

**INFLUENCE OF INTESTINAL COCCIDIA INFECTION OF RABBITS UPON PLASMA AND
FECAL PROTEIN LEVELS, AND PLASMA AND URINARY UREA AND CREATININE LEVELS**

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Two groups of rabbits, with 10 animals each, were artificially infected with varying doses of sporulated intestinal coccidia oocysts. The infectious material was composed of oocyst of several rabbit intestinal coccidia species: *Eimeria flavescens*, *Eimeria matsubayashi*, *Eimeria magna*, *Eimeria neoleporis*, *Eimeria perforans* and *Eimeria media*. A third group of 10 rabbits served as the control. Following the artificial infection and a 3-4 day incubation period, a subclinical form of the disease was induced in most rabbits while 3 animals developed full-blown disease with diarrhoea.

Shortly before and then on days 4, 7, and 10 after the infection, levels of the following plasma constituents were determined: total proteins, albumin, immunoglobulin G, urea and creatinine. Additionally, urea and creatinine were measured in urine samples, and protein levels were determined in feces. In plasma a decrease in total protein, albumin, urea and creatinine concentrations was found, whereas an increase in immunoglobulin G levels occurred. A rise in urinary urea and creatinine, and fecal proteins was found. The changes in concentration of the measured substances in blood, urine and feces were dependent on the number of inoculated infectious oocysts.

Key words: coccidiosis, rabbit, protein, urea, creatining

INTRODUCTION

Full production in the rabbit breeding industry is usually hampered by a number of diseases some of which are anthroozoonoses. Of parasitic diseases, the most prevalent is coccidiosis representing an important economic and health problem (Hoop et al. 1993, Polozowski 1993). Most studies of rabbit coccidiosis have been limited to the morphology of coccidia oocysts (Levin and Ivens 1972, Catchpole and Norton 1979). Few authors have described the alterations in levels

of certain constituents in blood, urine or feces. Ten days after the infection of rabbits with intestinal coccidia, the concentration of total plasma proteins was elevated to 59.0 ± 5.7 g/L compared to 57.5 ± 3.4 g/L in controls. At the same time point, levels of plasma urea were elevated to 11.1 ± 0.9 mM/L in comparison to 5.44 ± 2.62 mM/L in noninfected control rabbits (Peeters et al. 1984).

On day 10 following the infection, Coudert et al. (1978) observed a significant decrease in the percentage of albumin (-24%) and a simultaneous rise in relative amounts of all globulins, including γ globulins as well (+3.6%). Generally they found hypoproteinemia (-20%) which indicates general haemodilution. The observed 2.5-fold increase in plasma urea levels was explained as a derangement of intermediary metabolism.

Recently investigators have been increasingly dealing with the immunological aspects of intestinal coccidiosis in rabbits (Coudert et al. 1993, Licois et al. 1995).

In this study, we determined the concentrations of total proteins (TP), albumin (AL), urea (UR) and creatinine (CR) in blood, the concentration of urea (URU) and creatinine (CRU) in urine samples collected during 24 hours, and the concentration of total proteins in feces (PF). All the measurements were done following artificial infection of rabbits with sporulated intestinal coccidia oocysts.

MATERIALS AND METHODS

Male rabbits (Big Chinchilla breed) aged 52 days and weighing 1200 - 1300 g were used in the study. Prior to artificial infection, coprologic examination was consistently negative in all selected rabbits.

After an adaptation period of 10 days, the rabbits were transferred to metabolic cages and divided into three groups of 10 animals each. The first group served as the control (0) - noninfected rabbits. Rabbits of the second and third groups were infected by direct instillation of oocysts through a tube into the empty stomach. Oocysts of the following intestinal coccidia were used as the infectious material: *Eimeria flavescens* (7%), *Eimeria matsubayashii* (9%), *Eimeria magna* (21%), *Eimeria neoleporis* (19%), *Eimeria perforans* (21%) i *Eimeria media* (32%). Rabbits of the second group (A) were infected with 2×10^5 , whereas rabbits of the third group (B) were infected with 4×10^5 infectious oocysts.

Preparation of the infectious material: The stool specimens were prepared for examination by the flotation method using a saturated solution of sodium chloride. Sporulation of coccidia oocysts was induced by incubating the stool specimens with added 2% solution of potassium bichromate at 27°C for 5 days. The samples were observed under a light microscope (immersion, magnification 12×40). Coccidial oocysts were identified according to the following morphological criteria: length, width, shape, color, wall width, and the presence of oocyst's residual bodies. After the stool samples had been incubated in a thermostat at 27°C for 5 days, they were mixed with distilled water, centrifuged at 1500 rpm for 5 min, and the supernatant was discarded. The resulting pellet contained concentrated intestinal coccidia oocysts. The oocysts were counted in the McMaster

chamber. The infectious dose of the oocysts was determined on the basis of the number of oocysts in 1 mL of the pellet.

Blood samples were obtained immediately before the infection, then on the fourth, seventh, and tenth day following the infection with intestinal coccidia oocysts, 24-hour urine specimens and feces were collected. Blood was collected in syringes containing heparin sulphat (ICN-JUGOSLAVIJA) as an anticoagulant. Total proteins were determined by the biuret reaction and albumin was quantified spectrophotometrically. IgG was measured by immunodiffusion according to the method of Ouchterlony. Urea (conductometry) and creatinine (spectrophotometry) concentrations were measured by the device Astra-8 (Beckman). In figures the obtained results were reported as mean \pm standard deviation. Related values were statistically compared by using Student's *t* test for group comparasions or apaired *t* test. Statistical significance was set at $p < 0.05$.

RESULTS

Most infected rabbits displayed mild signs of coccidiosis such as polydipsia, bristling hair and moderate weight loss. Only three animals developed the complete clinical presentation of coccidiosis - diarrhoea, bristling hair, polydipsia, and weight loss. On day 10, coprological examination confirmed the presence of intestinal coccidia oocysts in stools of all the infected rabbits.

Total plasma proteins in rabbits with coccidiosis. - The concentration of total plasma proteins fell in both groups of infected rabbits (Figure 1). The decrease in total plasma protein levels on day 4 and 10 after the infection was more pronounced in the group of animals given the lower dose of infectious material

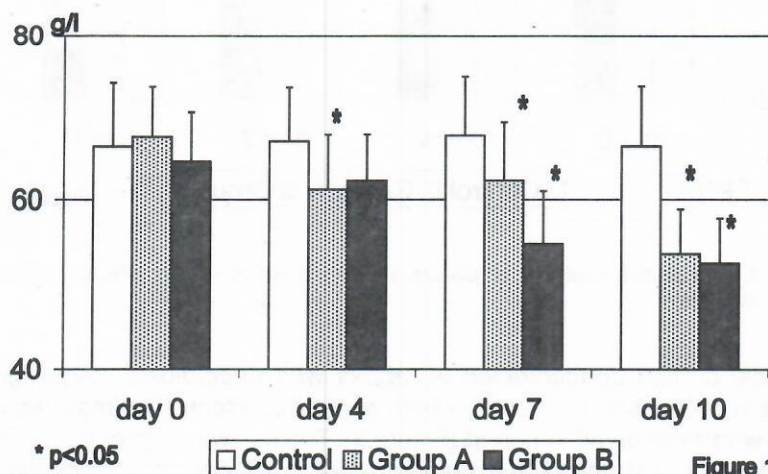


Figure 1

Figure 1. A comparative depiction of total plasma protein levels in rabbits infected with intestinal coccidia.

whereas on day 7 the decrease was greatest in the group infected with the higher dose of sporulated oocysts. Total plasma protein levels evenly declined and reached the lowest point on day 10 following the infection. At all observation time points except for group B on day 4, the decrease in total protein concentration was statistically significant. A significant intergroup variability was noted on day 4 and 7 after the infection.

Plasma albumin concentrations in rabbits with coccidiosis. - At all time points, a decrease in plasma albumin levels was found in both groups of rabbits infected with intestinal coccidia oocysts (Figure 2). The change in albumin concentration was parallel with the change in total protein concentration. Accordingly, the significant decline in albumin levels on days 4 and 10 was more apparent in the group of rabbits receiving the lower dose of the infectious material (A), whereas the decrease was most marked on day 7 in group B. The most pronounced and significant decrease was observed in both groups on day 10 after infection.

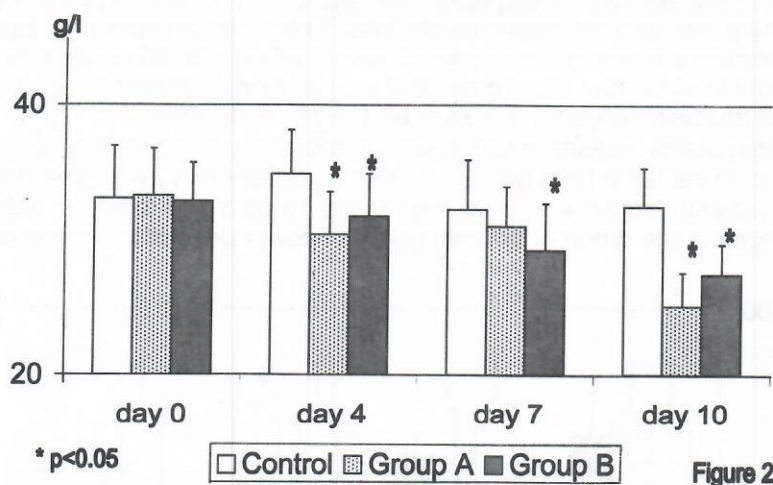


Figure 2. A comparative depiction of plasma albumin levels in rabbits infected with intestinal coccidia.

Fecal protein concentration in rabbits with coccidiosis - A progressive increase in proteins in the feces in both groups of infected animals was noted over the whole observation period (Figure 3). The rise was higher in the group of rabbits infected with the higher number of infection oocysts. Following a mild increase on day 4, a significant and more apparent elevation of protein levels in feces occurred on days 7 and 10 after infection.

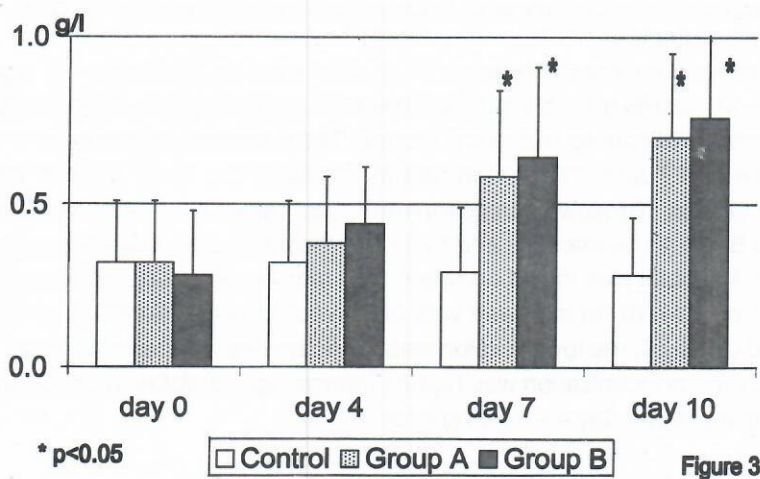


Figure 3. A comparative depiction of fecal protein levels in rabbits infected with intestinal coccidia.

Immunoglobulin G concentrations in plasma of rabbits with coccidiosis. - A progressive rise in plasma IgG levels in both groups of rabbits with coccidiosis was noted, being more apparent in animals infected with the higher dose of sporulated intestinal coccidia oocysts (Figure 4). A maximal and significant increase in IgG concentration in rabbits of groups A and B occurred on day 10.

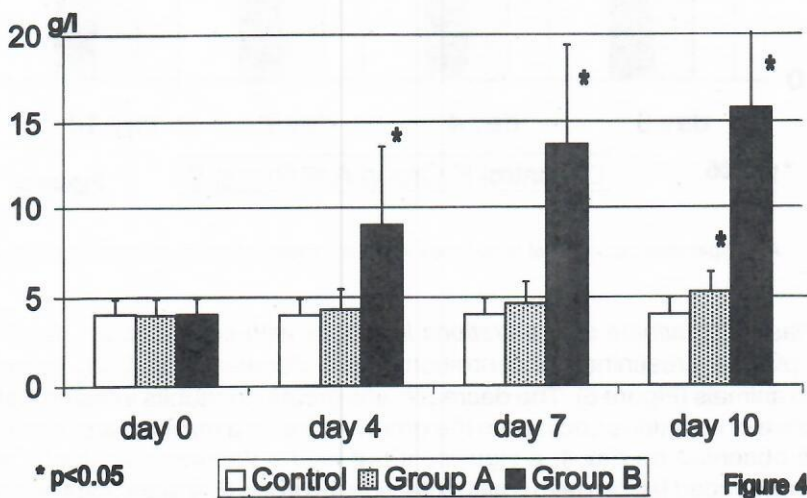


Figure 4. A comparative depiction of serum IgG concentrations in rabbits infected with intestinal coccidia.

Intergroup variability in plasma IgG concentration was observed on days 4, 7 and 10 following infection.

Plasma urea concentrations in rabbits with coccidiosis. - A significant decrease in plasma urea concentration was found during the whole study period in both groups of infected rabbits (Figure 5). The decrease was more pronounced on days 4 and 7 after infection in rabbits receiving the lower dose of infectious oocysts. On day 10 following infection the lowest values were observed in animals of group B. Thus the maximal and most significant decline in urea concentration in group A rabbits was found on day 4. The low value continued through day 7, whereas on day 10 an increase was observed. In group B rabbits a decrease occurred on day 4, the lowest value was found on day 7, whereas on day 10 after infection urea concentration was higher. Intergroup variability in urea concentrations was found on day 4 following infection.

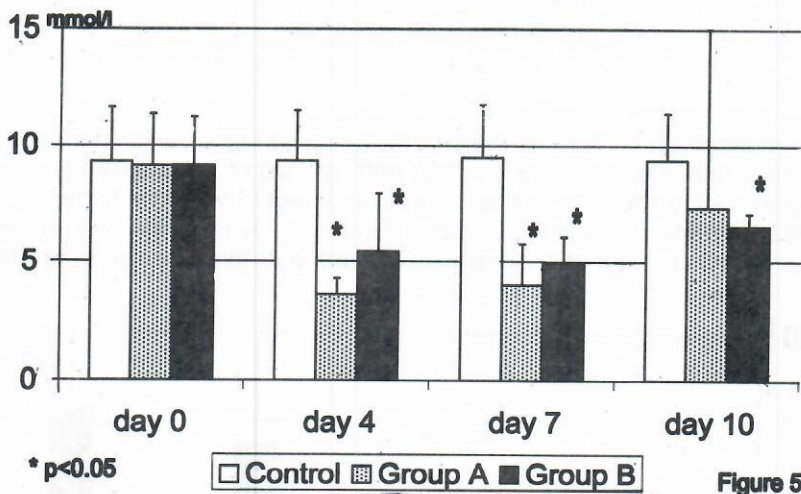


Figure 5. A comparative depiction of blood urea levels in rabbits infected with intestinal coccidia.

Plasma creatinine concentrations in rabbits with coccidiosis. - At all time points plasma creatinine concentrations were decreased in both groups of infected animals (Figure 6). The decrease was greater in rabbits infected with the lower dose of infectious oocysts. In the group A rabbits a maximal and significant fall was observed on day 4, a significant but milder decrease on day 7, and a more pronounced fall on day 10 after infection. In group B, a significant decrease was found on days 4 and 7 but a mild increase on day 10 following infection. On days 4 and 7, intergroup variability in creatinine concentrations was noted.

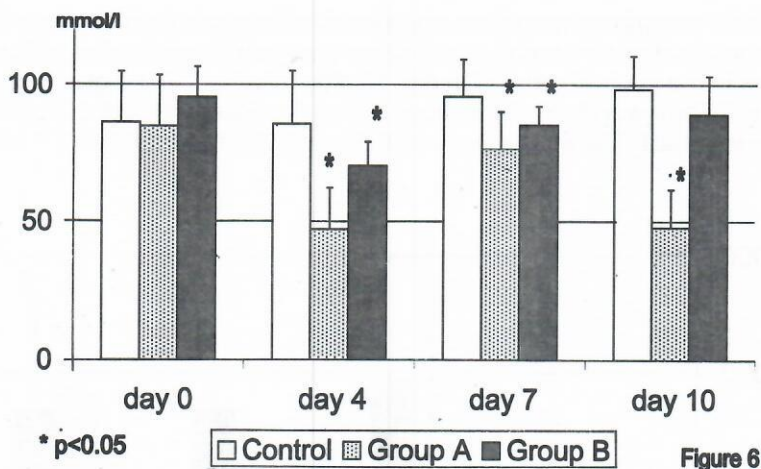


Figure 6. A comparative depiction of blood creatinine concentrations in rabbits infected with intestinal coccidia.

Urinary urea concentrations in rabbits with coccidiosis. - Urinary urea concentrations in rabbits with coccidiosis were elevated in both groups of animals but the effect was somewhat more pronounced in the group infected with the higher dose of infectious oocysts (Figure 7). On day 7 after infection, a significant

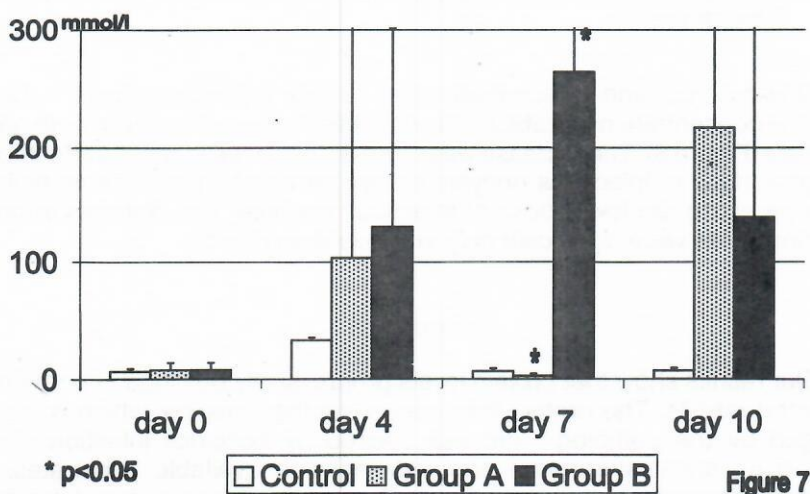


Figure 7. A comparative depiction of urinary urea concentrations in rabbits infected with intestinal coccidia.

fall in urea concentration was found in urine samples in rabbits receiving the lower dose of the infectious material. At the same time, a maximal and significant rise was noted in the second group. On day 10 after infection, in the group of rabbits infected with the lower dose of infectious material, urinary urea concentration was markedly elevated. The intergroup variability was significant on day 7 following infection.

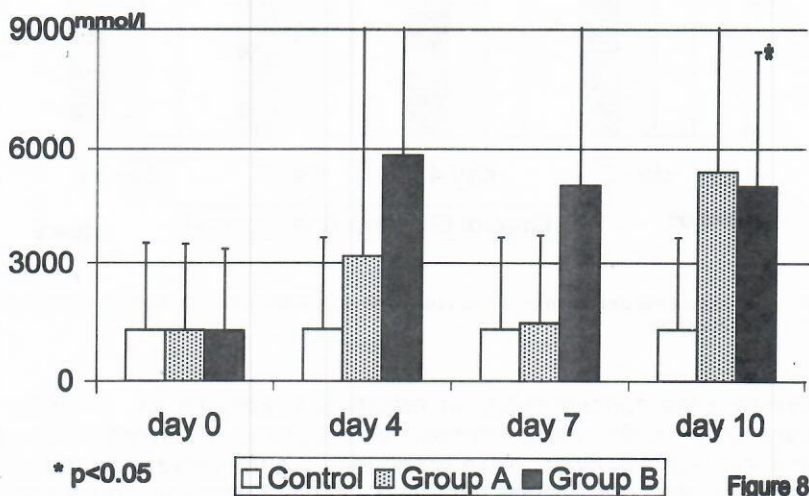


Figure 8

Figure 8. A comparative depiction of urinary creatinine concentrations in rabbits infected with intestinal coccidia.

Urinary creatinine concentrations in rabbits with coccidiosis. - Urinary creatinine concentrations in rabbits with coccidiosis were elevated in both groups of animals (Figure 8). The increase was more pronounced in rabbits infected with the higher dose of infectious oocysts except for day 10 when it was higher in rabbits receiving the lower dose of infectious material. The changes in urinary creatinine levels were significant only in group B on day 10.

DISCUSSION

Our results show that protein levels progressively declined in both groups of infected rabbits. The protein loss occurs via the intestinal which is mucosa damaged by the pathologic process caused by coccidial infection. Due to impaired intestinal absorption and deficiency in available substrates, i. e. aminoacids, protein synthesis declines, whereas, on the other hand, increased energetic requirements during the illness direct proteins towards metabolic degradation which produces energy. The increased synthesis of immunoglobulins in the course of the infection makes the lack of substrates for

albumin synthesis more pronounced. Being the largest fraction of total proteins, the dynamics of albumin changes parallels those of total proteins.

Data obtained by Peeters et al. (1984) show an increase in plasma total proteins on day 10 after infection. On day 10 too, Coudert et al. (1978) registered a significant decline in percentage of albumin in the plasma of rabbits with coccidiosis, and proteinemia reflecting a general haemodilution. Our results are in accord with theirs. A rise in protein concentration in feces was constantly observed throughout the study period. The protein loss via the stool in rabbits with coccidiosis is enormous and sometimes equals 15 g daily. This suggests that a disordered function of the intestinal barrier may represent the principal-pathogenetic mechanism in intestinal coccidiosis.

Rabbit coccidiosis is a primary parasitic disease so that the recovered animals asquire solid imunity. Hence in both groups of infected rabbits at all time points a rise in IgG levels occurs. The rise in IgG concentration results from activation of specific immunologic defence reactions. Coudert et al. (1978) reported a significant increase in total plasma globulins on day 10 after infection in rabbits suffering from the intestinal coccidiosis.

Throughout the study, sick rabbits had a decline in plasma urea and creatinine concentrations. Inasmuch as urea and creatinine are final metabolites of proteins we think that the fall in their plasma levels reflects a reduced turnover rate of proteins and their loss through the intestinal mucosa.

Our results confirm that larg amount of urea and creatinine are excreted via the kidneys so their concentrations in urine are increased many fold. Peeters et al (1984) found a two fold increase in plasma urea levels on day 10 after infection in rabbits with coccidiosis. Our results are therefore at variance with theirs.

The observed elevation of urea and creatinine levels in urine indicates preserved renal function during the whole period of illness.

Following the artificial infection of rabbits with various intestinal coccidia oocysts, the animals acquired a subclinical form of disease associated with changes in concentration of certian compounds in blood,urine and feces. We found a decrease in total proteins, albumin, urea and creatinine concentrations, and an increase in IgG concentration in plasma. In addition we observed a rise in total protein concentration in feces as well as an increase in urinary urea and creatinine concentrations.

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UTICAJ INFEKCIJE CREVNIM KOKCIDIJAMA KUNIĆA NA KONCENTRACIJU PROTEINA U PLAZMI I FECESU I KONCENTRACIJU UREJE I KREATININA U PLAZMI I URINU

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SADRŽAJ

Eksperimentalnu crevnu kokcidiozu smo izazvali kod dve grupe mladih kunića, rase velika činčila, s namerom da ispitamo uticaj te infekcije na koncentraciju nekih biohemijskih parametara u krvi, urinu i fecesu. Obe grupe kunića su brojale po 10 životinja, a treća grupa je bila kontrola. Prvu grupu smo inficirali sa 2×10^5 , a drugu sa 4×10^5 sporuliranih oocista crevnih kokcidija. Infektivni materijal su činile sporulirane oociste više vrsta crevnih kokcidija kunića. U plazmi kokcidioznih kunića smo registrovali smanjenje koncentracije ukupnih proteina i albumina, povećanje koncentracije imunoglobulina G, te smanjenje koncentracije ureje i kreatinina. U urinu smo zabeležili povećanje koncentracije ureje i kreatinina, a u fecesu povećanje koncentracije ukupnih proteina.