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Central histaminergic system interplay with suppressive effects of immune challenge on food intake in chicken

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Abstract
1. The aim of the current study was to investigate the interaction of the lipopolysaccharide (LPS) and histaminergic systems on appetite regulation in broilers. Effects of intracerebroventricular (ICV) injection of α-fluoromethylhistidine (α-FMH, histidine decarboxylase inhibitor), chlorpheniramine (histamine H1 receptor antagonist), famotidine (histamine H2 receptor antagonist) and thioperamide (histamine H3 receptor antagonist) on LPS-induced hypophagia in broilers were studied.
2. A total of 128 broilers were randomly allocated into 4 experiments (4 groups and 8 replications in each experiment). A cannula was surgically implanted into the lateral ventricle. In Experiment 1, broilers were ICV injected with LPS (20 ng) prior to α-FMH (250 nmol). In Experiment 2, chickens were ICV injected with LPS followed by chlorpheniramine (300 nmol). In Experiment 3, broilers were ICV injected with famotidine (82 nmol) after LPS (20 ng). In Experiment 4, ICV injection of LPS was followed by thioperamide (300 nmol). Then, cumulative food intake was recorded until 4 h post-injection.
3. According to the results, LPS significantly decreased food intake. Chlorpheniramine significantly amplified food intake, and LPS-induced hypophagia was lessened by injection of chlorpheniramine. α-FMH, famotidine and thioperamide had no effect on LPS-induced hypophagia.
4. These results suggest that there is an interaction between central LPS and the histaminergic system where LPS-induced hypophagia is mediated by H1 histamine receptors in 3 h food-deprived broilers.

INTRODUCTION

Eating behaviour is driven by a very complex interplay of hierarchically organised systems in the brain (Taati et al., 2011; Jonaidi et al., 2012; Mortezaei et al., 2013; Zendehdel et al., 2013, 2015b). Neurological networks exist between the immune system and appetite regulation centres in the central nervous system (CNS) (Zendehdel et al., 2012b). It is well known that immune challenge reduces exploratory behaviour, drinking and eating. Several brain centres and neurotransmitters act together to regulate food intake (Alimohammadi et al., 2015). The hypothalamic arcuate nucleus (ARC) and neural projections originating from the caudal brainstem play a prominent role in appetite regulation (Park et al., 2008). Also, a wide range of neurotransmitters have been identified in contributing to appetite regulation (Zendehdel et al., 2012b). Recent work on neurocircuitry of feeding behaviour implies that lipopolysaccharide (LPS) is responsible for voluntary food intake regulation (Volkoff and Peter, 2004; Zendehdel et al., 2012b). Endotoxins are LPS from the cell walls of Gram-negative bacteria. They regulate many physiological- and pathological-associated changes during bacterial infections (Walker et al., 2013). Pro-inflammatory cytokines include interleukin-1 (IL-1), tumour necrosis factor-α (TNF-α), IL-6, IL-10 and IL-1β produced by immune cells in response to bacterial LPS (Hollis et al., 2010). Several functions for these cytokines were identified like fever induction, neuroendocrine activation and hypophagia (Abe et al., 2001). Even though it is still unclear how LPS affects appetite regulation centres in the

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brain, it is well known that intracerebroventricular (ICV) injection of LPS or its activated cytokines attenuates food intake in rats (Volkoff and Peter, 2004).

Histamine is a monoamine in inflammatory cells which play an essential role in inflammation (Masaki et al., 2005). Also, the central histaminergic system is implicated in the modulation of various physiological aspects, e.g. locomotor activity, thermoregulation and appetite (Swiergiel et al., 1999). Feeding behaviour is not mediated via a single neurotransmitter but a variety of neurotransmitters in several pathways interacts over a widely distributed neural network (Olszewski et al., 2002). Food intake is modulated via central H1 receptors in rats (Sakata et al., 1991) and poultry (Meade and Denbow, 2001). In contrast, ICV injection of chlorpheniramine increases cumulative food intake in both rodents and birds (Meade and Denbow, 2001; Zendehdel et al., 2008). α-Fluoromethylhistidine (α-FMH, a histidine decarboxylase inhibitor) reduces histamine activity by blockade of L-histamine decarboxylase, thus increasing food intake in birds (Meade and Denbow, 2001). Central histaminergic neuron cell bodies are localised in the posterior hypothalamus in the tuberomammillary nucleus (TMN), and axons of these neurons project from the TMN to many areas of the brain (Rafiee et al., 2011).

In the ARC, two populations of neurons have been identified that play essential roles in the reward system. The neurons expressing pro-opiomelanocortin (POMC), cocaine- and amphetamine-regulated transcripts (CART) are associated with eating inhibition, whereas neuropeptide Y (NPY) and agouti-related protein (AgRP) increase food intake in poultry (Park et al., 2008; Saneyasu et al., 2011; Shiraishi et al., 2011). It is apparent that histamine expresses its role by stimulating POMC and CART, as well as blocking NPY and AgRP increase in the ARC (Morimoto et al., 2001). Presumably, orexigenic and anorexigenic neuropeptides play a key role in the hypophagic effect of LPS in the ARC (Hollis et al., 2010). Perhaps, LPS stimulates POMC gene expression and suppresses NPY and AgRP gene expression (Sergeyev et al., 2001; Scarlett et al., 2008; Iwasa et al., 2010).

Several cytokines are supposed to play a role as endogenous mediators for the hypophagic effects of LPS on the serotonergic, glutamatergic and histaminergic systems (Swiergiel et al., 1999; Zendehdel et al., 2012b). It is reported that IL-1β increases histamine turnover in rat hypothalamus. Swiergiel et al. (1999) described that histaminergic receptors (H1, H2 and H3) are not involved in IL-1-induced hypophagia in mice. Also, they reported that α-FMH was not able to attenuate LPS-induced hypophagia in mice. Based on the literature, it seems that systemic LPS does not traverse the blood–brain barrier. On the other hand, LPS impresses its effects through inducing proinflammatory cytokines by immune cells which influence the CNS (Abrehdari et al., 2013); ICV injection of bacterial LPS or cytokines reduces food intake in mammals (Langhans, 2000) and birds (Zendehdel et al., 2012b). No report was found on the involvement of the histaminergic system on LPS-induced anorexia in chickens. Because immune challenge is still one of the main hypophagia problems in poultry, the hypothesis of this study was to investigate a possible effect of centrally injected LPS and the histaminergic system in 3 h food-deprived (FD3) broilers.

MATERIALS AND METHODS

Animals

Four experiments were designed in this study. A total of 128, 8-d-old, Ross 308 broiler chickens (Eshragh Co., Iran) were used. In each experiment, birds (average live body weight 42 ± 2 g in each group) were randomly allocated into 4 treatment groups (8 chicks in each group) and reared in heated batteries with continuous lighting until 3 weeks of age. Chickens were offered ad libitum two experimental diets: a starter diet containing 200 g crude protein (CP)/kg and 12.1 MJ/kg of metabolisable energy (ME)/kg and a grower diet containing 190 g CP/kg and 12.4 MJ/kg of ME. All birds were offered ad libitum fresh water. At the age of 21 d, chickens were transferred to individual cages. Birds were reared in continuous light at a temperature of 22 ± 1°C with 50% humidity (Olanrewaju et al., 2006). Animal handling and experimental procedures were performed according to the Guide for the Care and Use of Laboratory animals by the National Institute of Health (USA) and the current laws of the Iranian government for animal care.

Experimental drugs

Experimental drugs included LPS from Escherichia coli, serotype 0111: B4 (No. L-2630; Sigma); α-FMH (histidine decarboxylase inhibitor), chlorpheniramine (histamine H1 receptor antagonist), famotidine (histamine H2 receptor antagonist) and thioperamide (histamine H3 receptor antagonist) were purchased (Tocris, UK). The drugs were dissolved in pyrogen-free 0.9% saline or first dissolved in absolute dimethyl sulphoxide and then diluted with saline at a ratio of 1/250. Doses of drugs were calculated based on previous (Langhans et al., 1989; Johnson et al., 1993; Johnson and Von Borell, 1994; Von Meyenburg et al., 2003; Zendehdel et al., 2008, 2015a; Taati...
et al., 2009, 2010) and pilot studies (not published).

**Surgical procedures**

At 21 d of age, cockerels were anesthetised with intramuscular (i.m.) injection of xylazine (1 mg/kg body weight) and 30 mg/kg body weight ketamine (Thurmon et al., 1996). Experimental injections were applied by a 29-gauge thin-walled stainless steel guide cannula (Razipakhsh, Iran) which was stereotaxically implanted into the right lateral ventricle. The stereotaxic specifications were anterior/posterior: 6.7 mm, 0.7 mm lateral of midline and 3.5 to 4 mm below the dura mater with the head oriented (Denbow et al., 1981). The cannula was immobilised using three stainless steel screws placed into the calvaria surrounding each guide cannula. Acrylic dental cement ( Pars Acryl, Iran) was applied to the screws and guide cannula. When birds were not used for injection (in the intervals between experiments), an orthodontic wire NO.014 (American Orthodontics) trimmed to the exact length of the cannula was inserted into the guide cannula. To hinder possible infection, lincospectin (Razak) was applied to the incision. Prior to receiving injections of solutions, all birds were allowed a recovery period of minimum 5 d.

**Experimental procedures**

To determine the possible role of the central histaminergic system on LPS-induced feeding behaviour, effect of central administration of α-FMH and histamine H₁, H₂ and H₃ receptor antagonists on food intake in chickens was investigated. All injections were carried out through a 29-gauge, thin-walled stainless steel injecting cannula (Razipakhsh, Iran) which extended 1.0 mm beyond the guide cannula. A 60-cm-long polyethylene tube (Persian tube, Iran) attached the injecting cannula to a 10-µl Hamilton syringe (Hamilton, Switzerland). All drugs had an injection period of 60 s. To make equipment pyrogen-free, all injection equipment was kept in 70% ethanol and the glassware was autoclaved. An additional 60 s was applied as a permit factor to allow the solution to diffuse from the tip of the cannula into the ventricle. Experimental procedures were made from 8:00 A.M to 3:30 P.M. In all experiments, chickens were taken from their individual cages, restrained by hand and then returned to the cages (Altromin, Germany) immediately after injections. Birds were handled and mock injected daily during the 5-d recovery period in order to adapt to the real experimental injection conditions (Jonaidi et al., 2002; Abbasnejad et al., 2005; Zendehdel et al., 2012a, 2012b; Mortezaei et al., 2013). At 3 h before the injection, birds were food-deprived and two injections with 15-min interval were applied for each broiler. Immediately after the second injection, birds were returned to their cages and cumulative food intake (g) was measured at 2 and 4 h after the second injection. In Experiment 1, chickens received the first ICV injection of 20 ng LPS in 5 µl saline. The second injection was α-FMH (250 nmol, in 5 µl) as summarised in the Table. All injections for the control group contained only 10 µl of saline. Experiments 2 and 3 were conducted in a manner similar to the first experiment except cockerels received chlorpheniramine (300 nmol), famotidine (82 nmol) and thioperamide (300 nmol) instead of α-FMH injection. Each bird was used once in each experiment. At the end of the experiments, to recognise accuracy of injection, chicks were killed by decapitation and the accuracy of placement of the guide cannula into the ventricle was confirmed via the incidence of cerebrospinal fluid and ICV injection of methylene blue (10 µl) followed by slicing the frozen brain tissue.

**Statistical analysis**

Cumulative food intake was analysed by 2-way ANOVA with repeated measures using SPSS 16.0 for Windows (SPSS, Inc., Chicago, IL, USA) and is presented as mean ± SEM. For treatments showing a main effect by ANOVA, means were compared using a post-hoc Bonferroni test. $P \leq 0.05$ was considered as significant differences between treatments.

### Table. Treatments in Experiments 1–4

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<th>Experiment 1</th>
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<td>Treatment</td>
<td>First injection (ICV)</td>
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<td>A</td>
<td>Saline</td>
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<td>B</td>
<td>LPS (20 ng)</td>
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<tr>
<td>A</td>
<td>Saline</td>
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<td>B</td>
<td>LPS (20 ng)</td>
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<td>C</td>
<td>Saline</td>
<td>Chlorpheniramine (300 nmol)</td>
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<td>B</td>
<td>LPS (20 ng)</td>
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<td>Famotidine (82 nmol)</td>
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<td>Treatment</td>
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<td>A</td>
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<td>Thioperamide (300 nmol)</td>
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Saline, 0.9% NaCl; LPS, lipopolysaccharide; α-FMH, fluoromethylhistidine. The interval between the two injections was 15 minutes.
RESULTS

The modulatory effects of the histaminergic system on LPS-induced hypophagia in chickens are presented in Figures 1–4. In all experiments, ICV injection of LPS (20 ng) significantly decreased food consumption in FD3 birds compared to the control group until 2 and 4 h post-injection ($P < 0.05$) (Figures 1–4).

In Experiment 1, ICV injection of $\alpha$-FMH (250 nmol) had no effect on food intake compared to the control group [$\alpha$-FMH, $F(3,27) = 2.31, P > 0.05$]. Interestingly, LPS-induced hypophagia was significantly decreased by $\alpha$-FMH (250 nmol) in comparison to the control group [Time, $F(3,27) = 16.13, P < 0.01$; LPS, $F(3,27) = 23.01, P < 0.01$; $\alpha$-FMH, $F(3,27) = 2.31, P > 0.05$; LPS × $\alpha$-FMH interaction, $F(3,27) = 18.72, P < 0.01$] (Figure 1).

In Experiment 2, ICV injection of chlorpheniramine (300 nmol) did not alter cumulative food intake compared with the control group [chlorpheniramine, $F(3,27) = 2.89, P > 0.05$]. Post-treatment with chlorpheniramine significantly diminished LPS-induced hypophagia in FD3 broilers [Time, $F(3,27) = 22.43, P < 0.01$; LPS, $F(3,27) = 27.14, P < 0.01$; chlorpheniramine, $F(3,27) = 2.89, P > 0.05$; LPS × chlorpheniramine interaction, $F(3,27) = 31.02, P < 0.01$] (Figure 2).

As seen in Figure 3, ICV administration of famotidine (82 nmol) in Experiment 3 had no effect on LPS-induced hypophagia compared with the control group in FD3 chickens [Time, $F(3,27) = 15.29, P < 0.03$; LPS, $F(3,27) = 24.63, P < 0.01$; famotidine, $F(3,27) = 4.05, P > 0.05$; LPS × famotidine interaction, $F(3,27) = 1.47, P > 0.05$] (Figure 3).

In Experiment 4, ICV injection of thioperamide (300 nmol) had no significant effect on cumulative food intake compared to the control group in FD3 cockerels [thioperamide, $F(3,27) = 2.79, P > 0.05$]. In addition, post-treatment with thioperamide (300 nmol) did not decrease LPS-induced hypophagia in FD3 chicks ($P > 0.05$) [Time, $F(3,27) = 17.21, P < 0.05$; LPS, $F(3,27) = 31.26, P < 0.01$; thioperamide, $F(3,27) = 2.79, P > 0.05$; LPS × thioperamide interaction, $F(3,27) = 2.93, P > 0.05$] (Figure 4).

Figure 1. Effect of intracerebroventricular injection of lipopolysaccharide (LPS) (20 ng) followed by histidine decarboxylase inhibitor ($\alpha$-FMH, 250 nmol) on cumulative food intake in chickens. Data are expressed as mean ± SEM. [F and P values are as follows: Time, $F(3,27) = 16.13, P < 0.01$; LPS, $F(3,27) = 23.01, P < 0.01$; $\alpha$-FMH, $F(3,27) = 2.31, P > 0.05$; LPS × $\alpha$-FMH interaction, $F(3,27) = 18.72, P < 0.01$]. Group means with no common superscript letter above a column differ significantly (a, b, and c; $P < 0.05$).

Figure 2. Effect of intracerebroventricular injection of lipopolysaccharide (LPS) (20 ng) followed by histamine H1 receptor antagonist (chlorpheniramine, 300 nmol) on cumulative food intake in chickens. Data are presented as the mean ± SEM. [F and P values are as follows: Time, $F(3,27) = 22.43, P < 0.01$; LPS, $F(3,27) = 27.14, P < 0.01$; chlorpheniramine, $F(3,27) = 2.89, P > 0.05$; LPS × chlorpheniramine interaction, $F(3,27) = 31.02, P < 0.01$]. Group means with no common superscript letter above a column differ significantly (a, b, and c; $P < 0.05$).

Figure 3. Effect of intracerebroventricular injection of lipopolysaccharide (LPS) (20 ng) followed by histamine H2 receptor antagonist (famotidine, 82 nmol) on cumulative food intake in chickens. Data are presented as the mean ± SEM. [F and P values are as follows: Time, $F(3,27) = 15.29, P < 0.03$; LPS, $F(3,27) = 24.63, P < 0.01$; famotidine, $F(3,27) = 4.05, P > 0.05$; LPS × famotidine interaction, $F(3,27) = 1.47, P > 0.05$]. Group means with no common superscript letter above a column differ significantly (a and b; $P < 0.05$).

Figure 4. Effect of intracerebroventricular injection of lipopolysaccharide (LPS) (20 ng) followed by histamine H2 receptor antagonist (famotidine, 82 nmol) on cumulative food intake in chickens. Data are presented as the mean ± SEM. [F and P values are as follows: Time, $F(3,27) = 17.21, P < 0.05$; LPS, $F(3,27) = 31.26, P < 0.01$; thioperamide, $F(3,27) = 2.79, P > 0.05$; LPS × thioperamide interaction, $F(3,27) = 2.93, P > 0.05$].
DISCUSSION

To the authors’ knowledge, this article is the first report on the specific role of histamine receptors on LPS-induced feeding behaviour and the possible involvement of histaminergic system on LPS-induced hypophagia in chickens. As predicted, and similar to mammals, LPS treatment reduced food intake in broilers (Von Meyenburg et al., 2003; Zendehdel et al., 2012b) and peripheral injection of LPS in chickens leads to reduced appetite (Kerryn et al., 2003). The new finding in the present study is the effects on central feeding behaviour with respect to the hypogaffic effect of LPS. Many of the physiological effects of LPS are mediated by IL-1, IL-6, IL-10 and IL-1α, and IL-1β and TNF-α potently reduce food intake after either peripheral or central administration in mammals, and are therefore implicated in the hypogaffic effect of LPS (Holli et al., 2010; Zendehdel et al., 2012b). LPS also decreased c-Fos expression in hypothalamic neurons associated with feeding (orexin-A and CART) in the rat (Park et al., 2008).

Cytokines induced by LPS increase satiety signals and may disturb the balance of neuropeptides that maintain homeostasis (Langhans, 2007) and contribute to anorexia during illness. Pro-inflammatory cytokines have also been shown to inhibit orexigenic activity and stimulate anorexigenic neuropeptides in the hypothalamic areas (Ramos et al., 2004). Based on previous findings, systemic LPS treatment leads to increased expression of CART, a peptide expressed in POMC/β-endorphin-containing neurons of the ARC that, when activated, suppress feeding (Sergeyev et al., 2001). In the present study, ICV injection of LPS diminished food intake until 4 h post-injection in chickens. Previously, Kovác et al. (2011) indicated that LPS-activated glial cells release cytokines via Toll-like receptor 4; receptor mRNA increases ~3 h post-ICV injection of LPS and remains elevated until 6 h in the brain. This result was similar to a former study on the potency of LPS on food intake regulation in chickens (Zendehdel et al., 2012b). Based on this evidence, systemic LPS treatment modulates key nodal points of feeding neurocircuitry (Park et al., 2008), and a possible modulatory role of the central histaminergic system on LPS-induced hypophagia in F3 broilers was investigated.

Previous researchers used effective doses of histamine receptor antagonists to fully block and investigate the effects of sole neurological systems on food intake responses (Taati, 2006), but in this study, sub-effective dose of antagonist drugs that block the receptors without affecting food intake was used in order to assay possible interactions of LPS and histamine on food intake. In Experiment 1, the involvement of α-FMH on food intake and LPS-induced hypophagia in broilers was studied. ICV injection of α-FMH increased cumulative food intake and diminished LPS-induced hypophagia. α-FMH is a suicide inhibitor of histidine decarboxylase which decreases central histamine level and amplifies food consumption in rats (Passani et al., 2011). In mice, α-FMH targets and disrupts histamine H1 receptors isolated not only in the TMN but also in PVN and VMH (Morimoto et al., 2001; Gotlo et al., 2007). Furthermore, ICV or intraperitoneal (i.p.) injection of IL-1β increased hypothalamic histamine turnover and/or histidine decarboxylase and suppressed food consumption (Swiergiel et al., 1999). By contrast, administration of α-FMH decreased the anorexic effects of IL-1β in rats (Kang et al., 1995; Swiergiel et al., 1999). The current study confirms a parallel result in poultry as in the rat model studies suggesting that α-FMH may exert its effect on LPS-induced hypophagia using similar pathways in both rats and chicken.

In Experiment 2, ICV injection of chlorpheniramine diminished the hypogaffic effect of LPS on food intake. Chlorpheniramine is a histamine H1 receptor antagonist. Research has shown that ICV injection of chlorpheniramine amplifies cumulative food intake in rats (Morimoto et al., 2001), layers and broiler chickens (Taati et al., 2009, 2010). Histamine may affect corticotropin-releasing hormone (CRH) in VMN and PVN neurons and may also affect AgRP, NPY and CART neurons. It seems that histamine suppresses food intake via neurochemical changes through an increase in CRH levels by stimulating POMC and CART, as well as blocking NPY and AgRP neurons in the ARC (Morimoto et al., 2001). Indeed, these centres act on ingestion regulation and energy.

**Figure 4. Effect of intracerebroventricular injection of lipopolysaccharide (LPS) (20 ng) followed by histamine H1 receptor antagonist (thioperamide, 300 nmol) on cumulative food intake in chickens. Data are presented as the mean ± SEM. (F and P values are as follows: Time, F(3,27) = 17.21, P < 0.05; LPS, F(3,27) = 31.26, P < 0.01; thioperamide, F(3,27) = 2.79, P > 0.05; LPS × thioperamide interaction, F(3,27) = 2.93, P > 0.05). Group means with no common superscript letter above a column differ significantly (a and b; P < 0.05).**
metabolism (Zendehdel et al., 2013). In the CNS, the most likely neurotransmitter reward mediators are histamine, glutamate, serotonin and cholinergic. Previous studies indicated that 5-HT and glutamate (via 5-HT2c and NMDA receptors, respectively) dependently regulate LPS-induced hypophagia in chickens (Zendehdel et al., 2012b). Interestingly, in the current study, post-treatment of chlorpheniramine weakened LPS-induced hypophagia in chickens. In rats, histamine decreases feeding responses through cerebral H1 receptors. Several lines of evidence indicate that central administration of H1 receptor antagonists (chlorpheniramine) decreases LPS or IL-1β depressed food intake in rats (Ookuma et al., 1993; Mercer et al., 1994), whereas Swiergiel et al. (1999) reported that H1 receptor antagonists had no effect on LPS or IL-1β-induced hypophagia in the rat. The results of the current study on modulatory roles of H1 receptors on LPS-induced food depression were similar to those reported in rats. It is suggested that circulating IL-1β and TNFα may reduce food intake by acting on the CNS. The PCR studies reported that IL-1β and TNFα mRNA and receptors are identified in the hypothalamus. Additionally, LPS inhibits c-Fos expression in food regulating neurons of the hypothalamus. The histaminergic system represents a critical hypothalamic element of the arousal-supporting neurocircuity (Gaykema et al., 2008). The exact mechanism for interaction of LPS and the histaminergic system is not fully clear. It seems that LPS or LPS-activated cytokines and histamine stimulates POMC gene expression whereas both block NPY and AgRP gene expression in the ARC (Morimoto et al., 2001; Iwasa et al., 2010) which leads to hypophagia in birds. Alternatively, IL-1β might directly stimulate hypothalamic CRF and reduce food intake. IL-1β causes a significant increase in CRF-IR release in rats. The same researchers reported that histamine rapidly upsurges CRF-IR release. These findings support the hypothesis that IL-1β and histamine enhance CRF secretion in the hypothalamus (Ohgo et al., 1991). The new findings of the current study imply that H1 receptor antagonists not only block histamine effects in the ARC, but also in an unknown manner may inhibit LPS effects on food intake in birds. Also, H1 receptor antagonist had the ability to increase food intake compared to only LPS-injected birds, but the dose of chlorpheniramine applied was not able to amplify food intake in comparison with chickens administered only chlorpheniramine. A neurological interaction might exist between histamine H1 receptors and cytokine receptors in the hypothalamus, but a possible pathway is unclear. Further research is needed to clarify any direct cellular and molecular signalling pathway between LPS and H1 receptors and appetite regulating centres.

Controversial reports exist on the role of the H2 receptors on feeding behaviour. Some researchers demonstrated that H2 receptors had no effect on eating behaviour in rats (Morimoto et al., 2001; Sindelar et al., 2004; Passani et al., 2011) and poultry (Taati et al., 2009). Interestingly, Meade and Denbow (2001) reported that H2 receptors have anorexic effects in broilers. It seems that hypophagia induced by LPS is not mediated by H2 receptors in FD3 broilers. Hence, post-treatment with thioperamide (H3 receptor antagonist) was not able to minimise LPS-induced hypophagia in birds. In a previous study on the role of histaminergic receptors on food intake in broilers, Taati et al. (2009) reported that ICV injection of thioperamide significantly decreased food intake in 12 h food-deprived broilers. Also, injection of 150 nmol thioperamide had no effect on cumulative food intake, whereas a dose-dependent decrease was observed by administration of different levels of thioperamide (300 and 600 nmol). Several lines of evidence suggest that ICV injection of thioperamide has no effect on cumulative food intake in neonatal chickens (Kawakami et al., 2000) or broilers (Zendehdel et al., 2008). In contrast, ICV injection of thioperamide had no effect on cumulative food intake in fasted or non-deprived rats in the lighting period, whereas thioperamide lessened appetite in the dark period when central histamine is at low levels. Thioperamide, therefore, has an effective role only when histaminergic system activity is negligible (Passani et al., 2011). Experiments in rodents showed that stimulation of hypothalamic H3 receptor antagonists causes a decline in feeding (Hancock and Brune, 2005; Malmlöf et al., 2005), whereas blockade of H3 receptors enhances food intake in rats (Chiba et al., 2009). It is also reported that administration of H3 receptor antagonists weakens feeding suppression induced by H3 antagonists in rats (Hancock and Brune, 2005). These results suggest that the suppressive effect of LPS on cumulative food intake was not mediated by histamine H3 receptors in poultry. It seems that there is a different regulatory role of H3 receptors among non-mammalian species such as poultry.

In conclusion, as discussed earlier, it is concluded that the histaminergic system mediates food intake via central H3 receptors in birds. In addition, H1 receptors regulate LPS-induced hypophagia in broilers. It was demonstrated that the inhibitory effect of LPS on food intake is modulated by pathway(s) linked to the H1 receptor. Thus, based on these data, there is perhaps an interaction between the histaminergic system and the immune system affecting the feeding response. The respective importance of these systems on regulating feeding in poultry remains to be elucidated. Also, the detailed
sites within the bird’s brain involved in the action of LPS, and therefore its interaction with other neurotransmitters, remain to be clarified. Perhaps, the hypoglycemic effects of ICV injection of LPS are the same as those seen with cytokines induced by systemic LPS. To conclude, we recommend further research to clarify direct interactions with other receptors affecting physiology of feeding behaviour and food intake regulation in poultry.

**DISCLOSURE STATEMENT**

The authors declare that there are no conflicts of interest.

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**REFERENCES**


Zendehdel, M., Hassanpour, S., Barapour, V., Charkhkar, S. & Madavi, M. (2015b) Interaction between endocannabinoid and opioidergic systems regulates food intake in...

