Lipid composition of blood plasma and epithelium of the jejunal mucosa in calves with dyspepsia and its correction

V. A. Gryshchenko*, O. O. Danchenko**, S. A. Tkachuk*, T. I. Fotina***, V. V. Zazharskyi****, V. V. Brygadyrenko*****

*National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine
**Dmytro Motorny Tavria State Agrotechnological University, Melitopol, Ukraine
***Samy National Agrarian University, Sumy, Ukraine
****Dnipro State Agrarian and Economic University, Dnipropetrovsk, Ukraine
*****Oles Honchar Dnipro National University, Dnipropetrovsk, Ukraine


Enteropathology in newborn ruminants significantly affects the functional formation of the digestive tract, which induces complications in other organs and systems, reducing the resistance and productivity in recovered animals. It is also characterized by metabolic disorders, particularly lipids, which adversely affects the regenerative processes at the cellular level. The material for the study was 2-day-old calves, from which 3 groups of 6 animals each were formed. 1st group – control was made up of clinically healthy animals, 2nd group – calves with toxic dyspepsia which received traditional treatment and 3rd group – calves which were additionally orally administered a liposomal form of a 1% solution of BAD “FLP-MD”. The lipid composition of blood plasma and the epithelium of the jejunal membrane of the jejunum of calves was studied by the method of thin-layer chromatography on standard Silufol plates. The lipidsogram of blood plasma of 30-day-old calves recovered from dyspepsia under traditional treatment regimen was characterized by dyslipidemia, which was manifested by an increase in total lipid content by 100.0% amid hypercholesterolemia and hypertriglyceridemia and a decrease in free fatty acids by 24.6% and phospholipids by 11.8%. Also, phosphatidylcholine and sphingomyelin content decreased in the blood plasma. In epithelium of the jejunal mucosa, total lipid content decreased due to total cholesterol, triacylglycerols, and phospholipids. The restoration of total lipid content and individual fractions was found in blood plasma of calves that received additionally phospholipid-containing bio-additive “FLP-MD”. Among the individual phospholipids phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine prevailed. In these calves, most of the lipid components in the epithelium of the jejunal mucosa were stabilized. In the future, we plan to investigate the content of total lipids in the blood and mucous membrane of the small intestine of calves.

Keywords: lipid fractions; phospholipid fractions; corrective therapy; phospholipids of milk.
Modern therapeutic drugs are not always highly effective when used in newborn animals with dyspepsia, which is explained by the absence of means with reparative actions in therapeutic schemes (Santrman-Berends et al., 2014; Peck et al., 2018; Gotsulya et al., 2020; Wang et al., 2022). This significantly decelerates the process of restoring the violated metabolism and structural and functional state of the intestinal mucosal epithelium in calves recovered from dyspepsia. This situation creates a problem of repeated recurrences of enteropathology in clinically healthy animals during the first month of life, the development of several complications in other organs and systems (bronchopneumonia, nephritis, hepatitis, etc.), reduced resistance and productive qualities in animals that recovered from dyspepsia (Zhukovsky et al., 2019; Zwierzchowski et al., 2020; Zazharskyi et al., 2021).

The main natural phospholipid source for restoring the lipid structure in cell membranes in the early postnatal period of animal life is colostrum, and later milk (Ellingsen et al., 2016; Gómez & Chamorro, 2017; Lopez & Heinrichs, 2022). Taking into account that phospholipids play not only a structural role, but also stimulate the biological activity of the vast majority of membrane receptors, activate membrane-bound enzymes, regulate numerous metabolic processes between the intra- and intercellular environment, and affect immune responses at the cellular level (Ontosouka et al., 2016; Kim & Wang, 2020), the biologically active additive (BAA) “FLP-MD” with reparative action based on natural phospholipids, the available raw material for which is butter milk (a by-product of processing the milk into butter), was created (Gryshchenko, 2020; Mohan et al., 2020; Wei et al., 2022). It is important that in their qualitative and quantitative spectrum they correspond to the structure of the plasma membrane, especially epithelioocytes: enterocytes and hepatocytes.

The purpose of the study is to establish the peculiarities of the lipid composition of the blood plasma and the epithelium of the mucous membrane of the jejunum in calves that have suffered non-infectious dyspepsia, and the corrective effectiveness of the dietary supplement “FLP-MD” based on milk phospholipids.

Materials and methods

During the experimental studies, the requirements were guided of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986), the Law of Ukraine “On the Protection of Animals from Cruelty” No. 3447 of 02/21/2006, and confirmed by the conclusion of the Bioethics Commission of the National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine.

The experiment was conducted on 2-day-old calves, which were divided into 3 groups of 6 animals each. At this age period, the development of non-infectious dyspepsia is mostly observed in newborn calves. The 1st group (control) includes animals that were clinically healthy during the first month of life; the 2nd group – calves with toxic dyspepsia, which were treated according to the traditional therapeutic scheme, and in the 3rd group were animals that were additionally orally administered liposomal form of 1% BAA “FLP-MD” solution. The bio-additive was fed to sick calves with milk once a day in the morning, from the jugular vein in calves. To obtain plasma, stabilized blood was centrifuged at 3000 rpm for 20 minutes. Isolated epithelial cells of the jejunal mucosa were obtained by chemical (citrate/EDTA-sodium) method. The total protein content was determined by the Lowry method (Lowry et al., 1951).

Extraction of lipids from blood plasma and samples of the intestinal mucosal epithelium was performed by the Folch method (Folch et al., 1957). The lipid spectrum was investigated by thin-layer chromatography on standard Silufol plates (Czech Republic). The following solvent system was used to separate the total lipids into fractions: petroleum ether/diethyl ether/acidic acid (90:70:1). Determination of the content of each phospholipid was carried out using the method of two-dimensional thin-layer chromatography on standard Sorbil plates (Vaskovsky et al., 1975) in solvent systems: chloroform/methanol/benzene/ammonia (65:35:10:6) and chloroform/methanol/benzene/acidic acid/water/acetone (70:50:10:5:1:4). Some classes of lipids were identified using well-known markers manufactured by “Sigma” (USA) and “Reanal” (Hungary).

Quantitative determination of individual lipid fractions was carried out spectrophotometrically using calibration graphs of their markers on an SF-56 spectrophotometer. The content of individual phospholipids was investigated by the amount of inorganic phosphorus (P) using a molybdate reagent by the Vaskovsky method (Vaskovsky et al., 1975). Individual phospholipids were identified using specific reactions and their markers, and their content – spectrophotometrically on a “Spekol 1300” spectrophotometer at λ = 850 nm using a calibration curve constructed according to P.

Statistical processing of the research results was carried out using the Statistica 6.0 program (StatSoft Inc., USA). Results are presented in the tables as x ± SD (mean ± standard deviation). Using ANOVA and Turkey’s test, the obtained results were compared in the control and experimental groups. The statistical significance of the results was assessed by P < 0.05 (taking into account the Bonferroni correction).

Results

The obtained results have shown that the lipidograms of blood plasma and epithelium of the small intestinal mucosa in calves with dyspepsia under different treatment regimens differ in their features.

Thus, the lipid spectrum of blood plasma in recovered calves under their traditional treatment regimen (2nd group) on the 30th day of life was characterized by hyperlipidemia. In particular, there was an increase in total lipids content in blood plasma by 10.1% compared with control. At the same time, a significant increase in total cholesterol by 19.5% amid an increase in free cholesterol by 33.6% and triacylglycerols by 87.5% was registered, whilst the decrease in levels of free fatty acids by 14.2% and phospholipids by 11.8% in blood plasma was detected (Table 1, Fig. 1). This is a typical picture for pathology with similar genesis, indicating the presence of lipid metabolism disorders in calves even three weeks after the disappearance of clinical symptoms. Meanwhile, this situation reflects the functional disorders of the organs involved in the regulation of lipid metabolism – the intestine and liver.

In addition, in blood plasma of calves of this group the content of phosphatidylcholine was significantly reduced by 17.1% and sphingomyelin – by 19.9%. These are the main structural components of the outer layer of cell membranes, which may decelerate the intensity of their recovery (Table 1, Fig. 1). The normalization of both total lipids’ content and some individual fractions was found in blood plasma of calves from the 3rd group (traditional treatment + BAA “FLP-MD”). As an exception, animals of this group had a significantly lower content of free fatty acids (by 26.2%). It is possible that free fatty acids are intensively used for phospholipid biosynthesis, which was confirmed by the increase in their level in blood plasma by 25% of these animals. Also, the high phospholipid content in blood plasma of calves recovered from dyspepsia under a comprehensive treatment regimen is explained by the effective absorption of exogenous phospholipid from BAA in the intestine. Among the indi-
vidual phospholipids in blood plasma of such animals, the content of phosphatidylcholine (by 23.7%), lysophosphatidylcholine (by 36.8%), phosphatidylethanolamine (by 24.8%), lysophosphatidylethanolamine (by 45.4%) and phosphatidyserine (by 25.2%) prevails in comparison with their level in the blood of calves from the 1st group (control).

Table 1 Lipid composition of blood plasma (mg/100 mL) in experimental calves on the 30th day of life (x ± SD, n = 8)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1st Group (control)</th>
<th>2nd Group (traditional treatment + BAA “FLP-MD”)</th>
<th>3rd Group (traditional treatment + BAA “FLP-MD”)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total lipids</td>
<td>4966±10.4</td>
<td>5463±5.59</td>
<td>5330±15.3</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>2501±4.6</td>
<td>2989±5.48</td>
<td>2486±3.7</td>
</tr>
<tr>
<td>– free cholesterol</td>
<td>1200±2.9</td>
<td>1603±6.74</td>
<td>1252±1.6</td>
</tr>
<tr>
<td>– esterified cholesterol</td>
<td>1301±4.9</td>
<td>1386±3.79</td>
<td>1234±6.8</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>37.8±0.8</td>
<td>28.5±1.1</td>
<td>279±0.7</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>35.1±1.9</td>
<td>65.8±1.0</td>
<td>39.7±2.8</td>
</tr>
<tr>
<td>Phospholipids, including</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>– phosphatidylcholine</td>
<td>58.7±3.7</td>
<td>48.7±1.2</td>
<td>72.6±4.4</td>
</tr>
<tr>
<td>– lysophosphatidylcholine</td>
<td>1.92±0.02</td>
<td>1.51±0.03</td>
<td>2.60±0.04</td>
</tr>
<tr>
<td>– phosphatidylethanolamine</td>
<td>52.7±1.8</td>
<td>48.4±3.2</td>
<td>65.8±2.1</td>
</tr>
<tr>
<td>– lysophosphatidylethanolamine</td>
<td>2.21±0.4</td>
<td>2.39±0.02</td>
<td>3.25±0.02</td>
</tr>
<tr>
<td>– sphingomyelin</td>
<td>113.6±3.8</td>
<td>153.1±4.3</td>
<td>216.8±7.8</td>
</tr>
<tr>
<td>– phosphatidylinositol</td>
<td>28.5±0.8</td>
<td>24.2±1.1</td>
<td>38.9±4.9</td>
</tr>
<tr>
<td>– phosphatidylserine</td>
<td>16.6±3.8</td>
<td>11.5±2.9</td>
<td>15.8±3.3</td>
</tr>
</tbody>
</table>

Note: different letters indicate values which reliably differ from one another within one line of Table according to the results of comparison using the Tukey test with Bonferroni correction (P < 0.05).

Fig. 1. Lipid composition of blood plasma in calves under their traditional treatment regimen

At the same time, in the calves from the 3rd group (traditional treatment + BAA “FLP-MD”), certain indicators of the lipid spectrum of the blood plasma underwent probable changes in relation to the animals of the 2nd group under traditional treatment (Table 1). In particular, a decrease in the content of total cholesterol by 16.8%, free cholesterol by 21.7%, and triglycerides by 39.7% was established, which is explained by the intensive restoration of lipid metabolism in their body. At the same time, there was a probable increase in the blood plasma concentration of total phosphatids by 41.6% and many phospholipid fractions, namely: phosphatidylcholine by 49.1%, lysophosphatidylcholine by 73.3%, phosphatidylethanolamine by 35.9%, phosphatidylethanolamine by 33.3%, sphingomyelin by 60.7%, and phosphatidylserine by 27.1%, which is explained by their additional exogenous supply as part of the bio-additive and effective assimilation at the level of the small intestine and liver.

The intensity of the exogenous phospholipids entering into animals significantly depends on the functional and structural state of enterocytes in the small intestine. Thus, in calves under the traditional treatment regimen for dyspepsia (2nd group), there was a significant decrease in the content of total lipids by 15.5%, total cholesterol by 18.9% and its bound form – esterified cholesterol – by 57.9%, triglycerides – by 18.4%, and phospholipids – by 12.3% in the jejunal epithelium (Table 2).

Table 2 Lipid composition (mg/100 g tissue) of the epithelium in the jejunal mucosa in experimental calves on the 30th day of life (x ± SD, n = 6)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1st Group (control)</th>
<th>2nd Group (traditional treatment + BAA “FLP-MD”)</th>
<th>3rd Group (traditional treatment + BAA “FLP-MD”)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total lipids</td>
<td>9465±5.30</td>
<td>9973±7.89</td>
<td>9106±5.94</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>1869±10.8</td>
<td>1518±8.4</td>
<td>1774±8.9</td>
</tr>
<tr>
<td>– free cholesterol</td>
<td>1358±4.0</td>
<td>1303±5.7</td>
<td>1373±6.2</td>
</tr>
<tr>
<td>– esterified cholesterol</td>
<td>511±3.7</td>
<td>215±1.6</td>
<td>401±1.4</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>130±0.6</td>
<td>101±2.6</td>
<td>92±4.0</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>85±3.4</td>
<td>700±6.1</td>
<td>67±2.3</td>
</tr>
<tr>
<td>Phospholipids, including</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>– phosphatidylcholine</td>
<td>5438±7.8</td>
<td>4767±8.7</td>
<td>5733±6.1</td>
</tr>
<tr>
<td>– lysophosphatidylcholine</td>
<td>217±9.0</td>
<td>178±7.6</td>
<td>244±5.2</td>
</tr>
<tr>
<td>– phosphatidylethanolamine</td>
<td>196±1.5</td>
<td>190±1.8</td>
<td>249±2.5</td>
</tr>
<tr>
<td>– sphingomyelin</td>
<td>1806±4.8</td>
<td>1756±4.1</td>
<td>194±4.5</td>
</tr>
<tr>
<td>– phosphatidylethanolamine</td>
<td>16±0.8</td>
<td>14±0.6</td>
<td>15±1.2</td>
</tr>
<tr>
<td>– phosphatidylserine</td>
<td>48.7±1.8</td>
<td>41±1.2</td>
<td>48.3±3.1</td>
</tr>
<tr>
<td>– phosphatidylinositol</td>
<td>23.5±2.9</td>
<td>25±1.8</td>
<td>21±9.1</td>
</tr>
<tr>
<td>“Lipid/protein”</td>
<td>0.88±0.11</td>
<td>0.87±0.10</td>
<td>0.68±0.08</td>
</tr>
</tbody>
</table>

Note: see Table 1.

Changes in the phospholipid composition of the jejunal epithelium in calves of the 2nd group have similar trends characterized by a significant decrease in the content of the main representatives of phospholipids: phosphatidylcholine – by 18.6%, phosphatidylethanolamine – by 7.4%, sphingomyelin – by 13.9%, and phosphatidylinositol – by 20.9% (Table 2).

The established patterns are shown in the Fig. 2. These trends may indicate insufficient recovery of lipid digestion and absorption in the gastrointestinal tract of recovered calves on the 30th day of life, which also indicates a violation of the structural and functional state of enterocytes of the small intestinal mucosa. However, the value of the lipid/protein ratio corresponds to the control values, which proves the fact of a simultaneous decrease in the intensity of protein synthesis in these cells.

Fig. 2. Lipid composition of the epithelium in the jejunal mucosa calves under their traditional treatment regimen

Meanwhile, the restoration of the quantitative characteristics of most indicators of the lipid spectrum in the small intestinal epithelium was detected in calves (3rd group) with dyspepsia under a comprehensive treatment regimen (Table 2). First of all, it concerned the total lipids’ content of the jejunal epithelium in recovered calves. Similar to the group of calves with the traditional treatment regimen, a significant decrease in the levels of esterified cholesterol – by 21.5% and triglycerides – by 21.3% was observed. The total cholesterol content corresponded to normal. There was also a significant decrease in the level of free fatty acids – by 28.9% in the jejunal epithelium of these animals with a significantly high phosphatidylcholine (by 5.4%). The designated quantitative redistribution of lipid fractions is probably the result of intensive use of lipid frac-
tions in restoring the structural and functional state not only of the intestinal epithelium but also other organs and tissues affected under the development of dyspepsia in newborn calves.

Among the investigated phospholipid fractions, the phosphatidylcholine content prevailed (by 11.4%), which positively characterizes the course of reparative processes in intestinal tissues (Table 2, Fig. 3). There is also a significant decrease in the lipid/protein ratio (by 22.7%) that may indicate the activation of protein-synthesizing processes in the cells of the small intestine.

In addition, in calves from the 3rd group (traditional treatment + BAA “FLP-MD”), certain indicators of the lipid spectrum of the jejunal mucosa were marked by probable changes in relation to the animals of the 2nd group under traditional treatment (Table 2). In particular, an increase in the content of esterified cholesterol by 86.5%, phospholipids by 20.3%, phosphatidylcholine by 36.8%, phosphatidylethanolamine by 10.9% and a decrease in the lipid/protein ratio by 21.8% were found, which indicates both positive changes in lipid metabolism and a significant improvement protein synthesizing function of enterocytes.

**Fig. 3. Corrective effect of BAA “FLP-MD”**

The corrective efficiency of the bio-additive is explained by the maximum correspondence of its lipid components in terms of a chemical structure and physicochemical properties to the lipid component of mammmalian cell membranes, which in conjunction gives higher biological and clinical effects.

**Discussion**

To obtain a positive result, during the treatment of toxic diarrhea in calves, a biologically active additive of reparative action "FLP-MD" based on natural phospholipids (phosphatidylcholine, sphingomyelin, phosphatidylethanolamine, phosphatidylserine and phosphatidylglycerol) was used (Li et al., 2023). Previous studies have shown that milk phospholipids exhibit significant antioxidant and reparative activity, which contributes to the effective restoration of damaged cell membrane structures and their physicochemical properties. This changes the rate constants of the lipidosis reaction (Huang et al., 2020).

As a rule, diseases that lead to intestinal inflammation affect changes in the lipid profile of blood plasma (Li et al., 2022). The level of lipids in the blood plasma of calves of the 2nd group indicates the occurrence of hyperlipidemia. At the same time, a high level of total cholesterol (due to the fraction of free cholesterol), triglycerides and a decrease in the content of free fatty acids is maintained (Coutinho et al., 2022).

In the blood plasma of calves of this group, the amount of phospholipids, which play an important role in cells as the main components of biological membranes, also decreases (Stoica et al., 2022). The most pronounced changes affect primarily the content of phosphatidylcholine and sphingomyelin (Hu et al., 2020; Lashkari et al., 2020). Like other biomolecules, phospholipids in the structure of cell membranes are frequent targets of non-enzymatic modifications upon contact with reactive substances, which leads to increased formation of end products of lipid peroxidation (Solís-Calero et al., 2015). In general, the percentage of calves requiring therapeutic interventions related to diarrhea (Ali et al., 2021; Duric & Samardzija, 2021) was higher in calves fed whole milk (Wuboren et al., 2021; Wilms et al., 2022). To date, it has been proven that diet has a significant effect on the lipid profile of the blood plasma of animals. So, animals fed with milk have a high concentration of cholesterol, low and high density lipoproteins in the blood plasma. At the same time, the composition of the milk replacer as a fat source is the main factor influencing the synthesis of proteins important for cholesterol metabolism and the lipid profile in artificially fed calves (Łepczyński et al., 2015; Ross et al., 2021).

In calves with diarrhea, significant changes in the content of total cholesterol, triglycerides, high-, low-, and very low-density lipoproteins (P < 0.01) were confirmed in the blood plasma, which also occurred according to the results of our studies of the lipid profile of the blood plasma. The secretion of very low density lipoproteins is increased due to the high content of lipids in the liver, which is the main cause of complex dyslipidemia. At the same time, the process of formation of cholesterol-enriched lipoproteins is less important for steatosis, but it is able to model inflammation (Bozulduhan et al., 2017; Poryvaeva et al., 2019; Heeren & Scheja, 2021).

It is possible to reduce the incidence of diarrhea in calves by experimentally evaluating feed additives of different composition, which effectively improve the immune status, antioxidant capacity and intestinal microflora of calves and also, as we have experimentally proven, by using corrective therapy based on milk phospholipids, which significantly accelerates reparative processes in damaged cell membranes of the small intestine (Jaruškevičius et al., 2018; Prakasita et al., 2019; Wang et al., 2021).

**Conclusions**

As a result of studying the lipid spectrum of blood plasma and the epithelium of the small intestinal mucosa in calves recovered from dyspepsia under the traditional treatment regimen, an imbalance of the lipid component was found three weeks after clinical recovery. Thus, the lipid spect-
runs of blood plasma in such calves is characterized by hyperlipidemia amid high levels of total cholesterol (due to the fraction of free cholesterol) and triacylglycerols with a simultaneous decrease in the levels of free fatty acids and phospholipids. Among the individual phospholipids in blood plasma of recovered calves, the content of phosphatidylcholine and sphingomyelin, which are the main structural components of the outer layer of cell membranes, significantly decreases, which may decelerate the intensity of their recovery.

In these calves, there is also a significant decrease in total lipid content in the epithelium of the intestinal mucosa due to the total and esterified cholesterol, triacylglycerols, and phospholipids. Among individual phospholipids, the deficiency of phosphatidylcholine, sphingomyelin, and phosphatidyl ethanolamine is observed. The described patterns may indicate insufficient restoration of lipid digestion and absorption in the gastrointestinal tract of calves recovered from dyspepsia on the 30th day of life. Meanwhile, the value of the lipid/protein ratio corresponds to the control level, which indicates a simultaneous decrease in the intensity of protein-synthesizing processes in enterocytes.

Additional inclusion in the traditional treatment of calves with dyspepsia of the bio-additive “FLP-MD” based on milk phospholipids has a positive effect on lipid metabolism, which allows us to recommend it for the correction of lipid metabolism disorders and acceleration of the recovery processes in the affected digestive system.

The authors declare no conflict of interest.

References


