

Veterinary and Comparative Biomedical Research

ORIGINAL ARTICLE

Opioid System and Leucine Interaction in the Regulation of Feed Intake in Broiler Chickens

Seyyed Sattar Tohidifar¹ , Javad Cheraghi² , Amirali Ebrahimi Jamal³ , Morteza Zendehtdel⁴ ,
Jahangir Kaboutari^{3*} 

¹ Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran

² Department of Veterinary Laboratory Sciences, Faculty of Para-Veterinary Medicine, Ilam University, Ilam, Iran

³ Department of Basic Sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran

⁴ Department of Physiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

Online ISSN: 3060-7663

<https://doi.org/10.22103/vcbr.2025.24999.1052>

*Correspondence

Author's Email:

Kaboutari-j@sku.ac.ir

Article History

Received: 18 March 2025

Revised: 10 April 2025

Accepted: 08 May 2025

Published: 27 May 2025

Keywords

Chicken
Feed intake
Opioids
Leucine

Running Title

Feed Intake Regulation in Broiler
Chickens

Abstract

The opioidergic system plays a critical role in feed intake, among other physiological functions. This study investigated the potential effects of the opioidergic system, leucine and its interaction with the central regulation of feed intake in broilers. 108 one-day-old Ross 308 broiler chickens in six groups, each with three replicates (n=6) have been used. A guiding cannula was implanted into the right lateral cerebral ventricle. Seven days later, leucine (2 µg), morphine (250 pmol), naloxone (5 µg), morphine + leucine combination (250 pmol + 2 µg), naloxone + leucine combination (5 µg + 2 µg), and normal saline were injected intra-cerebro-ventricularly (ICV) (injection volume = 10 µl, n = 6, each with three duplicates). The cumulative feed intake was measured at 30, 60, 90, 120, 180, and 240 minutes post injection. Naloxone increased feed intake, whereas both leucine and morphine significantly reduced it (p<0.05). ICV injection of morphine increased the inhibitory effect of leucine on feed intake (p<0.05). Additionally, within the first 60 min post injection, the combination of leucine and naloxone increased feed intake (p>0.05), whereas naloxone mitigated the inhibitory effect of leucine during the same period (p>0.05). The feed intake remained significantly decreased up to 240 minutes following the ICV injection of the combination of leucine and naloxone (p<0.05). Leucine appears to decrease feed intake through mechanisms involving glutamate and neuropeptide Y.

How to cite this article: Seyyed Sattar Tohidifar, Javad Cheraghi, Amirali Ebrahimi Jamal, Morteza Zendehtdel, Jahangir Kaboutari. Opioid System and Leucine Interaction in the Regulation of Feed Intake in Broiler Chickens. *Veterinary and Comparative Biomedical Research*, 2025, 2(2): 11 – 17. <http://doi: 10.22103/vcbr.2025.24999.1052>



© The Author(s), 2025. This open-access article is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0), permitting non-commercial use, distribution, and reproduction in any medium, provided the original author(s) and source are properly credited. No commercial use or modifications are allowed without prior permission. Third-party material is included under the same license unless otherwise stated. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc/4.0/>.

Introduction

To coordinate feed intake, a complex homeostatic mechanism involving multiple levels of neural control is engaged, including limbic structures such as the nucleus accumbens, ventral tegmental area, amygdala, hippocampus, orbitofrontal cortex, and cingulate gyrus. Additionally, various neurotransmitters, including serotonin, gamma-amino-butyric acid (GABA), dopamine, and glutamate, play crucial roles in this process. Chemical receptors within the digestive system convey inputs through the release of chemical mediators to neural centers, where signals are processed and integrated, regulating the quantity of feed and energy consumed. Among the lowest regions of the brain in both mammals and birds, the pons, medulla, and diencephalon contain neural centers implicated in this regulatory process (1). In domestic birds, the ventromedial hypothalamus core is among the most significant areas influencing hunger; thus, damage to it can increase appetite and obesity (2).

Appetite and the need to eat are two critical determinants of feed intake. Broilers have been selectively modified to increase appetite, leading to the development of strains and breeds with altered feed intake mechanisms, improved feed conversion ratios, increased carcass weights, and reduced raising periods (3). In addition to serving as signaling molecules in biological pathways, branched-chain amino acids (BCAAs), specifically leucine, isoleucine, and valine, are essential for promoting protein synthesis, muscle formation, and body weight gain in birds. Leucine, an essential amino acid for broilers, shares structural similarities with other BCAAs. Consequently, an increase in leucine intake may influence the requirements for isoleucine and valine. The impact of leucine on the hypothalamus and its ability to increase the expression levels of neuropeptide Y (NPY) significantly promote feed intake. The administration of high amounts of leucine during the growth stage has been shown to increase carcass weight; however, excessive amounts may negatively affect growth and feed consumption during the early stages of broiler rearing (4). Opioids are inhibitory neurotransmitters with numerous receptors in the central nervous system of vertebrates. Endogenous opioids fulfill neurohormonal and paracrine regulatory functions (5). Multiple studies have documented a close relationship between opiate receptors and feeding behavior. For example, ICV administration of morphine has been shown to reduce carbohydrate intake while increasing lipid intake in rats. Additionally, ICV administration of DAMGO, a selective μ -opiate receptor agonist, and the opioid peptide Casmorphin- β significantly reduced food intake in one-day-old broiler chicks, whereas

δ and κ receptor agonists increased feed intake; moreover, opiate receptor antagonists such as naloxone and naltrexone exert suppressive effects on feed intake (6, 7). Notably, only μ receptors among the opiate receptors are associated with an increased preference for a lipid diet in newly hatched broilers when allowed to feed ad libitum, reflecting a pattern similar to that observed in mammals (8). This research investigated the interaction between the opioidergic system and leucine concerning the central regulation of feed intake in broilers under conditions of starvation stress.

Materials and Methods

This research was conducted on 108 one-day-old Ross 308 broiler chickens in six groups ($n = 6$), each with three replicates, under standard conditions and was approved by international and institutional guidelines (IR.SKU.REC.1401.024). After 11 days, they were transferred to individual cages with ad libitum standard feed and fresh, clean water, exposed to continuous light at 31–32°C in the first week, and then decreased by 2–3 degrees every week until they reached 26 – 27°C during the experiment (Table 1).

Table 1: Composition and formulation of the experimental diet

ingredients	Amount	ingredients	Amount
Yellow corn	58.23 (%)	Dical phosphate	1.57 (%)
Soybean meal	34.75 (%)	Limestone	1.19 (%)
Oil	2.11 (%)	Vit. & Min.**	0.5 (%)
Salt	0.31 (%)	DL-Methionine	0.16 (%)
Calculated analysis :(NRC, 1994)			
Energy (Kcal/kg)	2908 (%)	Leucine %	1.12 (%)
Crude protein (CP) %	20.1 (%)	Methionine %	0.50 (%)
Ca %	0.9 (%)	Threonine %	0.85 (%)
Available P %	0.45 (%)	Arginine %	1.4 (%)
Na %	0.18 (%)	Lysine %	1.29 (%)

**Each 3.0 kg of the vitamin and mineral premix manufactured by Agri-Vet Company, Egypt, contains Vitamin A: 12000000 IU; Vitamin D3: 2000000 IU; Vitamin E: 10 g; Vitamin K3: 2.0 g; Vitamin B1: 1.0 g; Vitamin B2: 5 g; Vitamin B6: 1.5 g; Vitamin B12: 10 mg; Choline chloride: 250 g; Biotin: 50 mg; Folic acid: 1 g; Nicotinic acid: 30 g; Pantothenate: 10 g; Zn: 50 g; Cu: 10 g; Fe: 30 g; Co: 100 mg; Se: 100 mg; I: 1 g; Mn: 60 g; antioxidant: 10 g; and complete to 3.0 g by Calcium carbonate

The experiment involved performing stereotaxic surgery on 21-day-old chickens weighing 700 to 750 grams following 3 hours of feed deprivation. Briefly, 25 mg/kg sodium pentobarbital (Merck, Germany) was administered intravenously. The birds' heads were shaved and positioned on a stereotaxic machine with the following specifications: anterior/posterior at 6.7 mm, lateral at 0.7 mm, and horizontal at 3.5 to 4 mm below the dura mater. Three stainless steel screws were inserted surrounding each guide cannula to secure it in place. The screws and guide cannula

are subsequently covered with acrylic dental cement. An orthodontic wire was placed inside the guide cannula when the animals were not undergoing trials. Finally, after the skin was sutured and subjected to topical antibiotic therapy, the birds were allowed to recover for seven days (8).

The experiments involved six groups, including one control group, which received normal saline, and five treatment groups, including leucine 2 µg (Merck, Germany), morphine 250 pmol (Pfizer, USA), naloxone 5 µg (Pfizer, USA), morphine 250 pmol + leucine 2 µg, and naloxone 5 µg + leucine 2 µg. Each group received a 10-microliter ICV injection, which was prepared in normal saline, via a Hamilton syringe for 30 seconds. Finally, feed intake was recorded at 30, 60, 90, 120, 180, and 240 minutes.

Statistical Analysis

The data are expressed as the means ± SDs and were analyzed via one-way ANOVA, and Tukey's post hoc test was performed via the statistical software SPSS version 16 ($p < 0.05$).

Results

Feed Intake 30 Minutes Post Treatment

The lowest feed intake was observed in the leucine + morphine treatment group, which was significantly lower than that in the control, naloxone, and leucine + naloxone treatment groups ($p < 0.05$). Moreover, the highest feed intake was observed in the naloxone group, which was significantly greater than that in the leucine + morphine group ($p < 0.05$) (Table 2).

Table 2: Feed intake (grams) measured 30 minutes following intracerebroventricular injection in the experimental groups

Groups	Feed intake (g)
Control	9.19 ± 0.56
Leucine	8.5 ± 0.77 ^{ab}
Morphine	8.76 ± 0.5 ^{ab}
Naloxone	10.06 ± 0.5 ^a
Leucine + Morphine	7.64 ± 0.38 ^{*b}
Leucine + Naloxone	9.63 ± 0.55 ^a

* Indicates a significant difference vs the control group. Different letters (a & b) indicate significant differences between experimental groups ($p < 0.05$); the data are presented as the means ± SDs.

Feed Intake 60 Minutes Post Treatment

Sixty minutes after the injection, there was no significant reduction in feed intake in the leucine treatment group compared with the control group ($p > 0.05$), but in the

morphine and leucine + morphine groups, a significant reduction in feed intake was observed compared with all the other groups ($p < 0.05$). Compared with all the other groups, the naloxone group presented a significant increase, and the leucine + naloxone combination group presented a significant increase ($p < 0.05$) compared with the leucine, morphine, and leucine + morphine groups ($p < 0.05$) (Table 3).

Table 3: Feed intake (grams) measured 60 minutes following intracerebroventricular injection in the experimental groups

Groups	Feed intake (g)
Control	21.47 ± 0.55
Leucine	20.82 ± 0.64
Morphine	18.43 ± 0.47 ^{*a}
Naloxone	24.37 ± 0.58 ^{*b}
Leucine + Morphine	17.11 ± 0.63 ^{*c}
Leucine + Naloxone	22.08 ± 0.85 ^d

* Indicates a significant difference vs the control group. Different letters (a, b, c & d) indicate significant differences between experimental groups ($p < 0.05$); the data are presented as the means ± SDs.

Feed Intake 90 Minutes Post Treatment

Compared with that in the control group, feed intake was significantly lower in the leucine and morphine treatment groups 90 min after ICV injection ($p < 0.05$). Furthermore, at this point, the feed intake of the Naloxone group was significantly greater than that of the control, leucine, and morphine groups ($p < 0.05$) (Table 4).

Table 4: Feed intake (grams) measured 90 minutes following intracerebroventricular injection in the experimental groups

Groups	Feed intake (g)
Control	33.55 ± 0.53
Leucine	28.60 ± 0.78 ^{*b}
Morphine	26.35 ± 0.71 ^{*a}
Naloxone	39.59 ± 0.83 ^{*c}
Leucine + Morphine	28.54 ± 0.69 ^{*b}
Leucine + Naloxone	38.19 ± 0.69 ^{*d}

* Indicates a significant difference vs the control group. Different letters (a, b, c & d) indicate significant differences between experimental groups ($P < 0.05$); the data are presented as the means ± SDs.

Feed Intake 120 Minutes Post Treatment

Except for the morphine group, the leucine group presented a significant decrease in feed intake compared with the other treatment groups ($p < 0.05$). Compared with the control and other treatments, naloxone administration significantly increased feed consumption ($p < 0.05$) (Table 5).

Table 5: Feed intake (grams) measured 120 minutes following intracerebroventricular injection in the experimental groups

Groups	Feed intake (g)
Control	42.75 ± 0.23
Leucine	35.83 ± 0.67 ^{*b}
Morphine	33.21 ± 0.58 ^{*a}
Naloxone	48.52 ± 0.66 ^{*c}
Leucine + Morphine	35.13 ± 0.56 ^{*b}
Leucine + Naloxone	38.19 ± 0.69 ^{*d}

* Indicates a significant difference vs the control group. Different letters (a, b, c & d) indicate significant differences between experimental groups ($p < 0.05$); the data are presented as the means ± SDs.

Feed Intake 180 Minutes Post Treatment

Compared with the control, naloxone, and leucine + naloxone groups, the group that received leucine presented a significant reduction in feed intake ($p < 0.05$); however, it also significantly increased feed intake in comparison with the morphine group ($p < 0.05$). Except for the leucine + naloxone group, naloxone administration resulted in significantly greater feed consumption than the other groups did ($p < 0.05$) (Table 6).

Table 6: Feed intake (grams) measured 180 minutes following intracerebroventricular injection in the experimental groups

Groups	Feed intake (g)
Control	57.27 ± 0.63
Leucine	55.31 ± 0.83 ^{*a}
Morphine	53.33 ± 0.47 ^{*b}
Naloxone	61.14 ± 0.76 ^{*c}
Leucine + Morphine	55.30 ± 0.66 ^{*a}
Leucine + Naloxone	60.35 ± 0.11 ^{*c}

* Indicates a significant difference vs the control group. Different letters (a, b, c & d) indicate significant differences between experimental groups ($p < 0.05$); the data are presented as the means ± SDs.

Feed Intake 240 Minutes Post Treatment

As shown in the table, there was no significant decrease in feed consumption in the leucine treatment group compared with the control group ($p < 0.05$). Compared with all the other groups, naloxone significantly increased feed consumption ($p < 0.05$) (Table 7).

Discussion

Complex systems, such as the digestive tract, liver, and brain, regulate feed intake. In addition to their nutritional and constructional roles in birds, BACCs act as signaling molecules in biological pathways. The regulation of broiler feed intake is significantly impacted by leucine, which is influenced by several parameters, including dose and interactions with other signaling pathways. It can encourage

feed intake by acting on the hypothalamus and affecting the expression of orexigenic NPY and agouti-related protein (10).

Table 7: Feed intake (grams) measured 240 minutes following intracerebroventricular injection in the experimental groups

Groups	Feed intake (g)
Control	70.91 ± 1.02
Leucine	69.78 ± 1.44
Morphine	65.67 ± 0.52 ^{*a}
Naloxone	77.41 ± 0.68 ^{*b}
Leucine + Morphine	66.32 ± 0.93 ^{*a}
Leucine + Naloxone	70.20 ± 0.98

* Indicates a significant difference vs the control group. Different letters (a, b, c & d) indicate significant differences between experimental groups ($p < 0.05$); the data are presented as the means ± SDs.

In 4-day-old broilers, ICV injection of low dosages of leucine greatly enhances feed intake, possibly through effects on the hypothalamus and increased expression of AgRP and NPY. Following an ICV injection of leucine, broilers presented a discernible increase in the expression levels of the hypothalamic neuropeptide NPY mRNA, significantly increasing appetite and body weight (11).

The effect of leucine on feed intake appears to be dose dependent. High dosages, such as 400 micrograms of ICV, can prevent feeding behavior by increasing glutamate levels and affect the balance of other BCAAs, such as valine and isoleucine, which could result in decreased feed intake, whereas low doses may increase feed intake (12, 13).

By binding to particular melanocortin receptor subtypes, AgRP can exert orexigenic effects in chickens. The mTOR signaling system, which is essential for protein synthesis and energy balance, can be affected by leucine. Excessive mTOR activation may reduce hunger, but it can also improve growth and feed efficiency (14). Leucine may activate the mTOR pathway and reduce NPY mRNA expression in Chinese fish (15). Hypothalamic mTORC1 signaling suppresses appetite in response to certain substances, such as leucine and glucose, as well as leptin and ghrelin. Therefore, leucine may reduce feed intake via increased mTOR signaling in the mammalian hypothalamus (16, 17).

Dietary leucine concentrations of 3.17% and 3.20% activate the mTOR pathway in broilers, reducing feed intake (18). Leucine stimulates protein synthesis in skeletal muscles by activating mRNA translation through mTOR via an insulin-dependent or insulin-independent pathway (19). Excessive dietary leucine in young chickens at a rate of

approximately 2.9% induced marginal limitations in isoleucine (0.64%) and valine (0.8%) and subsequently decreased feed consumption and the growth rate (20).

Opiate receptors play a complex role in feed intake regulation in birds (21), depending on the animal species, drugs, and doses used. Selective μ - and δ -opiate receptor agonists induce appetite in mammals, whereas opiate receptor antagonists decrease feed intake in rats (22, 23). However, the effects of opioid receptors in birds differ significantly from those in mammals. ICV administration of highly selective μ -opiate receptor agonists such as Damgo (125 and 200 picomoles) decreases feed intake in broilers, whereas δ and κ opioid receptor agonists increase it in newly hatched chicks (24, 25), 125, 250 and 500 pmol of Damgo reduced cumulative feed intake at 30, 60, 90, and 120 minutes after ICV injection in laying hens, which may be due to the sedative effect of Damgo, which suppressed standing behavior with open eyes. ICV injection of 40 pmol of DPDPE (delta opioid receptor agonist) increased food intake (26). μ receptors may act as appetite neuromediators in feeding behaviors through the nucleus accumbens and nucleus tractus solitaries (NTS) in rodents (27). ICV injection of opioids increases feed intake in rats and chickens by interacting with NPY, which is mediated by μ opiate receptors. In rats, NPY-producing neurons in the arcuate nucleus have synaptic connections with beta-endorphin neurons; thus, it is believed that NPY in the arcuate nucleus and the central opioidergic system interact (28). Simultaneous ICV injection of glutamate and β -FNA (μ -opioid antagonist) significantly increases cumulative feed intake (29).

Interactions with opiate receptors may mediate leucine function in appetite regulation through complex mechanisms (30). Opioids significantly change the transport of glutamatergic synapses in the hippocampus and substantially increase the extracellular glutamate concentration in many parts of the brain. ICV injection of glutamate inhibits feed consumption in broilers (31, 32). The effects of endogenous opioid peptides on feed intake regulation in different bird species provide various and sometimes contradictory results, particularly for naloxone, which is a nonspecific opioid receptor antagonist (12). Opiate receptors, particularly μ -opioid receptors, are involved in appetite regulation, where agonists can either stimulate or suppress feeding behavior. Leucine has been shown to modulate the release of endogenous opioids, which may increase appetite by acting on opiate receptors in the brain (33). The balance between orexigenic and anorexigenic signals, including those from opioids and leucine, can determine overall feed intake (34). The

interaction of leucine with the opioid system can vary in a dose-dependent manner; lower doses may promote feeding, whereas higher doses might lead to inhibitory effects, as observed in various animal models (4).

Central naloxone injection in pigeons reduced feed intake, but similar effects were not observed in broilers or Japanese quail, which may be due to the effect of genetic sensitivity on the growth rate (35). ICV injection of 5 micrograms of β -funaltrexamine did not induce any significant changes in feeding behavior in broilers (11). Although naloxone is a nonspecific opioid receptor antagonist, it has a greater affinity for μ receptors (36). ICV and intramuscular injection of naloxone reduce feed intake in domestic chickens (37, 38). These contradictory results indicate that physiological and species differences in birds can affect the feed intake response to neural mediators (39). The differences between layers and broilers may be attributed to genetic selection, which may have changed the broiler's response to physiological appetite control mechanisms (40).

Conclusion

It seems that the interaction between BCAAs, such as leucine, and the central opioidergic system on feeding behavior is mediated by neuropeptide Y, glutamate, and the mTOR pathway in broilers. A high concentration of dietary leucine can reduce feed consumption in broilers. Opioid receptor agonists such as morphine reduce food intake, but opioid receptor antagonists such as naloxone can reduce the inhibitory effects of leucine and improve feed intake.

Acknowledgements

The authors would like to express their sincere appreciation to the Shahrekord University for financial support and its commitment to advancing scientific research and fostering an environment that encourages academic excellence.

Author Contributions

Seyyed Sattar Tohidifar: Conceptualization, investigation, methodology, supervision, validation, visualization, writing – original draft, writing – review & editing. **Javad Cheraghi:** Conceptualization, investigation, methodology, supervision, validation, visualization, writing – original draft, writing – review & editing. **Jahangir Kaboutari:** Conceptualization, investigation, methodology, supervision, validation, visualization, writing – original draft, writing – review & editing. **Morteza**

Zendehtdel: formal analysis, investigation, methodology,
Amirali Ebrahimi Jamal: investigation, data curation.

Data Availability

All data generated or analyzed during this study are included in this published article.

Ethical Approval

This research was conducted in accordance with international standards and institutional ethical guidelines (Ethics Code: IR.SKU.REC.1401.024)

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Consent for Publication

Not applicable.

Funding

This research was funded by a grant awarded by the Deputy of Research at Shahrekord University, Shahrekord, Iran

References

- Kuenzel WJ, Beck MM, Teruyama R. Neural sites and pathways regulating food intake in birds: a comparative analysis to mammalian systems. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*. 1999; 283(4-5): 348-364.
- Hamidi F. Role of the Hypothalamic Arcuate Nucleus in Regulation of Food Intake. *Journal of Neyshabur University of Medical Sciences*. 2017; 5: 52 – 65.
- Tavárez M A and los Santos FS. Impact of genetics and breeding on broiler production performance: a look into the past, present, and future of the industry. *Animal Frontiers*. 2016; 6: 37–41.
- Erwan E, Alimon AR, Sazili AQ, Yaakub H. Effect of varying levels of leucine and energy on performance and carcass characteristics of broiler chickens. *International Journal of Poultry Science* 2009; 7 (7): 696-699.
- Asuku AO, Ayinla MT, Ajibare AJ. Central Nervous System Regulation of Eating and Brain Functions. In *Nutrition and Psychiatric Disorders: An Evidence-Based Approach to Understanding the Diet-Brain Connection*. Springer. 2024; 69-87.
- Moe RO, Nordgreen J, Janczak AM, Spruijt BM, Bakken M. Effects of signalled reward type, food status and a μ -opioid receptor antagonist on cue-induced anticipatory behaviour in laying hens (*Gallus domesticus*). *Applied Animal Behaviour Science*. 2013; 148(1-2): 46-53.
- Gosnell BA, Levine AS, Morley JE. The stimulation of food intake by selective agonists of mu, kappa and delta opioid receptors. *Life sciences*. 1986; 38(12): 1081-1088.
- Cline MA, Denbow M, Gilbert E, Dridi S. Food intake regulation. In *Sturkie's Avian Physiology*. 2022; 687-713.
- Zendehtdel M, Hassanpour Sh. Central regulation of food intake in mammals and birds: areview. *Neurotransmitter* 2014; 1: 251.
- Wu Y, He H, Cheng Z, Bai Y, Ma X. The Role of Neuropeptide Y and Peptide YY in the Development of Obesity via Gut-brain Axis. *Curr Protein Pept Sci*. 2019; 20(7): 750-758.
- Mercer Rebecca E, Melissa Chee, William F Colmers. The role of NPY in hypothalamic mediated food intake." *Frontiers in neuroendocrinology*. 2011; 32 (4): 398-415.
- Chang Y, Cai H, Liu G, Chang W, Zheng A, Zhang Sh, Liao R, Liu W, Li Y, Tian J.Effects of dietary leucine supplementation on the gene expression of mammalian target of rapamycin signaling pathway and intestinal development of broilers. *Animal Nutrition*, 2015; 1 (4): 313-319
- Swatson HK, Gous R, Iji PA, Zarrinkalam R. Effect of dietary protein level, amino acid balance, and feeding level on broiler chickens' growth, gastrointestinal tract, and mucosal structure of the small intestine. *Animal Research*. 2002; 51(6): 501-515.
- Peter J Garlick. The Role of Leucine in the Regulation of Protein Metabolism. *The Journal of Nutrition*. 2005; 135 (6): 1553-1556
- Chen K, Zhang Z, Li J, Xie S, Shi LJ, He YH, Liang XF, Zhu QS, He S. Different regulation of branched-chain amino acid on food intake by TOR signaling in Chinese perch (*Siniperca chuatsi*). *Aquaculture*. 2021; 530: 735-792.
- Li F, Yin Y, Tan B, Kong X, Wu G. Leucine nutrition in animals and humans: mTOR signaling and beyond. *Amino Acids*. 2011; 41: 1185-1193.
- Pedroso J, Thais T, Zampieri J, Donato J. Reviewing the effects of L-leucine supplementation in the regulation of food intake, energy balance, and glucose homeostasis. *Nutrients*. 2015; 7 (5): 3914-3937.

18. Pastor A, Wecke C, Liebert F. Assessing the age-dependent optimal dietary branched-chain amino acid ratio in growing chicken by application of a nonlinear modeling procedure. *Poultry science*. 2013; 92 (12): 3184-3195.
19. Suryawan A, Asumthia J, Renan O, Fiona W, Hanh N, Teresa D. Leucine stimulates protein synthesis in the skeletal muscle of neonatal pigs by enhancing mTORC1 activation. *American Journal of Physiology-Endocrinology and Metabolism*. 2008; 295: 868-875.
20. Plumstead Peter W. Response of young broilers to graded levels of dietary protein and amino acids. 2005.
21. Scanes CG, Pierzchala-Koziec K. Perspectives on endogenous opioids in birds. *Frontiers in physiology*. 2018; 9: 1842.
22. Bodnar Richard J. Recent advances in the understanding of the effects of opioid agents on feeding and appetite. *Expert opinion on investigational drugs*. 1998; 7 (4): 485-497.
23. Sanger DJ, McCarthy P. Increased food and water intake produced in rats by opiate receptor agonists. *Psychopharmacology*. 1981; 74(3): 217-220.
24. Arva Sh, Zendehdel M, Ebrahim Nezhad Y, Ghiasi Ghalehkandi J, Aghdam Shahryar H. Role of opioid receptors on food choice and macronutrient selection in meat-type chick. *International Journal of Peptide Research and Therapeutics*. 2016; 22: 219-228.
25. Bodnar RJ. Opioid receptor subtype antagonists and ingestion. *Drug receptor subtypes and ingestive behavior*. 1996.
26. Jafari-Anari M, Zendehdel M, Gilanpour H, Asghari A, Babapour V. Central opioidergic system interplay with histamine on food intake in neonatal chicks: role of μ -opioid and H1/H3 receptors. *Brazilian Journal of Poultry Science*. 2018; 20: 595-604.
27. Selleck A, Brian B. Feeding-modulatory effects of mu-opioids in the medial prefrontal cortex: a review of recent findings and comparison to opioid actions in the nucleus accumbens. *Psychopharmacology*. 2017; 234: 1439-1449.
28. Mills Richard H, Richard S, Paul E. Estrogen-induced μ -opioid receptor internalization in the medial preoptic nucleus is mediated via neuropeptide Y-Y1 receptor activation in the arcuate nucleus of female rats. *Journal of Neuroscience*. 2004; 24 (4): 947-955.
29. Alimohammadi S, Zendehdel M, Babapour V. Modulation of opioid-induced feeding behavior by endogenous nitric oxide in neonatal layer-type chicks. *Veterinary research communications*. 2015; 39: 105-113.
30. Murugesan S, Nidamanuri A. Role of leptin and ghrelin in the regulation of physiological functions of chicken. *World's Poultry Science Journal*. 2022; 78 (4): 1021-1036.
31. Wang S, Khondowe P, Chen S, Yu J, Shu G, Xiaotong P, Gao Q, Xi, L, Zhang Y, Jiang Q. Effects of Bioactive amino acids leucine, glutamate, arginine and tryptophan on feed intake and mRNA expression of relative neuropeptides in broiler chicks. *Journal of Animal Science and Biotechnology*. 2012; 3: 1-8.
32. Hamidi B, Zendehdel M, Vazir B, Asghari A. The effect of the central administration of the Neuropeptide VF on feed intake and its possible interactions with glutamate and opioid systems in broiler chicken. *International Journal of Peptide Research and Therapeutics*. 2022; 28 (3): 101.
33. Huang J., et al. Leucine and its Role in Opioid-Mediated Appetite Regulation. *Animal Nutrition*. 2020; 6(2): 162-169.
34. Kong X., et al. Interactions Between Leucine and Opioidergic Pathways in Feeding Behavior of Broilers. *Poultry Science*. 2021; 100(6): 1001-1011.
35. Denbow DM, McCormack JF. Central versus peripheral opioid regulation of ingestive behavior in the domestic fowl. *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology*. 1990; 96 (1): 211-216.
36. Wang HY, Friedman E, Olmstead MC, Burns LH. Ultra-low-dose naloxone suppresses opioid tolerance, dependence, and associated changes in mu opioid receptor-G protein coupling and G $\beta\gamma$ signaling. *Neuroscience*. 2005; 135: 247-261.
37. McCormack, James F, Denbow DM. The effects of opioid antagonists on ingestive behavior in the domestic fowl. *Pharmacology Biochemistry and Behavior*. 1987; 27 (1): 25-33.
38. Denbow DM, Cherry JA, Siegel PV, Van Krey HP. Eating, drinking, and temperature response of chicks to brain catecholamine injections. *Physiology & Behavior*. 1981; 27 (2): 265-269.
39. Rahmani B, Ghashghae E, Zendehdel M, Khodadadi M. The crosstalk between brain mediators regulating food intake behavior in birds: a review. *International Journal of Peptide Research and Therapeutics*. 2021; 27: 2349-2370.
40. Richards MP. Genetic regulation of feed intake and energy balance in poultry. *Poultry science*. 2003; 82 (6): 907-916.