

DOI 10.1515/pjvs-2015-0010

Original article

# Biochemical parameters in Japanese quails *Coturnix coturnix japonica* infected with coccidia and treated with Toltrazuril

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## Abstract

The activity of aspartate aminotransferase, alanine aminotransferase and lactate dehydrogenase, total protein, albumin and cholesterol levels were determined in the blood serum of Japanese quails infected with coccidia and treated with Baycox (active ingredient: toltrazuril). Lower levels of AST and ALT activity were noted in treated birds regardless of the applied Baycox dose. The biochemical changes observed in the blood serum of Japanese quails point to coccidia-induced damage of digestive system tissues despite an absence of pronounced clinical symptoms. Significantly lower levels of AST activity and higher levels of LDH activity in treated birds indicate that coccidiosis treatment with toltrazuril contributed to the regeneration of digestive system tissues. An insignificant increase in cholesterol levels was noted, whereas the other serum biochemical parameters remained within the reference ranges.

**Key words:** *Coturnix coturnix japonica*, serum, toltrazuril, biochemical parameters

## Introduction

The Japanese quail (*Coturnix coturnix japonica*) is a poultry species that is reared mainly for eggs and meat. Intensive production systems where birds are kept on litter in confined spaces contribute to the spread of infections, including coccidiosis caused by *Eimeria uzura*, *E. bateri*, *E. taldykurganica* and *E. tsunodai* (Tsunoda and Muraki 1971, Tsutsumi 1972, Teixeira et al. 2004, Bashtar et al. 2010, Gesek et al. 2014). Young quails generally develop acute coccidiosis, sometimes without evident symptoms of diarrhea, whereas a subclinical form of the disease is noted

in older birds. In routine diagnosis of intestinal coccidiosis, the presence of oocysts is determined by parasitological examinations of fecal samples. Blood tests are generally not performed, although serum biochemical parameters are species-specific and are influenced by the birds' age, sex and diet (Scholtz et al. 2009). Japanese quails are small and highly mobile birds, therefore, blood sampling is technically difficult and requires sacrifice, therefore there are practically no publications describing the biochemical changes in the blood in the course of subclinical coccidiosis in this birds.

The aim of this study was to determine the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH), total protein (TP), albumin (ALB) and cholesterol levels in the blood serum of Japanese quails infected with coccidia and treated with Baycox 2.5% (active ingredient: toltrazuril, TOL). Blood for biochemical analyses was sampled at sacrifice after 14 days of treatment upon the consent of the Local Ethics Committee. Coccidia species were evaluated and subjected to a parasitological analysis, and histopathological changes in the internal organs of TOL-treated birds were determined in accordance with the methods described by Sokół et al. (2014).

## Materials and Methods

The quails were obtained from a commercial quail flock of 10,000 birds reared for eggs. The birds were kept on litter until 21 days of age, after which they were moved to cages with *ad libitum* access to water. They were fed a complete diet containing 27% total protein (TP) and 3000 kcal/kg metabolizable energy (ME) until the age of 21 days, 24% TP and 2900 kcal/kg between the age of 21 and 42 days, and 20% TP and 2800 kcal/kg ME beginning at the age of 42 days. There was no vaccination program in the flock.

Feces samples collected from birds aged 80 days were analyzed with the use of Darling's floatation solution (50% saturated sodium chloride + 50% glycerol) to reveal the presence of numerous oocysts (OPG  $7.4 \times 10^5$ ) of two coccidia species: *E. tsunodai* and *E. bateri* (natural infection). A post-mortem examination of tissue samples demonstrated inflammatory changes in the cecum (dilated intestinal lumen, mucosal lesions, blood in the intestinal contents) that scored 2 points on the scale proposed by Johnson and Reid (1970). A histopathological analysis revealed the presence of various developmental stages of the coccidia in duodenal, jejunal and cecal mucosa.

The birds were divided into four groups of 20 individuals per cage, and the experimental quails were administered Baycox in drinking water. Group I was administered treatment at 1.5 ml/1 l H<sub>2</sub>O (7 mg TOL/kg BW/day), and group II – at 3 ml/1 l H<sub>2</sub>O (14 mg TOL/kg BW). In groups I and II, the solutions were administered twice and were available *ad libitum* 24 h/24 h. Group III was administered treatment at 5 ml/1 l H<sub>2</sub>O (24.5 mg TOL/kg BW) on two occasions, available 8 h/24 h. Group C (control) was not treated with Baycox. After treatment, fecal samples were collected daily from every group to evaluate the effect of TOL on coccidia counts. A post-mortem test and a histopathological evaluation were performed at the end of the experiment.

Blood samples were collected by rapid decapitation into tubes containing EDTA. The time between removing the birds from cages and blood sampling was 20 seconds. Blood samples were centrifuged at 2500 x g for 10 minutes. AST and ALT serum activity were determined by the IFCC kinetic method, LDH serum activity – by the DGKC kinetic method, TP levels – by the biuret test, ALB levels – by the bromocresol green method, and cholesterol levels – by the colorimetric method with cholesterol esterase and oxidase. Measurements were performed with the use of the Cormay® ACCENT-200 chemistry analyzer and Cormay® reagents.

The results were processed by ANOVA – Duncan's test ( $p \leq 0.05$  and  $p \leq 0.01$ ) in the Statistica 9 for Windows application.

## Results

Between experimental days 3 and 12, the number of excreted oocysts decreased to  $0.1-2 \times 10^5$  OPG in all bird groups treated with Baycox. An insignificant increase in oocyst counts was noted on days 13 and 14. On day 14, a *post-mortem* examination revealed changes in the intestines (0-1 points), and a small number of *Eimeria spp.* at various developmental stages was noted in the histopathological analysis. In the control group, OPG reached  $17 \times 10^5$  at the end of the experiment and intestinal changes scored 2-3 points.

The results of biochemical analyses are presented in Table 1. A highly significant decrease in AST activity ( $p \leq 0.01$ ) was observed in treated birds relative to controls (140.50 U/l). AST activity were determined at 64.67 (U/l) in group I, 83.33 (U/l) in group II, and 74.33 (U/l) in group III.

LDH activities were significantly higher ( $p \leq 0.05$ ) in treated birds than in controls (416.50 U/l), and they were determined at 837.0 (U/l) in group I, 440.33 (U/l) in group II, and 624.0 (U/l) in group III.

ALT activity, TP and ALB levels differed insignificantly between groups and remained within the reference ranges (6).

Cholesterol concentrations were insignificantly higher in group C (5.15 mmol/l) than in group I (4.35 mmol/l), group II (4.81 mmol/l) and group III (4.79 mmol/l).

## Discussion

Biochemical profile blood tests are rarely performed in routine diagnosis of coccidiosis regardless of bird species. Serum chemistry reference values for male and female Japanese quails are cited in litera-

Table 1. Average values of selected serum biochemical parameters in Japanese quails administered various doses of TOL.

Parameter		TOL dose in ml/group				Reference range Scholtz et al. 2009
		1.5/I	3/II	5/III	0/C	
ALT (U/l)	$\bar{x}$	5.33	3.00	4.00	6.25	4.5-8.5
	SD	1.45	0.58	1.15	1.11	
AST (U/l)	$\bar{x}$	64.67 <sup>A</sup>	83.33 <sup>ABa</sup>	74.33 <sup>A</sup>	140.50 <sup>Bb</sup>	243-562
	SD	13.87	15.06	19.03	5.97	
LDH (U/l)	$\bar{x}$	837.00 <sup>a</sup>	440.33 <sup>b</sup>	624.00 <sup>ab</sup>	416.50 <sup>b</sup>	–
	SD	179.92	14.15	145.14	66.80	
Total protein (g/l)	$\bar{x}$	37.27	39.37	33.27	38.43	29.7-43.3
	SD	4.56	2.24	7.89	2.48	
Albumin (g/l)	$\bar{x}$	15.57	16.80	12.27	14.33	12.6-18.0
	SD	1.94	1.23	4.66	1.39	
Cholesterol (mmol/l)	$\bar{x}$	4.65	4.81	4.79	5.15	4.1-9.9
	SD	1.23	0.41	0.73	0.40	

Key: uppercase letters –  $p \leq 0.01$ , lowercase letters –  $p \leq 0.05$ .

ture (Scholtz et al. 2009). In this study, selected biochemical parameters were used to obtain reliable results that would contribute to effective diagnosis of coccidiosis. Since all examined birds were naturally infected with coccidia, obtained biochemical values were compared to reference ranges for quail hens proposed by Scholtz et al. (2009), and then the treated groups to the untreated one. In all groups of birds the values of TP, albumin, cholesterol, and ALT activity were within the normal range except AST activity which was lower in all examined birds.

A similar trend in the behavior of biochemical parameters was observed by Szweda et al. (2012) in dogs in the course of subclinical intestinal coccidiosis. AST activity was significantly increased during subsequent studies when oocysts were present in fecal samples, but significantly decreased to the reference value after treatment with Baycox. Koynarski et al. (2010) recorded the marked increase in AST and ALT in the course of a full-blown coccidiosis in chickens complicated by *E. coli* infection, but these results are difficult to compare with subclinical coccidiosis observed in examined quails due to the different severity of the disease.

AST and ALT are enzymes that are useful in detecting injury to liver parenchymal cells. They are also present in other tissues, including erythrocytes and striated muscles. In birds, elevated AST and ALT activity are indicative of damage to hepatocytes and intestinal mucosal cells (Scholtz et al. 2009, Koynarski et al. 2010), which also accompanies coccidiosis. LDH, an enzyme that belongs to the class of oxidoreductases, is present in the heart, kidneys, liver, muscles and other tissues. Large amounts of LDH enter the blood serum as a result of cell death

or increased permeability of cell membranes caused by anemia, electrolyte imbalance or toxin poisoning. Increased levels of LDH activity are observed in diseases accompanied by necrosis of intestinal mucosa resulting from intracellular proliferation of protozoa.

In the subclinical form of coccidiosis without evident symptoms (diarrhea), changes in enzyme activity levels can serve as additional indicators of damage to liver and intestinal cells. Enzyme activity levels in blood sampled during a *post-mortem* examination thus contribute to effective diagnosis of coccidia infections. In this study, lower AST activity in the experimental birds indicate that the TOL-induced elimination of coccidia contributed to the regeneration of mucosal membranes without causing hepatotoxic effects, which differentiates toltrazuril from other anti-parasitic drugs (Nazifi and Asasi 2001).

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