THEESIS SUMMARY

ANALYSIS OF THE HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS OF CHICKENS IN THE CONTEXT OF PHYSIO-PHARMACOLOGICAL INVESTIGATIONS OF SOME MEDICINAL PRODUCT FORMULAS

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INTRODUCTION

Hematological and biochemical analyses are essential to monitor the health status of poultry in general and Broilers in particular, although rarely offer sufficient information to outline an etiologic diagnosis. In many species of mammals and birds, clinical and laboratory diagnosis is based largely on the results of hematological and biochemical investigations.

Scientific research in the field often resorts to the use of chicken as experimental models; these results may be particularly relevant to the investigation of physiological mechanisms in different avian species. Although such studies are becoming more frequent, there is still little data available on the evolution of hematological and biochemical profiles in Broilers (WAKENELL, 2010; CLARK et al., 2009).

European legislation and national guidelines currently include special provisions relating to physio-pharmacological, preclinical and clinical testing necessary for approval and registration of medicinal products for human and veterinary use (** Directive 2010/63/EU). The main purpose of these tests consists in making assessments about drug tolerance in target species the detection of possible side effects and evaluating therapeutic efficacy.

An issue as important as the one created by the adverse effects became the exacerbated resistance following overuse of antibiotics as curatives or as growth promoters. In such circumstances become of grate concern, composition assessment, therapeutic efficacy and safety of new drug formulations TRÎNCĂ et al., 2013a,b).

Keywords: broiler chicken, hematological and biochemical profile, drug testing.
AIM, MOTIVATION AND RESERCH OBJECTIVES

The main purpose of this research is to evaluate the influence of physio-pharmacological factors in testing conditions of some veterinary medical products, on hematological and biochemical profiles in chickens, contributing thus to the development of avian veterinary medicine. Secondarily, in the present research we aim towards updating the information on the evolution of hematological and biochemical profiles in broilers and develop a relevant database for research in the field.

The originality elements of this study consist of analysis of the available data regarding the relevance of hematological and biochemical reference values in broiler research and adopt a suitable experimental model in generating them. Introducing physio-pharmacological component in the structure of the test protocols for evaluating pharmaceutical formulations on target species in the poultry sector is also a novel approach. An original character attribute and addressing the possibility of using mixed blood samples, formed by mixing individual samples that make up a experimental group. We believe that the implementation of mixed blood sample in evaluating hematological and biochemical profiles for avian species can greatly be beneficial by reducing costs and increasing the level of automation of the investigations.

General objectives:

1. Analysis of the European and national legislation on medicinal products tested on target species, with the implementation of a physio-pharmacological protocol on broiler chickens;
2. Optimization of avian blood samples collecting and processing procedures for hematological and biochemical tests;
3. Establish a minimum set of data regarding reference values that can be used to assess hematological and biochemical parameters in broiler chicken;
4. Hematological and biochemical analysis of tolerability and adverse effects in broiler chickens treated with two pharmaceuticals commonly used in poultry administered in therapeutic and multiple doses;
5. Implement a procedure based on the mixed blood sample for hematological and biochemical evaluation of the broiler chicken health.
STRUCTURE OF THE THESIS

The thesis entitled "Analysis of the hematological and biochemical parameters of chickens in the context of physio-pharmacological investigations of some medicinal product formulas" is detailed on a total of 182 pages and is composed of two parts, totaling 8 chapters. They are written and structured according to the requirements of the Doctoral School of the USAMV Cluj-Napoca.

**Part I** consists of four chapters, detailed on 53 pages, constituting a bibliographical study of the state of knowledge in the addressed areas: the legal framework in testing of medicinal products on birds as the target species, avian hematology and biochemistry, pharmacodynamics and pharmacokinetics of the active substances that make some medicinal products commonly used in poultry farming. Bibliographic documentation addresses the current state of knowledge, being a brief and relevant presentation of the principles and mechanisms that are the basis of the experimental models and investigations found in the own research.

**Part II** consist of the own research and it is organized into 5 chapters, developed on 106 pages. The first 4 chapters are divided into relevant biomedical sub chapters: objectives, materials and methods, results, discussion and partial conclusions. To these are added a last chapter, dedicated to the brief presentation of general conclusions and recommendations. The thesis continues with bibliography, totaling 195 references, selected based on their relevance to the research, and finishes with the summary in Romanian and English.

RESEARCH METHODOLOGY AND RESULTS

The own research plan is based largely on the joint physio-pharmacological protocol synthesized, presented in chapter II.1. Some of the methods included in this protocol and will be reflected in the conduct of the research found in chapter II.2.,II.3 and II.4. We also used a common set of tests for statistical analysis of the data resulted from the own research. Statistical analyzes were facilitated by the use of specialized computer applications (InGraphPad) used to perform the following biostatics tests: Tukey test for comparison of data framed by parametric distributions (Gaussian) and the Dunn test for non-parametric settings. These tests were the basis for calculating the mean, median, standard deviation, standard error and 95% confidence interval and the index of probability "p".

In the following, we present a brief development of the particular aspects of the methodology and the results of the own research chapters.

**Chapter II.1.** addresses the physio-pharmacological protocols implemented in testing two pharmaceutical formulations antibacterial on chickens, starting with a brief analysis of the requirements of European and national legislation regarding veterinary medical product testing and approval. The consulted information was the basis for establishing a general methodology of organizing the own research shaped by the physio-pharmacological components of a protocol, which contain current tests used to assess hematological, biochemical or clinical health in poultry. Under the current regulations, the tests were performed directly on the target species (broiler chicken) and the developed protocol included all the necessary aspects of its implementation for testing the investigated antibacterial products (Galiprotect and Eritrovit), with the following objectives: establishing a general physio-pharmaceutical protocol for drug testing which can provide conclusive data on the impact and effects of pharmaceuticals in poultry, analysis of the good management procedures for testing on target species of veterinary medical products, using the principles of replacement, reduction and refinement promoted at European level; assessing the applicability of the protocols used in drug testing in light of poultry farming conditions in our country, as
well as their relevance for the registration and re-registration of pharmaceutical products that have birds as target species.

We believe that facilitating producer information in regard to reviewing and clarifying dosage, tolerance and conditions that may lead to possible side effects, is a key feature of the protocol developed application in our research.

Chapter (II.2.) entitled “Analysis of the hematological parameters of broilers in the conditions of some medicinal product testing” has the following main objectives: assessing the erythrocyte and leukocyte parameters under physio-pharmacological testing of two antibacterial formulas on the line Ross broiler 308 and evaluating the influence of drug overdose on hematological profile in broiler chickens.

This chapter includes the essential arguments for involving hematological analyzes in the minimal set of screening tests used to monitor the health in broiler chickens. As is shown by the analyzed data, in this field there is much controversy on the diversity of factors influencing hematological and biochemical indices in chickens, which gives more of a orientation than a guideline to available information in this field.

The investigations carried out in this chapter consisted of a stepwise determination of blood count parameters in a control (n = 157) and 10 experimental groups (n = 15) of clinically healthy (9-14 days and 250 grams) broilers (Ross 308 line), from 2 commercial farms.

Blood sampling was preceded by the establishing the most accessible blood vessel (often basilar vein) in correlation to body weight, respectively the blood volume of the chicken. As anticoagulant, we used EDTA, knowing that heparin is not indicated for the determination of fibrinogen and leukocyte counts and may generate errors (CLARK et al., 2009).

Blood count parameters were determined by comparative usage of standard methods in avian hematology (detailed in the work).

The obtained data was processed and statistically analyzed by using of the aforementioned statistical tests. For evaluating the experimental data, we employed as references the obtained values in the initial investigations performed on the control group as well as those found in the specialized literature.

In agreement with Samour (2006), which shows that the topographic areas for accessing the blood stream in birds may be: the jugular, basilar and caudal tibial vain, we resorted to basilar vein puncture because of its superficial position, transcutaneous visibility and adequate size.

Among the significant findings made in this chapter, it is noteworthy to mention the jellification of blood plasma in some samples. This process is represented by the total or partial transformation of plasma into a gelatinous mass similar to the clot that can affect up to 25% of avian blood samples, compromising all or part of plasma (HARR, 2006). In our investigations, plasma jellification (shown in figure 1) affected 3-4 samples out of 15, reducing the number of adequate plasmas obtained in the experimental groups.

Figure 1. Partial plasma jellification of plasma from broiler chicken
For the assessment of leukocytes, we consider that using the Natt solution provided sufficient aspects regarding coloring, which allowed a better identification of this types of cells. The morphological evaluation of figurative elements, emphasize particular aspects related to the techniques of making and staining smears prepared from avian blood. In general, the technique used for the making the smears, with two microscope slides, provided good quality preparations, which were suitable for staining and examination. As artifacts, in the case of some smears, appeared acidophilic "inclusions" in the erythrocytes, caused by improper or incomplete drying or the total or partial degranulation of heterophils.

The investigation of chickens in the control group showed a statistically insured data set, which together with the bibliographical reference values led to the shaping of relevant images on the hematological parameters dynamics in broiler chicken. We believe that classification of the hematological values determined for the chickens in the control group within the physiological ranges, recorded by most valid bibliographical sources, justifies their use as experimental references.

Regarding the erythrocyte profile of chicken this group, it is sufficient to mention only the following mean values: 39.56 % for hematocrit (with standard error of 0.56 and 95 % confidence interval ranging from 38.45% and 40.67%), 9.04±3.30 g/dl for hemoglobin (with oscillations between 5.30 and 21.45 g/dl and 95 % confidence interval between 8.53 and 9.56 g/dl), for the total number of erythrocytes (with a standard error of 0.04 and 95% confidence interval limit of 2.38 and 2.52 T/l). The results of the statistical analysis showed that hemoglobin levels have not passed the normality test, this parameter lacking a Gaussian distribution. We noted also that in clinically healthy birds, there was a hematocrit/hemoglobin ratio (4.70±1.24) higher that the one found in mammals (3.00).

The development of the leukocyte parameters in the control group of chickens was characterized by mean values of 18.28±6.10 G/l for total leukocyte number, individual data ranging between 5.50 and 31.00 G/l, with a 95 % confidence interval within the limits of 17.33 and 19.24%. From the oscillations of the leukocyte subpopulations, we assigned relevance to the heterophils, which varied between 28% and 73%, fluctuating around the mean of 48.85±9.40 %; lymphocytes with individual variations from 16 to 60%, the 95% confidence interval being between 38.11 % and 35.43 % and monocytes, with a mean value of 11.59±4.89 % and a standard error of mean of 0.39 %

Also noteworthy are the results of investigations on the ratio between different types of blood cells, relevance for the control chickens the ratio between the erythrocytes and leucocytes of 1/155. In case of leukocyte counts, quantification of the subpopulations showed 1 lymphocyte to 1.47± 0.67 heterophiles, 1 monocyte to 5.70 ±4.91 lymphocyte and 1 monocyte to 4.25±3.06 lymphocytes.

Results obtained from the investigation of the chickens treated with Eritrovit. The statistical analysis indicated changes in the index of probability "p" depending on the dose as an experimental variable and the investigated parameter (table 1 and 2). As shown in table 1, the hematocrit variations in the mean values were lacking statistical significance (p=0.6864), being: 39.23±4.74%5 for the recommended dose, 37.00±5.71% for double dose, 38.10 ±5.66% and 38.12 ±3.63% for multiple doses (5x respectively 10x). More relevant developments were found in the case of hemoglobin, with very significant differences (p = 0.0001) 7.47±1.25 g/dl 7.47±1.22 g/dl, 6.35±0.73 and 6.47±1.00 g/dl. In case of the total number of erythrocytes, the mean values found were close 2.34±0.33; 2.47±0.34; 2.25±0.53 and 2.24±0.55 T/l. Noteworthy are also there are very significant differences (p < 0.0001), recorded MCHC, the recorded values reaching the highest level (20.68±4.88 g/dl) in the group treated with the double dose and lowest (17.17 ± 3.43 g/dl) in the one treated with the multiple dose of 10.

Morphological examination revealed, in addition to normal aspects, the presence of erythrocytes with a slight degree of hypochromia, but without changes in terms of shape and size.
Table 1.
Mean values of the erythrocyte parameters and probability index recorded in the evaluation of the erythromycin thiocyanate based product (Eritrovit)

<table>
<thead>
<tr>
<th>Group</th>
<th>Therapeutic dose (1E)</th>
<th>Double dose (2E)</th>
<th>Multiple dose 5x (3E)</th>
<th>Multiple dose 10x (4E)</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>Mean ± st. dev.</td>
<td>Mean ± st. dev.</td>
<td>Mean ± st. dev.</td>
<td>Mean ± st. dev.</td>
<td>Mean ± st. dev.</td>
</tr>
<tr>
<td>Ht (% )</td>
<td>39,23±4,74</td>
<td>37,00±5,71</td>
<td>38,10±5,66</td>
<td>38,12±3,63</td>
<td>39,56±7,09</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>7,47±1,25</td>
<td>7,47±1,22</td>
<td>6,35±0,73</td>
<td>6,47±1,00</td>
<td>9,04±3,30</td>
</tr>
<tr>
<td>Erit (T/l)</td>
<td>2,34±0,33</td>
<td>2,47±0,34</td>
<td>2,25±0,53</td>
<td>2,24±0,55</td>
<td>2,46±0,45</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>169,25±25,21</td>
<td>152,93±34,18</td>
<td>178,80±54,35</td>
<td>174,87±52,44</td>
<td>167,19±40,77</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>32,5±4,65</td>
<td>30,7±6,47</td>
<td>29,3±7,73</td>
<td>30,0±6,95</td>
<td>37,3±13,62</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>19,4±4,65</td>
<td>20,6±4,88</td>
<td>17,0±3,70</td>
<td>17,17±3,43</td>
<td>23,10±6,87</td>
</tr>
</tbody>
</table>

*p* = Value calculated by Dunn test for non-parametric distributions

Data referring to the leukocyte parameters development, as shown in table 2, shows very significant statistical oscillations (p = 0.0010) of the total leucocyte number, situated in the range of 11.88 ± 4.20 to 16.75 ± 3.40 G/l.

Distribution of leukocyte subpopulations showed statistically significant changes in most cases. In this context we would like to mention the heterophil subpopulation development between 38.91±11.03 to 51.33 ± 9.67%, with statistically significant differences, the non-Gaussian distribution of eosinophils, with mean values of 1.73 ± 1.35% for the group treated with the therapeutic dose to 4.00 ± 3.02% for the group treated with multiple dose of 10.

Important developments, with statistically significant differences (p <0.0001), presented also the lymphocyte subpopulation, whose mean values were situated in the range of 26.17 ± 10.94 to 43.45 ± 11.59%. In contrast, the mean monocyte population was predominantly higher, ranging from 12.55 ±3.27% to 19.25±7.20%, showing significant differences (p <0.0001).

Table 2.
Mean values of the leucocyte parameters and probability index recorded in the evaluation of the erythromycin thiocyanate based product (Eritrovit)

<table>
<thead>
<tr>
<th>Group</th>
<th>Therapeutic dose (1E)</th>
<th>Double dose (2E)</th>
<th>Multiple dose 5x (3E)</th>
<th>Multiple dose 10x (4E)</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>Mean ± st. dev.</td>
<td>Mean ± st. dev.</td>
<td>Mean ± st. dev.</td>
<td>Mean ± st. dev.</td>
<td>Mean ± st. dev.</td>
</tr>
<tr>
<td>Leuc. (G/l)</td>
<td>16,75±3,40</td>
<td>15,47±3,02</td>
<td>14,67±4,38</td>
<td>11,88±4,20</td>
<td>18,28±6,10</td>
</tr>
<tr>
<td>Heter. (%)</td>
<td>41,73±7,00</td>
<td>38,91±11,03</td>
<td>51,33±9,67</td>
<td>51,08±11,16</td>
<td>48,85±9,40</td>
</tr>
<tr>
<td>Eoz. (%)</td>
<td>1,73±1,35</td>
<td>1,73±1,35</td>
<td>2,83±1,47</td>
<td>4,00±3,02</td>
<td>2,40±1,97</td>
</tr>
<tr>
<td>Bazof. (%)</td>
<td>1,18±0,98</td>
<td>0,64±0,81</td>
<td>0,42±0,90</td>
<td>0,17±0,39</td>
<td>0,39±0,80</td>
</tr>
<tr>
<td>Limfo. (%)</td>
<td>42,82±8,10</td>
<td>43,45±11,59</td>
<td>26,17±10,94</td>
<td>31,33±8,94</td>
<td>36,77±8,54</td>
</tr>
<tr>
<td>Mono. (%)</td>
<td>12,55±3,27</td>
<td>15,27±3,29</td>
<td>19,25±7,20</td>
<td>13,42±5,14</td>
<td>11,59±4,86</td>
</tr>
</tbody>
</table>

*p* = Value calculated through Dunn test for non-parametric distributions

Results from the investigation of the chickens treated with Galiprot. According to the indicated posology of this product its use is differentiated by age. Thus with the therapeutic and double dose variables were performed two therapies, differentiated by age (2 respectively 7 consecutive days) and multiple doses variables (8x and 16x) treated only one age group (2, consecutive days).
Among the characteristics of evolution erythrocyte parameters (table 3) is noteworthy the lack of statistical significance (p = 0.9500) of the mean hematocrit values: 39.32 ± 4.12% respectively 39.59 ± 3.69 % for the groups treated with the therapeutic dose; 36.95 ± 5.57% respectively 39.45 ± 2.94% for those treated with double dose; 39.55 ± 4.90% for the group treated with multiple of 8 and 39 69 ± 8.36% for the one treated with multiple 16 of the dose.

Table 3.

Mean values of the erythrocyte parameters and probability index recorded in the evaluation of the oxytetracycline chlorhydrate based product (Galiprotect)

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>9-10 days</th>
<th>32-38 days</th>
<th>10-20 days</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Therapeutic dose (1AO)</td>
<td>Double dose (2AO)</td>
<td>Therapeutic dose (1BO)</td>
<td>Double dose (2BO)</td>
</tr>
<tr>
<td>Ht (%)</td>
<td>Mean ± st. dev.</td>
<td>Mean ± st. dev.</td>
<td>Mean ± st. dev.</td>
<td>Mean ± st. dev.</td>
<td>Mean ± st. dev.</td>
</tr>
</tbody>
</table>
| Hb (g/dl)              | 8.18±1.36 | 7.88±0.61 | 7.90±0.62 | 8.00±0.65 | 7.10±1.12 | 6.40±0.84 | 9.04±3.30 | 0.0027
| 167.19±40.77           | 167.19±40.77 | 167.19±40.77 | 167.19±40.77 | 167.19±40.77 | 167.19±40.77 | 167.19±40.77 | 167.19±40.77 | 167.19±40.77 | 0.4963 |
| Erit. (T/l)            | 24.40±3.33 | 24.09±2.22 | 24.44±0.36 | 24.53±1.99 | 27.2±1.01 | 22.54±2.47 | 24.60±4.45 | 0.2797 |
| MCV (fl)               | 169.61±22.53 | 148.7±4.33 | 164.96±25.37 | 150.77±15.78 | 172.67±64.90 | 177.00±19.38 | 167.19±40.77 | 0.4963 |
| MCH (pg)               | 35.49±7.61 | 31.85±3.09 | 33.08±5.71 | 30.53±2.92 | 29.50±8.29 | 29.65±7.59 | 37.37±13.62 | 0.2013 |
| MCHC (g/dl)            | 21.28±5.52 | 21.90±4.58 | 20.18±2.87 | 20.31±1.47 | 18.17±3.50 | 16.69±3.56 | 23.10±6.87 | 0.0007 |

Hemoglobin showed mean value oscillations ranging from a minimum of 6.40±0.84 g/dl (for the group treated with multiple 16 of the dose) and a maximum of 8.18±1.36 g/dl (for the group treated with therapeutic dose), with probability index "p" (0.0027) corresponding to statistically significant differences.

Total number of erythrocyte did not show statistical significant difference and was characterized by mean values of 2.34±0.33 T/l, respectively 2.44±0.36 T/l for the group treated with therapeutic doses; 2.49±0.22 T/l, respectively 2.72±1.01 T/l for those treated with duple dose and values of 2.72±1.01 T/l for the group exposed for the multiple of 8, respectively 2.25±0.47 T/l for the one treated with the multiple of 16 of the dose. The same dominant characteristic were found in the mean erythocyte constant, that showed close and statistically insignificant mean values, with the exception of MCHC that showed statistical significance in case of the comparison between the group treated of the multiple 16 of the dose and the control group (table 3).

The analyze leukocyte parameters highlighted more or less important individual oscillation, whose mean values and standard deviation are presented in table 4. Thus the total number of leukocyte the mean values presented some significant differences with oscillations situated in the interval 15.17±2.03-24.95±9.56 G/l, the minimal value being recorded in case of the group of 9-10 days treated with the double dose and the maximum value was found in the group aged 10-20 days treated of the multiple 8 of the dose. The development of heterophiles subpopulation indicated mean values with variation ranging between a minimum of 38.91±7.74% registered in case of the group of 9-10 days treated with the double dose and maximum of 61.55±5.91% for the group treated with the multiple of 8. The differences observed in the values of this parameter proved to be very statistical significant with a probability index p<0.0001.

The eosinophiles highlighted mean values situated between a minimum of 1.27±0.79%, established in case of the group of 38-39 days (given the therapeutic dose) and a maximum of 4.00±2.24% observed in the same age category in the case the group treated with the double dose. The levels of lymphocyte subpopulations where situated in the interval 21.27±5.78%-45.45±7.17%; the lowest observed value was found in the group treated with the multiple of
16 of the dose and the highest in case of the group age 9-10 days treated with the double dose, the registered differences being statistically significant (p<0.0001). Important increases were presented in proportion of monocytes, with mean values situated between a minimum of 12.73±2.76% and maximum of 17.00±6.47%, the observed differences being statistically significant in this parameter also (p=0.0053).

Table 4.

Mean values of the leukocyte parameters and probability index recorded in the evaluation of the oxytetracycline chlorhydrate based product (Galiprotect)

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>9-10 days</th>
<th>32-38 days</th>
<th>10-20 days</th>
<th>Control</th>
<th>&quot;p&quot; index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Therapeutic dose (1AO)</td>
<td>Double dose (2AO)</td>
<td>Therapeutic dose (1BO)</td>
<td>Double dose (2BO)</td>
<td>Multiple dose 8x (1CO)</td>
<td>Multiple dose 16x (2CO)</td>
</tr>
<tr>
<td></td>
<td>Mean ± st. dev.</td>
<td>Mean ± st. dev.</td>
<td>Mean ± st. dev.</td>
<td>Mean ± st. dev.</td>
<td>Mean ± st. dev.</td>
<td>Mean ± st. dev.</td>
</tr>
<tr>
<td>Leuc. (G/l)</td>
<td>16.95±3.64</td>
<td>15.17±2.03</td>
<td>21.53±2.68</td>
<td>24.37±2.93</td>
<td>24.95±9.56</td>
<td>22.95±4.91</td>
</tr>
<tr>
<td>Heter. (%)</td>
<td>41.73±6.84</td>
<td>38.91±7.74</td>
<td>41.27±5.14</td>
<td>40.45±2.70</td>
<td>54.55±9.84</td>
<td>61.55±5.91</td>
</tr>
<tr>
<td>Eoz. (%)</td>
<td>1.73±1.01</td>
<td>1.73±1.01</td>
<td>1.27±0.79</td>
<td>4.00±2.24</td>
<td>4.45±1.14</td>
<td>3.64±2.66</td>
</tr>
<tr>
<td>Bazopf. (%)</td>
<td>0.18±0.98</td>
<td>0.64±0.50</td>
<td>1.73±1.10</td>
<td>1.18±0.98</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Limfo (%)</td>
<td>42.82±6.27</td>
<td>45.45±7.17</td>
<td>42.45±4.48</td>
<td>37.36±6.74</td>
<td>25.64±12.46</td>
<td>21.27±5.78</td>
</tr>
<tr>
<td>Mono. (%)</td>
<td>12.73±2.76</td>
<td>13.27±3.74</td>
<td>13.27±4.52</td>
<td>17.00±6.47</td>
<td>15.36±7.24</td>
<td>13.55±4.89</td>
</tr>
</tbody>
</table>

\[\text{Value calculated through Dunn test for non-parametric distributions}\]

According to the presented data the overall development of the hematological indexes reviled less important alterations usually associated with a lack of statistical significance. This fact indicates that the therapeutic doses of the two tested products did not exercised relevant influences on the hematological parameters and hematopoesis. On the other hand the dynamic of hematological profile of the broiler chickens from the group treated with multiple doses highlighted important variation with statistical significance in case some hematological indices (figures 2-5). These modifications however showed that overdosing with antibiotics from macrolide and tetracycline group, possible in technical malfunction or automated therapy systems, can be tolerated without inducing acute symptoms and immediate clinical consences.

Figure 2. Hemoglobin dynamics of in the broiler groups treated erythromycin-based product, indicating significant declines in overdose
Thus, as shown in the figures 2 and 3 over dosage in case of the erythromycin tiocianat based product mainly influenced erythrocyte with statistically significant decreases of hemoglobin concentration attributed mainly to the presented of thiocyanate ion. The situation was different in the case of over dosage with the oxytetracycline chloralhydrate, which influenced mainly leukocytes inducing leukocytosis associated heterophilia (figure 4 and 5). The mentioned figures show decreases of lymphocyte population in both of the tested products. According to the data reported by Al-Mayah et al. (2005), Turcu et al. (2011), Fiţ et al. (2012a, b), we can conceder that this tendency towards lymphopenia can largely be attributed to the AD3E vitamin complex found in these formulas.

**Figure 3.** MCHC dynamics in broiler groups treated with erythromycin-based product, indicating significant declines in overdose

**Figure 4.** Dynamics of heterophil percentage in the groups of chickens treated with oxytetracycline-based product, indicating significant increases in overdose

**Figure 5.** Dynamics of lymphocytes percentage in groups of chickens treated with oxytetracycline based product, indicating significant reductions in overdose
In conclusion it is important to mention that the hematological parameters indicated some alterations that associated with other investigation may prove relevant for drug over dosage. The consulted bibliographic data shows little available information regarding the influence of medicinal substances on the hematological parameters of broiler chickens raised in the intensive system, which justified the investigations undertaken in this study, as well as their continuation in future research.

**Chapter II.3.** entitled “Assessment of some metabolic profile parameters of broiler chicken in the conditions of some medicinal product testing” had as aim the following major objectives: analyses bibliographical reference values for the main metabolic parameters and evaluating the relevance in monitoring the health of broiler chicken; evaluating the interval of variation of biochemical indexes in clinically healthy chickens and establishing the level of statistical reassurance; analyses of the relevance of the main metabolic profile indices in physio-pharmacological testing of the two medicinal products on Broilers as target species; evaluating the influence drug over dosage on the metabolic profile of broiler chickens; correlative analyses of the relevance of hematological and biochemical parameters in detecting accidental over dosage in broiler chickens.

According to the general protocol the research was focused on determining some metabolic profile parameters in broiler chickens (Ross 308 line), organized in a control group (n=35) and six experimental groups (n=5). The values established on the chickens from the control group were compared to the bibliographical references and utilized in evaluating the experimental groups. The investigation required a small amount of blood sample and consisted in implementing automated dry biochemical investigations using VetScan2 analyzer equipped with Avian and Reptilian profile kits. The method and the equipment were the basis for determining the following metabolic profile indices: aspartate transaminase, biliary acid, creatine phosphokinase, uric acid, glucose, calcium, phosphorus, total protein, albumin, globulins, potassium and sodium. Interpreting the obtained results also included statistical analyses of the individual data through the test mention in the general methodology.

**Regarding the results of the control group,** we would like the mentioned the following aspects utilized in characterization of the main biochemical indexes: protein levels situated near an average of 3.36±0.38 g/dl, with a minimum of 2.5 and a maximum 4.1 g/dl; albumin oscillations ranging in the interval 1.3-2.5 g/dl, the mean value being 1.93±0.39 g/dl. The mean values of globulin values of 1.12±0.39 g/dl, with oscillation between 0.5 and 2 g/dl and glycemia level situated in the interval 128-302 mg/dl with mean value of 210±39.99 mg/dl. Serum enzyme dynamics showed mean values of 188.69±2179 U/l for aspartate transaminase and 1321.29±482.71 U/l for creatin phosphokinasi; biliary acids levels were characterized by values situated under the minimal limit of 35 µmol/l; uric acid varying close the mean value of 4.6±0.73 mg/dl with oscillations in the interval 1.4-9 mg/dl. The development of serum ions was characterized by falling with in physiologic limits presenting mean values of 8.37±1.8 mg/dl for calcium, 6.02±1.15 mg/dl for phosphorus, 149.4±7.38 mmol/l sodium and 5.37±1.19 mmol/l for potassium.

It is important to mention that the consulted bibliographical references presented differences regarding the measurement unit of the reference values which hardens their global interpretation. In this context applying correction factors for uniforming measurement units becomes difficult especially due to the expression in the form mean and standard deviation. In conscience it becomes mandatory the utilization of own values obtained from control groups maintained in experimental condition for interpreting the obtained values and less on the data provided by bibliographical literature (BOWES, 1989).

As shown in the paper, the consulted bibliographical sources did not offer a wide enough range of reference values to cover all the parameters in this study.

**The results obtained from the experimental groups** highlighted some characteristic developments for Eritrovit. Regarding the protein metabolic profile was noticed oscillation in
physiological ranges for the following parameters: protein levels with the individual values from 3.2 to 3.7 g/dl; albuminemia, ranging between 1.6-2.3 g/dl (figure 6) and globulinemia with mean variations included in the interval 0.7-2.4 g/dl (figure 7). Statistically significant increase in globulinemia can be attributed mainly to dehydration or secondary inflammatory processes unassociated with medicinal treatment (HARR, 2006).

Glycemia varied within the physiological limits being framed by a minimum of 1.94 g/dl and a maximum of 2.74 g/dl. These oscillations without statistical significance show that the treatment with the erythromycin thiocyanate did not show adverse effects on glucose metabolism.

In evaluating the enzyme profile were observed normal variations of the levels of aspartate transaminase ranging in the interval 145-198 U/l and those of creatine phosphokinase framed by the interval 696-1263 U/l. The absence of the statistical significant differences in the main indexes of enzyme metabolism conforms the fact that exposure to therapeutical and double doses did not have any adverse effect on hepatic or muscular function of the investigated birds.

The concentration of bile acids constantly situated under the value of 35 µmol/l over the whole length of the investigation corresponds to a normal hepato-digestive function, increase of these parameters in broiler chickens being associated with hepatic disorders (BROMIDGE et al., 1985).

For evaluating renal functions were relevant the levels of uric acid which were included in the interval 2.7-5.7 mg/l.

In the general context observed in the investigated parameters also fell the following components of the ionogram: calcium (6.7-10.2 mg/dl), phosphorus (5.5-7.9 mg/dl), sodium (146-170 mmol/l) and potassium (3.4-7.8 mmol/l). The slight increasing tendency of some ionogram parameters registered in the experimental groups can be explained by possible dehydration of the individuals from those groups rather than a side effect of the tested product (GHERGARIU et al., 2000).

The analyses of the metabolic profile of the chickens treated with Galiprotect revealed as well as physiological developments and also alterations of some biochemical parameters. Regarding protein parameters are noticeable the following: relatively constant levels of proteins with oscillations subscribing in the interval 3-4.10 g/dl; wide oscillation of albuminemia (1.5-4 g/dl) with statistical significance differences (p<0.0001; important variations of globulinemia (0.10-2.30 g/dl) characterized by statistical significant differences (p=0.0191).

The oscillation observed in case of the protein metabolism with statistically significant differences in case of albumin can be attributed to the hiperproteic diet specific to the industrial raising of broiler chicken respectively to the metabolic strain specific to these birds as well as a possible dehydration (HARR, 2006). More so, Filipowic et al. (2007) show that albumin represents one of the main proteins that serves amino acid source for tissue synthesis in the periods rapid somatic growth of birds. The statistically significant differences observed in case of blood globulins can be attributed to the technique of the evaluation of these parameters which is based on the values of total proteins and albumins (HARR, 2006).

Noteworthy were also the homogenous decreasing tendency of the mean values of glycemia, individual values being situated in the interval 185-330 mg/dl. Similar to albumins, the statistical significances found in these parameter can be attributed to metabolic stress that in granivores is characterized by hypoglycemia (LUMEIJ, 1998).

In case of enzyme investigation was observed a development characterized by statistically significant differences in case of aspartate transaminase (71-220 U/l), with slightly increasing tendency in the investigated groups however inferior to the controls.

This aspect can be explained by the presence of vitamin C in the investigated product, vitamin known for it effect of reducing aspartate transaminase (WU, 2006).
Creatine phosphokinase presented a similar development to the aspartate transaminase, with individual oscillations (from 360.00 to 1264.00 U/l) lower in the control group and statistically significant differences (p=0.0043) (figure 8). This can be explained by the relatively low physical activity of broiler chickens. Wei et al. (1981) indicate that creatine phosphokinase may be influenced by physical activity, respectively that its levels are reduced when reducing physical effort.

Uric acid dynamics showed close oscillation to the control group that was lacking statistical significance and pathological important (2.00-6.52 mg/l).
The analysis of the iongram revealed a dynamic marked by variations lacking statistical significance that could not be attributed to pathological connotations, for calcium (7.20-11.00 mg/dl), sodium (146-170 mmol/l) and potassium (3-7 mmol/l). Phosphorus presented a dynamic dominated by individual oscillation framed by a minimum value of 3.1 and a maximum of 8 mg/dl with statistical significant differences (p=0.0469) (figure 9). The decreases observed in this parameter can be attributed to possible nutritional deficiencies associated with increased need caused by rapid growth of Broilers (HARR, 2006).

![Figure 9. Dynamics of blood phosphorus concentration in the broiler chicken from the experimental groups treated with oxytetracycline-based product](image)

**The Chapter II. 4. entitled “Relevance of mean multiple blood samples in investigations of hematological profiles in chicken” is characterized by unique aspects. In this chapter we followed as main objectives: verifying the degree of homogeneity of the investigated population of broiler chickens through evaluating blood compatibility resulting to Crossmatch type tests; morphological evaluation of the degree of hemolysis and/or hemaglutination of the mean combine blood sample in light of its adequacy to routine hematological investigations; establishing the level of statistical insurance of individual values from the mean combine sample; comparative evaluation of the level of conformity of the obtained values in the hematological and biochemical test performed on mixed and individual blood samples to the physiological reference physiological.**

According to the proposed objectives as a first experimental step we resorted to evaluating blood compatibility trough major and minor Crossmatch. After verifying the individual compatibility of the samples obtained from broiler chickens with high degree of homogeneity, hematological and biochemical tests were performed firstly on individual samples and then on mixed blood samples obtained from the individuals.

In order to perform the investigations from this chapter we resorted to utilization birds from two commercial farms presented in chapter II.2. The chickens were organized in two experimental groups, group I (n=9) and group II (n=20) which were subjected to the hematological and biochemical investigation presented in chapters II.2. and II.3., the latter on a reduced number of randomly selected samples: 9 from the first group and 6 from the second.

In the mean time individual sample compatibility was tested in order to obtain the mixed blood sample. In this regard we adopted the quick procedure of slide Crossmatch introduced by Ognean et al. (2009) for evaluating the hemotransfusional compatibility. Along the Crossmatch test we also performed the autoaglutination control test.

The results were evaluated through comparative analyses of the minimum and maximum levels, mean and standard deviations and 95% confidence interval of the individual and combine sample.
In regard to the procedure used for reading the results of the compatibility tests we considered that macroscopic evaluation of macroaglutination respectively microscopic evaluation of microaglutination or hemolysis offer good relevance for avian blood samples (figure 10). In the macroscopic evaluation we observed that the combined blood sample had a good degree of homogeneity without showing signs of clothing or hemolysis, being considered adequate for hematological and biochemical test (figure 11).

**Figure 10.** Negative crossmatch reaction, macroscopic and microscopic aspect (fresh preparation 10x)

**Figure 11.** Microscopic examination of an adequate combined sample (fresh preparation 20x)

The results regarding erythrocyte parameter development showed the following aspects (table 5): mean values for the number of erythrocytes for the chickens in group I (2.45±0.58 T/l) higher than that found in the mixed sample (2.09 T/l) and close to those found in group II (2.76 respectively 2.65±0.53 T/l); important differences were observed in case hemoglobin concentration in both experimental groups, for the combine sample the levels being 12.38 g/dl respectively 15.18 g/dl; important differences were also observed in case of the hematocrit of group II, in which the 60% level was situated above the superior trust threshold (52.27%).
as well as above the individual mean (48.98±7.04%); a slight increasing tendency of MCV in case of the combined sample.

The values of the erythrocyte indices of the experimental groups of broiler chicken

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No. of Erythrocytes (T/L)</th>
<th>Hemoglobin (g/dl)</th>
<th>Hematocrit (%)</th>
<th>Mean erythrocyte constants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MCV (fl)</td>
</tr>
<tr>
<td>Lot I (15 days)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>1.64</td>
<td>9.41</td>
<td>33.33</td>
<td>117.62</td>
</tr>
<tr>
<td>Maximum</td>
<td>3.60</td>
<td>13.53</td>
<td>54.55</td>
<td>277.16</td>
</tr>
<tr>
<td>Mean</td>
<td>2.45</td>
<td>11.55</td>
<td>44.93</td>
<td>193.75</td>
</tr>
<tr>
<td>St. dev.</td>
<td>0.58</td>
<td>1.30</td>
<td>5.91</td>
<td>53.86</td>
</tr>
<tr>
<td>Inferior 95%confidence interval</td>
<td></td>
<td></td>
<td></td>
<td>2.17</td>
</tr>
<tr>
<td>Superior 95%confidence interval</td>
<td></td>
<td></td>
<td></td>
<td>2.73</td>
</tr>
<tr>
<td>Mixed blood sample</td>
<td></td>
<td></td>
<td></td>
<td>2.09</td>
</tr>
</tbody>
</table>

| Lot II (20 days) |                         |                   |                |               |          |             |
| Minimum   | 1.76                      | 12.05             | 40.00          | 111.11       | 42.04    | 20.65       |
| Maximum   | 3.88                      | 21.45             | 66.67          | 285.71       | 96.56    | 53.63       |
| Mean      | 2.65                      | 15.89             | 48.98          | 192.38       | 58.95    | 33.48       |
| St. dev.  | 0.53                      | 3.08              | 7.04           | 49.33        | 15.04    | 9.74        |
| Inferior 95%confidence interval |               |                   |                | 2.40        | 14.45    | 45.68       |
| Superior 95%confidence interval |               |                   |                | 2.90        | 17.33    | 52.28       |
| Mixed blood sample |               |                   |                | 2.76        | 15.18    | 60.00       |

Analysis of white blood cell counts did not show important differences regarding the value of the total number of leukocytes, that for the combined sample were situated at 22.5 G/l (group I) respectively 19.5 G/l (group II) (table 6).

In case of the leucocyte populations were observed differences between the combined blood samples and individual means as well as towards 95% confidence interval. Thus in case of group II, were observed differences regarding the mixed blood sample for heterophiles (62%), compared to the group mean of 54.5±8.83%. Monocyte levels in case of the mixed blood sample (group I) reached a level of 16%, value that surpassed both the upper limit of the 95% confidence interval as well as the mean (9.21±3.99%) (table 6).

Comparative analyses of the biochemical parameters showed that the mixed blood samples presented similar values to those obtained in the individual sample (table 7). In fact in the comparison of the mixed blood samples to the individual means the only notable differences was given by the uric acid. In case of the mixed sample the value of this parameter (5.6 mg/dl respectively 5 mg/dl) surpassed the individual means (3.16±2.35 mg/dl respectively 4.8±1.25 mg/dl) registered in the experimental groups.

In light of the results obtained in this chapter we can conclude that Broilers have a high degree of blood compatibility respectively a high level of homogeneity in hematological and biochemical profiles, thus conforming the possibility of impractical implementation of the mixed blood sample for hematological and biochemical investigations that can bring important economic benefits.
### Table 6.
The mean values of the leukocyte parameters in the investigated groups of broiler chicken

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lot I (15 days)</th>
<th>Lot II (20 days)</th>
<th>Mixed blood sample</th>
<th>Inferior 95% confidence interval</th>
<th>Superior 95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Leukocytes (G/L)</td>
<td>Minimum</td>
<td>Maximum</td>
<td>Mean</td>
<td>St. dev.</td>
<td>Minimum</td>
</tr>
<tr>
<td>Heterophils (%)</td>
<td>9.00</td>
<td>31.00</td>
<td>18.45</td>
<td>6.55</td>
<td>15.289</td>
</tr>
<tr>
<td>Eosinophil (%)</td>
<td>30.00</td>
<td>63.00</td>
<td>50.05</td>
<td>8.85</td>
<td>45,788</td>
</tr>
<tr>
<td>Basophil (%)</td>
<td>0.00</td>
<td>7.00</td>
<td>2.05</td>
<td>1.75</td>
<td>1,210</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>0.00</td>
<td>1.00</td>
<td>0.16</td>
<td>0.37</td>
<td>0.06492</td>
</tr>
<tr>
<td>Monocyte (%)</td>
<td>27.00</td>
<td>60.00</td>
<td>38.53</td>
<td>9.94</td>
<td>33,738</td>
</tr>
</tbody>
</table>

### Table 7.
The values of the biochemical parameters in the investigated groups of broiler chicken

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TP g/dl</th>
<th>ALB g/dl</th>
<th>GLOB g/dl</th>
<th>GLU mg/dl</th>
<th>AST U/l</th>
<th>CK U/l</th>
<th>BA* µmol/l</th>
<th>UA mg/dl</th>
<th>CA mg/dl</th>
<th>PHOS mg/dl</th>
<th>Na mmol/l</th>
<th>K mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot I (15 days)</td>
<td>Minimum</td>
<td>3.00</td>
<td>2.00</td>
<td>1.00</td>
<td>128.00</td>
<td>161.00</td>
<td>1205.00</td>
<td>1.40</td>
<td>5.10</td>
<td>4.60</td>
<td>140.00</td>
<td>4.20</td>
</tr>
<tr>
<td>Maximum</td>
<td>4.10</td>
<td>2.50</td>
<td>2.00</td>
<td>238.00</td>
<td>299.00</td>
<td>183.56</td>
<td>1776.22</td>
<td>3.16</td>
<td>7.60</td>
<td>5.50</td>
<td>147.00</td>
<td>6.40</td>
</tr>
<tr>
<td>Mean</td>
<td>3.57</td>
<td>2.19</td>
<td>1.37</td>
<td>205.00</td>
<td>292.00</td>
<td>183.56</td>
<td>1776.22</td>
<td>3.16</td>
<td>7.60</td>
<td>5.50</td>
<td>147.00</td>
<td>6.40</td>
</tr>
<tr>
<td>St. dev.</td>
<td>0.29</td>
<td>0.15</td>
<td>0.28</td>
<td>34.65</td>
<td>151.2</td>
<td>521.25</td>
<td>143.33</td>
<td>0.37</td>
<td>2.65</td>
<td>0.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lot II (20 days)</td>
<td>Minimum</td>
<td>2.90</td>
<td>1.80</td>
<td>0.80</td>
<td>222.00</td>
<td>193.00</td>
<td>889.00</td>
<td>2.90</td>
<td>10.60</td>
<td>5.60</td>
<td>140.00</td>
<td>5.60</td>
</tr>
<tr>
<td>Maximum</td>
<td>3.70</td>
<td>2.60</td>
<td>1.90</td>
<td>302.00</td>
<td>326.00</td>
<td>2465.00</td>
<td>7.1</td>
<td>11.90</td>
<td>9.50</td>
<td>148.00</td>
<td>8.50</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>3.25</td>
<td>2.07</td>
<td>1.70</td>
<td>257.50</td>
<td>210.83</td>
<td>1378.67</td>
<td>4.96</td>
<td>7.45</td>
<td>5.50</td>
<td>145.37</td>
<td>6.27</td>
<td></td>
</tr>
<tr>
<td>St. dev.</td>
<td>0.27</td>
<td>0.25</td>
<td>0.39</td>
<td>28.09</td>
<td>19.98</td>
<td>628.71</td>
<td>1.25</td>
<td>0.49</td>
<td>1.45</td>
<td>3.28</td>
<td>1.07</td>
<td></td>
</tr>
<tr>
<td>Mixed blood sample</td>
<td>Minimum</td>
<td>3.90</td>
<td>2.30</td>
<td>1.00</td>
<td>211.00</td>
<td>181.00</td>
<td>1743.00</td>
<td>5.60</td>
<td>6.90</td>
<td>4.90</td>
<td>143.00</td>
<td>4.40</td>
</tr>
<tr>
<td>Superior 95% confidence interval</td>
<td>3.35</td>
<td>2.07</td>
<td>1.15</td>
<td>143.47</td>
<td>171.94</td>
<td>1375.6</td>
<td>6.20</td>
<td>11.00</td>
<td>7.22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lot II (20 days)</td>
<td>Minimum</td>
<td>2.97</td>
<td>1.80</td>
<td>0.76</td>
<td>228.02</td>
<td>189.86</td>
<td>717.72</td>
<td>3.49</td>
<td>10.48</td>
<td>5.69</td>
<td>140.03</td>
<td>6.14</td>
</tr>
<tr>
<td>Maximum</td>
<td>3.53</td>
<td>2.33</td>
<td>1.57</td>
<td>286.98</td>
<td>231.81</td>
<td>2039.6</td>
<td>6.11</td>
<td>11.52</td>
<td>8.74</td>
<td>145.97</td>
<td>8.39</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>3.20</td>
<td>2.10</td>
<td>1.10</td>
<td>253.00</td>
<td>208.00</td>
<td>1366.0</td>
<td>5.00</td>
<td>10.80</td>
<td>6.70</td>
<td>142.00</td>
<td>7.10</td>
<td></td>
</tr>
</tbody>
</table>
GENERAL CONCLUSIONS AND RECOMMANDATIONS

The own research finishes with a series of conclusions formulated in light of the obtained results from which we mentioned:

- According to national and European regulations and directives the implemented physio-pharmaceutical protocols for veterinarian medicinal product testing must excel in accuracy, relevance and uniformity;
- The conduct of the test protocols was based on clinical and laboratory evaluations focused primarily on the clinical evaluations in correlation with hematological and biochemistry findings;
- Some of the samples (about 20%) were affected by jellification (clotting), shortly after collection, process that resulted in their partial or total loss;
- Analysis of the leukocyte subpopulations revealed characteristic ratios for the age and species between lymphocytes and heterophiles (1:1.5), monocytes and heterophiles (1:5.7), monocytes and lymphocytes (1:4.3);
- Insignificant changes in the hematological profile, in birds treated with multiple doses revealed that in case of overdose, macrolides and tetracyclines are well tolerated, without immediate or delayed secondary symptoms;
- In explaining lymphopenia associated with overdose of the tested products importance must attribute and their content vitamin AD3E complex;
- Some hematological changes such as those expressed by monocytosis with multiple doses of erythromycin or anemic syndrome in the case of oxytetracycline, may underlie overdose of certain antibiotics in poultry.
- Changes in biochemical parameters, with statistically significant differences similar to those seen in our research can be attributed to the stimulatory effects of antibiotics on the metabolic processes involved in weight gain and feed consumption in Broilers;
- In the chicken from the experimental groups treated with double dose, we found no significant changes in the indices of metabolic profile, which indicates that the tested veterinary medical products had no significant metabolic influences.
- Crossmatch tests of the samples before creating the mixed samples showed no agglutination or hemolysis, both major and at the minor Crossmach test, indicating a high degree of blood compatibility between the line broilers investigated;
- Overall analysis of the data obtained for samples of mixed samples collected revealed values close to the individual mean of the groups as well as bibliographical references, confirming the possibility of practical implementation of this economically superior procedure.
From the obtained results the following recommendations could be made:

- We found particularly useful a prior analysis and clarification of the RCP in collaboration with the manufacturer who has to agree with any corrections,
- We believe that the exclusive use of patients with pathologies susceptible to the tested products is not appropriate to assess the tolerance of veterinary medical products, because in such circumstances it is difficult to create homogeneous groups;
- In conducting veterinarian medicinal product testing we recommend two separate protocols, one for pre-clinical (pharmacological and toxicological tolerance) and one for clinical investigations (to establish and confirm the dosage, resistance, therapeutic efficacy);
- We consider effective the collection of additional blood samples in case of broilers because some of them could be compromised by the jellification;
- In case of broiler research we recommend establishing a set of reference values for hematological and biochemical parameters by conducting investigations on the studied groups because bibliographical values may be questionable;
- In investigations of homogeneous groups broiler chickens we propose screening assessment through hematological and biochemical profiles for health surveillance, that can be useful if accidental drug overdose is suspected;
- For homogeneous lines of broiler chicken with high level of compatibility, we recommend the mixed blood sample because it is superior in terms of cost and workload.

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27. ***PROSPECT ROMVAC AD₃E.

ACRONIMS AND ABBREVIATIONS

Bazof.= Basophiles;
Dev.st.= Standard deviation;
EDTA= Etilen-diamin-tetra-acetic acid;
Eoz.= Eosinophils;
Erit.=Number of erythrocytes;
Hb= Hemoglobin;
Heter.= Heterophils;
Ht= Hematocrit;
Leuc.= Number of leucocytes;
Limfo.= Lymphocytes;
MCH= Mean cellular hemoglobin;
MCHC= Mean cellular hemoglobin concentration;
MCV= Mean cellular volume.
Mono.= Monocytes;
RCP= Summary of the products characteristics;