PH.D. Thesis Summary "Researches Concerning the Dynamics of the Main Metabolic Processes in Bitch during Lactation"

Keywords: Metabolism, Lactation, Bitch

The aim of this study is to determine the peculiarities of the main metabolic processes in the lactating female dog and to investigate the intraspecific relationships between these metabolic processes and size or lactation order. Specifically, we intend to examine the following variables: direct and derived erythrocyte constants, leukocyte series, platelets, main constituents of the protein, lipid and mineral profile, alkaline phosphatase activity and to determine the prolactin receptor gene expression.

The following were considered necessary steps in order to reach the intended goal of this thesis:

- studies on the dynamics of the metabolic profile's main constituents through morphological and biochemical blood investigations in the lactating female dog;
- identification and quantification of prolactin receptor expression by qRT-PCR;
- conclusion-drawing after the interpretation of metabolic profile by incorporation in a coherent set of data and comparison with reference values of the species; conclusion-drawing after analyzing changes in gene expression of receptors for prolactin.

Part I (Bibliographic Study) is divided into two chapters dealing with the current state of knowledge on the morphophysiology of the mammary gland in the female dog and the metabolic features in the lactating female dog.

Chapter I, "Particularities regarding the morphophysiology of the mammary gland in bitch during lactation", has five subchapters. The first subchapter is an analysis of the mammary gland morphology in the female dog. The second subchapter deals with the main aspects of the mammary gland physiology, namely: lactogenesis, galactopoiesis and lactation suppression. The third section presents the metabolism of the milk components (lactose, fat, protein, minerals and vitamins present in the milk), as well as the metabolic dynamics of the milk components during lactation.

Chapter II, "Current status of knowledge regarding the particularities of metabolic processes in bitch during lactation", has four subchapters, each dealing with the particularities of the metabolism of carbohydrates, lipids, proteins or minerals in the lactating female dog.

Part II (Personal Researches) is divided into six chapters regarding the place and period of the studies and researches, the animals, materials and methods used, the results, discussion and partial conclusions and the general conclusions.

Chapter III presents the location and duration of the research, which took place at the Internal Medicine Clinic within the Faculty of Veterinary Medicine and the Department of Molecular Biology, Biochemistry, and Hematology within the Institute of Diagnosis and Animal Health (IDAH) in Bucharest. The period of the research was 2004-2010.

Chapter IV, divided into eight subchapters, outlines the animals, materials and methods used in the research. Chapters V, VI and VII consist of the results of the personal research, their discussion and the preliminary findings.

Chapter V bears the title "Blood morphologic and biochimical evolution in bitch in late gestation and during lactation". The research described in this section was performed on lactating female dogs, of the German Shepherd breed, aged between 1 and 8 years. Their monitoring was done from day 42 of gestation through day 57 of lactation.

The tests performed in the morpho-hematological exam were: assessment of direct erythrocyte constants (number of red blood cells, hematocrit, hemoglobin), derived erythrocyte constants (MCV, MCH, MCHC) and WBC counts.

The evolution of the morphological constants of blood consisted in the decrease, at the onset of lactation, of RBC count, hemoglobin and hematocrit, to a value close to the minimum threshold of the reference range, followed by an increase over the next two weeks and stabilization at a relatively constant level until the end of lactation. The recorded changes were influenced by the dynamics of blood volume.

Leukocyte counts exceeded the maximum reference range in most weeks of the investigated period, consisting with the increased stress condition characteristic to the puerperal period. After approximately five weeks of lactation, WBC counts returned to levels comparable to those typical to the non-reproductive period.

The tests performed in the blood biochemical examination consisted of the dosing of the main constituents of the protein profile: total protein, albumin, globulin, albumino globulin ratio; of the main constituents of the lipid profile: cholesterol, lipids, triglycerides; and of the main constituents of the mineral profile: Ca, P, Mg; alkaline phosphatase activity.

Changes in blood chemistry showed significant decreases in serum protein and albumin concentrations, particularly in late pregnancy and early lactation as well as significant increases of globulin fractions. There is a decrease in protein anabolism in the first week after birth, followed by a peak during weeks 4-5 of lactation, which corresponds to an increasing tendency of the (hepatic) protein anabolism.

Triglyceride counts above the control values at the end of the last half of gestation and lactation, and their respective decrease at the onset of lactation are consistent with the increased lipid mobilization and intensification of the "de novo" synthesis which occur during postpartum. Lipidemia and cholesterolemia are characterized by high values at the beginning of lactation followed by a steady decline of these values during the first weeks of lactation, which illustrates a high lipid anabolism, probably imposed by the phenomenon of galactopoiesis.

Chapter VI, "Particularities of morphologic and biochemical profile in lactating bitch, according to the order of lactation (parity) and the breed size" contains two subchapters analyzing the influence of parity and breed size respectively on the metabolic profile of the lactating female dogs.

To study the effect of parity over hematology parameters, 14 clinically healthy German shepherd female dogs were divided into two groups: primiparous female dogs and multieven female dogs.

Results of the blood morphology examination reported an increase in erythrocyte count concurrent to the decrease of hemoglobin concentration due to parity, thus determining a positive correlation of this parameter with the order of parity in lactating female dogs. This correlation is also maintained in the case of indirect erythrocyte constants, pointing out increases in MCV values and the decrease of MCHC and MCH values.

Quantitative and qualitative examinations of the white line are influenced by physiological status and parity order. Thus, it appears that total leukocytes and lymphocytes present elevated counts in both groups of female dogs (primiparous and multiparous), compared to the maximum reference range, signaling an increase in their values inversely proportional to the parity order.

The mean values of total protein levels in the lots of primiparous and multiparous female dogs are located below the lower limit of the reference range, while decreased protein levels are inversely proportional to parity and those of albumin are directly proportional to it. In the multiparous lot, globulin values are located over the upper limit of reference for this parameter.

Lipemia dosage showed increased lipid values proportional to the order of lactation. In terms of cholesterolemia there is a slight increase in the mean values of the primiparous female dogs compared with the values from the multiparous lot. Serum triglycerides show a slight increase directly proportional to parity, but both triglycerides and cholesterol values recorded for primiparous and multiparous are very close.

The study of mineral profile elements with significance and relevance in lactation showed an increase in blood calcium levels and phosphoremy directly proportional to parity. Regarding the dosage of magnesium, the values of this parameter decrease with the order of lactation and are located near the upper limit of the reference range for this parameter.

Influence of size on hematological changes that occur during lactation was conducted on lactating female dogs of large, medium and small sized breeds.

Blood morphology investigations revealed the increase of erythrocytes, hemoglobin and hematocrit levels in a directly proportional amount to the breed size, although ranging within physiological limits. These changes reflect the high metabolic requirements of large breed, being known that milk productivity magnitude is analogous to weight.

Derived erythrocyte constants do not present any significant differences between the three lots of subjects divided according to size, with very similar values of MCV, MCH, MCHC in the cases of large and medium dog breeds.

White line investigation reveals leukocytosis, a change which cannot be correlated with body weight, as well as lymphocyte count increased proportionally to size and a percentage of monocytes and granulocytes with levels within the normal range.

Total serum protein profile as well as albumin and globulin fractions present higher values in the case of smaller size breeds compared to larger ones, which would advocate for a

greater mobilization and/or increased synthesis of proteins in smaller species, equating to an increase of the protein metabolism or to a genetic determinism of these differences in values.

As for the biochemical changes concerning the lipid profile, the total lipid and triglyceride levels rise proportionally to the size in the dogs from all three batches. This concurs with observations on the substantial decrease in the amount of body fat in large breeds, since it is well-known that increased mobilization of fatty acids from adipose tissue generates new resources for milk synthesis and that relatively greater physical dimensions have a greater contribution to resources.

The concentration of calcium, phosphorus and magnesium serum revealed increased levels, in a directly proportional amount to the size of the lactating female dogs. Increased alkaline phosphatase activity in a directly proportional amount to the size of the lactating female dogs can be correlated with the intensity of calcium metabolism.

The seventh and final part of the personal research, "Aspects regarding the RT-qPCR in the gene expression of the prolactin receptors in bitch during lactation" analyzes the change in the expression of prolactin receptor (PRLR) in the mammary gland of the female dogs during lactation.

Breast tissue was collected from a total of 16 clinically healthy female dogs, of German shepherd breed, aged between 1 and 5 years, with lactation being monitored from parturition throughout weaning. Total RNA was extracted and subsequently subjected to reverse transcription, and cDNA amplified using real-time PCR. Three experiments were designed according to the lactation period and the availability of biological material for analysis. Thus, the subjects for the first experiment consisted of sixteen individuals (clinically healthy female dogs, of German shepherd breed, aged between 1 and 5 years) at different stages in terms of the level of gene expression - postpartum (four subjects), day 8 postpartum (four subjects), day 24 postpartum (four subjects) and post-weaning (four subjects). For the other two experiments we used one set of samples taken from the same subject, on which we monitored the dynamics of the prolactin receptor gene expression (days 8, 16, 24, 32 postpartum and the level of post-weaning expression).

The first experiment results showed an increase gene expression for the receptors of prolactin during the lactation period compared with the postpartum and post-weaning periods, with a peak in the 8^{th} day of lactation. It is thus demonstrated that PRLR expression in mammary epithelium increases significantly as the mammary gland progresses from the non-reproductive stage to the lactation stage.

The other two experiments revealed a replication of the increase/decrease trends in prolactin receptors gene expression similar to that observed in the first experiment, this time related to the dynamic of expression for the same individual. Therefore, the findings show a replication of the increasing trend of the expression, starting from the postpartum period, with a maximum in the 8^{th} day, a subsequent regression trend, and a minimum level in the weaning period.

The individual variation observed in the analysis of gene expression can be explained by the fact that enhancing expression of the receptor gene during lactation is determined by removing the sucking milk from the mammary gland.

The individual variation observed in the analysis of gene expression can be explained by the fact that an enhanced expression of the receptor gene during lactation is the result of the milk removal from the mammary gland though sucking.

The experiments carried out in this study proved the possibility to validate the determination of prolactin receptor gene expression, therefore the methodology described can be successfully used in hormonal monitoring, both as stand-alone method and complementary to the well-proven techniques.

It is worth mentioning that, unlike serological techniques, the sensitivity of gene expression determination supplies real research possibilities of the stimulation/suppression mechanisms of the prolactin synthesis. Thus, since we are dealing with determinations at messenger RNA level, the accuracy of the response to external stimuli is much higher compared to indirect methods of hormonal dosage.

Chapter VIII presents the general conclusions of this study.

Chapter IX consists of bibliographical references.