Prezenta teză conţine:

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ABSTRACT

of the PhD thesis entitled

OBSERVATIONS ON THE VARIATION OF SOME SANGUINE BIOCHEMICAL AND BIOPRODUCTIVE PARAMETERS IN EGG-LAYING HENS IN RELATIONSHIP WITH BREEDING SYSTEM AND MAINTENANCE STATUS

This PhD thesis is structured in two parts: **Bibliographic study** and **Personal researches**.

PART I. BIBLIOGRAPHIC STUDY

The bibliographic study comprises 4 chapters, extended on 74 pages.

Chapter 1. The anatomic and physiological particularities of the digestive and reproductive apparatus in egg-laying hens. This approaches the digestive apparatus' particularities in poultry, et the levels of oral cavity, oesophagus, glandular stomach, jejunum, ileum, cecum, colon and cloacae. The subchapter describes poultry digestion within the oro-pharynx, gastric and intestinal compartments, including the deglutition terms and the mechanical, chemical and enzymatic processes exerted on fodder. It also deals with the absorption of the products resulted successively to protein, carbohydrate and lipid digestion, taken from feed. The major absorption headquarters is represented by jejunum and ileum.

The poultry reproductive apparatus is consisted of ovaries and oviducts. These two segments develop as symmetrical organs during the embryo period, while only the left ovary and oviduct develop after this. Adult poultry present the right ovary and oviduct as primitive, unfunctional formations.

Chapter 2. Poultry feeding norms. This chapter presents the most interesting methods of normed poultry feeding that meet the nutritive requirements by applying balanced ratios, adapted to poultry's productive and digestive particularities. It describes the reproductive poultry alimentation during growth and reproduction, the alimentation of young poultry destined for egg production and the alimentation of egg-laying hens. It also includes a description of the fodder used in poultry feeding.

Chapter 3. Neuro-hormonal activity in egg-laying hens. Poultry's hormonal activity is similar with the one of mammals, due to the three hypophysis gonadotropin hormones (FSH, LH and LTH), which present different chemical structures. This presents the egg formation stages in hen, at oviduct

segments level, starting with yolk capturing by infundibulum (the vitelino membrane formation occurs here, then the albumen proteins are released in magnum, the shell membranes appear in isthmus, the albumen get hydrated and the shell is released in the uterus) and completing with oviposition.

Chapter 4. Breeding systems. Several breeding systems of egg-laying hens have been used so far (conventional battery cages); these systems will be forbidden beginning with 1 January 2012, in compliance with the EU Directive 74/1999/EC.

This chapter presents a few breeding systems applied in our country: the free-range breeding system – including intensive farming on permanent bedding, and also the household breeding system, for farmers breeding pure breeds, important for exhibitions or genetic preservation, and also for ecologic egg production; the pyramid-type batteries (conventional and with enlarged space); and the hen breeding system with access to paddocks (greenhouses) or pastures, applied in countries with advanced aviculture, as alternative to battery systems, under the constraint of animal and environment protection associations and also under the ecologic production trend.

PART II. PERSONAL RESEARCHES

Comprises 6 chapters developed in 179 pages, including 85 tables and 94 figures.

The subchapters deal with the objectives of the study, materials and methods, results and discussions, egg chemical content, histological structure of aorta and internal organs, behavioural sequences in egg-laying hens and general conclusions.

Chapter 5. Objectives of the study.

The organism's health and balance status is determined by normal functioning of anabolic and catabolic processes that occur simultaneously. The apparition of nutrition and metabolism disorders in mammals and birds as well is generated by several organism-independent factors (nutrition, imbalances between different ratio components, modification of fodder composition, empirical or wrong ratio utilization, sudden and frequent ratio changes and various technical-organizatorical factors) and/or organism-dependent factors.

The objective of this study is to analyze and interpret the changes induced by breeding system and by the maintenance status on some sanguine biochemical parameters in egg-laying hens.

The objectives are:

1. supervision of microclimate conditions in all the three batches;
2. supervision and analysis of forage recipes according to pre-egg-laying and egg-laying stages;
3. determination of enzymatic biochemical parameters in the egg-laying hens from the three breeding systems (free-range, conventional batteries, enlarged batteries);
4. determination of haematological parameters for the three productive stages specific to egg-laying hens;
5. determination of bioproductive parameters in the egg-laying hens from the three breeding systems studied;
6. determination of meat chemical parameters;
7. determination of egg chemical parameters;
8. behaviour observation in compliance with poultry breeding system;
9. determination of correlations between the histological structure of internal organs (heart, lung, liver, spleen, kidney) and aorta artery, according to feeding regime.

Chapter 6. Materials and methods.

In concordance with the objective of this work, we supervised the effects exerted by three breeding systems (free-range, in conventional batteries and in batteries with enlarged space) on egg-laying hens (the hybrid ISA BROWN).

We created three groups of 50 hens each, namely one batch for each breeding system. Meanwhile, we studied the egg-laying hens (belonging to the same hybrid) from three production farms applying similar breeding systems.

The results were collected per three significant growth periods:

- stage I, started with 16-week chickens (transfer period) and continued until the age of 29 weeks,; this period corresponds to the beginning of egg-laying and reaching of maximal production;

- stage II, started with the age of 33 weeks and lasted until the age of 50 weeks, when hens should maintain the same egg-laying level;

- stage III consisted of the supervision of the biological and economic consequences and presents a special significance, because the duration of this period depends on the body stress degree during the first and second stages.

During each period, we determined meat's haematological, sanguine biochemical, bioproductive and physical-chemical parameters, and at the end of the experiment we analyzed the histological structure of internal organs (heart, lungs, liver, spleen, kidney), aorta, and also egg's chemical content.

The biochemical serum determinations were carried out with the help of specific procedures, relied on the kinetic and colorimetric methods; the specific methods of haemoglobin and haematological determination relied on the electric impedance and stream cytometry methods.

The primary data obtained successive to the investigations performed were mathematically and statistically processed (Statistics 7, non-parametric Mann-Whitney test), in order to underlie the interpretation of results.

Chapter 7. Results and discussions.

During the first experimental stage, we supervised the evolution of the haematological and sanguine biochemical parameters in 21 and 27-week old egg-laying hens, under the three breeding systems (free-range, conventional battery and enlarged-space battery).

According to the comparative analysis of results, we could draw several conclusions that confirm that, at this age, there are *significant differences* ($p \leq 0.05$) between the breeding systems:

- conventional battery - free-range: *amylase*'s enzymatic activity was more reduced in the hens bred in conventional batteries, and *aspartate aminotransferase* exerted more intense activity in the hens kept in free-range system; from a haematological viewpoint, the hens bred in conventional batteries recorded lower *hematocrit* values;

- conventional battery – enlarged space battery: *gamma glutamyl transferase* presented bigger values in the hens from the enlarged batteries; we obtained similar values in the case of *haemoglobin* and *mean erythrocyte values*;

- enlarged space battery – free-range: in the hens bred in enlarged-space batteries, we determined big values in the case of *gamma glutamyl transferase*, *erythrocyte number*, *hematocrit* and *leukocyte number*; in the case of *aspartate aminotransferase*, the hens bred on soil presented higher values than the hens bred in the other systems.

At 21 weeks old, there were *distinctly significant* differences ($p \leq 0.01$) between the breeding systems:

- conventional battery – free-range: the *leukocyte number* was more reduced in the hens bred on soil;

- conventional battery – enlarged-space battery: *hematocrit* value was bigger in the hens bred in enlarged-space batteries.

At the age of 27 weeks old, there were *significant differences* ($p \leq 0.05$) between the breeding systems:

- conventional battery – enlarged-space battery: the *mean erythrocyte value* (VEM) and the *mean haemoglobin erythrocyte concentration* (CHEM) presented bigger values in the hens bred in enlarged-space batteries;
- enlarged-space battery – free-range: the free-range breeding system induced a more intense *alanine aminotransferase* activity compared with the enlarged-space battery system; on the contrary, *haemoglobin* reached bigger values in the conventional battery system.

At the age of 27 weeks old, there were *distinctly significant* differences ($p \leq 0.01$) between the breeding systems:

- conventional battery – free-range: the hens bred on soil presented lower values of *alanine aminotransferase*, *haemoglobin*, *mean erythrocyte value* (VEM), *mean erythrocyte haemoglobin* (HEM) and *leukocyte number*;
- conventional battery – enlarged-space battery: *haemoglobin*, *hematocrit* and *the leukocyte number* had smaller values in the hens bred in conventional batteries;
- enlarged-space battery – free-range: the *mean erythrocyte value* (VEM) and the *leukocyte number* in the hens bred in enlarged-space batteries overtook much the normal values for this species; in the case of the *erythrocyte number*, the hens bred on soil presented smaller values than the other poultry group.

Egg-laying production started at 18 weeks in the hens from conventional and enlarged-space batteries, while in the case of free-range system, at the age of 19 weeks old. At 21 weeks, the free-range hens reached the egg-laying percentage of 32.6, while the hens from batteries reached 60.0 % (8.1 % less than the hybrid producer). At the end of the first stage, we recorded productions of 96.0 % in the case of batteries, 1.7 % more than the value obtained by the producer of the hybrid ISA Brown.

Significant differences ($p \leq 0.05$) between breast chemical content were observed in the case of *dry matter* (DM), between the hens bred on soil (S) and those bred in enlarged-space batteries (EB), and *crude ash* (CA), between the hens from conventional batteries (CB) and enlarged-space batteries (EB).

The cumulated *specific intake* was bigger in the hens bred on soil, 31.73 kg/kg compared with the hens from batteries, 27.30 kg/kg. The difference is 4.43 kg/kg.

At the age of 21 weeks, the *total protein* had the minimal value, because the batches presented a body weight deficit of 95 grams compared with the normal weight, and the egg production was smaller than the standard (50 % smaller in the hens bred on soil), a situation generated by protein intake for homeostasis and movement, in the disadvantage of egg production. This *hypoproteinemia* was maintained in week 27, too, because the hens from all the three breeding systems recovered their body weight deficit; in terms of production, they overtook the egg-laying percentage expected by the producer of the hybrid ISA Brown.

At the age of 21 weeks, amylasemia values were above the maximal limits in all poultry batches, being influenced by ratio (quantity and quality), in order to allow hens reach the age-specific weight.

At the age of 27 weeks, at the same time with the achievement of the weight specific to this age category, we observed that amylasemia fell back to the normal values.

Alkaline phosphatase, at the age of 21 weeks old, had values over the maximal limits in all the three breeding systems; this was generated by the productive enforcement.

The enzyme gamma glutamyl transferase, which expresses the hepatic activity, had constantly values above the maximal limit, with the peak in week 27 (as naturally). This enhancement corresponds to a great egg production.

By analyzing uricemia, we observed that, in week 21, the hens from the three breeding systems presented values above the normal limit; this situation is generated by the forage intake (20 % CP).

The second stage includes the moment when the egg production reaches its peak – the period of maximal production.

At the age of 38 weeks, there were *significant differences* ($p \leq 0.05$) between the breeding systems:

- conventional battery – free-range: *amylase*'s activity was reduced in the hens bred on soil compared with those bred in conventional batteries;
- conventional battery – enlarged-space battery: *haemoglobin* concentration was bigger, and the *mean erythrocyte volume* (VEM) was smaller in the hens from conventional batteries than in those with enlarged space.

At the age of 38 weeks, there were *distinctly significant* differences ($p \leq 0.01$) between the breeding systems:

- conventional battery – free-range: the *leukocyte number* was mostly reduced in the hens from conventional batteries;
- conventional battery – enlarged-space battery: the hens from enlarged-space batteries suffered *hyperproteinemia* and an *gamma glutamyl transferase* activity enhancement, and in the case of *alanine aminotransferase*, the reduction of enzymatic activity, where the hens from conventional batteries presented the lowest values; *hematocrit* concentration and the *leukocyte number* were smaller in the hens from conventional batteries, and the *mean haemoglobin erythrocyte concentration* value (CHEM) was bigger compared with the hens from the enlarged-space batteries;
- enlarged-space battery – free-range: the *proteinemia* and *gamma glutamyl transferase* values overtake the superior limits presented in the literature, while *alanine aminotransferase* was below the normal inferior limit, where the free-range bred poultry presented the lowest value.

At the age of 43 weeks, there were *significant differences* ($p \leq 0.05$) between the breeding systems:

- conventional batteries – free-range: the *erythrocyte number* was mostly reduced in the hens bred on soil.

At the same age category, we observed *distinctly significant* differences ($p \leq 0.01$) between the breeding systems:

- conventional battery – free-range: the hens from the conventional battery system presented a *mean erythrocyte haemoglobin* value (HEM) that was above the superior limits, and, in the case of *leukocyte number*, it reached almost the inferior limit;
- conventional battery – enlarged-space battery: from the viewpoint of the comparison between the two systems, the hens bred in conventional batteries presented lower *haemoglobin*, *mean haemoglobin erythrocyte concentration* (CHEM) and *leukocyte number* values;
- enlarged-space battery – free-range: the *mean haemoglobin erythrocyte concentration* (CHEM) is smaller in the hens bred on soil.

Regarding the egg-laying percentage, we observed that the hens farmed in conventional batteries presented a bigger percentage (98.5) than the hens bred on soil (94.5). The hens kept on soil had their maximal production extended on a 6-week period (over 94.0 %), while the egg production of the ones in conventional batteries (over 98.0 %) lasted for 11 weeks.

At the end of this stage, we observed *significant differences* ($p \leq 0.05$) of the *crude ash* (CA) and *crude fat* (CF) from the breast meat between the free-range (S) bred hens and the conventional battery system (CB). The *dry matter* (DM) and *crude protein* (CP) from drumsticks presented different values for the hens farmed in enlarged-space batteries (EB) and on soil (S), respectively in conventional batteries (CB).

In the case of battery-bred poultry, the reduction of egg production from 98.0 % to 92.8 % (in week 48) took place at the same time with the reduction of forage intake with 4 g/day/hen, from 118 g/day/hen (at the beginning of this stage) to 114 g/day/hen (at the end of the stage). The *specific intake* was 16.4 kg/kg.

The cumulated specific intake was bigger in the hens farmed on soil (like in the first stage), 17.88 kg/kg, 1.41 kg/kg more than in the hens farmed in batteries (16.47).

The third experimental stage deals with the enzymatic metabolic changes occurred in poultry body, induced by the breeding system, given that the productive effort is more reduced (the egg-laying percentage decreased to 84.0, respectively to 81.0 at the end of this stage).

At the age of 57 weeks, there were *significant differences* ($p \leq 0.05$) between the breeding systems:

- conventional battery – free-range: *amylase's* activity in the hens bred on soil was more intense compared with the hens from the other breeding systems;
- enlarged-space battery – free-range: *hematocrit* value and the *erythrocyte number* presented also bigger concentrations in the hens farmed on soil.

At the age of 63 weeks, there were *distinctly significant* differences ($p \leq 0.01$) between the breeding systems:

- conventional battery – free-range: *haemoglobin* and the *mean haemoglobin erythrocyte concentration (CHEM)* values were below the inferior limit of this species in the hens bred in conventional batteries;
- conventional battery – enlarged-space battery: *aspartate aminotransferase* activity was more intense in the hens bred in enlarged-space batteries, and *haemoglobin* and *the mean haemoglobin erythrocyte concentration* (CHEM) were more reduced in the hens from conventional batteries;
- enlarged-space battery – free-range: *uricemia* was bigger in the hens bred in enlarged-space batteries than in those bred on soil; *haemoglobin*, *hematocrit* and the *leukocyte number* presented similar haematological concentrations for both breeding systems. Regarding the concentrations obtained, the free-range bred hens present the most adequate physiological values.

There were also *significant differences* ($p \leq 0.05$) between the hens bred on soil (S) and those farmed in enlarged-space batteries in the case of *crude ash* (CA) and *crude fat* (CF) in breast and drumsticks.

At the end of this stage and of the productive period, the egg production becomes uniform for the three breeding systems, and the productive differences between the free-range and battery systems disappear. The *egg-laying percentage, body weight, forage intake* and the *specific intake* present values that are similar with the ones indicated by hybrid's producer.

The structural changes of the organs studied are not related to the breeding systems; they are the consequence of other microclimatic factors, effort or panic. According to our morpho-pathological observations, ratios were balanced and hens benefited by optimal farming conditions.

Regarding the *specific intake* cumulated at the end of the third stage, it was 16.43 kg/kg in the hens bred in free-range system and 15.29 kg/kg in those bred in batteries. The difference between systems was 1.14 kg/kg, favouring the battery-bred hens.

Chapter 8. Behavioural sequences in egg-laying hens.

Feeding behaviour. Land scratching and prehension, by picking, represent a particularity of poultry feeding behaviour. These two behavioural stages alternate and occur since the first day of life, indifferently of the breeding system.

The digestive behaviour is not influenced by forage's organoleptic features, only when their concentration is a very big one.

Social behaviour. In the intensive breeding units, the social behaviour of hierarchy establishment has suffered some changes compared with the natural breeding system, because of the permanent efforts of adaptation to the artificial life conditions. The almost permanent social stress cast adrift the hierarchy hostilities since the first two weeks of life, although these hostilities are mainly play behaviour. This social behaviour was observed in the poultry bred in batteries and in those bred in free-range system as well.

Sexual behaviour. Poultry intensive farming has changed a lot hens' sexual behaviour. Females present a characteristic syndrome of „immobility": they fall on earth and accept to be „stepped". During this period, the forage intake is reduced and the hens manifest a slight seclusion; near humans, they suddenly fall down and refuse to leave the location.

Chapter 9 presents 19 general conclusions and the recommendations that can be concluded from this PhD thesis.

- *Total proteins*
    a. At the age of 21 weeks, the *total protein* presented a minimal value, because the groups had a body weight deficit of 95 grams compared with the normal weight, and the egg production was smaller than the standard, with 50 % in the hens bred on oil; this determined protein consumption for homeostasis, disfavouring the egg production.
    b. *Hypoproteinemia* had the same value in week 27, because the hens in all the three breeding systems recovered their body weight deficit; in terms of production, they overtook the egg-laying percentage expected by the producer of the hybrid ISA Brown.
    c. In the second stage, when the egg production reached its peak and remained the same for 11 weeks in the hens from batteries, *proteinemia* presented maximal values due to the qualitative forage fed concomitantly with an adequate microclimate. We should mention that during the period of maximal productive effort (11 weeks), the hens consumed 2-4 g forage / day more than the standard values.
    d. At the end of this experiment, we observed a *total protein* reduction, although it did not reach the minimal values; this reflected on egg production and forage intake. Actually this represented egg-laying hen's physiological response to the end of the egg-laying period.

- *Amylase*
    a. At 21 weeks – Amylase values were above the maximal limits in all poultry groups, being influenced by ratio (quantity and quality) – to allow hens reach the weight specific to this age.
    b. At 27 weeks – We observed that amylasemia values came back to the normal limits at the moment when hens reached the weight specific to their age category.
    c. At 38 weeks, we observed that all groups presented minimal amilasemia values,

because egg production and the forage intake were big.

d. In week 43, we observed a slight amylase increase, corresponding to egg production decline. We may speak again of the dynamic balance refreshment, because, in week 48, the hens from the three breeding systems presented similar values.

- *Alkaline phosphatase*

  In week 21, the hens from the three breeding systems presented values above the maximal limits, and this is caused by the productive enforcement. This situation may be observed in week 38, too, when the egg production reaches its peak; starting with week 43, the alkaline phosphatase is between normal limits, until the end of the study.

- *Gamma glutamyl transferase*

  Since this is one of the enzymes expressing the hepatic activity, it presented constantly values above the maximal limit, with the peak (as normally) in week 27. This enhancement corresponds to a big egg production.

- *Aspartate aminotransferase*

  By analyzing aspartate aminotransferase values, we observed that, at 21 weeks, they were near the maximal limit; this corresponds to poultry preparation for production. These values were also high in week 27, remained relatively constant after that and did not fall below the minimal value. This dynamics allow us consider that the hens studied did not have to face an infectious pressure. This statement is supported by the histological examination of spleen, which presented primary lymphocyte follicles (areactives).

- *Alanine aminotransferase*

  This presented reduced values during the first stage, but, at the same with the reaching of production peak, the values fell below the inferior limits. These values were due to the intense hepatic activity, but only in terms of production.

- *Uric acid*

  By analyzing uricemia, we observed that, in weeks 21 and 38, the hens from the three breeding systems presented values above the normal limit, and this situation corresponds to forage intake (20 % CP). In the rest of the period, the values were between normal limits, suggesting that uricemia expresses accurately the degree of forage protein utilization.

- Successive to the haematological examination, we concluded that, for the entire period studied, the values of the *erythrocyte number* were between the accepted limits. We observed variations in the hens bred in free-range system, in week 27 (3.1 mil/mm^3) and during the last stage (2.7 mil/mm^3).

- The *hematocrit* values, in the hens bred in enlarged-space batteries (EB), were constantly bigger; they were once overtook by the *hematocrit* values of the hens bred in conventional batteries (CB), in week 43. On the whole, the hematocrit values were similar with the normal maximal limits, being in correlation with oxygen requirements and productive labour.

- *Haemoglobin* was between the normal limits, but on the whole it was nearby the medium-inferior limit. In the case of our experimental batches, the hens from the enlarged-space batteries (EB)

presented constantly bigger values, excepting week 38, when the free-range bred hens (S) and those from conventional batteries (CB) presented slightly bigger values, of 9.1 g/dl compared with 8.9 g/dl.

- During the experimental period, the *erythrocyte indices (VEM, HEM* and *CHEM)* made evident variations between the hens from the three breeding systems. These variations were due to the air volume available and to the adaptation capacity of the erythrocyte line to support the genetic line and ratio's protein and energetic background, materialized in great productive capacity.

- The *leukocyte number* values were constant during our study, between normal limits and slightly towards the normal inferior limit. Starting with week 38, we constantly observed that, in the hens bred on soil (S), this index presented significantly bigger values compared with the hens from conventional batteries (CB), respectively from enlarged-space batteries (EB). This fact may be explained with the role played by leukocytes in local defence reactions.

- *Specific intake*
    a. In the first stage, the specific intake was the biggest in the hens bred on soil (S), 31.73 kg/kg, followed by those from conventional batteries (CB), 27.3 kg/kg, and enlarged-space batteries (EB), 27.07 kg/kg. These values may be explained by the fact that this period was partially productive.
    b. In the second stage, the specific intake values are similar; there is insignificant difference between the hens from batteries (CB and EB), 16.47 kg/kg, and those bred in free-range system (S), 17.88 kg/kg. We should mention that, in this period, the results are cumulated, explaining in this way the specific intake reduction to a half.
    c. In the third stage, where the results are also cumulated, the differences between the breeding systems are still available: the free-range system (16.43 kg/kg) and batteries (15.29 kg/kg); the number of eggs obtained is similar in the case of battery-bred hens (CB and EB), and it is 90-pieces less in the free-range system (S).

- The *meat* in egg-laying hens represents a secondary product and, consequently, it does not have commercial value. For many times, it represents a problem in the moment of egg-laying livestock liquidation, when this reaches the final of the productive period. However, successive to meat's physical-chemical analysis (drumsticks and breast), at the end of the three productive stages, the dry matter, crude ash, crude protein, crude fat and pH values did not show any meat quality changes in the three breeding systems.

- The study of eggs' *chemical content* did not reveal any statistically assured *significant differences*. As we already know, egg's chemical content is directly influenced by forage composition. From this viewpoint, the forage was unique for all hens studied, and that is why we may consider that the breeding system, beside the egg-laying hen metabolism, represents a factor that can change egg's chemical content.

- From a *histological* point of view, the structural changes of the organs studied are not related to breeding systems; they represent the consequence of other microclimatic factors, effort or panic. According to our morpho-pathological observations, we may conclude that ratios were balanced and that we provided optimal farming conditions for poultry.

- These three breeding systems generate similar egg-laying results; it is not possible to determine the superiority of one of these systems. In history, the selection of one of these programs was determined by social-economic factors, and at the moment by the cultural factor (welfare) of the post-modern society.

- If we consider that the free-range breeding system with 2000 cm^2/hen would be the most comfortable variant for egg-laying poultry, and the breeding system in conventional batteries with 550 cm^2 / hen would be the most uncomfortable one, then we should conclude that the enlarged-space battery breeding system, 750 cm^2 / hen, represents the most common breeding system for egg-laying hens. As constant for the entire study period, we observed that this is also reflected by the biochemical data which represent an intermediate level between the values obtained in the hens bred on soil and those bred in conventional batteries.

- We do not consider that the enlarged-space battery system meets all the egg-laying hen requirements, but for sure it represents a much better alternative than the conventional battery system.