Biochemical Changes in Fowl Serum during Infection with *Salmonella* Typhimurium

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ABSTRACT. Two groups of White Leghorns were inoculated with *Salmonella* Typhimurium in the breast muscle. One group was injected with amikacin every 9 hr (8 times) from 24 to 96 hr after the bacterial inoculation and the other group was given no amikacin. In both groups, a significant increase in the levels of aspartate aminotransferase and creatine kinase, and a significant decrease in the levels of alkaline phosphatase, total cholesterol and glucose were found 96 hr after inoculation. There were, however, no significant differences in the blood chemical values between the amikacin-treated group and non-treated group. The results from the present study may provide basic data for further investigation of blood chemical values during *Salmonella* infection and amikacin therapy in fowls. — KEY WORDS: blood chemistry, fowl, *Salmonella* Typhimurium.

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Measurement of changes in the level of blood chemicals has become a useful technique for detecting and identifying avian diseases [2]. It is, however, often difficult for avian veterinarians to submit a serum sample to a laboratory and receive a numerical value for each test. Commercial laboratories provide analysis report values from a predetermined battery of tests that are established for human or domestic mammalian patients [6].

Changes in blood chemical values have been reported for various infectious diseases in poultry [8–11], but little is known about changes in blood chemical values during *Salmonella* infection, which is one of the most common infectious diseases in the poultry industry. There is also a lack of information concerning blood chemical changes during the treatment of infectious diseases.

The aim of this study was to clarify the changes in blood chemical values during *Salmonella* infection in poultry and to investigate the effects of antibiotic treatment on blood chemistry. Amikacin was chosen for the experiments as it is one of the most commonly used antibiotics in bird medicine [1, 7].

Experiments were carried out on 12 clinically normal White Leghorn fowls (average age: 6 months, average weight: 1,570 g), which were obtained from Sankyo Labo Service, Inc. (Tokyo). The birds were housed in cages at 23°C and had free access to food and water during the trial period. Amikacin sulfate (10 mg/m*l*) was obtained from Schering-Plough Co. (Osaka), and the drug was used without any dilution.

Salmonella Typhimurium strain No. 31, which was isolated from fowls in a aviary, was used in the experiments. The strain was inoculated in Trypticase soy broth and incubated at 37°C for 24 hr. The bacterial culture was centrifuged at 5,000 rpm for 15 min and washed with phosphate buffered saline (PBS). The bacterial pellet was suspended in PBS and diluted to approximately 10^9 CFU/m*l*. Diluted broth cultures (1.0 m*l*) were used for inoculation.

The birds were evenly divided in two groups: Group I and Group II. All birds were administered 1.0 m*l* of bacterial suspension in the right breast muscle. Twenty four hr after a bacterial inoculation, the birds in Group I were injected with 20 mg/kg of amikacin in the left pectoral muscle every 9 hr for 72 hr. The dosage rate and the injection interval were determined according to the usual therapeutic methods [1, 7]. No injections were given to the birds in Group II after bacterial inoculation. Blood samples (3 m*l*) were collected from the wing vein before *Salmonella* inoculation (pre-experiment) and 96 hr after inoculation (post-experiment). After coagulation of the blood, serum was collected and assayed for blood chemistry. The levels of total serum protein, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), total cholesterol, uric acid, and glucose were determined by the usual laboratory methods. The assays were carried out using a 7150 Automatic Blood Chemistry Analyzer (Hitachi Co., Ltd., Tokyo). Significant differences between pre-experiment and post-experiment values in Group I and Group II were determined by the Student's *t*-test. The general condition of the birds during the experiments was also investigated.

After inoculation, the fowls developed clinical signs of lethargy, anorexia, polydipsia and diarrhea. These general signs were more severe in Group II birds than in Group I birds. The birds in Group II showed a complete loss of appetite, while the birds in Group I maintained about one fourth of normal food intake.

A significant decrease in the level of ALP, total cholesterol and glucose, and a significant increase in the level of AST and CK in the sera of the infected fowls were observed in both groups (Table 1). No significant differences were observed between the pre-experiment and post-experiment levels of total protein, ALT, and uric acid.

No significant differences between Group I and Group II were observed in pre-experiment and post-experiment blood chemical values (Table 1). As the pre-experiment chemical values were similar to those reported in normal fowls [3], the pre-experiment data of Group I and Group II were considered to be normal.

According to previous reports, the TP levels in fowls with infectious diseases varies according to the disease. For example, the TP level was reported to be increased in

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Table 1. Blood chemical values in amikacin-treated group (Group I) and non-treated group (Group II)

Group I	Pre-experiment		Post-experiment	
	Average	SD	Average	SD
Total protein (g/dl)	4.58	0.41	4.48	0.44
ALP (KA-unit)	66.1	34.5	16.0	12.1[a)]
AST (IU/L)	207	40	504	219[b)]
ALT (IU/L)	2.33	0.47	3.33	1.97
CK (IU/L)	1840	730	11280	3800[a)]
Total cholesterol (mg/dl)	145	14	94	21[a)]
Uric acid (mg/dl)	5.30	0.65	4.67	2.17
Glucose (mg/dl)	243	12	174	22[a)]
Group II	**Pre-experiment**		**Post-experiment**	
	Average	SD	Average	SD
Total protein (g/dl)	4.70	0.44	4.23	0.60
ALP (KA-unit)	53.0	20.7	7.1	4.5[a)]
AST (IU/L)	205	38	802	434[b)]
ALT (IU/L)	3.00	1.53	5.00	2.83
CK (IU/L)	1970	700	11430	7280[b)]
Total cholesterol (mg/dl)	140	23	80	15[a)]
Uric acid (mg/dl)	5.42	1.10	6.80	2.22
Glucose (mg/dl)	244	19	189	25[a)]

a) Statistically significant ($P<0.01$) between pre-experiment and post-experiment.
b) Statistically significant ($P<0.05$) between pre-experiment and post-experiment.
No significant difference was seen between Group I pre-experiment, and Group II pre-experiment.
No significant difference was seen between Group I post-experiment, and Group II post-experiment.

spirochaetosis but decreased in Newcastle disease [9, 10]. In the present study, the TP level showed no significant change following *Salmonella* infection.

Previous reports have shown that the ALP level in fowls decreased with spirochaetosis or Newcastle disease, and increased with Marek's disease [8, 10, 11]. A decrease in the ALP level in spirochaetosis or Newcastle disease is attributed to damage in the intestine, anorexia or low food intake. S. Typhimurium-infected chickens in the present study developed signs of reduced food consumption and reduced ALP levels, which suggest that the reduced ALP levels is partially due to poor food intake.

AST and CK values increased significantly following *Salmonella* infection in both Group I and Group II, while AST showed no significant increase. Elevated AST levels reported in infectious diseases are considered to be due to damage of the infected organ [8, 10]. Itoh *et al.* [5] reported an increase in the level of some serum enzymes after organ injury. They reported that AST, ALT, and CK levels in budgerigars are sensitive markers of muscle injury. However in the present study, ALT did not increase during *Salmonella* infection, and this discrepancy may be due to species differences or the degree of organ injury. AST, ALT and CK levels were increased in the blood of normal budgerigars injected with gentamicin, which is an aminoglycoside like amikacin [4], but in the present study, there were no significant differences in blood chemical levels between the amikacin-treated group and non-treated group. This suggests that amikacin has no major effect on blood chemical levels in infected fowls.

As hypoglycemia from starvation was reported by Lewandowski *et al.* [6], the decrease in glucose in the present study was thought to be due to a reduction in food intake.

A decrease in the cholesterol level in fowls infected with spirochaetosis or a velogenic strain of Newcastle disease has also been reported [9, 10], although no change in cholesterol levels was observed in fowls infected with a mesogenic strain or lentogenic strain of Newcastle disease [9]. The decrease in cholesterol levels was previously thought to be due to a reduction in food intake, but recently, it has been found that the human monocyte colony-stimulating factor plays a role in lowering the blood cholesterol levels [12]. Further investigation is needed to clarify the changes in the cholesterol level in infectious fowls.

Elevated levels of uric acid were also observed in fowls with Newcastle disease, and this was considered to be due to damage of the kidney [9], but in the present study, the level of uric acid showed no change, suggesting the severity of renal damage from infectious diseases varies according to the infecting organism and/or the state of hosts.

Although blood collection was carried out only once after *Salmonella* inoculation in the present study, some blood chemical changes due to a direct or indirect effect of infection were detected. The data from this study can be used as a basis for further study to clarify blood chemical changes during *Salmonella* infection and during the treatment period.

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