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PHOSPHOLIPIDS CONTENT IN BULL SPERM UNDER THE INFLUENCE OF L-CARNITINE

The changes in the composition of phospholipids in the semen of bulls for the actions of different doses of L-carnitine in their diet were investigated. In bulls semen there were identified that phospholipids fractions of lizophosphatydylholin, sphingomyelin, phosphatidylinositol, fosfatydyl-serine, phosphatidylcholine, cardiolipin, phosphatidylethanolamine and phosphatidic acid. Adding to feed of L-carnitine causes a change in the ratio between different fractions of phospholipids. The probable increase in the relative content of phosphatidylcholine and phosphatidylethanolamine was established. The content of lizophosphatidylholin in semen of research bulls for the entire study period was significantly decreased, which is probably associated with a decrease in intensity of free radical oxidation of phospholipids. L-carnitine has a positive effect on the metabolism of spermatozoa, stabilizing their structure and increasing the protective capabilities of sperm.

Key words: sperm, bulls, carnitine, sphingomyelin, phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine, cardiolipin, lysophosphatidylcholine, phosphatidylinositol, phosphatidic acid.

Statement of the problem. Phospholipids are the main structural components of cell membranes. They regulate the mobility and activity of membrane proteins which determine the adaptation potential of the cells [4, 11]. Great heterogeneity and multiplicity of molecular forms within individual classes of lipids can show them as compounds that determine the ultrastructural organization and function of cellular structures. The evidence of that are the specific composition of phospholipids in different types of biological membranes and specificity of lipid and fatty acid composition [4].

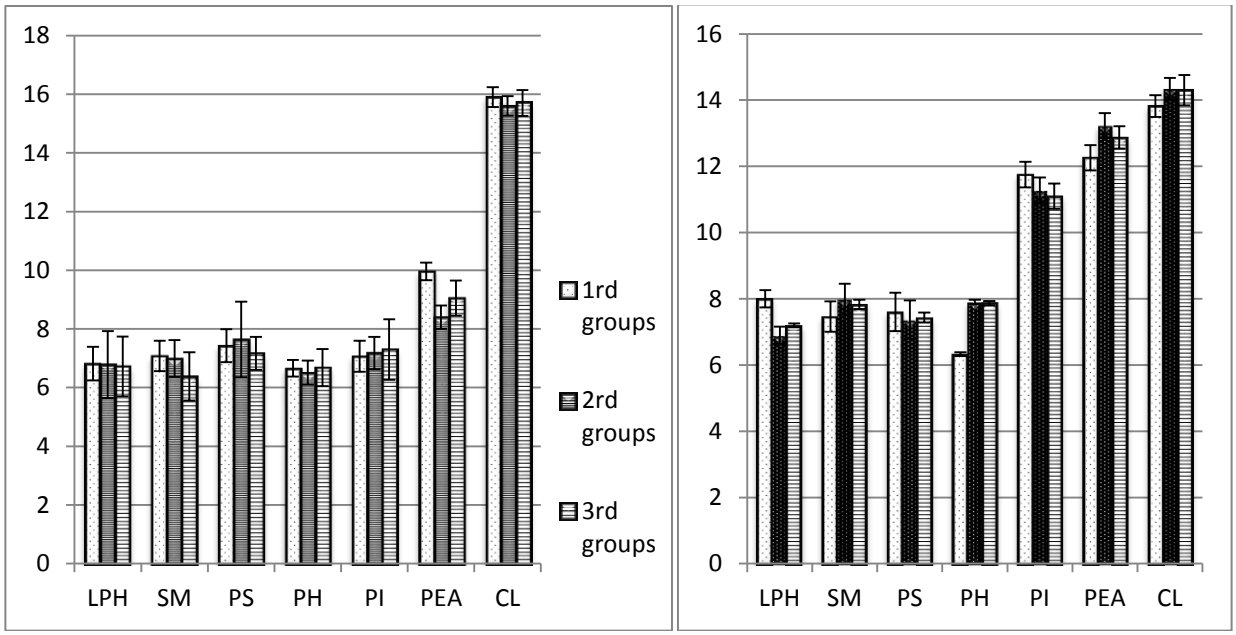
Phospholipids play an important role in the life of sperm as one of the most important biological effectors, regulators and mediators involved in almost all physiological processes. Value of certain subclasses of phospholipids, the level of unsaturation of fatty acids, which are included in their composition determine the fluidity of the lipid bilayer membrane affecting the ordering of the lipid molecules and the nature of lipid-lipid and protein-lipid interactions [5]. Increase of the lipid phase of the plasma membrane microviscosity leads to decrease of membrane-bound enzymes activity and violation of other important cell processes [8, 12].

It is known [4] that the exchange and recovery of membrane phospholipid fatty acid composition depends on the availability of long-chain acyl-CoA. In this respect, the role of L-carnitine is defined as acyl residue supply costs without ATP levels and support cellular CoA-SH at the required level. However, the available datas in the scientific literature of this kind are fragmentary and insufficient for broad generalizations. In particular, there is little information about the effects of L- carnitine as vitamin-like substance. In this way L-carnitine is the active metabolite of lipid metabolism which cause changes in the composition of phospholipids in the semen. The relevance of these studies is due to the central position of phospholipids in ensuring the ultrastructure and function of the sperm cell membrane [1]. In this regard, the aim of our study was to investigate changes in the composition of phospholipids in the bulls semen under different doses of L-carnitine in their diet.

Materials and methods of research. The study was conducted on the basis of the genetic Ukrainian company «UGC» and the Institute of animal biology at the NAAS. According to the analogues principle there were formed three groups of bulls with 4 heads each. Bitterns of the 1st group received standard feed (regular diet) and served as a control, and the 2nd and 3rd bitterns groups were fed for 75 days in addition to the basic diet with the L-carnitine (commercial name "Karnipas", produced by Loman animal health, Germany) in the amount of 20 g / head and 40 g / head accordingly.

The native sperm served as the material for the study. It was mixed with the Bioexel medium for dilution (1:1), the Folch's method was used to extract lipids [10]. Phospholipids were separated by one-dimensional thin chromatography layer[1], followed by their identification with the color reaction. The obtained statistical data was processed by Microsoft Excel.

Results and discussion. By the thin chromatography layer the following classes of phospholipids in the bulls semen were identified: lizophospfatidylholin (LPH), sphingomyelin (SM), phosphatidylserine (PS), phosphatidylcholine (PH), phosphatidylinositol (PI), phosphatidylethanolamine (PEA), cardiolipin (CL) (Fig. 1).



a) b)

Fig. 1. **The phospholipids fractions content in the bulls semen before the introduction of L-carnitine (a) and after 75 days of its use (b),** %.

The activation of phospholipids' anabolism was found in bulls semen with the influence of L-carnitine in both research groups. The activation goes mainly due to an increase in the relative content of phosphatidylcholine and phosphatidylethanolamine (Table 1).

At the beginning of the experiment the level of phospholipids was approximately the same in all groups of bulls, but later a gradual increase was noted in their content in groups treated with the investigational drug. Thus, the relative content of phosphatidylcholine (Fig. 2) significantly increased and after 75 days of feeding Karnipasu was higher compared to the control by 25% ($p < 0,001$) in the second group and by 24,3% ($p < 0,001$) – in the third. That probably is a consequence of reduced activity of phospholipase A2, an enzyme that catalyzes its hydrolysis from the phosphatidic acid [6, 7].

Table 1 – **Content of phospholipids in the bull-sires semen under the action of L-carnitine,** % (M ± m; n = 4)

Index	GROUPS		
	1 – control	2 – research (20 g/head)	3 – research (40 g/head)
1	2	3	4
Before the introduction			
Lysophosphatidylcholine	6,82±0,57	6,79±1,14	6,72±1,02
Sphingomyelin	7,08±0,52	6,99±0,63	6,38±0,82
Phosphatylserine	7,43±0,56	7,64±1,29	7,17±0,56
Phosphatidylcholine	6,66±0,28	6,51±0,41	6,68±0,63
Phosphatidylinositol	7,07±0,53	7,18±0,55	7,29±1,03
Phosphatidylethanolamine	9,96±0,30	8,35±0,40	9,05±0,59
Cardiolipin	15,91±0,34	15,6±0,33	15,71±0,44
Phosphatidic acid	39,83±0,27	40,95±1,16	40,99±0,49
After 27 days from the start of introduction			
Lysophosphatidylcholine	8,07±0,66	7,73±0,49	7,74±0,23
Sphingomyelin	7,5±0,43	7,87±0,24	7,94±0,30
Phosphatylserine	6,72±0,27	7,06±0,52	7,67±0,63
Phosphatidylcholine	7,78±0,27	8,46±0,13	8,96±0,67
Phosphatidylinositol	11,74±0,32	11,42±0,5	11,1±0,42
Phosphatidylethanolamine	11,7±0,59	12,81±0,57	12,82±0,58
Cardiolipin	13,11±0,21	12,95±0,26	11,96±0,52
Phosphatidic acid	33,37±0,83	31,69±0,38	31,8±0,05
After 75 days from the start of introduction			
Lysophosphatidylcholine	8,02±0,26	6,93±0,22*	7,25±0,06*

Sphingomyelin	7,46±0,46	7,99±0,18	7,83±0,14
Phosphatylserine	7,6±0,58	7,37±0,21	7,43±0,15
Phosphatidylcholine	6,33±0,06	7,91±0,04***	7,87±0,06***
Phosphatidylinositol	11,75±0,39	11,27±0,38	11,09±0,38
Phosphatidylethanolamine	12,26±0,35	13,23±0,34	12,87±0,32
Cardiolipin	13,82±0,33	14,34±0,08	14,31±0,46
Phosphatidic acid	32,65±0,85	30,94±0,92	31,35±0,27
After 22 days from the end of introduction			
Lysophosphatidylcholine	7,97±0,17	7,01±0,11**	6,98±0,06**
Sphingomyelin	7,43±0,24	7,57±0,19	7,70±0,14
Phosphatylserine	7,57±0,45	7,47±0,19	7,49±0,08
Phosphatidylcholine	6,42±0,08	7,79±0,07***	7,84±0,04***
Phosphatidylinositol	11,57±0,26	11,30±0,27	11,31±0,21
Phosphatidylethanolamine	12,13±0,09	13,08±0,33*	13,12±0,06***
Cardiolipin	14,00±0,21	14,46±0,05	14,46±0,28
Phosphatidic acid	32,88±0,38	31,31±0,28*	31,08±0,06**

Note. * – P <0,05; ** – P <0,01; *** – P <0,001, results are compared with the values of parameters in the control group.

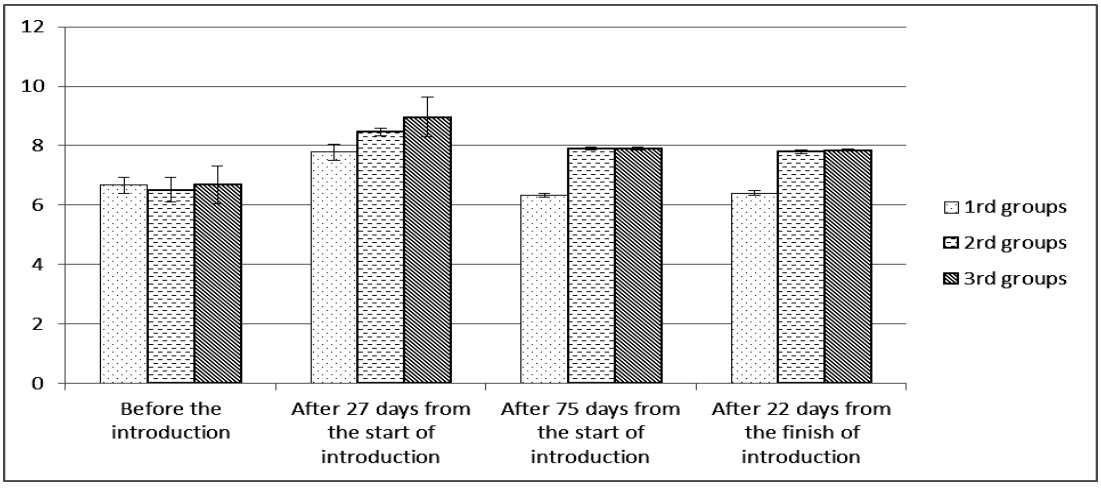


Fig. 2. **Dynamics of the relative content of phosphatidylcholine in the bulls' semen under the influence of L-carnitine (%).**

This view was confirmed by a significant reduction in the final product of enzymatic hydrolysis FH – lizophosphatidylholin (Fig. 3). Its number in the animals' semen of the 2nd and 3rd groups decreased respectively by 13,6% (p <0,05) and 9.7% (p <0,05). The same trend continued until the end of the experiment and it had a prolonged effect after feeding supplements. The increase of phosphatidylcholine in the 2nd and 3rd groups was by 21,3% (p <0,001) and 22,1% (p <0,001) with a parallel reduction of lizofosfatidylholin – 12 % (p <0,01) and 12,4% (p <0,01) respectively, compared to the control animal groups.

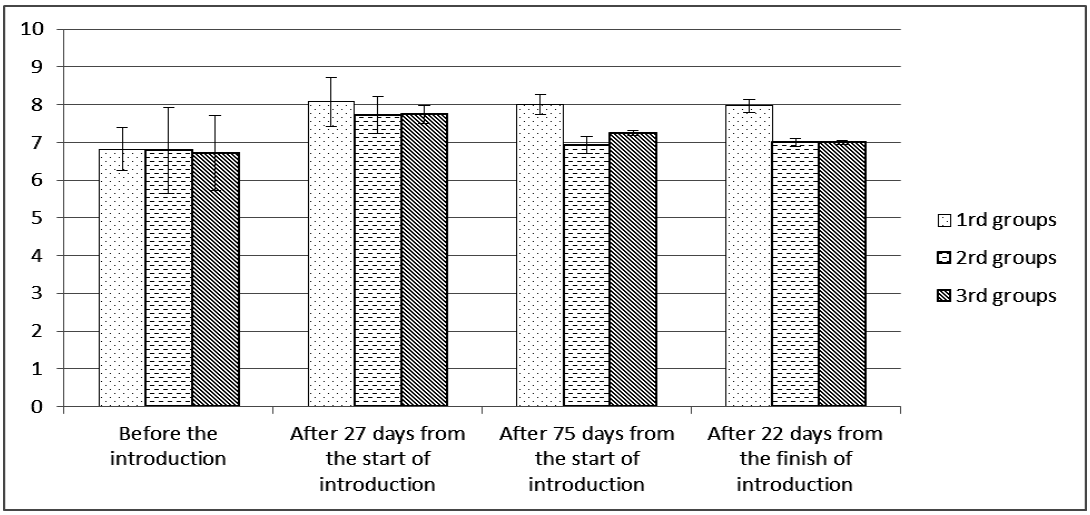


Fig. 3. **Dynamics of relative lizophosphatidylholin content in bull' semen under the L-carnitine action (%).**

During the experiment there was also found the tendency to increase the relative content of phosphatidylethanolamine, that reached a maximum value at the end of the experiment. Thus, the index values were significantly higher in the 2-nd group by 7,8% ($p < 0,05$) and in the 3rd group – by 8,2% ($p < 0,001$) compared with control after 22 days from the end of the feeding by specified supplements. The increase of the phosphatidylethanolamine content may be due to the fact that this phospholipid is involved in many physiological processes: reactions of detoxification, energy metabolism, activation and regulation of lipase activity of various transmembrane proteins consonant with the literature [13].

At the beginning of the experiment bulls' sperm showed the maximum relative cardiolipin content, which is the specific mitochondrial phospholipid (15.7 % of total phospholipids). During the first period of the experiment the cardiolipin content showed a downward trend in the animals of groups 1 and 2, and after 75 days from the start of supplements and before the end of the experiment gradually increased and it had the same values in both experimental groups, which was by 3.8 % higher compared to the control. During the experimental period, significant changes in the dynamics of the relative content of other fractions of phospholipids in the bulls' semen were not found.

Analysis of the research results found little variation phospholipid fractions in sperm that obviously connected with the influence of hot summer season, individual characteristics of bulls and technology to prepare them for semen collection.

An important aspect is to establish the fact of increasing the phosphatidylcholine and phosphatidylethanolamine number in sperm of experimental animals. These phospholipids contain highly sensitive to the action of AFO polyunsaturated fatty acids and therefore they are the important substrate for free radicals. Changes of their contents may indicate the direction of free-radical processes of lipid peroxidation in bulls semen. The intensity of lipid peroxidation processes influenced by other substances, including lizofosfatidylholin which can activate lipid peroxidation. Thus, the lizofosfatidylholin content in bulls' semen for the entire experimental period of the study was significantly decreased, which is probably due to the increase in the content of phosphatidylcholine and phosphatidylethanolamine and reduced intensity of free radical oxidation of phospholipids by the action of carnitine. This confirms the relationship of activation of lipid peroxidation with one of the manifestations of the lipid triad injury, which is called lizofosfatidylholin accumulation [2]. Reduction of phosphatidic acid in the semen was admited in animals fed with L-carnitine. It can be explained by the fact that it is a product of the phospholipase enzymes action, the activity of which is reduced by the action of additives, acting in cellular matrix as second messengers [10].

The addition to feed L-carnitine causes a change in the ratio between different fractions of bull sperm phospholipids. In particular, it reduced the lizofosfatidylholine content on background of relative increase of phosphatidylcholine, phosphatidylethanolamine and cardiolipin. This suggests that L-carnitine has a positive effect on sperm metabolism, stabilizing their structure and increasing the protective capabilities of sperm.

Prospects for further research. It is interesting to study the correlative patterns of changes in phospholipid composition and content of the lipid peroxidation products in blood and semen of animals in interrelation with physiological indicators of sperm.

LIST OF REFERENCES

1. Болдырев А. А. Введение в биохимию мембран / А.А. Болдырев. – М.: Высшая школа, 1986. – 109 с.
2. Климов А.Н. Обмен липидов и липопротеидов и его нарушения / А.Н. Климов, Н.Г. Никульчева. – СПб : Питер Ком, 1999. – 512 с.
3. Клиническая лабораторная диагностика в ветеринарии. Справочное издание / И.П. Кондрахин, Н.В. Курилов, А.Г. Малахов и др. – М. : Агропромиздат, 1985. – С. 102–106.
4. Копелевич В.М. Витаминоподобные соединения L-карнитин и ацетил-L-карнитин: от биохимических исследований к медицинскому применению // Укр. біохім. журн., 2005. – 77 (4). – S. 30–50.
5. Янович В.Г. Обмен липидов у животных в онтогенезе / В.Г. Янович, П.З. Лагодюк. – М. : Агропромиздат, 1991. – 317 с.
6. Abe A. The measurement of lysosomal phospholipase A_2 activity in plasma / A. Abe, R. Kelly, J.A. Shaymana // J. of Lipid Research, 2010. – Vol. 51. – P. 2464–2470.
7. Role of membrane lipids in developing insulin resistant diabetes mellitus type II in Caucasians and African Americans / H.G. Allen, J.C. Allen, L.C. Boyd, G. Fenner // Nutrition. – 2006, Nov-Dec. – Vol. 22. – C. 1096–1102.
8. Dynamics and function of phospholipase D and phosphatidic acid during phagocytosis / M. Corrotte, S. Chasserot-Golaz, P. Huang et al. // Traffic. – 2006. – N 7. – P. 365–377.
9. Polyunsaturated fatty acids in plasma lipids of obese children with and without metabolic cardiovascular syndrome / T. Decsi, G. Csabi, K. Torok et al. // Lipids. – 2000. – № 35. – P. 1179–1184.
10. Folch J. Asimple method for the isolation and purification of total lipids fromanimal tissues / J. Folch, M. Lees, G. Stauley // J. Biol. Chem. – 1957. – V. 226. – P. 497.
11. Diacylglycerols and phosphatidates: which molecular species are intracellular messengers? / M. Hodgkin, T. Pettitt, A. Martin et al. // Biochem. Sci. – 1998. – № 23 (6). – P. 387–393.
12.Laxalt A. M. Phospholipid signalling in plant defence / A. M. Laxalt, T. Munnik // Curr. Opin. Plant Biol. – 2002. – V. 5. – P. 332–338.
13. Nkondjock A. Specific fatty acids and human colorectal cancer: an overview / A. Nkondjock, B. Shatenstein, P. Maisonneuve // Ghadirian Cancer Detect Prev. – 2003.–№ 27. – C. 55–62.
14. Shivendra D. Shukla Metabolic turnover of ethanol into cellular lipids and platelet activating factor / D. Shivendra // Alcohol Clin. Exp. Res. – 2001. – Vol. 25, N 5, Suppl ISBRA. – P. 33–39.

REFERENCES

1. Boldyrev A. A. Vvedenie v bioximiyu membran / A.A. Boldyrev. – M.: Vysshaya shkola, 1986. – 109 s.
2. Klimov A.N. Obmen lipidov i lipoproteidov i ego narusheniya / A.N. Klimov, N.G. Nikulcheva. – SPb : Piter Kom, 1999. – 512 s.
3. Klinicheskaya laboratornaya diagnostika v veterinarii. Spravochnoe izdanie / I.P. Kondraxin, N.V. Kurilov, A.G. Malaxov i dr. – M. : Agropromizdat, 1985. – S. 102–106.
4. Kopelevich V.M. Vitaminopodobnye soedineniya L-karnitin i acetil-L-karnitin: ot bioximicheskix issledovanij k medicinskomu primeneniyu // Ukr. bioxim. zhurn., 2005. – 77 (4). – S. 30–50.
5. Yanovich V.G. Obmen lipidov u zhivotnyx v ontogeneze / V.G. Yanovich, P.Z. Lagodyuk. – M. : Agropromizdat, 1991. – 317 s.
6. Abe A. The measurement of lysosomal phospholipase A_2 activity in plasma / A. Abe, R. Kelly, J.A. Shaymana // J. of Lipid Research, 2010. – Vol. 51. – P. 2464–2470.
7. Role of membrane lipids in developing insulin resistant diabetes mellitus type II in Caucasians and African Americans / H.G. Allen, J.C. Allen, L.C. Boyd, G. Fenner // Nutrition. – 2006, Nov-Dec. – Vol. 22. – S. 1096–1102.
8. Dynamics and function of phospholipase D and phosphatidic acid during phagocytosis / M. Corrotte, S. Chasserot-Golaz, P. Huang et al. // Traffic. – 2006. – N 7. – P. 365–377.
9. Polyunsaturated fatty acids in plasma lipids of obese children with and without metabolic cardiovascular syndrome / T. Decsi, G. Csabi, K. Torok et al. // Lipids. – 2000. – № 35. – P. 1179–1184.
10.Folch J. Asimple method for the isolation and purification of total lipids fromanimal tissues / J. Folch, M. Lees, G. Stauley // J. Biol. Chem. – 1957. – V. 226. – P. 497.
11. Diacylglycerols and phosphatidates: which molecular species are intracellular messengers? / M. Hodgkin, T. Pettitt, A. Martin et al. // Biochem. Sci. – 1998. – № 23 (6). – P. 387–393.
12. Laxalt A. M. Phospholipid signalling in plant defence / A. M. Laxalt, T. Munnik // Curr. Opin. Plant Biol. – 2002. – V. 5. – P. 332–338.
13. Nkondjock A. Specific fatty acids and human colorectal cancer: an overview / A. Nkondjock, B. Shatenstein, P. Maisonneuve // Ghadirian Cancer Detect Prev. – 2003. – № 27. – C. 55–62.
14. Shivendra D. Shukla Metabolic turnover of ethanol into cellular lipids and platelet activating factor / D. Shivendra // Alcohol Clin. Exp. Res. – 2001. – Vol. 25, N 5, Suppl ISBRA. – P. 33–39.

Вміст фосфоліпідів у спермі бугаїв за дії L-карнітину

В.А. Коберська, С.І. Цехмістренко

Досліджували зміни складу фосфоліпідів у спермі бугаїв за дії різних доз L-карнітину в їх раціоні. У спермі бугаїв-плідників виявлені фосфоліпіди фракцій лізофосфатидилхоліну, сфінгомієліну, фосфатидилінозитолу, фосфатидилсерину, фосфатидилхоліну, кардіоліпіну, фосфатидилетаноламіну та фосфатидної кислоти. Додавання до комбікорму

L-карнітину спричинює зміни у співвідношенні між окремими фракціями фосфоліпідів. Встановлено вірогідне збільшення відносного вмісту фосфатидилхоліну та фосфатидилетаноламіну. Вміст лізофосфатидилхоліну в спермі дослідних бугаїв за весь період дослідження достовірно зменшувався, що, ймовірно пов’язано із зниженням інтенсивності процесів вільнорадикального окиснення фосфоліпідів. L-карнітин позитивно впливає на метаболізм сперміїв, стабілізуючи їх структуру та підвищуючи захисні можливості сперми.

Ключові слова: сперма, бугаї, карнітин, сфінгомієлін, фосфатидилсерин, фосфатидилетаноламін, фосфатидилхолін, кардіоліпін, лізофосфатидилхолін, фосфатидилінозитол, фосфатидна кислота.

Содержание фосфолипидов в сперме быков под действием L-карнитина

В.А. Коберская, С.И. Цехмистренко

Исследовали изменения состава фосфолипидов в сперме быков при действии различных доз L-карнитина в их рационе. В сперме быков-производителей обнаружены фосфолипиды фракций лизофосфатидилхолина, сфингомиелина, фосфатидилсерина, фосфатидилинозитола, фосфатидилхолина, кардиолипина, фосфатидилэтаноламина и фосфатидной кислоты. Введение в комбикорм L-карнитина изменяет соотношение между отдельными фракциями фосфолипидов. Установлено достоверное увеличение относительного содержания фосфатидилхолина и фосфатидилэтаноламина. Содержание лизофосфатидилхолина в сперме опытных быков во всем периоде исследования достоверно уменьшалось, что вероятно связано со снижением интенсивности процессов свободнорадикального окисления фосфолипидов. L-карнитин положительно влияет на метаболизм спермиев, стабилизируя их структуру и повышая защитные возможности спермы.

Ключевые слова: сперма, быки, карнитин, сфингомиелин, фосфатидилсерин, фосфатидилэтаноламин, фосфатидилхолин, кардиолипин, лизофосфатидилхолин, фосфатидилинозитол, фосфатидная кислота.

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