

USE OF SOME HEMATOLOGICAL AND BIOCHEMICAL TESTS TO MONITOR THE HEALTH STATUS OF ORNAMENTAL BIRDS THAT UNDERGO PHARMACEUTICAL TESTING USING PRODUCTS BASED ON METRONIDAZOLE AND OXYTETRACYCLINE HYDROCHLORIDE

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Abstract:

Metronidazole acts by preventing anaerobic microbial cell's production of hydrogen ions, while oxytetracycline, synthesis tetracycline, has only broad-spectrum bacteriostatic action. These active substances are used in compounded drug formulations for treatment of several forms of ornamental birds' enteropathies. As a consequence, the present study aims to assess the hematological and biochemical profile of ornamental birds under Enteroguard M-administration, a pharmaceutical product based on metronidazole and oxytetracycline.

The investigations consisted of hematological and biochemical testing of 3 groups of healthy ornamental hens, yellow Orpington breed, aged between 1 and 3 years. The evolution of investigated parameters showed insignificant differences between different lots of tested birds, that have shown a haematological- biochemical profile corresponding to a good level of health and welfare throughout the observation period, pre- and post-treatment.

Keywords: Hen, metronidazole, oxytetracycline, testing, hematology.

INTRODUCTION

Metronidazole is a synthetic chemotherapeutic which acts on the anaerobic microbial cell by preventing production of hydrogen ions. Oxitetracycline hydrochloride is a tetracycline and acts exclusively as a synthetic broad-spectrum bacteriostatic (Al-Mayah AS and JA Al-Ahmed, 2005). These active substances are a compounded drug formulation used in the treatment of several forms of enteropathy of ornamental birds (Ognean et al., 2011). In this context, the present study aims to assess the haematological and biochemical profile of ornamental birds under administration of Enteroguard M, pharmaceutical product based on metronidazole and oxitetracycline.

MATERIALS AND METHODS

Testing was performed on three groups of clinically healthy hens, aged between one and three years. The main exclusion criteria were hypersensitivity to metronidazole and

oxytetracycline or other chemical drugs from the same pharmacological group that could seriously affect vital signs, gastrointestinal tract, absorption, distribution, metabolism and excretion of substances used.

The hens were divided as follows:

Group M, represented by hens (n=10) observed in parallel with experimental groups in the same environmental conditions and feeding (control group);

Group 1 (n=10), represented by ornamental hens which received 6 mg of Enteroguard M powder / kg feed for 7 consecutive days;

Group 2 (n=10), represented by ornamental hens which received M Enteroguard 12 mg powder / kg of feed for 7 consecutive days.

Blood samples were collected by pre-treatment and post-treatment basilar vein puncture on EDTA for haematological tests: hematocrit, hemoglobin, total number of erythrocytes, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total number of leukocytes, respectively on lithium heparin

for determination of biochemical parameters using an automatic analyzer type VetScan with Avian Profile Plus kits: aspartate aminotransferase (AST), bile acids (BA), creatine phosphokinase (CK), uric acid (UA), glucose (GLU), calcium (CA), phosphorus (PHOS), total protein (TP), albumins (ALB), globulin (GLOB), potassium (K +) and sodium (Na +).

Data analysis was carried out following the methods described by Reece (2005) and Ghergariu et al. (2000). The individual and mean data were statistically analyzed following EMEA guides.

RESULTS AND DISCUSSIONS

The hematological and biochemical results from the animals that received Enteroguard M in normal dose and in a double dose, compared to the control group are expressed as mean \pm standard deviation and are presented in tables 1 and 2.

Table 1. Mean values of the erythrocyte parameters recorded at product testing

Parameters	Pre-treatment		
	Lot M	Lot 1	Lot 2
PCV (%)	36.88 \pm 4.6	40.25 \pm 3.25	39.13 \pm 3.16
Hb (g/dL)	7.03 \pm 1.07	7.73 \pm 0.53	7.55 \pm 0.76
Erit.(T/L)	2.72 \pm 0.36	2.37 \pm 0.12	2.50 \pm 0.19
MCV (fL)	136.80 \pm 27.29	170.23 \pm 11.96	157.01 \pm 15.62
MCH (pg)	26.18 \pm 5.7	32.78 \pm 3.47	30.36 \pm 3.95
MCHC (g/dL)	19.45 \pm 3.97	19.39 \pm 2.52	19.44 \pm 2.30
Leuk.(G/L)	19.32 \pm 8.6	20.82 \pm 3.11	22.31 \pm 12.23
Heter.(%)	38.29 \pm 15.7	39.57 \pm 4.06	38.00 \pm 23.04
Eos.(%)	1.00 \pm 0.05	2.11 \pm 1.38	1.59 \pm 0.99
Bas.(%)	1.14 \pm 1.13	1.75 \pm 1.18	1.84 \pm 1.55
Limph.(%)	43.14 \pm 18.6	46.12 \pm 3.15	43.00 \pm 29.84
Mono.(%)	16.43 \pm 6.85	10.29 \pm 4.54	15.57 \pm 7.54
Post-treatment			
PCV (%)	37.94 \pm 4.74	40.19 \pm 3.53	39.75 \pm 2.81
Hb (g/dL)	7.08 \pm 1.03	7.66 \pm 0.60	7.93 \pm 0.62
Erit. (T/L)	2.79 \pm 0.38	2.37 \pm 0.34	2.58 \pm 0.19
MCV (fL)	138.60 \pm 31.15	170.89 \pm 24.25	154.67 \pm 15.09
MCH (pg)	25.89 \pm 5.77	32.76 \pm 5.45	30.83 \pm 2.79
MCHC (g/dL)	18.94 \pm 3.62	19.28 \pm 2.75	19.99 \pm 1.41
Leuk.(G/l)	20.56 \pm 8.78	22.53 \pm 15.71	24.11 \pm 7.38
Heter.(%)	36.14 \pm 13.20	41.26 \pm 14.21	37.43 \pm 15.00
Eos.(%)	2.02 \pm 0.64	1.29 \pm 0.78	1.62 \pm 0.43
Bas.(%)	1.26 \pm 0.79	1.71 \pm 1.52	1.91 \pm 1.11
Limph.(%)	45.86 \pm 15.27	42.43 \pm 28.00	44.17 \pm 7.46
Mono.(%)	14.52 \pm 6.50	12.71 \pm 7.55	13.63 \pm 5.02

As shown in Table 1, individual and mean erythrocyte indices values showed wide variations, outlining the following characteristic haematological profile

evolution in this breed: fluctuations within the physiological limits and not statistically significant pre-and post-treatment hematocrit (40.25 \pm 3.25%, 39.13 \pm 3.16% and 40.19 \pm 3.53%, 39.75 \pm 2.81%), hemoglobin (7.73 \pm 0.53 g/dL, 7.55 \pm 0.76 g/dL, respectively 7.66 \pm 0.60 g/dL, 7.93 \pm 0.62 g/dL) and the total number of erythrocytes (2.37 \pm 0.12 T/L, 2.50 \pm 0.19 T/L respectively 2.37 \pm 0.34 T/L, 2.58 \pm 0.19 T/L).

We have observed similar ante-and post-treatment behavior in the case of erythrocytes constants with an average of 170.23 \pm 11.96 fL for group 1, 157.01 \pm 15.62 fL in pre-therapeutic group 2; 170.89 \pm 24.25 fL and 154.67 \pm 15.09 fL in post-treatment for MCV. For MCH pre-treatment mean values were 32.78 \pm 3.47 pg for group 1 and 30.36 \pm 3.95 pg for group 2, and post-treatment the values were 32.76 \pm 5.45 pg and 30.83 \pm 2.79 pg. Pre-treatment MCHC mean values were 19.39 \pm 2.52 g/dL for group 1, respectively 19.44 \pm 2.30 g/dL for group 2 and post-treatment 19.28 \pm 2.75 g/dL for group 1 and 19.99 \pm 1.41 g/dL for group 2.

Leukocyte parameters showed predominant developments within the physiological limits. Total number of leukocytes for pre-treatment group 1 was 20.82 \pm 3.11 G/L, and for group 2 of 22.31 \pm 12.23 G/L and post-treatment 22.53 \pm 15.71 G/L for group 1 and 24.11 \pm 7.38 G/L for group 2. Similar distributions were observed for eosinophils and basophils population. The proportion of lymphocytes pre-treatment was 46.12 \pm 3.15% for group 1 and 43.00 \pm 29.84% for group 2, respectively 42.43 \pm 28.00% for group 1 and 44.17 \pm 7.46% for group 2 post-treatment, and the proportion of monocytes pre-treatment was of 10.29 \pm 4.54% for group 1 and 15.57 \pm 7.54% for group 2 and respectively post-treatment 12.71 \pm 7.55% for group 1 and 13.63 \pm 5.02% for group 2.

The metabolic profile is outlined in Table 2. According to this summary, for group 1, the mean of total protein showed strong pre-treatment oscillations, in the range of 2.80 to 3.80 g/dL, mean 3.52 \pm 0.15 g/dL and post-therapeutic in the range of 3.10 to 3.80 g/dL, mean \pm 0.38 3.68 g/dL. For group 2, the total protein values varied pre-treatment in the range of 2.86 to 3.92 g/dL, with a mean of

3.54 ± 0.16 g/dl and post-treatment, in the range of 2.95 to 3.85 g/dL, with a mean of 3.56 ± 0.41 g/dL.

Table 2. Mean values of the metabolic parameters recorded in pre-clinical testing of the product

Parameters	Pre -treatment		
	Lot M	Lot 1	Lot 2
TP (g/dL)	3.48±0.17	3.52 ±0.15	3.54±0.16
ALB (g/dL)	1.75±0.27	1.92±0.54	1.96±0.15
GLOB (g/dL)	0.95±0.15	1.04±0.40	1.63±0.58
GLU (mg/dL)	215.60±15.63	254.60±33.32	261.80±16.80
AST (U/L)	185.40±20.12	185.80±24.23	192.80±31.62
CK (U/L)	954.40±62.73	925.40±64.21	836.80±86.32
BA (µmol/L)	< 35	< 35	< 35
UA (mg/dL)	6.18±0.62	7.02±1.04	6.26±1.48
CA (mg/dL)	8.22±1.50	8.26±1.23	9.58±0.73
PHOS (mg/dL)	7.55±0.52	7.56±0.74	6.35±0.94
NA ⁺ (mmol/L)	146.60±3.42	168.80±4.22	155.00±1.56
K ⁺ (mmol/L)	5.55±0.28	4.78±0.15	4.68±0.18
Post-treatment			
TP (g/dL)	3.36±0.42	3.68± 0.38	3.56±0.41
ALB (g/dL)	2.28±0.45	2.64±0.45	2.72±0.86
GLOB (g/dL)	1.12±0.21	0.95±0.23	0.78±0.52
GLU (mg/dL)	277.20±54.65	278.20±25.43	252.80±58.15
AST (U/L)	142.60±38.01	93.20±22.62	128.60±51.14
CK (U/L)	953.20±82.12	737.82±206.95	757.40±452.31
BA (µmol/L)	< 35	< 35	< 35
UA (mg/dL)	4.82±1.05	4.75±1.35	4.76±1.75
CA (mg/dL)	9.57±0.51	9.45±0.32	8.94±1.35
PHOS (mg/dL)	4.60±0.86	5.52±1.18	4.46±1.42
NA ⁺ (mmol/L)	156.60±6.58	157.20±5.72	157.40±8.96
K ⁺ (mmol/L)	3.36±0.42	4.82±0.85	4.95±1.26

Just as short variations were observed for pre- and post-treatment albumin levels (1.92 ± 0.54 g/dL, 1.96 ± 0.15 g/dL, respectively 2.64 ± 0.45 g/dL, 2.72 ± 0.86 g/dL), and globulinemia levels (0.40 ± 1.04 g/dL, 1.63 ± 0.58 g/dL respectively 0.95 ± 0.23 g/dL, 0.78 ± 0.52 g/dL). Glucose varied within physiological limits, with averages between 254.60 ± 33.32 mg/dL and 261.80 ± 16.80 mg/dL for group 1 and 278.20 ± 25.43 mg/dL and 252.80 ± 58.15 mg/dL in the case of group 2.

Mean values of pre-treatment aspartate aminotransferase were 185.80 ± 24.23 U/L for group 1 and 192.80 ± 31.62 U/L for group 2. Pre-treatment creatine phosphokinase values were 925.40 ± 64.21 U/L for group 1 and 836.80 ± 86.32 U/L for group 2, respectively post-therapeutic values were 737.82 ± 206.95 U/L for group 1 and 757.40 ± 452.31 U/L for group 2.

Bile acids were below 35 µmol/L throughout the investigation, and uric acid showed average pre-treatment values of 7.02 ± 1.04 mg/dL for group 1 and 6.26 ± 1.48 mg/dL for

group 2 and post-treatment values of 4.75 ± 1.35 mg/dL for group 1 and 4.76 ± 1.75 mg/dL for group 2.

Calcium reported pre-treatment average values were 8.26 ± 1.23 mg/dL for group 1 and 9.58 ± 0.73 mg/dL for group 2 and respectively post-treatment values were 9.45 ± 0.32 mg/dL for group 1 and 8.94 ± 1.35 mg/dL for group 2.

The average pre-therapeutic values obtained for phosphoric acid were 7.56 ± 0.74 mg/dL for group 1 and 6.35 ± 0.94 mg/dL for the second group. Post-treatment the average value obtained was of 5.52 ± 1.18 mg/dL for group 1 and 4.46 ± 1.42 mg/dL for the second group. Sodium pre-treatment values were 168.80 ± 4.22 mmol/L for group 1 and 155.00 ± 1.56 mmol/L for group 2, and post-treatment 157.20 ± 5.72 mmol/L for group 1 and 157.40 ± 8.96 mmol/L for group 2. Pre-treatment serum potassium showed an average value of 4.78 ± 0.15 mmol/L for group 1 and 4.68 ± 0.18 mmol/L for group 2, and a post-treatment average value of 4.82 ± 0.85 mmol/L for the first group and 4.95 ± 1.26 mmol/L for the second.

CONCLUSIONS

The behavior of physiological parameter of the treated birds with therapeutical doses and overdoses of Enteroguard M powder showed variable values, but within the physiological limits of specie and category, with minor individual deviations that did not influence the tested variables.

Thus, the comparative evaluation of erythrocyte indices and constants of the birds tested showed both a good tolerance of Enteroguard M in the used doses, and lack of negative effects on electrolyte balance and erythrocyte homeostasis, erythropoiesis and erythrocyte functions in general.

Therefore ornamental birds show high tolerance to the tested product expressed by lack of any type of adverse effect or toxicity secondary to curative doses and double doses.

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