IOSUD –„DUNĂREA DE JOS" UNIVERSITY OF GALATI

DOCTORAL SCHOOL OF INDUSTRIAL ENGINEERING

DOCTORAL THESIS

RESEARCH ON THE GENERAL ADAPTATION SYNDROME OF ANIMAL ORGANISMS - STUDY OF ENZYMATIC MARKERS

- Summary -

Candidate,

Cărădan Simona-Laura (Turcu)

Scientific leader,

Prof. dr. ing. Petru ALEXE

Seria I 4 Nr. 94

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Content

Introduction ... 3
I THE DOCUMENTARY PART ... 5
I.1. Documentation of the General Adaptation Syndrome (GAS) ... 5
I.1.1. Current research study ... 5
I.1.2. Stress in organisms – current concepts ... 6
I.1.2.1. Hypothalamic-pituitary-adrenal system (corticotropic axis) ... 6
I.1.2.2. Sympathetic system (adrenal medulla) ... 7
I.1.2.3. Glycemic variations - indicator of activation of the corticotropic axis ... 8
I.1.3. Enzyme markers ... 9
I.1.3.1. Sources of serum enzymes ... 9
I.1.3.2. Decreased activity of serum enzymes ... 10
I.1.3.3. Measurement of serum enzymes ... 10
I.1.3.4. Nomenclature of enzymes ... 10
I.1.3.5. Interpretation of increases in enzyme activities ... 11
I.1.3.6. Significance of increased serum enzyme activity ... 11
Lactate dehydrogenase (LD, LDH) ... 11
Alkaline phosphatase (ALP) ... 11
Creatine phosphokinase (CPK, CK) ... 12
Glycosylated hemoglobin (Hb. glic., HbA1c) ... 13
Fructosamine (Fru) ... 14
I.2. DISEASES INVOLVED IN HUMAN PATHOLOGY ... 14
I.2.1. Systemic Disease Inventory ... 14
I.2.2. Other types of diseases ... 16
I.3. DISEASES INVOLVED IN VETERINARY PATHOLOGY ... 16
II THE EXPERIMENTAL PART ... 17
II.1. MATERIALS AND METHODS ... 17
II.1.1. Creatine phosphokinase / Creatine kinase (CPK/CK) ... 17
II.1.2. Lactatedhydrogenase (LDH) ... 18
II.1.3. Alkaline phosphatase (ALP) ... 18

II.1.4. Glycosylated hemoglobin (HbA1c/Hb. glic.) 19
II.1.5. Fructosamine (Fru) 19
II.1.6. SPSS application 20
II.2 RESULTS AND DISCUSSION 20
II.2.1. Interpretation of human patient data 20
II.2.1.2. Lactatedhydrogenase (LDH) 22
II.2.1.3. Alkaline phosphatase (ALP) 23
II.2.1.4. Glycosylated hemoglobin (Hb. glyc.) 24
II.2.2 Interpretation of veterinary patient data 26
II.2.2.1. Creatine phosphokinase/Creatine kinase (CPK/CK) 26
II.2.2.2. Lactatedhydrogenase (LDH) 27
II.2.2.3. Alkaline phosphatase (ALP) 29
II.2.2.4. Fructosamine (Fru) 30
II.3. General conclusions, elements of originality and recommendations 32
II.3.1. Conclusions and interpretations of research results 32
II.3.2.1. Enzyme marker results 32
II.3.2.2. Representation of results – Venn diagrams 32
II.3.2.3. Statistical conclusions General Adaptation Syndrome of animal organisms 35
II.3.3. Final conclusions 37
Elements of originality 38
List of published and presented works 38
B ibliography 39

Introduction

The central elements of current scientific research are both the state of human health and the quality of the food we consume. The present work wants to bring a scientific contribution from several points of view, namely: obtaining animal products intended for human consumption - meat, of the best possible quality, as a raw material and of vital economic importance for the population. A consumption of animal foods of low nutritional quality presents more benefits to the human body, in the active population, but they even attract imbalances that affect the quality of life.

In choosing the theme of the present study, it was of real interest to expand the definition of the stress phenomenon, as a continuation of the effect it has on animal organisms (mammals and humans), especially the way organisms respond to the presence of certain factors of stress.

The intervention of stress generates normal response reactions of organisms from two points of view: *from a somatic point of view*, the element generating the state of discomfort produces signals that activate the Central Nervous System, which in turn starts two response pathways: one humoral/cellular and the second hormonal, each of them on different routes, with different effects on the body; *from a vegetative point of view*, involuntary signals are generated, the autonomous functions of the organisms being also in an imbalance or with visible variations, starting from the increase in heart rate or breathing to energy consumption through excessive muscle contraction when the reaction of " fight-flight".

The cascade of biochemical reactions of glycolysis was chosen as a fair way to highlight the General Adaptation Syndrome of the organisms (SGA) so that the moment of the action of the stress attracts the response reactions of the organism; these changes, constant in the long term or sudden but of strong intensity, produce visible effects by altering the state of physical health of the affected organisms. A series of enzymes that catalyze biochemical processes intervene in the chain of glycolytic reactions; the intervention of enzymes was considered a starting point for the study of various signs for the alteration of the physical health of organisms, more precisely through the appearance of pathological conditions. Broadly speaking, the enzymes that have a significant say in the assessment of stress in animals were considered those from the category of hydrolases, more precisely aldolase, later assimilated to lactate dehydrogenase (LDH), phosphatases (alkaline phosphatase ALP), creatine phosphokinase CPK and glycosylated hemoglobin (Hb .glyc).

The perspective of safe foods, of animal origin, was accurately launched by Luminița Coman and Alexe P. in the work *Transformarea animalului viu în carne*, a work in which the stress of animals subjected to slaughter had a chapter. [2]

The *general objectives* of the present work were:

1. Analysis of SGA and establishment of potential markers of an enzymatic nature involved in the adaptation process.

2. Investigations into human diseases through the prism of enzymatic markers fixed for analysis.

3. Investigations in animal diseases through the prism of enzymatic markers fixed for analysis.

4. Statistical analysis to determine the diagnostic power of the markers involved.

5. Recommendations regarding the use of enzyme markers in animal husbandry and meat utilization.

As working methods, it was proposed to identify the interrelationship between the enzymes involved and diseases from human subjects, translated to the animal world; enzymes that also reflect pathological conditions as well as interrelationships between diseases: diagnostic possibility limited to animals with an extended range in humans. The research was carried out following the compilation of data from the medical analysis bulletins of a large number of human patients, seconded by values of veterinary medical documents has on a smaller scale, within the Colentina Clinical Hospital Bucharest as well as the Veterinary Clinic Zet Diagnostic Bucharest, the Queen Maria Central Laboratory Bucharest.

The work is structured in two parts: Part I – documentary, where the current studies of stress research in animals and the general syndrome of adaptation of organisms to stress were brought, part containing a number of 74 pages, in which three sub-chapters are presented: First sub-chapter: Documentation of SGA – current research study, which includes Stress of organisms, enzyme markers analyzed (sources of serum enzymes, decreased enzyme activity, measurement serum enzymes, the nomenclature of enzymes, the interpretation of increases in enzyme activities as well as the significance of the increase in the activity of serum enzymes, taken individually: CPK, LDH, ALP, Hb. glyc. and fructosamine Fru, for veterinary samples).

The second subchapter represents an inventory with a description of all the diseases involved in human pathology, which we encountered in collecting data from human medical documents, an inventory that was followed by the classification of diagnoses by groups of conditions. The third sub-chapter is also an inventory of diseases encountered in clinical medicine, only it characterizes the spectrum of veterinary diagnosis and in the same way a ranking of diagnoses by groups of conditions was made.

Part II of the thesis includes the extensive presentation of the experimental contribution, structured on three other subchapters, contained in 117 pages: the first subchapter represents the presentation of materials and work methods, where the analysis methods are described for each of the enzyme markers analyzed for the characterization of SGA: CPK, LDH, ALP, Hb. Glycerin and fructosamine Fru, for veterinary samples, but also a description of the SPSS multivariate statistics application, with the help of which the interpretation of the data resulting from the values of the biological samples was carried out, with strict reference to those that had exceeded values of the reference intervals. The next subsection of this part contains a presentation of the results following the interpretation of the data from the values of the biological samples, both human and veterinary, results processed according to several criteria, for each marker studied: number of samples, number of patients, sex of patients.

Subchapter three of this part presents the conclusions of the research, for each analyzed marker reports were extracted from the statistical application, in the form of tables. Here, only the exceeded values of the main markers, grouped by categories of conditions, were taken into account, but only 17 groups of conditions that characterize SGA (metabolic, renal, hematological, hormonal, thyroid, neurological, cardiological, circulatory, digestive) were of interest

/gastrointestinal, diabetes, gynecological, hepatic, nutritional, muscular, bone, urogenital, autoimmune diseases). For an easy understanding of the results obtained, 15 Venn diagrams were also made here for the groups of conditions characteristic of SGA. From a statistical point of view, a series of conclusions were formulated where the confidence percentages of the enzyme markers or the degree of risk are explained, for each analyzed marker, according to the p-value (statistical tool used to assess the value of the certainty probability of markers in the diagnosis of disease groups) as well as the function of the statistical application Pearson correlation - coefficient used to measure the relationship and association between the data extracted from the enzyme marker values.

The final part of this work also contains the originality elements of the thesis as well as the list of works published in order to disseminate the research results. The work contains a number of 18 figures and 160 tables, together with a number of annexes, comprising a total of 242 pages.

I THE DOCUMENTARY PART

I.1. Documentation of the General Adaptation Syndrome (GAS)

I.1.1. Current research study

The General Adaptation Syndrome (GAS) of animal organisms is described by specialized literature as a series of reactions of the body, on a more or less long term, to environmental or endogenous factors - of the body, aggressive reactions also called stress. Regarding the changes, firstly behavioral, then biochemical, of animal organisms, stress has been carefully studied since the beginning of the 20th century, as a form of response of living things to a discomfort situation.

Endocrinologist Hans Selye (1907–1982) describes in its own way, the body's reaction to stress, SGA through three stages: 1. The alarm reaction - the immediate reaction of a stress factor - through which the body prepares to respond to a potential danger, in the case of certain organisms with different deficiencies, the immune system being able to be overcome by the situation and the disease or condition setting in with ease. 2. The stage of adaptation, some organisms equating it with the stage of resistance to stress. In the situation where the stress continues, the body produces several changes in order to adapt and to reduce the effect of the stress factor. A good example would be the case when the body does not receive food - nutrients, then a state is established in which it minimizes energy consumption, through the lack of physical effort. 3. Exhaustion stage. This is the last stage of an organism on which the stress has continued, its resistance capacity is exhausted and a state of collapse occurs, where the systems and organs no longer have the necessary resources to react and maintain the normal functioning of the organism. Severe infections that have already passed the barrier of the immune system, blood pressure and heart attack often occur in the case of people who persist in a state of mental and or physical stress.

Hans Selye's approach to GAS is a psychological one, this being emphasized by his publications and by the fact that he extended his research on this subject from a cognitive and

attitudinal perspective. The phasing of the effects of stress on the body, through his vision, was a good benchmark for the interdisciplinary nature of the General Adaptation Syndrome. [1]

I.1.2. Stress in organisms – current concepts

The present research aims to deepen the notion of stress from the point of view of the health of mammalian organisms, both the health of animals but especially the quality of life of people, an aspect that is also reflected in the quality of the food we consume.

The description and more precisely, the definition of the term stress in animal organisms, was made by Prof. Alexe Petru and Luminița Coman in the work "*Transformarea animalului viu în carne*" [2], where they explain, in an extremely well-argued way, how the phenomenon of stress occurs and the way in which animal organisms are affected through the prism of affecting the quality of meat as an essential element for human nutrition, an aspect to which Prof. S. Meica also refers, from the same point of view, a few years later [3]. Stress is defined as a state in which environmental conditions are unfavorable to the body, the body's regulatory systems are overcome by the action of environmental factors, leading to imbalances in the individual's state of adaptation.

Prof. P. Alexe describes the phenomenon of stress from several points of view, but emphasizes the description from a biological - physiological and metabolic point of view, at the level of animal organisms, namely: anatomical and metabolic structures with pathological involvement, occasion with which the present research is completed with its scientific data from its II part. The scientific approach to stress was also made from the point of view of psychologically, behaviorally and especially endocrine affected organisms.

In 2017, Habib Yaribeygi et al., within the Neuroscience Center of the Baqiyatallah University of Medical Sciences in Tehran, Iran, appreciate stress as a response state of organisms to the action of intrinsic or extrinsic stimuli that lead to a biological response. The actions caused by stress differ a lot depending on the moment of the stimulus application, the type of stimulus applied but also its severity, which can even lead to the impairment of the body's homeostasis, potentially putting life at risk. They analyzed several pathologies that could have occurred due to stress: complications of brain functioning (memory, reading, thinking – brain areas involved, structural and functional changes), immune function, cardiovascular system, gastrointestinal complications, damage to the immune system endocrine. Stressful situations can complicate the stages of the present diseases or can be a triggering factor. The conclusions of the authors in this study refer to the elimination of as many stress situations as possible both in clinical conduct and the own alternative of a healthy life [4].

I.1.2.1. Hypothalamic-pituitary-adrenal system (corticotropic axis)

For a better understanding of the functioning of the corticotropic axis, the composition and functioning of each organic component of this assembly of great relevance in defining stress in living organisms was presented. The *hypothalamus* - component of the brain, connects the nervous and endocrine systems through the pituitary gland, secretes the neurohormones that are necessary to mediate the functioning of the pituitary gland (among them gonadotropin), has a role in controlling emotions, sexual activity, body temperature , of thirst and hunger, of instinctual vegetative manifestations (fear, anger), regulates the sleep-wake state, influences metabolism

and by controlling endocrimal activity, which is why it is also called the center of vegetative activity [5]. The *pituitary gland* is a small gland with two lobes (one anterior and one posterior), the adenohypophysis and the neurohypophysis, with an important endocrine role. *Adrenal cortex* - component part of the adrenal glands; the adrenal glands are paired glands located at the anterior pole of the two kidneys, they are also called adrenal glands, being responsible for the secretion of corticosteroid hormones and catecholamines (adrenaline and noradrenaline). Adrenal secretion is responsible for regulating stress, infections and immune imbalances, but also the secretion of sex hormones androgens and estrogens [6].

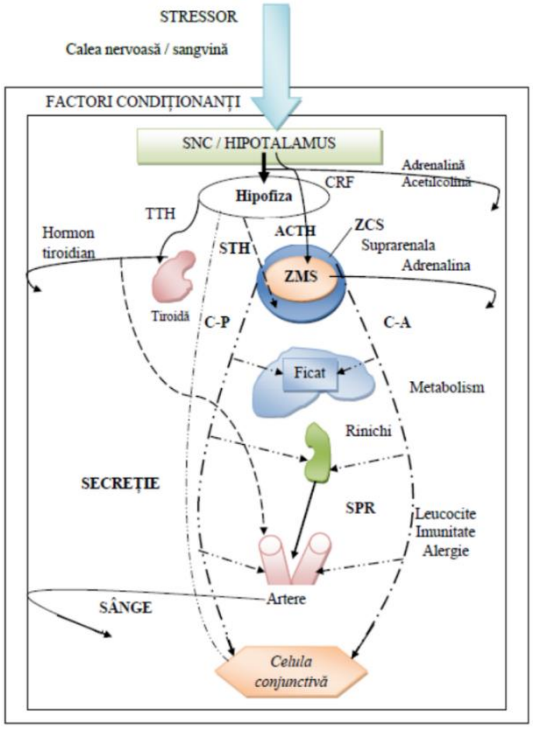


Fig. no. 1 Modulation of the General Adaptation Syndrome of living organisms (Source: *Transformarea animalului viu in carne*. Vol. I. Alexe P. pg. 133) [2]

The explanations: SNC-Central Nervous System; CRF- release factor for ACTH; ACTH- adrenocorticotropic hormone; TTH - thyrotropin hormone; ZCS - Cortico-adrenal zone; ZMS - Adrenal Medullary Zone; C-P - proinflammatory corticosteroids; C-A - anti-inflammatory corticosteroids; SPR - Renal pressor substances.

I.1.2.2. Sympathetic system (adrenal medulla)

The medullary zone - the adrenal medulla, represents the central zone of the gland and is surrounded by the cortical zone. The cells of the adrenal medulla secrete the hormones: adrenaline (epinephrine) and noradrelin (norepinephrine). These water-soluble hormones, derived from the amino acid tyrosine, act synergistically with the sympathetic nervous system [12].

In the situation where important stressors appear, in this area, certain physiological effects appear, mediated by the sympathetic nervous system, with the release of adrenaline and noradrenaline, when there is an increase in glycemic concentration, high values of plasma fatty acids, alteration of the functioning of the cardiac system and circulatory system of the animal. Noradrenaline is secreted by the nerve endings (of the neurons that describe the sympathetic nervous system) but also by the medullary area of the adrenal gland, along with adrenaline. The supervision of the secretion of adrenaline and noradrenaline from the adrenal medulla is done by the higher nerve centers in the spinal cord (pre- and postganglionic cholinergic and catecholaminergic neurons).

The general subject of this research is centered on the changes that occur in the activity of the corticotropic axis (GAS) and in the emergency response (sympathetic adrenal medullary system) also called the flight and fight syndrome, both systems being a fraction of what can be studied regarding the response of animals to stress.

I.1.2.3. Glycemic variations - indicator of activation of the corticotropic axis

The central nervous system (CNS) is known as the command center of the vital functions of organisms, it is the one that receives the information from the environment, from the receptors, processes the information and sends a response to the effectors in order to resolve the signal analyzed by the effectors. From a neurobiological point of view, the CNS activates two pathways of action:

- the peripheral nervous system (PNS) with its branches: somatic nerve fibers and vegetative nerve fibers that respond to the involuntary activity of glands and internal organs [16].

- the corticotropic axis and the sympathetic system - the adrenal medulla. Both structures are coordinated by the CNS. In the case of the corticotropic axis, the information starts from the hypothalamus which induces the secretion of ACTH with the role of controlling the glucocorticoid hormones secreted by the adrenal cortex. The sympathetic nervous system (SNS) is controlled by cholinergic preganglionic neurons (amplifies the effect of acetylcholine, favors the nervous control of skeletal muscles) that lead the information to the nerve fibers and the chromaffin cells of the adrenal medulla are responsible for the synthesis of catecholamines, only in the presence of tyrosine (amino acid synthesized by body, with great importance in the production of substances with the role of neurotransmitters).

Dysfunctions related to the activity of SNS neurotransmitters are observed by the appearance of variations in heart rate, blood pressure, piloerection in mammals, an increased concentration of fatty acids in the blood plasma and hyperglycemia. Hyperglycemia is already known as the increased value of glucose in the blood, an easy detection of this parameter is made from the peripheral blood, with high diagnostic accuracy.

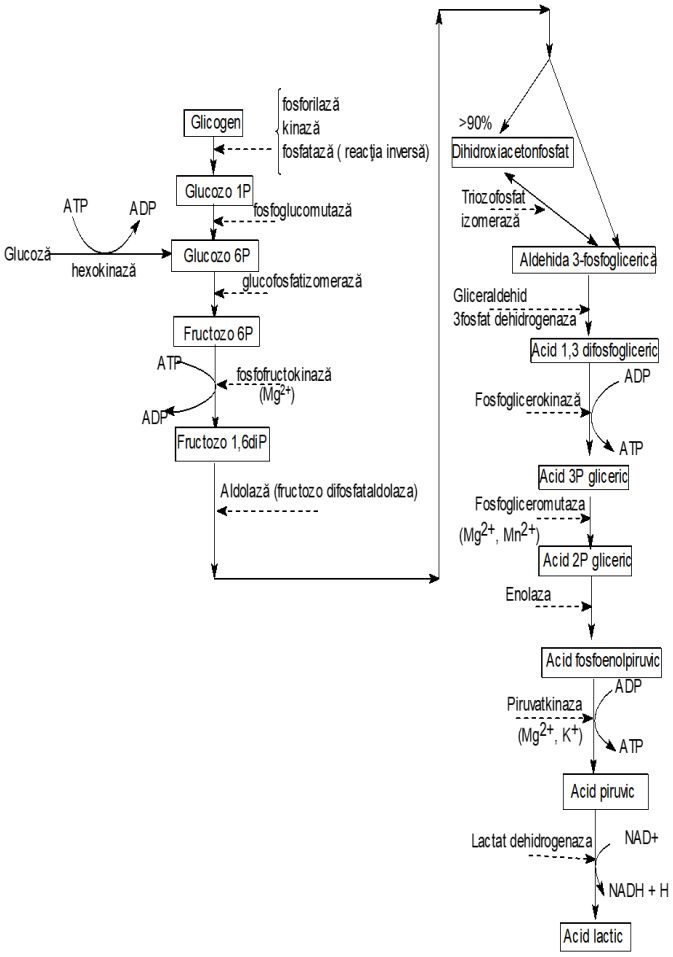


Fig. no. 2 Glycolysis (Source: Transformarea animalului viu in carne. Vol. II pg. 241. P. Alexe) [2]

I.1.3. Enzyme markers

L. Galluzzi et al. publishes a study in 2018 which states that at the cellular level, mammalian organisms respond to stress by maintaining the body's homeostasis, stressed cells emit signals in order to provide coordinated adaptive responses between tissues, organs up to the level of the entire organism. In the presence of evidence indicating the activation of mechanisms in response to various forms of cellular stress, Galluzzi believes that these mechanisms generate signals that cause microenvironmental and or systemic responses. Signals arising in response to cellular stress have the effect of changes in cell surfaces, the appearance of soluble factors or microvesicles, states of maladaptation and disease [21].

The study of stress in mammals is of great relevance because the effects on them reach the pathological sphere very easily, with serious consequences both from the economic point of view and the health of the population. The state of health of organisms is quantified in the first phase by their general state. When signs of alteration of the general state of health appear, the presence of some pathology is taken into account. The clinical examination is also conclusive in cases of disease, but the certainty of the conditions is given by laboratory tests, blood tests, on different compartments, where the presence of the disease can be certified (biochemistry, immunology, parasitology and others).

I.1.3.1. Sources of serum enzymes

Serum enzymes have different origins, from exogenous cells to blood serum, without well-established functions in blood. Some serum enzymes have been found to originate in the cytoplasm of some cells, in the mitochondria or even in some cell membranes. Physiological processes result in serum enzyme activity in healthy animals. Enzymes with low tissue specificity can come from several types of tissues and therefore from several types of cells. The presence of an enzyme in a tissue for which it has no specificity, does not necessarily mean that the

occurrence of lesions on that tissue, produce increases in the values or the action of the respective serum enzyme. The activity of a serum enzyme increases when the rate of enzyme entering the plasma exceeds the rate of enzyme inactivation or elimination from the plasma. [23]

Generalizing, there are five mechanisms by which the increase in serum enzyme activity is found: the increase in enzyme activity is launched by the appearance of affected cells, the start of enzyme synthesis, cell proliferation, the decrease in enzyme clearance, ingestion and absorption.

I.1.3.2. Decreased activity of serum enzymes

The decrease in the activity of most serum enzymes tested by blood biochemical analysis cannot give a relevant diagnosis. Measurement of enzyme activity may also be impaired due to poor handling of blood or plasma samples, enzyme degradation, presence of inhibitors such as anticoagulants, reference range that may be inappropriate for diagnosis, or a decrease in tissue mass, cell death or other processes physiological events occurring on the organism from which the biological samples were taken.

I.1.3.3. Measurement of serum enzymes

Activity per volume unit. The evaluation of routine measurements of enzyme activity is done by detecting the rate of substrate consumption or how quickly reaction products are formed. The most common method for evaluating serum enzymes is the spectrophotometric method.

Enzyme reactions for routine clinical evaluations - normal metabolic reactions of animal organisms where serum enzymes are involved as their catalysts.

The unit of measure used to express enzyme activity. U = international unit = the amount of enzyme that catalyzes the conversion of 1 micromole of substrate per minute, under known conditions. 1 U = 1µmol/min., are regularly expressed in U/L, occasionally mU/mL.

The influence of temperature correlated with the activity rate of serum enzymes. The common serum enzymes analyzed, including creatine phosphokinase, alkaline phosphatase and lactate dehydrogenase, led to obtaining percentage results on the same sample but at different temperatures.

Tabel no. 1 Correlation of temperatures with the activity rate of serum enzymes on the same analyzed sample

Relative activity of common serum enzymes				
Temperatures	25ºC	30 ºC	32 ºC	37 ºC
Serum enzymes	60-80%	100%	110-125%	130-210%

I.1.3.4. Nomenclature of enzymes

The International Union of Biochemistry and Molecular Biology, through the Nomenclature Committee, makes some recommendations regarding the naming of enzymes [25]. Most enzymes bear the name of the reaction of the first substrate (alanine, creatine), others have the name given by one up to the 6th category of enzymatically catalyzed reactions. Each enzyme is also identified by a unique code - Enzyme Commission number, given after the specific enzyme catalysis reaction, assigned in close connection with the enzyme nomenclature.

I.1.3.5. Interpretation of increases in enzyme activities

The measurement of enzyme activity shows variations between different assessments, in which case the enzyme activity of patients must be compared with appropriate reference intervals. In the case of increases in enzyme activity values, the degree of increase is determined by dividing the patients' enzyme value by the highest value of the upper reference limits (URL). The interpretation of the increase in serum enzyme activity combined with the potentially pathological processes and other information about the patient can be explanations and can help establish the diagnosis of a sick animal.

I.1.3.6. Significance of increased serum enzyme activity

Serum enzyme activity is a marker or indicator for pathological processes (hepatocyte damage or occurrence of cholestasis) and non-specific diseases. Many types of conditions can cause common pathological processes. For cytoplasmic enzymes (creatine phosphokinase CPK, Iditol dehydrogenase/sorbitol dehydrogenase ID, lactate dehydrogenase LDH and lipase LPS) the magnitude of the increase in activity may be relevant in the severity of the condition, a mild impairment may show an increase less than 2 x URL, more severe conditions have a increase greater than 50x URL. However, the rate of increase in enzyme activity does not differentiate reversible from irreversible or local from diffuse or systemic conditions.

Lactate dehydrogenase (LD, LDH)

Physiological processes and concepts. LDH is a cytoplasmic enzyme that characterizes a reversible reaction that converts pyruvate to lactate at the end of anaerobic glycolysis.

Pyruvate metabolism. Pyruvate, which is formed by the glycolytic degradation of glucose, occupies an important crossroads in glucose metabolism. In aerobic conditions: pyruvate is degraded by oxidative decarboxylation to acetyl-CoA, which will then be totally degraded in the Krebs cycle; in anaerobic conditions, pyruvate is reduced to lactate under the action of lactate dehydrogenase; Under conditions of activation of gluconeogenesis (synthesis of glucose from non-carbohydrate compounds), pyruvate is carboxylated to oxaloacetate, which will then transform into glucose [26].

Tissue origin responsible for increased serum LDH activity - cellular damage. The cellular source responsible for this increase is represented by hepatocytes, skeletal myocytes, cardiac myocytes and also erythrocytes.

Kumar Priti et al. published in Cold Spring Harbor Protocols in 2018 a study in which they explain the method of assessing cell cytotoxicity by measuring serum enzyme activity, more precisely LDH, released from affected cells. Kumar P. points out that after cell damage, LDH leaves the cell, a known fact in cases of apoptosis, necrosis or other forms of cell aggression. The method of analysis is based on tetrazolium salt, the assessment being made spectophotometrically, at a wavelength of 492nm. Quantitative results leading to the fact that the LDH value is directly proportional to the number of damaged cells [29].

Alkaline phosphatase (ALP)

Physiological processes and concepts. Alkaline phosphatase (ALP) - enzyme from the class of hydrolases (ortho-phospho-mono-ester-phosphohydrolase), has three isoenzymes

(hepatic, bone and intestinal) and during pregnancy in females a transient form appears - the placental isoenzyme. [30]

Several membrane cell types exhibit ALP activity, but only a few have sufficient activity to increase serum alkaline phosphatase. Hepatic ALP may be involved in the degradation of endotoxins while bone alkaline phosphatase has a role in the mineralization of bone structures [31].

In November 2022, Upstate Medical University through Dhruv Lowe et al., published a research in which they support the fact that ALP is cited in the clinical meanings in the case of liver diseases, especially in cholestatic ones (blockage of intra or extrahepatic bile flow), tissue damage toxic/drug-induced liver, severe chronic hepatitis leading to steatonecrosis, opportunistic infections in immunocompromised patients (AIDS) causing cholangiopathies – inflammation of the bile ducts. Many cases of increased ALP value occur as a result of non-specific inflammations, such as intra-abdominal infections, lymphomas (lymphatic carcinoma), myelomas (malignant degeneration of plasma cells - from the red bone marrow). Transient elevated ALP levels also occur in children, in growth spurts, after passing through a viral infection, without clinical significance. The exclusion of liver damage refers to dysfunctions of the bone system, simple bone fractures, bone metastases. The most common cases of ALP increase are those of hepatic origin, where the diagnosis is made by imaging, by observing the caliber of the bile ducts, later, as a final test, a liver puncture/biopsy is used for accurate diagnosis [32].

Tissue origin - increased serum enzyme activity are induced. The cellular source responsible for this increase is represented by hepatocytes - liver ALP (alkaline phosphatase induced by corticosteroids in dogs), biliary epithelium - liver ALP, osteoblasts - bone ALP and also the mammary epithelium.

Creatine phosphokinase (CPK, CK)

Physiological processes and concepts. CPK is an enzyme that reversibly catalyzes reactions involving transfers of the phosphate group PO4 to creatine-PO4 to adenosine diphosphate ADP to form ATP. CPK is a less commonly used name for this enzyme, which is why most scientific papers use the name creatine kinase CK. CK is a dimer; has four isoenzymes with a variable distribution with respect to affinity or tissue specificity: CK-1 is predominantly found in the brain; CK-2 and CK-3 occur in skeletal and cardiac muscle; CK-Mt occurs in mitochondria and other tissue types [44].

Tissue origin responsible for increased CK activity. - occurrence of cell damage. The cellular source responsible for this growth is represented by skeletal myocytes (muscle cells), cardiac myocytes, smooth muscle cells.

Guilherme Pereira Berriel et al., from the Federal University of Rio Grande do Sul. Porto Alegre, Brazil published in June 2020 a research referring to the variation of CPK in performance athletes, male volleyball players, in stages with different degrees of difficulty, up to maximal effort exercises, from the point of view of muscle demand skeletal. They found that stress levels increased shortly after the first weeks of training and had a steady trend in the pre-competitive stage. The variables used in monitoring physical effort during training showed a high level of stress and muscle damage, but also a positive adaptation to training stimuli, thus highlighting an improvement in athletes' performance. The findings of G. Pereira Berriel's study showed that

performance athletes showed adaptation to stress, fatigue and performance in an oscillating manner [48].

Glycosylated hemoglobin (Hb. glic., HbA1c)

Glycosylated hemoglobin (HbA1c) occurs as a result of the binding of a glucose molecule to the N-terminal of hemoglobin, it is a non-enzymatic process, it reflects an average of exposures of hemoglobin to glucose over a long period of time. The measurement of the blood glucose value allows the assessment of the current status of carbohydrate metabolism in diabetic patients, in particular. Determination of the value of the HbA1c test provides a retrospective estimate of the glycemic status, independent of the circadian rhythm, diet and other transient or permanent fluctuations in the concentration of glucose in the peripheral blood.

The appreciation of the HbA1c value as a marker of glycemic control in diabetic patients was highlighted by the results of two large studies - DCCT (Diabetes Control and Complications Trial) and UKPDS (United Kingdom Prospective Diabetes Study). They demonstrated the beneficial effect of intensive glycemic control both on metabolic parameters (glycemia and HbA1c) and on long-term complications (micro- and macrovascular) of type I or II diabetes and allowed the establishment of specific therapeutic targets based on HbA1c [56].

Hemoglobin glycosylation - enzyme-mediated process, is the addition of a sugar molecule to an organic molecule, especially a protein. It takes place intracellularly at the level of specific cellular organelles - the endoplasmic reticulum or the Golgi apparatus (post-translational), it is a regular process, it occurs in immature or unstable proteins whose stability increases and which it makes functional.

The term "glycated hemoglobin" describes a variety of compounds that have in common the glycation of hemoglobin - the non-enzymatic attachment reaction of glucose or other hexoses (fructose, galactose) to the hemoglobin molecule, is an irregular process and occurs in mature proteins which it can make non-functional and whose stability decreases. Glycation can occur at several amino acid residues in the hemoglobin structure and takes place in two stages:

- the condensation of glucose with the free amino group of hemoglobin generates a Schiff base (unstable aldimine);

- following the Amadori rearrangement phenomenon results in a stable ketoamine (in the case of glucose, fructosamine) [56].

Emily Eyth and Roopa Naik of the University of South Florida published in March 2023 a study Hb. Glycerin in which it specifies several aspects related to the interference factors of HbA1c in which inaccurate results may appear in the assessment of diabetes, namely: patients with sickle cell anemia, thalassemia, anemia, renal failure, kidney diseases or cases of patients who have had blood transfusions blood; in all these cases, the Hb values. Glycerin are inconclusive leading to peripheral blood glucose/glucose test. HbA1c can give falsely low results in pregnancy, hemorrhages, blood transfusions, erythropoietin treatments, Fe administration, hemolytic anemia, renal failure, liver damage, alcoholism. Treatment with Vit. C can give variations in Hb level. Glycerin HbA1c can give falsely high results due to a lack of Fe available in the blood (Fe deficiency anemia), anemia caused by infections or anemia resulting from some cases of tumors. Any kind of red blood cell damage, Vit deficiency. B_{12}, the increased value of triglycerides, organ

transplantation, in some ethnic groups the appearance of hyperglycation (the binding of glucose to Hb. by non-enzymatic means); exceptional cases are treatments with protease inhibitors or immunosuppressants. The study from the University of South Florida concludes that Hb. Glycerin is a valuable tool in the fight against diabetes and that all people involved in healthcare must know the variations of this indicator in order to correctly dose the medication so that the patients' quality of life is not affected [57].

Fructosamine (Fru)

Fructosamine appears as a result of the binding of glucose to the free amino groups of some serum proteins (this process is called glycosylation), especially albumin [58]. In the situation where the serum glucose concentration is constantly increased, as occurs in diabetes mellitus, increased glycosylation of serum proteins occurs, which leads to increased fructosamine values.

Verena Gounden et al. (University of KwaZulu Natal, University of Heidelberg Medical School Germany) publishes in August 2023 a paper in which they make a statistic of diabetes cases: in 2018 there were 34.2 million patients in the USA, with the prediction that in 2045 there would reaches 629 million patients globally. As accurate control measures, only plasma glucose and HbA1c are known to be universally accepted, with the clinical exceptions of Hb. glyc (chronic kidney disease, hemodialysis). The backup variant of HbA1c would be albumin, globulins and lipoproteins, of which only albumin is the most quantitatively present. Fructosamine is predominantly considered a measure of glycated albumin, both of which have a very important role in the diagnosis and control of diabetes. Regarding interference factors, Fructosamine can give erroneous values when temperature variations occur, the presence of reducing substances in the serum (Vit. C., bilirubin), conditions influencing serum albumin. If the serum albumin has values lower than 3g/dl. then the Fructosamine test is not conclusive (cirrhosis of the liver, loss of albumin due to renal or gastrointestinal diseases). Fructosamine and serum albumin are of great interest in the diagnosis and control of diabetes but also in the anticipation of vascular diseases, all the more so as the biological samples collected in this regard can be collected at any time during the day (not fasting) [59].

Dr. M. Codreanu and Alexandra Mihaela Popa argue for the use of the fructosamine test for diabetic patients, for blood glucose monitoring. Fructosamine is a glycosylated protein, evaluated in the monitoring and control of diabetes, used mainly in the evaluation of felines, but also in the case of canids, due to glycemic changes induced by cortisol, associated with stress, changes that can be determined by checking only glucose levels [60].

I.2. DISEASES INVOLVED IN HUMAN PATHOLOGY

The study of the General Adaptation Syndrome of organisms to various stress factors was carried out on the basis of assiduous documentation correlating the exceeded values of the studied markers with the diagnosis. The second part of this paper highlights the certainty of the values of the tracked indicators, as markers for GAS. This certainty is supported by an inventory of the conditions encountered during the acquisition of the research data.

I.2.1. Systemic Disease Inventory

Diseases with autoimmune damage - the body's immunity is a complex system with classical organization (organs, tissues, cells) whose function is to defend the body against

pathogens, of viral, bacterial, parasitic and fungal etiology. The diseases encountered in this thesis are: WEGENER's disease (granulomatosis), autoimmune hepatitis, thyrotoxicosis, autoimmune thyroiditis, rheumatoid arthritis, ankylosing spondylitis, SJÖGREN syndrome (SS), antiphospholipid syndrome (SAFL), systemic lupus erythematosus, overlap syndrome, Hyper IgE syndrome, systemic scleroderma, polymyositis and dermatomyositis, celiac disease, collagenoses or collagen vascular diseases, immune thrombocytopenia also called immune thrombocytopenic purpura.

Diseases with cardiovascular damage The conditions encountered in this thesis are: ischemic heart disease, ischemic coronary disease or ischemic heart disease (ICD), aortic disease - aortic stenosis, peripheral arterial disease (PAD) - peripheral vascular disease, right and left bundle branch block (BDR and BRS) bifascicular (BFAS), cardiomyopathies – dilatative, hypertrophic, restrictive; diastolic dysfunction, heart failure, atrial fibrillation, myocarditis, pericarditis, mitral valve prolapse, aortic stenosis, paroxysmal supraventricular tachycardia.

Diseases with circulatory impairment. This thesis contains the definition of the following conditions: acrocyanosis, aortic aneurysm, arteriopathy obliterans of the lower limbs or atherosclerosis obliterans of the lower limbs, Horton's arteritis, atheromatosis, stroke, varicose disease, arterial hypertension (HT), portal hypertension, pulmonary , arterial; vertebrobasilar circulatory insufficiency, venous insufficiency, HENOCH SCHONLEIN purpura, BEHÇET syndrome (BEHÇET disease - SB), RAYNAUD syndrome, thrombophlebitis (venous thrombosis), vasculitis.

Digestive and gastrointestinal diseases. The diseases encountered in the medical documents for the II part of the thesis are: parotid adenoma, angiodysplasias of the gastrointestinal tract, angiectasia, ascites, gastroesophageal reflux disease, hemorrhoidal disease, ulcer disease, colitis, irritable colon, colonic diverticulosis, duodenitis, BARETT's esophagus, esophagitis, gastritis, portal-hypertensive gastropathy, hiatal hernia, hypersplenism or splenomegaly, gallstones, sigmoid neoplasm, pancreatitis, colonic polyps, stomatitis, esophageal vvaricose veins.

Diseases with liver damage The diseases encountered in this paper are: biliary cirrhosis, liver cirrhosis, cholecystitis, cholestasis syndrome (hepato-biliary or jaundice syndrome), liver cysts, biliary dyskinesia, hepatic hemangioma, chronic hepatitis, chronic toxic ethanolic hepatitis, toxic hepatitis, hepatocytolysis, hepatomegaly, liver failure, liver nodule also called liver adenoma, hepatic steatosis, liver tumors.

Diseases with metabolic damage. This group of drugs contains the following diseases: phenylketonuria, dyslipidemia, prediabetes, diabetes, gout, hypercholesterolemia, hypertriglyceridemia (HTG), hypopotassemia / hypokalemia, obesity.

Diseases with neurological damage. The diseases encountered in this documentation are: cerebral atrophy, ALZHEIMER's disease, PARKINSON's disease, hemiparesis, vertigo syndrome or vertigo, anxiety, cerebral lacunarism, lumbosciatica, lumbago, ARNOLD's neuralgia, sensitive polyneuropathy, cervical radiculopathy or brachialgia, affective disorder, cognitive disorder, disorder depressed (depression).

Nutritional diseases This paper presents the following diseases: cachexia, diabetes, hypercholesterolemia, hypertriglyceridemia, hypoglycemia, lipodystrophies, obesity, anorexia, bulimia, iron deficiency anemia, hypochromic anemia, inadequate nutrition.

Diseases affecting the eye: blepharism, cataracts, iridocyclitis (anterior uveitis), SICCA syndrome (dry eye syndrome, keratoconjunctivitis sicca), glaucoma, maculopathy, presbyopia.

Diseases affecting Oto-Rino-Laryngology: otomastoiditis, hearing loss, chronic rhinitis, acute rhinosinusitis, sinusitis.

Diseases with bone damage Among the diseases encountered, we mention: kyphosis, kyphoscoliosis, popliteal cyst (BAKER cyst), coxarthrosis - arthrosis of the hip, nasal septum deviation, lumbar discopathy, disc herniation, Hallux Valgus (mounts), osteopenia, osteoporosis, scoliosis.

Diseases affecting joints: arthritis with microcrystals, arthritis, rheumatoid arthritis, psoriatic arthropathy, hip arthroplasty, arthrosis, gonarthrosis - arthrosis of the knee, DUPUYTREN's disease, enthesitis, scapulohumeral periarthritis (PSH), sacroiliitis, ankylosing spondylitis (Bechterew's disease), spondylosis, spondylodiscitis.

Diseases with kidney damage The diseases encountered are: chronic kidney disease, cystocele (bladder prolapse), renal cysts, renal colic, glomerulonephritis, hematuria, urinary incontinence, urinary infection, pyelonephritis, kidney stones.

Lung and respiratory diseases: asthma, chronic obstructive pulmonary disease (COPD), bronchiectasis, bronchitis, ventilatory dysfunction, respiratory failure, pulmonary fibrosis, pachypleuritis, pneumonia, sleep apnea syndrome SAS, tuberculosis or TBC.

Diseases affecting the thyroid: thyroid neoplasms (thyroid nodules / thyroid adenoma), nodular goiter, micronodular goiter, thyroidectomy, euthyroidism, thyroid insufficiency, hyperthyroidism, thyrotoxicosis, BASEDOW disease (Basedow-Graves, exophthalmic goiter), autoimmune thyroiditis, myxedema.

I.2.2. Other types of diseases

Dermatological affection: acne, dermatitis, intertrigo, keratosis, onychomycosis, panniculitis, papillomas, pityriasis, prurigo nodular, toxiderma, palmar tumor, vitiligo. *Gynecological/urological involvement:* prostate adenocarcinoma, breast cyst, ovarian cyst, uterine fibromatosis, mastectomy, uterine neoplasm, breast lump. *Lymphatic/inflammatory involvement:* Adenopathy - (lymphadenopathy).*Immunological impairment:* allergy to insect stings, hypergammaglobulinemia. *Endocrinological impairment:* iatrogenic hypercorticism or CUSHING syndrome, adrenal insufficiency. *Hematological damage:* megaloblastic anemia, normocytic anemias, lymphopenia, thalassemia. *Secondary parasitic or infectious diseases:* toxoplasmosis and toxocariasis, oral (oropharyngeal) candidiasis, herpes - Herpes simplex virus (HSV), athlete's foot (tinea pedis). *Muscle disorders: Fibromyalgia called (fibrositis), myopathy.*

I.3. DISEASES INVOLVED IN VETERINARY PATHOLOGY

Diseases with dermatological effects: dermatitis, infected parasitic dermatitis.

Diseases with metabolic effects: anemia.

Diseases affecting the ORL: parasitic pruritic otitis.

Diseases affecting muscles, bones and joints: hip dysplasia, muscle injuries, lameness.

Diseases with nutritional impairment: Low Ca and P, emaciation, Ca and P supplement, intensive growth system, energy substances.

Diseases with autoimmune involvement: CUSHING approximation.

Diseases with neurological damage: agitation, stress syndrome, prolonged agitation.

Hormonal diseases: hyperproduction of stress hormones, steroid treatment.

Renal/urological diseases: prostatic adenoma, dysuria, adrenal tumor.

Diseases affecting the thyroid: central hypothyroidism.

Diseases with gynecological effects: ovarian cyst, irregular, frequent estrus, 2-4 cilia compared to 2 normal, laborious parturition, lehuzia.

Clinical picture or distinct signs of the affected animal: low energy, moderate energy 15-20%, mobility - hyperactivity, starter feed (protein over 8%), protein in the diet over 10%, changed environment and nutrition.

II THE EXPERIMENTAL PART

II.1. MATERIALS AND METHODS

The working method is used for both - human and veterinary samples [298]:

II.1.1. Creatine phosphokinase / Creatine kinase (CPK/CK)

Creatine phosphokinase (CPK) catalyzes the reaction between creatine phosphate (CP) and adenosine-5-diphosphate (ADP) to form creatine and adenosine-5-triphosphate (ATP). Subsequently, glucose phosphorylates to glucose-6-phosphate (G6P) in the presence of hexokinase (HK), G6P is oxidized to gluconate-6P in the presence of nicotinamide adenine dinucleotide phosphate (NADP) in the reaction catalyzed by glucose-6phosphate dehydrogenase (G6P-DH). Conversion is monitored spectrophotometrically (kinetic) at 340 nm by the rate of increase in absorbance in the reduction of NADP to nicotinamide adenine dinucleotide phosphate (NADPH), proportional to CPK activity in blood samples. The spectrophotometer equipped with a thermostat is set at 25/30/37ºC, calibrated with distilled water at absorbance 0, the spectrophotometer cuvettes are prepared with the analysis quantities - 3 cuvettes with 1 ml of working reagent each for all 3 temperature thresholds and 3 other reference cuvettes, 2 cuvettes with 40 µl each for 25 and 30 ºC and 20 µl for 37 ºC. After 3 minutes of incubation, read the absorbance of the tubes - initial with a wavelength of 340nm, repeat the absorbance reading after exactly 1, 2 and 3 minutes. Calculate the difference between the measurements thus obtaining an average absorbance per minute.

Calculation of results: ΔA/min x 4127 = U/L CPK (25/30ºC); ΔA/min x 8095 = U/L CKP (37ºC). Reference intervals - Table no. 2

Table no. 2 Reference intervals for CPK

Temperature	25ºC	30 ºC	37 ºC
mens	≤ 65 U/L	≤105 U/L	≤174 U/L
womens	≤ 55 U/L	≤ 80 U/L	≤ 140 U/L
childrens	≤ 94 U/L	≤ 150 U/L	≤ 225 U/L

II.1.2. Lactatedhydrogenase (LDH)

Lactate dehydrogenase (LDH) catalyzes the reduction reaction of pyruvate to lactate in the presence of NADH at a pH of 7.5. The reaction is monitored in the same way as CPK, the rate of decrease in absorbance resulting from the oxidation of NADH to nicotinamide adenine dinucleotide (NAD+) being proportional to the activity of LDH in the blood samples. 2 reagents are used: R1 contains LDH substrate: TRIS buffer solution 100 mmol/L with pH 7.5, pyruvate 2.75 mmol/L, NaCl 222 mmol/L. R2 contains coenzyme LDH - NADH 1.55 mmol/L. The spectrophotometer equipped with a thermostat is fixed at 30/37ºC, calibrated with distilled water at 0 absorbance, the spectrophotometer cuvettes are prepared with the analysis quantities - 1 cuvette with working reagent of 1 ml and the other - standard 20 µl. Incubate initially for 30 seconds to obtain the initial absorbance, repeat the absorbance reading after exactly 1, 2 and 3 minutes. Calculate the difference between the measurements thus obtaining an average absorbance per minute.

Calculation of results: U/L = ΔA/min x 8095 Reference intervals - Table no. 3

Table no. 3 Reference intervals for LDH

Temperature	37 ºC	30 ºC
Adults	207-414 U/L	140-280 U/L

II.1.3. Alkaline phosphatase (ALP)

Alkaline phosphatase (ALP) catalyzes the hydrolysis of 4-nitrophenylphosphate (4-NPP) with formation of 4-phenol and free inorganic phosphate that alkalinizes the buffer solution as an acceptor phosphate group. The reaction is monitored spectrophotometrically (kinetic) at 405 nm by the rate of formation of 4-nitrophenol proportional to the activity of ALP in the analysis sample. The reagents used are: R1: ALP buffer solution - DEA buffer solution 1.25 mol/L pH 10.2. MgCl2 0.6 mmol/L. Biocides. R2: ALP substrate: 4-NPP 50 mmol/L. Biocides. ALP is stable in serum or plasma for 7 days at 2-8ºC.

The spectrophotometer equipped with a thermostat is set at 25/30/37ºC, calibrated with distilled water at absorbance 0, the spectrophotometer cuvettes are prepared with the analysis quantities - 3 cuvettes with 1 ml of working reagent each for all 3 temperature thresholds and 3 other reference cuvettes, 2 cuvettes with 40 µl each for 25 and 30 ºC and 20 µl for 37 ºC. After 3 minutes of incubation, read the absorbance of the tubes - initial with a wavelength of 340nm, repeat the absorbance reading after exactly 1, 2 and 3 minutes. Calculate the difference between the measurements thus obtaining an average absorbance per minute.

Calculation of results: U/L = ΔA/min x 2764 Reference intervals - Table no. 4

Table no. 4 Reference intervals for ALP

Temperature	25ºC	30 ºC	37 ºC
Childrens	≤ 480 U/L	≤590 U/L	≤800 U/L
Adults	≤ 180 U/L	≤ 220 U/L	≤ 270 U/L

II.1.4. Glycosylated hemoglobin (HbA1c/Hb. glic.)

Glycosylated hemoglobin (HbA1c) occurs as a result of the binding of a glucose molecule to the N-terminal of hemoglobin, it is a non-enzymatic process, it reflects an average of exposures of hemoglobin to glucose over a long period of time. [299] Three categories of substances are used: R1: Resin 25 x 2 mL. Ion exchange resin 8 mg/ml, buffer solution at pH 6.9. pre-distributed in tubes. R2: Lysis solution - potassium cyanide (KCN) 10 mM, surfactant. R3 – Standard solution (calibration-standard) HbA1c 10%.

The way it works is based on the use of a cation exchange resin, which has the role of separating HbA1c from the other Hb cells. For 5 minutes, the whole blood sample is homogenized with the resin, at which time only HbA1c remains free. The homogenate content is filtered to obtain the fraction containing HbA1c and to be subjected to spectrophotometry at a wavelength of 415 nm, the absorbance adjustment from 0 to 415 nm is made using deionized water as a standard. The average of two measurements represents the percentage of HbA1c present in the analyzed sample. The calculation of the results is done taking into account the fraction of total hemoglobin.

Table no. 5 Reference intervals for Hb1Ac/Hb. glic.

Samples analyzed	Sample values	Percent evidence	No. tested patients
Healthy patients	6.0 - 8.6 %	95%	100
Diabetic patients	8.4-16.00%	100%	31

II.1.5. Fructosamine (Fru)

Fructosamine appears as a result of the binding of glucose to the free amino groups of some serum proteins (this process is called glycosylation), especially albumin. [300] When the serum glucose concentration is constantly elevated, as occurs in diabetes mellitus, increased glycosylation of serum proteins occurs, leading to elevated fructosamine levels.

The principle of the analysis method is based on the ability to reduce ketoamines formed from non-enzymatic condensation products of glucose and proteins (fructosamine), by using tetrazolium salt (or tetrazolium chloride, nitrotetrazolium blue - NTB) in alkaline solution, when it produces a color shift. The presence of oxidized NTB can also act as a reaction initiator, in contact with fructosamine giving a purple-purple coloration. The concentration of fructosamine in the sample is visible by the intensity of the color shift of the reaction product solution.

Fructosamine reagent: carbonate buffer solution 0.2 mol/l with pH 10.35, NTB 0.5 mmol/l. Tension solution 2 g/l. Stabilizer. (the reagent is prepared industrially). Fructosamine calibration solution – optional glycated secondary standard solution. Samples with a concentration greater than 800 µmol/l are diluted 1:2 with a saline solution, analyzed using the Kroma analyzer, and the results are expressed after multiplying by 2.

Table no. 6 Reference intervals for Fru

Blood serum	Reference intervals
Children/babys	< 5%
Adults	205-285 µmol/l

II.1.6. SPSS application

SPSS multivariate statistical data processing application [301], [302]. The data extraction of this thesis was done with the help of a perfected set of techniques and univariate and multivariate analytical models, through mixed linear models to obtain deep information to simplify the processing of data extracted from medical documents. The statistical data with the SPSS application were processed closely, for all the analyzed markers, taken two at a time, with the aim of reaching the conclusions of the present research that lead to certain diagnoses of SGA following the values beyond the exceeded limits of the reference intervals of of the monitored enzyme markers. In order to structure the research conclusions, two functions of the SPSS application were used: the Pearson correlation coefficient and p-value.

The *Pearson correlation coefficient* helped to quantify the statistical relationship as well as the association between certain carefully selected data (numerical values assigned scales from 0 to 3); the results provided by this coefficient are expressed numerically, with values between 0 and 1, with the clear specification, where appropriate, that there is a significant 2-level correlation: 0.05 indicates a 5% risk, 95% being the confidence that that marker can be one of certainty for the condition for which it was calculated; the 0.01 level shows a risk of 1% which states that the diagnostic certainty of the respective marker is 99%, the correlation being strong between the marker and the respective group of conditions. The *p-value* (value per level) appears as a numeric expression, with values between 0 and 1, expressed to three decimal places.

II.2 RESULTS AND DISCUSSION

II.2.1. Interpretation of human patient data

The data values that will be presented in the following are the result of an initial centralization, data taken without prior processing. The groups of conditions present in the first column of the tables below were created according to the own way of synthesizing all the diagnoses found in the medical documents of the patients from whom the values of the biological samples were processed to study the SGC as a form of adaptation of the organisms to stress.

The percentage expression of the incidence of disease groups refers to the total number of biological marker sample values.

II.2.1.1. Creatine phosphokinase / Creatine kinase (CPK/CK)

Number of analyzed samples: 159, processed from a number of patients: 133. Number of samples from female patients: 101, number of samples from male patients: 32. Age of patients – age ranges: 16-88 years. Female patients – age groups: 16-88 years, male patients – age groups: 21-80 years. Interpretation of analyzed sample values: reference intervals (laboratory method): 0-26 (2 samples), 1-16 (2 samples), 7-25 (5 samples), 26-140 (106 samples), 38-174 (37 samples), 38-397 (6 samples), 55-170 1 sample). Analysis bulletin data: 14.12.2015-25.04.2016.

Interpretation of test values above the normal limit. In this category there are 51 (32%) samples out of a total of 159, of which 37 (23%) samples collected from women and 14 (8%) samples collected from men, highlighting the impact on groups as follows in table no. 9.

Table no. 9 CPK sample values above the normal limit

Group of conditions / affect	*no. samples ♀ CPK*	*% samples ♀ CPK*	*no. samples ♂ CPK*	*% samples ♂ CPK*
Autoimmune	21	13.21%	3	1.89%
Joint damage	5	3.14%	0	0.00%
Heart damage	15	9.43%	3	1.89%
Circulatory impairment	19	11.95%	5	3.14%
Dermatological affection	1	0.63%	0	0.00%
Digestive affection	7	4.40%	2	1.26%
Diabetes	6	3.77%	2	1.26%
Gynecological affection	4	2.52%	0	0.00%
Hormonal affect	1	0.63%	0	0.00%
Hematological impairment	0	0.00%	0	0.00%
Liver damage	16	10.06%	5	3.14%
Infectious affection	6	3.77%	2	1.26%
Muscle damage	1	0.63%	0	0.00%
Metabolic impairment	21	13.21%	5	3.14%
Neurological impairment	26	16.35%	1	0.63%
Nutritional diseases	17	10.69%	5	3.14%
Oto Rino Laryngology	3	1.89%	0	0.00%
Oncological affection	0	0.00%	0	0.00%
Ophthalmological	4	2.52%	0	0.00%
Bone damage	19	11.95%	3	1.89%
Pulmonary involvement	10	6.29%	2	1.26%
Renal impairment	13	8.18%	4	2.52%
Thyroid involvement	11	6.92%	1	0.63%
Urological involvement	0	0.00%	3	1.89%

Correlations of CPK sample values with the other enzyme markers studied

CPK – LDH from the total of 159 samples analyzed by CPK, 149 (93.71 %) are correlated with LDH samples, as follows: 86 samples (54.09 %) LDH with values above the normal limit; 3

samples (1.89 %) LDH with values below the normal limit; 62 samples (38.99 %) LDH with values within the normal range.

CPK – ALP from the total of 159 samples analyzed by CPK, 77 (48.43 %) are correlated with ALP samples, as follows: 7 samples (4.40 %) ALP with values above the normal limit; 6 samples (3.77 %) ALP with values below the normal limit; 64 samples (40.25 %) ALP with values within the normal range.

CPK – Hb. glic. from the total of 159 samples analyzed by CPK, 42 (26.42 %) are correlated with samples. Hb. glic., as follows: 21 samples (13.21 %) Hb. glic. with values above the normal limit; 1 sample (0.63 %) Hb. glic. with values below the normal limit; 20 samples (12.58 %) Hb. glic. with values within the normal range.

II.2.1.2. Lactatedhydrogenase (LDH)

Number of analyzed samples: 253, processed from a number of patients: 244. Number of female patient samples: 171, number of male patient samples: 73. Age of patients – age ranges: 16-88 years. Female patients – age groups: 16-88 years, male patients – age groups: 18-82 years. Interpretation of analyzed sample values: reference ranges (laboratory method): 35-104 IU/L (1 sample), 135-220 IU/L (252 samples). Analysis bulletin data: 14.12.2015-26.06.2016.

Interpretation of test values above the normal limit. In this category there are 104 (41%) samples out of a total of 253, of which 74 (29%) samples collected from women and 30 (12%) samples collected from men, highlighting the impact on groups as follows in table no. 12.

Table no. 12 LDH sample values above the normal limit

Group of conditions / affect	*no. samples ♀ LDH*	*% samples ♀ LDH*	*no. samples ♂ LDH*	*% samples ♂ LDH*
Autoimmune	35	13.83%	8	3.16%
Joint damage	24	9.49%	4	1.58%
Heart damage	41	16.21%	23	9.09%
Circulatory impairment	48	18.97%	21	8.30%
Dermatological affection	8	3.16%	1	0.40%
Digestive affection	21	8.30%	12	4.74%
Diabetes	7	2.77%	4	1.58%
Gynecological affection	8	3.16%	0	0.00%
Hormonal affect	7	2.77%	0	0.00%
Hematological impairment	7	2.77%	5	1.98%
Liver damage	39	15.42%	14	5.53%
Infectious affection	16	6.32%	8	3.16%
Muscle damage	2	0.79%	0	0.00%
Metabolic impairment	55	21.74%	16	6.32%
Neurological impairment	36	14.23%	7	2.77%
Nutritional diseases	36	14.23%	17	6.72%
Oto Rino Laryngology	7	2.77%	1	0.40%
Oncological affection	0	0.00%	2	0.79%

Ophthalmological	6	2.37%	0	0.00%
Bone damage	42	16.60%	7	2.77%
Pulmonary involvement	23	9.09%	13	5.14%
Renal impairment	34	13.44%	17	6.72%
Thyroid involvement	35	13.83%	1	0.40%
Urological involvement	0	0.00%	7	2.77%

Correlations of LDH sample values with the other enzyme markers studied

LDH – CPK from the total of 253 samples analyzed by LDH, 149 (49.41 %) are correlated with CPK samples, as follows: 39 samples (15.42 %) CPK with values above the normal limit; 6 samples (3.77 %) CPK with values below the normal limit; 80 samples (31.62 %) CPK with values within the normal range.

LDH – Alkaline Phosphatase from the total of 253 samples analyzed by LDH, 112 (44.27 %) are correlated with Alkaline Phosphatase samples, as follows: 15 samples (5.93 %) Alkaline Phosphatase with values above the normal limit; 6 samples (2.37 %) Alkaline phosphatase with values below the normal limit; 91 samples (35.97 %) Alkaline phosphatase with values within the normal range.

LDH - Hb. glic. from the total of 253 samples analyzed by LDH, 54 (21.34 %) are correlated with samples Hb. glyc., as follows: 37 samples (14.62 %) Hb. glyc. with values above the normal limit; 2 samples (0.79 %) Hb. glyc. with values below the normal limit; 15 samples (5.93 %) Hb. glyc. with values within the normal range.

II.2.1.3. Alkaline phosphatase (ALP)

Number of analyzed samples: 194, processed from a number of patients: 193. Number of female patient samples: 130, number of male patient samples: 61. Age of patients – age ranges: 16-88 years. Female patients – age groups: 16-88 years, male patients – age groups: 21-80 years. Interpretation of analyzed sample values: reference ranges (laboratory method): 00-187 (1 sample), 35-104 (132 samples), 40-129 (61 samples). Analysis bulletin data: 14.12.2012-26.04.2016.

Interpretation of test values above the normal limit. In this category there are 39 (20%) samples out of a total of 194, of which 29 (15%) samples collected from women and 10 (5%) samples collected from men, highlighting the impact on groups as follows in table no. 15.

Table no. 15 ALP sample values above the normal limit

Group of conditions / affect	*No. samples ♀ ALP*	*% samples ♀ ALP*	*No. samples ♂ ALP*	*% samples ♂ ALP*
Autoimmune	0	0.00%	0	0.00%
Joint damage	2	1.03%	2	1.03%
Heart damage	5	2.58%	5	2.58%
Circulatory impairment	8	4.12%	8	4.12%
Dermatological affection	1	0.52%	1	0.52%

Digestive affection	6	3.09%	6	3.09%
Diabetes	4	2.06%	4	2.06%
Gynecological affection	0	0.00%	0	0.00%
Hormonal affect	0	0.00%	0	0.00%
Hematological impairment	2	1.03%	2	1.03%
Liver damage	6	3.09%	6	3.09%
Infectious affection	2	1.03%	2	1.03%
Muscle damage	0	0.00%	0	0.00%
Metabolic impairment	8	4.12%	8	4.12%
Neurological impairment	3	1.55%	3	1.55%
Nutritional diseases	6	3.09%	6	3.09%
Oto Rino Laryngology	0	0.00%	0	0.00%
Oncological affection	1	0.52%	1	0.52%
Ophthalmological	1	0.52%	1	0.52%
Bone damage	2	1.03%	2	1.03%
Pulmonary involvement	7	3.61%	7	3.61%
Renal impairment	5	2.58%	5	2.58%
Thyroid involvement	2	1.03%	2	1.03%
Urological involvement	1	0.52%	1	0.52%

Correlations of ALP sample values with the other enzyme markers studied

ALP - CPK from the total of 194 samples analyzed by ALP, 64 (32.99 %) are correlated with CPK samples, as follows: 11 samples (5.67 %) CPK with values above the normal limit; 4 samples (2.06 %) CPK with values below the normal limit; 49 samples (25.26 %) CPK with values within the normal range.

ALP – LDH from the total of 194 samples analyzed by ALP, 114 (58.76 %) are correlated with LDH samples, as follows: 51 samples (26.29 %) LDH with values above the normal limit; 6 samples (3.09 %) LDH with values below the normal limit; 57 samples (29.38 %) LDH with values within the normal range.

ALP – Hb. glyc. from the total of 194 samples analyzed by ALP, 36 (18.56 %) are correlated with samples Hb. glyc., as follows: 25 samples (12.89 %) Hb. glyc. with values above the normal limit; 2 sample (1.03 %) Hb. glyc. with values below the normal limit; 9 samples (4.64 %) Hb. glyc. with values within the normal range.

II.2.1.4. Glycosylated hemoglobin (Hb. glyc.)

Number of analyzed samples: 79, processed from a number of patients: 79. Number of samples from female patients: 46, number of samples from male patients: 33. Age of patients – age ranges: 16-88 years. Female patients – age groups: 16-88 years, male patients – age groups: 41-85 years. Interpretation of analyzed sample values: reference intervals (laboratory method): 4.80-5.90. Analysis bulletin data: 14.01.2015-04.2016.

Interpretation of test values above the normal limit. In this category there are 62 (78%) samples out of a total of 79, of which 38 (48%) samples collected from women and 24 (30%) samples collected from men, highlighting the impact on groups as follows in table no. 18.

Table no.. 18 Hb. glyc. sample values above the normal limit

Group of conditions / affect	*no. samples ♀ Hb. glic.*	*% samples ♀ Hb. glic.*	*No. samples ♂ Hb. glic.*	*% samples ♂ Hb. glic*
Autoimmune	11	13.92%	2	2.53%
Joint damage	9	11.39%	4	5.06%
Heart damage	22	27.85%	18	22.78%
Circulatory impairment	27	34.18%	23	29.11%
Dermatological affection	1	1.27%	1	1.27%
Digestive affection	7	8.86%	4	5.06%
Diabetes	24	30.38%	18	22.78%
Gynecological affection	3	3.80%	0	0.00%
Hormonal affect	0	0.00%	0	0.00%
Hematological impairment	5	6.33%	1	1.27%
Liver damage	22	27.85%	15	18.99%
Infectious affection	13	16.46%	4	5.06%
Muscle damage	0	0.00%	0	0.00%
Metabolic impairment	29	36.71%	23	29.11%
Neurological impairment	14	17.72%	4	5.06%
Nutritional diseases	19	24.05%	18	22.78%
Oto Rino Laryngology	1	1.27%	1	1.27%
Oncological affection	0	0.00%	2	2.53%
Ophthalmological	2	2.53%	1	1.27%
Bone damage	19	24.05%	6	7.59%
Pulmonary involvement	11	13.92%	5	6.33%
Renal impairment	21	26.58%	18	22.78%
Thyroid involvement	17	21.52%	2	2.53%
Urological involvement	0	0.00%	7	8.86%

Correlations of Hb. glic. sample values with the other enzyme markers studied

Hb. glyc. - CPK from the total of 79 samples analyzed for Hb. glyc., 25 samples (31.65 %) are correlated with CPK samples, as follows: 7 samples (8.86 %) CPK with values above the normal limit; 2 samples (2.53 %) CPK with values below the normal limit; 16 samples (20.25 %) CPK with values within the normal range.

Hb. glyc. – LDH from the total of 79 samples analyzed for Hb. glyc., 55 (69.62 %) are correlated with LDH samples, as follows: 14 samples (17.72 %) LDH with values above the normal limit; 2 samples (2.53 %) LDH with values below the normal limit; 39 samples (49.37 %) LDH with values within the normal range.

Hb. glyc. – ALP from the total of 79 samples analyzed for Hb. glyc., 29 (36.71 %) are correlated with ALP samples, as follows: 4 samples (5.06 %) ALP with values above the normal limit; 3 samples (3.80 %) ALP with values below the normal limit; 22 samples (27.85 %) ALP with values within the normal range.

II.2.2 Interpretation of veterinary patient data

II.2.2.1. Creatine phosphokinase/Creatine kinase (CPK/CK)

Total analyzed samples: 47. 22 samples from individuals of the canine species and 25 samples from individuals of the porcine species.

The canine species. Number of samples analyzed: 22, processed from a number of patients: 22, of which 13 samples analyzed from females, 9 samples analyzed from males. Age of patients – age ranges: age groups: 4-12 years, male patients – 5-11 years, female patients: 4-12 years. Analysis bulletin data: 22.08.2016-23.10.2017. Interpretation of analyzed sample values: reference ranges (laboratory method): below 300 U/l.

Interpretation of test values above the normal limit. In this category there are 20 (90.8%) samples out of a total of 22, of which 13 (59%) samples collected from females and 7 (31.8%) samples collected from males, highlighting the impact on groups as follows in table no. 20.

Table no. 20 Canine veterinary CPK - sample values above normal limit

Group of conditions / affect	*No. samples ♀ CPK*	*% samples ♀ CPK*	*No. samples ♂ CPK*	*% samples ♂ CPK*
Dermatological affection	2	9.09%	3	13.64%
Hormonal affect	11	50.00%	0	0.00%
Nutritional diseases	4	18.18%	0	0.00%
Metabolic impairment	4	18.18%	2	9.09%
Infectious affection	0	0.00%	1	4.55%
Urological involvement	0	0.00%	3	13.64%
Gynecological affection	3	13.64%	0	0.00%
Oncological affection	2	9.09%	2	9.09%
Oto Rino Laryngology	0	0.00%	0	0.00%
Thyroid involvement	2	9.09%	0	0.00%
Bone damage	2	9.09%	1	4.55%
Joint damage	2	9.09%	1	4.55%
Muscle damage	0	0.00%	2	9.09%

Correlations of canine veterinary CPK test values with the other enzyme markers studied

CPK – LDH from the total of 22 samples analyzed by CPK, 10 (45.45 %) are correlated with LDH samples, as follows: 10 samples (45.45 %) LDH with values within the normal range.

CPK – ALP from the total of 22 samples analyzed by CPK, 7 (31.81 %) are correlated with ALP samples, as follows: 5 samples (22.72 %) ALP with values within the normal range; 2 samples (9.09 %) ALP with values above the normal limit.

Porcine species (farm pigs). Number of samples analyzed: 25, processed from a number of patients: 25, of which 15 samples analyzed from females, 10 samples analyzed from males. Age of patients – age intervals: 2, 10, 45, 90, 150 days, for both sexes: 2 days: 5 individuals (2 females, 3 males); 10 days: 5 individuals (2 females, 3 males); 45 days: 5 individuals (2 females, 3 males); 90 days: 5 individuals (2 females, 3 males); 150 days: 5 individuals (2 females, 3 males).

Interpretation of analyzed sample values: reference intervals (laboratory method): 280 U/l. Number of samples, percentage by group of conditions resulting from the diagnosis of patients, overall and by gender, as follows in table no. 21.

Table no. 21 CPK – swine veterinary samples (number and percentages) by disease groups

Group of conditions / affect	*no. samples CPK*	*% samples CPK*	*samples ♀*	*% samples ♀*	*samples ♂*	*% samples ♂*
Hormonal affect	15	60.00%	6	24.00%	9	36.00%
Nutritional diseases	25	100.00%	10	40.00%	15	60.00%
Metabolic impairment	25	100.00%	10	40.00%	15	60.00%

Interpretation of test values above the normal limit. In this category there are 9 (36%) samples out of a total of 25, of which 4 (16%) samples collected from females and 5 (20%) samples collected from males, in age categories 2, 45, 90 and 150 days, highlighting the impact on groups as follows in table no. 22.

Table no. 22 Porcine veterinary CPK - sample values above the normal limit

Group of conditions / affect	*no. samples ♀ CPK*	*% samples ♀ CPK*	*no. samples ♂ CPK*	*% samples ♂ CPK*
Hormonal affect	0	0.00%	0	0.00%
Nutritional diseases	2	8.00%	3	12.00%
Metabolic impairment	2	8.00%	2	8.00%

Correlations of porcine veterinary CPK sample values with the other enzyme markers studied CPK – values above the normal limit in 9 samples (36 %) in the following cases: 1 individual sample aged 2 days (after cutting fangs, during breastfeeding); 2 individual samples aged 45 days (at weaning); 1 individual sample aged 90 days (intensive feeding, weight 60 kg.); 5 individual samples aged 150 days (intensive feeding, weight 120 kg.).

II.2.2.2. Lactatedhydrogenase (LDH)

Total samples analyzed: 47, 22 samples from individuals of the canine species and 25 samples from individuals of the porcine species

The canine species. Number of samples analyzed: 20, processed from a number of patients: 20, of which 10 samples analyzed from females, 10 samples analyzed from males. Age of patients – age ranges: age groups: 1-13 years, male patients: 3-13 years, female patients: 1-12 years. Analysis bulletin data: 10.06.2016-23.09.2017. Interpretation of analyzed sample values: reference intervals (laboratory method): below 220 U/l.

Interpretation of test values above the normal limit. In this category there are 13 (65%) samples out of a total of 20, of which 9 (45%) samples collected from females and 4 (20%) samples collected from males, highlighting the effects on groups as follows in table no. 24.

Table no. 24 Canine veterinary LDH - sample values above the normal limit

Group of conditions / affect	*No. samples ♀ LDH*	*% samples ♀ LDH*	*No. samples ♂ LDH*	*% samples ♂ LDH*
Dermatological affection	0	0.00%	2	10.00%
Hormonal affect	9	45.00%	0	0.00%
Nutritional diseases	2	10.00%	2	10.00%
Metabolic impairment	3	15.00%	1	5.00%
Infectious affection	0	0.00%	1	5.00%
Urological involvement	1	5.00%	1	5.00%
Gynecological affection	3	15.00%	0	0.00%
Oncological affection	2	10.00%	1	5.00%
Oto Rino Laryngology	0	0.00%	0	0.00%
Thyroid involvement	0	0.00%	0	0.00%
Bone damage	0	0.00%	0	0.00%
Joint damage	0	0.00%	0	0.00%
Muscle damage	0	0.00%	0	0.00%

Correlations of canine veterinary LDH sample values with the other enzyme markers studied
LDH – CPK from the total of 20 samples analyzed by LDH, 10 (50 %) are correlated with CPK samples, as follows: 2 samples (45.45 %) CPK with values within the normal range; 8 samples (40 %) CPK with values above the normal limit.
LDH – Alkaline Phosphatase from the total of 20 samples analyzed by LDH, 18 (90 %) are correlated with Alkaline Phosphatase samples, as follows: 12 samples (60 %) Alkaline Phosphatase with values within the normal range; 6 samples (30 %) Alkaline phosphatase with values above the normal limit.

Porcine species (farm pigs). Number of samples analyzed: 25, processed from a number of patients: 25, of which 15 samples analyzed from females, 10 samples analyzed from males. Age of patients – age intervals: 2, 10, 45, 90, 150 days, for both sexes: 2 days: 5 individuals (2 females, 3 males); 10 days: 5 individuals (2 females, 3 males); 45 days: 5 individuals (2 females, 3 males); 90 days: 5 individuals (2 females, 3 males); 150 days: 5 individuals (2 females, 3 males). Interpretation of analyzed sample values: reference intervals (laboratory method): below 220 U/l.

Interpretation of test values above the normal limit. In this category there are 21 (84%) samples out of a total of 25, of which 8 (32%) samples collected from females and 13 (52%) samples collected from males, from individuals of all age categories, putting in evidence the impact on groups as follows in table no. 26.

Table no. 26 Pig veterinary LDH- sample values above the normal limit

Group of conditions / affect	*No. samples ♀ LDH*	*% samples ♀ LDH*	*No. samples ♂ LDH*	*% samples ♂ LDH*
Hormonal affect	5	20.00%	8	32.00%
Nutritional diseases	8	32.00%	13	52.00%
Metabolic impairment	8	32.00%	13	52.00%

Correlations of swine veterinary LDH sample values with the other enzyme markers studied
LDH– CPK of the total of 25 samples analyzed by CPK, 25 (100 %) are correlated with LDH samples as follows: 25 samples (100 %) LDH with values within the normal range.
LDH – ALP from the total of 25 samples analyzed by CPK, 25 (100 %) are correlated with Alkaline Phosphatase samples, as follows: 25 samples (100 %) Alkaline Phosphatase with values within the normal range.
LDH – blood glucose from the total of 25 samples analyzed by LDH, 25 (100%) of them are correlated with blood glucose samples as follows: 6 samples (24%) blood glucose with values above the normal limit; 19 samples (76%) blood sugar with values within the normal range.

II.2.2.3. Alkaline phosphatase (ALP)

Total samples analyzed: 45. 20 samples from individuals of the canine species and 25 samples from individuals of the porcine species

The canine species. Number of samples analyzed: 20, processed from a number of patients: 20, of which 11 samples analyzed from females, 9 samples analyzed from males. Age of patients - age ranges: age groups: 1-13 years, male patients - 5-13 years, female patients: 1-11 years. Analysis bulletin data: 04.06.2016-30.10.2017. Interpretation of analyzed sample values: reference ranges (laboratory method): below 200 U/l.

Interpretation of test values above the normal limit. In this category there are 14 (70%) samples out of a total of 20, of which 7(35%) samples collected from females and 7(35%) samples collected from males, highlighting the effects on groups as follows in table no. 28.

Table no. 28 Canine veterinary ALP - sample values above the normal limit

Grup afecțiuni /afectare	*nr. probe ♀ ALP*	*% probe ♀ ALP*	*nr. probe ♂ ALP*	*% probe ♂ ALP*
Dermatological affection	1	5.00%	2	10.00%
Hormonal affect	6	30.00%	2	10.00%
Nutritional diseases	0	0.00%	1	5.00%
Metabolic impairment	0	0.00%	3	15.00%
Infectious affection	0	0.00%	2	10.00%
Urological involvement	2	10.00%	1	5.00%
Gynecological affection	3	15.00%	2	10.00%
Oncological affection	1	5.00%	1	5.00%
Oto Rino Laryngology	0	0.00%	1	5.00%
Thyroid involvement	1	5.00%	1	5.00%
Bone damage	0	0.00%	1	5.00%
Joint damage	1	5.00%	1	5.00%
Muscle damage	0	0.00%	1	5.00%

Correlations of canine veterinary ALP sample values with the other enzyme markers studied
ALP - CPK from the total of 20 samples analyzed by ALP, 8 samples (40 %) are correlated with CPK samples, as follows: 3 samples (15 %) CPK with values within the normal range; 5 samples (25%) CPK have values above the normal limit.

ALP – LDH from the total of 20 samples analyzed by ALP, 10 samples (50%) are correlated with LDH samples, as follows: 4 samples (20%) LDH have values within the normal range; 6 samples (30%) LDH have values above the normal limit.

Porcine species (farm pigs). Number of samples analyzed: 25, processed from a number of patients: 25, of which 15 samples analyzed from females, 10 samples analyzed from males. Age of patients – age intervals: 2, 10, 45, 90, 150 days, for both sexes: 2 days: 5 individuals (2 females, 3 males); 10 days: 5 individuals (2 females, 3 males); 45 days: 5 individuals (2 females, 3 males); 90 days: 5 individuals (2 females, 3 males); 150 days: 5 individuals (2 females, 3 males). Interpretation of analyzed sample values: reference ranges (laboratory method): below 270 U/l.

Interpretation of test values above the normal limit. In this category there are 15 (60%) samples out of a total of 25, of which 6 (24%) samples collected from females and 9 (36%) samples collected from males, in all age categories, highlighting the impact on groups as follows in table no. 30.

Table no. 30 ALP veterinary swine - sample values above the normal limit

Group of conditions / affect	*No. samples ♀ ALP*	*% samples ♀ ALP*	*No. samples ♂ ALP*	*% samples ♂ ALP*
Hormonal affect	3	12.00%	4	20.00%
Nutritional diseases	6	30.00%	9	45.00%
Metabolic impairment	6	30.00%	9	45.00%

Correlations of swine veterinary ALP sample values with the other enzyme markers studied

ALP – CPK of the total of 25 samples analyzed by ALP, 25 (100 %) are correlated with CPK samples as follows: all 25 (100 %) CPK samples have values within the normal range.

ALP – LDH from the total of 25 samples analyzed by CPK, 25 (100 %) are correlated with LDH samples as follows: 25 samples (100 %) LDH with values within the normal range.

ALP – blood glucose from the total of 25 samples analyzed by ALP, 25 (100%) of them are correlated with blood glucose samples as follows: 6 samples (24%) blood glucose with values above the normal limit; 19 samples (76%) blood sugar with values within the normal range.

II.2.2.4. Fructosamine (Fru)

Total analyzed samples: 18, 6 samples from individuals of the canine species and 12 samples from individuals of the feline species.

The canine species. Number of samples analyzed: 6, processed from a number of patients: 6, of which 3 samples analyzed from females, 3 samples analyzed from males. Age of patients – age ranges: age groups: 6-12 years, male patients – 6-9 years, female patients: 6-12 years. Analysis bulletin data: 05.02.2019-05.09.2021. Interpretation of analyzed sample values: reference ranges (laboratory method): below 370 U/l.

Interpretation of test values above the normal limit. In this category there are 5 (83%) samples out of a total of 6, of which 3 (50%) samples collected from females and 2 (33.33%) samples collected from males, highlighting the effects on groups after as follows in table no. 32.

Table no. 32 Fructosamine - canine veterinary samples - sample values above the normal limit

Group of conditions / affect	*No. samples ♀ Fru*	*% samples ♀Fru*	*No. samples ♂ Fru*	*% samples♂ Fru*
Metabolic impairment/ diabetic Status	3	50.00%	2	33.33%
Electrolytes	0	0.00%	0	0.00%

Correlations of values of veterinary samples Fructosamine - canine

Fructosamine samples are tracked alongside glucose samples, but they are not relevant for the present research since glucose is not a relevant diagnostic marker for the general syndrome of adaptation of organisms to different forms of stress. From the total of 6 samples of Fru, 1 sample (16%) of them shows Glucose (Glu) with values above the normal limit.

Feline species. Number of samples analyzed: 12, processed from a number of 12 patients, of which 7 samples analyzed from females, 5 samples analyzed from males. Age of patients – age ranges: age groups: 3-15 years, male patients – 3-15 years, female patients: 6-15 years. Analysis bulletin data: 12.03.2019-21.05.2021. Interpretation of analyzed sample values: reference ranges (laboratory method): below 340 U/l (9 samples), 165-240 (2 samples), 191-349 (1 sample).

Interpretation of test values above the normal limit. In this category there are 10 (83%) samples out of a total of 12, of which 7 (58.33%) samples collected from females and 3 (25%) samples collected from males, highlighting the effects on groups after as follows in table no. 34.

Table no. 34 Fructosamine - feline veterinary samples - sample values above the normal limit

Grup afecțiuni /afectare	*No. samples ♀ Fru*	*% samples ♀Fru*	*No. samples ♂ Fru*	*% samples♂ Fru*
Metabolic impairment/ diabetic Status	7	58.33%	3	25.00%
Electrolytes	0	0.00%	0	0.00%

Correlations veterinary test values Fructosamine - feline

Fructosamine samples are tracked alongside glucose samples, but they are not relevant to the present research since glucose is not a relevant diagnostic marker for the general syndrome of adaptation of organisms to different forms of stress.

From the total of 12 samples of Fructosamine analyzed in felines, 1 sample (8.3%) of them has Glucose (Glu) with values above the normal limit.

From the total of 12 Fructosamine samples analyzed from individuals of the feline species, 2 (17%) samples had values within the normal range, although they appear in diabetic status, one of them also appearing with electrolyte values at the upper limit.

II.3. General conclusions, elements of originality and recommendations

II.3.1. Conclusions and interpretations of research results

After processing the data with the SPSS multivariate statistical application, the following data centralizations of the samples with values above the limit of the reference intervals were obtained, in the case of groups of conditions that strictly characterize GAS.

It should be mentioned that in the assessment of the results of this research, only the samples with exceeded values of all the markers taken into analysis were used so that the assessment of the markers as indicators of certainty is in conditions of maximum safety - minimum scientific risk.

The four enzyme markers were analyzed in turn, which were assigned correlations with the exceeded values of the other markers, taken in pairs, two each.

The overlap of the values of the main markers with the other markers not being in the mirror as there are situations in which the main marker shows increased values compared to the reference interval without having overlaps with the other markers for some groups of conditions, a fact that leads to differences in the expression of the percentage in what concerns statistical expression (crosstabs).

II.3.2.1. Enzyme marker results

Grouping of conditions by main markers.

In tables no. 155-158 are presented the real correlations of the values above the allowed limit, superimposed with the help of the statistical application of each main marker with the other markers taken into analysis. The increased values of the biological samples were grouped according to the categories of conditions that help to characterize SGA, the percentage being one compared to the total number of samples with values above the allowed limit of the reference interval.

II.3.2.2. Representation of results – Venn diagrams

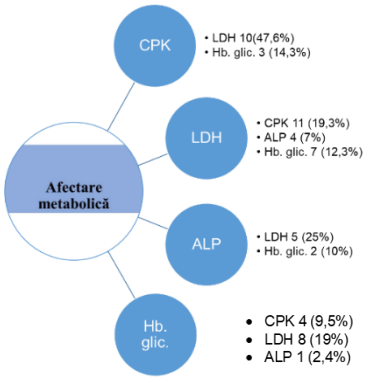


Fig. no. 3 Percentage representation of the incidence of enzymatic markers of GAS in metabolic diseases

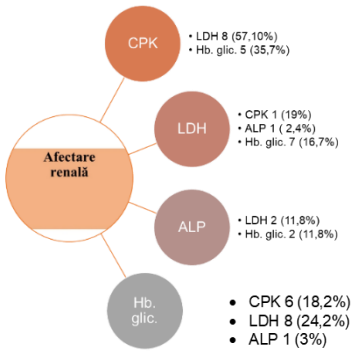


Fig. no. 4 Percentage representation of the incidence of GAS enzyme markers in kidney diseases

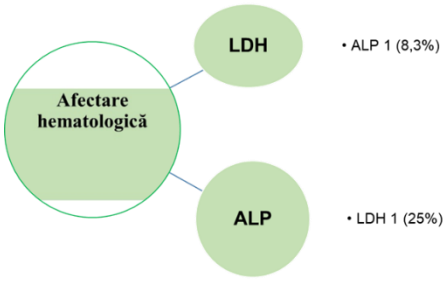


Fig. no. 5 Percentage representation of the incidence of GAS enzyme markers in hematological disorders

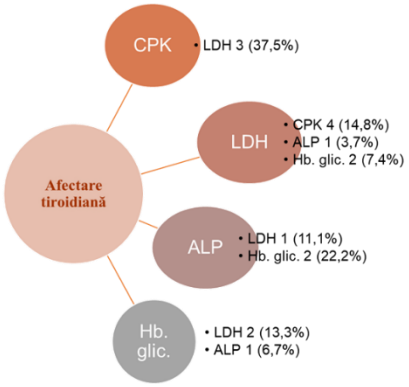


Fig. no. 6 Percentage representation of the incidence of enzymatic markers of GAS in thyroid diseases

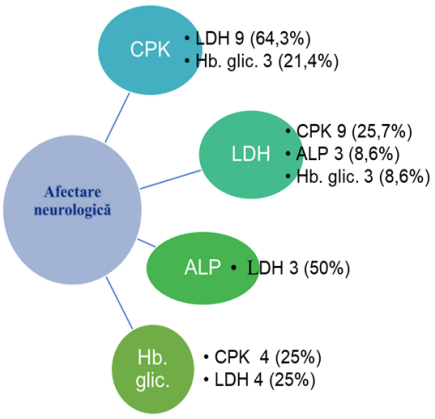


Fig. no. 7 Reprezentarea procentuală a incidenței markerilor enzimatici ai GAS în afecțiunile neurologice

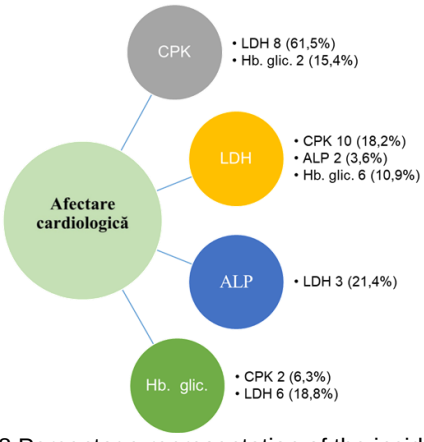


Fig. no. 8 Percentage representation of the incidence of GAS enzyme markers in cardiological conditions

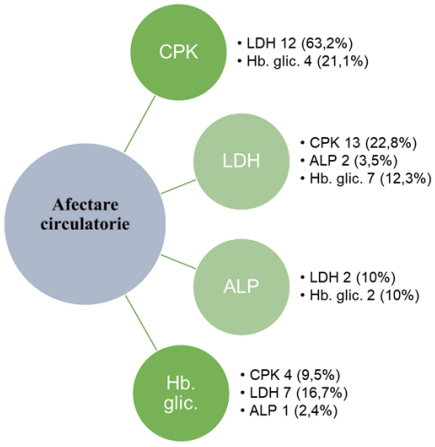


Fig. no. 9 Percentage representation of the incidence of enzymatic markers of GAS in circulatory diseases

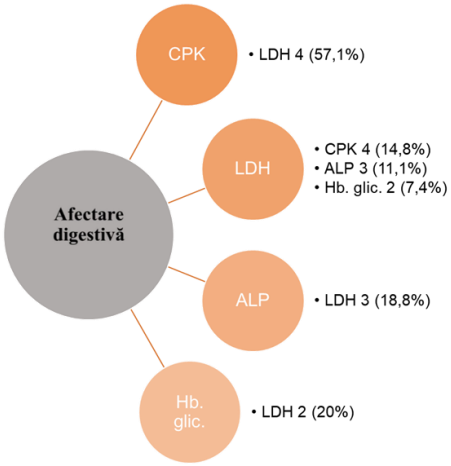


Fig. no. 10 Percentage representation of the incidence of enzymatic markers of GAS in digestive disorders

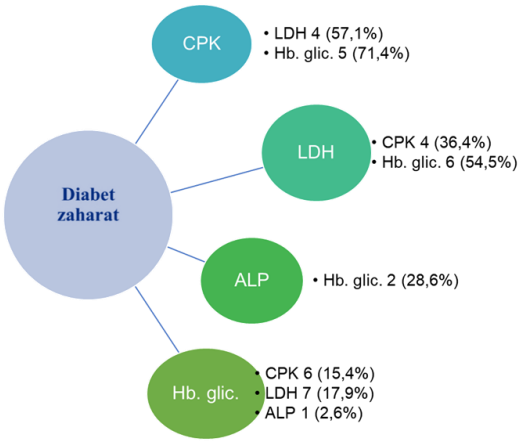


Fig. no. 11 Percentage representation of the incidence of enzymatic markers of GAS in diabetes mellitus

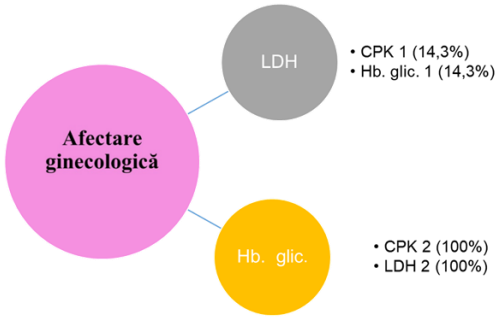


Fig. no. 12 Percentage representation of the incidence of enzymatic markers of GAS in gynecological conditions

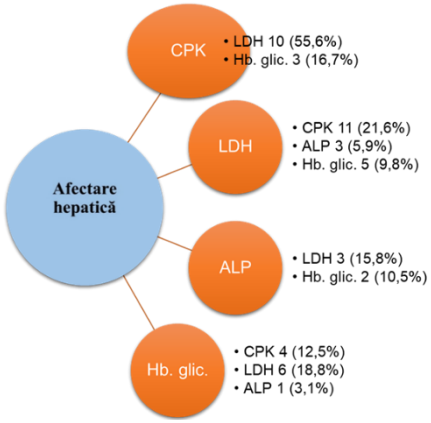


Fig. no. 13 Percentage representation of the incidence of enzymatic markers of GAS in liver diseases

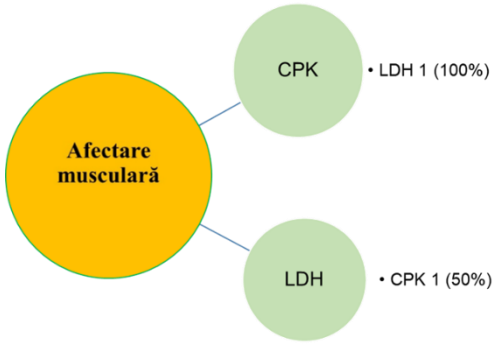
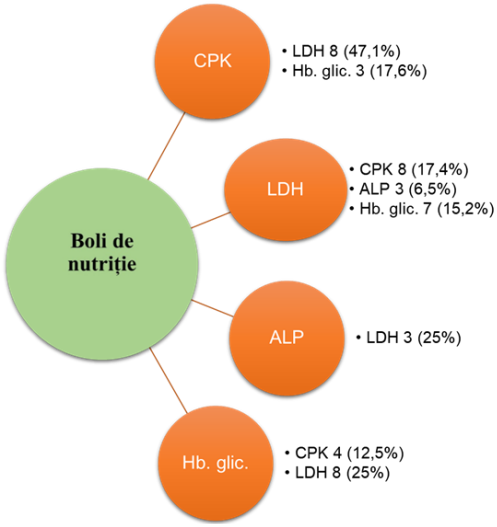


Fig. no. 14 Percentage representation of the incidence of enzymatic markers of GAS in muscle diseases



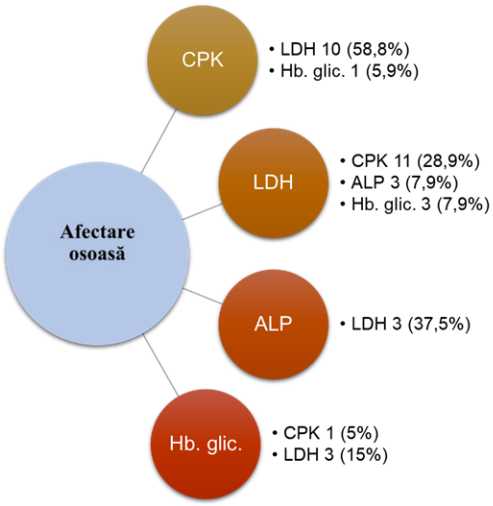
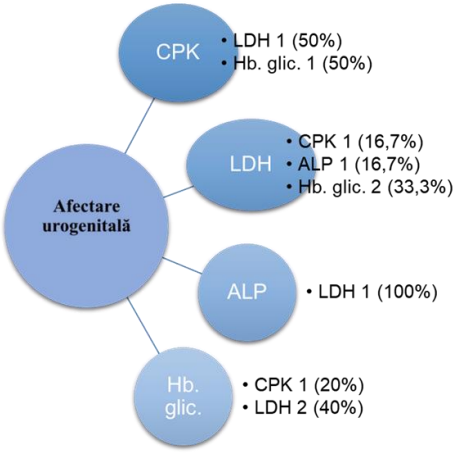


Fig. no. 15 Percentage representation of the incidence of enzymatic markers of GAS in nutritional diseases

Fig. no. 16 Percentage representation of the incidence of enzymatic markers of GAS in bone diseases



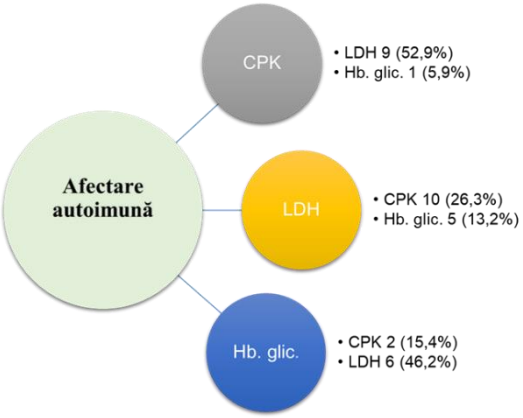


Fig. no. 17 Percentage representation of the incidence of enzymatic markers of GAS in urogenital diseases

Fig. no. 18 Percentage representation of the incidence of enzymatic markers of GAS in autoimmune diseases

II.3.2.3. Statistical conclusions General Adaptation Syndrome of animal organisms

After processing the data with the statistical application SPSS and interpreting them, the following conclusions resulted:

1. Biological markers taken independently cannot certify stress in animal organisms;
2. The diagnostic certainty of the markers taken together, as a nucleus, is given by the superimposition of the sample values of three markers from the initial table regarding the diagnosis of the groups of conditions that characterize GAS (CPK, LDH, Hb. glyc), on its directions (nervous, hormonal, humoral, cellular/tissue/organ or system);
3. The greatest and most consistent weight in stress certification from the point of view of the GAS is the range of values of CPK and LDH and Hb. glyc..; an inconclusive weight is represented by ALP values;
4. The initial table that characterizes GAS includes a number of 17 groups of conditions, which fulfill their certainty and or uncertainty with the help of the 4 biological markers taken into analysis: CPK, LDH, ALP and Hb. glyc.;
5. The exceeded values of the markers subjected to statistical analysis led to the following observations, according to the Pearson statistical correlation coefficient, the statistical relationship was measured between the values above the limits allowed by the reference intervals of the enzyme markers that lead to the description of GSA from the point of view of groups of conditions. The statistical analysis of the values above the limits of the reference intervals given by the analyzed biological samples, enrolls the 4 analyzed markers in the initial table as a definition of stress studied independently, by directly correlating 2 or 3 of them with exceeded

values for the same condition, as the table shows no. 160, the 99% percentile indicates a 1% risk of diagnostic carrying of the markers in this correlation, while the 5% risk is described by a 95% probability of the excess of values above the accepted limits of the reference intervals of the analyzed markers .

Table no. 160 Representation of the degree of certainty of enzyme markers by disease groups in the diagnosis of GAS

Metabolic impairment	Hb. glic. – CPK	99%
	Hb. glic. – LDH	99%
	Hb. glic. –ALP	95%
Kidney damage	CPK – Hb. glic.	95%
	Hb. glic. – CPK	99%
	Hb. glic. – LDH	99%
	Hb. glic. –ALP	95%
Hematological impairment	Hb. glic. – CPK	99%
	Hb. glic. –ALP	95%
Thyroid involvement	Hb. glic. – CPK	99%
	Hb. glic. – LDH	99%
	Hb. glic. –ALP	95%
Neurological damage	Hb. glic. – CPK	99%
	Hb. glic. – LDH	99%
	Hb. glic. –ALP	95%
Cardiological damage	ALP-CPK	95%
	Hb. glic. – CPK	99%
	Hb. glic. – LDH	99%
	Hb. glic. –ALP	95%
Circulatory damage	CPK -LDH	95%
	CPK-ALP	95%
	Hb. glic. – CPK	99%
	Hb. glic. – LDH	99%
	Hb. glic. –ALP	95%
Digestive / Gastrointestinal affection	Hb. glic. – CPK	99%
	Hb. glic. – LDH	99%
	Hb. glic. –ALP	95%
Diabetes	CPK -Hb. glic.	99%
	LDH-Hb. glic.	99%
	ALP-Hb. glic.	99%
	Hb. glic. – CPK	99%
	Hb. glic. – LDH	99%
	Hb. glic. –ALP	95%
Gynecological affection	Hb. glic. – CPK	99%
	Hb. glic. – LDH	95%
	Hb. glic. –ALP	95%
Hepatic impairment	ALP-Hb. glic.	95%
	Hb. glic. – CPK	99%
	Hb. glic. – LDH	99%
	Hb. glic. –ALP	95%
Nutritional diseases	ALP-CPK	95%
	Hb. glic. – CPK	99%
	Hb. glic. – LDH	99%
	Hb. glic. –ALP	95%
Bone damage	LDH-ALP	95%
	Hb. glic. – CPK	99%
	Hb. glic. – LDH	99%
	Hb. glic. –ALP	95%
Urogenital affection	Hb. glic. – CPK	99%
	Hb. glic. – LDH	99%
	Hb. glic. –ALP	95%
Autoimmune disease	LDH-CPK	95%

II.3.3. Final conclusions

1. The investigations focused on the factors that shape glycolysis, because it is consecutive every time a stressor intervenes and the body prepares for fight or flight. Enzymes from the beginning of the glycolytic cycle - phosphorylases, phosphatases, energy-providing enzymes from the fast source of ATP - creatine phosphokinase, myokinase, from the modulatory point of glycolysis - aldolase and from the glycolytic end - lactate dehydrogenase, when pyruvic acid, in instead of entering the cycle of tricarboxylic acids, it passes into lactic acid. Along with these enzymes, two global indicators of high blood sugar were targeted - glycosylated hemoglobin and fructosamine. The following were considered - alkaline phosphatase, creatine phosphokinase, lactate dehydrogenase, glycosylated hemoglobin and fructosamine.

2. Research in the area of human conditions was carried out with the expected determinations in mind. Variations were entered according to a specific condition. They clearly did not provide any consistent individual marker.

3. The investigations done in the human area (on a smaller picture of conditions) led to the same conclusion, that there is no relevance of an individual marker.

4. The statistical analysis confirmed the observations of our research, but provided a special conclusion, that there is a group of markers that can be absolutely significant - creatine phosphokinase, lactate dehydrogenase and glycosylated hemoglobin. Statistical relationships confirm the constant existence of three indicators with diagnostic power.

5. The creatine phosphokinase-lactate dehydrogenase-glycosylated hemoglobin complex can characterize the influence of stress on an animal organism, with the possibility of diagnosis before dealing with abnormal meat unsuitable for industrial processing.

6. It is recommended to introduce the determination of glycosylated hemoglobin in meat animals. This indicator best reflects glycemic variations, during the erythrocyte life span, 100-120 days.

7. The results of our research can also be communicated to researchers in the human medical world, as a gesture of reciprocity and convergence.

8. The creation of a digital application that allows the prediction of a risk or a diagnostic certainty, following the implementation of algorithms that, when meeting two different marker values and with exceeded limits, signal the diagnostic susceptibility to the clinician.

9. Recommendations for animal husbandry and meat processing specialists.

1. The introduction into the research portfolio of the creatine phosphokinase-lactate dehydrogenase-glycosylated hemoglobin triplet to detect early the stress state of animals and prevent the appearance of abnormal meats (DFD-dark, fiirm dry- i.e. a dark, hard and dry meat, PSE pale-soft-exudative, i.e. pale, soft and good dry or hyperacidic meat).

2. Determining the animals susceptible to stress would lead to the avoidance of abnormal meat processing in the industrial network..

3. Replacing the determination of fructosamine with the determination of glycosylated hemoglobin, the latter being a more accurate measure of glycemic variations over time.

4. By using our research triplet, the influence and consequence of stress can be associated more faithfully in the operation and treatment of some diseases.

Elements of originality

1. The dual approach, human-animal, in the General Adaptation Syndrome, considering the similarity of the action towards stress and the activity of the enzymes involved to find the resonance between their actions so that we can quickly and efficiently characterize the intervention of stress in a certain pathology.

2. Selection of enzymatic markers involved in an investigation with multiple human and animal pathologies (alkaline phosphatase, creatine phosphokinase, lactate dehydrogenase, glycosylated hemoglobin or/and fructosamine), markers targeting glycolytic degradation following the general stress reaction.

3. The transition from the interpretation of the results in enzyme investigations, with a potential role as an enzyme marker, carried out according to human and animal pathologies to the in-depth statistical analysis, which expresses the certainty of the observed correlations.

4. Recommendations to breeders and processors in the meat industry to avoid abnormal meats that may end up in processing. These recommendations are concrete consequences of the cumulative evaluation of some enzyme markers (creatine phosphokinase, lactate dehydrogenase and glycosylated hemoglobin).

List of published and presented works

1. Biologist Ph.D Student Turcu Simona Laura, Prof. Ph. D. Petru Alexe, *Research on general adaptation syndrome in animals bodies to different forms of stress*. Scientific Conference Of Doctoral Schools-UDJG 2023, Galați;

2. Biolog drd. Simona Laura Turcu, Prof. univ. dr. Sergiu Meica Prof. univ. dr. Petru Alexe, Şef lucrări dr. Mihai CORNILĂ, Asistent şef Mihai PREDA, *Efectele metabolismului animal la diferitele forme de stress sub aspectul sindromului general de adaptare*. Al XII-lea Congres Național de Medicină Veterinară.Cluj-Napoca 2017;

3. Biologist Ph.D Student Simona Laura Turcu, Assoc. Prof. Ph.D. Marian Nicolae Prof. Ph. D. Petru Alexe, Lecturer Prof. Ph. D. Mihai Cornila, Head assistant Mihai Preda. *Research on general adaptation syndrome in animals bodies to different forms of stress*. SGEM, Section Advances in Biotechnology . ISI Albena, Bulgaria 2017;

4. Simona Laura Turcu, Petru Alexe, Marian Nicolae, Mihai Cornilă, Mihai Preda. *Reacțiile metabolice și afecțiunile organismelor animale la diferitele forme de stress - sindromul general de adaptare* The 13th Annual meeting “Durable Agriulture-Agriculture of the Future”,B+, Craiova, 2017.

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