

The Evaluation of Therapeutic Doses of Erythromycin on the Main Hematological Parameters of Broiler Chickens

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Abstract. Monitoring of hematologic profile in a group of broiler chickens (n = 33) was the basis for the evaluation of tolerance and the influence of various doses of erythromycin thiocyanate. The investigations required the formation of the 3 homogeneous groups (2 experimental and 1 control), each consisting of 11 individuals, 12-19 days of age and weighing around 500 g, kept and fed under identical conditions. The individuals from the experimental groups were treated for 7 consecutive days with therapeutic (10 mg/individual/day) and double (20 mg/individual/day) doses of erythromycin. During the experiment were performed two sets of hematological investigations (pre-and post-treatment) on blood samples collected (on heparin) from the three groups of chickens, consisting in determining: the total number of erythrocytes and leukocytes, packed cell volume (PCV) hemoglobin concentration (Hb), mean erythrocyte constants, white blood cell counts and leucogram. The results were interpreted statistically and compared with reference values. Global analysis of the developments registered in the investigated physiological parameters of the groups of broiler chickens revealed mean values variations from one group to another, falling within physiological limits of the species and category, or with slight deviations from some references, respectively the intervals established in the control group. Comparative developments of hematological parameters pre-and post-treatment outlined a normal profile for the investigated category of broiler chickens, indicating a good tolerance of therapeutic and double doses of erythromycin thiocyanate with no negative effects on electrolyte balance and erythrocyte homeostasis, erythropoiesis and general erythrocyte functions. An overview of the determined leukocyte parameters data, outlined specific aspects of leukocyte configuration with a good defensive potential, for the group treated with therapeutic dose and also for the one treated with the double dose of erythromycin thiocyanate. Statistical analysis of the obtained data showed significant differences ($p < 0.05$) only in case of the control group, consisting of a decrease in the percentage of heterophiles from (43.18 ± 2.71) to (38.36 ± 6.50) and an increase in the percentage of lymphocytes (from 37.36 ± 4.20 to 42.64 ± 6.65), due to individual cases of slight heteropenia and/or lymphocytosis. The results of the clinical and hematological monitoring of the health status, have led to the identification of no potential risks associated with doubling the dose of erythromycin thiocyanate. The investigated doses of 10 or 20 mg/individual/day, for a period of 7 consecutive days, lacked immediate or delayed adverse reactions respectively signs of immunosuppression.

Keywords: hematology, immunosuppression, adverse reactions, tolerance, drug

INTRODUCTION

The use of hematological investigations in monitoring the health status of birds has grown in extent, becoming an indispensable component of the protocols used for testing bioequivalence, safety and tolerance of active substances on the target species (Ognean *et al*, 2004). Raising poultry in the industrial system, such as broilers, limits the possibility of individual administration of medicinal products, the main limiting factor being the increased number of birds from these types of facilities (Ognean and Cernea, 2006).

As an alternative procedure, were developed, for industrial poultry complexes, management methods based on administering medicinal products in feed or drinking water, with the major disadvantage individual dose uncertainties.

Erythromycin thiocyanate as active substance is found in the composition of several medical products-as a veterinary antibiotic with rapid digestive absorption and main elimination in bile and faeces in birds. This antibiotic is fixed in part (84%) to protein and has a bioavailability of approximately 35% in birds, achieving peak plasma concentrations of 0,028 µg/ml (at 4 hours for a dose of 10 mg) and has a half-life of 1.5 hours. Erythromycin penetrates well into most tissues and fluid compartments, except for cerebrospinal fluid, concentrating in the liver, where is excreted through bile in high concentrations up to 50 times the level found in plasma (8.9-anhidro erythromycin A 6.9-hemiacetals 3 metabolite) because it is inactivated in a small extent by demethylation in the liver (Cristea, 2011).

Since erythromycin is a broad spectrum antibiotic with curative and preventive use in poultry, the purpose of this investigation was to evaluate the influences of various doses of orally administered erythromycin thiocyanate on hematological profile of broilers, in view the current issue of immunosuppression, tolerance or drug residues (Pop *et al.*, 2002).

MATERIALS AND METHODS

The present research has been integrated into a comprehensive experimental protocol, being conducted on a group of broilers (n = 33) coming from an intensive exploitation farm. In the experiment were introduced only clinically healthy chickens with hematological and clinical parameters within the physiological limits, following an initial evaluation (conducted over a period of 7 days pre-treatment). The birds were divided into 2 experimental and 1 control groups: group I (n = 11), subject to oral therapy with therapeutic dose of erythromycin thiocyanate, 10 mg/individual/day for 7 consecutive days, group II (n = 11) subject to oral treatment with double dose of erythromycin thiocyanate, 20 mg/individual/day for 7 consecutive days, group III (n = 11) included untreated controls. The individuals of the 3 investigated groups were kept in identical feeding and environmental conditions.

Pre-and post-treatment blood samples were collected on heparin, samples which were subject to the following hematological investigations: determination of the total number of erythrocytes and leukocytes (hemocytometric method), packed cell volume (microhematocrit method), hemoglobin (spectrophotometric method), mean erythrocyte constants and leucogram on smears stained panoptically (Dia panoptic method).

Individual data was statistically analyzed using specialized biostatistics applications (Graph Pad Instant V3.0, Microsoft Excel), which allowed calculation of the mean value, standard deviation and probability index "p". These parameters were the basis of the final statistical analysis of the obtained data.

RESULTS AND DISCUSSIONS

During the course of the experiment, clinical and laboratory monitorization of health development in the groups of investigated chicken ethics and welfare standards were respected, which ensured the relevance of the data and its interpretation in the general context of medical and veterinary practice. Thus, the overall analysis of the physiological parameters evolution in the groups showed mean values oscillations from one group to another, falling within physiological limits of the species and category, with few deviations from certain sources, or to the ranges established in the control group, kept in identical conditions to experimental groups (table 1 and 2).

Comparative assessments of hematological indices and erythrocyte mean constants in the investigated birds, showed characteristic features of normal profile of broilers, indicating a good tolerance of therapeutic and double doses of erythromycin thiocyanate and thus the lack of obvious side effects on electrolyte balance and erythrocyte homeostasis, erythropoiesis and erythrocyte function in general. All this was supported by individual developments, which were reflected in the average values, given in table 1. Thus, the mean recorded values of the packed cell volume oscillated between a minimum of $37.00 \pm 5.44\%$, registered post-treatment in group II and maximum of $39.68 \pm 3.48\%$, pre-treatment in group I, values considered high compared to some reference intervals (22.00 to 35.00%) (Bounous and Stedman, 2000) and within physiological intervals by others (24.00 to 43.00%) (Gylstorff, 1983). Hemoglobin concentration also showed wide fluctuations, mainly falling within the physiological interval of 7-13 g/dL (Bounous and Stedman, 2000), except for the control group, which was below the lower limit. Compared to other bibliographic sources (Gylstorff, 1983) the obtained mean values were situated below the physiological range (8.90 to 13.50 g/dL), hemoglobin concentration also showing a decreasing trend in the experimental and increasing the control groups.

Tab. 1.

Mean values registered for the main hematological indices in groups of investigated chicken

Group/variable		PCV (%)	Hb (g/dL)	Erythrocytes (T/L)	Mean erythrocyte constants		
					MCV (fL)	MCH (pg)	MCHC (g/dL)
I	Pre	39.68±3.48	7.77±1.03	2.28±0.19	174.21±13.24	34.36±5.78	19.75±3.12
	Post	39.23±4.52	7.47±1.25	2.34±0.31	169.25±24.04	32.51±7.34	19.44±4.43
II	Pre	37.91±3.42	7.27±1.16	2.31±0.27	166.48±25.95	31.95±6.98	19.35±6.98
	Post	37.00±5.44	7.47±1.22	2.47±0.32	152.93±32.59	30.70±6.98	20.68±4.65
III	I	37.00±5.17	6.88±1.07	2.68±0.36	141.18±29.49	26.19±5.66	19.03±4.12
	F	38.00±4.86	6.92±1.03	2.67±0.40	146.71±32.67	26.53±5.79	18.55±3.73
R ¹		22.0-35.0	7.0-13.0	2.50-3.50	90.0-140.0	33.0-47.0	26.0-35.0
R ²		24.0-43.0	8.9-13.50	2.20-3.30	120. 0-137.0	-	-

Note: Pre= ante- treatment; Post = post- treatment; I=initial; F=final;

R¹ -Bounous DI, Stedman NL: Normal avian hematology (2000);

R² - Gylstorff I: Handbuch der Geflügelphysiologie (1983).

The total number of erythrocytes presented developments in correlation hemoglobin concentration, with mean values of ranging from 2.50 to 3.50 T/L, below the lower limit for

experimental groups, the same limits were considered physiologic by Bounous and Stedman (2000). However, the decreases observed in this parameter were not considered important; because they fell within the physiological range (2.20 to 3.30 T/L) reported by other sources (Gylstorff, 1983).

Oscillations more or less important were present also in the mean erythrocyte constants which exceeded the reference values (established by Bounous and Stedman, 2000, respectively Gylstorff, 1983) for MCV (90.00 to 140.00 fL) and were situated below the lower limit for MCH (33.00 to 47.00 pg) and MCHC (26.00 to 35.00 g/dL).

The statistical analysis of the values of the hematological parameters registered pre- and post-treatment were not of significant differences, the value of the probability index "p" was greater than 0.05 for each of the determined parameters (table 3).

An overview of obtained data in the determined leukocyte parameters outlined specific aspects with a good defensive potential, both for the group treated with therapeutic and double dose of erythromycin thiocyanate.

The distribution of leukocyte subpopulations proportions also indicated a level high tolerance to therapeutic and double doses of erythromycin thiocyanate, and the lack of sensitization or adverse effects on leukocyte functions in general and leucopoiesis (table 2, 3).

Tab. 2.

The mean values of the leucogram in the investigated groups of broilers

Lot/variable		Leukocytes (G/L)	Leucogram (%)				
			heterophile	eosinophile	basophile	lymphocyte	monocytes
I	Pre	16.69±3.82	41.00±6.10	1.36±0.92	0.82±1.08	43.18±7.77	13.64±3.14
	Post	16.75±3.40	41.73±7.00	1.73±1.35	1.18±0.98	42.82±8.10	12.55±3.27
II	Pre	15.16±2.52	41.55±8.31	1.91±1.30	0.82±0.87	40.64±8.90	15.09±3.91
	Post	15.47±3.02	38.91±11.03	1.73±1.35	0.64±0.81	43.45±11.59	15.27±3.29
III	I	20.35±2.52	43.18±2.71	1.64±1.43	1.27±1.01	37.36±4.20	16.55±4.57
	F	20.21±2.78	38.36±6.50	1.36±1.03	1.27±1.01	42.64±6.65	16.36±4.46
R ¹		19.80-32.60	19.80-32.60	1.50-2.70	1.70-4.30	45.00-75.00	8.10-16.50
R ²		20.00-30.00	13.00-49.00	2.00-14.00	1.00-17.00	31.00-72.00	1.00-4.00
R ³		13.00-32.00	20.00-31.00	1.00-3.50	1.50-4.00	50.00-70.00	6.00-11.00
R ⁴		25.00	25.00	4.00	2.00	62.00	4.00
R ⁵		-	13.00-35.00	1.20-2.50	2.10-3.10	58.00-76.00	1.10-6.50

Note: Pre= ante- treatment; Post = post- treatment; I=initial; F=final.

R¹-Gylstorff I., 1983, Handbuch der Geflügelphysiologie. Diseases of Exotic Animals Wallach JD, Boever WJ;

R²-Hoffmann G., 1961, Abriss der Laboratoriumstierkunde. Veb Gustav Fisher Verlag Jena;

R³-Pîrvu Gh., I. Barna, A. Caprarin, 1984, Hematologie veterinară practică. Ed.Ceres, Bucuresti;

R⁴-Ghegariu S., Al. Pop, L. Kadar, Marina Spânu, 2000, Manual de laborator clinic veterinar. Ed. All București;

R⁵-Uray Z., 1992, Ghid de date biologice și fiziologice ale animalelor de laborator. Biologia animalelor de laborator și oncologie comparată, Vol 19.

The abundance of reference data and the controversies between them increased considerably the difficulty of interpreting and framing the physiological ranges of individual and mean values for the leucogram parameters (table 2).

Thus, the total number of leukocytes varied in the interval between $15.16 \pm 2.52 - 20.35 \pm 2.52$ G/L situated within the physiological limits (13.00 to 32.00 G/L) only by one source (Pîrvu et al, 1984), the rest placing the observed means of the experimental groups under physiological limit (Uray, 1992; Ghergariu *et al.*, 2000; Hoffmann, 1961; Gylstorff, 1983). Moreover, post-treatment decreases the total number of leukocytes in the experimental groups (16.75 ± 3.40 G/L, respectively 15.47 ± 3.02 G/L) compared to the control group (20.21 ± 2.78 G/L) were statistically significant ($p < 0.05$).

Likewise developments in the leukocyte subpopulations highlighted large variations falling within some of the mentioned reference intervals and some situated beyond, of greater or lesser importance than others. Thus, the proportions of PMN leukocytes showed oscillations between 38.36 ± 6.50 and $43.18 \pm 2.71\%$ in case of heterophiles, 1.36 ± 0.92 and $1.91 \pm 1.30\%$ for eosinophils, respectively 0.82 ± 0.87 and $1.27 \pm 1.01\%$ for basophils. Lesser variations presented the proportion of lymphocytes (37.36 ± 4.20 to $43.45 \pm 11.5\%$) and monocytes ($12.55 \pm 3.27 - 16.55 \pm 4.57\%$).

Particular relevance was given to the statistical analysis to the leucogram values, which showed significant differences ($p < 0.05$) only for the control group, consisting of a lower percentage of heterophiles (from 43.18 ± 2.71 to 38.36 ± 6.50) and increase in lymphocytes (from 37.36 ± 4.20 to $42.64 \pm 6.65\%$), due to individual cases of mild heteropenia and/or lymphocytosis.

Also it is important to emphasize that no significant decreases in post-treatment total number of leukocytes or leukocyte subpopulations was observed to suggest the presence of hypersensitivity or immunosuppression drug phenomena.

Tab. 3.

The values of the probability index "p" in the investigated groups of broilers

Parameter	I		II		III		I/II		I/III		II/III		
	Pre	Post	Pre	Post	I	F	Post	Post	Post	F	Post	F	
PCV	0.7963		0.6437		0.6453		0.3082		0.5457		0.6543		
Hb	0.5459		0.6977		0.9297		> 0.9999		0.2734		0.2667		
Erythrocytes	0.5902		0.2196		0.9515		0.3447		0.0429		0.2101		
Mean erythrocyte constants	MCH	0.5556		0.2935		0.6813		0.2935		0.0802		0.6596	
	MCH	0.5188		0.6611		0.8906		0.5384		0.0466		0.1178	
	MCHC	0.8514		0.4569		0.773		0.5292		0.6119		0.2477	
Leukocytes	0.9693		0.7965		0.9027		0.3617		0.0167*		0.0010*		
Leucogram	heterophile	0.7969		0.5333		0.0344*		0.4823		0.2557		0.8881	
	eosinophile	0.4613		0.7534		0.6040		> 0.9999		0.4782		0.4782	
	lymphocyte	0.9163		0.5309		0.0376*		0.8840		0.9551		0.8427	
	monocytes	0.4346		0.9082		0.9224		0.0660		0.0334*		0.5216	

Note: Pre= ante- treatment; Post = post- treatment; I=initial; F=final;

*= statistically significant.

Comparative analysis of the values obtained for the investigated parameters with bibliographic sources indicated some uncertainty in interpreting the results, with potential controversy arising from physiological ranges reported by various authors.

However, in the absence of statistically significant changes between the mean pre-and post-therapeutic values in the main hematological indices of the experimental groups, we considered that no alterations occurred after treatment with erythromycin thiocyanate.

CONCLUSIONS

- Analysis of the development of physiological parameters in the investigated groups of broilers revealed mean value variations from one group to another, falling within physiological intervals for the species and category, or slight deviations from some references, and the ranges established in the control group;
- Comparative developments pre-and post-treatment of hematological parameters have outlined a normal profile for the category of investigated broilers, indicating a good tolerance of therapeutic and double doses of erythromycin thiocyanate and no negative effects on electrolyte balance and erythrocyte homeostasis, erythropoiesis and general erythrocyte functions;
- Mean variations of the PCV (37.00 ± 5.44 - $39.68\pm 3.48\%$), hemoglobin concentration (6.88 ± 1.07 - 7.77 ± 1.03 g/dL) and total number of erythrocytes (2.28 ± 0.19 - 2.68 ± 0.36 T/L) were within the physiological intervals reported in some reference sources, or situated at the lower limits of others, showing a decreasing trend;
- MCV mean values (141.18 ± 29.49 - 174.21 ± 13.24 fL) exceeded some physiological intervals in the experimental groups, while MCH (26.53 ± 5.79 - 34.36 ± 5.78 pg) and MCHC (18.55 ± 3.73 - 20.68 ± 4.65 g/dL) were below the values found in most references also showing a decreasing trend;
- An overview of obtained data in the determined leukocyte parameters outlined specific aspects with a good defensive potential, both for the group treated with therapeutic and double dose of erythromycin thiocyanate;
- Evolution of the mean values of total number of leucocytes (15.16 ± 2.52 - 20.35 ± 2.52 G/L) and leukocyte subpopulations distribution, indicated a high level of tolerance to therapeutic and double doses of erythromycin thiocyanate confirming and lack of sensitization or adverse effects on leukocyte functions in general and leucopoiesis;
- Statistical analysis highlighted significant differences ($p<0.05$) only for the control group, consisting of a lower the percentage of heterophiles (from 43.18 ± 2.71 to 38.36 ± 6.50) and increase in lymphocytes (from 37.36 ± 4.20 to $42.64\pm 6.65\%$), due to individual cases of mild heteropenia and/or lymphocytosis;
- Clinical and hematological monitoring of some physiological health indicators has identified no potential risks associated with doubling the dose as an experimental variable; doses of 10 and 20 mg erythromycin/individual/day, administered for 7 consecutive days, were not followed by immediate or delayed side effects, or any sign of immunosuppression.

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