

Research paper

The effect of spexin injection and its interaction with nitric oxide, serotonin, and corticotropin receptors on the central regulation of food intake in broilers

Mohaya Farzin^a, Shahin Hassanpour^a, Morteza Zende del^{b,*}, Bitavazir^a, Ahmad Asghari^c

^a Division of Physiology, Department of Basic Sciences, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran

^b Division of Physiology, Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran 14155-6453, Iran

^c Department of Clinical Sciences, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran

ARTICLE INFO

Keywords:

Spexin
Serotonin
Corticotropin
Nitric oxide
Food intake
Broilers

ABSTRACT

Complex homeostatic control mechanisms are tools to adjust the food birds eat and their appetite. Birds and mammals differ in several ways considering food intake regulation. Therefore, this study aimed to investigate the special effects of the intracerebroventricular (ICV) injection of spexin and its interaction with nitric oxide, serotonin and corticotropin receptors on central food intake regulation in broilers. In the test 1, Broilers received ICV injection of saline, PCPA (p-chlorophenylalanine, 1.25 µg), spexin (10 nmol) and PCPA+spexin. In test 2–7, 8-OH-DPAT, SB-242084 (5-HT_{2C}, 1.5 µg), L-arginine (Precursor of nitric oxide, 200 nmol), L-NAME (nitric oxide synthetize inhibitor, 100 nmol), Astressin-B (30 µg) and Astressin2-B (30 µg) were injected to Broilers instead of the PCPA. Then, the amount of food received was measured up to 2 h after the injection. The food consumption was significantly decreased by Spexin (10 nmol) ($P < 0.05$). Concomitant injection of SB-242084+spexin attenuated spexin-induced hypophagia ($P < 0.05$). Co-injection of L-arginine+spexin enhanced spexin-induced hypophagia and this effect was reversed by L-NAME ($P < 0.05$). Also, concomitant injection of Astressin-B + spexin or Astressin2-B + spexin enhanced spexin-induced hypophagia ($P < 0.05$). Founded on these observations, spexin-induced hypophagia may be mediated by nitric oxide and 5-HT_{2C}, CRF1, and CRF2 receptors in neonatal broilers.

1. Introduction

The brain regulates energy homeostasis in response to the signals from the adipose tissue and the gastrointestinal tract (Boguszewski et al., 2010). There have been many progress and advancements made in the description of hypothalamic neural networks and neuropeptide transmitters, as well as the discovery of novel peptides and the association they have with the signal system they send to the brain regarding the nutritional status of the body (Fry et al., 2007). Complex homeostatic mechanisms and neural systems can potentially control food intake. Food intake influences the synthesis of these neurotransmitters and can significantly affect feeding behaviors (Berthoud and Morrison, 2008; Fernstrom, 1981). Important neurotransmitters have which been discovered exceed 40,

including Acetylcholine(ACh), epinephrine, norepinephrine, histamine, gamma-aminobutyric acid (GABA), glycine, serotonin or 5-

hydroxytryptamine (5-HT), and glutamate which are among the most important neurotransmitters influencing behavioral-control nutrition pointed (Shojaei et al., 2020). Given the importance of studying food consumption in farmed birds, in 1983 it was reported that an increase in growth rate in broilers is mainly due to increased food consumption and also food quality has a minimal role in this regard (Neves et al., 2014). In general, diet-independent weight gain was gotten in broilers. While the share of conversion in feed conversion ratio was small due to improved food quality in weight gain. Therefore, diet-independent weight gain was obtained in broilers. While the share of changes in feed conversion ratio is small due to improved food quality in weight gain (Niknafs and Roura, 2018). Therefore, understanding the mechanisms of food intake is important both in terms of improving methods of increasing appetite in broilers and turkeys (Niknafs and Roura, 2018). The most newly discovered member of the galanin/kisspeptin/spexin family of peptides is Spexin (Tran et al., 2021). This 14-amino acid peptide is highly

* Corresponding author.

E-mail address: zendedel@ut.ac.ir (M. Zende del).

<https://doi.org/10.1016/j.ibneur.2024.04.010>

Received 6 December 2023; Received in revised form 16 April 2024; Accepted 30 April 2024

Available online 3 May 2024

2667-2421/© 2024 Published by Elsevier Inc. on behalf of International Brain Research Organization. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

unspoiled and is also involved in homeostatic functions including, metabolism, energy homeostasis, and reproduction. Spexin is expressed by neurons in the hypothalamus, which coordinates energy homeostasis and reproduction (Lim et al., 2019a). Spexin is expressed in the islets of the human pancreas and is secreted by insulin into the secretory pathways and can be released with this hormone. In addition, a negative feedback loop has been found between spexin and insulin, indicating a multifaceted association between glucose, insulin, and spexin in the pancreatic islets (Sassek et al., 2019). Spexin also plays an important role in regulating serotonin-induced behavior, as long-term use of a serotonin reuptake inhibitor reduces spexin expression in the rat hypothalamus. However, it increases spexin levels in the stratum corneum and hippocampus (Jeong et al., 2019). Spexin is closely related to nitric oxide (NO) (Pałasz et al., 2016). Interestingly, it has been shown in internal mouse habenula and orthologous dorsal habenula in zebrafish that spexin is able to regulate depression and anxiety (Yun, Reyes-Alcaraz, Lee, Yong, Choi, Ham, Sohn, Kim, Son, Kim, et al., 2019). The internal habenula travel to the intercellular nucleus, which subsequently reaches the raphe nucleus where serotonergic neurons are located, indicating a potential interaction between the Spexin and the serotonergic system. Increased c-Fos expression of dorsal raphe nucleus 5-HT neurons was observed in mice implanted with corticosterone following injection of the spexin -based GALR₂ agonist, denoting a potential spexin modulatory mechanism in 5-HT neurons (Lim et al., 2019b). Although there have been researches done on spexin effects and the interactions it possesses with other neurotransmitters, its characteristics in bird's central regulation have not been investigated. Despite many similarities that seem to exist between mammalian and avian species considering food regulatory systems, some differences have been reported (Richards, 2003). The results demonstrate some different mechanisms in the central regulation of food intake between birds and mammals, which can be physiologically absolutely essential, although there are many similarities between birds and mammals in the mechanisms of appetite regulation (Richards et al., 2010). Therefore, the aim of the present study was to investigate the effects of ICV injection of spexin and its interaction with NO and serotonin and corticotropin receptors on the central food intake regulation in neonatal Broilers.

2. Material and methods

2.1. Animals

In the present study, 308 meat-type broilers (aged one-day old) (Ross- 308) obtained from a domestic hatchery were used (Mahan Co. Iran). They were kept in groups for two days with 23 h light and 1 h dark schedule at 31 ± 2° C with 50 % ± 2 humidity. They were then placed in solitary confinement for 5 days (Mahan Co. Iran). Poultry care was done based on an entirely randomized design during the study, the birds were given free access to fresh water and commercial diet containing 21 % crude protein and 2850 kcal/kg of metabolizable energy (Iranian Institute for Animal Science Research, Table 1). All experimental procedures were done based on animal care and experimental methods were founded by the National Institute of Health (USA) Instructions for the Care and Use of Laboratory Animals (Olanrewaju et al., 2017; Zendeheel et al., 2017). All test procedures were done based on animal care (Sikes and Gannon, 2011).

2.2. Experimental drugs

Drugs which contained spexin (CAS No.: 1370290-58-6), PCPA (p-chlorophenylalanine, Serotonin synthesis inhibitor, CAS No.: 14173-40-1), 8-OH-DPAT (5-HT_{1A} auto receptor agonist, CAS No.: 87394-87-4), SB-242084 (5-HT_{2C} receptor antagonist, CAS No.: 1260505-34-7), L-arginine (NO precursor, CAS No.: 74–79–3), L-NAME (NO synthetase inhibitor, CAS No.: 51298-62-5), Astressin-B (Corticotropin releasing factor (CRF), (CRF₁/CRF₂ receptor antagonist, CAS No.: 170809-51-5),

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
			1.21
Acidifier	0.15	Lysine (%)	0.49
DL-Methionine	0.10	Methionine (%)	0.83
Toxin binder	0.10	Methionine β cystine (%)	0.70
L-Lysine HCl	0.05	Threonine (%)	0.20
Vitamin D3	0.1	Tryptophan (%)	0.78
Multi enzyme	0.05	Valine (%)	

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 selenium from 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.

Astressin2-B (CRF₂ receptor antagonist, CAS No.: 681260-70-8) and Evans Blue were bought. The drugs were diluted using 0.85 % saline which contained Evans blue at a ratio of 1/250. The saline mixture containing Evans blue was utilized for the control group. (Jonaidi et al., 2012; Motaghi et al., 2022; Yousefi et al., 2019). The effective dose for spexin in neonatal broilers was obtained from our recently published study (Farzin et al., 2022).

2.3. Intracerebroventricular injection protocol

ICV was injected at 5 days of age. In order to inject broilers in a cerebral ventricular way, the conscious chick's head was held by an acrylic device with a 45-degree tip angle and the skull surface was parallel to the surface of the workbench. A hole was made in the stencil, so it was immediately placed on the skull in the right ventricular region. The solutions were injected through a hole which was made using a Hamilton syringe. Then, the needle head was inserted just 4 mm into the skin and skull. Needless to say that this procedure was not stressful in broilers. (Saito et al., 2005). The capacity of injections in each collection was 10 microliters (Furuse et al., 1999). Satisfactory to identify the injection at the end of the test, decapitation was done and finally, the correct placement of the injection in the ventricle was confirmed by the presence of Evans Blue in the brain tissue (Jonaidi et al., 2012; Motaghi et al., 2022; Yousefi et al., 2019).

2.4. Food intake measurement procedure

In this study, seven test were designed with 4 groups of 11 broilers each (n= 44) broiler in each experiment). In the first experiment, birds were injected with ICV of saline, PCPA (1.25 µg), spexin (10 nmol) and

PCPA + spexin. In the second experiment, the broilers were given saline, 8-OH-DPAT (15.25 nmol), spexin and 8-OH-DPAT + spexin. In the third experiment, saline was injected to chicks, SB-242084 (1.5 µg), spexin and SB-242084 + spexin. In the fourth experiment, the birds were injected with L-arginine (200 nmol), spexin and L-arginine + spexin. In the fifth experiment, injection of the saline, L-NAME (100 nmol), spexin and L-NAME + spexin were done in broilers. In the sixth experiment, the injection of the saline, Astressin-B (30 µg) spexin and Astressin-B + spexin were done in chicks. In the seventh experiment, injection of the saline, Astressin2-B (30 µg), spexin and Astressin2-B (30 µg) + spexin were done in chicks (Table 1). The chicks were deprived of food for 180 min (FD₃) both before the trial and after the injection, they were returned to their cages and had free access to food and water. Then, the amount of cumulative feed was measured at 30, 60 and 2 h after the injection. Food intake was expressed as a percentage of body weight in order to minimize the effect differences in weight between broilers regarding food intake. The injections dosage was determined based on a pilot study (not-published) and previous studies (Lv et al., 2020; Tian et al., 2020b).

2.5. Statistical analysis

All data were analyzed using SPSS software version 22. The cumulative food intake was analyzed using the repeated measurements and data were presented as mean ± SEM. Tukey-Kramer test was used to compare the means of any treatment. Treatment was by considering the significant level less than 0.05.

Table 2
Treatment procedure in experiments1-7.

Exp.1	ICV Injection	Exp.5	ICV Injection
<i>Treatment groups</i>		<i>Treatment groups</i>	
I	CS*	I	CS*
II	spexin (10 nmol)	II	spexin (10 nmol)
III	PCPA (1.25 µg)	III	L-NAME (100 nmol)
IV	Spexin + PCPA	IV	Spexin+ L-NAME
Exp.2	ICV Injection	Exp.6	ICV Injection
<i>Treatment groups</i>		<i>Treatment groups</i>	
I	CS*	I	CS*
II	spexin (10 nmol)	II	spexin (10 nmol)
III	8-OH-DPAT (15/25 nmol)	III	Astressin-B)30 µg (
IV	Spexin + 8-OH-DPAT	IV	Spexin+ Astressin-B
Exp.3	ICV Injection	Exp.7	ICV Injection
<i>Treatment groups</i>		<i>Treatment groups</i>	
I	CS*	I	CS*
II	spexin (10 nmol)	II	spexin (10 nmol)
III	SB-242,084(1.5 µg)	III	Astressin2-B)30 µg (
IV	Spexin + SB-242,084	IV	Spexin+ Astressin2-B
Exp.4	ICV Injection		
<i>Treatment groups</i>			
I	CS*		
II	spexin (10 nmol)		
III	L-arginine (200 nmol)		
IV	Spexin + L-arginine		

CS: control solution, Physiological serum with Evans Blue 0.1 %: PCPA (serotonin synthesis inhibitor): 8-OH-DPAT (5-HT_{1A} receptor antagonist): SB-242,084 (5-HT_{2C} receptor antagonist): L-arginine (nitric oxide precursor): L-NAME (nitric oxide inhibitor): stressin-B CRF₂ receptor antagonist: stressin2-B: CRF₁ / CRF₂ receptor antagonist

3. Results

3.1. Food intake

Intraventricular injection PCPA (1.25 µg) did not have a significant effect on food consumption in broilers compared to the control group (P >0.05). Spexin was able to significantly decrease food ingestion in broilers associated with the control group. Simultaneous injection of the PCPA + Spexin showed no significant effect on hypophagia in broilers (P >0.05) (Fig. 1).

They were not significant alterations observed on food intake in broilers after they were injected with Intraventricular of the 8-OH-DPAT (15.25 nmol) compared to control group. Spexin was able to significantly decrease food consumption in broilers as compared with control group (P <0.05). Co-administration of the 8-OH-DPAT + spexin had no significant effect on spexin-induced hypophagia in broilers (P >0.05) (Fig. 2).

The injection of the SB-242084 (1.5 µg), showed not significant effect on broiler's food intake compared to control group. Food intake decreased significantly following the injection of the Spexin. Co-administration of the SB-242084 + spexin significantly attenuated spexin induced hypophagia in broilers (P <0.05) (Fig. 3).

The injection of L-arginine (200 nmol) showed not significant effect on food intake in broilers compared to control group (P >0.05). Food intake decreased significantly following the injection of the Spexin in broilers (P <0.05). Co-administration of the L-arginine + spexin significantly enhanced Spexin-induced hypophagia in broilers (P <0.05) (Fig. 4).

The injection of L-NAME (100 nmol), showed no significant effect on food intake in broilers compared to control group (P >0.05). Food intake decreased significantly following the injection of the Spexin (P <0.05). Co-administration of the L-NAME + spexin decreased the Spexin-induced hypophagia significantly in broilers (P <0.05) (Fig. 5).

The injection of Astressin-B (30 µg), showed not significant effect on food intake in broilers compared to control group (P >0.05). Food intake decreased significantly in broilers following the injection of Spexin (P <0.05). Co-administration of the Astressin-B + spexin significantly decreased Spexin-induced hypophagia in broilers (P <0.05) (Fig. 6).

The injection of Astressin2-B (30 µg) showed not significant effect on food intake in broilers compared to control group (P >0.05). Food intake was significantly decreased in broilers following the injection of the Spexin (P <0.05). Co-administration of the Astressin2-B + spexin significantly attenuated Spexin-induced hypophagia in broilers (P <0.05) (Fig. 7).

4. Discussion

This is the first report on spexin receptors effects on food intake and its interaction with NO, 5-HT and corticotropin receptors in broiler. Spexin is a kind of adipokine which is potential to be applied in obesity treatment by working on energy balance (Agha and Agha, 2017). Studies have shown that spexin possesses anorexic effect on Siberian sturgeon (Tian et al., 2020a). In the current study, there was a reduction of food intake by ICV injection of spexin (Tian et al., 2020b). The hypothalamic and supraventricular nuclei of the hypothalamus were able to play a crucial role in spexin development while contributing to water regulation, food intake, energy consumption, and reproductive behaviors. Spexin genes expression in the paraventricular and supraoptic nuclei of the hypothalamus similar to the nesfatin-1 receptor, were shown to be associated with oxytocin and corticotropin system (Pałasz et al., 2021). Also, Recent studies have shown the interaction of GAL upsurges the polarization of serotonergic neurons outstanding to the receptor-receptor interaction with 5-HT_{1AR} and decreases the binding affinity of the auto receptor after binding to serotonin (Wang et al., 2016).

Our findings showed that spexin hypophagia effect could be

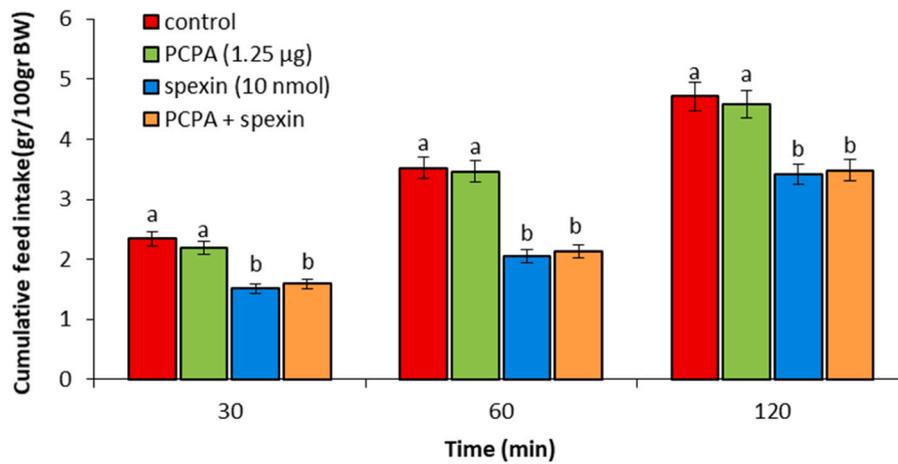


Fig. 1. Effect of ICV injection of PCPA (1.25 µg), spexin (10 nmol) and their combination on cumulative food intake in neonatal chicken (n=44). PCPA: parachlorophenyl-alanine, serotonin depletive. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments (P < 0.05).

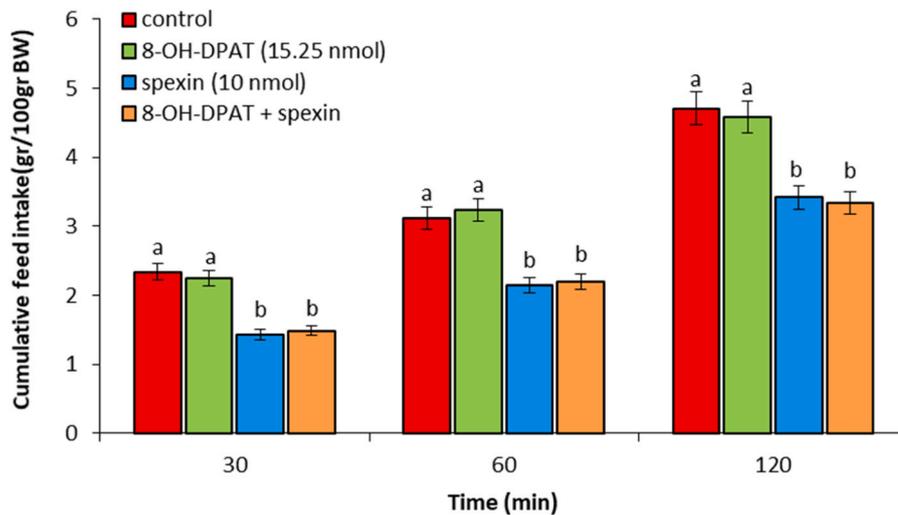


Fig. 2. Effect of ICV injection of 8-OH-DPAT (15.25 nmol), spexin (10 nmol) and their combination on cumulative food intake in neonatal chicken (n=44). 8-OH-DPAT: 5-HT1A autoreceptor agonist. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments (P < 0.05).

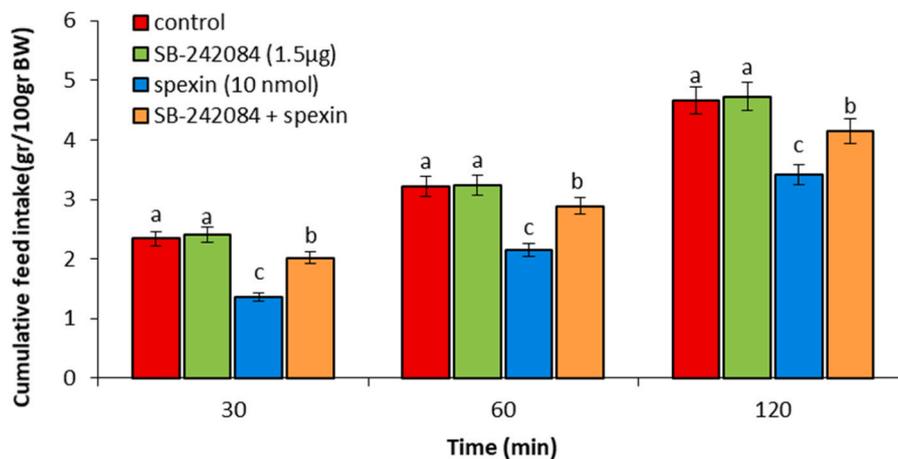


Fig. 3. Effect of ICV injection of SB-242084 (1.5 µg), spexin (10 nmol) and their combination on cumulative food intake in neonatal chicken (n=44). SB-242084: 5-HT2C receptor antagonist. Data are expressed as mean ± SEM. Different letters (a, b and c) indicate significant differences between treatments (P < 0.05).

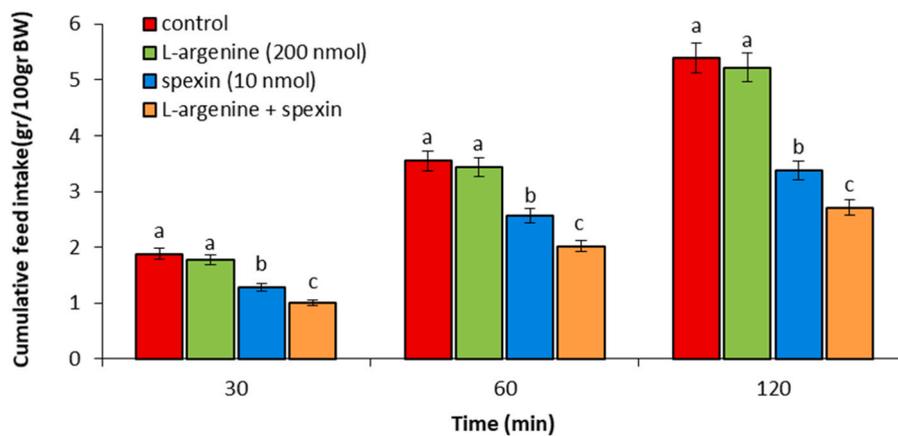


Fig. 4. Effect of ICV injection of L-arginine (200 nmol), spexin (10 nmol) and their combination on cumulative food intake in neonatal chicken (n=44). L-arginine: nitric oxide precursor. Data are expressed as mean ± SEM. Different letters (a, b and c) indicate significant differences between treatments (P < 0.05).

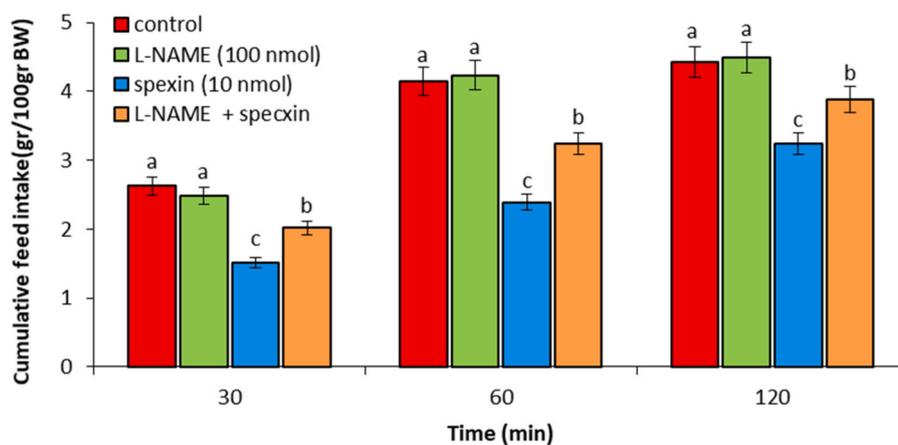


Fig. 5. Effect of ICV injection of L-NAME (100 nmol), spexin (10 nmol) and their combination on cumulative food intake in neonatal chicken (n=44). L-NAME: nitric oxide synthetase inhibitor. Data are expressed as mean ± SEM. Different letters (a, b and c) indicate significant differences between treatments (P < 0.05).

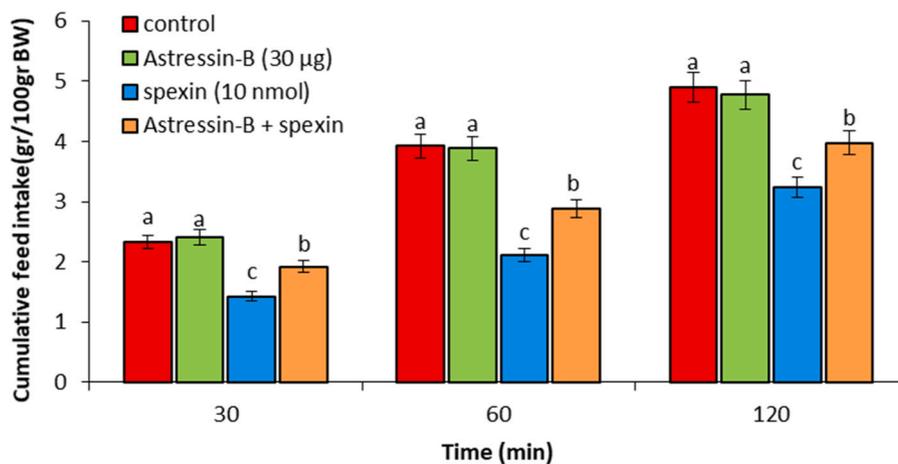


Fig. 6. Effect of ICV injection of Arestressin-B (30 µg), spexin (10 nmol) and their combination on cumulative food intake in neonatal chicken (n=44). Arestressin-B: CRF₁/CRF₂ receptor antagonist. Data are expressed as mean ± SEM. Different letters (a, b and c) indicate significant differences between treatments (P < 0.05).

mediated by NO in broilers (Zendehdel et al., 2021). NO induces anorexigenic responses in hypothalamic neurons and tissue by increasing the anorexigenic ratio of proopiomelanocortin and cocaine and amphetamine regulated transcript to neuropeptide Y and agouti related peptide (Tran et al., 2020). Notably, some previous studies

indicate an inconsistent finding regarding the effect of the nitroergic system on food intake in Broilers. For example, there is evidence showing that the intraperitoneal (IP) injection of the L-NAME could decrease feeding behavior in both layers and broilers (Khan et al., 2007). Similar results have been shown in rats (De Luca et al., 1995). Probably

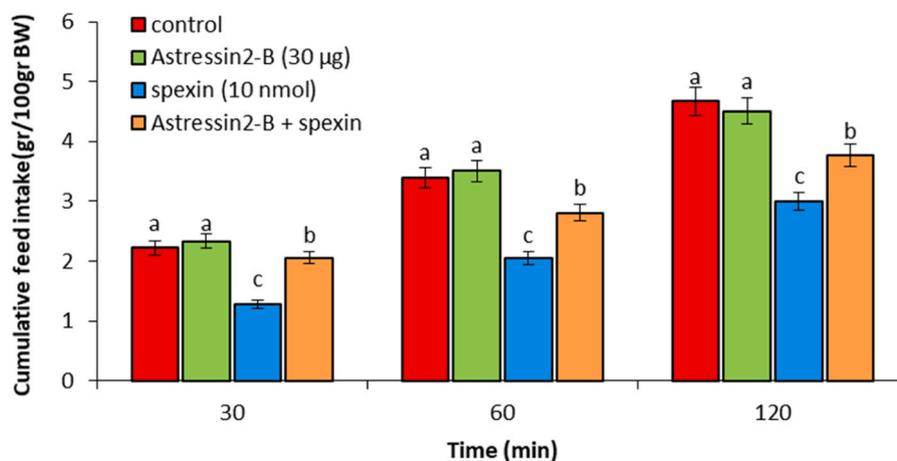


Fig. 7. Effect of ICV injection of Astressin2-B (30 µg), spexin (10 nmol) and their combination on cumulative food intake in neonatal chicken (n=44). Astressin2-B: CRF₂ receptor antagonist. Data are expressed as mean ± SEM. Different letters (a, b and c) indicate significant differences between treatments (P < 0.05).

the main reason for this difference (hyperphagic effect of the NO in mammals and broilers but hypophagia role in layer broiler) is related to different pathways, which can cause the involvement of peripheral NO receptors in IP administration and thus activate different cascades in regulating food intake (Alimohammadi et al., 2015). Previous study revealed that the ICV injection of L-NG-Nitro arginine (L-NNA), as a competitive NOS inhibitor, diminished food intake in Broilers which was in agreement with our study (35). In addition, different neurochemical pathways in nutritional behavior are influenced by different genetic factors that can ultimately elicit different nutritional responses to the same neurotransmitter (Denbow, 1994; Khan et al., 2007; Yousefvand and Hamidi, 2021; Zendehdel et al., 2015). Studies have shown that a spexin-based Galanin receptor agonist (SG₂A) simultaneously induces mood and body weight behaviors in low-weight corticosteroid (CORTI) mice with increased symptoms of anhedonia, anxiety, and depression (Yun, Reyes-Alcaraz, Lee, Yong, Choi, Ham, Sohn, Kim, Son, and Kim, 2019). In addition, the activation of excitatory amino acid receptors, especially NMDA receptors, increases the activity of the enzyme NO synthase, resulting in various cognitive and non-cognitive effects, while NO inhibitors can greatly inhibit the effects mediated by NMDA receptors (Wan et al., 1994; Xu et al., 1998). As an immunonutrition, feeding arginine shows some immunostimulatory and thermotropic roles and improves both humoral and cellular immunity. L-Arginine also improves insulin, growth hormone, and thyroid hormone secretion and by which, improves growth in a dose-dependent manner (Ghamari Monavvar et al., 2020).

Our study also showed a correlation between spexin, Astressin-B and Astressin2-B in terms of food intake in broilers, suggesting that CRF1/2 receptor activation may be involved in the feeding inhibition induced by spexin. Studies have shown that not only CRF₁ but also CRF₂ are involved in inhibiting stress-induced food intake, and Two subtypes of the CRF) receptor are likely to act within one hour of exposure to stress (Sekino et al., 2004). ICV injection of these CRF-related peptides inhibits food intake in rats; however, daily food intake of CRF-deficient in CRF₁-deficient transgenic mice does not differ from that in wild-type mice (Bale et al., 2000; Jacobson, 1999). Another study showed that CRF₁ is at least partially mediated by the inhibition of nutritional behavior induced by emotional stress and increased motor activity (Hotta et al., 1999). CRF is also involved in the mechanism of stress-induced food intake through the CRF receptor type 1, as well as opioid and dopaminergic systems. Cerebral CRF also has dual effects on food intake, hyperphagia, and anorexia (Samarghandian et al., 2003) CRF₁ receptors are involved in regulating motor activity during the dark period, but not in regulating feeding behavior under non-stressful conditions (Tabarin et al., 2007). CRF₁ receptors do not affect

hypothalamic-pituitary-adrenal axis activity, either under baseline or after acute stress (Ohata et al., 2002).

In tilapia, a large population of CRF neurons has been reported in the telencephalon where spexin expressing cells are localized. Similarly, low density of CRF fiber projections and glucocorticoid receptors has been reported in the semicircular torus area where spexin expressing cells are localized. The upregulation of spexin mRNA expression levels in chronic social defeat stress fish (elevated plasma cortisol) suggested that spexin may be under the feedback regulation of cortisol (Lim et al., 2020). The increase in gene expression of spexin observed during chronic social defeat evoked by prolong high level of plasma cortisol may also be indirectly mediated by an unknown factor. One of the possible regulators of spexin might be 5-HT (Lim et al., 2020). Spexin mRNA expression was decreased in hippocampus, while CRF mRNA expression was increased (Zhuang et al., 2020). Local hippocampus spexin mRNA was found to be decreased by injecting CRF into mouse hippocampus (Zhuang et al., 2020). The inhibitory effects of CRF on spexin promoter activity in HEK293 cells with CRFR2 over-expression were dose-dependently mimicked by cAMP analog (Zhuang et al., 2020). In different brain regions, neurons have different CRF expressions and responses to behavioral responses and it seems that spexin-related anxiety behavior may be regulated by CRF through the MEK signaling pathway (Zhuang et al., 2020).

The findings of the present study showed that there was no significant relationship between spexin-induced food intake and 5HT_{1A} receptor agonist receptors in broilers. However, there was a significant difference in the 5-HT_{2C} receptor antagonist. Serotonin is considered to be the most important neurotransmitter in hemostatic systems (Soslau, 2022). serotonin can play the metabolic roles in different tissues. Central serotonin suppresses appetite, reducing nutrient intake. In the periphery, serotonin promotes nutrient storage by increasing gut motility to facilitate absorption after feeding. Serotonin elevates insulin secretion from pancreatic islets, which raises nutrient storage in different tissues (Yabut et al., 2019). These results suggested that 5-HT hypophagia and hyperdipsia were mediated by different mechanisms in the central nervous system (Zendehdel et al., 2012a). It is showed that there is a neural interaction between serotonergic systems and melanocortin in terms of nutritional behavior in broilers (Zendehdel and Hassanpour, 2014). The 5-HT affects the centers that regulate nutrition and energy balance (Zendehdel et al., 2012b). Other studies have shown clear support for a hypothetical source of the brainstem for hypophagia serotonin function (Lee et al., 1998). Preliminary observations show that the area where spexin expressing neurons are localized is densely innervated by 5-HT fiber projections. Since neurons of the ventral periventricular pretecal nuclei (PPV) project to the semicircular torus in

the brain of tilapia, this suggests an indirect neuroanatomical link between the 5-HT neurons in the PPV and spexin neurons localized in the ventromedial nucleus of semicircular torus can be speculated (Lim et al., 2020). Hypothalamic spexin expression could be down-regulated by serotonin-dependent actions of antidepressants like selective serotonin reuptake inhibitors (Pałasz et al., 2021). Also, amygdala spexin expression was increased by classical antipsychotics. Haloperidol and chlorpromazine were associated with decreased anxiety in rats (Pałasz et al., 2021)

5. Conclusion

Founded on observations, spexin can mediate hypophagia through NO, 5-HT_{2C}, CRF₁, and CRF₂ receptors in broilers. In actual fact, there was not any comparable study to compare our results in a poultry model. It is also suggested that more extensive research is needed for the direct effects of hypophagia caused by spexin with nitric oxide, serotonin, and corticotropin receptors in broiler chickens.

Ethical approval

All steps of experimentation were performed in accordance with the regulations of the Science and Research Branch of Islamic Azad University, Tehran, Iran Guide for the Care and Use of Laboratory Animals (Ethics code: IR.IAU.SRB.REC. 1400.345). Full determinations were made to reduce the use of animals and to advance their comfort.

Funding

This manuscript did not contain any studies with human subjects performed by any of the authors. All test was performed in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Ethics Committee. The study protocol was approved by Animal Ethics Committee of the Science and Research Branch of Islamic Azad University, Tehran, Iran (IR.IAU.SRB.REC. 1400.345).

CRedit authorship contribution statement

Shahin Hassanpour: Writing – review & editing, Writing – original draft, Supervision, Project administration. **Morteza Zendehtdel:** Project administration, Methodology. **Mohaya Farzin:** Writing – original draft, Data curation. **Bitva vazir:** Resources. **Ahmad Asghari:** Validation.

Conflicts of Interest

The authors have no conflicts of interest to declare regarding the study described in this article and preparation of the article.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Acknowledgments

The authors would like to thank the Razi Clinical Research Development Unit and the central laboratory (Dr. Restegar Lab) the University of Tehran, Iran for their cooperation in publishing the research. Islamic Azad University. This paper was obtained from a Ph.D. thesis performed by the first author.

Consent to publish

All authors reviewed and approved the manuscript

References

- Agha, M., Agha, R., 2017. The rising prevalence of obesity: part A: impact on public health. *Int. J. Surg. Oncol.* 2 (7), e17.
- Alimohammadi, S., Zendehtdel, M., Babapour, V., 2015. Modulation of opioid-induced feeding behavior by endogenous nitric oxide in neonatal layer-type chicks. *Vet. Res. Commun.* 39 (2), 105–113. <https://doi.org/10.1007/s11259-015-9631-8>.
- Bale, T.L., Contarino, A., Smith, G.W., Chan, R., Gold, L.H., Sawchenko, P.E., Koob, G.F., Vale, W.W., Lee, K.-F., 2000. Mice deficient for corticotropin-releasing hormone receptor-2 display anxiety-like behaviour and are hypersensitive to stress. *Nat. Genet.* 24 (4), 410–414.
- Berthoud, H.-R., Morrison, C., 2008. The brain, appetite, and obesity. *Annu. Rev. Psychol.* 59, 55–92.
- Boguszewski, C.L., Paz-Filho, G., Velloso, L.A., 2010. Neuroendocrine body weight regulation: integration between fat tissue, gastrointestinal tract, and the brain. *Endokrynol. Pol.* 61 (2), 194–206.
- De Luca, B., Monda, M., Sullo, A., 1995. Changes in eating behavior and thermogenic activity following inhibition of nitric oxide formation. *Am. J. Physiol. -Regul. Integr. Comp. Physiol.* 268 (6), R1533–R1538.
- Denbow, D.M., 1994. Peripheral regulation of food intake in poultry. *J. Nutr.* 124 (suppl. 8), 1349S–1354S.
- Farzin, M., Hassanpour, S., Zendehtdel, M., Vazir, B., Asghari, A., 2022. Effects of intracerebroventricular injection of spexin and its interaction with NPY, GalR(2) and GalR(3) receptors on the central food intake regulation and nutritional behavior in broiler chickens. *Neurosci. Lett.* 777, 136589 <https://doi.org/10.1016/j.neulet.2022.136589>.
- Fernstrom, J.D., 1981. Effects of the diet on brain function. *Acta Astronaut* 8 (9-10), 1035–1042. [https://doi.org/10.1016/0094-5765\(81\)90076-x](https://doi.org/10.1016/0094-5765(81)90076-x).
- Fry, M., Hoyda, T.D., Ferguson, A.V., 2007. Making sense of it: roles of the sensory circumventricular organs in feeding and regulation of energy homeostasis. *Exp. Biol. Med.* 232 (1), 14–26.
- Furuse, M., Ando, R., Bungo, T., Shimojo, M., Masuda, Y., 1999. Intracerebroventricular injection of orexins does not stimulate food intake in neonatal chicks. *Br. Poult. Sci.* 40 (5), 698–700.
- Ghamari Monavvar, H., Moghaddam, G., & Ebrahimi, M. (2020). A review on the effect of arginine on growth performance, meat quality, intestine morphology, and immune system of broiler chickens.
- Hotta, M., Shibasaki, T., Arai, K., Demura, H., 1999. Corticotropin-releasing factor receptor type 1 mediates emotional stress-induced inhibition of food intake and behavioral changes in rats. *Brain Res.* 823 (1-2), 221–225. [https://doi.org/10.1016/S0006-8993\(99\)01177-4](https://doi.org/10.1016/S0006-8993(99)01177-4).
- Jacobson, L., 1999. Glucocorticoid replacement, but not corticotropin-releasing hormone deficiency, prevents adrenalectomy-induced anorexia in mice. *Endocrinology* 140 (1), 310–317.
- Jeong, I., Kim, E., Seong, J.Y., Park, H.-C., 2019. Overexpression of spexin 1 in the dorsal habenula reduces anxiety in zebrafish. *Front. Neural Circuits* 53.
- Jonaidi, H., Abbassi, L., Yaghoobi, M., Kaiya, H., Denbow, D., Kamali, Y., Shojaei, B., 2012. The role of GABAergic system on the inhibitory effect of ghrelin on food intake in neonatal chicks. *Neurosci. Lett.* 520 (1), 82–86.
- Khan, M.S.I., Tachibana, T., Hasebe, Y., Masuda, N., Ueda, H., 2007. Peripheral or central administration of nitric oxide synthase inhibitor affects feeding behavior in chicks. *Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol.* 148 (2), 458–462.
- Lee, M.D., Aloyo, V.J., Fluharty, S.J., Simansky, K.J., 1998. Infusion of the serotonin1B (5-HT1B) agonist CP-93,129 into the parabrachial nucleus potently and selectively reduces food intake in rats. *Psychopharmacology* 136 (3), 304–307. <https://doi.org/10.1007/s002130050570>.
- Lim, C.H., Lee, M.Y.M., Soga, T., Parhar, I., 2019a. Evolution of structural and functional diversity of spexin in mammalian and non-mammalian vertebrate species. *Front. Endocrinol. (Lausanne)* 10, 379.
- Lim, C.H., Lee, M.Y.M., Soga, T., Parhar, I., 2019b. Evolution of structural and functional diversity of spexin in mammalian and non-mammalian vertebrate species [Review]. *Front. Endocrinol. (Lausanne)* 10. <https://doi.org/10.3389/fendo.2019.00379>.
- Lim, C.H., Soga, T., Levavi-Sivan, B., Parhar, I.S., 2020. Chronic social defeat stress up-regulates spexin in the brain of Nile Tilapia (*Oreochromis niloticus*). *Sci. Rep.* 10 (1), 7666. <https://doi.org/10.1038/s41598-020-64639-4>.
- Lv, S., Zhou, Y., Feng, Y., Zhang, X., Wang, X., Yang, Y., Wang, X., 2020. Peripheral Spexin Inhibited Food Intake in Mice. *Int. J. Endocrinol.* 2020, 4913785 <https://doi.org/10.1155/2020/4913785>.
- Motaghi, S., Jonaidi, H., Talebifard, H., Chavoshzadeh, M., 2022. The avian central roles of alpha and beta oestrogen receptors on food intake. *J. Anim. Physiol. Anim. Nutr.* 106 (2), 308–312.
- Neves, D., Banhazi, T., Nääs, I., 2014. Feeding behaviour of broiler chickens: a review on the biomechanical characteristics. *Braz. J. Poult. Sci.* 16 (2), 01–16.
- Niknafs, S., Roura, E., 2018. Nutrient sensing, taste and feed intake in avian species. *Nutr. Res. Rev.* 31 (2), 256–266.
- Ohata, H., Arai, K., Shibasaki, T., 2002. Effect of chronic administration of a CRF1 receptor antagonist, CRA1000, on locomotor activity and endocrine responses to stress. *Eur. J. Pharmacol.* 457 (2), 201–206. [https://doi.org/10.1016/S0014-2999\(02\)02663-8](https://doi.org/10.1016/S0014-2999(02)02663-8).
- Olanrewaju, H., Purswell, J., Collier, S., Branton, S., 2017. Effects of light ingress through ventilation fan apertures on selected blood variables of male broilers. *Int. J. Poult. Sci.* 16 (8), 288–295.
- Pałasz, A., Suszka-Świtek, A., Filipczyk, L., Bogus, K., Rojczyk, E., Worthington, J., Krzysztanek, M., Wiaderekiewicz, R., 2016. Escitalopram affects spexin expression in the rat hypothalamus, hippocampus and striatum. *Pharmacol. Rep.* 68 (6), 1326–1331.

- Pałasz, A., Suszka-Świtek, A., Kałkosz, A., Plewka, D., Bogus, K., Filipczyk, L., Blaszczyk, I., Bacopoulou, F., Worthington, J.J., Piowowarczyk-Nowak, A., 2021. Spexin-expressing neurons in the magnocellular nuclei of the human hypothalamus. *J. Chem. Neuroanat.* *111*, 101883.
- Pałasz, A., Żarczyński, P., Bogus, K., Mordecka-Chamera, K., Della Vecchia, A., Skałbania, J., Worthington, J.J., Krzysztanek, M., Żarczyńska, M., 2021. Modulatory effect of olanzapine on SMIM20/phoenixin, NPQ/spexin and NUCB2/nesfatin-1 gene expressions in the rat brainstem. *Pharm. Rep.* *73* (4), 1188–1194. <https://doi.org/10.1007/s43440-021-00267-7>.
- Richards, M., 2003. Genetic regulation of feed intake and energy balance in poultry. *Poult. Sci.* *82* (6), 907–916.
- Richards, M., Rosebrough, R., Coon, C., McMurtry, J., 2010. Feed intake regulation for the female broiler breeder: In theory and in practice. *J. Appl. Poult. Res.* *19* (2), 182–193.
- Saito, E.-S., Kaiya, H., Tachibana, T., Tomonaga, S., Denbow, D.M., Kangawa, K., Furuse, M., 2005. Inhibitory effect of ghrelin on food intake is mediated by the corticotropin-releasing factor system in neonatal chicks. *Regul. Pept.* *125* (1–3), 201–208.
- Samarghandian, S., Ohata, H., Yamauchi, N., Shibasaki, T., 2003. Corticotropin-releasing factor as well as opioid and dopamine are involved in tail-pinch-induced food intake of rats. *Neuroscience* *116* (2), 519–524. [https://doi.org/10.1016/s0306-4522\(02\)00712-1](https://doi.org/10.1016/s0306-4522(02)00712-1).
- Sassek, M., Kolodziejcki, P.A., Szczepankiewicz, D., Pruszyńska-Oszmialek, E., 2019. Spexin in the physiology of pancreatic islets—mutual interactions with insulin [Article]. *Endocrine* *63* (3), 513–519. <https://doi.org/10.1007/s12020-018-1766-2>.
- Sekino, A., Ohata, H., Mano-Otagiri, A., Arai, K., Shibasaki, T., 2004. Both corticotropin-releasing factor receptor type 1 and type 2 are involved in stress-induced inhibition of food intake in rats. *Psychopharmacol. (Berl.)* *176* (1), 30–38. <https://doi.org/10.1007/s00213-004-1863-1>.
- Shojaei, M., Yousefi, A., Zendehelel, M., Khodadadi, M., 2020. Food intake regulation in birds: the role of neurotransmitters and hormones. *Iran. J. Vet. Med.* *14* (1), 99–115.
- Sikes, R.S., Gannon, W.L., 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *J. Mammal.* *92* (1), 235–253.
- Soslau, G., 2022. Cardiovascular serotonergic system: Evolution, receptors, transporter, and function. *J. Exp. Zool. Part A: Ecol. Integr. Physiol.* *337* (2), 115–127.
- Tabarin, A., Diz-Chaves, Y., Consoli, D., Monsaingeon, M., Bale, T.L., Culler, M.D., Datta, R., Drago, F., Vale, W.W., Koob, G.F., Zorrilla, E.P., Contarino, A., 2007. Role of the corticotropin-releasing factor receptor type 2 in the control of food intake in mice: a meal pattern analysis. *Eur. J. Neurosci.* *26* (8), 2303–2314. <https://doi.org/10.1111/j.1460-9568.2007.05856.x>.
- Tian, Z., Xu, S., Wang, M., Li, Y., Chen, H., Tang, N., Wang, B., Zhang, X., Li, Z., 2020a. Identification, tissue distribution, periprandial expression, and anorexigenic effect of spexin in Siberian sturgeon, *Acipenser baeri*. *Fish. Physiol. Biochem.* *46* (6), 2073–2084. <https://doi.org/10.1007/s10695-020-00856-y>.
- Tian, Z., Xu, S., Wang, M., Li, Y., Chen, H., Tang, N., Wang, B., Zhang, X., Li, Z., 2020b. Identification, tissue distribution, periprandial expression, and anorexigenic effect of spexin in Siberian sturgeon, *Acipenser baeri* [Article]. *Fish. Physiol. Biochem.* *46* (6), 2073–2084. <https://doi.org/10.1007/s10695-020-00856-y>.
- Tran, A., He, W., Chen, J.T.C., Belsham, D.D., 2021. Spexin: Its role, regulation, and therapeutic potential in the hypothalamus. *Pharmacol. Ther.*, 108033 <https://doi.org/10.1016/j.pharmthera.2021.108033>.
- Tran, A., Loganathan, N., McIlwraith, E.K., Belsham, D.D., 2020. Palmitate and Nitric Oxide Regulate the Expression of Spexin and Galanin Receptors 2 and 3 in Hypothalamic Neurons. *Neuroscience* *447*, 41–52. <https://doi.org/10.1016/j.neuroscience.2019.10.028>.
- Wan, R.-Q., Pang, K., Olton, D.S., 1994. Hippocampal and amygdaloid involvement in nonspatial and spatial working memory in rats: effects of delay and interference. *Behav. Neurosci.* *108* (5), 866.
- Wang, P., Li, H., Barde, S., Zhang, M.-D., Sun, J., Wang, T., Zhang, P., Luo, H., Wang, Y., Yang, Y., 2016. Depression-like behavior in rat: Involvement of galanin receptor subtype 1 in the ventral periaqueductal gray. *Proc. Natl. Acad. Sci.* *113* (32), E4726–E4735.
- Xu, X., Boshoven, W., Lombardo, B., Spranger, J., 1998. Comparison of the amnesic effects on NMDA receptor antagonist MK-801 and nitric oxide synthase inhibitors: L-NAME and L-NOARG in goldfish. *Behav. Neurosci.* *112* (4), 892.
- Yabut, J.M., Crane, J.D., Green, A.E., Keating, D.J., Khan, W.I., Steinberg, G.R., 2019. Emerging roles for serotonin in regulating metabolism: new implications for an ancient molecule. *Endocr. Rev.* *40* (4), 1092–1107.
- Yousefi, A., Shojaei, M., Zendehelel, M., 2019. Evaluation the role of central serotonin and 5HT_{2c} serotonin receptor on feed intake in female layer-type Bovans chicken by intracerebroventricular (ICV) injection of Para-chlorophenylalanine and SB242084. *Vet. Res. Biol. Prod.* *32* (1), 55–62.
- Yousefvand, S., Hamidi, F., 2021. The role of ventromedial hypothalamus receptors in the central regulation of food intake. *Int. J. Pept. Res. Ther.* *27* (1), 689–702.
- Yun, S., Reyes-Alcaraz, A., Lee, Y.-N., Yong, H.J., Choi, J., Ham, B.-J., Sohn, J.-W., Kim, D.-H., Son, G.H., Kim, H., 2019. Spexin-based galanin receptor type 2 agonist for comorbid mood disorders and abnormal body weight. *Front. Neurosci.* *13*, 391.
- Yun, S., Reyes-Alcaraz, A., Lee, Y.-N., Yong, H.J., Choi, J., Ham, B.-J., Sohn, J.-W., Kim, D.-H., Son, G.H., Kim, H., Kwon, S.-G., Kim, D.S., Kim, B.C., Hwang, J.-I., Seong, J.Y., 2019. Spexin-Based Galanin Receptor Type 2 Agonist for Comorbid Mood Disorders and Abnormal Body Weight [Original Research]. *Front. Neurosci.* *13* <https://doi.org/10.3389/fnins.2019.00391>.
- Zendehelel, M., Hamidi, F., Babapour, V., Mokhtarpouriani, K., Fard, R.M.N., 2012a. The effect of melanocortin (Mc3 and Mc4) antagonists on serotonin-induced food and water intake of broiler cockerels. *J. Vet. Sci.* *13* (3), 229–234.
- Zendehelel, M., Hamidi, F., Babapour, V., Mokhtarpouriani, K., Fard, R.M.N., 2012b. The effect of melanocortin (Mc3 and Mc4) antagonists on serotonin-induced food and water intake of broiler cockerels. *jvs* *13* (3), 229–234. <https://doi.org/10.4142/jvs.2012.13.3.229>.
- Zendehelel, M., Hamidi, F., Hassanpour, S., 2015. The effect of histaminergic system on nociceptin/orphanin FQ induced food intake in chicken. *Int. J. Pept. Res. Ther.* *21* (2), 179–186.
- Zendehelel, M., Hassanpour, S., 2014. Central regulation of food intake in mammals and birds: a review. *Neurotransmitter* *1*.
- Zendehelel, M., Khodadadi, M., Zandiyeh, H., Mokhtarpouriani, K., Rahmani, B., Baghbanzadeh, A., 2021. A newly discovered interference of the central nitrenergic system on oxytocin-induced hypophagia in layer-type chickens. *Iran. J. Vet. Sci. Technol.* *13* (1), 22–33.
- Zendehelel, M., Parvizi, Z., Hassanpour, S., Baghbanzadeh, A., Hamidi, F., 2017. Interaction between nociceptin/orphanin FQ and adrenergic system on food intake in neonatal chicken. *Int. J. Pept. Res. Ther.* *23* (1), 155–161.
- Zhuang, M., Lai, Q., Yang, C., Ma, Y., Fan, B., Bian, Z., Lin, C., Bai, J., Zeng, G., 2020. Spexin as an anxiety regulator in mouse hippocampus: Mechanisms for transcriptional regulation of spexin gene expression by corticotropin releasing factor. *Biochem. Biophys. Res. Commun.* *525* (2), 326–333. <https://doi.org/10.1016/j.bbrc.2020.02.023>.