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# Clinical and hematological effects of *Salmonella gallinarum* endotoxin in cockerels

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**ABSTRACT**

A study was undertaken to investigate the effect of *Salmonella gallinarum* endotoxin on clinical symptoms and some haematological changes in mature six-month-old cockerels. *Salmonella gallinarum* endotoxin caused characteristic clinical symptoms, severe olygocytic hypochromic anaemia, leucocytosis and increased phagocytic function of leucocytes. Heterophilia was associated with relative lyphopenia. The endotoxin manifested pyrogenic activity. The demonstrated signs were similar to those produced in chickens with experimental fowl typhoid. Together, these features suggest that *Salmonella gallinarum* endotoxin has been a contributor to the main manifestation of experimental fowl typhoid in birds.

**Key words:** *Salmonella gallinarum*, endotoxin, haematological changes, pyrogenic activity, cockerels

## Introduction

It is well recognized that bacterial endotoxin release during sepsis stimulates the production of a variety of inflammatory mediators, resulting in dramatic pathophysiological reactions (KLUGER, 1991; HOLST et al., 1996;

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COLLINS, 1996). The study of bacterial endotoxin involves a broad spectrum of goals to understand in detail the molecular mechanism(s) by which this unique microbial product contributes to the changes observed in patients with Gram-negative infections. Little is known about lipopolysaccharide (LPS) activity in poultry, and experimental data are conflicting and divergent (IVANOV, 1977; SMITH et al., 1978; JONES et al., 1983).

Fowl typhoid remains a disease of worldwide significance (SHIVAPRASAD, 1997). Investigations into the interaction between poultry and *Salmonella gallinarum* (*S. gallinarum*) endotoxin will elucidate the pathways by which LPS manifests its potentially deleterious effect and will provide essential information for effective therapeutic intervention in the treatment of this disease.

In this study, we show results of the action of *S. gallinarum* endotoxin on clinical symptoms and changes in some haematological parameters in cockerels.

## Materials and methods

Seven cockerels, salmonella-free six-month-old SL, bred for egg laying and weighing approximately 2 kg each (purchased from a commercial farm) were used. The cockerels were given an intravenous (i.v.) injection of 50 mg/kg body mass *S. gallinarum* endotoxin. Endotoxin from *S. gallinarum* was extracted by the phenol method (WESTPHAL et al., 1952). Clinical observation and blood samples were obtained at 0, 1, 3, 5 and 24 hours (h) after inoculation and red blood cells (RBC), haemoglobin concentration (Hb), total white blood cells (WBCs) and phagocytosis, were determined as previously described (KOKOSHAROV and TODOROVA, 1987; KOKOSHAROV, 1998). Body temperature was recorded daily. All glassware, syringes, and needles were heated for 30 min. at 25 °C to exclude pyrogenic activity.

The trial was conducted in two replicates. All results were statistically analyzed using the Student-Fisher test.

## Results

The cockerels became obviously ill within one hour of i.v. injection of *S. gallinarum* endotoxin. They sought the corners of the cages, were

depressed, reluctant to move, manifested somnolence, loss of appetite and inhibition in drinking. A thin, yellowish diarrhoea appeared. These behavioural changes were pronounced for about 4-5 hours and then disappeared gradually, recovery being complete within 24 hours. None of the cockerels died. After one hour latency period the cockerels developed fever, and temperature increased significantly by 0.7, 0.6 and 0.5 $^{o}$C and 24 h post injection of LPS (Table 1).

The RBC count dropped significantly as early as the 3$^{rd}$ h following experimental endotoxemia, with a gradually lowering in values up to 5 h, inclusive (2.24 ± 0.35 - 1.79 ± 0.34×10$^{6}$ ml, on 3$^{rd}$ - 5$^{th}$ h respectively.). The values of the haemoglobin also diminished after the 3$^{rd}$ h (Table 1).

Table 1. Dynamics of temperature reaction, red and white blood cells for cockerels, injected i.v. with *Salmonella gallinarum* endotoxin (n=7 in each group).

| Index | Hours after experimental endotoxemia | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 3 | 5 | 24 |
| Tb $^{o}$C (1) | 41.5 ± 0.3 | 41.6 ± 0.3 | 42.2 ± 0.2 * | 42.1 ± 0.5 * | 42.0 ± 0.2 |
| RBC 10$^{6}$/ml (2) | 3.84 ± 0.12 | 2.83 ± 0.37 | 2.24 ± 0.35 * | 1.79 ± 0.34 * | 2.79 ± 0.18 |
| Hb (3) | 12.57 ± 0.64 | 13.33 ± 1.11 | 10.91 ± 1.45 * | 11.68 ± 1.84 * | 9.75 ± 1.49 |
| WBCs10$^{9}$/L (4) | 2.69 ± 0.60 | 5.27 ± 2.85 * | 7.03 ± 2.18 * | 7.62 ± 2.42 * | 4.01 ± 0.34 |
| Myelocites % | 5.50 | 9.83 | 14.00 | 16.00 | 11.66 |
| Bandnuclear % | 3.83 | 8.00 | 15.60 | 17.20 | 10.50 |
| Segment nuclear % | 9.50 | 16.66 | 20.60 | 29.20 | 19.00 |
| Total heterophils % | 17.83 | 34.49 | 50.20 | 62.40 | 41.16 |
| Monocytes % | 1.33 | 1.16 | 0.60 | 1.20 | 0.60 |
| Lymphopcytes % | 79.82 | 64.83 | 49.20 | 36.40 | 56.50 |

* - significantly different from control (P<0.05), 1 - body temperature, 2 - red blood cells, 3 - hemoglobin concentration, 4 - white blood cells

Leucocytosis was observed on the 1$^{st}$ h after injection of *S. gallinarum* endotoxin and this continued until the 24$^{th}$ h, inclusive (5.27 ± 2.85, 4.01 ± 0.34×10$^{9}$/L). Changes were significant. The percentages of total heterophils rose at the 1$^{st}$ h and continued to augment until the 5$^{th}$ h post injection. Observed granulocytosis was characterized by myelocytosis and an enhanced rate of the young white blood cells from the 1$^{st}$ to the 24$^{th}$ h. The

peak occurred at the 5th h. The level of lymphocytes was less than the baseline value.

Monocytes remained in normal range (Table 1). Basophilic and eosinophilic cells did not change their values (data not shown).

Phagocytes by the circulating leucocytes after experimental endotoxemia from *S. gallinarum* were elevated in the period between the 3rd and 5th h. The difference was significant. The values of these indexes re-established after the 5th h (Table 2).

Table 2. Changes in the values of phagocytosis in cockerels with *Salmonella gallinarum* endotoxemia (n=7 in each group)

| Index | Hours after experimental endotoxemia | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 3 | 5 | 24 |
| P A | 0.79 ± 0.15 | 0.88 ± 0.10 | 0.98 ± 0.06 * | 0.98 ± 0.02 * | 0.75 ± 0.22 |
| P N | 2.98 ± 1.00 | 3.09 ± 1.36 | 5.03 ± 1.04 * | 4.77 ± 0.22 * | 2.55 ± 1.25 * |
| P I | 2.44 ± 1.25 | 3.07 ± 1.61 | 4.93 ± 1.18 * | 4.61 ± 0.13 * | 2.78 ± 1.10 |

P A = phagocytic activity; P N = phagocytic number; P I = phagocytic index; * = significantly different from control ($P<0.05$)

## Discussion

Our results corresponded to those of other authors (IVANOV, 1977; SMITH et al., 1978; JONES et al., 1983) who found similar clinical symptoms in poultry treated with LPS from other Gram-negative bacteria than *S. gallinarum.* The current study indicated a well demonstrated pyrogenic reaction in mature six-month-old cockerels after a single i.v. injection of *S. gallinarum* endotoxin. Immature birds treated with bacterial endotoxin usually developed a non-significant hypothermia, followed later by moderate hyperhermia (SMITH et al., 1978; JONES et. al., 1983). They concluded that the thermoregulatory ability of younger chickens was not firmly established. However, when endotoxin was given for 3 to 6 consecutive days, significant biphasic increases in temperature were observed (SMITH et al., 1978). It is possible that a prior sensitization is a requisite for fever reaction. Endotoxin

Table 1. Specification of myomorphus mammals examined by renoculture and microscopic agglutination acording to the trapping area with corresponding results



of intestinal origin already present in host tissue in older animals and man could contribute to the toxic effect of additional exogenous endotoxin (WALKER et al., 1975). It is thought that this was the cause of hyperthermic response in mature birds to *S. gallinarum* endotoxin. Fever is the key symptom in all definitions of septic shock. Whereas fever is the most frequent thermoregulatory manifestation of sepsis and septic shock, hypothermia occurs in the most severe cases of shock and is believed to significantly worsen the patient's prognosis (ROMANOVSKI et al., 1996). The mortality of the animals was inversely survival-evident at body temperature (KLUGER, 1991). Used in our study, *S. gallinarum* endotoxin was not lethal.

We established progressive declines in RBC count and a hypochromic nature of anaemia in mature birds. The effect of LPS from *S. gallinarum* on RBC values was probably responsible for the severe anaemia detected in endotoxemic cockerels. The olygocytic hypochromic nature of anaemia in the cockerels indicated that *S. gallinarum* LPS provokes a sudden loss of red cells in the circulation, and perhaps membrane damage in the circulating red cells. Total WBC count was very rapidly enhanced to two and more times control values as early as the 1$^{st}$ to the 24$^{th}$ h. Leucocytosis after a single injection of *S. gallinarum* endotoxin in mature birds was significant. This was in agreement with the experimental model, where peak level of myelocytes and young white blood cells were enhanced one hour after the experimental endotoxemia in cockerels. This showed that the bone marrow was still able to produce young granulocytes and was good prognostic sign. The differentiation, proliferation and function of haematopoietic cells are controlled and regulated by a network of cytokines (SACHS and LOTEM, 1994). TNF-alfa and IL-1 have been determined as central mediators (LONG, 1996) and induced clinical symptoms and haematological changes in animals with endotoxemia. TNF-alfa-induced inhibition in water consumption (CALAPAI et al., 1991), also a colony-stimulating factor (CSF) to regulate and stimulate granulopoesis (KOGUT et al., 1997). CSF leads to the production of IL-1 and enhancement in the production of interferon in the presence of endotoxin. This was linked to the increased production of endorphins, which paralysed gastric emptying. The intestinal tract became hypermotile and diarrhoea appeared (BERRY and GASKA, 1983). Endogenous pyrogen in the cytokine IL-1 produced by macrophages and

monocytes, circulating in the blood stream and impinged on thermosensitive neurons in the anterior hypothalamus (TIMONEY et al., 1992).

Heterophils were associated with a relative lymphopenia. Lymphopenia during endotoxemia is likely to be the result of the increase of ACTH and subsequent increase in endogenous cortisol (DELGAR et al., 1984). At the same time, in this article we report for the first time enhancement of the phagocytic function of the leucocytes in *S. gallinarum* endotoxemic cockerels. From the 3rd h post i.v. injection we observed a growth in the percentage of active phagocytes, and the number of engulfed bacteria from one Lc, and engulfed function of the Lc. Some cytokines also enhance phagocytosis and bacterial eradication (LONG, 1996).

Our results showed that i.v. injection of purified *S. gallinarum* endotoxin cause similar signs to those produced in chickens with experimental fowl typhoid (KOKOSHAROV and TODOROVA, 1987; KOKOSHAROV et al., 1997; KOKOSHAROV, 1998). Our experimental observations make a strong case for the importance of *S. gallinarum* endotoxin as a key factor in the pathogenesis of fowl typhoid. This function of *S. gallinarum* endotoxin is related directly to the virulence of this microorganism.

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**SAŽETAK**

Istraživanje je provedeno radi utvrđivanja učinka endotoksina bakterije *Salmonella gallinarum* na neke kliničke i hematološke odrednice u šestomjesečnih pijetlova. Ustanovljeno je da endotoksin uzrokuje karakteristične kliničke znakove i oligocitnu hipokromnu anemiju, leukocitozu, te pojačanu sposobnost fagocitoze leukocita. Osim toga učinak se endotoksina očituje i pojavom heterofilije i limfopenije te povišenom tjelesnom temperaturom. Zbog velike sličnosti znakova uzrokovanih endotoksinom i onih koji prate kokošji tif, autori ističu povezanost endotoksina s kliničkim znakovima bolesti.

**Ključne riječi:** *Salmonella gallinarum*, endotoksin, hematološke promjene, pirogeni učinak, pijetlovi