



Original Article

Central and Peripheral Effects of Lipopolysaccharide on Food Choice and Macronutrient Selection in Meat-Type Chick

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Abstract

This report aimed to determine the effect of lipopolysaccharide (LPS) on food intake in broiler chicks with different rations. All birds received a starter diet until five days of age, but experimental diets were provided on days of injections. In experimental group one, chickens received an intracerebroventricular (ICV) injection of LPS (25, 50, and 100 ng) with a standard diet. In experimental group two, chickens received intraperitoneal (IP) injections of LPS (50, 100, and 200 µg) with a standard diet. In experimental group three, birds received ICV injections of saline and different diets. Accordingly, a standard diet without fat, a diet containing 20% higher nutrient energy than the standard, a diet containing 20% less nutrient energy than the standard, and a standard diet containing fat were offered to them to investigate the desire of chickens for the diets. Experimental groups four, five, and six were similar to experimental group three, except that the chickens received ICV injections of LPS. In experimental groups seven, eight, and nine, chickens received IP injections of LPS with different diets. Afterward, their cumulative food intake was measured until 180 min post-injection. According to the results, ICV and IP injections of LPS decreased food intake ($P<0.05$). However, the ICV injection of saline increased the desire of chickens for the standard diet with fat ($P<0.05$). The ICV injection of the LPS (50 and 100 ng) increased the appetite for a standard diet with nutrient energy 20% higher than the standard and a standard diet containing fat, at 120 and 180 min after the injection ($P<0.05$). In addition, IP injection of LPS (200 µg) significantly increased the desire for a standard diet with nutrient energy 20% higher than the standard and a standard diet containing fat ($P<0.05$). These results suggested the desire of chickens for different types of rations is affected by central or peripheral administration of the LPS.

Keywords: Broiler chicken, Food intake, Lipopolysaccharide

1. Introduction

Neurological networks exist between appetite regulation centers in the central nervous system (CNS) and the immune system, concerning the modulation of voluntary food intake during bacterial infections (1). Lipopolysaccharide (LPS), also termed endotoxins, comprises a considerable proportion of the outer layer of the biologically active gram-negative bacterial cell walls, which are released during prompt proliferation periods or bacteriolysis and provoke several acute-

phase responses (2). Their effects are influenced by inducing interleukin-1 (IL-1), tumor necrosis factor- α (TNF- α), IL-10, IL-6, and IL-1 β , which are pro-inflammatory cytokines production.

In this regard, LPS affects the CNS of birds and rats and plays roles in various disorders of the body, like anorexia, fever induction, and neuroendocrine activation; however, it is unknown how it influences central appetite regulation (3, 4). Previous studies have insinuated that endotoxins regulate voluntary dietary

consumption (3, 5). Moreover, it has been shown that IP injection of LPS induces hypophagia and increases mRNA levels for the proopiomelanocortin (POMC) of anorexigenic messenger and cocaine and amphetamine-regulated transcript (CART) (6).

It has been reported that modification in caloric intake and dietary preferences was observed when animals were allowed to choose their diet from pure macronutrient sources. Among the neuropeptides, endogenous opioids play a role in diet selection in rats (7). Opioids have been shown to increase fat consumption while decreasing or not affecting carbohydrate or protein intake (8). In addition, endogenous opioids govern preferences for fat-rich foods in broiler chicken (7).

According to previous studies, there is also a link between LPS and preference for ration choice. According to this view, IP injection of the LPS decreases dietary intake and the total amount of required amino acids, but does not affect the use of lysine and threonine to increase protein in chicken (9). Furthermore, acute-phase responses to LPS injection are much more significant in chicks with a low-protein diet, compared to those with a high-protein diet (10).

Based on the literature, despite the reported hypophagic effect of LPS, there is no report on its possible impact on the desire of chickens for different rations. Therefore, this study aimed to determine the effect of the central or peripheral injection of the LPS on food intake in broiler chicks with extra rations.

2. Materials and Methods

For the purposes of the study, 396-day-old meat-type chickens (Ross 308) were purchased from a local hatchery (Morghak Co., Tehran, Iran). The chickens were kept as flocks for 2 days; afterward, they were randomly transferred into individual cages and kept at a temperature of 30 ± 1 °C with $50\pm 2\%$ humidity (11). Four experimental diets used in this study included a starter diet without fat (2970 Kcal metabolizable energy), a starter diet containing 20% higher nutrient energy than the standard, a starter diet containing 20% less nutrient energy than the

standard, and a starter diet containing fat (Table 1). They were used based on UFFDA to determine the role of central and peripheral LPS on the feeding behavior of birds. The composition of diets is presented in table 1. All birds received a starter diet for 5 days, but experimental diets were provided on days of injections. All chicks were offered *ad libitum* food and fresh water during the study. Just 3 h before the intracerebroventricular (ICV) injections, the chickens were food deprived (FD₃) but had free access to fresh water. The injections were administered to all birds at 5 days of age.

2.1. Intracerebroventricular Injections

Birds were randomly divided into nine experimental groups with four sub-groups (n=44). The birds were weighed and divided into experimental groups based on their body weight (BW), as the mean BW of diverse experimental groups was the same. The ICV injections were performed once for each group by a microsyringe (Hamilton, Switzerland) without anesthesia according to the techniques of Davis, Masuoka (12), (13).

In summary, the chicken head was held using an acrylic device with a bill holder at an angle of 45°. Calvarium was in a parallel position relative to the table surface, as described by van Tienhoven and Juhasz (14). Next, an orifice was made in a plate over the skull of the right lateral ventricle, through which a microsyringe was inserted. The needle tip perforated 4 mm under the skull skin (15), and the volume of injections was 10 µL (13). It should also be mentioned that the animals in the control group received 10 µL of the control solution (13).

It should be noted that the above-mentioned method does not cause physiological stress for newly hatched chickens (16). Decapitation was carried out to identify injection accuracy at the end of experiments. The accuracy of the injection site in the ventricle was confirmed based on the presence of Evans blue and the slicing of frozen brain tissues. All birds in each experimental group received injections. However, only the data from 11 birds in each group that had dye in their lateral ventricle were analyzed. All the test procedures were performed from 8 am to 3 pm.

Table. Ingredient and nutrient analysis of experimental diets

Ingredient	A standard diet without fat	A diet containing nutrient energy 20% higher than standard	A diet containing nutrient energy 20% lower than standard	A standard diet containing fat
Corn	59.78	66.35	50.81	50
Soybean meal, 44% CP	24.67	24.53	25.07	40.43
Gluten meal, CP	9.7	4.17	17.13	0
Soybean oil	0	0	0	4
Oyster shell	1.39	1.21	1.63	1.34
Di-calcium phosphate	1.98	1.62	2.44	1.87
Sodium chloride	0.23	0.18	0.27	0.23
Sodium bicarbonate	0.26	0.22	0.31	0.27
Mineral premix	0.25	0.25	0.25	0.25
Vitamin premix	0.25	0.25	0.25	0.25
DL-Methionine	0.30	0.28	0.34	0.39
L-Lysine HCl	0.66	0.45	0.92	0.28
DL- Threonine	0.48	0.44	0.53	0.64
Salinomycin	0.05	0.05	0.05	0.05
ME, kcal/g	2970	2970	2970	2970
Crude protein (%)	23.07	19.97	27.31	23.07
Calcium (%)	1.05	0.9	1.24	1.05
Available phosphorus (%)	0.5	0.4286	0.59	0.5
Sodium (%)	0.18	0.15	0.21	0.18
Potassium (%)	0.71	0.707	0.717	0.961
Chlorine (%)	0.1795	0.15	0.2039	0.1786
Lysine (%)	1.43	1.23	1.7	1.43
Methionine + cystine (%)	1.07	0.93	1.27	1.07
Tryptophan (%)	0.253	0.236	0.277	0.329
Threonine (%)	0.94	0.81	1.11	0.94
Linoleic Acid (%)	1.739	1.88	1.549	1.795

ME: metabolizable energy, CP: crude protein. Per kg of diet, the mineral supplement contains 60 mg manganese; 60 mg iron; 51.74 mg zinc; 4.8 mg copper; 0.69 mg iodine; 0.16 mg selenium. The vitamin supplement contains 7040IU vitamin A, 2000 IU D₃, 8.8 IU of Vitamin E, 1.6 mg K₃, 1.2 mg B₁, 3.2mg of vitamin B₂, 6.3 mg of B₃, 28 mg of vitamin B₅, 1.97 mg of B₆, the 0.008 mg of B₁₂, 0.12 mg of Biotin, 320 mg of choline chloride

2.2. Intracerebroventricular Injections

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In summary, the chicken head was held using an acrylic device with a bill holder at an angle of 45°. Calvarium was in a parallel position relative to the table surface, as described by van Tienhoven and Juhasz (14). Next, an orifice was made in a plate over the skull

of the right lateral ventricle, through which a microsyringe was inserted. The needle tip perforated 4 mm under the skull skin (15), and the volume of injections was 10 µL (13). It should also be mentioned that the animals in the control group received 10 µL of the control solution (13).

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the data from 11 birds in each group that had dye in their lateral ventricle were analyzed. All the test procedures were performed from 8 am to 3 pm.

2.3. Food Intake Measurement Procedure

In experimental group one, the FD₃ chickens received an ICV injection of saline besides LPS (25, 50, and 100 ng) with a standard diet. In experimental group two, the FD₃ chickens received IP injection of saline, LPS (50 µg), LPS (100 µg), and LPS (200 µg) with a standard diet. In experimental group three, the FD₃ birds received ICV injections of saline and different diets. They were offered a standard diet without fat, a diet containing nutrient energy 20% higher than the standard, a diet containing nutrient energy 20% lower than the standard, and a diet containing fat to investigate their desire for diets.

In experimental group four, the FD₃ chickens received ICV injection of LPS (25 ng), and four different diets were offered to them, including a standard diet without fat, a diet containing 20% more nutrient energy than the standard, a diet with 20% less energy than the standard, and a standard diet containing fat. In experimental group five, the FD₃ birds received ICV injection of LPS (50 ng), and four different diets were offered to them, including a standard diet without fat, a diet containing 20% more nutrient energy than the standard, a diet with 20% less energy than the standard, and a standard diet containing fat.

Experimental group six received an ICV injection of LPS (100 ng) and were offered four different diets, including a standard diet without fat, a diet containing 20% more nutrient energy than the standard, a diet containing 20% less nutrient energy than the standard, and a standard diet containing fat. In experimental group seven, FD₃ chickens received IP injection of LPS (50 µg) and were offered four different diets, namely a standard diet without fat, a diet containing 20% more nutrient energy than the standard, a diet with 20% less energy than the standard, and a standard diet containing fat.

In experimental group eight, the FD₃ chickens

received IP injection of LPS (100 µg) and were offered four different diets, including the standard diet without fat, a diet containing 20% more nutrient energy than the standard, a diet with 20% less energy than standard, and a standard diet containing fat. The experimental group nine received the IP injection of LPS (200 µg), and four different diets as a standard diet without fat, a diet containing 20% more nutrient energy than the standard, a diet containing 20% less nutrient energy than the standard, and a standard diet containing fat.

After injection, FD₃ fowls were returned to their cages and supplied with fresh water and food (pre-weighed). Cumulative food intake (gr) was measured at 30, 60, 120, and 180 min post-injection. Food consumption (plus any food spillage) was calculated as a percentage of body weight to minimize the impact of body weight on the amount of food intake. Each bird was just used once in each experimental group. The doses of drugs were established by the pilot and previous studies (3, 17).

2.4. Statistical Analysis

Cumulative food intake (% BW) was analyzed by two-way analysis of variance (ANOVA) for repeated measurement in SPSS software (version 16.0). The results were presented as mean±SEM. For the experimental groups showing the main effect by ANOVA, means were compared using the post-hoc Bonferroni test ($P<0.05$).

3. Results

As seen in figure 1, the ICV injection of LPS (50 and 100 ng) significantly decreased food intake at 120 min post-injection, compared to the control group ($P<0.05$). However, no difference was observed in food consumption by 25 ng of the LPS ($P>0.05$). Based on figure 2, the IP injection of the LPS (200 µg) significantly decreased food intake at 120 min post-injection, compared to the control group ($P<0.05$). However, no difference was observed between the chickens who received 50 and 100 µg of the LPS in terms of food consumption ($P>0.05$).

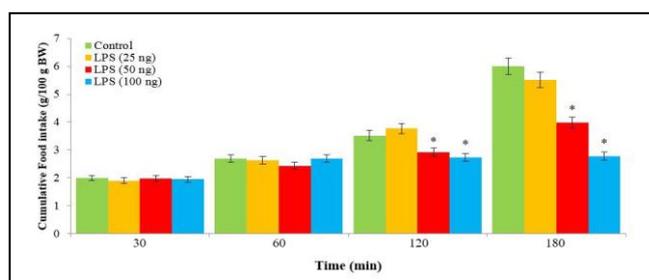


Figure 1. Effect of ICV injection of LPS (25, 50, and 100 ng) on cumulative food intake in neonatal chickens (n=44). Data are expressed as mean \pm SEM. LPS: Lipopolysaccharide. * $P < 0.05$ compared to control group

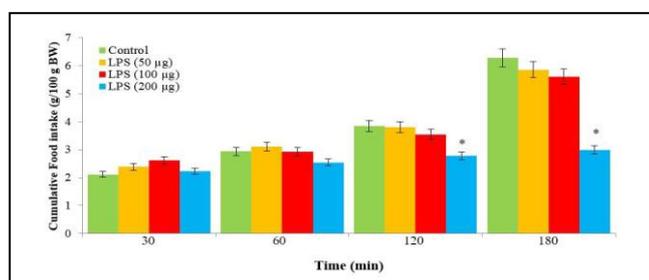


Figure 2. Effect of IP injection of LPS (50, 100, and 200 µg) on cumulative food intake in neonatal chickens (n=44). LPS: Lipopolysaccharide. Data are expressed as mean \pm SEM. * $P < 0.05$ compared to control group

As seen in figure 3, chickens had a higher desire for the standard diet with fat and a lower appetite for a diet containing nutrient energy (20% higher than standard) at 30 min post-injection ($P < 0.05$). However, there was no significant difference between the diets containing less and more nutrient energy than the standard ($P > 0.05$). Moreover, it was found that at 60, 120, and 180 min after ICV injection of saline, chickens had a higher desire for the standard diet with fat ($P < 0.05$).

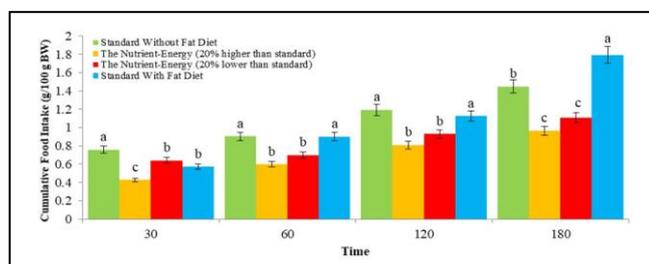


Figure 3. Effect of ICV injection of saline in neonatal meat-type chickens fed different diets: standard diet without fat, diet containing nutrient energy 20 % higher than standard, diet containing nutrient energy 20 % lower than standard, and a standard diet containing fat. Data are expressed as mean \pm SEM. There are significant differences between groups with different superscripts in each time (a, b, and c; $P < 0.05$)

In experimental group four, ICV injection of the LPS (25 ng) significantly decreased the desire for standard diets with 20% less and more nutrient energy than the standard at 30, 60, and 120 min post-injection ($P < 0.05$). However, birds had a higher desire for a standard diet with fat, but it was not statistically significant ($P > 0.05$). At 180 min post-injection, a significantly increased appetite for a standard diet with fat and a diet containing 20% less energy than the standard was observed (Figure 4).

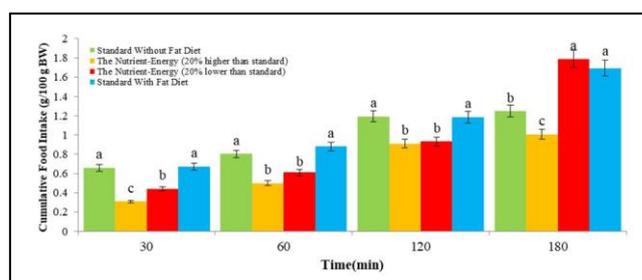


Figure 4. Effect of ICV injection of LPS (25 ng) in neonatal meat-type chickens fed different diets: standard diet without fat, diet containing nutrient energy 20 % higher than standard, diet containing nutrient energy 20 % lower than standard, and a standard diet containing fat. LPS: Lipopolysaccharide. Data are expressed as mean \pm SEM. There are significant differences between groups with different superscripts in each time (a, b, and c; $P < 0.05$)

According to figure 5, the ICV injection of LPS (50 ng) significantly increased the desire for a standard diet with fat and a standard diet without fat ($P < 0.05$). In addition, there was a decrease in the appetite for the normal diet containing 20% more or 20% less energy than the standard level, compared to the control group ($P < 0.05$). At 120 and 180 min post-injection, a significant increase was observed in the desire for a standard diet with fat and a diet containing 20% more energy than the standard ($P < 0.05$). Despite the hypophagic effect of the LPS, a significant difference was observed in the desire for the standard diet at 120 and 180 min post-injection ($P < 0.05$).

The ICV injection of LPS (100 ng) significantly increased the desire for a standard diet with fat

and a standard diet without fat ($P < 0.05$), but decreased the appetite for standard diets containing 20% more or 20% less energy than the standard, compared to the control group ($P < 0.05$). At 120 and 180 min post-injection, a significant increase was observed in the desire for a standard diet with fat and a diet containing 20% more energy than the standard ($P < 0.05$). Despite the hypophagic effect of the LPS, a significant difference was observed in the desire for a standard diet at 120 and 180 min post-injection ($P < 0.05$) (Figure 6).

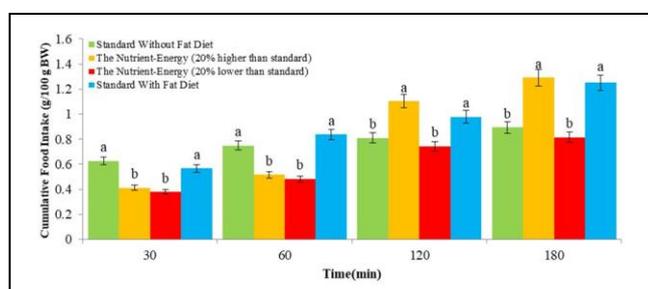


Figure 5. Effect of ICV injection of LPS (50 ng) in neonatal meat-type chickens fed different diets: standard diet without fat, diet containing nutrient energy 20 % higher than standard, diet containing nutrient energy 20 % lower than standard, and a standard diet containing fat. LPS: Lipopolysaccharide. Data are expressed as mean \pm SEM. There are significant differences between groups with different superscripts in each time (a and b; $P < 0.05$)

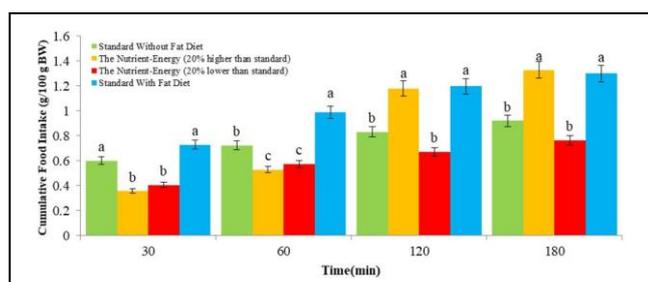


Figure 6. Effect of ICV injection of LPS (100 ng) in neonatal meat-type chickens fed different diets: standard diet without fat, diet containing nutrient energy 20 % higher than standard, diet containing nutrient energy 20 % lower than standard, and a standard diet containing fat. LPS: Lipopolysaccharide. Data are expressed as mean \pm SEM. There are significant differences between groups with different superscripts in each time (a and b; $P < 0.05$)

According to figure 7, IP injection of LPS (50 μ g) significantly decreased the desire for a standard diet containing 20% more energy at 30 min post-injection. It also decreased the appetite for standard diets containing 20% more and 20% less energy than the standard at 60, 120, and 180 min post-injection ($P < 0.05$). The IP injection of LPS (50 μ g) increased the desire for a standard diet with fat, but no significant difference was observed, compared to the diets containing 20% more or less energy than the standard at different time points ($P > 0.05$).

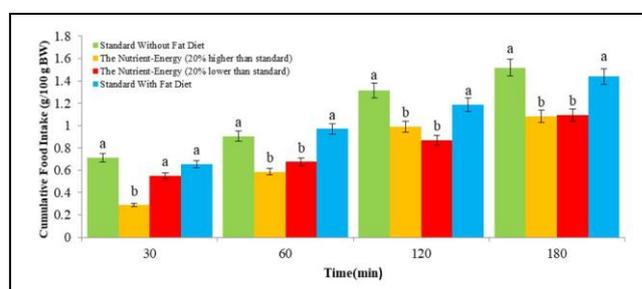


Figure 7. Effect of IP injection of LPS (50 μ g) in neonatal meat-type chickens fed different diets: standard diet without fat, diet containing nutrient energy 20 % higher than standard, diet containing nutrient energy 20 % lower than standard, and a standard diet containing fat. LPS: Lipopolysaccharide. Data are expressed as mean \pm SEM. There are significant differences between groups with different superscripts in each time (a and b; $P < 0.05$)

As seen in figure 8, the IP injection of LPS (100 μ g) significantly diminished the desire for a diet containing 20% more nutrient energy than the standard at 30 min after the injection. It also reduced the appetite for a diet containing 20% less energy at 60, 120, and 180 min post-injection ($P < 0.05$). In addition, LPS (100 μ g) increased the desire for a standard diet with fat ($P < 0.05$). Based on the experimental group nine, the IP injection of LPS (200 μ g) significantly increased the desire for a standard diet with fat and a standard diet containing 20% higher energy at 60, 120, and 180 min post-injection ($P < 0.05$) (Figure 9).

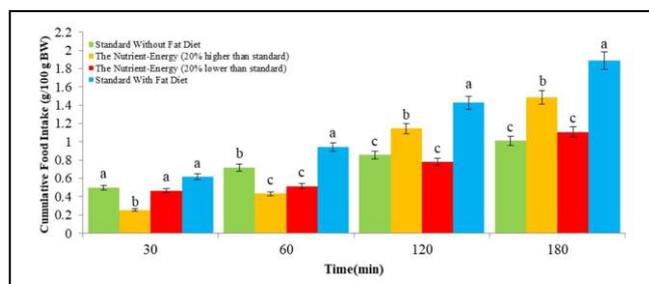


Figure 8. Effect of IP injection of LPS (100 µg) in neonatal meat-type chickens fed different diets: standard diet without fat, diet containing nutrient energy 20 % higher than standard, diet containing nutrient energy 20 % lower than standard, and a standard diet containing fat. LPS: Lipopolysaccharide. Data are expressed as mean ± SEM. There are significant differences between groups with different superscripts in each time (a, b, and c; $P < 0.05$)

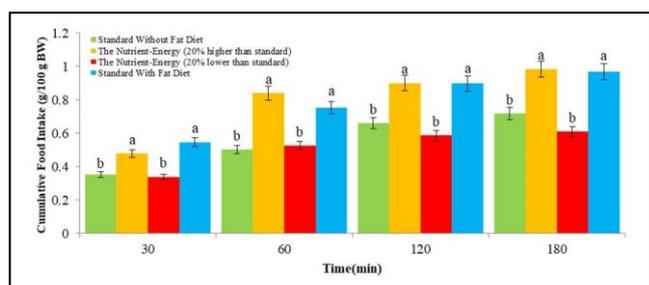


Figure 9. Effect of IP injection of LPS (200 µg) in neonatal meat-type chickens fed different diets: standard diet without fat, diet containing nutrient energy 20 % higher than standard, diet containing nutrient energy 20 % lower than standard, and a standard diet containing fat. LPS: Lipopolysaccharide. Data are expressed as mean ± SEM. There are significant differences between groups with different superscripts in each time (a and b; $P < 0.05$)

4. Discussion

This is the first report on the effects of the central and peripheral injection of the LPS on food intake using different rations in chicken. During bacterial infections, neurological networks exist between appetite regulation centers in the CNS and the immune system to modulate voluntary dietary intake. The LPS, which is released during rapid proliferation terms or bacteriolysis, stimulates several acute-phase reactions by inducing pro-inflammatory cytokine production, such as IL-1, TNF- α , IL-10, IL-6, and IL-1 β . Pro-inflammatory cytokines prevent orexigenic activity and stimulate anorexigenic neuropeptides in the brain (18). Moreover, systemic LPS treatment amplifies CART, a

peptide expressed in POMC/ β -endorphin-containing neurons of the arcuate nucleus that, when activated, suppresses feeding (6).

Furthermore, the LPS-induced hypophagia is decreased when the corticotropin-releasing factor (CRF) receptors are blocked (17). Moreover, it has been demonstrated that many anorectic agents exert their impacts through CRF neurons in chickens (17). The LPS is thought to play a role in food intake regulation and has an anorexigenic effect on chickens (1, 3, 19). This is in line with the findings of the present study, which indicated that LPS had anorexigenic activity in chicks.

Besides, based on the literature, there is a relationship between LPS and preference for ration choice. It has been reported that the IP injection of the LPS (100-400 µg) decreased food consumption and the absolute quantity of amino acids required but did not influence lysine and threonine consumption for protein accretion in chickens (9). According to the results of the current study, ICV injection of the LPS decreased the desire for the standard diets with 20% less and 20% more nutrient energy than the standard at 30 and 60 min post-injection. However, it increased the appetite for a standard diet with fat and a diet containing 20% higher energy than the standard at 120 and 180 min post-injection. Despite the hypophagic effect of the LPS, a significant difference was observed in the desire for the standard diet without fat and the standard diet containing 20% less energy than the standard. The IP injection of LPS significantly increased the desire for a standard diet with fat and a standard diet containing 20% more energy at 60, 120, and 180 min post-injection.

Previously, increased fat consumption has also been indicated to prefer the LPS-containing gram-negative bacteria at the expense of gram-positive bacteria in the gut (20). In human studies, an increase in postprandial endotoxemia was observed after a single high-fat meal (21). In addition, a protein-deficient diet has been seen to lower the metabolic responses to endotoxin and the capability of monocytes to create cytokines (10).

In most investigations performed on mammals, the impacts of IL-1 on animals with a low-protein or protein-free diet were evaluated by thermos effector responses to IL-1 from macrophage culture or endotoxin. Production of IL-1-like substances following the single injection of LPS was enhanced to a greater extent in chicks provided with a low-protein diet rather than the chicks fed on a high-protein diet, regardless of the LPS injection program (10). Results of the present study suggest that ICV and IP injection of LPS decreased food intake in chicks after 2 h. Moreover, LPS increased the desire of chickens for a standard diet with fat and a standard diet containing 20% more energy than the standard.

Finally, the findings showed that the desire of chickens for different types of rations is affected by central and peripheral administration of the LPS. More research projects are needed to underline the cellular and molecular signaling pathways of the effect of LPS on the desire of chickens for various types of rations.

Authors' Contribution

Study concept and design: H. H., M. Z., and A. A.

Acquisition of data: S. G.

Analysis and interpretation of data: M. Z.

Drafting of the manuscript: M. Z.

Revision of the manuscript: M. Z., H. H., A. A., and N. S.

Ethics

All experiment procedures were approved by the Faculty of Veterinary Medicine, Islamic Azad University, Science and Research Branch, Tehran, Iran.

Conflict of Interest

The authors declare that they have no conflict of interest.

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