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### **Distribution, etiology, course and diagnosis specificity of polymorbid internal pathology in cows**

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**Abstract.** There is introduced the idea as to polymorbidity of internal pathology in high-producing cows, experimentally and theoretically substantiated its pathogenesis. For the first time in Ukraine there was used complex approaching as to the study of clinical and functional status of high-producing cows in different keeping and feeding systems, there were determined physiological limits of biochemical indexes that are indicative to infringement of rumen digestion, carbohydrates and lipid exchanges, functional condition of liver that are the basis for elaborating informative diagnostic methods.

Polymorbid pathology was diagnosed in 32% of cows. For the diagnosis of polymorbid internal pathology in high-producing cows represented by ketosis, hepatodystrophy, rumen hypotony the most informative indexes are determining the ketone body in urine, the level of pH in rumen content, the content of butyric acid, general protein and albumins in blood serum, the results of mercury chloride test, hyperfermentemia of AsAT.

**Key words:** polymorbid internal pathology, rumen distonia, ketosis, hepathodystrophy, etiology, diagnosis, dairy cow.

**Introduction.** For the most part, the diseases of animals, including those of non-contagious etiology, are studied as mono-diseases. At the same time, in veterinary medicine, there may be found associated infectious diseases with two or more pathogens (for example, rotavirus enteritis and escherichiosis, escherichiosis and cryptosporidiosis, etc.) (Verbitskii P. et al., 2004). Internal non-infectious diseases are mostly polyetiologic, and therefore, having the same or similar causative factors, run parallel. Furthermore, because the pathology of any organ or metabolic disorders may cause complications, the pathological processes mostly extend to other organs and systems. In the national and world scientific literature there are many examples of parallel and combined course of many internal animal diseases, obstetric, surgical pathology, etc. (Levchenko V. et al., 1998; Sokoluk V. et al., 2011; Chapinal N. et al., 2012; Raboisson D. et al., 2014). Such a combination of several diseases is called a polymorbide internal pathology. Consequently, polymorbide internal pathology (Greek *poly* means many; Latin *morbus* means - a disease) is a series of diseases that have a simultaneous course and common or similar etiology and interdependent pathogenetic links, symptoms, and syndromes. At the core of the development of polymorbidity in high-yielding cows are ketosis, hepatodistrophy and forestomach hypotony (dystony) (Sakhniuk V., 2006).

**The purpose of the work** was to study the etiology, course specificity and changes in the clinical and biochemical status in cows with polymorbid internal pathology (PIP).

**Materials and methods.** Clinical and experimental studies were carried out in the winter-spring period. The Holstein cows of 1st-3rd lactation, 1–14 and 20–80 days after calving there were used in the study.

The diagnosis of PIP was based on anamnesis, clinical data, ration analysis, laboratory tests results of blood, urine and rumen content (Sakhniuk V., 2006). Biochemical parameters of blood were determined by unified methods on the BioChemical analyzer Stat Fax-4500 (USA), fraction of volatile fatty acids (PFAs) – using chromatograph "Hrom-5" (Czech Republic).

**Research results.** The polymorbid internal pathology (PIP) is represented by the development of rumen dystonia, ketosis and hepatodystrophy. Simultaneous course of the diseases was diagnosed in 217 cows, which makes up 32.0% of the cows studied. It included 34.7% (8.4–65.7) of cows 1–14 days after delivery and 31.2% (11.254.8) of cows in early lactation. Significant fluctuations in the distribution of pathology we contributed to the different structure of feeding rations and energy supply.

The main causes of the development of PIP were: a) violation of the structure of the cows rations: the share of coarse (hay and straw) fodder by the exchange energy was 6,0-16,1%, concentrates - 48,0-61,5, succulent fodder - 32,9-48,5%; the rations often lack exchange energy and dry matter content; b) a deficit in the diet of highly-fermented carbohydrates - sugar and starch, and their ratio with digestible protein was low - 1.3-1.8:1, versus 2.0-2.5:1 normally; c) feeding a large amount of concentrated feed (3.5-4.0 kg per meal, two or three meals per day. Most of the concentrates were distributed during the first half of the day; d) an imbalance of rations with macro- and microelements and vitamins; e) obesity in the dry period; e) hypodynamia.

In the most of sick cows the pathology was manifested by inhibition of general state, decreased milk production and appetite, refusal of concentrates, taste distortion, in some animals - anorexia and forestomach hypotonia (3-6 times per 5 min). One third of cows at the beginning of the disease showed a sharp decrease in milk yield (1.5-2 times), anorexia, hyperesthesia, shaky stroke, tremor of muscles, grinding of teeth. With the development of the disease there were noted further oppression, drowsiness, the animals stood with their head lowered, the reaction to the external stimuli was minimal, difficulties while standing up. The affected cows quickly lose their body condition score (up to 60-80 kg per cow). Body temperature in sick cows was at the upper limit or slightly increased. In the vast majority of the affected cows there were found the weakening of pulse and tones of the heart, tachycardia, tachypnoe. 36.4% of the cows showed an increase in the liver size (in the 12th intercostal space, the liver dropped below the hook line by 8-13 cm, and in the 11th - much lower of the horizontal line of the middle of the shoulder blade, sometimes coinciding with the edge of the costal arch, and the width of the dull zone reached 20-30 cm. Fecal masses in affected animals were often not formed, semi-liquid and even watery consistency.

The infringement of the ration structure and the feeding regime caused the development of forestomach dystonia, the reduce of the pH of the rumen content to 5.7-6.3 ( $5.9 \pm 0.02$ ), the decrease of the number of infusorians in 1.83 times and lowering the reductase activity (table 1).

Table 1 – **The indexes of rumen content in high-yielding cows, (n=391)**

Indexes	Biometric indicator	Groups		
		healthy cows	PIP cows	p <
Hydrogen Index (pH)	Lim	5,9–7,2	5,7–6,3	0,001
	M ± m	6,7±0,02	5,9±0,02	
Number of infusorians, thsd / ml	Lim	200–1350	125–975	0,001
	M ± m	657,7±14,7	358,5±36,70	
Reductivase activity, s	Lim	10–280	15–600	0,001
	M ± m	90,5±3,64	137,3±10,11	

The synthesis of volatile fatty acids (VFA) decreased ( $p < 0.001$ ) in the rumen of affected animals. In particular, the level of acetic acid, decreased to  $42.0 \pm 0.48\%$ , the propionic acid level

raised to  $28.8 \pm 0.33$  and butyric acid raised notably up to  $28.0 \pm 0,33\%$ . This lead to a decrease in the ratio between propionic and butyric acids to 1.03:1 (in healthy cows - 1.3-1.4:1) and stimulated the ketogenesis (Sakhniuk V., 2006). Digestive disorders in the cows forestomach deteriorates because of lack of fiber in the ration (77.1-85.7% of the need), which, in turn, suppresses their motor function, and also reduces the synthesis of acetic acid in the rumen (table 2).

Table 2 - **The content of volatile fatty acids and their fractions in the rumen of high-yielding cows, (n=391)**

Indexes	Biometric indexes	Groups		
		healthy cows	PIP cows	p <sub>1</sub> <
VFA content, mmol/L	Lim	50,0–175,0	45,0–195,0	0,001
	M ± m	111,4±1,53	102,6±2,14	
Including, acetic acid, %	Lim	31,7–66,9	27,2–55,0	0,001
	M ± m	47,9±0,45	42,0±0,48	
Propionic acid, %	Lim	18,8–34,9	20,9–44,2	0,001
	M ± m	27,0±0,24	28,8±0,33	
Butyric acid, %	Lim	12,6–33,4	14,2–38,0	0,001
	M ± m	21,4±0,44	28,0±0,33	
Izo-valeric acid, %	Lim	0–7,2	0–10,1	0,001
	M ± m	1,5±0,17	0,67±0,13	
Valeric acid, %	Lim	0–7,9	0–7,1	0,001
	M ± m	2,2±0,12	0,61±0,11	

In 59,3-84,8% of cows with PIP develops hyperproteinemia ( $91,6 \pm 0,71$  g/l). In 20,9% of the affected cows the total protein content was greater than 100 g/l, and its average the value was 1.2 times greater compared to clinically healthy animals (p<0.001; table 3).

Table 3 - **Protein exchange, functional state of the liver and kidneys in cows with PIP, (n=897)**

Indexes	Biometric indexes	Groups		
		Healthy cows, (n=680)	Cows with PIP, (n=217)	p <
Total protein, g/L	Lim	70,–86,0	59,4–117,7	0,001
	M ± m	77,8±0,24	91,6±0,71	
Albumines, %	Lim	36,0–50,0	11,0–38,0	0,001
	M ± m	39,2±0,19	26,7±0,73	
Mercury chloride test, ml	Lim	1,52–1,74	0,8–2,0	0,001
	M ± m	1,63±0,004	1,28±0,009	
Urea, mmol/L	Lim	3,0–5,0	0,9–9,84	0,1
	M ± m	4,0±0,05	3,72±0,19	
Kreatinine, mkmol/L	Lim	83,0–180,0	53,4–332,0	0,001
	M ± m	131,3±3,80	157,2±3,24	
AsAT, mmol/Lxhr	Lim	1,42–2,14	1,36–5,2	0,001
	M ± m	1,78±0,02	2,46±0,06	
ALAT, mmol/Lxhr	Lim	0,45–1,03	0,13–3,46	0,001
	M ± m	0,74±0,01	0,94±0,03	
GGTP, mkkat/Lxhr	Lim	0,25–0,45	0,11–1,25	0,001
	M ± m	0,35±0,004	0,47±0,01	

The deep alterations of the exchange of simple proteins and liver pathology were confirmed by the development of hypoalbuminemia (11.0-38.0%), positive coagulation tests with mercury chloride ( $1.28 \pm 0.009$  ml), copper sulfate ( $1.84 \pm 0.02$  ml) and formaldehyde (97.2% ) solutions, decreased urea (less than 3.0 mmol/l) in 39.6% of affected cows.

The increase in the activity of AsAT was established in 97.7% of with with PIP( $2.46 \pm 0.06$  mmol/Lxhr), hyperfermantemia of AlAT - in 32.3%, which is an indicator of the membrane disruption of plasmolema and mitochondria of hepatocytes. The increase of GGTP activity was found in blood serum of 49.8% of affected cows, indicating the damage to the intrahepatic bile ducts and the development of cholestasis. In 41.4% of cases, the activity of GGTP and AsAT was combined (table 3).

The profound nature of metabolic changes in the body of affected cows is confirmed by the results of glucose metabolism studies: hypoglycemia was diagnosed in 35.0-62.2% of animals ( $2.18 \pm 0.04$  mmol/L), with 37.2% of them having glucose level less than 2.0 mmol/L, and in some cases - within 1.3-1.1 mmol/L. The liver damage and metabolic abnormalities are also verified by the changes of lipid metabolism indices (table 4). In severe cases the content of total lipids and cholesterol in blood serum increased. In affected cows there was diagnosed ketonuria and in 58.6% of the animals the concentration of acetic bodies in the urine was 5-7 to 13-15 mmol/L, and in 17.2% of freshly calved cows - higher than 15 mmol/l (normally not above 1,5 mmol/L). The results attests the acute and subacute course of ketosis. In one third of the cows during severe liver disease, ketonuria is negligible (up to 1.7-3.0 mmol/L), which should be taken into account during the diagnosis of ketosis (Levchenko V., Sakhniuk V., 2004; LeBlanc S.J., Reprod Dev., 2010; Chapinal N. et al., 2012).

Table 4 - **Indicators of carbohydrate-lipid metabolism in high-yielding cows, (n=897)**

Indexes	Biometric indexes	Groups		
		Healthy cows, (n=680)	Cows with PIP, (n=217)	p <
Glucosa, mmol/L	Lim	2,04–3,32	0,64–3,66	0,001
	M±m	2,7±0,02	2,2±0,04	
Total lipids, g/L	Lim	4,50–8,50	2,12–12,6	0,01
	M±m	6,5±0,14	5,8±0,22	
Cholesterol, mmol/L	Lim	2,30–4,0	1,35–6,32	0,05
	M±m	3,1±0,15	2,7±0,15	
Triacilglicerols, mmol/L	Lim	0,18–0,64	0,05–0,58	0,001
	M±m	0,4±0,02	0,2±0,04	
β-lipoproteins, un.exten.	Lim	0,25–0,50	0,08–0,31	0,1
	M±m	0,4±0,08	0,3±0,02	

The PIP of high-yielding cows reduces the activity of thyroid follicular cells, which in turn reduces the secretion of thriiodothyronine and thyroxine in 1.6 and 2.1 times, respectively, compared to clinically healthy animals ( $p < 0.001$ ). The decrease in the concentration of T3 and T4 hormones was combined with the increase in the synthesis of thyroid stimulating hormone pituitary (TSG). The content of TSG was 4.2 times greater ( $100-850$  nMO/ml;  $330.0 \pm 88.24$ ) than in clinically healthy cows (Levchenko V., Sakhniuk V., 2004; Schulz K., 2014).

Thus, the violation of the feeding regimen and the structure of rations for dry and lactating cows predetermine the development of multiply or polymorbide internal pathology, which are mainly presented by forestomach dystonia, ketosis and hepatodistrophy.

The most informative laboratory tests for the diagnosis of multiply internal pathology in highly productive cows are determining the content of ketone bodies in the urine, pH values of the

rumen content, the proportion of butyric acid (an increase of 66.9% of affected cows), total protein and blood serum albumin (decrease in 97.8%), mercury chloride tests (positive in 97.2 and 94.9%), increased activity of AsAT (hyperfermentemia in 97.7% of affected cows). There was established direct correlative dependence ( $r = + 0.59$ ) between a violation of the albumins exchange and a positive Mercury chloride test.

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