Biochemical Analysis of Animal Blood Parameters

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Pig Breeds and Their Hybrids

By

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Cambridge
Scholars
Publishing

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This book first published 2025

Cambridge Scholars Publishing

Lady Stephenson Library, Newcastle upon Tyne, NE6 2PA, UK

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

ISBN: 978-1-0364-5759-4
ISBN (Ebook): 978-1-0364-5760-0

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PREFACE

The variety of methods for biochemical analysis of biological fluids of animals are used for a comprehensive assessment of the processes of development, vital activity and physiological-biochemical status (PhBS) of the animal's body. Animal blood is the most important biological fluid for assessing the PhBS of animals, monitoring their feeding, maintenance and treatment. Currently, a lot of data has been accumulated on the biochemistry of the blood of both humans and some animals (mainly dogs and cats). However, there is no comprehensive monograph that systematically describes data on the biochemistry of the blood of both pigs of different breeds and the main commercial hybrids.

The monograph is compiled on the basis of modern data and research methods using rich material accumulated by domestic and foreign scientists, and can be recommended to researchers at academic institutions, graduate and senior students of universities, as well as specialists in the field of biology (primarily biochemistry) of animals, research and development (R&D) personnel of production facilities, firms, farms and companies working in the field of pig farming.

The chapters 1 and 4 of this book were carried out within the framework of state assignment No. 124020200032-4 (FGGN-2024-0016 for the years 2024-2026 by the Ministry of Science and Higher Education of the Russian Federation), the chapters 2 and 3 of this book were supported by a grant from the Russian Science Foundation (project no. 20-16-00032–P for the years 2023-2024) in the Federal Research Center for Animal Husbandry named after Academy Member L.K. Ernst.

INTRODUCTION

"*It is difficult to express generally known things*
in your own way".
Horace, "The Science of Poetry"

In a comprehensive assessment of the processes of development and vital activity of organs, tissues, specialized systems and the animal's body as a whole, the determination of its physiological and biochemical status (PhBS) becomes of great importance. For this purpose, numerous approaches are used, the basis of which is the methods of biochemical analysis of biological fluids (blood, urine, lymph, saliva, etc.) of animals. Here, the key biochemical indicators are the concentrations and ratios of various types of proteins, lipids, low-molecular regulators and other biologically active compounds (BAS) in these biological fluids (primarily in the blood) and other tissues and organs during a certain period of functioning of the body. Animal blood is the most important biological fluid for assessing the PhBS of animals, monitoring their feeding, maintenance and treatment. Blood contains cells and many plasma components that make blood testing very complex. In addition to standard methods, new technologies are being developed, such as PCR analysis of certain markers, dynamic tensiometry of blood plasma (serum), and others. In the body, many physiological and biochemical processes occur through the interaction between BAS with the participation of cell membranes, lipoproteins and other supramolecular blood systems. Examples of such processes are blood coagulation and fibrinolysis, oxygen binding and carbon dioxide release, stages of adsorption and desorption of BAS (in particular, lipids and proteins on the surface of lipoproteins, other particles or blood cells), the functioning of enzyme systems in plasma membranes, in the respiratory chain of mitochondria and others.

Currently, a large number of methods of biochemical analysis have been described and a lot of data has been accumulated on the biochemistry of the blood of both humans and some animals (mainly small domestic animals - dogs and cats). However, there is a lack of generalizing works describing data on the biochemistry of the blood not only of pigs of different breeds, but also of the main commercial hybrids. Taking into account this focus of the work, the authors begin the monograph by

outlining the methodological foundations of biochemical blood analysis, then describe and discuss specific results for various breeds and hybrids of pigs. In conclusion, the correlations obtained for the first time between different groups of data from biochemical analysis of pig blood are presented. On the other hand, the authors considered it necessary to include a description of a number of data on the biochemical analysis of pig blood, especially voluminous tables, as well as some reference data in various sections in all chapters of this monograph.

The chapters 1 and 4 of this book were carried out within the framework of state assignment No. 124020200032-4 (FGGN-2024-0016 for the years 2024-2026 by the Ministry of Science and Higher Education of the Russian Federation), the chapters 2 and 3 of this book were supported by a grant from the Russian Science Foundation (project no. 20-16-00032–P for the years 2023-2024) in the Federal Research Center for Animal Husbandry named after Academy Member L.K. Ernst (Dubrovitsy 60, Podolsk Municipal District, Moscow Region, 142132 Russia).

CHAPTER 1

METHODOLOGICAL BASICS OF ANALYSIS OF PLASMA AND SERUM OF ANIMALS

1.1. Introduction

The use of standard biochemical tests to analyze the blood of animals is of great importance for the prevention and early detection of diseases, timely diagnosis, confirmation of the correctness of treatment or its adjustment [1-5]. Blood biochemistry data can help in developing a plan for therapeutic and other measures [4-9]. The interpretation of the main biochemical indicators, as a rule, is the same for related animal species, but there are always some features in each specific case [1-4, 6, 7, 10]. It is important to emphasize that when interpreting data from biochemical analyzes, well-known "normal" or "standard" values can only be used in a wide range of values, which is noted even in laboratory diagnostics of human blood [11-20]. Of course, each individual laboratory and each animal species has its own values (the so-called "reference" values), which more accurately describe the normal state of the body and allow one to judge deviations from the norm [2-4, 6, 7].

Typically, the standard biochemistry of animal blood includes from 10 to 15 indicators: total protein (TP), albumin (A), globulins (G), urea, creatinine, bilirubin, glucose, triglycerides (TG), cholesterol (CHOL), a number of enzymes (usually aminotransferases, amylase, lipase) a number of cations and anions (primarily potassium, sodium, calcium, phosphorus, chlorine).

Advanced biochemistry includes more than 30 indicators: TP, A, G, albumin/globulin ratio (A/G), total bilirubin (Bil.T), direct bilirubin (Bil.D), urea, creatinine, alpha-amylase, glucose, pyruvate, lactate, lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), de Ritis ratio (AST/ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase or glutamyltransferase (γ-GT or GGT), creatine kinase or creatine phosphokinase (CK or CPK) , lipase, cholinesterase, TGs, CHOL, phospholipids (PL), chylomicrons, high-

density lipoproteins (HDL), low-density lipoproteins (LDL), very low-density lipoproteins (VLDL), potassium, sodium, phosphorus, total calcium, ionized calcium, iron, chlorine, magnesium, acidity (pH), uric acid, "osmolarity" and some others. The description of the analytical procedures for about 30 biochemical indicators (with the basic description of the measurements of each biochemical indicator) will be presented and discussed below.

1.2. Biochemical analysis of protein metabolism indicators in the blood of animals

1.2.1. Total protein

The level of total protein (TP) in blood plasma or serum is determined very often at the first place (even in healthy animals) and, as a rule, is of great importance when interpreted together with hematological parameters [4, 8, 10, 21-24].

Proteins are "nitrogen-containing biopolymers" (or macromolecular "organic compounds"), consisting of alpha-amino acid residues linked together by "peptide bonds" [8]. Proteins are widely present in the body; carry out structural, catalytic, regulatory, protective, transport and other functions [8-10]. Protein metabolism, according to most biochemists [1-11], is one of the dominant metabolic processes in the body, "ensuring the continuity of breakdown and renewal of the entire variety of protein and peptide structures" [8-11]. In the body of many mammal species, "about half of all proteins" are renewed in six months [8-11]. The quantitative and qualitative composition of an animal's diet has an important influence on protein metabolism. Proteins of animal origin usually contain all the essential amino acids (AAs) and are therefore considered as "proteins of full biological value", i.e. with "complete set of 20 AA residues" [8]. Thus, the biological value of "proteins of animal origin is 70–90%, and proteins of plant origin – 60–65%" [8, 10], because the latter, as a rule, do not have a complete set of α-amino acids in the required proportions. The fact is that the animal's body does not have the ability to synthesize some amino acids at a rate sufficient to ensure metabolism (especially in newborns), or does not synthesize them at all, i.e. such amino acids must be supplied in the diet for normal growth and development of the animal. These amino acids are called "essential" or "partially essential" and make up about half of the well-known 20 "proteinogenic" α-amino acids [8].

In the simplest case, for the purposes of general diagnostics of animal physiological and biochemical status (PhBS), all blood serum proteins can

be divided into two fractions: albumins and globulins, although there are dozens of protein fractions, which can be separated at particular conditions [8-10]. The value of measuring total protein for clinical diagnosis is determined by the deviation of protein levels from the "normal values": high level (hyperproteinemia) - due to blood dehydration or increased concentration of specific proteins; low level (hypoproteinemia) - due to the loss of some blood volume, impaired synthesis or excessive catabolism of proteins, "chronic anemia, hemolytic anemia", etc. [8-10, 21-24]. It is important to emphasize that the general condition of the animal and medical history play no less important role than the main biochemical and hematological indicators when making a diagnosis and prescribing specific treatment. Thus, determining TP is essential for correct diagnosis and treatment of animals [9, 24].

For the qualitative and quantitative determination of protein in biological and model liquids, various methods are used, primarily spectrophotometric, colorimetric and spectrofluorimetric methods [1-4, 25]. However, the "biuret method" has long been used to quantify the concentration of total protein in blood serum (especially, using automatic analyzers) [1-4, 25].

Biuret method

The bases of this method are as follows: the protein reacts in an alkaline medium with copper sulfate in a mixed solution with other salts (the "biuret" reagent), forming a violet-blue complex. Sodium and potassium iodides are often included in the mixture as antioxidants. The color intensity of the complex is directly proportional to the concentration of total protein in the test liquid. By measuring the optical density of the resulting complex, it is possible to determine the protein concentration in the sample with high accuracy [1-4, 25].

To calculate a concentration of the total protein ($\mathbf{C_{TP}}$), use the formula:

$$\mathbf{C_{TP}}^{*} = \mathbf{C_{calibrator}}^{**} \times (\mathbf{A_{probe}}^{***} / \mathbf{A_{calibrator}}^{***}),$$

where *(g/L); **$\mathbf{C_{calibrator}}$ – protein concentration in the calibrator solution (i.e. 70 g/L); ***$\mathbf{A_{probe}}$ or $\mathbf{A_{calibrator}}$ – optical density of the probe or calibrator solutions (in optical units); for conversion factors, see the relevant publications [1-3, 8-10].

Measuring range: from the minimum reliable detection level of 2.0 g/L (0.2 g/dL) to the linearity limit of 150 g/L (15.0 g/dL). Lists of some substances, that affect the determination of TP concentration in animal serum, are presented in the works of Young D.S. and others [16-18].

1.2.2. Albumins

Albumins (A) are the most important serum proteins produced in the liver [1, 4, 8-10, 21-24]. Albumins in the presence of bromocresol green in a medium with pH 4.2 [1-3] cause a change in the color of the indicator from yellow-green to green-blue. The color intensity is directly proportional to the concentration of albumins in the serum [1-3].

To calculate a concentration of albumins (C_A), use the formula:

$$C_A^* = C_{calibrator}^{**} \times (A_{probe}^{***} / A_{calibrator}^{***}),$$

where *(g/L); **(50 g/L); ***(optical units).

Measuring range: from detection level of 0.4 g/L (0.04 g/dL) to linearity limit of 60 g/L (6 g/dL).

1.2.3. Total bilirubin

Total bilirubin (Bil.T) is determined by the colorimetric method using dimethyl sulfoxide (DMSO). Bilirubin is a product of hemoglobin decomposition, has water-soluble and water-insoluble fractions, is transported from the spleen to the liver and excreted in bile [1-4, 8]. Bilirubin is converted to colored azobilirubin by reaction with diazotized sulfanilic acid (DSA) and measured photometrically. The color intensity at a wavelength of 540 nm is directly proportional to the concentration of total bilirubin (in optical units) in the sample [1-3].

To calculate total bilirubin ($C_{Bil.T}$), use the formula [1-3]:

$$C_{Bil.T}\ (\mu mol/l) = (A_{probe} - A_{calibrator}) \times 326.61,$$

conversion factor: mg/dL x 17.1 = µmol/L.

1.2.4. Urea

Urea is most often determined using the Berthelot colorimetric method [1-3]. Urea is the major end product of protein nitrogen metabolism; it is synthesized in the liver from ammonia, which, in turn, is formed by deamination of a number of amino acids [8]. Urea is hydrolyzed in the presence of urease to form ammonium and carbon dioxide. Ammonium ions react with salicylate and hypochlorite to form a green chromophore (indophenol). The color intensity is proportional to the concentration of urea in the sample [1-3]:

$$C_{urea} + H_2O\ (\text{with urease}) \rightarrow 2\ NH_3 + CO_2.$$

To calculate urea (C_{urea}) use the formula [1-3]:

$$C_{urea}^* = C_{standard}^{**} \times (A_{probe}^{***} / A_{standard}^{***}),$$

where *(mmol/L); **(mmol/L); ***(optical units). The test is linear at urea concentrations up to 200 mg/dL or 33.33 mmol/L.

1.2.5. Uric acid

Uric acid is determined by the enzymatic method (with uricase) for in vitro diagnostics [1-3]. Uric acid and its salts are the end product of purine metabolism [8]. Uric acid is oxidized by uricase to form allantoin and hydrogen peroxide (H_2O_2), which, under the influence of peroxidase (POD), converts 2,4-dichlorophenol sulfonate (DCPS), 4-aminophenazone (4-AP) (or their analogues such as 4-aminoantipyrine) into final colored product as quinone complex (QC):

Uric acid + O_2 + 2 H_2O (with uricase) → Allantoin + CO_2 + H_2O_2.

2 H_2O_2 + 4-AP + DCPS + (with POD) → QC + 4 H_2O.

The color intensity of the quinone imine complex (with a maximum at a wavelength of 500±5 nm) is directly proportional to the concentration of uric acid in the sample [1-3].

For calculation (when determining uric acid in serum or plasma), use the formula [1-3]:

$$\textbf{Uric\_acid (µmol/L)} = (A_{probe} / A_{standard}) \times C_{standard},$$

where $S_{standart}$ = 357 µmol/L.

Measuring range: from detection level of 1.8 µmol/L to linearity limit of 1487 µmol/L.

1.2.6. Creatinine

Serum creatinine is usually determined by the Jaffe kinetic method (without deproteinization). Creatinine is the result of the breakdown of creatine, a component of muscle, which can be transformed into ATP, which is an energy source for cells [8]. The test is based on the reaction of creatinine with sodium picrate, as described by some authors [1-3]. Creatinine reacts with alkaline picrate to form a red complex. The change in the optical density of the resulting complex is proportional to the concentration of creatinine in the sample [1].

For calculation (when determined in serum or plasma), use the formula [1-3]:

$$\textbf{Creatinine (µmol/L)} = (\Delta A_{test} / \Delta A_{standard}) \times C_{standard},$$

where $C_{standart}$ = 176.8 µmol/L.

Measuring range: from detection level of 8 µmol/L to linearity limit of 1326 µmol/L.

1.3. Analysis of low molecular metabolites of carbohydrate metabolism in the blood of animals

1.3.1. Introduction

Carbohydrates are important components of the body, and their determination (primarily glucose) in the blood of animals provides significant information about the state of carbohydrate metabolism in the body [1-4, 8-10]. Formally, carbohydrate metabolism begins with the hydrolysis of glycogen and starch in the oral cavity of humans and some other mammals (primates, pigs, rats, mice), which is carried out by salivary α-amylase. But the main events occur in the gastrointestinal tract and then in the blood of animals [8-10].

1.3.2. Glucose

Glucose is the main carbohydrate present in the peripheral blood [8-10]. Elevated glucose levels can be associated with many diseases [1-4, 8-10]. Glucose oxidase (GOD) catalyzes the oxidation of glucose to gluconic acid. The hydrogen peroxide (H_2O_2) formed during the reaction reacts in the presence of peroxidase (POD) with phenol and 4-aminophenazone (4-AP) and forms a red product as quinone complex (QC), which can be easily "photo-detected" [1-3, 26-28]:

$$\textbf{Glucose} + O_2 + H_2O + \textbf{(with GOD)} \rightarrow \textbf{gluconic acid} + H_2O_2,$$

$$H_2O_2 + \textbf{phenol} + \textbf{4-AP} + \textbf{(with POD)} \rightarrow \textbf{QC} + H_2O.$$

The intensity of the resulting color (with a maximum at a wavelength of 500±5 nm) is proportional to the concentration of glucose ($\mathbf{C_{glucose}}$) in the sample [1-3]. The formula used for calculation is:

$$\mathbf{C_{glucose}\ (mmol/L) = (A_{probe} / A_{standard}) \times C_{standard}},$$

where $\mathbf{C_{standard}}$ = 5.55 mmol/L. Unit conversion factor: mg/dL × 0.0556 = mmol/L.

Measuring range: from detection level of 0.002 mmol/L to linearity limit of 27.8 mmol/L.

1.3.3. Lactate

Lactate is determined by the enzymatic colorimetric method (with lactate oxidase and peroxidase). Lactate (the organic anion of lactic acid) is a product of cellular metabolism resulting from the anaerobic glycolysis (formerly called "lactic fermentation" or "lactic fermentation") of glucose. Its precursor in glycolysis is pyruvate (see next section). Lactate is

oxidized by lactate oxidase (LO) to pyruvate and hydrogen peroxide (H_2O_2), which, under the influence of peroxidase (POD), 4-aminophenazone (4-AP) and 4-chlorophenol, forms a red quinone complex (QC) in accordance with the following reaction:

Lactate + O_2 + H_2O (with lactate oxidase) → pyruvate + H_2O_2

H_2O_2 + 4-AP + 4-chlorophenol (with POD) → QC + H_2O

The color change of the quinone complex (QC) is directly proportional to the concentration of lactate in the serum.

For calculation of the concentration of lactate ($C_{lactate}$) use the formula:

$$C_{lactate}\ (\mu mol/L) = C_{calibrator} \times (A_{probe} / A_{calibrator}),$$

conversion factor: mg/dL × 0.111 = mmol/L.

Measuring range: from detection level of 0.043 mmol/L to linearity limit of 16.7 mmol/L.

1.3.4. Pyruvate

Determination of pyruvate is carried out according to the modified method of Freedman and Haugen [1-3]. Pyruvate (the organic anion of pyruvic acid) is a product of cellular metabolism formed as a result of the first stage (of 10 reactions) of aerobic glucose glycolysis. Pyruvate, with the addition of 2,4-dinitrophenylhydrosine, is converted into 2,4-dinitrophenylhydrosone pyruvate, which then forms a brown-red compound with alkali, determined by a spectrophotometer.

For calculation use the formula:

$$C_{piruvate} = (A_{probe} / A_{calibrator}) \times C_{calibrator}$$

The conversion factor when converting from mg% to μmol/l is 113.6; Moreover, an alternative calculation can be carried out using a calibration curve, which is described in detail in [1].

The lactate/pyruvate ratio can be used to judge the relative intensity of the processes of anaerobic and aerobic glycolysis. The lactate/pyruvate ratio is of great importance for fundamental biochemistry and assessment of the PhBS of the animal body and can be useful in animal husbandry practice.

1.4. Biochemical analysis of lipid metabolism indicators

1.4.1. Introduction

Lipids are chemically diverse (both in structure and function) organic compounds that have biological activity, are part of organs and tissues, the common property of which is their insolubility in water or

"hydrophobicity" [1-4, 8-10]. Among all lipids, the most important ones are triglycerides (more precisely, triacylglycerols), phospholipids, steroids and others [8-10].

1.4.2. Total lipids

The indicator of total lipids (TL) is often determined in fundamental biochemistry and is of great importance when interpreted together with hematolytic indicators [1-4, 8-10]. To determine total lipids in serum and plasma, the colorimetric method (with phosphovanillin reagent) is often used [1-3]. This process consists of two stages: 1) in the first stage, unsaturated fatty acids (FA) of lipids react with concentrated sulfuric acid; 2) at the second stage, oxidized FAs react with phosphovanillin, forming a pink color, and the intensity of the color is directly proportional to the concentration of total lipids in the sample.

To calculate the concentration of total lipids ($\mathbf{C_{TL}}$) in serum and blood plasma, use the formula [1-3]:

$$\mathbf{C_{TL} = C_{standart} \times (A_{probe} / A_{standard}),}$$

where $\mathbf{C_{standard}}$ = 750 mg/dL.

1.4.3. Triglycerides

An enzymatic colorimetric test is used to quantify triglyceride (TG) concentrations in serum and plasma. Triglycerides are enzymatically hydrolyzed into glycerol and free fatty acids by some lipase [4, 8-10]. The concentration of glycerol is determined by the enzymatic method, using the Trinder reaction with the formation of the final product – quinone complex (QC):

Triglycerides + H_2O + (with lipase) → glycerol + 3 fatty acids,

Glycerol + ATP + (with glycerol kinase) → glycerol-3-phosphate + ADP,

Glycerol-3-phosphate + O_2 + (with GPO) → dihydroxyacetone phosphate + H_2O_2,

H_2O_2 + 4-AP + p-chlorophenol + (with peroxidase) → QC + H_2O.

The intensity of the developing color of quinone imine, measured at 500±5 nm, is directly proportional to the concentration of triglycerides (TG) in the sample.

To calculate the concentration of triglycerides ($\mathbf{C_{TG}}$) use the formula:

$$\mathbf{C_{TG} = C_{standard}\ (2.28\ mmol/L) \times (A_{probe} / A_{calibrator}),}$$

where conversion factor: mg/dL × 0.0113 = mmol/L.

Measuring range: from a detectable level of 0.008 mmol/L to a linearity limit of 11.30 mmol/L.

1.4.4. Phospholipids

The enzymatic colorimetric method is used for the determination of phospholipids (PL) in serum and plasma. Phospholipids are a class of lipids containing a complex of phosphate with choline (to a greater extent) or with ethanolamine and other BAS (to a lesser extent), glycerol and free fatty acids [1-4, 8-10]. Phospholipids (PL) are hydrolyzed by phospholipase D, and the released choline is then oxidized by choline oxidase (COD) to betaine to form hydrogen peroxide. In the presence of peroxidase (POD), hydrogen peroxide binds with 4-aminophenazone (4-AP) and dichlorophenol to form a quinone complex (QC) [29]:

Phospholipids + H_2O + (with phospholipase) → choline + phosphatidic acid,

Choline + O_2 + H_2O + (with choline oxidase) → betaine + H_2O_2,

H_2O_2 + 4-AP + dichlorophenol + (with POD) → QC + H_2O.

The intensity of the resulting color is proportional to the concentration of phospholipids in the sample. Store at 2–8°C.

To calculate the content of phospholipids (C_{PL}), use the formula:

$$C_{PL}\ (mg/dL) = C_{standard}\ (300\ mg/dL) \times (A_{probe} / A_{standard}),$$

conversion factor: mg/dL × 0.0129 = mmol/L.

Measuring range: from a detectable level of 0.033 mmol/L to a linearity limit of 7.74 mmol/L.

1.4.5. Cholesterol

The enzymatic colorimetric method is used to quantify total cholesterol (CHOL) in blood serum [30-35]. Cholesterol is a major representative of the lipids (previously called "lipid-like" substances) based on cyclopentane perhydrophenanthrene, which is found in all animal cells [4, 8-10, 30]. Cholesterol and its esters are isolated from lipoproteins under the influence of detergents. Cholesterol esterase hydrolyzes cholesterol esters, and the resulting cholesterol is oxidized by cholesterol oxidase. During the reaction, the indicator quinone complex (QC) is formed from hydrogen peroxide and 4-aminophenazone (4-AP) in the presence of phenol and peroxidase [30]:

Cholesterol ester + H_2O + (with cholesterol esterase) → cholesterol + fatty acids,

Cholesterol + O_2 + (with cholesterol oxidase) → 4-cholestenone + H_2O_2

H_2O_2 + 4-AP + phenol + (with peroxidase) → QC + H_2O

The intensity of the resulting color (at 500±5 nm) is directly proportional to the cholesterol concentration [30]. To calculate the concentration of cholesterol (C_{CHOL}) in the blood, use the formula [1-3]:

$$C_{CHOL} = C_{standars} \times (A_{probe} / A_{standart}),$$

where $C_{standard}$ = 200 mg/dL or 5.17 mmol/L.

Conversion factor: mg/dL × 0.0258 = mmol/L. Measuring range: from a detectable level of 0.015 mmol/L to a linearity limit of 15.48 mmol/L.

1.5. Enzyme diagnostics (method for determining enzymes)

1.5.1. Alpha-amylase

Alpha-amylase is one of the key enzymes of carbohydrate metabolism, has a highly specific effect on the rupture of α-1,4-glucosidic bonds of polysaccharides [1-3, 8-10]. A kinetic method for determining alpha-amylase activity in blood serum (plasma) for diagnosis is carried out using p-nitrophenyl-D-maltoheptaoside (PNPDMH7) in accordance with the following reactions:

PNPDMH7 (with α-amylase) → PNPDMH2-5 + Maltotetrose,
PNPDMH2-5 (with gluco-amylase) → PNP-glucoside + glucose,
PNP-glucoside (with α-glucosidase) → p-nitrophenol (PNP) + glucose.

It was shown that the rate of change in optical density during the formation of p-nitrophenol (PNP) in the system (measured at 405 nm) is directly proportional to the alpha-amylase activity in the sample.

To calculate α-amylase activity (C_{AA}) in international units (IU/L), the obtained values (ΔA/min.) are multiplied by the following coefficients:

$$C_{AA}\ (IU/L) = \Delta A/min. \times 2690\ (at\ 25\text{-}30°C);$$
$$C_{AA}\ (IU/L) = \Delta A/min. \times 5125\ (at\ 37°C).$$

Factor for converting international units (IU/L) to SI units (cat/L):
1 IU/L = 16.67 nkat/L or 1 μkat/L = 60 IU/L.

1.5.2. Alanine aminotransferase

Alanine aminotransferase (ALT) is determined in vitro by the UV kinetic method (ultraviolet measurements). ALT is a cellular enzyme present in high concentrations in the liver and kidneys [36]. ALT catalyzes the reversible transfer of the amino group from alanine to α-ketoglutarate with the formation of glutamate and pyruvate [1-3, 8]. The resulting

pyruvate is reduced into lactate by lactate dehydrogenase (LDH) with $NADH+H^+$ [1-3, 8]:

L-alanine + α-ketoglutarate (with ALT) → L-glutamate + pyruvate,

Pyruvate + NADH + H^+ (with LDH) → lactate + NAD^+

The rate of decrease in $NADH(+H^+)$ concentration, measured photometrically at 340 nm, is directly proportional to the catalytic activity (concentration) of ALT present in the sample.

To calculate ALT activity in samples, use the formula [1-3]:

ALT (IU/L) = ΔA/min. × (1750),

with the following conversion factors (factors). Units: 1 international unit (IU/L) is the amount of enzyme that transforms 1 μmol of substrate per minute under standard conditions.

1.5.3. Aspartate aminotransferase

Aspartate aminotransferase (AST) is also determined by the UV-kinetic method. AST is a cellular enzyme present in high concentrations in cardiac muscle, liver cells, skeletal muscle and in smaller quantities in other tissues [1-3, 8-10, 37]. Accordingly, by increasing AST activity in the blood, one can judge the degree of cell destruction. It is important to emphasize that the amino acid sequence of aspartate aminotransferase was the first in the world to be established in joint work by two groups of Soviet scientists under the leadership of academicians A.E. Braunstein. and Yu.A. Ovchinnikov [8]. AST catalyzes the reversible transfer of an amino group from aspartate to α-ketoglutarate to form glutamate and oxaloacetate. The resulting oxaloacetate is reduced to malate under the action of malate dehydrogenase (MDH) and $NADH(+H^+)$ [1-3, 8]:

Aspartate + α-ketoglutarate (with AST) → L-glutamate + oxaloacetate

Oxaloacetate + NADH + H^+ (with MDH) → malate + NAD^+

The rate of decrease in $NADH(+H^+)$ concentration, measured photometrically at 340 nm, is directly proportional to the catalytic activity (concentration) of AST present in the sample.

To calculate AST activity in samples, use the formula [1-3]:

AST (IU/L) = ΔA/min × (1750),

with the following conversion factors (factors). Units: 1 international unit (IU/L) is the amount of enzyme that transforms 1 μmol of substrate per minute under standard conditions.

1.5.4. Creatine kinase

Creatine kinase or creatine phosphokinase (CK or CPK) is determined by the UV-kinetic method. CPK is a cellular enzyme widely present in the cells of all organs and tissues [1-3, 8-10]. Its physiological role is to create adenosine triphosphate (ATP) for the contractile and transport systems [8, 38]. CPK catalyzes the reversible transfer of a phosphate group from creatine phosphate (phosphocreatine) to ADP. This reaction is associated with subsequent ones, in which ATP and glucose are converted to ADP and glucose-6-phosphate (G-6-P) under the action of hexokinase, and glucose-6-phosphate dehydrogenase (G-6-P-DG) oxidizes G-6-P and restores NADP+ [38]:

Creatine phosphate + ADP (with CPK) → creatine + ATP,
ATP + Glucose (with hexokinase) → glucose-6-phosphate + ADP,
Glucose-6-phosphate + NADP+ (with G-6-P-DG) →
6-Phosphogluconate + NADPH + H^+

The rate of NADPH(+ H^+) formation, measured photometrically at 340 nm, is directly proportional to the catalytic activity of CPK present in the sample. To calculate creatine phosphokinase activity in a sample, use the formula:

CPK (IU/L) = ΔA/min. × factor (F)

by multiplying ΔA/min. to the next factor (F): 4127 (25/30°C) or 8095 (37°C). The evaluation is carried out in international units (IU/L), which corresponds to the amount of enzyme that transforms 1 μmol of substrate per minute under standard conditions. Factors for converting international units (IU/L) to SI units (cat/L) are as follows: 1 IU/L = 16.67×10^{-9} cat/L or 16.67×10^{-3} μkat/L; 1 μkat/L = 60 IU/L.

Measuring range: from detectable level of 1 IU/L to limit frostiness 1000 IU/L (manual method) or 1800 IU/L (automatic method). If the results obtained exceed the linearity limit, the samples should be diluted 1:10 with saline solution (9.0 g/L NaCl) and the test repeated, and the result obtained multiplied by 10. No interference was observed with glucose up to 7 g/L, hemoglobin up to 5 g/L and triglycerides up to 7 mmol/L.

1.5.5. Lactate dehydrogenase

Lactate dehydrogenase (LDH) is determined by the UV-kinetic method ("UV-test") with a liquid reagent. LDH is an enzyme widely present in the cells of all organs and tissues [1-3, 8, 38]. LDH catalyzes the reversible reaction of converting pyruvate into lactate [1-3, 39]:

Pyruvate + NADH + H^+ (with LDH) ↔ Lactate + NAD^+

The rate of decrease in NADH($+H^+$) concentration, measured photometrically at 340 nm, is proportional to the catalytic activity of LDH present in the sample.

To calculate LDH activity in a sample, use the formula:

LDH (IU/L) = ΔA/min. × factor (F)

by multiplying ΔA/min. to the following factor (F): 4925 (25/30°C) or 9690 (37°C).

Measuring range: from a detectable level of 5.5 IU/L to a linearity limit of 1453 IU/L.

1.5.6. Alkaline phosphatase

Alkaline phosphatase (ALP) is determined by the kinetic method. ALP is an enzyme widely present in all tissues and organs, especially in bones, liver, placenta, intestines and kidneys [1-3, 8-10]. ALP catalyzes the hydrolysis of p-nitrophenyl phosphate at pH 10.4 to form p-nitrophenol and phosphate according to the following reaction equation [40]:

p-nitrophenyl phosphate + H_2O + (with ALP) → p-nitrophenol + phosphate

The rate of p-nitrophenol formation, measured photometrically at 405 nm, is directly proportional to the catalytic activity (concentration) of alkaline phosphatase present in the sample.

To calculate alkaline phosphatase (ALP) activity in a sample, use the formula:

ALP (IU/L) = ΔA/min. × factor (F)

by multiplying ΔA/min. to the following factor (F): 3300. Units: 1 international unit (IU/L) is the amount of enzyme that transforms 1 μmol of substrate per minute under standard conditions.

Measuring range: from a detectable level of 4.26 IU/L to a linearity limit of 825 IU/L.

1.5.7. Acid phosphatase

Acid phosphatase (AP) is determined by the kinetic method. AP is an enzyme widely present in all tissues and organs, especially in the prostate, stomach, liver, muscles, spleen, erythrocytes and platelets [1-3, 8-10]. AP activity in serum is determined by the modified Hillman reaction [41]:

α-naphthylphosphate + H_2O + (with AP) → α-naphthol + phosphate,
α-naphthol + FRTR–salt → colored product,

where FRTR salt (Fast Red TR Salt) is, as a rule, 4-chloro-2-methylphenyl diazonium chloride.

Tartrate is used as a specific agent for the prostatic acid phosphatase fraction. The increase in light absorption at a wavelength of 405 nm is proportional to the activity of total acid phosphatase in the sample.

To calculate the activity of acid phosphatase (total AP) in a sample, use the formula:

$$\textbf{Total AP (U/l)} = 750 \times \Delta A/\text{min}.$$

Specified fraction of AP (IU/L) = 750 × (ΔA/min of total AP – ΔA/min of fraction not inhibited by tartrate). The method is linear up to 150 IU/L.

1.6. Biochemical analysis of mineral metabolism indicators in the blood of animals

1.6.1. Calcium

Calcium (Ca) is determined by a colorimetric test. Calcium (Ca) is the most abundant and one of the most important microelements ("minerals") in the body of animals and humans [1-3, 8-10]. Calcium ions react with the o-cresolphthalein complex in an alkaline medium and form red complexes [1-3, 42]:

Ca^{2+} + o-cresolphthalein (in alkaline medium) → Colored complex

The intensity of the resulting color of the complex is directly proportional to the calcium concentration in the sample.

To calculate the concentration of calcium (C_{Ca}) in a sample, use the formula:

$$C_{Ca}\ (\text{mmol/L}) = C_{standard}\ (2.5) \times (A_{probe}/A_{standard}),$$

where conversion factor: mg/dL × 0.25 = mmol/L.

Measurement range: detectable level – 0.0425 mmol/L, linearity limit – 3.75 mmol/L.

1.6.2. Magnesium

Magnesium (Mg) in serum is quantified using a colorimetric method [1-3, 43]. Magnesium is the second most common intracellular cation in the body of animals and humans (after potassium); it is involved in a large number of enzymatic (metabolic) processes [1-3, 8-10]. It is a cofactor for all enzymatic reactions involving ATP and proteins that provide electrical excitability of cells, etc. Magnesium forms a colored red complex with

calmagite in an alkaline environment. The color intensity (at 520 nm) is directly proportional to the magnesium concentration in the sample.

To calculate the concentration of magnesium (C_{Mg}) in a sample, use the formula:

$$C_{Mg}\ (mmol/L) = C_{standard}\ (0.824) \times (A_{probe} / A_{standard}),$$

conversion factor: mg/dL × 0.412 = mmol/L or 0.5 mmol/L = 1.0 mEq/L = 1.22 mg/dL = 12.2 mg/L. Measurement range: detectable level – 0.0824 mmol/L, linearity limit – 2.06 mmol/L.

1.6.3. Chlorides

Chloride (Cl^-) in serum is quantified using a colorimetric method. Chloride ions form a soluble non-ionized complex with mercury ions and displace thiocyanate ions (SCN^-) $HgCl_2$ [44]. The released thiocyanate ions react with iron ions, forming a colored complex (absorbance at 480 nm):

$$Hg(SCN)_2 + 2\ Cl^- \rightarrow HgCl_2 + 2\ SCN^-,$$
$$3\ SCN^- + Fe^{3+} \rightarrow 4\ Fe(SCN)_3\ \text{red complex.}$$

To calculate the concentration of chlorides in a sample, use the formula:

$$C_{Cl}\ (mmol/L) = C_{standard}\ (125) \times (A_{probe}/A_{standard})$$

Conversion factor: mmol/L = mEq/L; mmol/L × 3.55 = mg/dL; mg/dL × 0.282 = mmol/L. Measurement range: detectable level – 1.13 mmol/L, linearity limit – 130 mmol/L (mEq/L).

1.7. Conclusions

In general, the biochemical profile of serum or plasma is a valuable diagnostic tool that can be used to evaluate body PhBS [1-3, 6-9, 11-15]. When used in combination with anamnesis, physical and chemical examinations and other laboratory tests (for example, complete blood cell counts, urinalysis and others), blood biochemistry allows you to assess the body's PhBS; may be useful for formulating a list of problems or exceptions, confirming a diagnosis, determining a treatment prognosis; to plan therapeutic options and monitor response to veterinary treatment. drugs [45-47].

When conducting biochemical testing, it is strongly recommended that a quality control program be used to minimize “laboratory” errors (or “analytical stage” errors) and ensure that the results of blood sample analysis are reliable. A quality control program can help identify problems with the instrument, test method, reagents, and the technician performing

the test. A typical quality control program involves the use of at least two controls on a daily basis. Controls are solutions that are available in low, normal, and high concentrations for the analyte of interest and can be purchased commercially, often from the instrument manufacturer. Planned maintenance of the device should include cleaning and replacement of components (worn out or exhausted); periodic calibration of the device; and written documentation of daily inspection results, maintenance records, problems encountered and repair calls is also part of a good quality control program, as are a number of other activities that are difficult to describe briefly. One of the most important concepts in laboratory (clinical) biochemistry is the concept of “reference interval”. This interval represents the values that would be expected from the blood parameters of a healthy animal and are necessary for interpreting the test results. The authors [45-47] do not recommend the use of terms such as “normal range” or “normal values” for several reasons: 1) a value may fall within the specified reference interval (i.e., appear “normal”), but still may not correspond to the patient’s actual condition; 2) there is difficulty in defining what is truly “normal”; 3) the use of the term “range” is also not approved by some foreign clinical biochemists (since the statistical definition of “range” is the difference between the smallest number and the largest number, which is often not suitable for clinical parameter interpretation [45-47]).

Ideally, the "reference interval" should be based on a large number of samples obtained from healthy animals (reference population), and theoretically calculated to cover 95% of the healthy population. It is generally recommended to use at least 60 clinically healthy animals to establish a “reference interval” [45–47]. The reference population should be selected based on predefined clinical criteria, such as species, breed, age, sex, stage of pregnancy or lactation, and should be representative of the animal population. In reality, to be truly reliable, the “reference interval” must be established using instruments and methodology that are used in samples from the relevant population. “Balance values,” although useful for many hematological parameters, are of limited use for interpreting the biochemical properties of serum [45–47]. For example, many published reference intervals (that are established for pigs and other farm animals) often do not distinguish between sex, age, feeding, housing conditions, and direction of animal productivity. However, if “reference intervals” are not available, then there is no other choice but to use previously published results with their clarification and the formation of new databases. In addition, the previous obtained values obtained for the same animals before disease are particularly useful in subsequent response

to treatment or monitoring of the disease process. On the other hand, some selected methods, mentioned above, are used for biochemical analysis of the pig blood and meat, especially for monitoring of all pork production cycle (Figure 1).

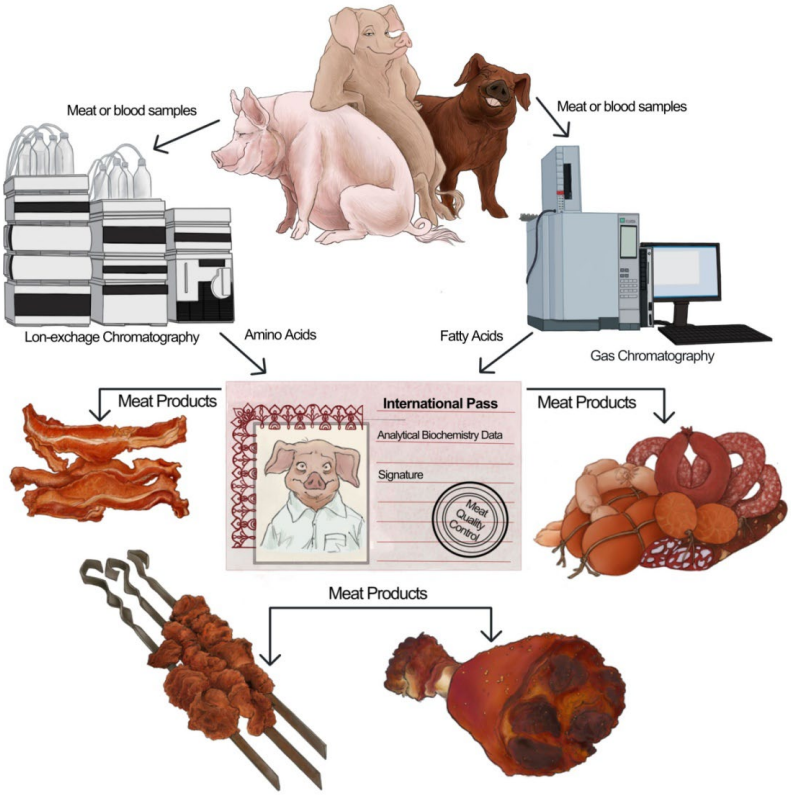

Figure 1. General scheme of the selected methods of biochemical analysis of the pig blood and meat (adapted from the author's preprints, doi:10.20944/preprints202012.0220.v1).

In most cases, it is necessary to determine the overall biochemical profile, but not individual indicators, especially when the animal is examined for the first time. It is believed that the "reference interval" is intended to include 95% of healthy animals; whereas the remaining 5% may have values outside the range for any individual test. When

performing a group of tests, the probability that at least one test will be outside the reference interval is much greater than 5%; Therefore, caution is advised when interpreting a single value that is even slightly outside the reference interval. Finally, if a laboratory value does not appear reasonable in the context of the overall biochemical profile of the animals, then it should be treated as incorrect and requiring another round of measurements.

Despite some overlap with fundamental analytical data, a practical approach to assessing the blood biochemistry profile is to group the analyzed indicators by body system and accordingly, i.e. systematically, to interpret the results obtained.

If the above recommendations are followed, the values of the obtained biochemical blood parameters not only form the basis of evidence-based animal medicine, but complement the existing “reference interval” or can even become the basis of a new “reference interval” for a certain animal species. The “totality” of the obtained biochemical blood parameters, compiled into a specific database for a certain type of animal, is of particular value in animal husbandry.

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