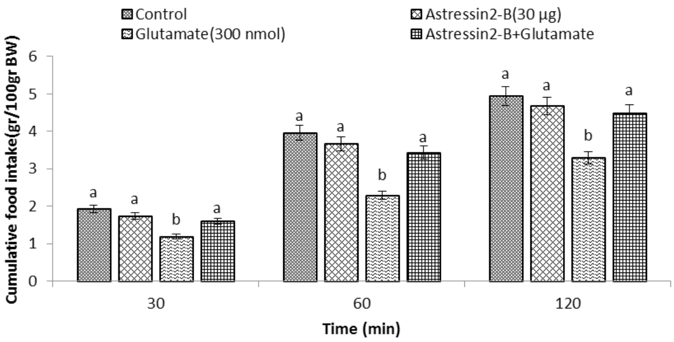


Figure 3 – Effects of intracerebroventricular injection of control solution(PBS), astressin2-B (CRF_2 receptor antagonist; 30 µg), glutamate (300 nmol) and co-injection of the astressin2-B + glutamate on cumulative feed intake (gr/100gr BW) in neonatal meat--type chicks. Data are expressed as mean ± SEM. PBS: phosphate- buffered saline. Different letters (a and b) indicate significant differences between treatments at each time (p<0.05).

In experiment 4, ICV administration of the 0.5 nmol MC3/MC4 antagonist (SHU9119) had no effect on feeding behaviour (P >0.05) while the glutamate injection (300 nmol) significantly decreased feed intake compared to control group (p<0.05). Co-injection of the SHU9119 + glutamate decreased the hypophagic effect of the glutamate in neonatal chicks [treatment effect: F (3, 80) = 240.27, p<0.0001; time effect: F (2, 80) = 628.15, p<0.0001; treatment and time interaction: F (6, 80) = 51.17; p<0.0001; Fig. 4].

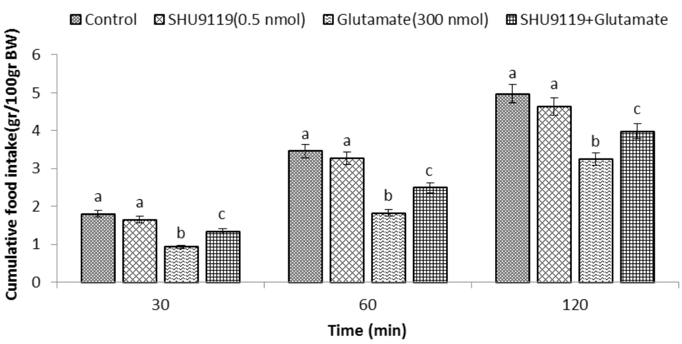


Figure 4 – Effect of ICV injection of control solution(PBS), SHU9119 (MC3/MC4 antagonist, 0.5 nmol), glutamate (300 nmol) and their combination on cumulative feed intake (gr/100gr BW) in neonatal meat-type chicks. PBS: phosphate- buffered saline. Data are expressed as mean ± SEM. Different letters (a, b and c) indicate significant differences between treatments at each time (p<0.05).

In experiment 5, MCL0020 (0.5 nmol) had no effect on feeding behavior in FD_3 neonatal broilers compared to the control group (P >0.05) while the glutamate injection (300 nmol) had hypophagic effect in FD_3 neonatal broilers compared to the control group (p<0.05). Co- administration of the glutamate + MCL0020 significantly diminished glutamate-induced hypophagia in chicks [treatment effect: F (3, 80) = 163.72, p<0.0001; time effect: F (2, 80) = 407.13, p<0.0001; treatment and time interaction: F (6, 80) = 11.06; p<0.0001; Fig. 5].

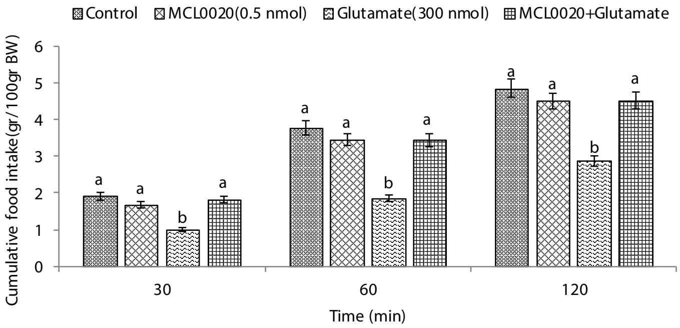


Figure 5 – Effect of ICV injection of control solution(PBS), MCL0020 (MC4 receptor antagonist, 0.5 nmol), glutamate (300 nmol) and their combination on cumulative feed intake (gr/100gr BW) in neonatal meat-type chicks. PBS: phosphate- buffered saline. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments at each time (p<0.05).

In experiment 6, sole ICV injection of the MTII (2.5 ng) or glutamate (75 nmol) had no effect on feeding behavior in FD_3 neonatal broilers compared to the control group (P >0.05). Co-injection of the MTII + glutamate significantly decreased feed intake compared to glutamate or MTII alone [treatment effect: F (3, 80) = 439.52, p<0.0001; time effect: F (2, 80) = 376.08, p<0.0001; treatment and time interaction: F (6, 80) = 6.27; p<0.0001; Fig. 6].

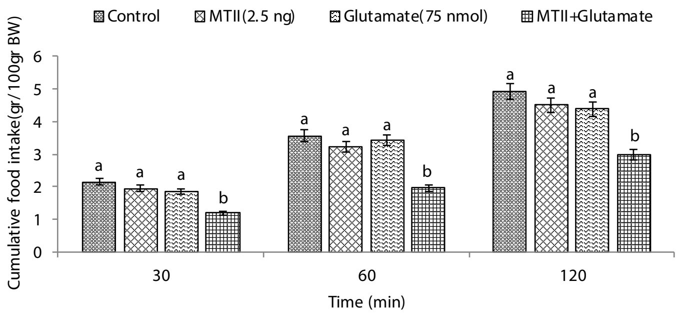


Figure 6 – Effect of ICV injection of control solution(PBS), MTII (MC3/MC4 agonist, 2.5 ng), glutamate (75 nmol) and their combination on cumulative feed intake (gr/100gr BW) in neonatal meat-type chicks. PBS: phosphate- buffered saline. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments at each time (p<0.05).

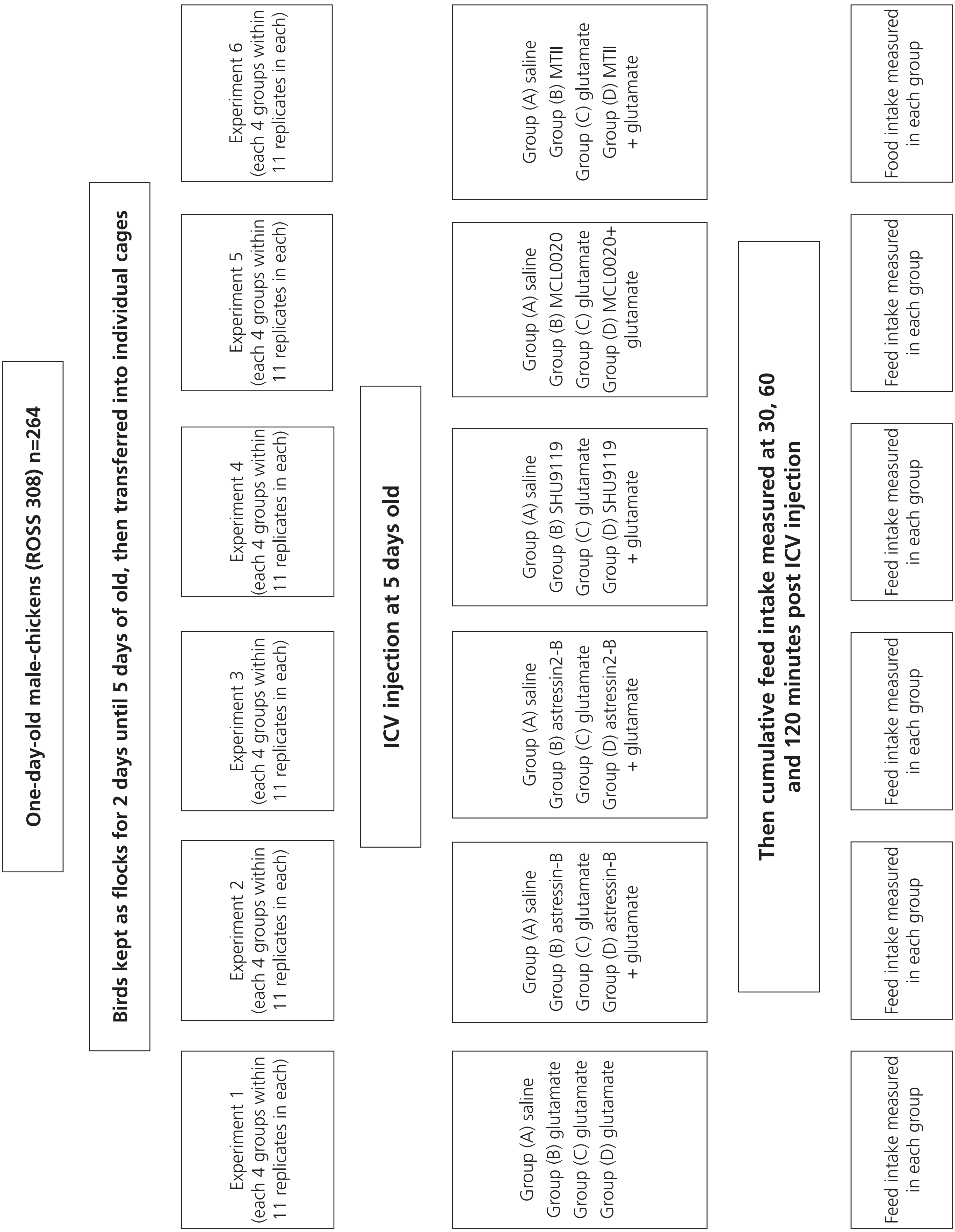
DISCUSSION

To the best of our knowledge, this is the first report on the role of the glutamate with melanocortin and corticotrophin systems on feed intake in FD_3 neonatal meat-type chicks. Based on the findings of the current study, dose dependent hypophagia was observed by ICV injection of the glutamate (75, 150 and 300nmol) in neonatal broiler chicken (figure 1). ICV injection of the metabotropic glutamate receptors antagonist increased food intake in broilers (Baghbanzadeh and Babapour, 2007). ICV injection of the NMDA receptor antagonist (DL-AP5) increased cumulative feed intake in FD_3 cockerels (Taati *et al.*, 2011). Injection of NMDA and AMPA-kainite receptor antagonists into ventral pallidal and ventral striatal nuclei decreased feed intake in the pigeon (Da Silva *et al.*, 2003). The effect of the glutamatergic system on feed intake is mediated via NMDA, AMPA and $mGLU_1$ receptors in FD_3 neonatal chicken (Torkzaban *et al.*, 2017). It seems, glutamate metabotropic receptors have partial interaction with other neurotransmitters in feed intake regulation in broilers (Torkzaban *et al.*, 2017). The $mGluR_1$ mechanically acts via phospholipase C activation which leads to the formation of the IP_3 and diacylglycerol, intracellular release of Ca^{2+} and stimulation of protein kinase C, while $mGLUR_2$ and $mGLUR_3$ coupled to adenylyl cyclase and cyclase respectively (Mortezaei *et al.*, 2013).

Co-injection of the astressin-B + glutamate and astressin2-B + glutamate decreased glutamate-induced hypophagia in neonatal broiler chicken (figures 2 and 3). The interconnection is reported between CRF_1 and CRF_2 receptors with glutamatergic system. Activation of the CRF_1 and CRF_2 receptors have opposed actions on glutamatergic transmission in amygdala and the lateral septum mediolateral nucleus (Liu *et al.*, 2004). Both CRF_1 and CRF_2 receptors are highly expressed in amygdala and the lateral septum mediolateral nucleus and influences neuronal properties (Liu *et al.*, 2004). In the VTA, excitatory glutamatergic transmission is potentiated by CRF_2 modulation of the NMDA transmission (Liu *et al.*, 2004). In the current study, co-injection of the glutamate + MC_3/MC_4 receptors antagonist decreased hypophagic effect of the glutamate in chicken (figures 4 and 5). In this regard, Lu *et al.*, (2003) reported MC_3 and MC_4 receptors decrease feed intake in rats and nonhuman primates. Both CRF and MC_4R mRNA are expressed in the PVN. MTII-induced plasma corticosterone was abolished by injection of the HS014 (0.25–1.0 nmol, selective MC_4 antagonist) (Lu *et al.*, 2003). In MC_4-/- mice, because of the inability of MTII to suppress feed intake, MTII-induced anorexia mediates by the MC_4R (Marsh *et al.*, 1999).

The CRF has two side effects on glutamatergic synaptic transmission in the central nucleus of the amygdala which CRF_1 and CRF_2 receptors has inhibitory and facilitatory roles, respectively (Liu *et al.*, 2011). Mice lacking CRF_1 receptors in glutamatergic neurons reduced anxiety and impaired neurotransmission in the hippocampus while increased anxiety-like behavior and reduced dopamine release in the prefrontal cortex (Inda *et al.*, 2017). Under physiological conditions CRF1 controlled dopaminergic and glutamatergic systems and could function in an antagonistic manner to retain adaptive responses to stressful situations in balance (Kratzer *et al.*, 2013). Presynaptic glutamatergic neurotransmission increases by CRF in the central amygdala (Silberman & Winder, 2013). Extracellular glutamate levels increase after ICV injection of CRF into the central amygdala (Skorzewska *et al.*, 2009). CRF_1 has a higher affinity for CRF which suggested CRF increases glutamatergic neurotransmission in the central amygdala via CRF2 even after CRF1 receptors become saturated (Silberman & Winder, 2013). Corticotropin-releasing factor neurons express the NMDA receptor and this protein is transported to intracellular locations in dendrites (Beckerman *et al.*, 2013).

Co-injection of MC_3/MC_4 receptors agonist with NMDA glutamate receptors antagonist, decreased melanocortine-induced hypophagia in neonatal chicken (Ahmadi *et al.*, 2017). Hypophagic effect of melanocortin is mediated by glutamatergic system in rats (Campos *et al.*, 2015). ICV administration of the MTII into the NTS decreased feed intake and this effect was weakened by glutamatergic system (Carlos *et al.*, 2015). Activation of the presynaptic MC_4 receptors in the central vagal afferent terminals by MTII reduces feed intake in rats (Campos *et al.*, 2014). Glutamate release is required for phosphorylation of synapsin (I) in afferent vagal endings (Carlos *et al.*, 2015). Synapsin (I) activation due to glutamate release is required for MTII-induced hypophagia in the rat (Carlos *et al.*, 2015). Blockade of the glutamate receptor attenuated MTII-induced hypophagia in rats (Carlos *et al.*, 2015). It seems, hypophagic effect of the melanocortin neurons acts by a mediatory role of the pro-opiomelanocortin (POMC) on the glutamate receptors (Dicken *et al.*, 2012). Both POMC and MC_3 and MC_4 receptors have distribution on ARC and NTS which agouti related protein (AgRP) and neuropeptide Y (NPY) express (Gautron *et al.*, 2010). The POMC neurons mediate glutamate release in the NTS which contribute to the reduction in food intake in the presence of MC_4 receptors agonists (Gautron *et al.*, 2010). POMC and AgRP neurons are located proximate to each other parallel to the glutamate, MC_3 and MC_4 receptors in the ARC. Based on the literature, there was no previous report on interaction between glutamate


One-day-old male-chickens (ROSS 308) n=264
Birds kept as flocks for 2 days until 5 days of old, then transferred into individual cages
Experiment 1 (each 4 groups within 11 replicates in each)
Experiment 2 (each 4 groups within 11 replicates in each)
Experiment 3 (each 4 groups within 11 replicates in each)
Experiment 4 (each 4 groups within 11 replicates in each)
Experiment 5 (each 4 groups within 11 replicates in each)
Experiment 6 (each 4 groups within 11 replicates in each)
ICV injection at 5 days old
Group (A) saline Group (B) glutamate Group (C) glutamate Group (D) glutamate
Group (A) saline Group (B) astressin-B Group (C) glutamate Group (D) astressin-B + glutamate
Group (A) saline Group (B) astressin2-B Group (C) glutamate Group (D) astressin2-B + glutamate
Group (A) saline Group (B) SHU9119 Group (C) glutamate Group (D) SHU9119 + glutamate
Group (A) saline Group (B) MCL0020 Group (C) glutamate Group (D) MCL0020+ glutamate
Group (A) saline Group (B) MTII Group (C) glutamate Group (D) MTII + glutamate
Then cumulative feed intake measured at 30, 60 and 120 minutes post ICV injection
Feed intake measured in each group
Feed intake measured in each group
Feed intake measured in each group
Feed intake measured in each group
Feed intake measured in each group
Food intake measured in each group

with melanocortin and corticotrophin systems on feed intake. So, we were not able to compare our results with it. In conclusion, these results suggested hypophagic effect of the glutamate mediates via CRF_1/ CRF_2 and MC_3/MC_4 receptors in chickens. However, further investigation is required to elucidate the underlying cellular and molecular signaling pathways in the interconnections between glutamate with melanocortin and corticotrophin systems on food intake in neonatal chicks.

COMPLIANCE WITH ETHICAL STANDARDS

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

INFORMED CONSENT

This manuscript does not contain any studies with human subjects performed by any of the authors.

HUMAN AND ANIMAL RIGHTS

All experiments were executed according to the Guide for the Care and Use of Laboratory Animals and were approved by the institutional animal ethics committee.

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