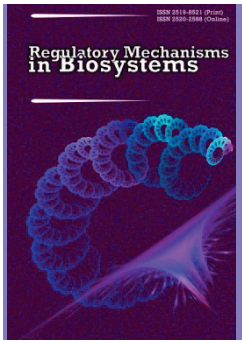

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Features of the lactate dehydrogenase isoenzymes spectrum in animal tissues and organs

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Activity and content of lactate dehydrogenase (LDH) isoenzymes is one of the objective criteria for assessing oxidative metabolism in organs and tissues and the physiological state of the whole organism. Lactate dehydrogenase (LDH) is a key enzyme of energy metabolism. LDH is a heterotetrameric protein consisting of subunits of two types H (cardiac) and M (muscular). This enzyme exists in five isoforms: LDH1, LDH2, LDH3, LDH4 and LDH5. Each of the isoforms is characterized by different physicochemical and catalytic properties and is found in most tissue cells. Content of LDH isoenzymes is not constant and depends on the intensity of oxidative metabolism in tissue cells and on the influence of various factors, including new therapeutic drugs and biogenic compounds. Therefore, the aim of the research was to study and establish the limits of isoenzyme content and LDH activity in individual tissues and organs of rats, pigs and cows to characterize and detect changes in the intensity of metabolism in the body of the animals under different physiological state. Tissue samples of rat, pig and cow organs were taken for research. Isoenzymes of lactate dehydrogenase were detected by staining according to Garbus in our modification. The content of isoenzymes (%) was determined by the TotalLab TL120 software, the activity of LDH in blood plasma (μkat/L) and in tissue homogenates (μkat/mg protein) – by the rate of NADH oxidation. Brain, spleen, liver, kidney, testis, muscle and blood plasma of rats and pigs were characterized by 5 catalytically active LDH isoenzymes. In muscle and liver tissues, LDH5 prevailed, and in the brain, testis and kidney – LDH1. LDH3 was most abundant isoenzyme in spleen tissue. It was found that the same activity of LDH in the tissues of the liver and testis of rats (lim 4.5–4.6 μkat/mg of protein) was provided by different LDH isoenzymes. In addition to tissue characteristics, the content of LDH isoenzymes depended on the physiological state of the organ. Thus, in the cow ovaries the activity of LDH was the same (2.8–3.0 μkat/mg of protein) under the physiological state of "follicular growth" and pathology – "hypofunction". LDH activity was lower by 0.6 and 1.0 μkat/mg of protein under "early" and "late" corpus luteum (2.0 and 2.2 μkat/mg of protein). The highest content of LDH1 was in the physiological state of "late corpus luteum", and LDH3, LDH4 and LDH5 – in "early corpus luteum". Content of LDH2 was high in the state of ovarian dysfunction – "hypofunction". Content of LDH2 was lower and had no statistical difference (19.8% and 22.1%) in other physiological states. Isoenzyme spectrum of LDH was characterized by tissue specificity. Tissues with a predominance of anaerobic processes (muscle and liver) were characterized by a high content of LDH5, and with a predominance of aerobic processes (brain, testis, kidneys) – by a high content of LDH1. The spleen was characterized by a high content of LDH3. It was established, that the same activity of the enzyme in the organs could be ensured by different content of LDH proteins. It has been proven that the content of individual LDH isoenzymes depended on the physiological state of the ovary.

Keywords: energy metabolism, enzyme, polyacrylamide gel, skeletal muscles, heart, liver, kidneys, spleen, ovaries, rats, pigs, cows.

Introduction

Lactate dehydrogenase (L-Lactate: NAD-oxidoreductase, ECN 1.1.1.27, M.m. 140 kDa, LDH) is an intracellular enzyme found in various tissues and organs, such as skeletal muscles, heart, liver, kidneys, spleen, organs of the reproductive system, etc. LDH is a key enzyme in the control of energy metabolism, as it catalyzes the mutual conversion of pyruvate into L-lactate using NAD+ as a hydride acceptor, and regulates the levels of these metabolites depending on the availability of oxygen (Schurr et al., 2006; Adeva-Andany et al., 2014; Szrejder et al., 2023). Thus, LDH is on the border between glycolysis, the metabolic pathway that converts glucose to pyruvate, and the tricarboxylic acid cycle, in which pyruvate is oxidized (Hui et al., 2017; Khan et al., 2020).

LDH consists of four subunits of two types: H (heart) and M (muscle). Different combinations of these subunits lead to formation of five different molecular LDH forms: HHHH (LDH1), HHHM (LDH2), HHMM (LDH3), HMMM (LDH4) and MMMM (LDH5), which are separated by 1d electrophoresis in a polyacrylamide gel on five separate lanes (Takehana et al., 2023). The emergence of different isoenzymes of LDH is due to the peculiarities of tissue oxidative metabolism. Isoenzymes LDH4 and LDH5 (M-types of LDH) are catalytically active in anaerobic conditions, and LDH1 and LDH2 (H-types) – in aerobic conditions, when pyruvate is quickly oxidized in the Krebs cycle to CO_2 and H_2O rather than being reduced to lactic acid.

In particular, the LDH1 isoenzyme (H4), found in heart muscle and the kidneys, consists of four H subunits and is the lightest in molar weight, and LDH5 (M4) is the most abundant in skeletal muscle and the liver and is the heaviest of all isoenzymes, consisting of four subunits of M. LDH2 (3H1M), LDH3 (2H2M) and LDH4 (1H3M) are characterized by intermediate mobility and are found in most tissues (Duka et al., 2014).

The activity of total LDH in whole blood has no diagnostic value, since it is not known due to which isoenzyme it increases or decreases

and, accordingly, where the pathological process is located (Corrigan, 2011; Levchenko & Vlizlo, 2019). In contrast, the determination of LDH isoenzymes in blood serum or plasma has clinical and diagnostic significance for detecting damage to membrane structures that are characteristic of various diseases (damage to the heart, liver, muscles, kidneys, etc.) (Nagy et al., 2020). In particular, LDH1 and LDH2 are localized in large quantities in the heart muscle, so their increased content in the blood characterizes myocardial damage (Aydin et al., 2019; Niu et al., 2024). The detected high content of LDH4 and LDH5 in the blood indicates destructive changes in the cells of skeletal muscles and liver, since these tissues have the highest content of these isoenzymes (Brancaccio et al., 2010; Jeong et al., 2019; Tokinoya et al., 2020). The study of LDH isoenzymes is important not only for enzyme diagnostics. In particular, the study of the isoenzyme spectrum of LDH can be used to establish the effect of any kind of new therapeutic drugs and biogenic compounds on the organism, since it is possible to detect changes in the oxidative metabolism of individual tissues and organs, for which a certain ratio of molecular forms of the enzyme is established, which are characterized by a certain ratio of enzyme molecular forms (Khan et al., 2020).

LDH activity and content of its isoenzymes in the organism are not constant and depend on endo- and exogenous factors. The study and establishment of their limits will ensure the characterization and detection of the peculiarities of metabolism in individual tissues and organs of animals. In particular, this applies to studies of the activity and content of LDH isoenzymes in the organisms of rats, pigs and cows. When testing new therapeutic drugs and biogenic compounds, the content of LDH isoenzymes and enzyme activity can indicate changes in metabolism and provide the possibility of predicting the feasibility of using active substances in the fields of biology and medicine (Zhou et al., 2020).

The goal of work is to study and establish the limits of LDH isoenzyme content and activity in individual tissues and organs of rats, pigs, and cows in order to characterize and detect changes in the intensity of metabolism in the organism under different physiological conditions.

Materials and methods

All manipulations with animals were carried out in accordance with the International Convention for the Protection of Animals and the Law of Ukraine "On the Protection of Animals Against Cruel Treatment" (Research expertise was conducted on the meeting of the bioethics commission of the Institute of Animal Biology of the National Academy Agrarian Sciences No. 139a dated September 20, 2023).

For research were used: male rats *Rattus norvegicus var. Alba*, Wistar line, weighting 200–250 g (n = 45), which were kept in the Institute of Animal Biology NAAS vivarium. The animals were housed in cages, four individuals per cage, with access to drinking water and food – complete combined diet and standard sanitary and hygienic norms (with ventilation and temperature of 22 ± 2 ℃, relative humidity – 55–70%, daily light cycle light/dark – 12/12 hours). In the vivarium, temperature and humidity were controlled according to the recommendations of the Guide for the Care and Use of Laboratory Animals (www.ncbi.nlm.nih.gov/books/NBK54039). Also used in the research were hybrid Large White breed and Landrace pigs (6 months old; weighing 100–120 kg; n = 30) and 4–6-year-old Ukrainian Black-spotted Dairy cows (weighing 400–450 kg). The pigs were kept in standard rooms in pens measuring 4.1 × 6.0 m on a slatted floor, with microclimate support systems, water supply and removal of faeces. The pigs were fed with full-ration compound feed with balancing of the ration with the vitamin-mineral additive Maxcare Fattening EX 2.5%. Feed was distributed to the pigs using the Osuch feeding system, and the amount of feed in the feeders was adjusted depending on the rate at which the animals consumed it. The cows, according to their physiological state and age, were kept in pens on deep straw bedding in typical premises for cows, using the latest technologies for keeping dairy cattle. The diet of the animals was balanced in terms of nutrients and corresponded to the productivity and physiological state. The cows were housed in a free-standing box system on deep straw bedding in typical head facilities converted from poultry houses using the latest dairy cattle housing technologies. The cows were grouped in sections according to physiological status and age.

The rats were decapitated under light chloroform anesthesia, and the pigs and cows were slaughtered at meat processing enterprises according to the technology of the enterprises (by electrocution). The extraction of tissues and organs of pigs and cows was carried out with permission of the private owner of the meat processing enterprise. The selected tissues and organs were delivered to the laboratory in a thermos at a temperature of 2–4 ℃. After decapitation and slaughter of the animals: blood was collected from the rats and pigs into tubes with K2EDTA and organs were extracted (from all investigated tissues and organs – 100 mg): liver, spleen, kidneys, testicles and brain – in rats; liver, spleen, kidneys and the longest back muscle (latissimus dorsi) – in pigs; in cows ovaries in different physiological states were extracted: with "fresh" ovulation, there was a hole at the site of the ovulated follicle, the corpus luteum was absent or the diameter was up to 5 mm, the color was red (n = 14); with an "early" corpus luteum, diameter 10–20 mm, red or brown color (n = 41); with a "late" corpus luteum, diameter 5–15 mm, color yellow (n = 32); "follicular growth", without corpus luteum (n = 84). In addition, gonads were selected from cows with a deviated sexual cycle – ovarian hypofunction (n = 14). The diagnosis was conducted on the basis of the anamnesis (absence of sexual cycles) and, after slaughtering the cows, a visual assessment of the ovaries was conducted – there were no dominant and antral follicles on the surface of the gonadal tissue. Plasma was obtained from blood by centrifugation (5 min. at 2.5 thousand rpm). The selected tissues were washed with a 0.9% sodium chloride solution at a temperature of 2–4 ℃. Tissue homogenates were prepared: minced tissues were washed with 0.9% sodium chloride solution and 1:1 (weight: volume) 0.25 M sucrose solution was added cooled to 4 °C and homogenized in a Potter homogenizer. The obtained tissue homogenate was centrifuged at 8000 rpm. for 15 min. at a temperature of 4°C. The supernatant was collected for further research. The content of isoenzymes was determined after vertical electrophoresis of protein samples in 7.5% polyacrylamide gel (PAAG) plates. Samples were prepared for electrophoresis: blood plasma and tissue homogenates were diluted 1:1 with Tris-glycine buffer (pH 8.5) and 0.05 ml of 40% sucrose was added, 0.02 mL of sample (protein concentration ~ 100 μg) was added to the wells of the stacking gel. Electrophoresis was carried out at 200 V, 30 mA. After electrophoresis, PAAG was stained according to Garbus et al. (1971) in our modification (Kuzmina et al., 2020): gels were incubated for 60 min in the dark at temperature of 37 ℃ in the incubation medium: 0.1 mg/mL PMS (phenazine metasulfate), 0.2 M lactate, 0.5 mg/mL NAD+ and 0.5 mg/mL NBT (nitroblue tetrazolium) in 0.1 M Tris/HCl buffer (pH 8,5). In the places where the enzyme proteins are localized, the gel acquires a purple color due to the reduction of NBT to formazan. Cow erythrocyte hemolysates were used as a marker of LDH isozyme composition (Robin et al., 2023).

To determine the content of isoenzymes (%) in their total spectrum, the PAAG plates were scanned and their content was calculated with TotalLab TL120 software. LDH activity was determined by the rate of NADH oxidation and was calculated in μkat/L in blood plasma and μkat/mg of protein in tissue homogenates (Vlizlo et al., 2012). The data were analyzed using Statistica 6.0 software pack (StatSoft Inc., USA). The data are presented in the diagrams as x ± SD (standard deviation). Differences between the values of the control and the experimental groups were determined using ANOVA, where the differences were considered statistically reliable at $P < 0.05$ (taking into account the Bonferroni correction). The correlation was calculated using Pearson's coefficient.

Results

After specific PAAG staining, five bands of catalytically active enzyme proteins were detected in all the examined tissues: LDH1, LDH2, LDH3, LDH4, and LDH5. These proteins were characterized by different mobility in the electric field, the size of the bands, and the intensity of staining (Fig. 1, Tables 1, 2). High content of LDH4 and LDH5 isoenzymes was found in rat liver – LDH4+LDH5 – 65.40%; pig muscle and liver – LDH5 – 55.00 ± 5.97% and 40.54 ± 3.07%. The largest content of LDH1 was in rats: brain – 26.10 ± 2.15%, testis – 32.70 ± 2.40% and kidneys – 31.31 ± 3.14%; pigs: kidneys – 58.25%). Animal spleen tissue was characterized by a high content of LDH3 (in rats – 39.01 ± 5.89% and in pigs – 33.57 ± 1.79%).

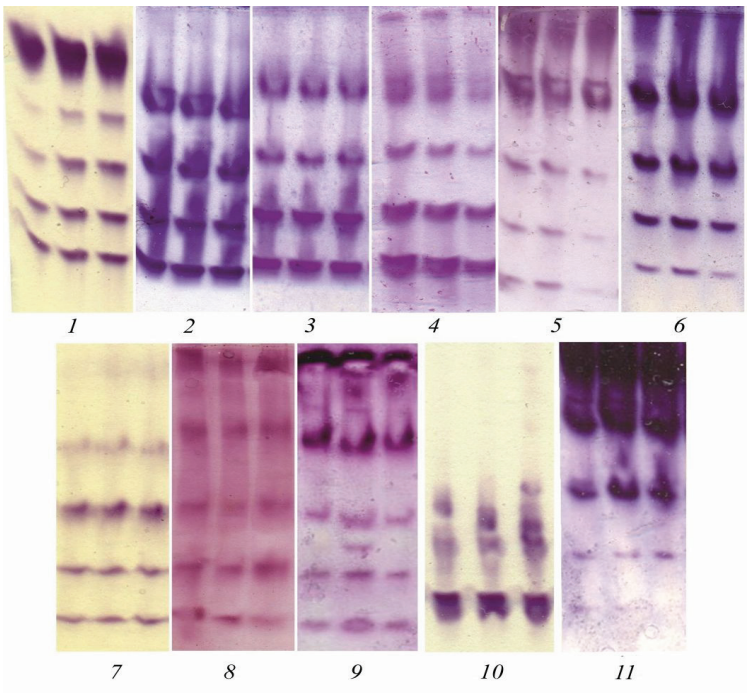

Fig. 1. LDH isoenzymes in individual tissues and organs of animals:
rats: *1* – plasma, *2* – brain, *3* – kidney, *4* – testes, *5* – liver, *6* – spleen; pigs: *7* – plasma, *8* – spleen, *9* – muscle, *10* – kidney, *11* – liver

Table 1
Activity and content of LDH isoenzymes in organs and tissues of rats and pigs (x ± SD)

Animals	Tissues and organs	Activity	Isoenzyme content, %				
			LDH_5	LDH_4	LDH_3	LDH_2	LDH_1
Rats (n = 45)	Blood plasma	2.87 ± 0.28^{1a}	28.71 ± 5.52^{a}	40.10 ± 6.65^{a}	10.00 ± 2.94^{a}	10.40 ± 2.61^{a}	10.81 ± 2.26^{a}
	Rats' brain	3.98 ± 0.48^{2b}	0.64 ± 0.34^{b}	31.80 ± 3.81^{a}	26.94 ± 2.56^{b}	14.67 ± 2.93^{a}	26.10 ± 2.15^{bc}
	Spleen	1.62 ± 0.18^{2c}	4.77 ± 2.92^{c}	14.37 ± 1.78^{b}	39.01 ± 5.89^{b}	22.49 ± 5.86^{b}	19.33 ± 3.48^{c}
	Liver	4.54 ± 0.36^{2b}	27.12 ± 7.17^{a}	38.28 ± 5.57^{a}	17.22 ± 6.68^{ab}	9.24 ± 3.60^{a}	8.24 ± 3.95^{a}
	Kidney	7.63 ± 0.13^{2d}	1.61 ± 1.23^{b}	27.43 ± 3.19^{ac}	16.73 ± 1.50^{a}	23.01 ± 2.07^{b}	31.31 ± 3.14^{b}
	Testicle	4.57 ± 0.42^{2b}	4.76 ± 0.76^{c}	22.40 ± 4.25^{c}	14.11 ± 2.74^{a}	26.04 ± 3.26^{b}	32.70 ± 2.40^{b}
Pigs (n = 30)	Blood plasma	4.50 ± 0.54^{1b}	16.36 ± 1.42^{a}	10.44 ± 0.54^{c}	21.21 ± 0.99^{c}	23.51 ± 5.68^{a}	30.11 ± 2.98^{b}
	Pigs' muscle	6.73 ± 0.75^{2bc}	55.00 ± 5.97^{d}	7.79 ± 2.72^{cd}	12.33 ± 2.58^{a}	13.04 ± 1.83^{a}	11.84 ± 1.82^{a}
	Liver	6.13 ± 0.88^{2bc}	40.54 ± 3.07^{c}	25.22 ± 3.80^{a}	6.09 ± 3.74^{a}	11.10 ± 3.14^{a}	16.30 ± 6.28^{a}
	Spleen	2.78 ± 0.38^{2a}	12.81 ± 3.12^{a}	17.90 ± 1.88^{b}	33.57 ± 1.79^{b}	19.52 ± 3.49^{a}	16.08 ± 2.39^{a}
	Kidney	9.40 ± 0.93^{2c}	2.03 ± 0.76^{b}	4.20 ± 1.01^{d}	14.30 ± 4.64^{a}	24.59 ± 9.03^{a}	58.25 ± 9.17^{c}

Note: values with different superscripts in each column are significantly different according to Tukey's multiple comparison procedure; *1* – μkat/L; *2* – μkat/mg of protein.

In addition, it should be noted that almost the same activity of the enzyme was found in the tissues of the liver and testis of rats (4.54 and 4.57 μkat/mg protein) provided by different isoenzymes of LDH.

Along with the dependence of the content of LDH isoenzymes on the oxidative metabolism in tissues, there are also individual features of the content of LDH isoenzymes, which are manifested by different content of the same isoenzyme in different animals of the same species. In particular, depending on the individual characteristics of the animals, in the liver tissue of pigs, the difference between the content of LDH1 in individual animals can be up to 21.14%, and LDH5 – 10.23%, in muscle, respectively, up to 6.35% and 20.23% (Table 2).

Table 2
Limits of fluctuations of LDH isoenzymes in organs and tissues depending on the individual characteristics of metabolism of rats and pigs

Animals	Tissues and organs	Content of LDH isoenzymes, %				
		LDH_5	LDH_4	LDH_3	LDH_2	LDH_1
Rats (n = 45)	Blood plasma	20.81–43.10	24.84–31.55	10.31–16.50	7.20–14.11	8.82–20.91
	Rats' brain	1.03–6.23	25.81–37.02	17.03–30.42	10.12–19.17	21.47–36.03
	Spleen	7.40–19.55	27.00–44.64	24.40–33.81	14.19–20.12	3.74–13.15
	Liver	27.42–62.30	17.73–45.85	9.78–34.01	2.35–14.88	2.67–21.85
	Kidney	3.57–12.91	16.45–33.33	11.00–19.73	15.37–24.10	24.70–32.78
	Testicle	4.00–7.64	16.68–28.78	9.85–18.67	18.51–30.89	29.31–38.74
Pigs (n = 30)	Blood plasma	15.10–18.67	9.90–11.22	20.50–23.64	16.90–35.89	27.81–35.80
	Pigs' muscle	47.92–68.15	4.23–11.45	6.09–15.07	8.67–14.91	7.19–13.54
	Liver	34.13–44.36	19.45–32.88	1.04–10.56	3.72–15.54	3.89–24.03
	Spleen	8.52–16.16	15.20–19.92	30.82–36.14	16.10–26.56	13.82–19.33
	Kidney	1.15–3.33	3.10–5.75	10.21–23.59	19.83–47.31	50.51–78.78

Similar results were obtained from the study of the content of isoenzymes in rat tissues: in the liver the difference between the content of LDH1 was up to 19.18%, and LDH5 – up to 34.88%.

In the brain, testis and kidney tissue, the dependence of the content of LDH isoenzymes on the individual characteristics of the animal organism was also established. In particular, the value of the LDH1 content varies,

depending on the individual characteristics of the intensity of the metabolic processes in the organism. In particular, in rats, the content of the specified isoenzyme in the brain tissue differed by 14.56%, testicles by 9.43%, and kidneys by 8.08% (Table. 2). At the same time, for the LDH5 isoenzyme, the difference between the values in the specified tissues was 5.20%, 3.64%, and 9.34%, respectively. Similarly, in the kidney tissue of pigs, depending on the individual characteristics of the intensity of metabolic processes, the difference between the content of LDH1 was 28.27%, and LDH5 – 2.18%. For spleen tissue, depending on the individual characteristics of the metabolic processes intensity, the content of LDH4 and LDH3 differed, respectively, in rats – by 17.64% and 9.41% and in pigs – by 4.72% and 5.32%. The content of LDH isoenzymes changed dynamically and depends on the physiological (pathological) processes in a specific organ or tissue, which was confirmed by the results registered in a composition study of LDH enzyme content in cow ovarian tissues (Fig. 2).

In particular, ovarian tissue was characterized by a high content of LDH1 (36.56–49.34%), lower content of LDH2 and LDH3 (11.07–27.45%), and the lowest content of LDH4 and LDH5 (6.80–13.21%; Table 3). The established values of LDH isoenzymes indicated the predominance of the aerobic pathway of glycolysis in ovarian tissue.

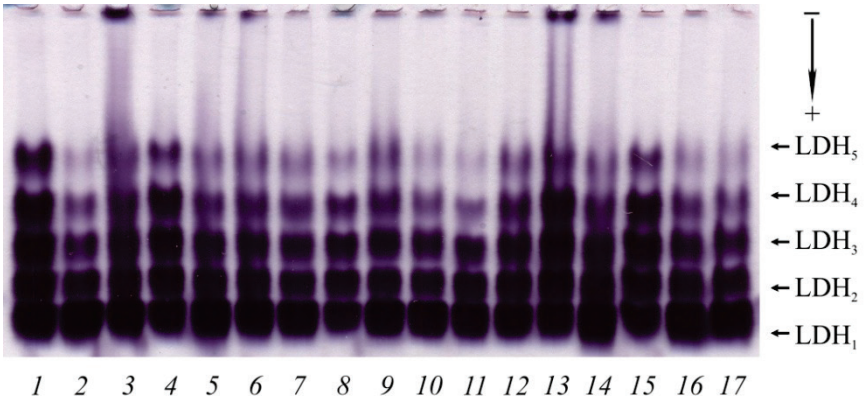

Fig. 2. LDH isoenzymes in ovarian tissues at time of:
hypofunction – *1–5*; "early corpus luteum" – *6–9*; "late corpus luteum" – *10–13*; "follicular growth" – *14–17*

Table 3
Activity and content of LDH isoenzymes in ovarian tissue (x ± SD)

Indicators	Physiological state of the ovary			
	corpus luteum		follicular growth, n = 84	hypo function, n = 14
	early, n = 41	late, n = 32		
Activity[1]	2.03 ± 0.33^{a}	2.20 ± 0.55^{ab}	3.04 ± 0.21^{b}	2.78 ± 0.52^{ab}
Isoenzyme content[2]				
LDH_5	13.21 ± 3.06^{b}	6.80 ± 0.77^{a}	10.82 ± 2.53^{ba}	8.61 ± 2.53^{a}
LDH_4	11.23 ± 2.42^{ab}	10.09 ± 1.00^{b}	6.78 ± 1.36^{a}	11.04 ± 3.91^{ab}
LDH_3	16.40 ± 1.70^{ac}	14.00 ± 0.36^{a}	11.07 ± 1.27^{b}	16.24 ± 1.64^{c}
LDH_2	22.13 ± 3.55^{a}	19.78 ± 0.34^{a}	21.94 ± 2.63^{a}	27.45 ± 4.26^{a}
LDH_1	36.67 ± 4.81^{a}	49.34 ± 1.64^{b}	46.90 ± 2.54^{b}	36.56 ± 7.93^{a}

Note: values with different superscripts in each row are significantly different by Tukey's multiple comparison procedure ($P < 0.05$); *1* – μkat/mg protein; *2* – %.

At the same time, a low value of LDH1 was found in "early corpus luteum" (36.67 ± 4.81%) and on 12.67%, 10.23% ($P < 0.05$) higher in "late corpus luteum" and "follicular growth".

The content of LDH2 almost did not depend on the physiological state of the ovary, the value ranged from 19.78% to 22.13%. On the contrary, LDH3 content was high (16.40 ± 1.70%) in "early corpus luteum", lower by 2.40% in "late corpus luteum" and the lowest was in "follicular growth" – 11.07 ± 1.27%.

A similar result was obtained in the analysis of LDH4 – a high level was found in "early corpus luteum" (11.23 ± 2.42%) and lower by 4.45% ($P > 0.05$) in "follicular growth". The LDH5 value tended to be lower in the ovarian tissue for "late corpus luteum" (6.80 ± 0.77%), and higher by 4.02% for "follicular growth" and by 6.41% ($P > 0.05$) for "early corpus luteum". In the pathological condition (hypofunction) of the ovary, a high content of LDH2 (27.45 ± 4.26%) was manifested in the tissue, the value of which was higher, respectively, by 5.32%, 5.51% and 7.67% than in "early corpus luteum", "follicular growth" and "late corpus luteum".

At the same time, no significant difference was found between the activity of the enzyme in ovarian tissue during "follicular growth" and hypofunction (3.04 and 2.78 μkat/mg protein). Similarly, the value of the indicator did not change in "early" and "late corpus luteum" – 2.03 and 2.20 μkat/mg protein. Correlation analysis shows moderate dependence of LDH activity and content of enzyme isozymes on the physiological state and hypofunction of the gonad (LDH – r = 0.387, LDH1 – r = 0.465; LDH3 – r = 0.551; LDH4 – r = 0.336; LDH5 – r = 0.347).

Discussion

The greatest percentage of LDH1 was found in brain and testes – tissues that have high oxygen consumption, which is logical, because LDH1 mainly turns lactate in pyruvate, that later is included in Crebs cycle that is active in brain and testes tissue. LDH3 – an isoenzyme that characterizes the high lability and, regardless of substrate oxidation conditions (under conditions of aerobic or anaerobic oxidation of carbohydrates), provides cells with resynthesized ATP was found in the spleen – hematopoietic organ that needs to function regardless of oxygen quantity. The highest content of LDH5 and LDH4 were found in organs and tissues that are characterized mainly by anaerobic oxidation of substrates for ATP resynthesis liver and muscles (Augoff et al., 2004).

The wide ranges of fluctuations in the isoenzyme content indicate that the established LDH proteins spectrum, which depends on the type of oxidative metabolism in tissues (aerobic or anaerobic pathway of substrate oxidation for ATP resynthesis), can change dynamically. At the same time, the revealed individual features of the LDH isoenzymes' content can be influenced by content of substrates, microelements, hormones, biologically active substances, diseases, which in turn determine the features of metabolism and the intensity of oxidation processes which are typical only for a particular animal. The conclusion is confirmed by research conducted to study the dependence of LDH activity on the content of glucose and insulin (Hsieh et al., 2022), adrenaline (Radaković et al., 2018), Magnesium salts (Ksendzovsky et al., 2022), hormones (Radaković et al., 2018), biologically active substances (Ostapiv et al., 2015) and malignancies (Augoff et al., 2004). Therefore, the content of LDH isoenzymes characte-

rizes the intensity and advantages of aerobic or anaerobic ways of carbohydrate oxidation in tissues of an individual animal.

It is known that the growth, development and maturation of oocytes in the ovary, the formation of the corpus luteum is under the control of gonadotropic and steroid hormones. Follicle-stimulating hormone (FSH) promotes follicle growth and increases estrogen production. In turn, increased estrogen levels suppress FSH secretion and stimulate the release of luteinizing hormone (LH) and cause ovulation and development of the corpus luteum. With the development of the corpus luteum under the influence of luteotropic hormone (prolactin), the corpus luteum produces progesterone. If fertilization did not occur, the corpus luteum dissolves and the concentration of FSH increases, the next sexual cycle begins (Kosenko et al., 2005). Changes in the concentration of hormones in the cow's body during the growth of follicles intensify the blood supply to the organs of the reproductive system and, in particular, the ovaries (Ying et al., 2011). Activation of blood circulation and increased oxygen and substrate supply with blood stimulates oxidative metabolism and ATP resynthesis in ovarian tissue, which is important for the growth and maturation of oocytes in follicles (Józwik et al., 2007, Dupont et al., 2016). Therefore, there is an increase in LDH1 content, an isoenzyme that characterizes the aerobic pathway of carbohydrate oxidation, with a gradual change in the physiological state: "early corpus luteum"→ "late corpus luteum"→ "follicular growth". This ensures the physiological process of growth and maturation of oocytes in the follicles in the ovarian tissue. The following changes in the physiological state of the ovary: "early corpus luteum" → "late corpus luteum", which are under the control of progesterone and prolactin, lead to a decrease in the intensity of oxidative metabolism in the gonad and an increase in the content, respectively, of LDH4 and LDH5 isoenzymes (Clark et al., 1971; Vanithakumari et al., 1980). Changes in the content of LDH isozymes due to hypofunction of the gonads are obviously due to reduced gonadotropic activity of hypophysis and reduced reactivity of ovarian tissue to gonadotropins, as well as factors of the level and completeness of feeding, unfavorable conditions of maintenance and external environment (Kosenko et al., 2005).

Conclusion

Brain, spleen, liver, kidney, testis, muscle and blood plasma of rats and pigs were characterized by 5 catalytically active LDH isoenzymes. In muscle and liver tissue, LDH5 prevailed (limits of fluctuations (lim) 27.42–68.15%), and in the brain, testis and kidney – LDH1 (lim 21.47–78.78%). LDH3 was most abundant isoenzyme in spleen tissue (lim 24.40– 36.14%). It was found that the same activity of LDH in the tissue of the liver and testis of rats (lim 4.54–4.57 μkat/mg of protein) was provided by different LDH isoenzymes. In addition to tissue characteristics, the content of LDH isoenzymes depended on the physiological state of the organ. Thus, in the cow ovaries the activity of LDH was the same (2.78–3.04 μkat/mg of protein) under the physiological state of "follicular growth" and pathology – "hypofunction". LDH activity was lower by 0.58 and 0.78 μkat/mg of protein under "early" and "late" corpus luteum (2.03 and 2.20 μkat/mg of protein). The highest content of LDH1 (49.34 ± 1.64%) was in the physiological state of "late corpus luteum", and LDH3, LDH4 and LDH5 (16.40 ± 1.70, 11.23 ± 2.42 and 13.21 ± 3.06%) – in "early corpus luteum". Content of LDH2 was high (27.45 ± 4.26 %) in the state of ovarian dysfunction – "hypofunction". Content of LDH2 was lower and has no statistical difference (19.88 and 22.13 %) in other physiological states. Conclusion: isoenzyme spectrum of LDH was characterized by tissue specificity. Tissues with a predominance of anaerobic processes (muscle and liver) were characterized by a high content of LDH5, and with a predominance of aerobic processes (brain, testis, kidneys) – by a high content of LDH1. Spleen was characterized by a high content of LDH3. It was established that the same activity of the enzyme in the organs could be ensured by different content of LDH proteins. It has been proven that the content of individual LDH isoenzymes depends on the physiological state of the ovary.

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