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***Original Article***

# Role of Dopaminergic and Cannabinoidergic Receptors on Ghrelin-Induced Hypophagia in 5-Day-Old Broiler Chicken

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**Abstract**

The present study aimed to identify the role of dopaminergic and cannabinoidergic systems in the ghrelin-induced hypophagia among meat-type chickens. In the first experiment, intracerebroventricular (ICV) injection was applied to birds with control solution, $D_1$ receptor antagonist (5 nmol), ghrelin (6 nmol), and $D_1$ receptor antagonist plus ghrelin. The second to sixth experiments were similar to the first one, with the difference that $D_2$ receptor antagonist (5 nmol), $D_3$ receptor antagonist (6.4 nmol), $D_4$ receptor antagonist (6 nmol), the precursor of dopamine (125 nmol), and 6-hydroxy dopamine (150 nmol) instead of $D_1$ antagonist were injected into the broiler chickens. In experiment 7, control solution and different levels of ghrelin antagonists (5, 10, and 20 nmol) were injected. In experiment 8, the chickens were ICV injected with control solution, ghrelin antagonist (10 nmol), dopamine (40 nmol), and ghrelin antagonist plus dopamine. In experiments 9 and 10, $CB_1$ and $CB_2$ receptors antagonist (6.25µg and 5µg) were co-injected with ghrelin (6 nmol), respectively, measuring the food intake for 120 min after the injection. It was observed that ghrelin ICV injection considerably reduced food intake, whereas ghrelin antagonist increased food intake, depending on the dose ($P<0.05$). In addition, ghrelin-induced hypophagia was significantly attenuated by $D_1$ receptor antagonist and 6-hydroxy dopamine ($P<0.05$), while the dopamine precursor considerably elevated the ghrelin-induced food intake ($P<0.05$). The dopamine-induced feeding behavior was diminished by the co-administration of [D-Lys-3]-GHRP-6 (10 nmol)+dopamine (40 nmol) ($P<0.05$). In addition, $CB_1$ receptor antagonists enhanced the ghrelin influence on food intake ($P<0.05$). The results implied that the hypophagic impact of ghrelin was probably mediated by $D_1$ and $CB_1$ receptors within neonatal broilers.

**Keywords:** Ghrelin, Cannabinoid, Dopamine, Broiler chicken, Food intake

## 1. Introduction

Appetite and energy expenditure are controlled using central and peripheral neurons to modulate the ingestion response. The feeding behavior of avians is regulated by several peptides (1). However, a number of these regulatory mechanisms are different among animals. As a basic catecholamine neurotransmitter within the brain, Dopamine (DA) plays an essential role in appetite regulation. A total of five distinct Dopaminergic (DAergic) receptor subtypes have at least been recognized ($D_1$-$D_5$) in the ventral tegmental area, hypothalamus, and substantia nigra (2). The most abundant DA receptors within the brain are the $D_1$ and $D_2$ receptors. The DAergic system engages in numerous physiological functions (e.g. cognition, locomotor activity, food intake, and emotion) (3). Food intake is reduced in rats by $D_1$ and $D_2$ receptors (4). Moreover, $D_1$ receptors in chickens mediate DA-

induced hypophagia, while other receptors ($D_2$-$D_4$) may influence appetite regulation (5). The central feeding behavior is documented to be not regulated by a single neuropeptide. Furthermore, other neurotransmitters on feeding status are in interaction with a wide distributed neural network (6).

Ghrelin was detected in the brain, lung, stomach, proventriculus, spleen, (7), breast muscle, small intestine, and abdominal fat (8), provoking the release of the growth hormone (GH) via GH secretagogue receptors (GHS-R) (9). The subtypes of the ghrelin receptor detected in broilers include GHS-R1aV (homologous to mammalian GHS-R1b), GHS-R1tv, and GHS-R1a ad (10). Ghrelin ICV or systemic injection enhances the feeding behavior of laboratory animals (11), while it inhibits that of meat-type chicken (12-14) and Japanese quail (15). It has been 19 years since the discovery of Ghrelin; however, its role has not yet been fully investigated because of the numerous physiological functions in animals. Moreover, the interaction between ghrelin and other neurotransmitters remains yet to be revealed (15).

The endocannabinoids (ECB) are expressed in the peripheral nervous system, immune cells and tissues, and central nervous system (CNS) (16). The ICV administration of CB agonists raises the food intake of rodents, whereas selective $CB_1$ receptor antagonist (SR141716A) diminished that I of rats (17). The ICV injection of $CB_1$ or $CB_2$ cannabinoid receptors agonists (2-AG or JWH015) enhances the feeding behavior in layers (18), whereas only $CB_2$ receptors can be effective for broilers (19). In addition, the injection of the AM251 (inverse $CB_1$ receptors agonist) attenuates broiler food intake (20).

The ghrelin and DAergic receptors are co-expressed in the CNS neurons (21). GHS-R1a forms heteromers with $D_1$ receptors in hypothalamic neurons and GHS-$R_1$a. $D_2$ receptor signaling is essential in $D_1$ receptors agonist-induced hypophagia (22). GHSR KO mice treated with a selective GHS-R1a antagonist are resistant to the anorexigenic effects of the $D_2$ receptors agonist (22). By modulating the DAergic reward pathway from VTA to nucleus accumbens (NAcc), the central ghrelin system engages in food intake (23). In the mice, the hippocampal $D_1$ receptor signaling is dependent upon the ghrelin receptor (21). Ghrelin injection to the VTA and NAcc increases the food intake and extracellular dopamine in mice (24). The mesolimbic dopamine system's ghrelin-induced activation in mice is decreased by CB receptor antagonist ICV injection (25). The hypothalamic ECB content of $CB_1$ KO or in rimonabant-treated mice is elevated by ghrelin (26).

To date, several reports have been provided on the neurotransmitter impacts on food intake among mammalian; however, the food intake aspects of avians remain yet to be revealed (14, 20). For instance, ghrelin plays an anorexigenic role in rats (8). For birds, however, ghrelin serves as an anorexigenic neurotransmitter (13, 14).

It is essential to understand the interaction between ghrelin in avian and other neurotransmitters. According to the literature, the engagement of DAergic and cannabinoidergic (CBergic) systems in ghrelin-induced hypophagia in broiler avian has not been reported. To shed light on the mechanism that underlies the unique function of ghrelin in chicks, the relationship of ghrelin with the DAergic and CBergic systems is demonstrated for the first time in the present study by investigating the roles of $D_1$-$D_4$ and $CB_1$/$CB_2$ receptors in the ghrelin-induced food intake suppression of some 5-day-old broiler chickens.

## 2. Material and Methods

### 2.1. Animals

In total, 440 one-day-old broiler chickens of a local hatchery (ROSS 308) were obtained (Mahan Co., Iran). The chickens were kept within stabilizing electrically-heated batteries at 32±1ºC and relative humidity of 40%-50% for a 23:1 light/dark schedule (27) as flocks for two days. Following that, they were randomly grouped, moved to individual cages, and provided with a commercial diet provided. During the study, the entire broilers were provided with diets and freshwater

*ad libitum* access. The birds were deprived of food ($FD_3$) three hours before injections; however, water was available. At the age of five days, ICV injections were applied.

**2.2. Experimental Drugs**

The drugs included ghrelin, AMI-193 ($D_2$ receptor antagonist), SCH 23390 ($D_1$ receptor antagonist), L-741,742 ($D_4$ receptor antagonist), NGB2904 ($D_3$ receptor antagonist), 6-OHDA (6-hydroxy dopamine), L-DOPA (precursor of dopamine), 6-OHDA (6-hydroxy dopamine), AM630 ($CB_2$ receptor antagonist), [D-Lys-3]-GHRP-6 (ghrelin antagonist), and Evans blue which was obtained from U.S. Sigma-Aldrich Company and U.K. Tocris Company. The drugs were all dissolved in absolute dimethyl sulfoxide (DMSO) before they were diluted with 0.85% saline with a 1/250 ratio (0.4% DMSO) of the Evans blue (0.1%) content. This DMSO ratio has no cytotoxic impacts (28, 29). Evans blue-containing DMSO/Saline was utilized as the control group.

**2.3. ICV Injection Protocol**

The birds were divided randomly into 10 groups, each of which was composed of four sub-groups (i.e., n=44). In each experiment, an ICV injection was performed on each chicken through a microsyringe (Hamilton, Switzerland) with no anesthesia according to the methods proposed by Davis, Masuoka (30), (31). In summary, the chicken heads were held using an acrylic device with a 45-degree bill holder and a parallel calvarium to the table 1 surface according to Van Tienhoven and Juhasz (32). No physiological stress was induced in neonatal chickens in this technique (33). Eventually, the chickens were sacrificed in order to measure injection accuracy.

**Table 1.** Treatment procedure in experiments 1-10

| **Exp. 1** | **ICV Injection** |
|---|---|
| Treatment groups | |
| I | CS* |
| II | SCH23390 (5 nmol) |
| III | ghrelin (6 nmol) |
| IV | SCH23390+ghrelin |
| **Exp. 2** | **ICV Injection** |
| Treatment groups | |
| I | CS * |
| II | AMI-193 (5 nmol) |
| III | ghrelin (6 nmol) |
| IV | AMI-193+ghrelin |
| **Exp. 3** | **ICV Injection** |
| Treatment groups | |
| I | CS * |
| II | NGB2904 (6.4 nmol) |
| III | ghrelin (6 nmol) |
| IV | NGB2904+ghrelin |
| **Exp. 4** | **ICV Injection** |
| Treatment groups | |
| I | CS * |
| II | L-741,742 (6 nmol) |
| III | ghrelin (6 nmol) |
| IV | L-741,742+ghrelin |
| **Exp. 5** | **ICV Injection** |
| Treatment groups | |
| I | CS * |
| II | L-DOPA (125 nmol) |
| III | ghrelin (6 nmol) |
| IV | L-DOPA+ ghrelin |

| **Exp. 6** | **ICV Injection** |
|---|---|
| Treatment groups | |
| I | CS * |
| II | 6-OHDA (150 nmol) |
| III | ghrelin (6 nmol) |
| IV | 6-OHDA+ghrelin |
| **Exp. 7** | **ICV Injection** |
| Treatment groups | |
| I | CS * |
| II | [D-Lys-3]-GHRP-6 (5 nmol) |
| III | [D-Lys-3]-GHRP-6 (10 nmol) |
| IV | [D-Lys-3]-GHRP-6 (20 nmol) |
| **Exp. 8** | **ICV Injection** |
| Treatment groups | |
| I | CS * |
| II | [D-Lys-3]-GHRP-6 (10 nmol) |
| III | dopamine (40 nmol) |
| IV | [D-Lys-3]-GHRP-6 (10 nmol)+ dopamine (40 nmol) |
| **Exp. 9** | **ICV Injection** |
| Treatment groups | |
| I | CS * |
| II | ghrelin (6 nmol) |
| III | SR141716A (6.25 µg) |
| IV | ghrelin (6 nmol)+SR141716A (6.25 µg) |
| **Exp. 10** | **ICV Injection** |
| Treatment groups | |
| I | CS * |
| II | ghrelin (6 nmol) |
| III | AM630 (5 µg) |
| IV | ghrelin (6 nmol)+AM630 (5 µg) |

CS: control solution, SCH 23390: $D_1$ receptor antagonist, AMI-193: $D_2$ receptor antagonist, NGB2904: $D_3$ receptor antagonist, L-741,742: $D_4$ receptor antagonist, L-DOPA: precursor of dopamine, 6-OHDA: 6-hydroxy dopamine, [D-Lys-3]-GHRP-6: ghrelin antagonist, SR141716A: $CB_1$ receptor antagonist, AM630: $CB_2$ receptor antagonist.

## 2.4. Feeding Experiments

The present study designed 10 experiments in order to identify the possible impacts of particular dopaminergic receptors (i.e., $D_1$, $D_2$, $D_3$, and $D_4$) on the hypophagic impact of ghrelin for $FD_3$ neonatal broiler chickens. In the first experiment, ICV injections were applied to the chickens with a control solution, ghrelin (6 nmol), SCH 23390+ghrelin, and SCH 23390 (5 nmol). In the second experiment, the control solution, ghrelin (6 nmol), AMI-193 (5 nmol), and their combination were applied by ICV injections. In the third experiment, ICV injections were carried out on the $FD_3$ birds by using the control solution, ghrelin (6 nmol), NGB2904 (6.4 nmol), and the co-injection of NGB2904+ghrelin. For experiment four, ICV injections were performed on the $FD_3$ chickens using the control solution, ghrelin (6 nmol), L-741,742 (6 nmol), and the co-injection of L-741,742+ghrelin. Experiment five involved the ICV injection of the control solution, ghrelin (6 nmol), L-DOPA (125 nmol), and their combination into the

birds. Experiment six was the ICV injection of the control solution, ghrelin (6 nmol), 6-OHDA+ghrelin, and 6-OHDA (150 nmol). Experiment seven included the ICV injection of the control solution, [D-Lys-3]-GHRP-6 (10 nmol), [D-Lys-3]-GHRP-6 (5 nmol), and [D-Lys-3]-GHRP-6 (20 nmol). In experiment eight, the ICV injection of the control solution, dopamine (40 nmol), [D-Lys-3]-GHRP-6 (10 nmol), and their combination was performed on the $FD_3$ birds. The control solution, ghrelin+SR141716A, SR141716A (6.25 µg), and ghrelin (6 nmol) were injected in experiment nine. Experiment ten involved the ICV injection of the control solution, AM630 (5 µg), ghrelin (6 nmol), and ghrelin+AM630. The chickens were fed immediately after they were injected, measuring the cumulative food intake (g) when 30, 60, and 120 min elapsed from the injection. The drug doses were determined based on previous studies (14, 34, 35).

### 2.5. Statistical Analysis

The repeated measure two-way analysis of variance (ANOVA) was used to analyze the cumulative food intake. The results were provided as mean value±SEM. The mean values of the treatments that were found to have posed an effect based on the ANOVA were compared to the Bonferroni test. It was considered that p-values smaller than 0.05 represented significant differences between the treatments. Moreover, the variance analysis was carried out using the following model (36):

$$Y_{ijk}= \mu + \alpha_j + \beta_k + (\alpha\beta)_{jk} + \varepsilon_{ijk}$$

In which $Y_{ijk}$ denotes the individual observation variable value, $\mu$ is the grand mean, $\alpha_j$ signifies the treatment impact based on time, $\beta_k$ represents the treatment impact for the drug, $(\alpha\beta)_{jk}$ indicates the interaction impact for time and drugs, and $\varepsilon_{ijk}$ denotes the error.

## 3. Results

Figures 1-8 illustrate the central DAergic system's impacts on the ghrelin-induced hypophagia of the $FD_3$ neonatal chickens. For the first experiment, the ICV injection of SCH 23390 (5 nmol) had no effects on the cumulative food intake in comparison to the control group at 30, 60, and 120 min post-injections ($P>0.05$). The ICV injection of ghrelin (6 nmol) considerably diminished food intake in comparison to the control group ($P<0.05$). Furthermore, the ghrelin-induced hypophagia of the neonatal chickens was considerably attenuated by the co-injection of ghrelin and SCH 23390 ($P<0.05$), as shown in figure 1.

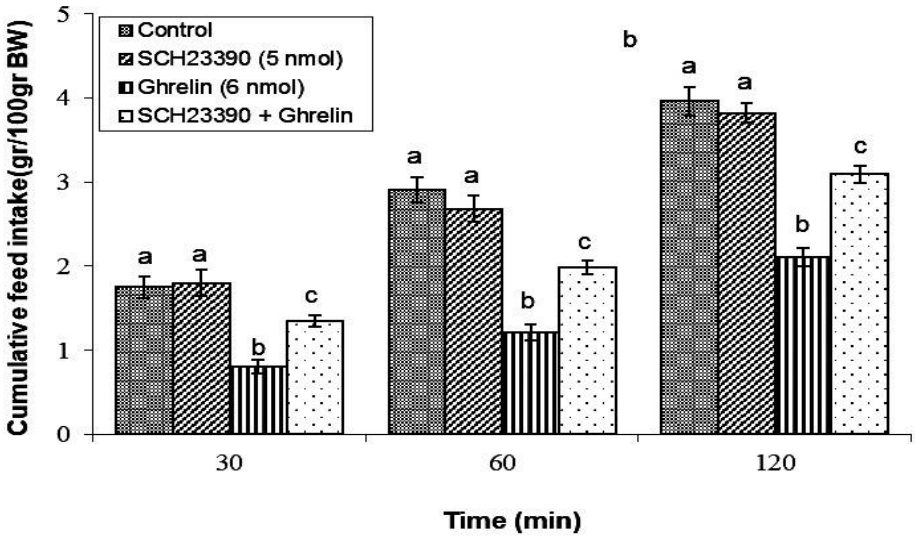


**Figure 1.** Effect of ICV injection of SCH 23390 (5 nmol), ghrelin (6 nmol), and their combination on cumulative food intake in neonatal chicken (n=44). SCH 23390: $D_1$ receptor antagonist; Data are expressed as mean±SEM. Different letters (a, b, and c) indicate significant differences among treatments ($P<0.05$).

In the second experiment, the AMI-193 (5 nmol) ICV injection had no significant impacts on the cumulative food intake (P>0.05), whereas ghrelin (6 nmol) substantially reduced the cumulative food intake in comparison to the control group (P<0.05). Furthermore, the co-injection of AMI-193 (5 nmol)+ghrelin (6 nmol) had no effects on ghrelin-induced hypophagia in comparison to the control group (P>0.05), as shown in figure 2.

In the third experiment, the NGB2904 (6.4 nmol) ICV injected birds exhibited no significant influence on the cumulative food intake (P>0.05). The ICV injection of ghrelin (6 nmol) considerably induced hypophagia within broilers (P<0.05). In addition, the co-administration of NGB2904+ghrelin showed no significant compact, compared to the control group (P>0.05), as shown in figure 3.

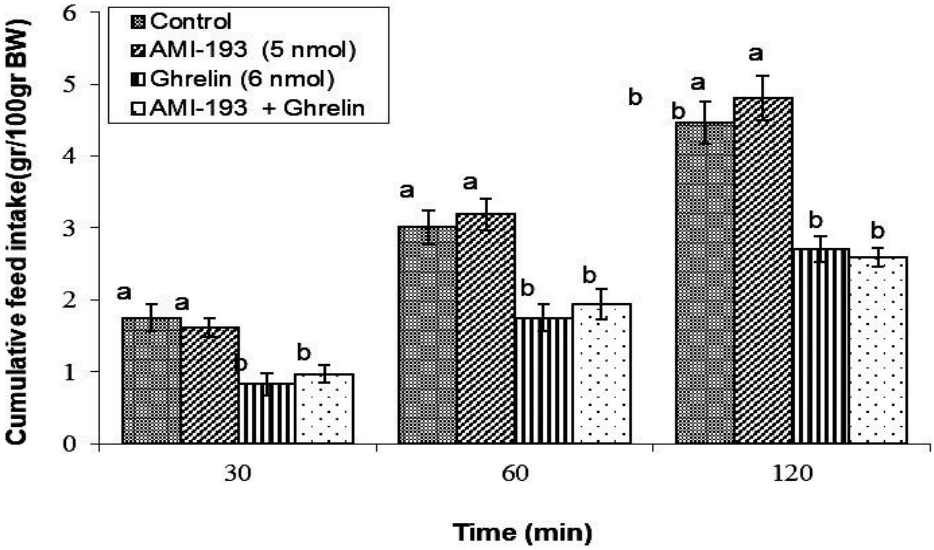


**Figure 2.** Effect of ICV injection of AMI-193 (5 nmol), ghrelin (6 nmol), and their combination on cumulative food intake in neonatal chicken (n=44). AMI-193: $D_2$ receptor antagonist; Data are expressed as mean±SEM. Different letters (a and b) indicate significant differences among treatments (P<0.05).

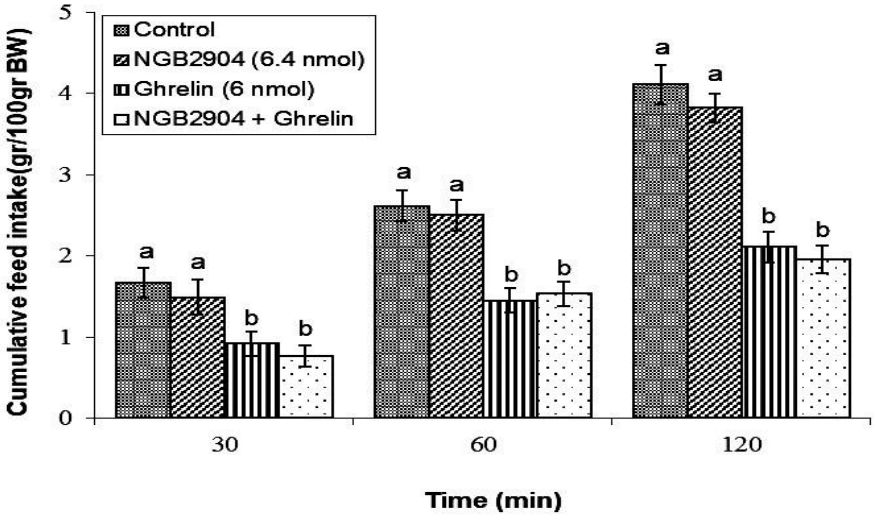


**Figure 3.** Effect of ICV injection of NGB2904 (6.4 nmol), ghrelin (6 nmol), and their combination on cumulative food intake in neonatal chicken (n=44). NGB2904: $D_3$ receptor antagonist; Data are expressed as mean±SEM. Different letters (a and b) indicate significant differences among treatments (P<0.05).

In experiment four, the L-741,742 (6 nmol) ICV injection exhibited no impact on the food intake of the neonatal broilers (P>0.05). The ICV injection of ghrelin (6 nmol) substantially reduced the cumulative food intake (P<0.05). Additionally, no significant impacts were induced by the co-injection of L-741,742 (6 nmol) and ghrelin (6 nmol) on ghrelin-induced hypophagia (P>0.05), as shown in figure 4.

For experiment five, the ICV injection of L-DOPA (125 nmol) was observed to have no significant impacts on the cumulative food intake in comparison to the control group (P>0.05). The ICV injection of ghrelin (6 nmol) considerably diminished the cumulative food intake in comparison to the control group (P<0.05). Furthermore, the co-injection of L-DOPA+ghrelin substantially enhanced the ghrelin effect on food consumption (P<0.05), as shown in figure 5.

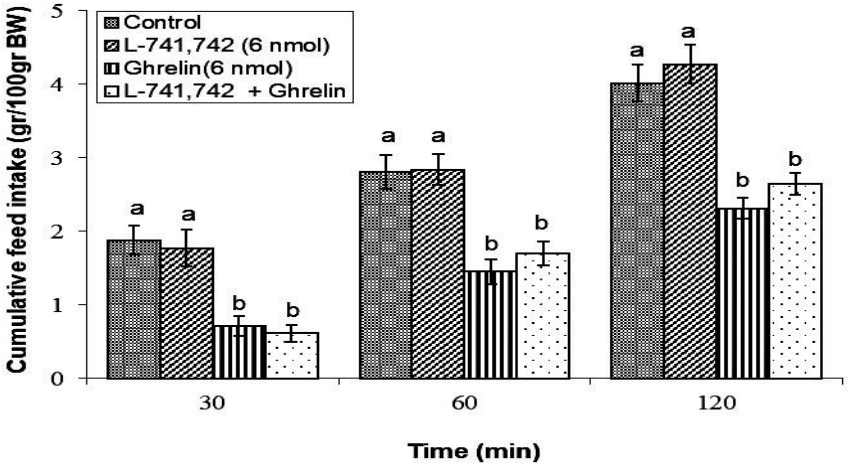


**Figure 4.** Effect of ICV injection of L-741,742 (6 nmol), ghrelin (6 nmol), and their combination on cumulative food intake in neonatal chicken (n=44). L-741,742: $D_4$ receptor antagonist; Data are expressed as mean±SEM. Different letters (a and b) indicate significant differences among treatments (P<0.05).

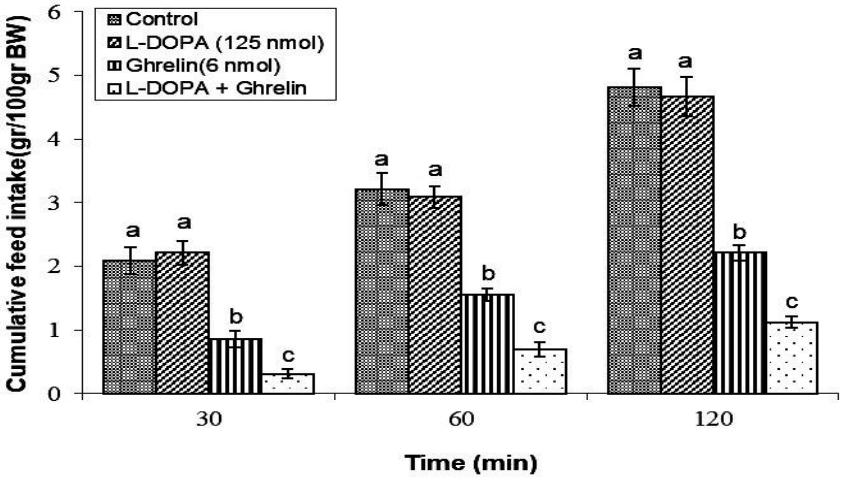


**Figure 5.** Effect of ICV injection of L-DOPA (125 nmol), ghrelin (6 nmol), and their combination on cumulative food intake in neonatal chicken (n=44). L-DOPA: precursor of dopamine; Data are expressed as mean±SEM. Different letters (a, b, and c) indicate significant differences among treatments (P<0.05).

For experiment six, the ICV injection of 6-OHDA (150 nmol) (P>0.05) into the birds showed no considerable effects on the cumulative food intake. The ICV injection of ghrelin (6 nmol) substantially diminished the cumulative food intake in comparison to the control group (P<0.05). Furthermore, the co-injection of 6-OHDA (150 nmol) and ghrelin (6 nmol) considerably enhanced the ghrelin hypophagic impact (P<0.05), as shown in figure 6.

For experiment seven, in comparison to the control group at 30, 60, and 120 min post-injections, the ICV injection of [D-Lys-3]-GHRP-6 (5, 10, and 20 nmol) exhibited a dose-dependent rise in the cumulative food intake (P<0.05), as shown in figure 7.

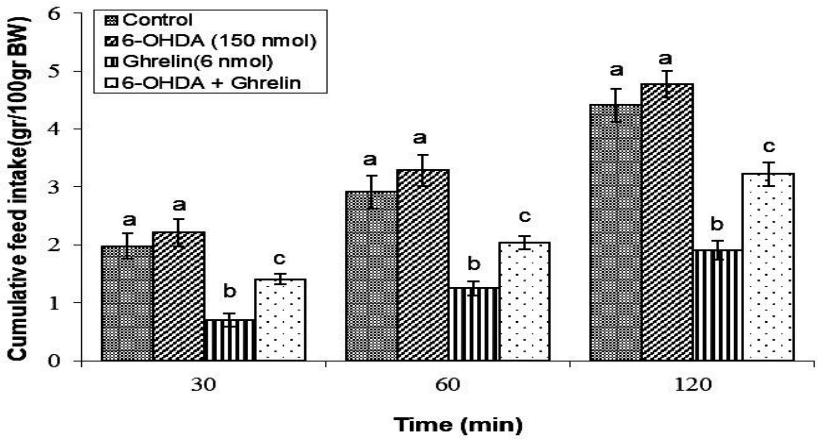


**Figure 6.** Effect of ICV injection of 6-OHDA (150 nmol), ghrelin (6 nmol), and their combination on cumulative food intake in neonatal chicken (n=44). 6-OHDA: 6-hydroxy dopamine; Data are expressed as mean±SEM. Different letters (a, b, and c) indicate significant differences among treatments (P<0.05).

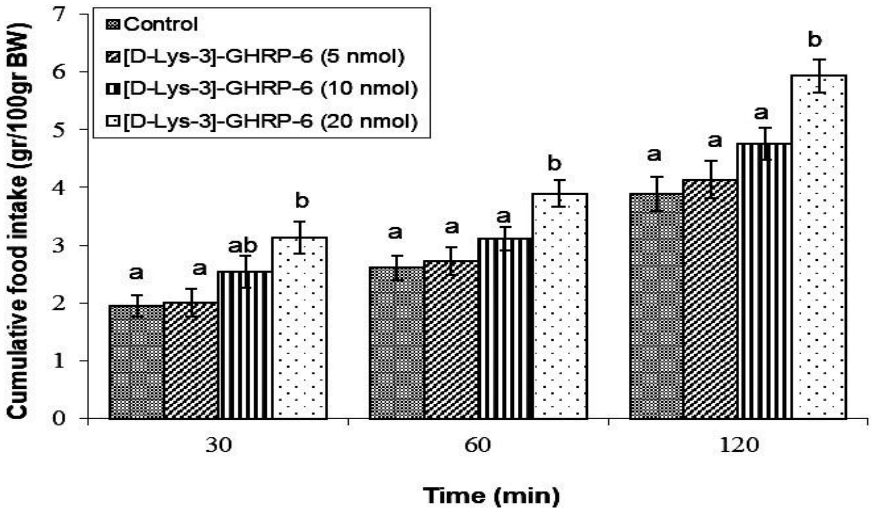


**Figure 7.** Effect of ICV injection of [D-Lys-3]-GHRP-6 (5 nmol), [D-Lys-3]-GHRP-6 (10 nmol), and [D-Lys-3]-GHRP-6 (20 nmol) on cumulative food intake in neonatal chicken (n=44). [D-Lys-3]-GHRP-6: ghrelin antagonist; Data are expressed as mean±SEM. Different letters (a, b, and c) indicate significant differences among treatments (P<0.05).

For experiment eight, the ICV injection of dopamine (40 nmol) substantially diminished the food intake (P<0.05), whereas [D-Lys-3]-GHRP-6 (as ghrelin antagonist; 10 nmol) had no effects on the cumulative food intake (P>0.05). The co-administration of [D-Lys-3]-GHRP-6 (10 nmol)+dopamine (40 nmol), however, decreased the dopamine-induced feeding behavior in comparison to the control group (P<0.05), as shown in figure 8.

Concerning experiment nine, the ICV injection of ghrelin (6 nmol) considerably reduced the cumulative food intake (P<0.05). Moreover, the ICV injection of SR141716A (6.25 µg) was observed to have no significant impacts on the cumulative food intake (P>0.05), whereas the co-injection of AMI-SR141716A (6.25 µg)+ghrelin (6 nmol) enhanced the ghrelin-induced feeding behavior in comparison to the control group (P<0.05), as shown in figure 9.

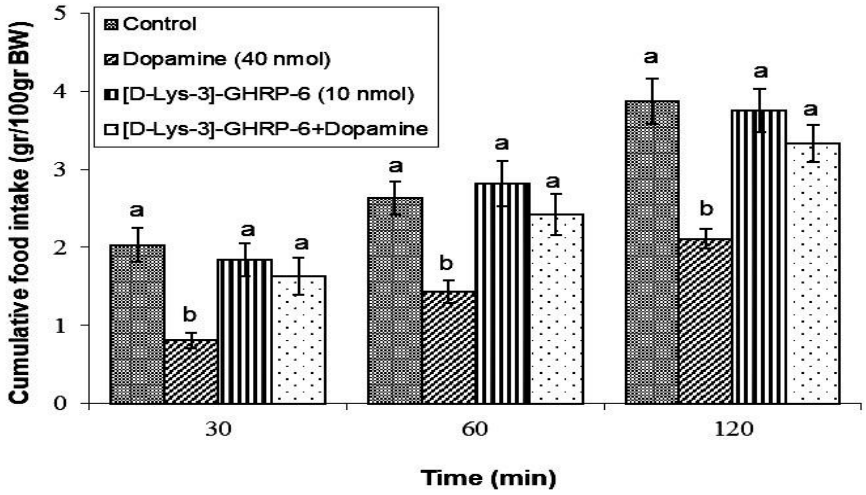


**Figure 8.** Effect of ICV injection of [D-Lys-3]-GHRP-6 (10 nmol), dopamine (40 nmol), and their combination on cumulative food intake in neonatal chicken (n=44). [D-Lys-3]-GHRP-6: ghrelin antagonist; Data are expressed as mean±SEM. Different letters (a and b) indicate significant differences among treatments (P < 0.05).

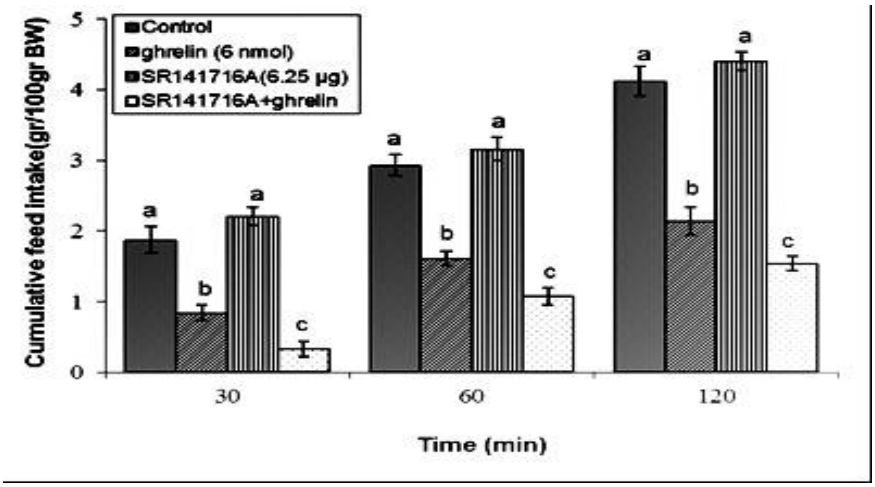


**Figure 9.** Effect of ICV injection of ghrelin (6 nmol), SR141716A (6.25 µg), and their combination on cumulative food intake in neonatal chicken (n=44). SR141716A: $CB_1$ receptor antagonist; Data are expressed as mean±SEM. Different letters (a, b, and c) indicate significant differences among treatments (P<0.05).

Concerning experiment ten, the ICV injection of ghrelin (6 nmol) considerably induced hypophagia within the birds (P<0.05). Furthermore, the ICV injection of AM630 (5 µg) (P>0.05) into the birds exhibited no significant impact on the cumulative food intake. Eventually, the co-administration of AM630+ghrelin was found to have no considerable effect on ghrelin feeding behavior, compared to the control group (P>0.05), as shown in figure 10.

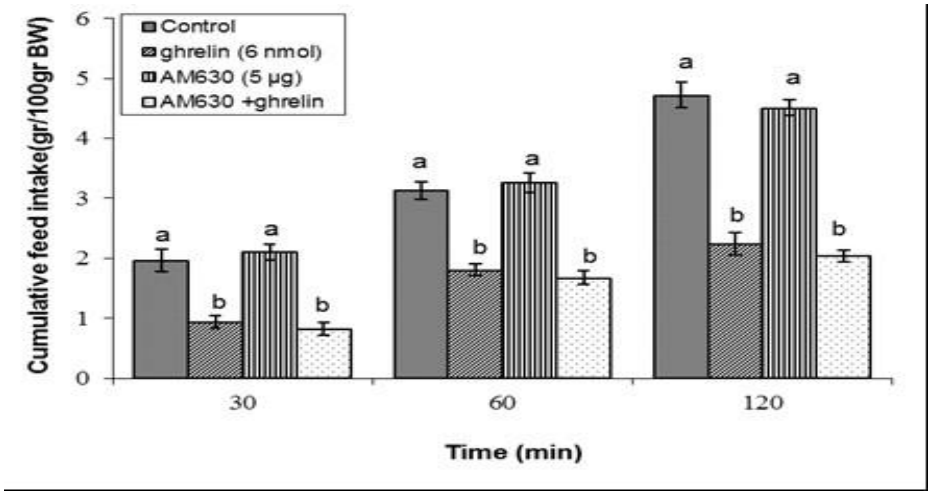


**Figure 10.** Effect of ICV injection of ghrelin (6 nmol), AM630 (5 µg), and their combination on cumulative food intake in neonatal chicken (n=44). AM630: $CB_2$ receptor antagonist; Data are expressed as mean±SEM. Different letters (a and b) indicate significant differences among treatments (P<0.05).

## 4. Discussion

To the best of the authors' knowledge, the present study is the first to report the impacts of the DAergic system on ghrelin-induced hypophagia for the broiler chickens. It was observed that the ICV injection of ghrelin (6 nmol) considerably reduced the food intake of the $FD_3$ chickens. The ICV injection of the ghrelin antagonist into the $FD_3$ chickens induced a dose-dependent rise in the cumulative food intake ([D-Lys-3]-GHRP-6), which was different from previous studies on mammalian (9, 12, 15). The ghrelin ICV injection reduced the avian food intake (13). The ghrelin ICV injections at the concentrations of 0.3, 1.1, 4.3, and 6.2 nmol diminished the food intake for 8-week-old broilers (37). Additionally, the ghrelin ICV injection (1 nmol) reduced the Japanese quail food intake (15). The ghrelin-induced hyperphagia mediates by activating agouti-related protein (AgRP) and neuropeptide Y (NPY) neurons in mammals, whereas it is completely different in avians (14). Ghrelin receptors are more expressed in NPY than the pro-opiomelanocortin neurons that are expressed in rats. Ghrelin injection enhanced the dopamine β-hydroxylase expression of the nucleus tractus solitarious (38).

Our results showed that the co-injection of the SCH 23390 and ghrelin significantly diminished the ghrelin-induced hypophagia, while the ICV injection of L-DOPA considerably enhanced the food intake by ghrelin within the neonatal chickens. In addition, the administration of the 6-OHDA as a dopamine synthesis inhibitor significantly decreased ghrelin-induced food intake. The ICV injection of the ghrelin antagonist diminished dopamine-induced hypophagia in chickens. Based on the previous studies, ghrelin receptors are expressed on VTA dopamine neurons. The ghrelin injection into the VTA increases the dopamine in the NAcc shell (39). Both $D_1$ and ghrelin (GHS-R1a) receptors are coexpressed in hippocampal neurons (21). Ghrelin receptor is quite important in $D_1$ receptor-induced hippocampal synaptic plasticity and memory formation (21). G-protein-coupled receptors heteromerization between ghrelin and $D_1$ receptors

initiates a cAMP-independent signaling pathway that regulates DAergic regulation of hippocampal memory (21).

The administration of the ghrelin into the NAcc increases the dopamine release and induces locomotor activity (40). The reward system, particularly the mesolimbic dopamine system, enhances food-seeking behaviors (40). The mesolimbic DAergic pathway is an essential component of the ghrelin-responsive network (40). Peripherally, the injected ghrelin can access the mesolimbic reward and stimulate the mesolimbic DAergic system (41). The ICV injection of the GHS-R1A antagonist blocks the hyperphagic effect of the ghrelin, and the dopamine release suggest that the ghrelin activates GHS-R1A expressed on DAergic cell bodies (42). In mice hippocampal, $D_1$ receptor signaling is dependent on the ghrelin receptor (21). Ghrelin injection to the VTA and NAcc increases the food intake and extracellular dopamine in mice (24). Therefore, the role of ghrelin is undeniable in the midbrain DAergic system in food intake and energy balance to include reward-seeking behavior (40). Dopamine-containing projection to the dorsal vagal complex has a potent role in ghrelin release in the CNS (43). Furthermore, it is known that there is a correlation between DA and corticotrophin-releasing hormone (CRH) neurons in the CNS. The possible heteromerization among $CRH_2$ and $D_1$ receptors is responsible for the synergism between DA and CRH transmission (44).

In addition, $CRH_1$ and $D_1$ receptors are expressed in low levels in the VTA (45). It is known that ghrelin-induced hypophagia mediates via CRH system (46); moreover, the interaction of DA and CRH plays a mediatory role in ghrelin via CRH system. The ICV injection of the $D_2$ receptor agonist induced a vagal cholinergic-dependent increase in plasma ghrelin levels (43). Despite direct cellular and molecular mechanism, the interaction between DAergic and ghrelin is not fully elicited; however, the activation of GHS-R via ghrelin is reported to enhance $D_1$ receptor-induced cAMP accumulation and change the G protein coupling of the GHS-R from G-11/q into G-i/o through a mechanism that is consistent with the agonist-dependent formation of GHS-R/$D_1$ receptor heterodimers (47). The VTA dopamine cells are not only sensitive to direct ghrelin but also innervated by ghrelin-sensitive lateral hypothalamic hypocretin/orexin neurons (48).

As it was observed, the co-injection of the $CB_1$ receptor antagonist along with the ghrelin enhanced ghrelin-induced hypophagia in comparison to the control group. The $CB_1$ receptor peripheral blockade diminished food intake. This effect declines gastric ghrelin secretion in food-deprived rats (49). The observed results are apparently mediated by the mTOR pathway (49). $CB_1$ and $CB_2$ receptor agonists are reported to raise the food intake of neonatal broiler chickens (16). Furthermore, ghrelin has a hypophagic effect in broilers (14). However, it seems that receptors have almost no effects on the interactions with the other neurotransmitters in the food intake regulation of broilers (16). As observed in our findings, $CB_1$ had a regulatory impact on ghrelin-induced hypophagia, while $CB_2$ had no interaction with ghrelin on feeding behavior in broiler chicken. Ghrelin had no orexigenic role in $CB_1$-knockout mice (50). In our explanations, the $CB_1$ receptor increased the food intake, while the $CB_1$ receptor blockade diminished food intake. On the other hand, ghrelin reduced food intake, and hypophagic effect of the ghrelin was enhanced when the $CB_1$ receptor was blocked. It seems that $CB_1$ receptor inhibition has yielded to amplify the effect of ghrelin. However, more investigations are required to determine the interactions of these neurons in central food regulation centers in avians.

Hypothalamic AMP-activated protein kinase activity plays a considerable role in the mediating impact of $CB_1$ receptors on the hypophagic role of ghrelin (50). Ghrelin hinders the excitatory inputs of $CB_1$ receptors on the paraventricular nucleus. It is eliminated by the ICV injection of $CB_1$ antagonists in mice (50). Ghrelin impresses its impacts via growth hormone secretagogue

receptor type-1 and probably elevation on the ECBS synthesis (50). Rimonabant administration blocks the ghrelin impact stimulatory on 2-AG in mice. Therefore, the interconnection between CBergic and ghrelin mediates through $CB_1$ receptors on the food intake (51). Furthermore, major DAergic cell activation within the VTA of the midbrain is reward processing. Such DAergic neurons project the NAc, prefrontal cortex, and other brain areas, such as the hippocampus and hypothalamus (52). It is worth noting that ghrelin interacting with ECB and GABA also has an interaction with ECBs and dopamine (21).

In conclusion, this study demonstrated that the ICV injection of ghrelin suppressed food intake in chicks and was probably mediated by the DAergic and CBergic systems within the brain. The co-administration of the ghrelin antagonist+dopamine diminished the dopamine-induced feeding behavior. Therefore, $D_1$ and $CB_1$ receptors may be involved in the inhibitory impact of ghrelin on food intake in the brain feeding centers of neonatal broiler chickens. Accordingly, ghrelin-induced anorexia is mediated by the $D_1$ and $CB_1$ receptors of the broiler chickens. Furthermore, ghrelin, dopamine, cannabinoids, and their receptors have been demonstrated to exist in several areas of the chicken brain (5, 6, 18, 20). However, the precise sites in the chicken brain are engaged in the action of ghrelin and dopamine or in that of ghrelin and cannabinoids. As a result, their interaction is yet to be revealed.

## Authors' Contribution

Study concept and design: R. F. and V. B.
Acquisition of data: R. F. and V. B.
Analysis and interpretation of data: M. Z.
Drafting of the manuscript: A. A.
Critical revision of the manuscript for important intellectual content: V. B.
Statistical analysis: H. G
Administrative, technical, and material support: R. F. and V. B.

## Ethics

This manuscript did not contain any studies with human subjects performed by any of the authors. All experiments were executed in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Ethics Committee.

## Conflict of Interest

The authors declare that they have no conflict of interest.

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