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HAEMATOBIOCHEMICAL PARAMETERS IN BROILER CHICKENS EXPERIMENTALLY INFECTED WITH *EIMERIA SPP* IN VARIABLE CHEMOPROPHYLAXIS AND THERAPY CONDITIONS

PARAMETRII HEMATOLOGICI ȘI BIOCHIMICI LA PUII BROILER INFECTAȚI EXPERIMENTAL CU *EIMERIA* SPP ÎN CONDIȚII DIFERITE DE CHIMIOPROFILAXIE ȘI TERAPIE

Olimpia C. IACOB[1)], Mona CIORNOHAC[2)], C. CHIRUȚĂ[1)], Geta PAVEL[1),*)]

ABSTRACT | REZUMAT

Eimeriosis is a severe parasitic infection of the intestinal tract of chickens, causing death by digestive haemorrhage. The research aimed to induce the digestive syndrome and to assess the induced changes on hematological and serum biochemistry parameters under different conditions of chemoprophylaxis and therapy. Cobb 500 broilers, aged 15 days old, were organized into four groups (n=15/group) of which three experimental groups and one control group. The experimental groups were infected for two days with a dose of 25000 oocysts of *Eimeria spp.* per day, administered by gavage. Thus, group I received simple diet associated to therapy (Sulfaquinoxaline) at the occurrence of hemorrhagic enteritis, group II, dietary chemoprophylaxis (Salinomicyn), without therapy and group III, dietary chemoprophylaxis and therapy. Hematological parameters (RBC, WBC, HGB, HCT, MCHC, MCV), and serum biochemistry parameters (total proteins, glucose, lipids, cholesterol, urea, alkaline phosphatase) were determined using automatic analysers. The results were statistically analyzed by Mann-Whitney U test and Wilcoxon signed rank sum test. Hematological and serum biochemistry results show very significant differences ($P< 0.0001$) in group I compared with control group, where enteral blood and electrolyte losses have been massive since the 5th day post-infection; very significant differences ($P<0.0001$) were recorded in group II, in which chemoprophylaxis attenuated the schizogony multiplication ability of *Eimeria* and delayed the clinical onset, but did not stop the emergence of hemorrhagic enteritis. In group III, the changes were not significant ($P>0.05$), suggesting remission of symptomes due to immediate therapy. These results recommend chemoprophylaxis associated with specific therapy, immediately, to avoid economic losses by mortality, in chicken farms.

Keywords: *Eimeria* spp., broiler, blood parameters, chemoprophylaxis, therapy

Eimerioza este o infecție parazitară severă a tractusului intestinal la puii de găină, cauzând moartea prin hemoragie digestivă.

Scopul cercetării a fost inducerea sindromului digestiv și aprecierea modificărilor hematologice și biochimice ale sângelui, în condiții diferite de chimioprofilaxie și terapie.

În acest context, pui broiler Cobb 500 în vârstă de 15 zile au fost organizați în patru loturi (n = 15/lot) dintre care, trei experimentale și unul martor.

Loturile experimentale au fost infectate cu doza de 25.000 oochiști de *Eimeria* spp. pe zi, două zile consecutiv, administrată prin gavaj.

Astfel, lotul I a primit o dietă simplă asociată cu terapie (Sulfaquinoxalină) la apariția enteritei hemoragice; lotul II, dietă cu chimioprofilaxie (Salinomicină) fără terapie; lotul III, dietă cu chimioprofilaxie și terapie. Parametrii hematologici (Eritrocite, Leucocite, Hemoglobină, Hematocrit, CHEM, VEM) și biochimici (proteine totale, glucoză, lipide, colesterol, uree, fosfataza alcalină) s-au determinat folosind analizoare automate.

Rezultatele au fost asigurate statistic prin testele neparametrice Mann-Whitney U și Wilcoxon.

Rezultatele hematologice și biochimice arată diferențe foarte semnificative ($P<0.0001$) la lotul I, comparativ cu lotul martor, pierderile enterale de sânge și electroliți fiind masive începând cu a 5-a zi post-infecție; diferențe foarte semnificative ($P< 0.0001$) s-au înregistrat și la lotul II, la care chimioprofilaxia a atenuat capacitatea de multiplicare schizogonică a eimeriilor și a întârziat debutul clinic, fără a stopa apariția enteritei hemoragice. La lotul III, diferențele au fost nesemnificative ($P>0.05$), sugerând remiterea simptomatologiei, ca urmare a terapiei imediate.

Aceste rezultate recomandă chimioprofilaxia asociată imediat cu terapia specifică, pentru a evita pierderile economice prin mortalitate la pui.

Cuvinte cheie: *Eimeria* spp., broiler, parametri sanguini, chimioprofilaxie, terapie

1) University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Iași, Romania
2) Food Safety and Sanitary Veterinary Directorate, Vrancea, Romania
*) *Corresponding author: geta_pavel@yahoo.com*

Eimeriosis is recognized as the major parasite infection of the intestinal tract in poultry husbandry. It is caused by a complexan protozoa belonging to at least seven different species of *Eimeria*. *E. acervulina, E. brunetti, E. maxima, E. mitis, E. necatrix, E. praecox* and *E. tenella* are considered economically relevant due to their pathogenic effects and ubiquitous distribution (12). Chicken eimeriosis may cause digestive disturbance of variable degrees, depending on the pathogenicity and virulence of the species and/or strains involved. Bloody diarrhea and high mortality may be observed in *E. tenella* infection and immune suppression or increased susceptibility to other diseases (13). The blood loss in the digestive tract induced by *E. tenella, E. acervulina* and *E. brunetti* highlights the severity of anemia in eimeriosis (9, 11, 13). The white blood cells, the lymphocytes, monocytes, basophils and neutrophils significantly increased in *E. tenella* and *E. brunetti* infected broilers, compared to the reference values (2). A significant decrease in red blood cells and changes of biochemical parameters (total protein, glucose, cholesterol, triglycerides) were found in the blood of broilers experimentally infected with *E. tenella* with and without prophylactic therapy (17,18). Dietary conditions can modulate susceptibility to infection, but eimeriosis may reduce the feed intake and the growth of chickens (3, 4).

The control of eimeriosis is mainly based on prophylactic administration of anticoccidial drugs. However, this has led to a widespread occurrence of anticoccidial resistance to almost all drugs currently applied (6). Annual costs related to eimeriosis are estimated at more than £500 million worldwide, which includes the costs of prophylaxis by in-feed medication or vaccination or treatment in case of outbreaks, mortality, reduced performance etc. (4, 7). In Romania, eimeriosis is a severe parasitic infection which affects the growth of chicken in both intensive and extensive systems, causing huge economic losses. This is attributed to intestinal pathology and, consequently, to an impaired function of the digestive tract, but also to general metabolic alteration (14, 15). This study analyses haematological and biochemical parameters during experimentally induced eimeriosis, in variable chemoprophylaxis and therapy conditions.

METHODS AND MATERIALS

The project reported here was conducted in the premises of Food Safety and Sanitary Veterinary Laboratory biobase in Focsani, Romania, on Cobb 500 broiler chickens. The chickens were kept under electric lighting, natural ventilation, ad libitum conventional watering systems, permanent litter of shavings during the experimental period. The feed was conceived in order to meet the requirements of broilers at all stages of growth (21). The research has been complied with all the relevant national regulations and institutional policies for the care and use of animals (Ethical I of the Faculty of Veterinary Medicine from Iași, no.12/2019).

Experimental design

Sixty broilers of 15 days old, both sexes, were allocated to four groups of 15 chickens each: three experimental groups and one control group.

The infective material was represented by a suspension of oocysts isolated from *Eimeria tenella, E. acervulina* and *E. brunetti* naturally infected chickens, following a spontaneous infection. Ingesta was collected from dead chickens and suspended with distilled water then filtered through 4-layers sieves. After that, 2.5% potassium dichromate was added in a ratio of 1:1 to the obtained suspension. This suspension was kept at 20-25ºC for 4 days and repeatedly aired to provide sufficient oxygen for oocysts sporulation. Sporulated oocysts were collected by repeated washing and subsequent unforced sedimentation for 2-3 hours, repeated three times. The number of sporulated oocysts was determined using the Mc Master method.

The chickens were infected for two days with a dose of 25000 oocysts per day (50.000 oocysts/ chicken), administered in 1 ml of water, by gavage, using a 1 ml syringe.

The daily diet of chickens differed by group as follows: group I received plain food, and after the clinical onset of the haemorrhagic diarrhea, the chickens were treated with Coccistop (Sulfaquinoxaline) tablets; group II received dietary chemoprophylaxis (Salinomycin), but no additional treatment was applied after the onset of haemorrhagic diarrhoea; group III was similarly treated with Kokcisan (Salinomycin) – 0.5 g/kg, but moreover these chickens were treated with Sulfaquinoxaline, administered after the onset of diarrhoea. Coccistop (Sulfaquinoxaline) tablets were administered in a dose of 1 tablet/day/chicken, for two consecutive days, repeated after a period of three days. One tablet contains 2 mg sulfaquinoxaline, 4 mg vitamin K3 and 10 mg vitamin C. All chickens were clinically observed and monitored daily, for 30 days after the infection.

Blood examination

The blood was collected from the axillary vein into standard vacutainers of 1 ml with K 3 EDTA (1mg/ml) for haematological determinations and in dry vacutainers of minimum 1 ml for biochemistry.

Because of the variable onset of the disease in the three experimental groups, blood samples were collected at different times: on 6 days post infection (p.i.) in group I, on 12 days p.i. in group II, on 9 days p.i. in group III and at the end of experimental period in control group.

Table 1

Haematological parameters in broilers, experimental groups versus control group and their statistical significance

Parameter/group	WBC x10^3/μL	RBC x10^6/μL	Hb g/dL	PCV (Hct) %	MCHC %	MCV fL
Control group	25.093 ± 0.72	2.82 ± 0.17	7.59 ± 0.30	27.93 ± 1.45	29.21 ± 1.63	100.01 ± 3.28
Group I	15.24 ± 2.57***	1.86 ± 0.40***	5.19 ± 0.32***	18.82 ± 3.25***	28.52 ± 6.49**	102.54 ± 8.81*
Group II	13.45 ± 1.49***	1.85 ± 0.19***	3.87 ± 0.86***	16.78 ± 2.45***	22.85 ± 2.88***	90.25 ± 9.57*
Group III	24.65 ± 0.93	2.78 ± 0.32	8.04 ± 0.38*	27.33 ± 1.70	29.44 ± 1.85	99.14 ± 8.84

Group I – therapy with Coccistop; Group II - prophilaxis with Salinomycine; Group III -both chemoprophylaxis and therapy.$05.0=\alpha$; P value < α; P value > 0.05: not significant (ns); P value < 0.01: significant (*); P value< 0.001: distinctively significant (**); P value < 0.0001 very significant (***)

Table 2

Serum biochemistry parameters in broilers, experimental groups versus control group and their statistical significance

Parameter/ group	Total proteins g/dL	ALP IU	Glucose mg/dL	Urea mg/dL	Lipids mg/dL	Cholesterol mg/dL
Control group	3.60 ± 0.30	601.36 ± 21.52	129.78 ± 3.76	3.66 ± 0.26	376.62 ± 10.59	101.16 ± 6.55
Group I	1.99 ± 0.13***	292.70 ± 14.51***	95.60 ± 4.11***	14.95 ± 1.25***	285.90 ± 26.29***	78.60 ± 6.99***
Group II	1.84 ± 0.12***	291.00 ± 18.63***	89.33 ± 8.52***	14.29 ± 1.08***	273.66 ± 18.50***	70.83 ± 6.83***
Group III	3.14 ± 0.19**	600.67 ± 14.70	126.8 ± 6.78	3.38 ± 0.30**	619.27 ± 16.76**	99.40 ± 2.89

Group I – therapy with Coccistop; Group II - prophilaxis with Salinomycine; Group III -both chemoprophylaxis and therapy; P value < α; P value > 0.05: NS; P value < 0.01: significant (*); P value< 0.001: distinctively significant (**); P value < 0.0001 very significant (***)

Haematological and biochemical analyses were performed in the Food Safety and Sanitary Veterinary Laboratory Vrancea, Romania. The analysed haematological parameters were: WBC, RBC, Hb, Hct (PCV), MCV and MCHC, for which MS 4-5 Vet Haematology Analyzer (Melet Schloesing Laboratories,Osny,France) was used. The biochemical parameters: total proteins, glucose, lipids, cholesterol, urea and ALP were determined using the IDEXX Vet Test 8008 and IDEXX Vet auread biochemical analyzers.

Statistical analysis

The results were statistically processed using Mann-Whitney U test, for independent selections, applicable for groups I and II (because these groups have a different number of chickens), and Wilcoxon signed rank sum test, for dependent selections, applicable for group III and control group with a significance level of 0.05.

RESULTS AND DISCUSSION

The haematological and biochemical results were obtained from *Eimeria* spp. experimentally infected broilers depending on the experimental group. Tables 1 and 2 show the haematological and biochemical results obtained in group I, II and III and their statistical significance compared with control group. Chickens from group I have been exposed to eimerian infection without any drug protection, providing an ideal environment for eimeries biological cycle. The infection was acute for the group. The first clinical cases of haemorrhagic diarrhoea occurred six days after the infection, initially in five chickens, then gradually, over the same day, for the remaining broilers. Mortality occurred after the onset of haemorrhagic diarrhoea and was 66.6% (10 chicks/15).

In the second group, the rate of multiplication of eimeries was slowed by the constant administration of

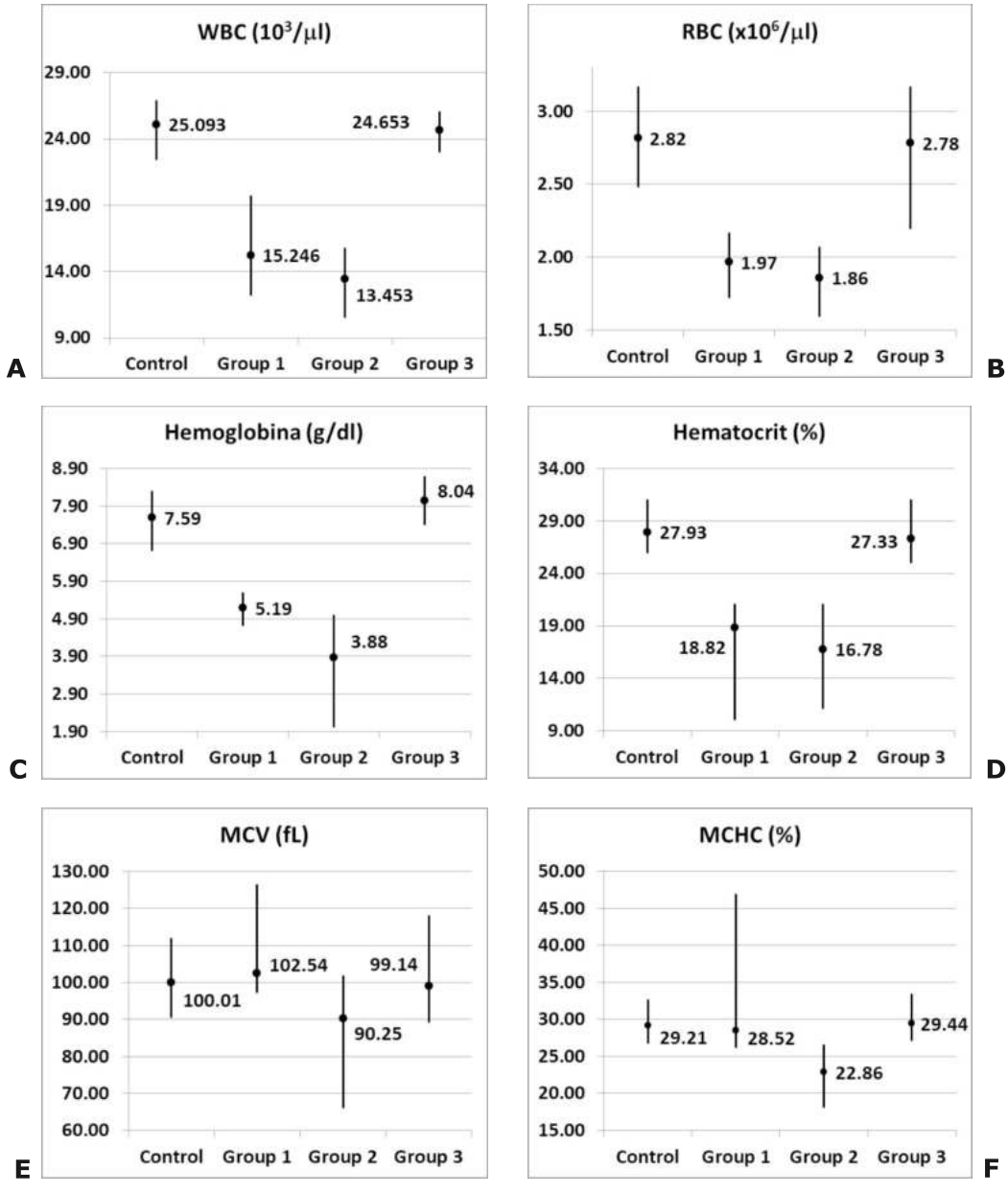

Fig. 1. (A-F). Comparative dynamics of hematological parameters between control group versus experimental groups in broiler chickens infected with *Eimeria spp* (group 1 treated with Coocistop; group 2 treated with Salinomycine; groupe 3 treated with Salinomycine and Coccistop). WBC - white blood cells (x 10^3/μl) (A), RBCs – red blood cells (x 10^6/μl) (B), Hemoglobin (g/dl) (C), Hematocrit (%) (D), MCV – medium cell volume (fL) (E) MCHC – medium cell hemoglobin concentration (%) (F)

the eimeriostatic in the diet. The digestive signs occurred at 12 days p.i., characterised by deviation, capricious appetite, polydipsia, whitish faeces diarrhoea and blood streaks, then haemorrhagic diarrhoea. Mortality occurred after the onset of clinical signs and presented the following dynamics: 3 chickens on the 13th day p.i.; 2 chicks on the 14th day p.i.; 2 chickens on the 15th day p.i.; 1 chick, on the 16-th day p.i.; 2 chicks on the 19th and the 20thday p.i.; and 1 chick on the 21th day p.i. Mortality occurred on the 13th day p.i. (28 days of age) and was maintained throughout the experimental period, reaching 86.6% (13 chicks out of 15).

In group III, chickens benefited from both Salinomicyn (Kokcisan x 0.5 mg/kg) prophylaxis and Sulfaquinoxaline (Coccistop tablets) specific therapy instituted after the onset of diarrhoea without allowing the occurrence of haemorrhagic diarrhoea. The first clinical signs occurred 9 days p.i (24 days of age) characterised by deviation, capricious appetite, polydipsia, whitish faeces and fine blood streaks diarrhoea.

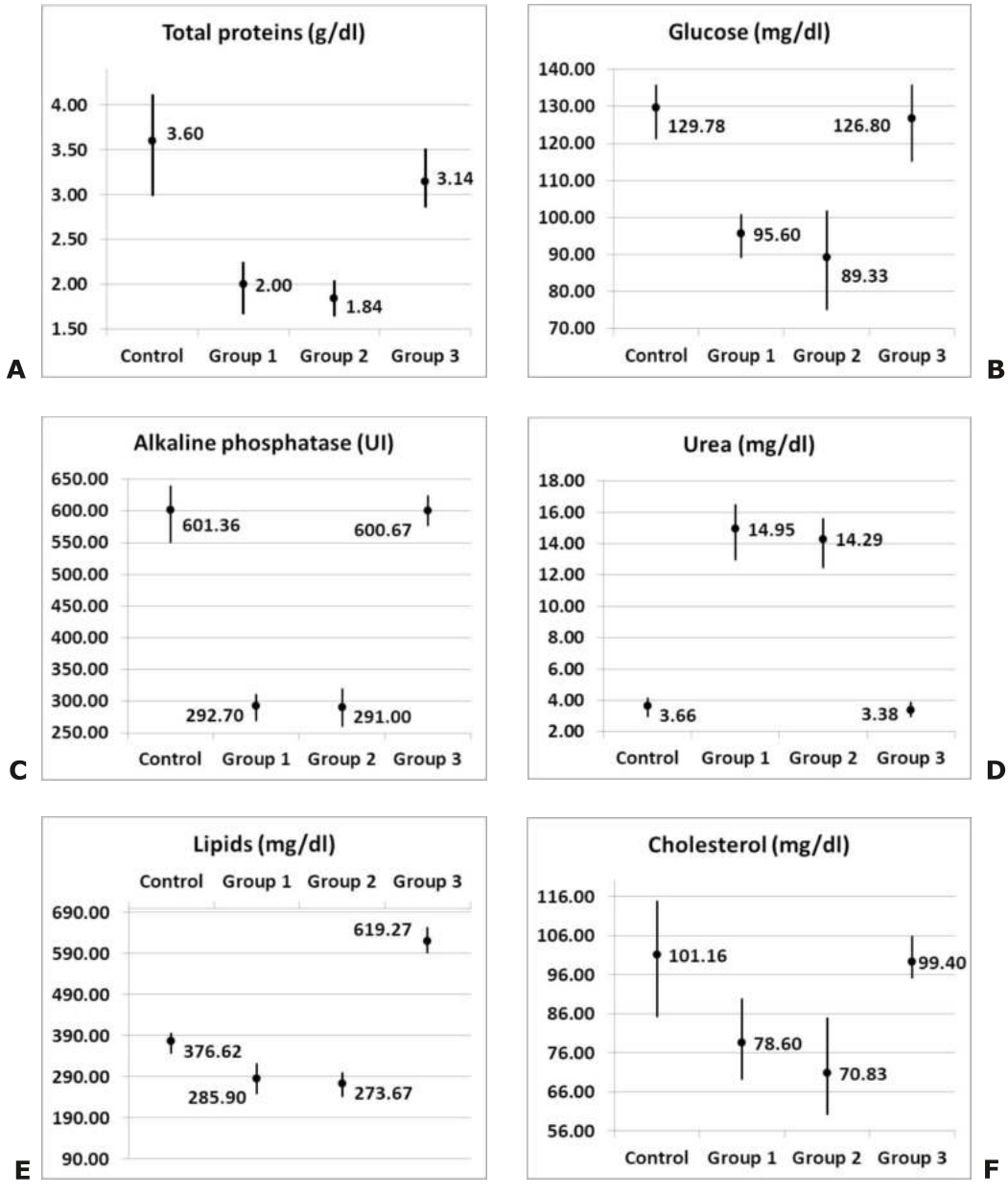

Fig. 2. (A-F). Comparative dynamics of serum biochemistry parameters between control group versus experimental groups in broiler chickens infected with *Eimeria* spp (group 1 treated with Coccistop; group 2 treated with Salinomycine; groupe 3 treated with Salinomycine and Coccistop). Total proteins (g/dl) (A), Glucose (mg/dl) (B), Alkaline phosphatase (UI) (C), Urea (mg/dl) (D), Lipids (mg/dl) (E), Cholesterol (mg/dl) (F)

The therapy with Sulfaquinoxaline diminished the clinical signs after the first administration and completely stopped those after the second treatment. In this case, the haematological and biochemical results revealed minimum oscillations for the studied parameters, close to the reference values (20). Comparative dynamics of haematological and bio-chemical parameters between experimental groups and control group are represented in Figures 1 (A-F) and 2 (A-F).

In group I, (sampled 6 days p.i.) the eimerian infection caused severe alteration of haematological and biochemical data expressed by a very significant decrease ($P<0.0001$) of analyzed haematological and biochemical values confirming a massive loss of plastic substances and blood through digestive bleeding. Toxicity was expressed by a very significant increase ($P<0.001$) in urea compared both to the control group and also, to literature reference data. The haemorrhagic diarrhoea induced haematological and biochemical changes in the blood because of the caused bleeding and haemorrhagic enteritis, changes stopped by applying specific therapy with Coccistop (Sulfaquinoxa-

line). The results are similar to those in the literature (2, 15, 16, 17, 18, 19). Decrease of plasma proteins (hypoproteinemia) was observed in the acute phase because of the loss through the intestinal wall until the 10th day post infection (10). All classes of lipids are reduced in *E. acervulina* infected chickens due to jejunal mucosal damage and lipid absorption reduction, in addition, the reduced pH delaying the intestinal hydrolases action, including lipase (9).

The excessive increase of alkaline phosphatase (ALP) could be associated with lesions induced by *Eimeria* spp in the intestinal epithelium (2). *E. tenella* experimentally infected broilers, revealed significant changes after 5-, 7-, and 9-day post infection in terms of biochemical parameters, including glucose, total protein, cholesterol and triglycerides (TG) (18). Unlike our results that highlighted low values for blood glucose due to intestinal malabsorbtion, Mondal et al. (2011) revealed a significant increase in serum glucose levels; the peak of glucose increase is considered to be achieved 7 days post infection.

In group II, haematological and biochemical results have shown low mean values compared to the control group and literature data (5, 20). Very significant differences (P value <0.0001) were recorded both in haematological and biochemical post infective results. Salinomicyn (Kokcisan) administered in the diet of the chickens slowed the multiplication rate of schizogonic eimeries, delaying the appearance of clinical signs until the 12 days p.i. but did not stop the onset of haemorrhagic diarrhoea. The lack of specific therapy was followed by severe clinical manifestations associated with significant losses, as previously noted. Hypoproteinemia in eimerian infection, found in our study as in other studies, may be due to acute stress and corticosteroids secretion associated with protein catabolism acceleration. Acute haemorrhage 7-day p.i., determines the transfer of interstitial fluid to vascular compartment in order to restore volemia, which induces acute hypoproteinemia (18).

Mohammed (2012) revealed a significant decrease in red blood cells, even after the administration of antieimerian probiotics (Salinomicyne, 60 ppm and Poltrystar, 0.05 %) in broiler's food for 33 days before *E.tenella* experimental infection.Lymphocyte's growth is generally attributed to long-term antigen stimulation (12). În group III, haematologic parameters indicate a significant reduction (P<0.05) of haemoglobin and packed cell volume (PCV), but within the physiological limits. Within determined biochemical parameters, total proteins, lipids and urea have increased very significant (P<0.001). The results obtained in this group show that the constant presence of Salinomycin (Kokcisan) in the diet and the application of Sulfaquinoxaline (Coccistop) therapy after the onset of diarrhoea limited eimerian aggression, resulting in a mild evolution of the infection with minimum indirect losses and no mortality. The white blood cells (WBC) count reveals significantly reduced post infection values in groups I and II, while in group III the leukocytes value remains almost unchanged. Thus, in groups I and II, the inflammatory process is surprised when the number of eimeries exceeded the local defense barrier, either earlier (6 days p.i.), in the case of group I without eimeriostatic prophylaxis or later (12 days p.i.), in the case of group II, which benefited from eimeriostatic prophylaxis. In both situations a massive migration of polymorphonuclear leukocytes (PMN) into tissues occurred, by phenomena of chemotaxis, margination, diapedesis and focal accumulation.

In group III, eimeriostatic prophylaxis associated with specific therapy instituted after the onset of the first clinical signs, prevented systemic pro inflammatory changes, the number of blood leukocytes remaining unchanged. The erythrocytes parameters revealed the presence of slightly macrocytic, normochromic post haemorrhagic anemia in group I, with a possible bone marrow regeneration of the young cells of the erythrocyte line. In group II, the prolonged period of infection induced an intensification of the anemia, diminution of medullary regenerative activity with microcytosis and hypochromia, characteristic aspects to the plastic elements insufficiency required for hematopoiesis.

In group III, antieimerian therapy applied after the first week of eimeriostatic prophylaxis prevented the occurrence of post-haemorrhagic or spoliation anemia, all erythrocyte parameters falling within the reference limits, although some of those have significantly changed compared to the the control group decreasing to the lower limit of the species. The parasitic spoliation is reflected by the very significant reduction of serum biochemical parameters in groups I and II, with or without chemoprophylaxis, as well as by a less significant variation for some of the biochemical parameters in experimental group III, with antieimerian therapy. Thus, total protein, glucose, alkaline phosphatase, as well as lipids and cholesterol in *Eimeria* spp. experimentally infected chickens have very significantly decreased (p<0.0001), both in the experimental group I and II, while urea showed a very significant increase (p<0.0001) in the same experimental groups. In experimental group III, biochemical parameters showed a significant decrease (p<0.001) in total protein and urea, as well as a distinctively significant increase (p<0.001) in total lipids, while blood glucose, alkaline phosphatase and cholesterol levels did not show statistically significant changes (p>0.05) compared to the control group. The increase in serum urea levels in chickens in groups I and II can be attributed to a massive reabsorption of urea in the urinary tubes caused by tissue dehydration that accompanies almost any disease condition.

Serum urea is considered an indicator of dehydration in birds without being useful in the diagnosis of kidney disease (11). In group III, increased lipids values associated with low protein levels can be explained by the absence of amino acids needed to block Acetyl-CoA-carboxylase involved in the synthesis of fatty acids, so that the pathway of triglyceride synthesis remains free (17).

Antieimerian prophylaxis and therapeutic intervention are widely accepted and applied in all intensive systems (1, 6, 8, 14). Prophylaxis by itself does not stop the occurrence and evolution of eimeriosis. Lack in the association of prophylaxis and therapy measures determines the onset of severe clinical symptoms and economic losses by failing to fulfill broilers' growth indices and high mortality.

These results confirm the prophylactic protection against intestinal and caecal infection in *Eimeria* spp infected broilers and also the therapeutical intervention in the case of clinical symptoms and haemorrhagic enteric syndrome occurrence. The use of Salinomycin at the proposed concentration (accepted by the European Food Safety Authority) is considered safe for chickens as well as for the aquatic and terrestrial ecosystem due to metabolisation and the rapid degradation in the environment (19).

CONCLUSIONS

Eimeria spp experimental infection of broilers permitted the comparison of the haematological and biochemical changes induced by parasites, reflecting the parasitic spoliation of plastic substances, the anemia produced by digestive haemorrhages and also, the toxicity induced by parasites metabolism. The results obtained in eimerian experimental infection in broilers recommend chemoprophylaxis associated with specific therapy, to avoid economic losses through mortality.

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