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THE HYPOTHESIS OF A SPECIFIC RELATIONSHIP BETWEEN PEROXISOMAL, MITOCHONDRIAL, AND CYTOPLASMIC PROCESSES IN METABOLIC REGULATION OF HIGHLY PRODUCTIVE RUMINANTS

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Abstract

The authors believe that the explanation of the accumulated data on metabolic processes in highly productive ruminant animals, which for the time being remains within the framework of the existing physiological and biochemical paradigm, requires an in-depth interpretation on a fundamentally new experimental and conceptual basis, which assumes an analysis of the complex interconnections of the set of objects and their functions that were previously not considered. First, , it is necessary to consider the biochemistry of intracellular compartmentalization from a different point of view based on the strict mutual complementarity of the mitochondrial Krebs cycle and the cytoplasmic glycolysis and gluconeogenesis with a peroxisomal glyoxylate cycle. The possibility of glyoxylate cycle functioning in highly productive ruminants was postulated by the authors for the first time following from experimental data on catalytic activity of isocitrate lyase (EC 4.1.3.1) and malate synthase (EC 4.1.3.2) (V.P. Galochkina et al., 2012). The presence of these enzymes allows the synthesis of glucose from acetic acid, which comes in large quantities from the contents of the rumen. Ruminants are physiologically hypoglycemic. Phylogenetically, they mainly eat coarse vegetable food which increases the proportion of acetate in the rumen content. Easily hydrolyzed carbohydrates in the rumen content reduce the percentage of acetate and increase the proportion of propionate and butyrate, which results in a decreased pH (M. Oba et al., 2015). Permanent glucose deficiency causes an increase in the somatotropin to insulin level indicating an increase in the metabolically ineffective gluconeogenesis. Simultaneously, the blood concentration of unesterified fatty acidы increases, indicating an increase in lipolysis in fat depots. There is a low ratio of insulin to glucagon with an increase in urea concentration. Milk fat content reduces (F. Piccioli-Cappelli et al., 2014). Peroxysomes are partially capable of beta-oxidation of fatty acids to C 13, which facilitates Krebs cycle and allows changes in its metabolic orientation. The authors consider the glyoxylate cycle as a chance which enables the animal to improve metabolism and intensify productivity. Bicarbon acid oxidation is energetically more effective compared to tricarboxylic acid cycle, since the glyoxylate cycle is a shortened of tricarboxylic acid cycle capable of functioning without limiting isocitrate dehydrogenase and alpha-ketoglutarate dehydrogenase reactions (V.P. Galochkina et al., 2011). Secondly, one must considered hypothetical provisions on the leading regulatory role of multifactorial interrelationships between mono- and multimolecular constellations of mono- and polymeric biologically active substances, hormones and enzymes, both temporarily formed and constant. This extensive group of specific agents includes insulin, peroxisomal cysteamine, glyoxylic acid, oxygen, hydroperoxide and D-amino acid oxidases. The theoretical positions stated in the article have passed primary validation in model experiments on intensively fattened bulls with the use of clenbuterol, the agonist of beta-adrenergic receptors.

Keywords: regulation of metabolism, peroxisomes, glyoxylate cycle, D-amino acid oxidase, glyoxylate, cysteamine, insulin, hydroperoxide, oxygen

A large number of organic acids formed in a fore stomach of highly productive ruminant animals and serving as the main source of metabolic energy have a multiple positive effect on the Krebs cycle and on specific features of the pro-

duction and metabolic processes in vivo. Glyoxylate cycle in majority of the living beings plays a role of the main assistant in the main metabolic cycle. Due to the expressed biochemical features of metabolism in highly productive ruminant animals, glyoxylate cycle evolutionally plays a role of the auxiliary link to ensure the improved metabolism and, accordingly, to increase the productivity in animals. It must be understood that organism of highly productive ruminant animals operates in a specific mode of chronic metabolic stresses and requires suitable meeting of the specific metabolic needs for realization of the high productivity potential. It results in a continuing need for the reasoned biologization of all technical feeding and growing aspects of highly productive animals.

Highly proactive animals not only produce more products of better quality, but also consume less nutritional substances for production thereof. Their metabolism is characterized by different speed and different metabolic trend. Highly productive milking cow shall have productive longevity while maintaining its reproductive function. A cow yielding 30 kg of milk daily eliminates at average of 1200 g of fat, 1000 g of protein, and 1400 g. of lactose. Herewith, no more than 10% of the glucose formed in the gastrointestinal tract due to the hydrolysis of feed carbohydrates is used in the metabolic process. The remaining glucose required for synthesis of the milk components and to address all metabolic needs of the organism are synthesized de novo [1, 2].

Recently, many farming units in Russia get an average annual milk yield per stock of 10000 kg per cow and more. Ruminant animals are phylogenetically adapted to consumption of a coarse vegetable food rich in fiber, which is hydrolyzed in the rumen content predominantly formed with acetate. In practice, high-concentrate hydrolyzing rations, hydrolysis products of which change the evolutionally developed fermentation processes are applied. They decrease proportion of acetate and propionate in rumen content and results in decrease of pH and systemic disturbance of acid-base balance [3], acidulation of tissues and decrease of fat biosynthesis in the breast gland. Consequently, syndrome of a low content of fat in milk is an issue of a particular concern in the world, and pertains to so-called nutritional disorders in highly productive cows.

In our view, the existing visions of metabolic processes in highly productive cows do not yield the necessary level of understanding of how to achieve high productivity while maintaining the fat content in milk. A revolutionary new physiologic and biochemical basis for rethinking it shall involve knowledge on glyoxylate cycle in peroxisomal reactions in combination with oxidation of D-amino acids, glycolysis, gluconeogenesis, liposysis and bioenergetic processes in cytoplasm and mitochondria [4, 5]. Before proceeding with presentation of the subject matter of the proposed conceptual approach, we are going to consider the role of some critical sub-cellular organelle, metabolic cycles, and molecular compounds participating in the regulatory processes.

Peroxisome. Being the oldest intracellular subunit and the last one discovered in recent years, it is considered to be the key organelle of intracellular, intercellular, and inter-organ communication, cooperation, and regulation of the biochemical processes [6]. Peroxisome got its name because it always contains enzymes by use of the molecular oxygen for detachment of hydrogen atoms from the organic substrates in the oxidation reactions with formation of hydrogen peroxide [7]. Along with mitochondria, peroxisome serves as the main oxygen utilization center in a cell [8]. Peroxisome, having metabolic systems of formation and decomposition of hydrogen peroxide, generation and quenching of superoxide radicals, may influence on many processes in a cell. Quantity of intracellular hydrogen peroxide defines the intensity of morphogenetic and biochemical processes, and peroxisome serves as the regulator of oxidation-reduction peroxide-

dependent reactions controlling both the speed of biosynthesis and biodegradation [9]. As distinguished from the processes in mitochondria, hydrogen peroxide, rather than high-energy compounds, is formed due to the peroxisomal oxidation. Peroxisome just like the mitochondria performs the biological oxidation function, save that the oxidation process in peroxisome is not associated with generation of the nicotinamide adenine dinucleotide, reduced form (NADN) and adenosine triphosphate (ATP) [10]. If mitochondrial Krebs cycle is publicly recognized as the main metabolic and energetic "tank" utilizing the end products of all principal metabolic flows in vivo and simultaneously performing a function of a regulatory center of such processes, then peroxisomes with their glyoxylate cycle shall be apparently seen as antioxidant "reactors" and "dispatching nods" in vivo. It should be noted than both pro- and antioxidant reactions simultaneously proceed in both most important cell compartments – mitochondria and peroxisome.

Noble Prize Winner C. De Duve was the first to suggest that it was the peroxisomal metabolism that played an important role in development of new biochemical transformation ways. For that end, peroxisome required enzymatic machinery for performance of the metabolic reactions (in addition to electron carrier system) ensuring cooperation with mitochondria [11]. Interoperation between peroxisome and mitochondria is due to the organization of metabolite flows comprising an integral regulated structure specific for this or other tissue, organ, and organism in general [12].

D-amino acid oxidase and production of reactive oxygen forms. Peroxisome is the only sub-cellular organelle where D-amino acid oxydase (DAAO) consume oxygen in the catalytic process of right-handed amino acid hydrolysis and produce hydroperoxide [13]. Oxygen is indispensable for all aerobic organisms and plays the paramount role in generation of high-energy compounds at oxidative phosphorylation. Reactive oxygen forms (ROF), including superoxide anion and H_2O_2, are formed in these reactions, which, inter alia, are required for signal transduction in metabolic pathways, regulating the cell growth and oxidation-reduction status [14]. The oxygen role in vivo is dualistic. ROFs, being natural and absolutely essential metabolites, are involved in a number of natural physiological processes, including killing of microbes and viruses. However, during stress periods of any etiology ROF concentration may drastically grow, thus, causing many pathological states in structures and functions of a cell [15].

Low production of H_2O_2 in astrocyte peroxisome protects neurons from the oxidation stress, whilst high concentration of H_2O_2 is neurotoxic [16, 17]. Inhibition of astrocyte enzyme of D-amino acid oxydase protects neurons from the oxidation loss allowing using the H_2O_2-based neuroprotection machinery [18]. These discoveries show implication of D-amino acid oxydase in control of intracellular concentration of hydrogen peroxide across time and space, and represent opportunity for decoding of the most interesting and novel astrocyte-dependent neuroprotection mechanism. In pursuance of the above-listed factors, it was assumed that for combat with oxidation stress all neurons are linked together in a metabolic complex with astrocytes. The same assumption was made regarding the substrates of peroxisomal oxydase, which could represent an unstable and, rather, temporary structures — unstable non-enzymatic complexes of glyoxylic acid with various nucleophilic agents, including D-amino acids [19]. Hydrogen peroxide plays the key role in a cell signal system, and is deemed to be the predominant candidate to adaptive reaction mediators in astrocytes. Thus, it implies the importance of hydrogen peroxide in maintenance of astrocyte-dependent neuron protection from the oxidation stress and the role of H_2O_2 in in-

duction of astrocyte activation of specific neurotropic transcriptional nuclear factor (Nrf-2). Such results presuppose the availability of specific regulating mechanisms for neuronal cells with involvement of the hydrogen peroxide [20]. The organism constantly requires regulating and neutralization of ROFs with participation of a number of special and indirectly involved enzymes, including DAAO, superoxide dismutase, xanthine oxidase, glutathione peroxidase, hemoxygenase, and etc. [21].

Hypothesis on interoperation of glyoxylate cycle, D-amino acid oxidase and insulin in regulation of the cell metabolism. *Metabolic function of cysteamine.* For a long time, cysteamine was regarded as a traditional antioxidant and standard radio protector. Cysteamine is a thiol-containing product of cysteine decarboxylation with participation of panthotenic acid serving as the primary component of coenzyme A synthesis. In case of deficit of the panthotenic acid in the organism, insulin treatment results in sharp reduction of coenzyme A synthesis [22]. Insulin decreases the degradation speed of coenzyme A and cysteamine synthesis. It is quiet natural since coenzyme A is required for insulin activation of acetate acylation and residues of other fat acids upon synthesis of the fat acids with longer carbon chain [23]. Cysteamine is regarded as an intracellular negative messenger of insulin. To this end, insulin and cysteamine have diametrically opposite effects on a number of metabolic processes. For instance, insulin promotes activity of pyruvate dehydrogenase (PDH), glycogen synthase, hexokinase, phosphorylase, and phosphatase. Cysteamine have an inhibition effect on these enzymes [24]. Insulin inhibits fructose-1,6-bisphosphatase, while cysteamine activates thereof. Decrease of cysteamine concentration has positive effect on the processes flowing under the action of insulin. Krebs cycle, glycolysis, lipogenesis, and synthetic processes are activated [25].

Spontaneously formed nucleophilic cysteamine-glyoxylate complex is a good substrate for D-amino acid oxidase. This complex is formed in the physiological conditions in presence of oxygen [23]. Reaction of oxydase with cysteamine-glyoxylate complex flows significantly faster than with complexes of glyoxylate with acetaldehyde-ammonia, putrescine, aminopropanol, octapamine, ethylendiamine, and ethyl ether of cysteine. Normal physiological amines, including histamine, serotonin, adrenalin, noradrenalin, spermine, spermidine, and cadaverine do not practically react with glyoxylate by D-amino acid oxidase [26, 27]. It is assumed that product of glyoxylate-cysteamine reaction may serve as a metabolic effector which, following formation of a covalent link, may intensify reactivity of enzymes and may modify nucleic acids [28].

Role of glyoxylate acid. Glyoxylate is a quiet toxic intracellular compound for the animals [29]. It controls many reactions: it inhibits mitochondrial carriage of phosphorus, carriage of electrons through cytochrome chain and transfer of mitochondrial substrates, and suppresses activity of enzymes of Krebs cycle. Glyoxylate, having inhibited phosphatase of pyruvate dehydrogenase complex (PDC) (its regulatory subunit), decreases the flow of pyroracemic acid through the pyruvate dehydrogenase complex. It is crucial for the organism to neutralize such highly-reactive hazardous two-carbon intermediate product of peroxisomal reactions. As it was already noted before, the best substrates of peroxisomal oxidase are unstable non-enzymatic glyoxylate complexes with different nucleophilic agents, including the D-amino acids [19, 20].

Inhibitors of D-amino acid oxidase. D-amino acid oxidase is found in peroxisome only. It plays the specific role in the central nervous system due to involvement in maintenance of the cognitive functions, which we associate with animal aggressiveness, temper, and adaptiveness to feeding and keeping conditions. Many studies in activation and inhibition of D-amino acid oxidase are devoted to decoding of the interoperation mechanisms of such enzyme category with

specific receptors, particularly, in the brain tissues [27, 30, 31].

Strong non-specific oxydase inhibitors, including D-amino acid oxidase, involve adenosine diphosphate (ADP), adenosine diphosphate ribose (ADP-ribose), nicotineamide adenine dinucleotide, reduced form (NAADN), nicotineamide adenine dinucleotide phosphate, reduced form (NAADP), diphosphocoenzyme A (intermediary product of CoA synthesis, the most effective inhibitor of such type of inhibitors), which at physiological concentration inhibit under the Flavin Adenine Dinucleotide (FAD) – concurrent mechanism. Weak non-specific inhibitors also include CoA, acetile-CoA, ATP, and etc. [32, 33]. The fact that ADP is an effective inhibitor whereas ATP is not, expressly points out to influence of the energetic cell state on the oxidase activity. Restored nicotinamide coenzyme forms inhibit the oxidase, while the oxidated forms activate it. Accordingly and what is very important, the oxidation-reduction cell systems, are predominantly exposed to effect of O_2 (by all means, along with other substrate-metabolic and cofactor effects) [34, 35].

Insulin. Insulin is one of the important poly-functional and thoroughly studied anabolic hormones. By activation of Na^+-K^+-dependable adenosine triphosphatase it strengthens carriage of both glucose and amino acids to a cell. Along with that, insulin is in charge for biosynthesis and degrading of proteins, growth of muscle mass, depositing and consumption of energetic material in form of lipids and glycogen (23). As we have already noted before, cysteamine has an anti-insulin effect on metabolism. Insulin may be considered to be involved in practically all main processes in vivo: it actively promotes pyruvate dehydrogenase, it advances glycogen synthesis, it activates glycolysis, proteo-synthesis and lipogenesis, at the same time suppressing glycogenolusis and gluconeogenesis, and reducing liposysis. As it infers from the preceding paragraph, cysteamine has positive effect on all the above-listed processes [23].

According to the available information on oxidase inhibition, reactions catalyzed by D-amino acid oxidase are engaged in regulatory insulin system in a cell. Earlier we have considered in detail the possibility of specific induction mechanisms and interrelated operation of peroxisome and insulin in ruminant animals, as apart from such mechanisms in monogastric animals [36, 37]. We believe that studying of the functional activity of insular apparatus, its interoperation with the general hormone status, value and efficiency of metabolic and productive response reaction to practically all influencing factors, serves as the pacing factor for description of the metabolic control mechanisms in animals [36]. Insulin and somatotropin production value and dynamics were studied depending on the qualitative and quantitative type of the animal diet [36]. Based on such studies, we have emerged to a justified opinion that concentration of insulin and somatotropin in blood varies upon strengthening of hydrolysis in rumen and intestinal tract, whereas it is increased in the first one and decreased in the second one. In other words, the increased secretion of insulin induced by the breeding inhibits biosynthesis and secretion of the growth hormone produced by adenohypophysis. However, serious biochemical explanation of the paradox of multidirectional change of concentration of such two anabolic hormones important for the organism ("insulin-somatotropin scissors") is still missing. We have proposed that high concentration of insulin after breeding results in its higher connectivity, and insulin in a bound state does not have any effect on proteolytic activity and is unable to activate somatotropic hormone which, in pursuance thereof, requires participation of a specific protease. Consequently, after feeding most part of somatotropin is in inactive form and is scarcely found. Most probably, such mechanism may partially explain the multidirectional action of such an-

abolic hormones. Insulin and somatotropin are synergic by their effect on somatomedin and are antagonists by their effect on metabolism of glucose and fat acids. Such relations are precisely regulated by "insulin-somatotropin scissors". After feeding, not only accumulation of the plastic and energetic substances in form of the muscle mass is required, but also accumulation of carbohydrates and fats in form of glycogen and deposited lipids. Somatotropin promotes lipolysis and gluconeogenesis, of which from the amino acids. Therefore, after feeding the insulin, having activated the synthesis of glycogen and lipogenesis, presents activation of somatotropin. At that, insulin activates glycolysis for use of the produced energy in synthesis of the muscular proteins.

Tests on bull-calves with various feeding schedules and use of hormones and clenbuterol - agonist of β-adreno receptors. We have compared metabolism of Kholmogory bull calves at different feeding intensity, different levels of dietary protein, lysine, and methionine not digestible in a rumen, upon use of androgenic and estrogenic preparations and betazine thyrostatic and upon use of different dosages of clenbuterol, the agonist of β-adrenoreceptors [36]. The results were conclusive, but unexpected. Thus, use of synthetic clenbuterol anabolic just like the intensive feeding had resulted in suppression of insular apparatus of pancreas, adrenal, and thyroid glands. Compensative strengthening of functions of such glands was noted after withdrawal of the medicines. We have drawn our attention to such paradoxical and unexplainable fact that insulin concentration in blood is reduced, whereas this hormone is primarily in charge for growth of the muscular mass and accumulation of energy sources for maintenance of the growth.

It is a well-known that both clenbuterol and insulin promote biosynthesis of a muscle protein and reduce degrading thereof [36]. Although insulin increases lipogenesis and suppresses lipolysis, clenbuterol reduces deposition of a fat. In other model test at feeding of the bull-calves by exogenous anabolic sex steroids and betazine, activity of all dehydrogenases of citrate cycle was reduced in liver with sharp increase of pyruvate carboxylase activity in combination of the high intensity of growth and significant decrease of concentration of 11-oxycorticosteroids, insulin, and somatotropin in blood [37, 38. Herewith, the most intensive growth and the best results of carcass yield, yield of additional meat in carcass, and content of the protein in meat were found in bull-calves with the lowest basal level of insulin and thyroid hormones in blood. Such direction of metabolic processes and formation of the productive features in animals is absolutely not in line with the existing biochemical paradigm. In our opinion, such facts may have physiological and biochemical explanation only accounting for the processes flowing in peroxisomes. In particular, we believe that ability of such structures to supply the additional acetate-based glucose to the organism, thus resulting in the increased activity of Krebs cycle, and to participate simultaneously in induction of the synthesis and secretion of insulin plays an important role in the considered studies [1, 4, 37].

G.A. Hamilton [23] had shown the ability of peroxisomal oxidase to catalyse formation of H_2O_2 with consumption of molecular oxygen. Besides, it is well-known that reduction of intracellular partial oxygen pressure results in inhibition of oxidation-reduction reactions. Cysteamine-glyoxylate complex serves as an active substrate form of D-amino acid oxidase. Complexes are formed spontaneously and are present in the physiological conditions in concentrations leading to the significant growth of reaction speeds catalyzed by D-amino acid oxidase. Glyoxylate itself is considered as an inhibitor of oxidative metabolism and breath in vitro. It has non-enzymic reaction with oxaloacetate and produces oxalomalonat (keto-isocitrate), which simultaneously serves as an inhibitor of aconitase, NAADP-dependent isocitrate dehydrogenase and α-ketoglutarate dehydrogenase.

Besides, it inhibits mitochondrial carriage of phosphorus and electrons along the cytochrome chain. As we have already noted before, D-amino acid oxidase acidifies glyoxylate in form of glyoxylate-cysteamine complex, and cysteamine performs a function of a negative intracellular insulin messenger. In its turn, insulin may slow down formation of cysteamine by inhibition of CoA metabolism leading in formation of cysteamine. Insulin, having linked with its receptors on membranes, increases hardness of the later and slows down the access of intracellular CoA metabolites and phospho-pantetheine to relevant enzymes — nucleotide pyrophosphatase and alkaline phosphatase located on surface of the membrane exposed to the cytoplasm [23].

Besides, it is known that hypoglucemia is developed in animals with deficit of pantothenate and such animals are substantially more sensitive to insulin than animals sufficiently supplied with such vitamin. It is also known that concentration of CoA does not forego any significant changes upon additional insulin injections. However, the speed of its biosynthesis from the pantothenic acid is sharply decreased (90 % of inhibition in perfused heart). Insulin reduces the CoA degrading and cysteamine synthesis speed. As it was noted before, insulin promotes activity of pyruvate dehydrogenase, glycogen synthase, hexokinase, phosphorylase, and phosphatase. Cysteamine has an inhibition effect on such enzymes. At the same time, insulin inhibits fructose-1,6-bisphosphatase, while cysteamine activates thereof. Since insulin decreases concentration of intracellular oxygen, it shall, in our opinion, decrease the activity of peroxysomal oxydase, thus, leading to accumulation of glyoxylate. At the same time, decrease of the partial oxygen pressure promptly inhibits the activity of Krebs cycle enzymes. Decrease of activity in peroxysomal oxydase results in increased concentration of glyoxylate, which also serves the active inhibitor of Krebs cycle enzymes and cytochrome system enzymes. In our opinion, the resulting metabolic state of a cell shall result in activation of glyoxylate cycle in peroxisomes.

According to G.A. Hamilton [23], H_2O_2, it may have insulin-like effect by free access through membranes. However, this idea was not taken seriously by major part of scientific world, regardless of the firm empirical support of fast phosphorylation of insulin receptors in fat tissue in presence of the hydrogen peroxide. Similar mechanism was confirmed for insulin-like growth factor (IGF). It is notable that in purified plasmatic membranes (as compared to homogenates) hydrogen peroxide does not have an adverse effect. It is assumed that the said insulin-like effect of H_2O_2 may not be intermediated by stimulation of phosphorylation of insulin receptors by hydrogen peroxide. It results in the increased membrane tension and activation of Na^+/K^+-ATP in tissues, including miocytes, which promotes biosynthesis of muscular proteins and growth of muscular mass. The above-listed factors confirm the ability of H_2O_2 to render insulin-like effects. Herewith, activation of receptors by hydrogen peroxide requires a number of cell components [22, 39-41]. We assess these facts in support of our hypothesis, subject to which the processes similar to processes flowing at insulin stimulation may flow under the effect of H_2O_2 in the intensively fed bull-calves at low concentration of insulin in blood and activation of peroxisomal reaction not only in adipocytes, but also in other tissues, including the muscular tissue. These may, in particular, involve the observed strengthening of biosynthesis of the muscular proteins. Besides, even if role of H_2O_2 as an intracellular insulin messenger (the similar function is performed by cysteamine) was not confirmed, its ability to render insulin-like effects was already demonstrated [23, 24].

For ruminant animals, when large quantity of acetic acid is formed at chronic deficit of glucose, it is metabolically important to use part of acetic acid for synthesis of organic acids, from which glucose is formed in furtherance. This

is justified by the need for glyoxylate cycle operation in such animals, which would allow achieving the higher productivity without metabolic disorders in form of ketosis and acidosis, provided sufficient quantity of easily-digestible fiber in a diet of such animals [4]. In complex crosslinks in the clenbuterol test we have seen the action of specific insulin-peroxisome interaction mechanisms. According to our concept, it could be assumed that application of clenbuterol results in activation of glyoxylate cycle with strengthening of peroxisomal β-oxidation of fat acids. Herewith, two molecules of the activated acetate acid with formation of one molecule of succinate, malate, and oxaloacetate are used for one turn of glyoxylate cycle [37].

Glyoxylate cycle. All biochemistry study guides point out to the lack of glyoxylate cycle in higher animals. However, we have demonstrated that key glyoxylate cycle enzymes of isocitrate lyase (EC number 4.1.3.1) and malate synthase (EC number 4.1.3.2) are functioning along with Krebs cycle dehydrogenase and pyruvate carboxylase (key enzyme of gluconeogenesis) in hepatic tissues of bull-calves intensively fed for meat [1, 37]. Therefore, we believe that it is possible to assume that in metabolism securing the high productivity in ruminant animals glyoxylate cycle not only functions by strengthening of metabolite flow through the cycle of tri-carbon acids to terminal oxidation chain, but also renders regulatory effect on the general trend of metabolite flow simultaneously with Krebs cycle dehydrogenase [2, 4]. Two of the above-mentioned cycles, by performance of the intracellular endoecological function, integrate carbohydrate and lipid metabolism regulating the gluconeogenesis, Krebs, and glyoxylate cycles, and electron carriage chains. This demonstrates the quantitative coherence of carbon flows, processes in various sub-cellular compartments and hormone, substrate, cofactor, and enzyme regulation of such processes. In our opinion, glyoxylate and Krebs cycles are two complementary, interchangeable, interrelated, and mutually regulating processes.

Works of the national scientists show functioning of the glyoxylate cycle in laboratory animals — new-born, starving, diabetic, and placed in extreme conditions (stress) [42-45]. In support of our hypothesis, it is principally important that four of the above-described states may be unified by a common consistent pattern – metabolic deficit of glucose, which is also peculiar to ruminant animals with hypoglycemia – physiological norm. The above considered factors allow us to assume that ruminant animals in process of their evolution may have produced and philogenetically fixed the response reaction of organism to chronically lowered concentration of glucose in blood. One of the assumed adaptations is functioning of the glyoxylate cycle is constantly induced by glucose deficit for the additional metabolic support by such essential metabolite. To sum up, it should be concluded that glyoxylate cycle in ruminant animals - first of all (in order of priority, not in order of importance), directly relates to cell endoecology, secondly, relates to growth of metabolic productivity of the tri-carbon acid cycle (upon interoperation thereof with bi-carbon acid cycle) for improvement of the high production substrate support, and thirdly, participates in coordination of the principal intracellular, intercellular, and inter-organ metabolic processes across the time and space.

To conclude, we again draw our attention to potential metabolic role of D-amino acid oxidase in ruminant animals. We have considered the ability of such enzyme to hydrolyse the substrates with the use of molecular oxygen and production of hydrogen peroxide. As it was noted before, reduction of intracellular partial oxygen pressure results in inhibition of the oxidation-reduction reactions, and growth of such pressure results in activation thereof. Accordingly, D-amino acid oxidase may contribute to intracellular regulation at two levels: by decreasing of oxygen concentration and by increasing the concentration of H_2O_2.

Thus, we suggest a hypothesized regulation of the metabolic processes in highly productive ruminant animals accounting for the role of pro-oxidation systems, hormones, cell compartments, related cycles of tri-carbon and bi-carbon cycles. Known laws of biochemical logic allow assuming the existence of the assumed hypothetic chain of metabolic transformations, while the proposed approach allows adjusting the traditional paradigm. Besides, it may contribute to perception of the whole number of received paradoxical and empirical factors pending their interpretation and discovered by us and many authors in considering the specific aspects of metabolism in ruminant animals. Volume of accumulated knowledge allows adjusting the existing physiological and biochemical perspectives at principally new conceptual platform. It is based on subtle entanglement of mitochondrial Krebs cycle, cytoplasm glycolysis and gluconeogenesis processes with peroxisomal glyoxylate cycle. The complex of multi-factor interlinks between the insulin, peroxisomal cysteamine, glyoxylate, Krebs cycle dehydrogenase, oxygen, hydroperoxide, and D-amino acid oxidase should be accounted for understanding of their leading role in regulation of the metabolic processes in highly productive ruminant animals. Naturally, the proposed hypothetic concept requires supplementary understanding, updating, and broad empirical verification.

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