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D-AMINO ACIDS AND THEIR OXIDASE IN FARM ANIMALS — THE ROLE AND OPPORTUNITIES (review)

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Abstract

In the article, the basic physiological and biochemical functions and the importance of D-amino acids and D-amino acid oxidase in mammals are described. Serious attention is paid to metabolic role of D-amino acid oxidase in health and disease. D-amino acids and D-amino acid metabolizing enzyme has been discovered by Krebs 1935 (H. Krebs, 1972). Nowadays, most attention is given to the signal, communication and regulatory role of peroxisomes in metabolism. The peroxisomes are considered as candidate agents able to provide a relationship between the nervous and endocrine systems and participate in metabolism regulation in cells, organs and the body. Significant amounts of D-amino acids, the L-amino acids stereoisomers, are found in the peroxisomes of various organs and tissues: in the nervous system (J. Sasabe et al., 2014.), endocrine glands (A. D'Aniello et al., 2000), liver, kidney, breast, etc. (S.V. Khoronenkova, V.I. Tishkov, 2008). These active molecules provide communications in the neuronal network via the synapses of nerve endings (C.W. Morgans et al., 2013) and are involved in cell aging and apoptosis (A.V. Worms, 2010), biosynthesis and secretion of hormones, blood pressure regulation, maintenance of cell osmotic pressure (Y. Nishina, 2008), anti-inflammatory reactions and anti-carcinogenesis (G.H. Fisher, 1998). D-amino acid oxidase affects the activity of body as a whole in very diverse ways due to simultaneous engagement in diametrically opposite multi-parametric processes, such as regulation of cell level of D-amino acids and amino acid D-amines; the activity of central and peripheral nervous system; the maintenance of cell ecology; biosynthesis and secretion of epiphyseal melatonin (H.K. Park et al., 2007), hypothalamic releasing factors, pituitary, thyroid and steroid hormones (A. Santillo et al., 2014); defense against xenobiotics, microorganisms, viruses, stresses, and malignant tumors (R. Rana et al., 2012). Special attention the authors of this paper pay to the role of D-amino acids and D-amino acid oxidase in farm animals. World publications are mostly devoted to physical and chemical properties and the involvement of these agents in a number of neurodegenerative diseases and human clinical pathology of the central and peripheral parts of brain. As to farm animals, the data are almost completely absent. Recently, a growing body of in-deep examinations appear of the impact of D-amino acid oxidase and D-amino acids on the entire hierarchy of the endocrine system from the pineal gland, the hypothalamus and the pituitary gland to the ovaries and testes (S. Yasuaki et al., 2012). The studies are mostly focused on the regulation mechanisms of reproductive function in humans and animals. Elucidation of the involvement of D-amino acids and D-amino acid metabolizing enzyme in excitation and inhibition processes in the central nervous system is of a particular interest for farm animal biology. Cognitive function in farm animals is of a separate interest as it is tightly associated with animal adaptability to commercial production, formation of the nervous system type and a reduced aggressiveness. Eventually, these will result in control of farm animal behavior and performance.

Keywords: D-amino acids, D-amino acid oxidase, peroxisomes, nervous and endocrine regulation of metabolism

The phenomenon of optical isomerism was discovered in the XIX century by Louis Pasteur, who discovered the left and right rotations of polarization in culture media. Pasteur associated it with the ability of individual molecules to exist in the form of two mirror-antipodal “dissymmetric” spatial configurations, and

called them chiral (Gr. "hand"). Pasteur defined this property of molecules as fundamental and universal, by this attracting close attention to stereoisomerism (tautomerism). There still are undiscovered spots in this field. Discussions continue; the hypotheses are put forward and require theoretical and experimental justification [1]. Since the middle XX century it is known that the use of a small amount of D-amino acids instead of L-amino acids in feeding laboratory and farm animals leads to negative consequences. There are also forecasts that chiral pollutants may become a global environmental problem [1]. Interest in D-amino acids and their oxidases has grown into an interdisciplinary issue [1]. The majority of scientific works deal with these substances' functions in the central and peripheral nervous system in humans. However, the studies on the issue with special reference to agricultural animals are extremely few and have not been conducted in Russia.

The aim of this review is to systematize all available data on the role of D-amino acids and their oxidases in metabolism in agricultural animals in normal and pathological ways, describing their specific functions in metabolic pathways in various systems.

Special interest is drawn to studying D-amino acids and their oxidase (D-amino-acid oxidase, DAAO) due to the processes of excitation and inhibition in the central nervous system, adaptability to industrial conditions, the formation of the type of the nervous system, the reduction of aggressiveness, the establishment of the social rank of an animal and, finally, with the knowledge and management of behavioral responses in agricultural animals [2]. This field should expect the emergence of brainstorm and the consequent development of ways to increase the quantity and quality of livestock products. Scientists still cannot count the amino acids existing in living nature in the form of D-isomers, and how many oxidases of D-amino acids are found in the body. All known amino acids undergo racemization reactions, but their rates vary greatly. As for the oxidase, the answer is also ambiguous. In all the world literature, D-amino acid oxidase was called a singular, and it had the classification number EC 1.4.3.3. However, the official nomenclature has another enzyme, D-asparaginic acid oxidase (EC 1.4.3.1). It is mentioned in a much smaller number of papers [3]. The first enzyme has broad substrate specificity with the corresponding imino acid, ammonia and hydrogen peroxide as the final products of the catalyzed reactions. The byproducts of the second enzyme (also being a deaminating one) are oxaloacetate, ammonia and hydrogen peroxide. To some extent, the D-amino acids oxidase is quantitatively capable of exhibiting activity on D-aspartate and vice versa. But this fact still needs to be specified. Scientific literature does not have clear physiological and biochemical justification for these two enzymes functioning in the body. Perhaps it is predetermined by the exclusive part of D-aspartate in the N-methyl-D-aspartic receptor, the specific value of oxaloacetate in the metabolism and the functions of D-aspartic acid in the endocrine system. This work refers to the oxidase of D-amino acids in the singular, as it is generally accepted in the world scientific literature.

D-amino acids play a key role in the regulation of a number of processes in a cell. DAAO is one of the main enzymes responsible for maintaining the required amount of D-amino acids in the body. The mechanism of this regulation is multidirectional and has multiple parameters. The same enzyme simultaneously affects the content of all D-amino acids and D-derivatives of biologically active amines, which can initiate various metabolic pathways and physiological effects [4]. As a rule, with advancing age of animals and in case of pathological conditions of different nature, the concentrations of D-serine in blood and cerebrospinal fluid decrease. However, at the same time there always is a simultane-

ous decrease in the number of D-amino acids and an increase in the activity and expression of D-amino acid oxidase [5].

For quite a long time, they believed that D-amino acids were not synthesized in the mammalian body, and their presence was explained by the hydrolysis carried out by commensal microorganisms. However, due to modern analytical methods, significant amounts of D-amino acids were found in mammals. D-serine and D-aspartate are the most common. D-serine, which is synthesized by serine racemase and degraded by DAAO, is found in the brain and is considered as one of the two leading neurotransmitters. D-aspartate, synthesized by the aspartate racemase and degraded by oxidase D-aspartate, is present in significant amounts in neuroendocrine structures and organs of the endocrine system. It is known as a significant regulator of synthesis and secretion of hormones and spermatogenesis. D-serine and D-aspartate join the glycine site of the corresponding subtype of the N-methyl-D-aspartate receptor (NMDA) and operate as two major agonists and co-agonists to accelerate signal transfer between glia and neurons [6].

D-amino acid oxidase is a flavoenzyme that contains flavin adenine dinucleotide (FAD) in the active site. D-amino acid oxidase mainly catalyzes the oxidative deamination of the basic and neutral D-amino acids to the corresponding imino acids responsible for optical inversion. The reaction also results in forming hydrogen peroxide, the FAD is reduced, and the imino acid is non-enzymatically decomposed to the α -keto acid and the ammonium ion. The active center has SH-groups and ferrum atom. The optimal pH for both oxidases ranges from 8.5 to 11; the medium should be oxygenated. D-amino acids oxidase was found in all eukaryotic cells, except for the cells of higher plants. In mammals, it is found in brain, kidneys, liver, lungs, etc. It is remarkable that DAAO concentration in liver and kidneys of pigs is particularly high [7]. Multiple enzyme functions indicate the relation between changes in DAAO activity in the cell with different regulatory processes in the body [8, 9]. A characteristic feature of DAAO is the almost absolute specificity to D-isomers with different specificity to various D-amino acids, while catalytic activity on L-forms is almost not detectable [10].

DAAO performs a variety of physiological functions which rank from anabolic one in yeast cells, that allows them to grow only on D-amino acids as sources of carbon, nitrogen and energy, to the regulatory one in brain of higher animals, where DAAO controls the amount of D-serine neuromodulator (11). The enzyme that oxidizes D-amino acids was discovered by H.A. Krebs in 1935 and has become the object of numerous studies [11]. This enzyme is considered as a general model to describe the functioning flavoproteins of the dehydrogenase-oxidase class. Structural and functional research suggests that a specific physiological function is performed through various structural elements that control access to the active center and substrate or byproducts [12]. The kinetic characteristics of pig DAAO indicate that the enzyme binds FAD more rapidly than L-amino acid oxidase and exists as a stable homodimer even in apoprotein form. The chemical properties of oxidase are determined by the specificity of hydrophobic bonds of short fragments of amino acid sequences. These fragments are associated with the surface of the flavin nucleous, forming a unique stable conformation with specific kinetic characteristics [13].

The interest of scientists in DAAO has been rapidly growing since the mid-1990s. Firstly, this was due to numerous experimental data that justified the extremely important role of D-amino acids in the vital activity of an organism. Secondly, genetic engineering create recombinant producers of D-amino acid oxidase and allows obtaining the enzyme in quantities sufficient for studying.

Thirdly, the sequencing of entire genomes of prokaryotes and eukaryotes has provided the background for the search and cloning of the new DAAO genes. Fourth, the methods of enzymological engineering allow constructing an enzyme capable of easy and strong fixation on insoluble carriers, for use as sorbents, disinfectants, enzyme electrodes, etc. [14].

A number of metabolic conditions are associated with increased expression of DAAO and its specific activator, the G72 protein, discovered in 2002. It is still discussed, by what mechanisms G72 protein plays a regulatory role in the activity of D-amino acid oxidase. It was found that amino acid residues at positions 123-153 and 138-153 in the long G72 isoform bind to DAAO and increase its activity by 22 and 32 %, respectively. More detailed studies [15] showed that these regions of G72 interact with special loops in the DAAO molecule and create a tunnel that facilitates the substrate and cofactor's penetration into the active center of the enzyme. Molecular mechanism involving the interaction between the C-terminal fragment of G72 and DAAO that can affect the properties of the N-methyl-D-aspartate receptor potentiating neurotransmission, is also considered. Similar channels, or tunnels, are also described for hydrogen peroxide and oxygen [15]. Protein G72, activating DAAO, promotes oxidative deamination of D-3,4-dihydroxy phenylalanine and D-serine. D-3,4-dihydroxy phenylalanine is converted to L-3,4-dihydroxyphenylalanine, a precursor of dopamine, while D-serine is involved in the glutamatergic system. These results show that variations in the D-amino acid oxidase gene affect the turnover of dopamine in healthy adult individuals. Perhaps the dopamine metabolism disorder serves as a mechanism responsible for the relationship between the genetic variations of D-amino acid oxidase and the behavioral phenotype.

Since the discovery in mammals, D-serine has become the most studied "unnatural amino acid". This transmitter-like amino acid, found in brain in a significant amount, plays a major role in the central nervous system in higher animals by modeling the activity of the subtype of the glutamine N-methyl-D-aspartate receptor. Normal brain development and functioning requires the physiologically optimal content of D-serine. Any changes in the amount of this neuromodulator may cause pathologies [16]. About a half of research works on D-amino acids published in the last few years deals with the role of D-serine as a high-grade neurotransmitter, the neuromodulator and allosteric agonist of glycine-binding site at the glutamine receptor of N-methyl-D-aspartate, which plays an important role in many physiological and pathophysiological processes [17].

A decrease in D-serine content is dangerous because of high affinity of D-serine interaction with the glycine-binding site of NMDA receptors [18]. The decrease in the amount of D-serine reduces the functional activity of NMDA receptors, which is considered to be one of the main causes of abnormalities development in the central and peripheral nervous system. Recently, this hypothesis has been accepted as the main one, since the long-term potentiation of the synaptic transmission between the two neurons can maintain a prolonged activation of the synapses activity. By acting on the synaptic path, D-serine participates in mechanisms that provide synaptic plasticity, allowing the nervous system to adapt to changing environment [19].

In the brain of mutant mice and rats with DAAO-associated enzyme defect, the amount of D-amino acids, particularly D-serine, significantly increases. DAAO catabolizes D-serine and thus modulates neurotransmission. Mutant mice behave like animals with altered activity of the glutamate N-methyl-D-aspartate receptor subtype due to the increased content of D-serine. D-serine and DAAO play an important role in cerebral activity and synaptic mobility. They are involved in various physiological and pathological responses of the central nervous

system [20]. D-serine performs the NMDA functions and is known as an enhancer of the negative impact and cognitive symptoms in nervous disorders, unlike the weak effects of standard D-2 antagonists. The inhibition of DAAO is of significant interest as an effective way to increase the amount of D-serine in brain. Over the past few years, a large number of DAAO inhibitors have been discovered. Some of them have significantly higher activity than that of conventional D-amino acid oxidase inhibitors. Many of the recently described DAAO inhibitors show activity in increasing the amount of D-serine. They are efficient in laboratory animals when modeling behavioral responses, aggressiveness and adaptability [21].

Mice with partial defects in serine racemase and D-aspartate oxidase are capable of reproduction and growth. They have mutations in D-amino acid oxidase gene which is found in rats and mice. Behavioral responses in mutant animals differ from those of normal animals due to increased or decreased activity of the NMDA receptor [22].

Oxidase of D-amino acids catalyzes oxidative deamination of D-amino acids including the D-serine, the agonist of glycine modulatory site in the receptor for N-methyl-D-aspartate. It is shown that DAAO is important in the D-serine clearance during blood circulation. Therefore, DAAO inhibitors with a longer biological half-life can maintain a higher D-serine blood level for a long time, which can have a significant effect in the treatment of systemic hypertension [23].

According to the recent findings, D-3,4-dihydroxy-phenylalanine (D-DHPA) is the best DAAO substrate in laboratory animals compared to D-serine [24]. The catalytic efficiency denoted as k_{cat}/K_M (the catalytic constant to the Michaelis constant ratio) for DHPA was 14 times higher than that for D-serine. That is, DAAO provides an efficient alternative metabolic pathway for the conversion of D-DHP to dopamine, which may be interesting for controlling behavioral responses and reducing animal aggressiveness.

G.H. Fisher [25], while studying the physiological role of high D-alanine concentrations in the intracellular space of tissues and organs, suggested that this amino acid is involved in the regulation of intracellular osmotic pressure.

According to the concentration in the cells of higher animals, D-proline and D-leucine are on the fourth place after D-serine, D-asparagine and D-alanine. Currently, the physiological role of D-proline is being discussed. In the organism, unidirectional optical inversion of amino acids and analogues of D-amino acids is carried out. Exogenous NG-Nitro-D-arginine and endogenous D-phenylalanine and D-methionine are inverted much faster than other D-amino acids. In the cells of long-lived tissues (dentin, tooth enamel, crystalline lens, etc.), there is a clear correlation between the age of the animal and the D-hydroxyproline and D-aspartate concentrations [25]. Data on neuro-, hepato- and renal toxicity of D-proline for rats are actively discussed [26]. The study of the content of D-proline in various tissues in normal and mutant (absence of the gene responsible for the synthesis of DAAO) in mice showed that in the latter, D-proline accumulates in larger amounts in the kidneys, and its excess is naturally excreted [26].

D-aspartate oxidase found in peroxisomes of the liver, kidneys and many other tissues and organs in humans, cattle, pigs, and sheep serves as a peroxisome marker enzyme together with catalase and DAAO.

D-aspartic acid is recognized as one of the most important regulators of the synthesis and secretion of steroid hormones and releasing factors of protein hormones [27]. High content of D-aspartic amino acid is observed in the glands of internal secretion, in the nervous and endocrine tissues. For this amino acid,

the highest concentration is characteristic of embryonic period. With advancing age, D-aspartic acid level gradually decreases in the nervous tissue, but increases in the endocrine glands, especially in the pineal gland, pituitary gland, adrenal glands and testes. In the pineal gland, D-aspartate reduces the secretion of melatonin, in the testes it stimulates Leydig cells, as well as the biosynthesis and secretion of testosterone that occurs through the activation of steroidogenesis by expression of a specific regulatory protein. This discovery shows that D-aspartic acid is a new type of messenger in the mammalian body [28].

Regulation of the formation and secretion of the epiphyseal hormone melatonin is a specific physiological function of D-aspartate. Various mechanisms for the response to noradrenaline stimulation of pinealocytes which were previously treated with D-asparagine [29] are discussed. Significant amounts of D-aspartate are found in the mammary gland. L-aspartic acid demonstrates an inversely proportional dose-dependent effect on prolactin secretion. Testosterone, cortisone and thyroid hormones increase the activity of oxidases. In the kidneys of rats fed with a diet limited in Na, oxidase activity also increases. Hypolipidemic drugs reduce the formation of peroxisomes in liver and the DAAO activity (the latter is more noticeable than expected). In the neuroendocrine system, decreasing D-aspartate concentrations are found as follows: NMDA > anterior pituitary gland > hypothalamus > pineal gland. In intraperitoneal injections of amino acids, the greatest accumulation occurs in these glands. It is accompanied by a dose-dependent increase in the concentration of prolactin and somatotropin [30-32].

The dependence was found of hormonal activity and the onset of maturity on the D-aspartate level in the pituitary gland and testicles. Immunocytochemical studies have shown that this enantiomer is localized in Leydig and Sertolli cells, and have confirmed its important role in steroidogenesis [33]. In various animals, the authors demonstrated the regulating effect of D-aspartic acid on the biosynthesis and secretion of testosterone and luteinizing hormone, but not in estradiol secretion. The mechanism underlying the effect of D-aspartate injections on testicular androgen receptor proteins is described in detail. The content of these proteins increases substantially, while the amount of estrogen α -receptors and P-450 aromatase receptors decreased [33].

In studying the molecular mechanisms of the evolutionary transition from asexual to sexual reproduction, a hypothesis was put forward that DAAO inhibits the formation of immature ovaries by degradation of D-amino acids [34]. As a result of sexual induction, the oxidase expression in ovaries increases. Absent oxidase slows the maturation of all reproductive organs besides the ovaries. This circumstance allowed admitting that oxidase boosts the maturation of the ovaries that determine the complete sexual induction. The authors also suggested that oxidase which is formed in somatic cells prevents the reduction of sexual induction in asexual status. After sexual induction, female reproductive cells specifically produce DAAO to induce complete maturation

A combination of accurate and sensitive methods of fluorimetry and high-performance liquid chromatography not only confirmed the presence of D-aspartate in the nervous tissue and endocrine glands, but also showed the induction of the secretion of growth hormone and luteinizing hormone in the anterior pituitary gland. It is interesting that the induction of luteinizing hormone was recorded only in the case of a joint incubation of the anterior pituitary gland with the hypothalamus. It could occur due to the induction of the gonadotropin hypothalamic releasing, which is responsible for the secretion of the luteinizing hormone of the pituitary gland, by D-aspartic acid. Compared to NMDA, D-aspartate concentration to release the hormone is a 100 times less [34].

As early as in 1939, it was shown that tumor proteins contain significant amounts of D-amino acids, especially D-glutamic acid, D-valine, D-leucine, and D-lysine. Later, the D-serine content was found to be extremely high. In precancerous cells and malignant tumors, DAAO activity is extremely low, which points at their important role in metabolic control, including possible participation in the control of cell growth. It was assumed that the initiation and autonomic nature of the uncontrolled growth of tumors depends on D-amino acid contents in cellular proteins. This postulate remains controversial for many years. The published scientific papers were both confirming and refuting this hypothesis. The discussion was heating among scientists from the Netherlands, England and Germany. However, many years later, with the development of analytical methods for the detection of amino acids enantiomers, more convincing evidence has appeared. It claimed that D-amino acids do not serve as a common indicator for tumors and, apparently, are not responsible for malignant transformation processes (at least if yes, then not for all tissues) [35]. It is known that tumor cells grow very fast, so many researchers believe that L-amino acids do not have enough time to be racemized into D-isomers, as this process is rather slow. However, the use of the DAAO test to diagnose cancer continues to be one of the interesting and promising areas, as experiments in laboratory animals show almost complete absence of the DAAO activity in cancer cells. Some D-amino acids may come in small amounts with food intake, but it is not known which enzyme systems are capable of incorporating D-amino acids into tumor tissues. However, significant amounts of D-amino acids are found in tumor proteins. There is no reliable argument for their emergence here, but studying this phenomenon is certainly important for understanding the mechanisms of autogenesis and supporting the development of tumors [36].

Prostaglandin D-synthetase is a bifunctional protein acting as both an enzyme producing PGD₂ (prostaglandin D2), and as a lipid transporter. This enzyme is a member of the lipocalin superfamily of proteins that can bind to a large number of lipophily molecules. Expression of the enzyme is increased in neuronal cells treated with H₂O₂. The content of the prostaglandin-producing enzyme is closely interrelated with apoptosis induced by hydrogen peroxide. This enzyme protects neuronal cells from death from hydrogen peroxide. The cell viability test shows a dose-dependent effect of the enzyme [37]. Moreover, the titration of free thiols in the prostaglandin synthetase treated with hydrogen peroxide proves that in case of exposure to H₂O₂ per cell, the thiol is oxidized to sulfinic acid. The affinity of the oxidized form of the enzyme for lipophilic molecules is comparable to that of the untreated form. These results show that the enzyme protects neuronal cells from death by neutralizing reactive oxygen species without lowering the ligand-binding function. This property of prostaglandin synthetase may be useful for suppressing stress-induced neurodegenerative oxidative pathologies [38, 39].

Since D-amino acid oxidase generates H₂O₂ and uses D-amino acid as a substrate, it has been suggested that it can prevent bacterial infections. Indeed, D-amino acid oxidase inhibits the growth of *Staphylococcus aureus* in vitro in a dose-dependent concentration. Adding catalase to the incubation medium prevented the bacteriostatic effect. However, the presence of myeloperoxidase in the same system increased bactericidal activity. This paradoxical observation needs clarification, since both enzymes are capable of decomposing H₂O₂, but the physiological effect of their combined action is antipodal. Nevertheless, it is reliably known that an increase in the D-serine level and a decrease in DAAO activity are efficient in treating various diseases, including mental disorders and oncological ones [40-45].

Thus, this work systematized and analyzed a wide range of functions of D-amino acids and their oxidase, with an emphasis on complex competitive physiological and biochemical relationships in different systems (from the epiphysis, hypothalamus and pituitary gland to the ovaries and testes). The peroxisomes contain D-amino acids and their oxidase which are involved in providing the communication between the nervous, endocrine and immune systems in the regulation of metabolism in cell, organ and organism. The signal, communicative and regulatory functions of the peroxisome is discussed. As it comes from the publications in the world academic and medical literature, researchers prefer to clarify the role of D-amino acids and their oxidase in the central and peripheral parts of the human nervous system. In our opinion, it is necessary to raise interest in this problem in biological livestock science. The confidence in the prospects of such works is based on the experimental findings on the kinetic characteristics of DAAO which indicate the ability to provide an efficient alternative metabolic pathway for the conversion of D-dihydrophenylalanine to dopamine. It may be useful for finding mechanisms to manage behavioral responses and reducing the aggressiveness of animals.

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