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Interference of Salmonella typhimurium Lipopolysaccharide on Performance and Biological Parameters of Broiler Chickens

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ABSTRACT

This study was conducted to determine the interference of *Salmonella typhimurium* lipopolysaccharide (sLPS) on the performance, biological parameters, and histological evaluations of 198 one-day-old male broiler chickens divided into three treatments according to sLPS dose (0, 250, or 500 µg/application/bird) that was applied to the birds every other day, from 15 to 27 days of age. At the end of the experiment (28 days), significant effects were observed on body weight (R= -0.17 and P=0.05), total cholesterol serum levels(R=0.43 and p<0.01), phosphorus (R=0.53 and P<0.01), uric acid (R= -0.38 and P<0.01), C-reactive protein (R=0.68 and p<0.01), serum activity of aspartate aminotransferase (R=0.39 and p<0.01) and alkaline phosphatase (R= -0.39 and p<0.01). According to these results, sLPS mainly affect broiler biological parameters, but also their live performance.

INTRODUCTION

Lipopolysaccharide (LPS or Endotoxin) is the main component of Gram-negative bacterial cell wall, covering approximately 40% of the bacterial surface. The specific structure of LPS is different among bacterial species. However, in general, they have very similar molecular structures and can be divided in: Lipid A, Inner Core, Outer Core, and O Antigen. This antigen is responsible for immunogenic responses, and it is also the most variable part, which can be used to differentiate bacterial serotypes. Lipid A is a lipid portion of LPS, non variable among bacterial species (or serotypes), and it is responsible for LPS's toxicity (Liebers *et al*., 2008; Tuin, 2007).

The bacterial LPS is one of the most powerful activators of the immune system, leading to a non-specific inflammatory response (Mueller *et al*., 2004; Luyendyk *et al*., 2002). The environment of a poultry house can be highly contaminated with LPS in suspension, since the contamination of the facilities with Gram-negative bacteria is very common (Fernandes, 2005). Nevertheless, the presence of Gram-negative bacteria in the intestinal tract and the use of anti-bacterial drugs can increase poultry exposure to LPS. Extensive research has been conducted to determine the effects of LPS exposure in humans (Liebers et at., 2008; Roth *et al*., 1997) and mammals (Mueller *et al*., 2004; Luyendyk *et al*., 2002; Barton *et al*., 2000; Roth *et al*., 1997). However, there are few studies on the effects of LPS on broiler performance using *in vivo* procedures and with different inoculation doses.

The aim of this study was to provide information about the effects of *Salmonella typhimurium* Lipopolysaccharide (sLPS) on the performance, biochemical parameters, and histological evaluation in broilers.

MATERIAL AND METHODS

The experiment was conducted with the approval and according to the recommendations of the Ethics Committee on the Use of Animals on Research of the Federal University of Rio Grande do Sul under the process number 19847.

Birds, facilities, and management

This study was carried out using 198 one-day-old male Cobb broilers purchased from a local hatchery. The experiment was conducted at Instituto SAMITEC's experimental poultry farm, in Santa Maria, RS, Brazil (geographic coordinates: 29°42'44" South and 53°38'25" West).

The facilities consisted of a 20-m² room, under full-time air-conditioning and exhaustion to maintain the ideal environmental temperature and air quality for the birds, and a 24-h of light lighting program during the entire experiment. Birds were kept in 18 electrically-heated battery brooders, with wired floors from 1 to 28 days of age.

Feed and water were provided *ad libitum*, and the feed was formulated to provide the minimum levels (NRC, 1994) of crude protein (20.00%), energy (3,050 kcal/g), calcium (0.95%), phosphorus available (0.48%), methionine (0.39%), methionine+cystine (0.74%), and lysine (1.19%).

Experimental design

Birds were randomly divided into three completely randomized treatments with six replicates of 11 birds each, according to the dose of lipopolysaccharide applied as presented below:

Treatment 1: birds received 0.5 mL of sterile saline solution (control group);

Treatment 2: birds received 0.5 mL of a 500 µg/mL sLPS solution. This inoculation gave a total of 250 µg of sLPS/bird per application;

Treatment 3: birds received 0.5 mL of a 1,000 µg/mL sLPS solution. This inoculation gave a total of 500 µg of sLPS/bird per application.

Lipopolysaccharide administration

The *Salmonella typhimurium* lipopolysaccharide (sLPS) was purchased from Sigma USA (item L7261) and diluted in sterile saline solution to reach the desired concentration and dose per treatment. Each bird received 0.5 mL of its treatment solution every other day, starting on day 15 until day 27 (seven applications). The sLPS solution was administered to the birds using a variable-volume calibrated micropipette, disposing the solution into the crop.

The dose of sLPS used in this study was established as a fixed amount of sLPS/bird per application.

Evaluated variables and laboratorial analyses

All birds were weighed at the beginning and on 14 day of the experiment to determine the homogeneity of the groups, and at the end of the experiment for performance evaluation. Feed intake was also measured on days 14 and 28 of the experiment.

At the end of the experiment, birds were euthanized by cervical dislocation after CO_2 desensitization, for blood and tissue collection. Blood samples (18 per treatment) were collected from the cervical veins (after desensitization), centrifuged and the serum was stored at -20° C until analysis. Serum samples were analyzed for total plasma proteins (TPP), albumin (ALB), total cholesterol (COL), triglycerides (TRI), calcium (Ca), phosphorus (P), uric acid (UA), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyltransferase (GGT), alkaline phosphatase (AP), and C-Reactive Protein (CRP). All serum analyses were performed using commercial test kits (Labtest Diagnóstica S.A. – Brazil).

Livers from all birds were collected for relative weight calculation. For histological evaluation, one fragment of each tissue (bursa of Fabricius, thymus, spleen, heart, liver, pancreas, small intestine, kidney, and lung) was collected from six birds per treatment and processed according to the usual histopathological techniques (Luna, 1968).

Statistical analyses

All data obtained were submitted to simple regression analysis. Parameters that presented significance level higher than or equal to 90% ($p \leq 0.10$) were submitted to analysis of variance (one-way ANOVA), and their means were compared by Bonferroni's Multiple Range Test ($p \leq 0.05$). All statistical analyses were carried out using Statgraphics Centurion XV computer statistical program.

RESULTS AND DISCUSSION

On days 1 and 14 of the experiment, there were no differences ($p > 0.05$) in body weight or feed intake among the evaluated treatments, indicating the homogeneity required for performance evaluations.

Birds did not show any clinical signs of sLPS exposure, probably due to the low levels of sLPS applied or

the route of administration selected. Xie *et al*.(2000) described some clinical signs in broilers receiving sLPS as 5.0 mg/kg of body weight (average of 3.45 mg/bird), such as drowsiness, lethargy, and reduced feed and water intake. Those signs began within 1 hour after application of sLPS and persisted for at least four hours.

Performance results of broilers at 28 days of age are presented in Table 1. Body weight showed a significant (P=0.05) but low correlation (R= -0.17) with the sLPS doses used in this experiment. The comparison among treatments did not show any differences (p>0.05) in body weight, feed intake, or feed conversion rate. Guaiume (2005) obtained similar broiler performance results when intraperitonially injecting 0, 200, or 400 µg *E. coli* LPS/application/bird (applications occurred every other day, from 7 to 21 days of age). However, Xie *et al*. (2000) observed a significant reduction in relative body weight gain 12, 24, and 48 hours post injection. Mireles *et al*. (2005), in a series of experiments applying *E. coli* LPS subcutaneously, observed body weight reduction and a worsening of feed conversion rate of broilers at 24, 28, 30, 32, or 34 days of age.

Table 2 and Table 3 present the results of clinical biochemistry parameters measured in the serum of broilers per treatment. Table 2 shows the results for total cholesterol (mg/dL), phosphorus (mg/dL), and uric acid (mg/dL) levels, whilst Table 3 shows the results for aspartate aminotransferase (U/L) and alkaline phosphatase (U/L) activities, and C-reactive protein (mg/L) levels. The levels of total cholesterol, phosphorus, uric acid, and C-reactive protein, and the activity of aspartate aminotransferase and alkaline phosphatase were significantly affected (p≤0.10) by the levels of sLPS used in this experiment.

Total plasma protein, albumin, triglycerides, and calcium levels, and the activity of alanine aminotransferase and gamma glutamyltransferase, as well as the liver relative weight (data not shown) were not influenced (p>0.10) by the evaluated sLPS levels.

Total cholesterol (R=0.43) and phosphorus (R=0.53) levels significantly (p<0.01) increased in the presence of sLPS, whilst uric acid levels (R= -0.38) significantly (p<0.01) decreased. Aspartate aminotransferase activity (R=0.39) also significantly (p<0.01) increased with sLPS inoculation.

Hypercholesterolemia and hypouricemia are commonly associated with liver damage, hyperphosphatemia with kidney damage, and increased AST activity with both liver and kidney damage (Hochleithner, 1994). According to Harr (2002), most of the synthesis of uric acid occurs in the liver, and reduced serum levels of this metabolite suggest liver damage. Concomitant exposure to LPS reduced the capacity of the liver to metabolize substances that have their primary target at this organ. It is established that the co-administration of chlorpromazine, ethionine, deoxynivalenol, monocrotaline (Roth *et al*., 1997), or aflatoxin B_1 (Roth *et al*., 1997; Barton *et al*., 2000; Luyendyk *et al*., 2002) with small doses of LPS makes non-hepatotoxic doses of those substances potentially hepatotoxic, suggesting that some of the systemic effects of LPS are due to hepatic injury.

Xie *et al*. (2000) observed similar behavior of phosphorus levels in broilers receiving sLPS, and attributed this result to some transient impairment of kidney function during the acute phase of inflammatory reaction.

Table 1 – Performance of male broilers inoculated with Salmonella typhimurium lipopolysaccharide (sLPS), every other day, from 15 to 27 days of age.

sLPS[1]	BW[2]	(CV)[3]	FI[4]	(CV)	FCR[5]	(CV)
0	1,450.1[a]	(7.5)	2,375.8[a]	(3.1)	1.64[a]	(3.4)
250	1,422.0[a]	(9.4)	2,379.5[a]	(2.2)	1.67[a]	(2.7)
500	1,395.7[a]	(10.7)	2,326.3[a]	(3.4)	1.66[a]	(1.9)
p_{anova}	0,14		0.37		0.57	
Correlation Matrix						
Model	BW=1,449.8-0.109*LPS		NS[6]		NS	
R	-0.17					
p	0.05		0.23		0.49	

[a] Means in the same column with common superscript are not different by Bonferroni's test (p≤0.05).
[1] sLPS= Dose of *Salmonella typhimurium* lipopolysaccharide applied to each bird, every other day, from 15 to 27 days of age (µg/application/bird).
[2] Body weight (g).
[3] Coefficient of variation (%).
[4] Cumulative feed intake (g/bird).
[5] Feed conversion ratio (g/g).
[6] Not significant in simple regression analysis (p>0.10).

Table 2 – Total cholesterol, phosphorus, and uric acid serum levels of male broilers inoculated with *Salmonella typhimurium* lipopolysaccharide (sLPS), every other day, from 15 to 27 days of age.

sLPS[1]	CHOL[2]	(CV)[3]	P[4]	(CV)	UA[5]	(CV)
0	115.25[b]	(12.7)	11.59[b]	(15.7)	7.33[a]	(27.2)
250	123.91[ab]	(17.8)	13.40[a]	(12.2)	6.92[ab]	(14.4)
500	136.38[a]	(13.1)	14.52[a]	(16.6)	5.88[b]	(19.0)
p_{anova}	0.01		<0.01		0.03	
Correlation Matrix						
Model	COL=114.66+0.042*LPS		P=11.70+0.006*LPS		UA=7.43-0.003*LPS	
R	0.43		0.53		-0.38	
p	<0.01		<0.01		<0.01	

[a - b] Means in the same column with different superscripts are different by Bonferroni's test (p≤0.05).
[1] sLPS= Dose of *Salmonella typhimurium* lipopolysaccharide applied to each bird, every other day, from 15 to 27 days of age (µg/application/bird).
[2] Mean cholesterol serum levels (mg/dL).
[3] Coefficient of variation (%).
[4] Mean phosphorus serum levels (mg/dL).
[5] Mean uric acid serum levels (mg/dL).

There is little information on the reduction of AP activity; however, increased AP activity in avian species has been associated with increased cellular activity (rather than cell damage) predominantly in the duodenum and kidney, indicating that decreased activity of this enzyme can be related to reduced cellular activity, either in the small intestine or in the kidney (Hochleithner, 1994).

The levels of CRP (R=0.68) significantly (p<0.01) increased with sLPS doses. CRP is an acute-phase protein in inflammatory responses and it is stimulated by the presence of both interleucine-1 (IL-1) and interleucine-6 (IL-6) (Tizard, 2008). It is associated with various inflammatory conditions as a positive acute-phase protein and plays an important role in the protection against infections, clearance of damaged tissue, prevention of autoimmunity, and regulation of inflammatory responses (Chamanza *et al*., 1999; Juul-Madsen *et al.*, 2008). After entering the cell, LPS induces particularly, the production and secretion of IL-1, IL-6, and tumor necrosis factor (TNFα). The main cells involved in the production and secretion of those cytokines are macrophages, neutrophils, and endothelial cells (Fernandes, 2005; Tuin, 2007; Liebers *et al*., 2008). The observed increase in CRP levels in this experiment demonstrates that LPS is one of the most powerful activators of the immune system.

At histological evaluation, no lesions were found in any tissue of the birds of the control group. In birds receiving sLPS, only the liver and the kidney were affected. Microscopic kidney changes consisted of necrosis of the tubular epithelium and were more pronounced in birds receiving 500 µg sLPS, indicating a dose-response behavior. Alterations observed in the liver were hepatocellular vacuolization, hyperplasia of biliary ducts, hepatocellular degeneration, proliferation of biliary ducts, and lymphoid hyperplasia, and the severity of these lesions appeared to be the same in birds receiving either 250 or 500 µg sLPS. Barton *et al*. (2000) also showed microscopic changes in the liver of rats receiving LPS.

Table 3 – Aspartate aminotransferase, alkaline phosphatase, and C-reactive protein serum levels of male broilers inoculated with Salmonella typhimurium lipopolysaccharide (sLPS), every other day, from 15 to 27 days of age.

sLPS[1]	AST[2]	(CV)[3]	AP[4]	(CV)	CRP[5]	(CV)
0	248.92[b]	(15.7)	2,756.58[a]	(22.7)	144.0[b]	(35.6)
250	297.80[a]	(11.7)	2,066.17[ab]	(44.9)	163.2[b]	(28.4)
500	294.79[a]	(17.9)	1,872.00[b]	(47.5)	312.0[a]	(31.4)
p_{anova}	<0.01		0.02		<0.01	
Correlation Matrix						
Model	AST=257.7+0.091*LPS		AP=2,659-1.735*LPS		CRP=119.1+0.34*LPS	
R	0.39		-0.39		0.68	
p	<0.01		<0.01		<0.01	

[a - b] Means in the same column with different superscripts are different by Bonferroni's test (p≤0.05).
[1] sLPS= Dose of *Salmonella typhimurium* lipopolysaccharide applied to each bird, every other day, from 15 to 27 days of age (µg/application/bird).
[2] Mean aspartate aminotransferase activity in the serum (U/L).
[3] Coefficient of variation (%).
[4] Mean alkaline phosphatase activity in the serum (U/L).
[5] Mean C-reactive protein levels in the serum (mg/L).

CONCLUSIONS

The results of the present study demonstrate the impact of *Salmonella typhimurium* lipopolysaccharide on broilers. Despite the lack of statistical differences in performance among treatments, the presence of sLPS was significantly and negatively correlated with body weight at 28 days of age. Clinical biochemistry and inflammatory parameters were affected by the presence of sLPS, demonstrating the biological effect of this substance. As LPS is widely disseminated in poultry houses, some losses in broiler production may be currently overlooked due to a lack of understanding of the impact of LPS on broilers.

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