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μ-Opioid Receptor Blockade Potentiates NPW23-Induced Hyperphagia in Neonatal Broiler Chicks

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A B S T R A C T

Neuropeptide W (NPW) is an endogenous ligand for GPR7 and GPR8 receptors and has recently been identified as an orexigenic factor in birds. However, the involvement of the central opioidergic system in NPW-induced feeding has not been investigated. The aim of this study was to determine the role of μ-, δ-, and κ-opioid receptors in hyperphagia elicited by intracerebroventricular (ICV) injection of NPW23 in neonatal broiler chicks. A total of 176 male chicks (Ross 308 strain), aged 24 hours post-hatching, were utilized as experimental subjects across four distinct investigations. In Experiment 1, chicks received ICV injections of control solution or NPW23 (0.75, 1.5, and 3 nmol) to establish a dose-response curve. In the second experiment, subjects received injections of either a control vehicle, NPW23 at a dosage of 3 nanomoles, β-funaltrexamine (β-FNA; a selective μ-opioid receptor antagonist), or a combination of NPW23 and β-FNA. The third experimental trial employed naltrindole (NTI; a δ-opioid receptor antagonist), whereas the fourth investigation utilized nor-binaltorphimine (nor-BNI; a κ-opioid receptor antagonist). Following the administration of treatments, cumulative feed consumption was measured at three discrete time intervals; 30, 60, and 120 minutes post-injection; and subsequently normalized as a proportion of each subject's body weight. NPW23 at 1.5 and 3 nmol dose-dependently increased food intake at all time points (p<0.05). Co-administration of β-FNA with NPW23 significantly potentiated NPW23-induced hyperphagia (p<0.05). In contrast, co-injection of NTI or nor-BNI with NPW23 did not alter the orexigenic response (p>0.05). These findings indicate that μ-opioid receptor blockade potentiates NPW23-induced feeding, suggesting that central μ-opioid receptor signaling may exert an inhibitory modulatory influence on NPW23-induced hyperphagia in neonatal broiler chicks.

Keywords: *Neuropeptide W; Opioid receptor; Food intake; Broiler chicken; Hyperphagia*

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1 Introduction

The control of feeding behavior constitutes an intricate biological mechanism necessitating accurate integration between systemic metabolic cues and neural pathways within the central nervous system, particularly within the hypothalamus (Brüning & Fenselau, 2023; Campos et al., 2022). In poultry, understanding the mechanisms governing appetite is of particular importance for optimizing growth performance and feed efficiency (Gao et al., 2025). Although considerable progress has been made in identifying appetite-regulating neurotransmitters in birds, differences exist between avian and mammalian species in the functional roles of several mediators, including ghrelin, melanin-concentrating hormone, and galanin, underscoring the need for species-specific research (Kuenzel et al., 1999).

Neuropeptide W (NPW) was originally purified from porcine hypothalamic tissue and identified as an endogenous agonist for two orphan G-protein-coupled receptors, namely GPR7 (also referred to as NPBWR1) and GPR8 (designated NPBWR2) (Singh & Davenport, 2006). NPW is found in two distinct molecular forms, designated NPW23 and NPW30, each of which exhibits a high degree of evolutionary conservation among different vertebrate species (Bu et al., 2016). Within the central nervous system, both Neuropeptide W (NPW) and its corresponding receptor populations are localized in neural areas essential to the maintenance of energy balance, such as the arcuate nucleus, paraventricular nucleus, lateral hypothalamic region, and the amygdaloid complex (Takenoya et al., 2010). Central infusion of NPW has been reported to increase meal consumption in rats under light-phase conditions (Levine et al., 2005; Mondal et al., 2003), although suppressive effects have been observed during the dark phase (Mondal et al., 2003). Recent studies have demonstrated that central infusion of NPW23 stimulates feed consumption in neonatal broilers, an effect mediated in part by corticotropin-releasing factor (CRF1/CRF2) and neuropeptide Y (NPY1) receptors (Mahdavi et al., 2023). However, the potential involvement of other central neurotransmitter systems, particularly the opioidergic system, remains unexplored.

The opioidergic system is a well-established regulator of feeding behavior in both birds and mammals (Morley, 1995). Three principal classes of opioid receptors; designated μ (mu), δ (delta), and κ (kappa); are abundantly distributed throughout the nervous system and have been established as key modulators of feeding behavior (Sandoval-Caballero et al., 2023). In broilers, μ-opioid receptor agonists have been reported to suppress feeding (Bungo et al., 2005), while κ- and δ-opioid receptor agonists consistently increase food consumption (Alimohammadi et al., 2015). However, the endogenous μ-opioid ligand β-endorphin has been shown to enhance feeding in broilers (McCormack & Denbow, 1988; Yanagita et al., 2008), suggesting a more complex functional involvement for the μ-opioid system in appetite regulation. Importantly, structural and signaling similarities exist between NPW receptors (GPR7/GPR8) and opioid receptors. Both belong to the Gi/o-coupled GPCR family, share the ability to inhibit cAMP production and activate ERK1/2, and GPR7 has been shown to bind β-endorphin with low affinity (O'Dowd et al., 1995; Sakurai, 2013). Moreover, previous studies have documented functional links between opioid-mediated pathways and additional anorexigenic or orexigenic peptides in birds, including serotonin (Rahmani et al., 2022), ghrelin (Baghaeikia et al., 2022), and spexin (Badri et al., 2025).These observations suggest the possibility of bidirectional interaction between the NPW pathway and opioidergic networks in the control of feeding behavior.

Although indirect evidence exists, no study has directly tested whether central opioid receptors mediate NPW-induced hyperphagia in any species. Hence, the present study examined the hypothesis that NPW23-evoked feeding in neonatal broiler chicks involves μ-, δ-, and κ-opioid receptors, using selective antagonists delivered by ICV injection.

2 Materials and Methods

2.1 Animals

A cohort comprising 176 male broiler chicks of the Ross 308 strain, aged 24 hours post-hatching (mean body weight: 42.3±1.2 g), was sourced from a commercial supplier (Mahan Company, Tehran, Iran). For the initial 48 hours, the birds were reared in collective pens under standardized environmental parameters (temperature: 30±1°C; relative humidity: 50–55%; photoperiod: 23L:1D). Feed and water were provided without restriction, utilizing a conventional starter ration (metabolizable energy: 2850 kcal/kg; crude protein: 21%) formulated with corn (52.85%), soybean meal (31.57%), wheat (5%), gluten meal (2.50%), wheat bran (2.47%), di-calcium phosphate (1.92%), oyster shell (1.23%), soybean oil (1%), and mineral/vitamin supplements (0.5%). On day 3 post-hatch, each animal was relocated to an individual enclosure to facilitate acclimatization. All experimental manipulations involving

intracerebroventricular (ICV) administration were carried out on the fifth day post-hatch. All ICV administrations were carried out on day 5 post-hatch following a 3-hour fasting period with free access to water.

2.2 Drugs

The following drugs were used: NPW23 (Tocris, USA), β-funaltrexamine (β-FNA, μ-opioid receptor antagonist, Sigma, USA), naltrindole (NTI, δ-opioid receptor antagonist, Sigma, USA), nor-binaltorphimine (nor-BNI, κ-opioid receptor antagonist, Sigma, USA), and Evans Blue dye (Sigma, USA). All drugs were dissolved in sterile saline (0.9% NaCl) containing 0.1% Evans Blue to verify injection site accuracy. The control solution consisted of saline with 0.1% Evans Blue. All drug solutions were prepared to a final volume of 10 μL per chick, and the control group received the vehicle (saline+Evans Blue) alone. Drug doses were selected based on previous studies in neonatal chicks and were confirmed in our preliminary experiments to be sub-effective doses that did not alter baseline feeding when administered alone (Hamidi et al., 2022; Mahdavi et al., 2023).

2.3 Intracerebroventricular (ICV) injection

ICV administrations were carried out according to a previously validated technique specifically adapted for neonatal chicks, which does not require anesthetic agents or surgical intervention (Davis et al., 1979; Furuse et al., 1997). In brief, each chick's cranium was secured within an acrylic stereotaxic apparatus, with the beak holder adjusted to a 45° inclination such that the dorsal surface of the skull became horizontally aligned with the laboratory bench. A miniature aperture was subsequently drilled through the calvarium above the right lateral cerebral ventricle. A Hamilton microsyringe equipped with a 30-gauge needle was then introduced to a depth of 4 mm to access the ventricular cavity. The test solution was infused slowly over a 30-second interval. This methodology has been demonstrated not to induce appreciable physiological distress in avian subjects (Saito et al., 2005). All procedures were performed by an experienced investigator, and chicks were monitored for signs of distress throughout the procedure. Any bird showing signs of distress, discomfort, or abnormal behavior was humanely euthanized and excluded from the study (none occurred). Upon completion of each experimental trial, the animals were humanely euthanized via cervical dislocation, in accordance with the American Veterinary Medical Association (AVMA) Guidelines. Thereafter, the brains were harvested and inspected for the presence of Evans Blue dye. Any subject in which the dye was not detected within the ventricular system was excluded from subsequent data analysis; a total of 4 chicks died during or immediately after the injection procedure across all experiments (2 chicks in Experiment 2, 1 chick in Experiment 3, and 1 chick in Experiment 4; no mortality occurred in Experiment 1), and these birds were excluded from the final analysis.

2.4 Experimental design

Four experiments were conducted with the following sample sizes: Experiment 1: 44 chicks divided into 11-bird treatment groups (no mortality; final n=11 per group); Experiment 2: 44 chicks divided into 11-bird treatment groups (2 chicks died, resulting in n=11 for control and NPW23 groups, and n=10 for β-FNA and NPW23+β-FNA groups; total n=42); Experiment 3: 44 chicks divided into 11-bird treatment groups (1 chick died, resulting in n=11 for control, NPW23, and NTI groups, and n=10 for the NPW23+NTI group; total n=43); Experiment 4: 44 chicks divided into 11-bird treatment groups (1 chick died, resulting in n=11 for control, NPW23, and nor-BNI groups, and n=10 for the NPW23+nor-BNI group; total n=43). Chicks were randomly assigned to treatment groups using a stratified randomization procedure based on body weight to ensure balanced group means. Feed-intake measurements were performed by an investigator blinded to treatment allocation. Injections were performed in randomized order to minimize any potential time-of-day effects. Post-infusion feed intake was measured at 30, 60, and 120 minutes and normalized as a percentage of body weight (%BW). Following injection, chicks were individually housed with pre-weighed feeders. Cumulative consumption was calculated by subtracting residual feed from initial weight and expressed as grams per 100 g body weight. Detailed information regarding treatment groups, drug doses, and injection protocols for all experiments is presented in Table 1.

Table 1. Intracerebroventricular (ICV) injection protocols for experimental groups

Experiment	Group	ICV Injection
1	A	Control solution
	B	NPW23 (0.75 nmol)
	C	NPW23 (1.5 nmol)
	D	NPW23 (3 nmol)
2	A	Control solution
	B	β-FNA (5 μg)
	C	NPW23 (3 nmol)
	D	NPW23 (3 nmol)+β-FNA (5 μg)
3	A	Control solution
	B	NTI (5 μg)
	C	NPW23 (3 nmol)
	D	NPW23 (3 nmol)+NTI (5 μg)
4	A	Control solution
	B	nor-BNI (5 μg)
	C	NPW23 (3 nmol)
	D	NPW23 (3 nmol)+nor-BNI (5 μg)

ICV, intracerebroventricular; NPW23, neuropeptide W (23 amino acid isoform); β-FNA, β-funaltrexamine (μ-opioid receptor antagonist); NTI, naltrindole (δ-opioid receptor antagonist); nor-BNI, nor-binaltorphimine (κ-opioid receptor antagonist). Control solution: 0.9% saline containing 0.1% Evans Blue. Injection volume: 10 μL per chick.

2.5 Statistical analysis

All data are shown as Mean±SEM. A two-way repeated-measures ANOVA was applied for each experiment, with Tukey's test used for post hoc comparisons. SPSS 26 (IBM, USA) served for statistical analysis, and SigmaPlot 14.0 for graphing. Significance was defined as P $p \leq 0.05$.

3 Results

3.1 Dose-dependent influence of NPW23 on feeding behavior

As shown in Fig. 1, ICV administration of NPW23 resulted in a dose-related elevation of cumulative feed consumption across every measured post-injection interval. Two-way repeated-measures ANOVA revealed significant effects of treatment [$F(3,40)=17.23$, $p<0.05$] , time [$F(2,80)=138.91$, $p<0.05$] , and treatment×time interaction [$F(6,80)=4.67$, $p=0.003$]. At the lowest dose (0.75 nmol), no statistically significant change in feed consumption was observed relative to the control condition, regardless of the time point examined ($p>0.05$). Delivery of NPW23 at a dosage of 1.5 nmol led to a statistically significant enhancement of feed consumption at 60 and 120 minutes ($p<0.05$), and the highest dose (3 nmol) increased feeding at 30, 60, and 120 minutes ($p<0.05$). The 3 nmol dose was selected for subsequent antagonist studies because it produced the most robust and sustained hyperphagic effect.

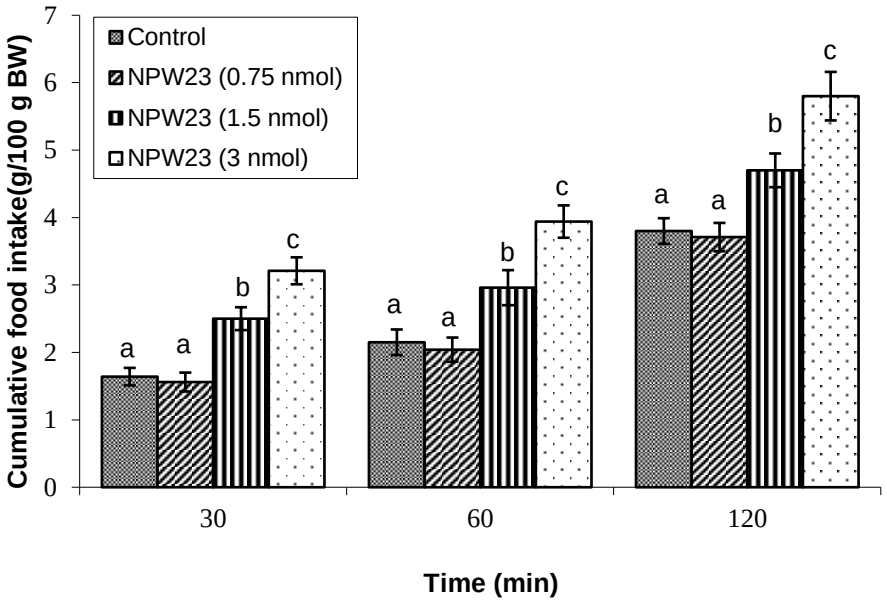


Figure 1. Effect of ICV injection of NPW23 (0.75, 1.5, and 3 nmol) on cumulative food intake in neonatal chickens. Data are expressed as mean ± SEM. Each group included 11 chicks, with no mortality recorded during the experiment (total N = 44). Different letters (a, b, and c) indicate statistically significant differences among treatments at the same time point ($p < 0.05$).

3.2 Effect of μ-opioid receptor blockade on NPW23-induced hyperphagia

Fig. 2 illustrates the effect of μ-opioid receptor blockade on NPW23-induced hyperphagia. Two-way repeated-measures ANOVA revealed significant effects of treatment [$F(3,38)=20.45$, $p<0.05$] , time [$F(2,76)=148.92$, $p<0.05$] , and treatment×time interaction [$F(6,76)=5.21$, $p=0.002$]. ICV infusion of NPW23 alone (3 nmol) significantly increased cumulative food intake at all intervals compared to the control group ($p<0.05$). Administration of β-FNA (5 μg) alone had no significant effect on feeding behavior ($p>0.05$). Notably, co-administration of NPW23 with β-FNA significantly potentiated the hyperphagic response to NPW23 ($p<0.05$), indicating that blockade of μ-opioid receptors enhances NPW23-induced feeding.

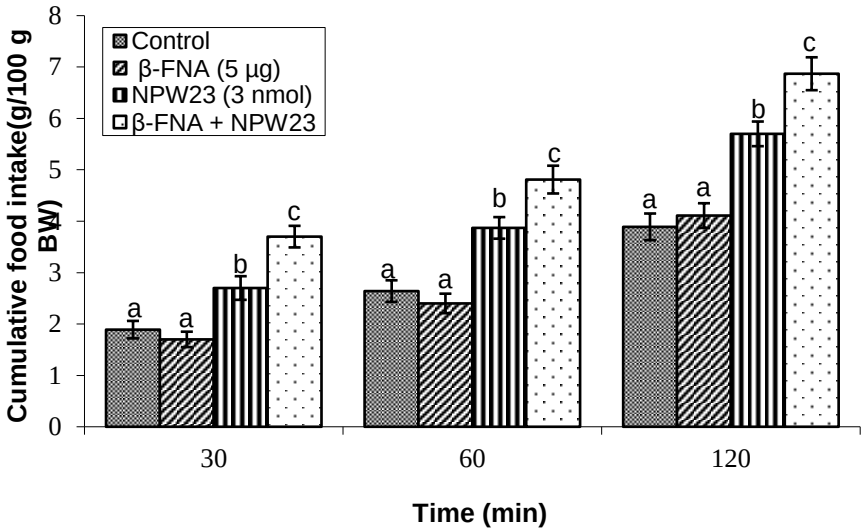


Figure 2. Effect of ICV injection of β-FNA (5 μg), NPW23 (3 nmol), and their combination on cumulative food intake in neonatal chickens. β-FNA: mu-opioid receptor antagonist. Each of the control and NPW23 groups included 11 chicks, whereas the β-FNA and NPW23 + β-FNA groups each included 10 chicks (two chicks were excluded due to mortality during injection; total N = 42). Different letters (a, b, and c) indicate statistically significant differences among treatments at the same time point ($p < 0.05$).

3.3 *Effect of δ-opioid receptor blockade on NPW23-induced hyperphagia*

In Fig. 3, treatment with NPW23 alone once again elicited a marked elevation in feed consumption relative to the control condition. Two-way repeated-measures ANOVA revealed significant effects of treatment [$F(3,39)=18.76$, $p<0.05$] , time [$F(2,78)=145.23$, $p<0.05$] , and treatment × time interaction [$F(6,78)=4.12$, $p=0.009$]. The δ-opioid receptor antagonist NTI (5 µg) administered alone had no effect on baseline meal consumption ($p>0.05$). Simultaneous infusion of NPW23 together with NTI failed to produce any statistically significant modification of the NPW23-induced hyperphagic effect ($p>0.05$), indicating that δ-opioid receptors are not involved in mediating NPW23-induced feeding under the present experimental conditions.

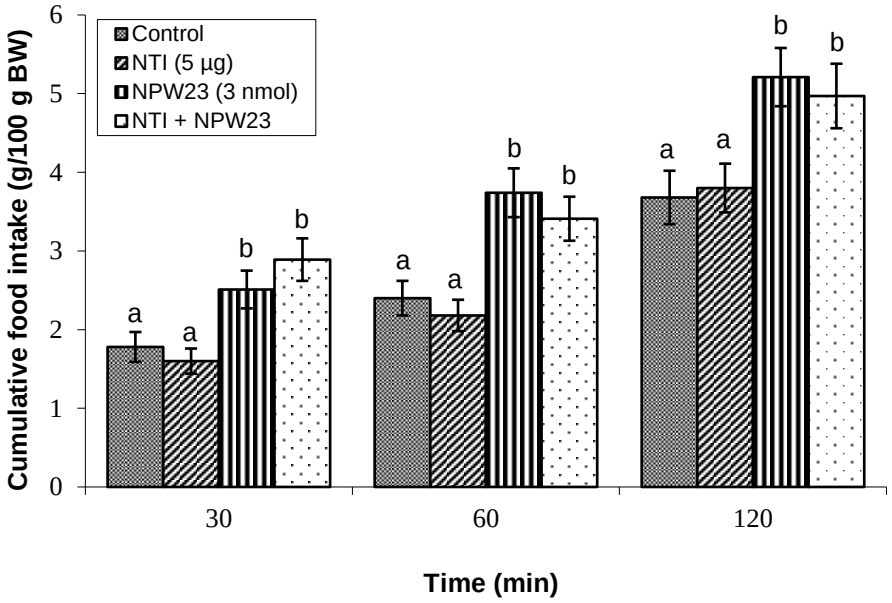


Figure 3. Effect of ICV injection of NTI (5 µg), NPW23 (3 nmol), and their combination on cumulative food intake in neonatal chickens. NTI: delta-opioid receptor antagonist. Data are expressed as mean ± SEM. Each of the control, NPW23, and NTI groups included 11 chicks, whereas the NPW23 + NTI group included 10 chicks (one chick was excluded due to mortality during injection; total N = 43). Different letters (a and b) indicate statistically significant differences among treatments at the same time point ($p < 0.05$).

3.4 *Effect of κ-opioid receptor blockade on NPW23-induced hyperphagia*

As shown in Fig. 4, blocking κ-opioid receptors failed to affect the orexigenic response to NPW23. Two-way repeated-measures ANOVA revealed significant effects of treatment [$F(3,39)=19.45$, $p<0.05$] , time [$F(2,78)=149.67$, $p<0.05$] , and treatment×time interaction [$F(6,78)=4.38$, $p=0.006$]. A significant rise in feed intake was observed following NPW23 (3 nmol) at every measured interval ($p<0.05$), while nor-BNI administered alone did not alter consumption ($p>0.05$). Co-infusion of nor-BNI with NPW23 did not significantly alter the orexigenic effect of NPW23 at any time point ($p>0.05$), indicating that κ-opioid receptors do not participate in the mediation of the orexigenic effect induced by NPW23 under the present acute co-injection conditions.

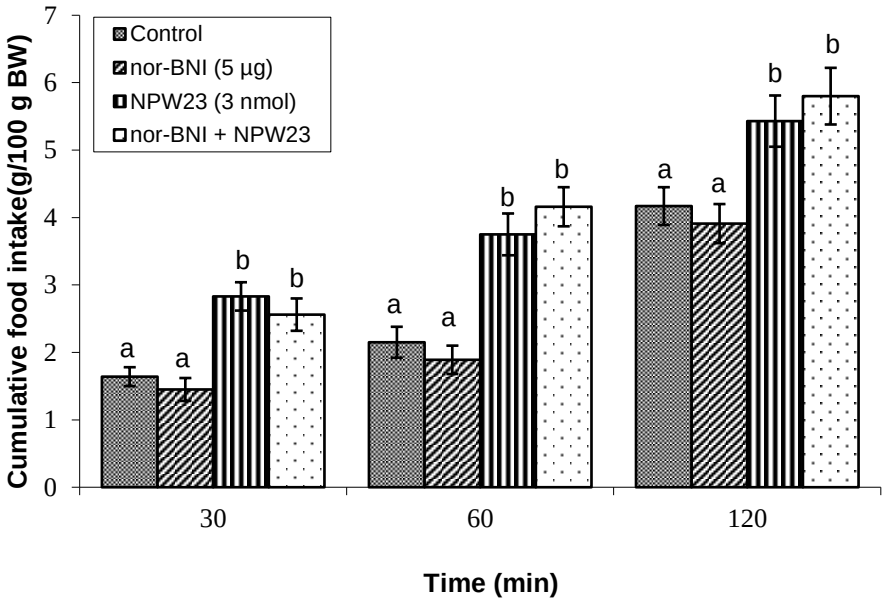


Figure 4. Effect of ICV injection of nor-BNI (5 µg), NPW23 (3 nmol), and their combination on cumulative food intake in neonatal chickens. nor-BNI: kappa-opioid receptor antagonist. Data are expressed as mean ± SEM. Each of the control, NPW23, and nor-BNI groups included 11 chicks, whereas the NPW23 + nor-BNI group included 10 chicks (one chick was excluded due to mortality during injection; total N = 43). Different letters (a and b) indicate statistically significant differences among treatments at the same time point ($p < 0.05$).

4 Discussion

The present study offers the initial demonstration that the hyperphagic effect of central NPW23 in neonatal broiler chickens is modulated through µ-opioid receptors, with blockade of these receptors potentiating the feeding response. In contrast, δ- and κ-receptors do not appear to participate in this effect under the present experimental conditions. These data implicate a convergent pathway wherein NPW signaling interfaces with opioidergic networks to influence appetite in birds, with µ-opioid receptor signaling playing an inhibitory modulatory role.

4.1 NPW23 dose-dependently stimulates food intake in broilers

Our dose-response data confirm and extend the recent findings of Mahdavi et al. (2023), who reported that central infusion of NPW23 at 2 and 4 nmol increased meal consumption in broiler chicks. In the present study, 3 nmol NPW23 produced a clear and sustained hyperphagic effect, while 0.75 nmol was ineffective and 1.5 nmol produced an intermediate response. This dose-dependent orexigenic action aligns with mammalian studies in which NPW injected into the paraventricular nucleus increased feeding (Levine et al., 2005; Mondal et al., 2003). The rapid onset of action (as early as 30 minutes post-injection) suggests a direct central mechanism, likely involving activation of hypothalamic feeding circuits. The similarity in amino acid sequence between chicken NPW and its mammalian counterparts (93% homology with human NPW)) supports the functional conservation of this peptide's orexigenic properties across vertebrates (Bu et al., 2016).

4.2 µ-Opioid receptors mediate NPW23-induced hyperphagia

Co-infusion of β-FNA significantly potentiated NPW23-induced hyperphagia, indicating that µ-opioid receptor signaling normally limits the orexigenic effect of NPW23. In other words, activation of µ-opioid receptors may exert an inhibitory brake on NPW23-induced feeding. This observation aligns with previous studies indicating that direct µ-opioid receptor activation reduces feeding in chickens (Alimohammadi et al., 2015; Bungo et al., 2004). Notably, β-endorphin (an endogenous µ-opioid agonist) has been demonstrated to increase feeding in broilers (McCormack & Denbow, 1988; Yanagita et al., 2008).

Taken together, these observations suggest the following hypothesis: NPW23 may act as a secretagogue for β-endorphin or other endogenous opioids, which then act through μ-opioid receptors to influence food intake. The potentiation of NPW23-induced hyperphagia by β-FNA could be explained by the removal of an inhibitory feedback mechanism that normally limits feeding. However, we acknowledge that this model is speculative and requires direct confirmation through measurement of opioid peptide release, receptor activation, or downstream signaling. An alternative explanation is that NPW23 and opioid signaling converge on common downstream pathways without direct peptide interaction. Anatomically, dense μ-receptor localization characterizes the arcuate nucleus, paraventricular nucleus, and nucleus accumbens (Zheng et al., 2005), regions that also contain NPW fibers and receptors (Chottova Dvorakova, 2018; Mondal et al., 2003). Thus, it is possible that NPW23 acts directly on μ-opioid receptor-expressing neurons or indirectly through promoting the secretion of endogenous substances that activate μ-opioid receptors. The partial nature of the potentiation observed with β-FNA may reflect that NPW23 acts through multiple parallel orexigenic pathways beyond the μ-opioid system.

4.3 *δ- and κ-Opioid receptors do not participate in this process*

The failure of NTI and nor-BNI to alter NPW23-induced hyperphagia suggests that δ- and κ-opioid receptors are not involved in mediating this feeding response under the present experimental conditions. This finding is noteworthy because κ-opioid receptor agonists (e.g., U-50488H, dynorphin) are well-documented to increase meal consumption in broilers (Bungo et al., 2004). The absence of κ-opioid involvement in NPW-induced hyperphagia suggests that NPW does not engage this orexigenic pathway, despite its robust feeding-stimulatory effect. Similarly, the lack of δ-opioid involvement, despite the known orexigenic effect of δ-agonists such as DPDPE in broilers (Bungo et al., 2004), indicates that NPW selectively recruits μ-opioid signaling without affecting δ- or κ-pathways. This selectivity is not without precedent; Baghaeikia et al. (2022) similarly reported that ghrelin-induced anorexia in layers was conveyed through μ-opioid receptors, whereas δ- and κ-opioid receptor subtypes did not contribute to this effect. Thus, the opioid receptor subtype dependence appears to be specific to the orexigenic or anorexigenic peptide involved. However, we note that nor-BNI has a distinctive delayed and long-lasting antagonist profile in many experimental systems. The acute co-administration used in the present study may not fully establish functional κ-receptor blockade at the time of NPW23 action. Therefore, the absence of effect with nor-BNI should be interpreted cautiously.

4.4 *Comparison with other appetite-regulating systems*

Recent work by Mahdavi et al. (2023) demonstrated that NPW23-induced hyperphagia in broilers is additionally facilitated via CRF1/CRF2 and NPY1 receptors. The present findings add μ-opioid receptors to this complex regulatory network. The interplay between opioidergic and NPY systems is well documented (De Pedro et al., 2000; Desai et al., 2013). Given that NPY1 receptor blockade suppressed NPW-induced hyperphagia (Mahdavi et al., 2023) and μ-opioid receptor blockade potentiated this effect (present study), it is plausible that NPY1 and μ-opioid receptors function in a common downstream pathway, with μ-opioid receptors exerting inhibitory modulation. Alternatively, NPW may act independently on both systems. It is worth noting that a study in rats reported that the non-selective opioid antagonist naltrexone did not alter NPW23-induced feeding but significantly reduced deprivation-induced hyperphagia (Levine et al., 2005). This suggests that the interaction between NPW and the opioid system may be species-specific or context-dependent, potentially reflecting differences in the relative contribution of opioid tone to feeding behavior between altricial (rats) and precocial (chicks) species. Furthermore, evidence from pain studies indicates that co-administration of NPW23 with morphine reduces the required analgesic dose of morphine while mitigating its side effects (likely via GPR7-mediated pathways), suggesting the existence of a more extensive functional crosstalk among these pathways that extends beyond the control of feeding behavior (Xing et al., 2021). Future studies should explore the hierarchical relationship between these mediators; for example, whether NPY1 receptor blockade affects μ-opioid receptor activation following NPW administration, or vice versa.

4.5 *Limitations*

A number of constraints inherent to the present investigation warrant recognition. First, we only used one dose of each antagonist based on previous literature; dose-response studies for antagonists could reveal more nuanced interactions. Second, while the ICV injection method is well-established, it does not allow site-specific localization within

the brain. Future studies using microinjection into specific hypothalamic nuclei would provide greater anatomical resolution. Third, we did not measure expression of opioid peptides or receptors following NPW administration; molecular studies (qPCR, in situ hybridization, or Western blot) would help determine whether NPW alters opioid gene expression or signaling. Fourth, the present research did not include a positive control group using a known opioid agonist to confirm the efficacy of the antagonists under our experimental conditions, although the doses were selected based on published effective and sub-effective doses. Fifth, the use of cumulative food intake measurements creates non-independent time points; although repeated-measures ANOVA accounts for within-subject correlation, future studies might consider reporting incremental intake or area under the curve. Sixth, the nor-BNI acute co-injection protocol may not be sufficient to fully exclude κ-opioid involvement due to the known delayed and long-lasting antagonist profile of this drug. Seventh, baseline control values varied slightly across experiments, which is expected given that each experiment was separately performed, but this could be addressed in future studies by including an internal control in each experimental block.

5 Conclusion

ICV administration of NPW23 dose-dependently increased cumulative feed intake in neonatal broiler chicks. Acute blockade of μ-opioid receptors with β-FNA potentiated the feeding response to NPW23, suggesting that central μ-opioid receptor signaling may exert an inhibitory modulatory tone on NPW23-induced hyperphagia. Under the present acute co-injection conditions, δ- and κ-opioid receptor antagonists did not significantly alter the NPW23 response. These findings improve basic understanding of central appetite regulation in neonatal broiler chicks. Further studies using antagonist dose-response designs, site-specific brain injections, positive-control opioid agonists, and molecular assessment of opioid signaling are needed to clarify the mechanism.

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Use of Artificial Intelligence

During the preparation of this work, the author(s) used Perplexity AI in order to identify and correct potential grammatical errors and improve the overall flow and readability of the manuscript. After using this tool, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

H.Z. and M.Z. conceived and designed the research and methodology. R.F. conducted the experiment, performed the literature search, and drafted the original manuscript. H.Z. and K.H. supervised the research. M.Z. performed the statistical analyses and data interpretation. H.Z., M.Z., and K.H. edited and finalized the manuscript. All authors read and approved the final version of the manuscript before submission for publication.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethical Considerations

All experimental procedures were conducted in strict accordance with the guidelines for the care and use of laboratory animals established by the Research Ethics Committee of the Islamic Azad University. The animal study protocol was reviewed and formally approved by the Institutional Animal Care and Use Committee (IACUC) of the Islamic Azad University, Garmsar Branch (official name: Research Ethics Committee of the Islamic Azad University, Garmsar Branch), under approval code IR.IAUGB.REC.1403.737 on 1 May 2024. All efforts were made to minimize animal suffering and to reduce the number of animals used, in compliance with the ARRIVE guidelines and national regulations for animal research.

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