

HAEMATOLOGIC AND BLOOD ELECTROLYTE CHANGES IN BROILER CHICKENS, EXPERIMENTALLY INVADED WITH *EIMERIA TENELLA*

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Summary

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The influence of *Eimeria tenella* infestation on haematological parameters and potassium, sodium, inorganic phosphate, calcium and magnesium serum concentrations in 3-week-old broiler chickens was studied. The experiment was performed on 120 one-day old chickens divided into 2 groups – experimental and control. The parameters were followed prior to the invasion and at days 3, 5, 7 and 9 afterwards.

A decrease in red blood cell counts by 29–59%, in haemoglobin level by 30% and haematocrit was observed within the period from the 5th to the 7th day following artificial infestation. Simultaneously, an increase in eosinophils (by 49–102%) and lymphocytes was registered. The concentrations of serum sodium, calcium and inorganic phosphate in experimental birds tended to decrease during the whole period of the study. At the same time, a statistically significant elevation in serum potassium levels between days 7–9 occurred.

Key words: blood electrolytes, *Eimeria tenella*, haemoglobin, red blood cells, white blood cells

INTRODUCTION

Eimeriosis is a disease, causing considerable economic losses to modern poultry breeding industry. *Eimeria tenella* is the most pathogenic species for chickens (Lozanov, 1980; Weber, 1997). In previous studies of ours we reported that it is the commonest *Eimeria* species, spread on chickens in our country (Koinarski et al., 1997). The mortality in invaded chickens can reach up to 60–80%.

The pathogenic influence of *E. tenella* on avian organism has been studied by numerous authors (Natt, 1956; Mukkur and Bradley, 1969; Kogut et al., 1984; Ruff and Augustine, 1982). The studies

included the haematological changes and those in serum macro- and trace elements. The results in most cases were contradictory, most probably because of strain, age- and resistance-related differences among birds.

The complex interactions between the protozoa and the host are still unclear as well as the pathogenetic mechanism of the influence of eimeriae on avian organism. Thus our aim was to elucidate some points of the pathogenesis of *E. tenella* invasion and in particular, the influence upon some haematological and serum biochemical parameters in chickens.

MATERIALS AND METHODS

The experiment was performed on 120 one day-old broiler chickens (Cornish & Plimouthrock crossbreds) from both genders. They were housed in cages, on a grilled floor and under conditions excluding an additional invasion with *Eimeria*. The light and temperature regimens were suitable for their age. The birds were fed combined forage (starter composite for broiler chickens) without antibiotics and coccidiostatics. At the age of 15 days the birds were divided into 2 groups with 60 birds* each – experimental (invaded with *E. tenella*) and control (healthy – intact) housed separately under uniform conditions. Each experimental chicken was challenged orally with 6.10^4 sporulated *E. tenella* oocysts. The strain was isolated from naturally invaded birds and enriched via transmission through susceptible birds. Blood samples were obtained from the wing vein prior to the invasion and at post invasion days 3, 5, 7, and 9 from different experimental birds at each interval. Similarly, blood was sampled from control birds.

The haematological parameters – red blood cells (RBC) counts, haemoglobin concentrations (Hb), thrombocytes (Thr), total and differential white blood cells (WBC) counts were determined in 1 ml individual blood samples obtained from ten birds. Serum electrolytes were determined in 2 ml individual blood samples in another 10 birds. Total calcium and magnesium concentrations were determined by the complexometric method (Ibrishimov and Lalov, 1984), inorganic phosphate – by a modified method of Kuttner

* The number of chickens in both groups was higher than the number needed to obtain blood samples because some lethal issues were anticipated.

abd Cohen (Karakashov and Pandov, 1970); sodium and potassium levels – by flame photometry on Spektromom 381 L (Ibrishimov and Lalov, 1984). Haemoglobin levels were determined by the cyanhaemoglobin method and haematocrit (Hc) – by microcentrifugation. RBC and total WBC were counted in the Bürker's chamber. The differential WBC counts were determined on a blood smear.

The results, presented as mean $\pm$ SEM were statistically processed using one-way analysis of variance.

RESULTS

The haematological data are presented in Table 1 and 2. The total RBC and Thr counts (Table 1) were lower in the experimental group, especially at post invasion days 5, 7 and 9. The RBC counts were statistically lower at day 7 (13 T/l vs 32 T/l in controls) by 59%. Thr were significantly lower at days 5 (by 12%) and 7 (by 42%) after the challenge. Haemoglobin concentrations were the lowest at day 7 after the invasion – by 31% compared to controls ($p<0.05$). A similar changes was observed in Hc that decreased by 6.9% 7 days after the invasion ($p<0.05$).

The total WBC counts tended to increase (Table 2) during the period of the study, being statistically different than controls at days 5, 7 and 9 after the challenge. The highest WBC counts were reached at day 7 (increase by 28.3%). The changes in differential WBC counts were characterized by a significant increase (by 102%) in eosinophil percentages at day 7 following the invasion (8.11% in invaded birds vs 4.00% in controls, $p<0.01$). The lymphocytes were slightly increased vs controls – by 10%, $p<0.05$. Segmented neutrophils and monocytes did not change considerably.

Table 1. Changes in haemoglobin (Hb), red blood cell (RBC) counts, haematocrit (Hc) and thrombocyte (Thr) counts in healthy (controls) and *E. tenella* invaded (experimental) broiler chickens (mean $\pm$ SEM)

Period/Groups		n	Hb, g/l	RBC, $10^{12}/l$	Hc, l/l	Thr, $10^9/l$
Prior to invasion		10	126 $\pm$ 7.9	28.0 $\pm$ 3.1	0.30 $\pm$ 0.02	39.1 $\pm$ 2.5
Day 3	Healthy	10	121 $\pm$ 9.2	33.0 $\pm$ 2.3	0.32 $\pm$ 0.04	38.2 $\pm$ 1.5
	Invaded	10	122 $\pm$ 5.7	28.0 $\pm$ 1.5	0.29 $\pm$ 0.07	37.5 $\pm$ 2.2
Day 5	Healthy	10	131 $\pm$ 7.4	31.0 $\pm$ 4.2	0.31 $\pm$ 0.01	41.5 $\pm$ 1.2
	Invaded	10	120 $\pm$ 2.1*	22.0 $\pm$ 5.3	0.28 $\pm$ 0.03	36.5 $\pm$ 1.8**
Day 7	Healthy	10	128 $\pm$ 3.2	32.0 $\pm$ 2.1	0.29 $\pm$ 0.01	38.1 $\pm$ 2.5
	Invaded	10	89 $\pm$ 1.1*	13.0 $\pm$ 1.1**	0.27 $\pm$ 0.02*	22.0 $\pm$ 1.15*
Day 9	Healthy	10	117 $\pm$ 3.2	32.1 $\pm$ 2.1	0.31 $\pm$ 0.01	39.0 $\pm$ 8.11
	Invaded	10	110 $\pm$ 2.5	22.2 $\pm$ 1.0*	0.28 $\pm$ 0.02	36.5 $\pm$ 2.81

* $p < 0.05$; ** $p < 0.01$ vs controls.

The changes in serum electrolyte concentrations are shown in Table 3. Serum calcium and inorganic phosphate tended to decrease. Significant differences were observed in serum Ca levels at day 7 and 9 following the invasion – by 14.4% and 14.7% respectively ($p < 0.05$ vs controls). The inorganic phosphate levels decreased significantly at days 5, 7 and 9. The highest difference was observed at day 9: 1.7 ± 0.2 mmol/l in invaded and 2.0 ± 0.15 mmol/l in control birds ($p < 0.05$).

Serum Na concentrations were significantly lower in invaded birds at post challenge days 7 and 9 (by 16.2% and 13.7% respectively, $p < 0.05$). At the same time, serum K levels increased with statistically significant differences ($p < 0.01$) vs controls at days 7 (by 71.3%) and 9 (by 36%).

Serum Mg concentrations showed a tendency towards elevation, although the differences were not significant.

The results of haematological investigations in chickens invaded with *E. tenella* showed that within 5-7 days after the invasion, a clear anaemia accompanied by thrombocytopenia occurred. Similar data are reported by Pascalon-Pekelnizky and Chauve (1994) in *Eimeria*-invaded ducklings – decreased RBC counts, Hb and Hc. According to Natt (1956) in the early days of *E. tenella* invasion in chickens (days 3-5), Hb, Hc and RBC counts increased because of the diarrhoea and the severe systemic dehydration of infested birds. In the subsequent days (days 6-7) the author observed anaemia explained by considerable haemorrhages due to *Eimeria*-damaged intestinal mucosa. We agree with the opinion of Lozanov (1980), Musaev and Elchiev (1983) that it could be due to haemorrhages in the intestinal mucosae as well as to possible haemopoietic disturbances. The thrombocytopenia,

DISCUSSION

Table 2. Total and differential white blood cell (WBC) counts in healthy (controls) and *E. tenella* invaded (experimental) broiler chickens (mean $\pm$ SEM)

Period/Groups	n	WBC, $10^9/l$	Eosinophils, %	Segmented neutrophils, %	Lymphocytes, %	Monocytes, %
Prior to invasion	10	23.50 ± 1.51	5.00 ± 0.11	33.0 ± 0.21	60.0 ± 1.81	2.0 ± 0.01
Day 3						
Healthy	10	21.81 ± 1.85	5.00 ± 0.21	30.0 ± 0.25	64.0 ± 5.71	1.0 ± 0.01
Invaded	10	26.51 ± 1.10	6.00 ± 1.81	30.0 ± 2.21	62.0 ± 3.81	2.0 ± 0.01
Day 5						
Healthy	10	22.82 ± 8.71	4.10 ± 1.51	39.9 ± 5.31	54.6 ± 1.71	1.5 ± 0.02
Invaded	10	$28.52 \pm 5.21^{**}$	6.10 ± 1.52	29.9 ± 3.20	$61.0 \pm 2.56^*$	3.0 ± 0.03
Day 7						
Healthy	10	24.21 ± 3.22	4.00 ± 0.55	34.0 ± 1.51	60.0 ± 5.61	2.0 ± 0.01
Invaded	10	$31.07 \pm 3.13^*$	$8.11 \pm 0.65^{**}$	23.9 ± 3.22	$66.0 \pm 3.20^*$	2.0 ± 0.02
Day 9						
Healthy	10	23.81 ± 1.81	3.00 ± 0.31	37.0 ± 5.10	59.0 ± 3.80	1.0 ± 0.01
Invaded	10	$27.51 \pm 0.54^*$	4.00 ± 0.25	30.0 ± 2.50	$64.0 \pm 3.11^*$	2.0 ± 0.01

* $p < 0.05$; ** $p < 0.01$ vs controls.**Table 3.** Serum electrolyte concentrations in healthy (controls) and *E. tenella* invaded (experimental) broiler chickens (mean $\pm$ SEM)

Period/Groups	n	Na, mmol/l	K, mmol/l	Total Ca, mmol/l	Inorganic phosphate, mmol/l	Total Mg, mmol/l
Prior to invasion	10	150.5 ± 9.6	3.98 ± 0.66	2.31 ± 0.16	2.04 ± 0.17	0.92 ± 0.07
Day 3						
Healthy	10	145.0 ± 10.0	4.05 ± 0.70	2.50 ± 0.15	2.02 ± 0.21	0.80 ± 0.11
Invaded	10	130.0 ± 10.0	4.71 ± 0.50	2.33 ± 0.17	1.80 ± 0.15	0.91 ± 0.10
Day 5						
Healthy	10	152.2 ± 7.8	3.78 ± 0.53	2.40 ± 0.20	2.10 ± 0.20	0.86 ± 0.13
Invaded	10	139.2 ± 8.2	4.42 ± 0.46	2.25 ± 0.20	$2.00 \pm 0.20^*$	0.96 ± 0.10
Day 7						
Healthy	10	156.2 ± 8.7	3.55 ± 0.51	2.43 ± 0.09	$2.10 \pm 0.20^*$	1.03 ± 0.10
Invaded	10	$130.9 \pm 5.2^*$	$6.08 \pm 0.79^{**}$	$2.08 \pm 0.08^*$	$1.86 \pm 0.20^*$	1.18 ± 0.09
Day 9						
Healthy	10	161.0 ± 10.0	3.69 ± 0.48	2.52 ± 0.20	2.00 ± 0.15	0.90 ± 0.10
Invaded	10	$138.9 \pm 9.6^*$	$5.02 \pm 0.38^{**}$	$2.15 \pm 0.20^*$	$1.70 \pm 0.20^*$	0.96 ± 0.11

* $p < 0.05$; ** $p < 0.01$ vs controls.

observed in our experiment is due, in our opinion, to the loss of blood from the damaged intestinal mucosa and the activation of haemocoagulation, changes, noticed in previous studies in ours in turkeys invaded by *E. adenoides* (Koinarski and Nikolov, 1987). The observed eosinophilia and lymphocytosis corresponded to the results of Kogut et al. (1984). The increase in eosinophil counts could be due to a systemic allergisation under the influence of toxic substances released by parasites (Withlock et al., 1981) as well as to the absorption of toxic products obtained following the destruction of devitalized cells in caecal lumen (Lozanov, 1980). The increased lymphocyte percentages are probably related to the involvement of those cells in the realization of the immune response, triggered by the antigenic structures, as we observed in *E. tenella* invasion (Koinarski and Matev, 1994).

The decrease in serum Ca, P and Na in infested birds could result from the diarrhoea and the excretion of significant amounts of electrolytes with faeces. The renal tubular failure, evidenced by Lozanov (1980) in *E. tenella* invaded chickens, could impair the reabsorption of Ca, P and Na. Acido- and ammoniogenesis in damaged kidneys are impaired that result in retention of H^+ and acid metabolites, excretion of Ca, P and Na and therefore, decrease in their blood concentrations. The increase in serum K in invaded birds was most emphatic at post invasion day 7 and was probably due to the release of K ions in extracellular pool following the increased cellular wall permeability (Allen et al., 1973). The hyperpotassaemia could be related to secondary acidosis in invaded birds, observed in previous studies in ours (Angelov and Koinarski, 1997) as a consequence of impaired renal function (Lozanov, 1980). On one part, hyper-

potassaemia is characterized by reduced renal K^+ excretion and on the other – by translocation of K salts that move from the intracellular pool, replaced by increased H^+ concentrations in this acid-base disorder.

CONCLUSIONS

Eimeriosis, caused by *Eimeria tenella* results in significant haematological changes in chickens, manifested by decreased RBC counts, haemoglobin and haematocrit values and increased eosinophil and lymphocyte percentages.

The invasion with *E. tenella* increased the serum potassium levels and decreased serum sodium, calcium and inorganic phosphate concentrations.

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