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The role of cyclooxygenase inhibitors in lipopolysaccharide-induced hypophagia in chicken

M. Zendehdel[1], A. Baghbanzadeh[1], B. Yeganeh[1], S. Hassanpour[2]

[1]Department of Physiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[2]Department of Physiology, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran

ABSTRACT: Previous studies showed that cyclooxygenase 1 (COX) enzyme has an important role in lipopolysaccharide (LPS)-induced hypophagia in mammals but the effect of COX on LPS-induced hypophagia has not been studied in avian species. The current study was designed to investigate the effects of Indomethacin, a non-selective cyclooxygenase inhibitor, Aspirin (irreversible cyclooxygenase inhibitor), Piroxicam (a selective COX-1 inhibitor), and Celecoxib (a selective COX-2 inhibitor) on LPS-induced hypophagia in 3-h food-deprived (FD_3) cockerels. One hundred and sixty ROSS 308 chickens were randomly divided into 5 experiments and 4 treatment groups (8 replicates in each group of experiments). Guide cannula was surgically implanted into the lateral ventricle of chickens. In Experiment 1, birds received LPS (5, 10, and 20 ng) intracerebroventricularly (ICV). In Experiment 2, chickens were intraperitoneally (i.p.) injected with Indomethacin (5 mg/kg) prior to LPS injection (20 ng; ICV). In Experiment 3, birds were i.p. injected with Aspirin (50 mg/kg) followed by LPS injection (20 ng; ICV). In Experiment 4, chickens were given LPS (20 ng; ICV) after Piroxicam injection (10 mg/kg; i.p.). In Experiment 5, chickens were injected with Celecoxib (10 mg/kg; i.p.) prior to LPS injection (20 ng; ICV). Cumulative feed intake was determined until 8 h post-injection. According to the results, LPS significantly decreased feed intake at 4 and 8 h post injection in birds ($P \leq 0.05$). Furthermore, LPS-induced hypophagia was attenuated by pre-injection with Indomethacin, Aspirin, and Celecoxib ($P \leq 0.05$). However, Piroxicam had no effect on LPS-induced hypophagia ($P \geq 0.05$). These results suggest that presumably COX-2 mediates LPS-induced hypophagia in broilers.

Keywords: prostaglandins; cytokines; food intake; broiler

INTRODUCTION

Regulation of feed intake is a complex phenomenon which is in part controlled by central nervous system (CNS) (Zendehdel et al. 2012c). The brain, mainly the hypothalamus, plays undeniable role in the regulation of energy homeostasis (Volkoff et al. 2009). A number of major central neurotransmitters regulating appetite have been identified (Zendehdel et al. 2012b). Literature review shows that a positive correlation exists between immune, neural, neurohumoral, endocrine, and neuroendocrine systems in the CNS and modulates voluntary feed intake during bacterial infections (Johnson 1998; Volkoff and Peter 2004; Zendehdel et al. 2012d).

Lipopolysaccharide (LPS) constitutes a large proportion of the outer layer of the biologically active Gram-negative bacterial cell walls which release during rapid proliferation periods or bacteriolysis and initiates a number of acute-phase responses (Langhans 2000; Walker et al. 2013). Lipopolysaccharide administration is a routine assay to investigate a variety of disorders including systemic infection fever, anorexia, and increased slow wave sleep in laboratory animals (Abe et al. 2001). Bacterial LPS impresses its effects through induc-

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ing proinflammatory cytokines production such as interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), IL-6, IL-10, and IL-1β by immune cells which influence the CNS in rat and birds. These proinflammatory products have different role in the body such as fever induction and neuroendocrine activation (Hollis et al. 2010; Nadjar et al. 2010; Abrehdari et al. 2013).

However, the mechanisms by which peripheral LPS affects the brain have not been fully understood (Singh and Jiang 2004). Systemic injection of LPS changes the nociceptive threshold as well. The intraperitoneal (i.p.) or intravenous (i.v.) injections of LPS in a dose-dependent manner cause hyperalgesia or analgesia (Abe et al. 2001). Recently, it has been suggested that central injections of LPS and IL-1β have important effects on feed intake regulation and energy utilization (Hollis et al. 2010; Nadjar et al. 2010). It has also been suggested that intracerebroventricular (ICV) injection of bacteria LPS or cytokines reduces food intake in mammals (Langhans 2000) and birds (Zendehdel et al. 2012d). Previously, Langhans et al. (1989) reported that i.p. injection of LPS (125, 100, 75, and 50 μg/kg) suppressed feed retake in rat. The same results were reported on food intake after administration of LPS in mammals (Inui 2001; Plata-Salaman 2001; Inui 2002).

The direct pathways by which LPS acts on the CNS level are not fully elicited (Singh and Jiang 2004). Previous studies suggest that i.v. or i.p. injection of interleukins induces expression of the prostaglandin-producing enzymes in the brain (Nadjar et al. 2010) which catalyze arachidonic acid to prostaglandins (PGs) and thromboxane (Choi et al. 2008). It is well documented that PGs have a key role in mediating LPS-induced behavioral and physiological effects in chicken (Johnson et al. 1993). Indomethacin, a nonselective COX inhibitor, blocks eicosanoid synthesis and attenuates anorectic effect of LPS in rat (Langhans et al. 1989) and chicken (Johnson et al. 1993). Hence, it is reported that peripheral injection of COX-2-specific inhibitor decreases LPS-induced anorexia in rat (Parsadaniantz et al. 2000; Li et al. 2001; Swiergiel and Dunn 2002).

On the other hand, a wide series of neuropeptides has been identified in the hypothalamus translating immune signals into metabolic changes. Also, orexigenic and anorexigenic neuropeptides are verified in the arcuate nucleus (ARC) which can play a key role in LPS-induced hypophagia and energy expenditure (Hollis et al. 2010). By contrast, it is reported that LPS promotes pro-opiomelanocortin (POMC) genes expression while suppresses gene expression of the classically orexigenic neuropeptide Y (NPY) and agouti-related peptide (AGRP) (Sergeyev et al. 2001; Scarlett et al. 2007, 2008; Iwasa et al. 2010).

Following up the previous research on the interaction of LPS with COX enzyme, the present study was aimed at evaluating the effects of selective and non-selective COX inhibitors on LPS-induced hypophagia in chicken. We used LPS treatments to examine the effect of LPS on feeding response in cockerels.

MATERIAL AND METHODS

Animals. To investigate possible effects of cyclooxygenase inhibitors on LPS-induced hypophagia, 5 experiments were designed in this study. All experimental procedures and animal handling were done based on 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. One hundred and sixty ROSS 308 chickens (Eshragh Co., Tehran, Iran) were used in this study. In each experiment, birds (average live body weight (LBW) 42 ± 2 g in each group) were randomly divided into 4 treatment groups (eight birds in each replicate) and reared in heated batteries with continuous lighting until 3 weeks of age. The chickens were provided with a starter diet containing 20% crude protein (CP) and 2900 kcal/kg of metabolizable energy (ME) and grower diet included 19% CP and 2950 kcal/kg of ME. Fresh water was offered *ad libitum* during the study. At approximately 21 days of age, cockerels were transferred to individual cages. Birds were maintained at a continuously lighted condition at 22 ± 1°C with 50% humidity (Olanrewaju et al. 2006).

Experimental drugs. The drugs used included LPS *Escherichia coli*, serotype 0111: B4 (No. L-2630), Indomethacin (a non-selective COX inhibitor), Aspirin (acetylsalicylic acid, ASA) (irreversible COX-1 and COX-2 inhibitor), and Celecoxib (a selective COX-2 inhibitor) (all produced by Sigma-Aldrich, St. Louis, USA) and Piroxicam (a selective COX-1 inhibitor) (Tocris Bioscience, Bristol, UK). All solutions were prepared in pyrogen-free 0.9% NaCl solution (saline) that served as control. Doses of drugs were calculated based

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on our previous and pilot studies (Langhans et al. 1989; Johnson et al. 1993; Johnson and von Borell 1994; Von Meyenburg et al. 2003; Zendehdel et al. 2012d).

Surgical procedures. At 21 days of age, broilers were anaesthetized with xylazin (1 mg/kg body weight, intramuscular (i.m.) injection) and ketamin (30 mg/kg body weight, i.m.) (Thurmon et al. 1996). A 23-gauge thin-walled stainless steel guide cannula (Razipakhsh Co., Tehran, Iran) was stereotaxically inserted into the right lateral ventricle in accordance with the previous method described by Denbow et al. (1981). The stereotaxic coordinates were anterior/posterior: 6.7 mm, lateral: 0.7 mm, and horizontal: 3.5–4 mm below the dura mater with the head oriented (Van Tienhoven and Juhaz 1962). To immobilize the guide cannula, three stainless steel screws were placed into the calvaria surrounding each guide cannula. Then acrylic dental cement (Pars Acryl Co., Tehran, Iran) was applied to the screws and guide cannula. In the periods between experiments when there was no injection, an orthodontic #014 wire (American Orthodontics, Sheboygan, USA) trimmed to the exact length of cannula was inserted into it. Lincospectin (Razak Co., Tehran, Iran) was applied to the incision to prevent possible infections. The chickens were allowed a minimum of 5 days to recover prior to receiving injections of solutions (Zendehdel et al. 2013a, b).

Experimental procedures. To evaluate the possible involvement of COX1 and COX2 enzymes on LPS-induced eating responses, the effects of LPS, Indomethacin, Aspirin, Piroxicam, and Celecoxib on feed intake in broiler cockerels were investigated. Injections were applied by a 29-gauge (thin-walled) stainless steel injecting cannula (Razipakhsh Co.) and extended 1.0 mm beyond the guide cannula. This injecting cannula was connected through a 60-cm long polyethylene-20 tubing (Parsian Az Teb, Tehran, Iran) to 10-μl syringe (Hamilton, Biel/Bienne, Switzerland). The injection equipment was kept in 70% ethanol and the glassware was autoclaved to make materials pyrogen-free. LPS was centrally injected over a period of 60 s. In addition, an extra 60 s injection was applied to allow the solution to diffuse from the tip of the cannula into the ventricle. All experimental processes were performed from 9:00 to 17:00 h. The cockerels were removed from their individual cages, restrained by hand, and after receiving the injections put back to the cages. To acclimate birds to injection process and lessen palpation stress, a 5-day recovery period was used (Zendehdel et al. 2012a, b; Mortezaei et al. 2013). Before initiating the experiments, birds had been fasted for 3 h (FD_3). In this study, five experiments were designed, each of them containing four treatment groups (n = 8 per group). In Experiment 1, each bird received one ICV injection of LPS. Control groups were ICV injected with 10 μl of saline as vehicle whereas treatment groups received 5, 10, and 20 ng LPS in 10 μl saline. In Experiment 2, each bird received two injections as described in Table 1. The first injection consisted of Indomethacin (5 mg/kg, i.p.). The second injection consisted of LPS (20 ng, ICV) in 10 μl saline. The interval time

Table 1. Treatments procedure in Experiments 1–5[1]

Treatment groups	1st injection (i.p.)	2nd injection (ICV)
Experiment 1		
A	Saline	–
B	LPS (5 ng)	–
C	LPS (10 ng)	–
D	LPS (20 ng)	–
Experiment 2		
A	Saline	Saline
B	Indomethacin (5 mg/kg)	Saline
C	Saline	LPS (20 ng)
D	Indomethacin (5 mg/kg)	LPS (20 ng)
Experiment 3		
A	Saline	Saline
B	Aspirin (5 mg)	Saline
C	Saline	LPS (20 ng)
D	Aspirin (5 mg)	LPS (20 ng)
Experiment 4		
A	Saline	Saline
B	Piroxicam (10 mg)	Saline
C	Saline	LPS (20 ng)
D	Piroxicam (10 mg)	LPS (20 ng)
Experiment 5		
A	Saline	Saline
B	Celecoxib (10 mg)	Saline
C	Saline	LPS (20 ng)
D	Celecoxib (10 mg)	LPS (20 ng)

Saline = 0.9% NaCl, LPS = lipopolysaccharide, i.p. = intraperitoneal application, ICV = intracerebroventricular application
[1]time interval betwen the two injections was 15 min, n = 8 in each group

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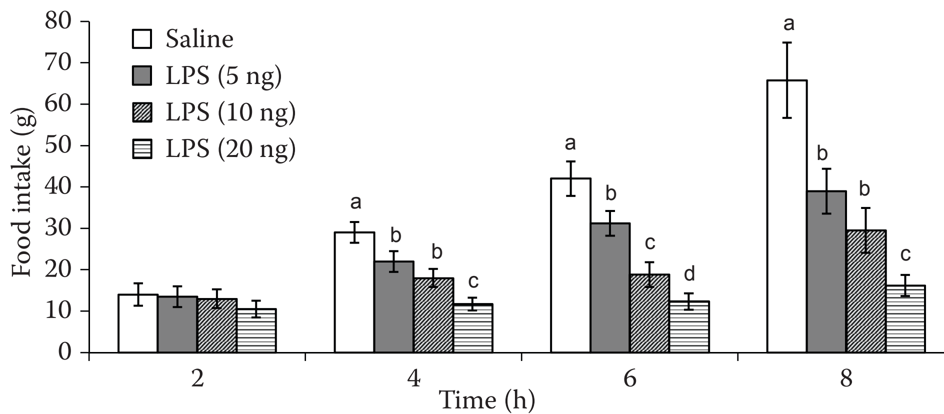

Figure 1. Effect of intracerebroventricular (ICV) injection of lipopolysaccharide (LPS) (5, 10, and 20 ng) on cumulative feed intake in broiler cockerels

[a–d]significant differences between groups with different superscripts in a column

data are presented as mean ± SEM; $P \leq 0.05$

between two injections was 15 min. Experiments 3–5 were similar to Experiment 2 except that the cockerels received Aspirin (50 mg/kg, i.p.), Piroxicam (10 mg/kg, i.p.), and Celecoxib (10 mg/kg, i.p.) instead of Indomethacin injection. After the second injection, birds were returned to their cages and feed intake (g) was recorded at 2, 4, 6, and 8 h. Each bird was used in one experiment only. Broilers were slaughtered painlessly subsequently, according to the mentioned guidelines. The placement of guide cannula into the ventricle was confirmed via the presence of cerebrospinal fluid in the guide cannula (CSF) and ICV injection of methylene blue followed by slicing the frozen brain tissue at the end of the experiments.

Statistical analysis. Cumulative feed intake was analyzed by Two-Way Analysis of Variance (ANOVA) for repeated measurement using SPSS software (Version 16.0, 2007) for MS Windows, and was presented as mean ± SEM. For treatments showing a main effect by ANOVA, means were compared using the post hoc Bonferroni test. $P \leq 0.05$ was considered as significant difference between treatments.

RESULTS

The effect of cyclooxygenase inhibitors on LPS-induced hypophagia in chicken is presented in Figures 1–5. According to the results in Figure 1, the ICV injection of LPS significantly induced hypophagia at 4, 6, and 8 h post injection in broiler cockerels [Time, $F(2, 67) = 24.72$, $P < 0.001$; LPS, $F(3, 27) = 18.05$; $P \leq 0.05$] (Figure 1). Also, LPS

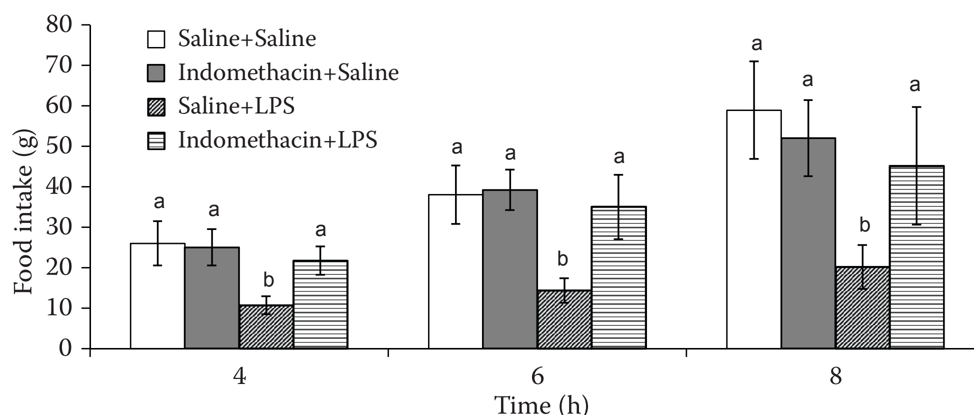

Figure 2. Effect of intraperitoneal (i.p.) injection of Indomethacin (5 mg) followed by intracerebroventricular (ICV) injection of lipopolysaccharide (LPS) (20 ng) on cumulative feed intake in broiler cockerels

[a,b]significant differences between groups with different superscripts in a column

data are presented as mean ± SEM; $P \leq 0.05$

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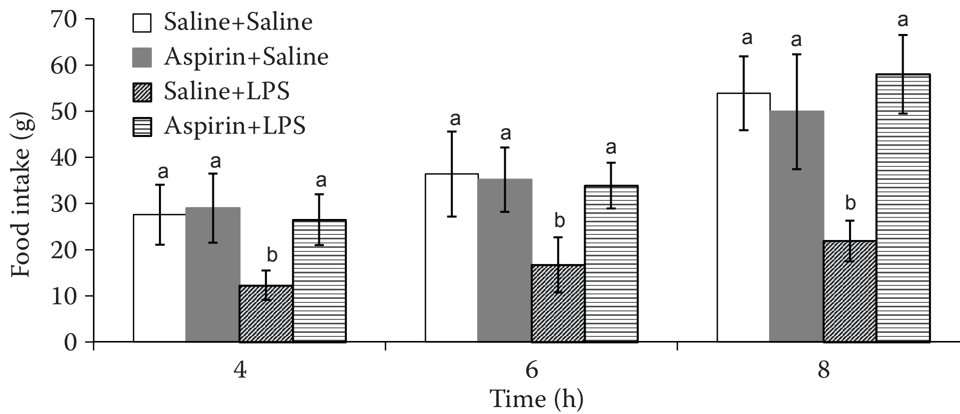

Figure 3. Effect of intraperitoneal (i.p.) injection of Aspirin (50 mg) followed by intracerebroventricular (ICV) injection of lipopolysaccharide (LPS) (20 ng) on cumulative feed intake in broiler cockerels

[a,b]significant differences between groups with different superscripts in a column

data are presented as mean ± SEM; $P \leq 0.05$

decreased feed intake in a dose-dependent manner. Additionally, no significant change in feed intake was observed after ICV injection of different levels of LPS compared to control group (vehicle) at 2 h post injection [$F(3, 27) = 1.04$; $P \geq 0.05$] (Figure 1). The result suggests that the initiation time of hypophagia is 4 h post injection of LPS.

In Experiment 2, i.p. pre-treatment with Indomethacin (5 mg/kg) significantly decreased hypophagic effect of LPS (20 ng; ICV) at 4, 6, and 8 h post injection in cockerels [Time, $F(2, 91) = 35.15$, $P < 0.01$; Indomethacin × LPS $F(3, 27) = 15.85$; $P \leq 0.05$] (Figure 2). Likewise, Indomethacin alone (5 mg/kg; i.p.) had no effect on feed intake [$F(3, 27) = 2.18$; $P \geq 0.05$] (Figure 2). It seems that the inhibitory effect of LPS on feed intake is attenuated by i.p. injection of Indomethacin (a nonselective COX inhibitor) in broilers.

In Experiment 3, we determined the effect of Aspirin on LPS-induced hypophagia in chicken. Pre-treatment with 50 mg/kg of Aspirin (i.p., irreversible COX-1 and COX-2 inhibitor) significantly suppressed hypophagia induced by LPS (20 ng; ICV) at 4, 6, and 8 h post injection [Time, $F(2, 31) = 28.03$, $P < 0.01$; Aspirin × LPS $F(3, 27) = 21.64$; $P \leq 0.05$] (Figure 3). Furthermore, Aspirin (50 mg/kg; i.p.) had no effect on feed intake [$F(3, 27) = 1.98$; $P \geq 0.05$] (Figure 3). The result suggests that suppressive effect of LPS on cumulative feed intake is mediated via COX pathway in chickens.

The effect of i.p. injection of Piroxicam (a selective COX-1 inhibitor) followed by LPS on cumula-

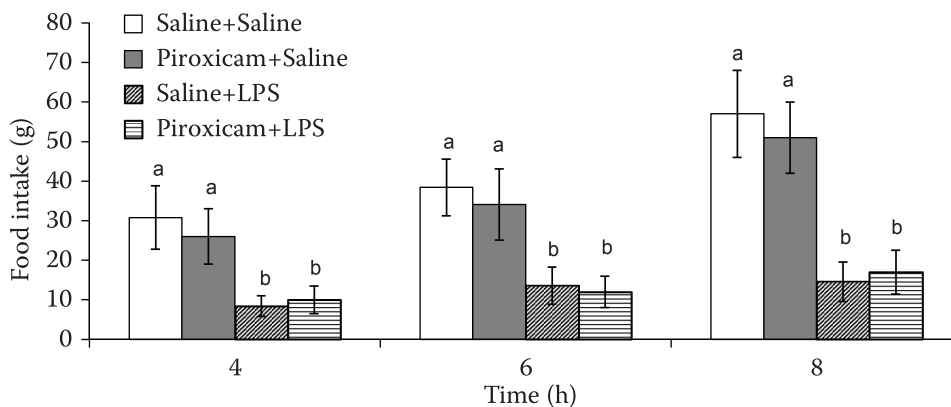

Figure 4. Effect of intraperitoneal (i.p.) injection of Piroxicam (10 mg) followed by intracerebroventricular (ICV) injection of lipopolysaccharide (LPS) (20 ng) on cumulative feed intake in broiler cockerels

[a,b]significant differences between groups with different superscripts in a column

data are presented as mean ± SEM; $P \leq 0.05$

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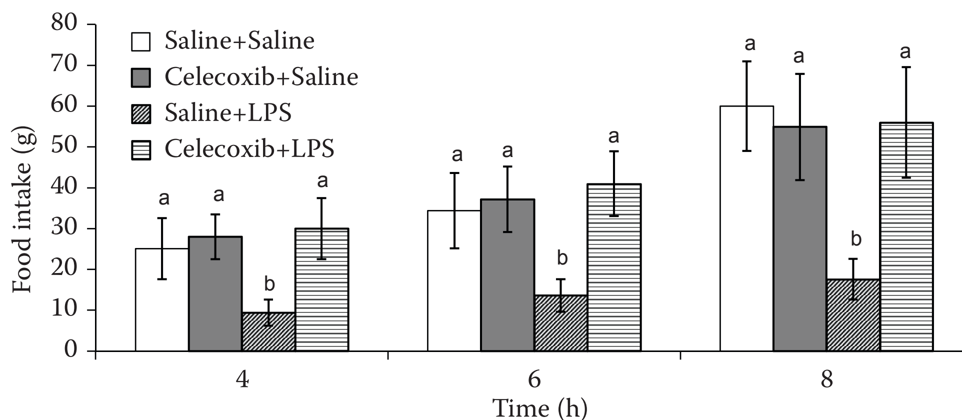

Figure 5. Effect of intraperitoneal (i.p.) injection of Celecoxib (10 mg) followed by intracerebroventricular (ICV) injection of lipopolysaccharide (LPS) (20 ng) on cumulative feed intake in broiler cockerels

[a,b]significant differences between groups with different superscripts in a column

data are presented as mean ± SEM; $P \leq 0.05$

tive feed intake in broiler cockerels is shown in Figure 4. Interestingly, i.p. injection of Piroxicam (10 mg/kg) had no effect on the inhibitory effect of LPS (20 ng, ICV) on feed intake [$F(3, 27) = 3.16$; $P \geq 0.05$] (Figure 4). Also, Piroxicam alone had no effect on cumulative feed intake at any times post injection [$F(3, 27) = 1.70$; $P \geq 0.05$] (Figure 4). Perhaps LPS-induced hypophagia is not mediated via COX-1 enzyme in broilers.

In Experiment 5, we determined the effect of pre-treatment with Celecoxib (a selective COX-2 inhibitor) followed by LPS on cumulative feed intake in cockerels. As shown in Figure 5, single i.p. injection of 10 mg/kg Celecoxib had no effect on feed intake compared to control group [$F(3, 27) = 5.08$; $P \geq 0.05$] (Figure 5). Furthermore, hypophagic effect of LPS (20 ng, ICV) was significantly attenuated by pretreatment with Celecoxib (10 mg/kg; i.p.) at 4, 6, and 8 h post injection in birds [Time, $F(2, 41) = 26.83$, $P < 0.01$; Celecoxib × LPS $F(3, 27) = 17.04$; $P \leq 0.05$] (Figure 5). Presumably, COX-2 plays the main role in LPS-induced hypophagia in chickens.

DISCUSSION

Our study was aimed at revealing the possible role of COX in LPS-induced hypophagia in chicken. Data in Experiment 1 indicate that the ICV injection of LPS decreases feed intake 4 h post injection in FD_3 chicks which was in agreement with previous reports in rat (Langhans et al. 1989), goldfish (Volkoff and Peter 2004), mammals (Inui 2001, 2002; Plata-Salaman 2001), and chicken (Johnson et al. 1993; Zendehdel et al. 2012d). Previous as well as recent studies proved the hypophagic effect of peripheral and central injection of LPS. The direct hypothesis which reveals the role of COX-1 and COX-2 enzymes on LPS-induced hypophagia has not been well studied in avian. The suggested mechanism is that LPS activates glial cells via Toll-like receptor 4 (TLR4) which leads to the release of IL-6, IL-1β, and TNF-α in CNS. Interleukins receptor mRNA increases 3.5 h post ICV injection of LPS in the brain (Kovacs et al. 2011). Moreover, at 6 h after LPS injection TLR4 mRNA levels in CNS remain high (Singh and Jiang 2004). In the current study, cumulative feed intake decreased in a dose-dependent manner at 4 h post LPS injection. This result was in agreement with our previous report that LPS reduces feed intake in chickens from 4 h post LPS injection (Zendehdel et al. 2012d).

In Experiments 2 and 3, LPS-induced hypophagia was significantly attenuated by administration of Indomethacin and Aspirin. Consistently with previous reports, administration of Indomethacin diminished LPS-induced hypophagia in chicken and rat. Indomethacin is a specific inhibitor of COX enzyme which blocks PGs synthesis endogenously from arachidonic acid by consecutive reaction of COX in the chicken brain (Langhans et al. 1989; Johnson et al. 1993; Ohinata et al. 2009b). The anorectic role of PGs and other eicosanoids (e.g. ILs) is well established (Langhans et al. 1989). In fact, there are multiple interactions between PGs and ILs on anorexia caused by LPS. Lately it has been suggested that PGE_2 suppresses feed intake

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via a novel anorexigenic pathway (EP_4 receptor) in CNS (Ohinata et al. 2008, 2009a).

Two pathways are suggested for involvement of LPS on feeding behaviour (Konsman et al. 2002). It is reported that LPS amplifies formation of cytokines binding sites in numerous parts of the brain endothelial cells (Singh and Jiang 2004). The arcuate nucleus of hypothalamus is the main region of the brain where the inflammatory cytokines act (Scarlett et al. 2008). This nucleus is responsible for controlling feed intake behaviour and energy homeostasis (Zendehdel et al. 2013a, b). For instance, LPS increases C-Fos expression in the pro-opiomelanocortin (POMC) and corticotropin releasing factor (CRF) neurons in the arcuate nucleus (ARC) and paraventricular nuclei (PVN) of the hypothalamus which are involved in feed intake regulation (Zendehdel et al. 2012d). Previously, Volkoff and Peter (2004) reported that LPS decreases neuropeptide Y (NPY) and agouti related peptide (AgRP) expression and upsurges cholecystokinin (CCK) and cocaine and amphetamine regulated transcript (CART) expression in ARC nucleus. By contrast, LPS leads to the release of α-MSH in POMC neurons. Interleukin-1 receptor terminated from LPS is responsible for these alterations. It is proved that α-MSH decreases cumulative feed intake via melanocortin receptors (MC_4-R) in broiler cockerels (Zendehdel et al. 2012d). It seems that LPS-induced hypophagia is more complicated and is controlled via numerous neural systems in poultry. In this regard, in our previous study, we showed that LPS-induced hypophagia is mediated by glutamatergic and serotonergic systems in chickens (Zendehdel et al. 2012d). Among five receptor subtypes for NPY, the Y_1 receptor primarily mediates orexigenic activity. Currently, it is suggested that PGE_2 may suppress feed intake by Y_1 receptor blockade in mice (Ohinata et al. 2008). To our knowledge, we think LPS may impress its effects using this mechanism in broilers. The most abundant PG in mammals' CNS is PGD_2 (a positional isomer of PGE_2). It appears that unlike PGE_2, PGD_2 stimulates cumulative feed intake in rat (Ohinata et al. 2008). The accuracy of it is still controversial in avian species.

In Experiment 4, Piroxicam (a selective COX-1 inhibitor) was not able to decrease LPS induced-hypophagia in FD_3 broilers. Piroxicam is a widely used non-steroidal anti-inflammatory drug (NSAID). Cyclooxygenase-1 is predominantly responsible for homeostatic PG synthesis (Choi et al. 2008). In this regard, Dunn and Swiergiel (2000) reported that treatment with Piroxicam attenuated the hypophagic responses to LPS in rat. Probably, Piroxicam administration inhibits IL-1β production or vagal afferents. Our result was not in line with previous results in rat. It seems that LPS-induced hypophagia is not mediated by COX-1 in birds.

According to the results obtained from Experiment 5, LPS induced-hypophagia was significantly attenuated by pre-treatment with Celecoxib. Cyclooxygenase-2 is as the isoform induced in response to inflammatory stimuli and most appropriate target for anti-inflammatory drugs (Choi et al. 2008). It is a highly specific inhibitor of COX-2 which decreases proinflammatory cytokines production and is able to improve cachexia (Mantovani et al. 2010). It is suggested that the delayed effect of LPS is attenuated by Celecoxib whereas it is not affected by the inhibition of COX-1 in rat (Swiergiel and Dunn 2002). Our data is in line with previous researches and pharmacological approach strongly suggests COX-2 controls LPS-induced hypophagia in chickens. Cyclooxygenase-2 expression is high in cortex, hippocampus, amygdala, and hypothalamus. LPS cannot passively cross the blood-brain barrier (BBB). Intracerebroventricular injection of LPS induces the expression of COX-2 in endothelial cells, catalyzes the formation of PGs, and influences neural activity (second pathway). It seems PGs and inflammatory cytokines reinforce the LPS effects on feeding response (Konsman et al. 2002; De Paiva et al. 2010). For instance, Johnson (1997) reported that human IL-1 injection induces PG synthesis in neonatal pig brain. There is evidence of differences in central feed intake regulation mechanisms in layer- and meat-type chickens, because of genetic differences between the breeds. Broilers have higher feed consumption and energy expenditure whereas layers are selected for egg production. For instance, ICV injection of AgRP increases feed intake in broilers, but not in layers. Also, administration of NPY increases feed intake by both broiler and layer chickens. Genetic selection for meat or egg production presumably genetically altered in central neurological pathways associated with food-intake control mechanisms (Zendehdel and Hassanpour 2014). Because of the lack of investigations on the possibility of LPS and COX effect on food intake in avian, the major finding in our study is that presumably COX-2

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mediates LPS-induced hypophagia in broilers. To our knowledge there was no previous study on the effect of COX on LPS-induced feeding behaviour in layer-type chicken. So we think it would be helpful to investigate the effect of COX on LPS-induced feeding behaviour in layers. Further researches identifying the direct interaction of LPS and COX receptors in feeding behaviour in poultry are recommended.

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Corresponding Author

Dr. Morteza Zendehdel, Associate Professor, University of Tehran, Faculty of Veterinary Medicine, Department of Physiology, 14155-6453, Tehran, Iran; Phone: +98 21 61 117 186, e-mail: zendedel@ut.ac.ir