

Chapter

Research of Fat Component Safety and Pre-Clinical Evaluation of Infant Adapted Dry Milk Mixtures Physiological Effect

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Abstract

The aim of the study deals with determination of fat component safety and quality key indicators of adapted infant dry milk formulas provided by various manufacturers. The most popular in Russia adapted infant dry milk formulas were selected as study objects. It was found that the qualitative composition of the fat component of dry milk mixtures corresponds to the information placed on the package. However none of the samples under study in terms of the average composition of the prevailing fatty acids fully corresponds to human breast milk. The regulation documents of the Customs Union (TR CU 021/2011, TR CU 024/2011, TR CU 033/2013) establish only the organoleptic evaluation of the adapted breast milk formulas quality indicators. Among the fat component safety indicators only the determination of the peroxide value characterizing the accumulation of primary fat oxidation products. It was also found that the peroxide values of the studied mixtures do not exceed the regulated values. Meanwhile the samples of infant milk food made from dry milk mixtures almost all have unsatisfactory organoleptic characteristics. Defects of taste and smell are associated with the accumulation in the original adapted milk mixtures of a significant amount of secondary products of fat oxidation, which in a biological experiment on animals lead to a decrease in the content of leukocytes and a change of its blood count.

Keywords: infant adapted dry milk formulas, safety, fat component, physiological effect, histological pattern

1. Introduction

The optimum food product for an infant in the first months of life is the breastmilk which corresponds to the characteristics of its digestive system and

metabolism which ensures the adequate development of an infant's body with a rational diet of a lactating woman. Lactotrophic nutrition (breastfeeding) is the postnatal equivalent of fetal hemotrophic nutrition. It has a unique biological and emotional impact on the health of both mother and baby. All nutrients in human milk are easily absorbed if their composition and ratio correspond to the functional capabilities of the gastrointestinal tract of an infant. After the birth of a child, the "mother-placenta-fetus" system transforms into its postnatal analog "mother-mammary gland-breast milk-infant" with the preservation of the genetic link formed during prenatal development. Thus breastfeeding being an important factor in the formation of health has a multifaceted effect on infants' physical and mental development as well as the formation of their behavior and intellectual development [1, 2].

However, a significant proportion of women by means of various reasons are unable to provide their infant with natural feeding. In these cases, it is important to organize optimum artificial feeding which cannot replace breast milk. But with the proper approach may ensure the correct growth and development of the child [3]. The modern trend of bottle-feeding is connected with the use of specialized industrial baby food products, modern breast milk substitutes, so-called baby "mixtures"—adapted milk formulas. The main goal of its production is the maximal adaptation to the composition and properties of breast milk as well as taking into account the nature of the child's digestion. Approximation of the composition provided by addition of the following components: carbohydrate, mineral, vitamin, protein, and fat [4]. The need for the fat component in an infant is a maximum—44–49% of the energy value of the diet [3]. A number of studies have shown that approximating the composition of mixtures fat components closer to the composition of breast milk can contribute to a more harmonious development of the child and reduce the risk of infection [5, 6], improving the psychoemotional and physical growth and development of children at the age of 4 months [6], improves cognitive development and also has a significant impact on the intellectual development [7, 8]. Therefore, it is extremely important that the child continues to receive the fat component of the optimal composition either with breast milk or with infant formula [9].

One of the expected results of the implementation of global strategies in the field of healthy nutrition is the reduction of alimentary-dependent diseases among children providing them with adequate nutrition from the first days of life. Due to the immaturity of protective mechanisms, physiological and metabolic characteristics of young children, increased hygienic requirements are imposed on the safety and quality of infant food [10].

In the scientific literature, studies of infant formula are widely presented. They are dedicated to their compliance with the requirements of regulatory documents in terms of the quantitative composition of the components of the mixture [11], toxicological safety [12, 13], weight gain of the child, tolerance of the mixture [14]. At the same time, there're a limited number of studies dedicated to the change in the qualitative characteristics of the lipid fraction of infant formula during storage [15, 16]. However, thermal treatment of native raw materials at the stage of spray drying, a sufficiently long shelf life can provoke the oxidation of the fat component which is a potential source of danger to the body. This is due to the fact that during the oxidation process such undesirable oxidation products as glycidol ethers [17–20], epoxides, secondary oxidation products insoluble in petroleum ether are formed which have teratogenic, mutagenic, and carcinogenic effects [21, 22].

Thus, the safety requirements for the fat component of adapted milk formulas, in our opinion, are not sufficiently presented in the legal requirements of the Russian Federation and the Customs Union.

The aim of this work was a pilot study of some indicators of the safety and quality of the fat component of adapted infant dry milk formulas from various manufacturers.

2. Objects of research and research methods

The research was carried out in two stages. At the first stage among the wide range of infant formulas available on the market, the popular in Russia adapted infant dry milk formulas “IS” (Denmark), “IM”, “IN”, “IL” (Russian Federation), “IX” (Germany) were selected as research objects. The shelf life of infant formula according to the manufacturers’ documentary standards is equal to 18 months. The packages purchased through retail chains were unsealed in the initial period of their shelf life—before 6 months of expiration. The study of physical and chemical indicators was carried out immediately after opening the package. Fat for analysis was isolated from dry milk mixtures by the extraction-weight method. In the extracted fat the fatty acid composition was determined by gas-liquid chromatography of fatty acid methyl esters. Methyl esters of fatty acids were prepared according to and the compliance with the average fatty acid composition of the studied mixtures to the composition of human milk was assessed.

The legal requirements of the Customs Union (TR CU 0211/2011, TR CU 033/2013) regulate only organoleptic indicators of the quality indicators of adapted infant dry milk mixtures. However, among the safety indicators of the fat component, it regulates only the determination of the peroxide value, which characterizes the accumulation of primary products of fat oxidation. The peroxide number of the fat component of the mixtures was determined by the iodometric method. Infant milk food for organoleptic analysis was prepared according to the recommendations indicated on the package with a hydromodule of 1–3. Organoleptic analysis was carried out by a descriptive method. All experiments were carried out in triplicate.

At the second stage, *in vivo* experiments were carried out. *In vivo* biomodels allow obtaining multifactorial data on the effect on various organ systems and the entire body as a whole. The advantages of *in vivo* methods are connected with their adequacy and reliability, a high degree of correlation with the human, and the possibility of a comprehensive evaluation of properties. In this regard, the samples of mixtures were studied with the participation of biomodels which were Wistar rats. The experiment involved six groups of Wistar rats 65 days old with an initial weight of 130–210 g, corresponding to the Minimal Diseases quality standard for the control and experimental groups. In the first experimental group, the usual diet was replaced with mixture 1; in the second experimental group the usual diet was replaced with mixture 2; in the third experimental group the usual diet was replaced with mixture 3; in the fourth experimental group the usual diet was replaced with mixture 4; in the fifth experimental group, the usual diet was replaced with mixture 5 accordingly. Research on animals was carried out in accordance with the “Rules for work on experimental animals”, with Directive 2010/63/EU of the European Parliament and of the Council of the European Union of September 22, 2010, on the protection of animals used for scientific purposes, and was carried out on the basis of a certified vivarium of the Scientific and Technological Center of Veterinary hospital and laboratories of

the Department of Morphology, Pathology of Animals and Biology of the Saratov State Agrarian University named after N.I. Vavilov. All experimental studies were carried out on groups of clinically healthy rats, formed according to the principle of analogs, taking into account the breed, sex, age, live weight, and clinical condition. The animals were fed for 40 days. During the experiment, the rats were kept in individual cages (10 individuals in each). Before the introduction of the studied mixtures into the diet, animals were kept in quarantine for 21 days (to detect latent forms of diseases) and transferred to the experimental diet in accordance with the experimental plan. After the experiment, the rats were sacrificed by the optimum and universal method which was given anesthesia overdose, also known as the introduction of an anesthetic in a lethal dose (dosage for anesthesia $\times 3$), in compliance with the rules of euthanasia for animals (order of the USSR Ministry of Health No. 755 of 08/12/1977), then autopsy was performed. Samples of tissues of the liver, kidneys, spleen, small and large intestines were taken from biomodels, after fixation of which morphological and statistical studies were carried out. From paraffin blocks on a sled microtome model 2712 (Reichert Wien), histological sections with a thickness of 8 μm were obtained, processed according to conventional techniques, and stained with Ehrlich's hematoxylin and eosin, followed by microscopy [23, 24]. The morphological structure was studied on various histological sections. Histological examination of the prepared preparations was carried out under different magnifications with detailed logging and photographing of the studied areas. Microphotography of histological preparations was carried out using a CANON Power Shot A460 IS camera. Statistical processing of the results was carried out using standard methods. The work was carried out on the basis of research laboratories of the Saratov State Agrarian University named after N.I. Vavilov.

Statistical data analysis was carried out using the licensed computer software package STATISTICA 10, StatSoft, Inc. (Series 0411-R) using the methods of biomedical statistics with the calculation of the mean and standard error of the mean. Checking the normality of the distribution of quantitative data was carried out using the Shapiro-Wilks test. For all indicators including behavioral studies, with normally distributed data intergroup comparisons were made using Student's *t*-test for independent samples at $p < 0.05$.

3. Discussion of the results

Preliminary study of the ready-to-use samples of mixtures showed that almost all samples had an unsatisfactory taste and smell which is characteristic of the relatively oxidized fat components of mixtures. Therefore, for a more complete evaluation of the hydrolysis degree and oxidation of the fat component in the mixtures, the acid number of the released fat was determined by the titrimetric method, the anisidine number was determined by a method based on measuring the optical concentration of the analyzed solution after reaction with an acetic acid solution of paraanisidine. The measurement takes place in the presence of 350 nm waves. The content of secondary oxidation products insoluble in petroleum ether (CIPE) according to an increase in the intensity of the absorption band of ultraviolet radiation at a wavelength of 232 nm, which corresponds to the absorption of conjugated diene-chromophores. The content of epoxides was determined according to reactions with concentrated phosphoric acid. All studies were carried out immediately after opening the cans with mixtures.

Determination of the acid number is important for determining the degree of fat hydrolysis. This indicator is of particular importance when analyzing mixtures containing coconut and palm kernel oils, since a soapy aftertaste occurs when the mixture contains appreciable amounts of free lauric acid. In addition, during intensive hydrolysis of fats, di- and monoglycerides accumulate, which, according to modern data, can be precursors of the dangerous toxicant 3-chloropropanediol (3-MCPD), which is detected in infant milk formulas at concentrations up to 1.0 mg/kg of fat extracted from the product.

The anisidine number correlates in a certain way with the accumulation of free aldehydes (hexenal, nonenal, 2,4-decadienal), which give the product foreign smells of fish, beans, etc.

The accumulation of thermostable secondary oxidation product mixture in the fat component is characteristic of the oxidation of fats during their heat treatment. The formation of highly polar compounds, insoluble in petroleum ether (CIPE), and epoxides were found in the study of deep-fried dough products as well as during the storage of confectionery products with a high-fat content—Kurabie cookies, shortbread cookies, Chak-chack, Creamy cake, and others. In a biological experiment on animals in our previous works, it was shown that fats containing more than 1% of CIPE with systematic consumption have a negative effect on the organs of the gastrointestinal tract, sharply reduce the level of erythrocytes and leukocytes in the blood, cause the accumulation of cholesterol and bilirubin [25]. The potential for such compounds to be formed in baby foods is a major concern.

Edible fats are an essential part of an infant's diet. Reproduction of the fatty acid composition of breast milk in the creation of infant formula is a complex scientific, technological and medical problem.

According to the requirements of Article 4 of TR CU 021/2011, adapted infant milk powder should be as close in chemical composition to human milk as possible in order to meet the physiological needs of infants for the necessary substances and energy. The fatty acid composition of breast milk is characterized by a relatively high content of polyunsaturated fatty acids (PUFA), the concentration of which in mature human milk is 12–15 times higher than in cow's milk (0.4–0.5 g/100 ml versus 0.009 g/100 ml). In the infant's body, unsaturated fatty acids are either synthesized to a limited extent (monounsaturated) or not synthesized at all (PUFA) while these compounds perform the most important plastic and metabolic functions. Of the greatest importance for young children are representatives of the ω -3 and ω -6 PUFA families, of which the most significant are α -linolenic and linoleic acids. In breast milk, the ratio of PUFAs of the ω -6 and ω -3 families is optimum and ranges from 10:1 to 7:1. Under the influence of the enzyme delta-6 desaturase, these compounds are converted into long-chain polyunsaturated fatty acids which play a leading role in the development of the central nervous system of infants, the visual analyzer, the immune system, and the regulation of metabolic processes and inflammatory reactions [26, 27]. Comparative characteristics of the average composition of the predominant fatty acids of adapted infant milk formulas and human milk are presented in **Table 1**.

The data presented in **Table 1** indicate the presence of gadoleic acid C20: 1 and a sufficiently high amount of linolenic acid C18: 3 in the "IN", "IM", "IL" mixtures, which might contain non-erucid rapeseed oil. According to the analysis of the fatty acid composition, the "IN", "IM", "IL" mixtures may have almost identical recipes.

From the data presented it can be seen that the mixtures were selected, first of all, according to the content of oleic and palmitic acids. "IX" sample contains 15–20% of coconut oil, "IS" sample contains 35% of coconut oil, the rest contain 30% of coconut oil. On the contrary, the "IS" mixture contains about 20% of palm oil, the "IX" sample contains 40–45% of palm oil, the rest contain 35–40% of palm oil.

The trivial name for a fatty acid, the number of carbon atoms in the chain, and the number of double bonds	Mass fraction of fatty acid in samples, %					
	Breast milk	IS	IN	IM	IL	IX
Caprylic C8: 0	0.17	3.2	2.2	2.2	2.0	0.9
Capric C10: 0	1.66	2.5	1.8	1.7	1.6	0.8
Lauric C12: 0	5.8	17.9	13.0	12.7	11.9	9.3
Myristic C14: 0	8.6	6.6	5.4	5.3	5.2	3.6
Palmitic C16: 0	21.0	8.4	18.9	18.6	19.2	24.4
Palmitoleic C16: 1	3.4	0.1	0.2	0.2	0.2	0.2
Stearic C18: 0	8.0	2.8	3.1	3.3	3.4	3.3
Oleic C18: 1	36.5	39.3	38.0	38.3	38.7	38.1
Linoleic acid C18: 2	10.8	16.9	14.1	14.1	14.2	16.2
Linolenic C18: 3	1.0	1.5	2.4	2.7	2.6	2.2
Arachidic C20: 0	0.21	0.2	0.3	0.3	0.3	0.3
Gadoleic C20: 1	0.20	0.2	0.4	0.4	0.4	0.40
Behenic C22: 0	0.10	0.4	0.2	0.2	0.3	0.2
Mass fraction of fat, %	4.4	3.6	3.4	3.3	3.3	3.6

Table 1. Comparative characteristics of the average fatty acid composition of breast milk [11] and adapted milk powder.

The highest content of polyunsaturated acids (linoleic and linolenic) is in the “IS” and “IX” mixtures, while the most favorable ratio $\omega6/\omega3 = 11: 1$, corresponding to the characteristics of breast milk, was found in the “IS” mixture.

Mass fraction of the fat component ranges from 24.1% (“IM”) to 27.7% (“IX”).

Based on the research results, the fatty acid composition of adapted infant dry milk formulas by various manufacturers corresponds to the information indicated on the labels of the tested products.

Table 1 shows that the proposed mixtures contain an excessive amount of lauric acid and linoleic acid, with a relatively lower mass fraction of stearic acid. None of the studied samples in terms of the average composition of fatty acids fully corresponds to breast milk.

Infant formula was prepared for sensory analysis according to the recommendations indicated on the package, with a hydro module of one to three. Dispersion of dry mixtures in water occurred with the same intensity. The results of sensory analysis are presented in **Table 2**.

Baby food made from “IS”, “IN”, “IM”, and “IL” mixtures had a fishy smell of varying intensity; “IX” mixture was more herbaceous, leguminous. The sweet taste was most explicit in the “IN” infant formula and least of all in the “IM” infant formula. The consistency of food systems varied slightly with colors ranging from white to cream.

Generally, it should be noted the unsatisfactory results of sensory analysis of varying degrees of food compositions intensity. The composition of the “IX”

Sample name	Taste	Odor	Consistency	Color
IS	Fishy aftertaste	Slightly fishy	Homogeneous, light, watery	Baked milk
IN	Sweet-salty with a fishy aftertaste	Poorly expressed fishy	Homogeneous, light, non-watery	Creamy white
IM	Slightly sweetish, creamy	Slight fishy smell	Homogeneous, light, non-watery	Creamy white
IL	Sweet and salty, with a metallic aftertaste	Slight fishy smell	Homogeneous, light, slightly watery	Creamy white
IX	Bean, with a cold synthetic flavor	Herbaceous	Homogeneous, light, non-watery	Light cream

Table 2.
 The results of the sensory analysis of mixture samples.

Sample name	Acid number, mg KOH/g	Peroxid number, meq/kg	Anisidine number, cu	Mass fraction of oxidation products insoluble in petroleum ether, %	Mass fraction of oxidation products insoluble in petroleum ether, %
IS	0.5	0.4	1.9	0.3	25.6
IN	0.2	0.9	2.2	0.8	27.1
IM	0.6	1.4	3.6	1.0	31.1
IL	0.7	0.8	2.6	0.7	27.9
IX	0.4	1.1	2.9	0.6	26.3

Table 3.
 Physicochemical indicators of the safety of adapted infant dry milk formulas.

mixture has the worst performance. Thus, a negative result of sensory analysis of the prepared infant formula indicates an unacceptable level of oxidation of the fat component and the need to change the shelf life of powdered infant formula. It has been proven that the weakening of antioxidant protection and uncontrolled enhancement of lipid oxidation is one of the important links in the pathogenesis of autonomic dysfunction, atopic dermatitis, dental pathology, diabetes mellitus, arthropathies, diseases of the gastrointestinal tract, urinary tract, etc. [28–30].

Physicochemical indicators of the safety of adapted infant dry milk formulas are presented in **Table 3**.

Analyzing the data in **Table 3** we can conclude that the safety indicator standardized by TR CU 033/2013 “On the safety of milk and dairy products” known as the peroxide number is within the normal range for all samples (no more than 4 meq/kg).

The limit value of the acid number for adapted milk powder is not determined by the legal requirements of the Customs Union. According to TR CU 024/2011 “For fat and oil products” for refined oils and their fractions, mixtures of refined oils, the acid number is normalized to no more than 0.6 mg KOH/g. For three of the five studied dry milk mixtures, the acid number does not exceed this indicator. In the “IM” and “IL” samples, the content of free fatty acids in the fat component is respectively 0.6 and 0.7 mg KOH/g.

The permissible value of the anisidine number (that shows the concentration of secondary oxidation products, i.e., aldehydes) is also not regulated by the legal requirements for adapted infant dry milk formulas. In GOST 1129-2013 “Sunflower oil.” Specifications (as amended) this indicator is not more than 3 conventional units for Premium oils and Highest grade oils. In “IM” sample the anisidine number is 3.6 conventional units. The anisidine number shows the relative content of aldehydes. First of all, α - and β -unsaturated aldehydes which form yellow condensation products with reagents enter into the reaction with anisidine.

In addition, not only primary oxidation products are a risk factor but also oxidized fatty acids and their copolymerization products (CIPE), that is, secondary oxidation products. Therefore, in prototypes, this indicator was studied to identify a complete safety picture. The mass fraction of compounds insoluble in petroleum ether is regulated for waste frying fats according to SP 2.3.6.1079-01 at no more than 1%. This indicator has not yet been reflected when ensuring the safety of the fatty component of other food products, although there are a number of studies indicating the need for its regulation [31, 32]. In “IM” sample, immediately after opening, the mass fraction of CIPE was 1%. In biological studies on animals [33] it was noted that with the systematic consumption of fats containing more than 0.88% of CIPE, there are progressive negative pathological changes in the body (granular degeneration and hyperemia of the liver, desquamation processes in the intestinal wall, edema of the submucosal layer of the intestine, change in blood picture). Such changes indicate an increase in the number of eosinophils and compounds that are leukotoxins which commonly leads to disruptions in the work of the antioxidant defense systems of the body.

Some studies [34] have shown that it is the isomers of epoxyoleic acid that have the properties of leukotoxins. According to some researchers [35], the formation of an epoxy ring occurs through the reaction of a double bond in a fatty acid chain with a hydroperoxide formed in another adjacent fatty acid chain. This mechanism explains the presence of an epoxy ring at the site of the double bond, and the concomitant formation of a hydroxy function from hydroperoxide in the adjacent fatty acid chain. The formation of epoxides is accelerated by the thermal treatment of unsaturated fats which is used in spray drying of dry milk formulas.

Estimating the concentration of epoxides can be proposed as an operational method for monitoring the safety of the fat component of dry milk mixtures and other food products. In the studied infant dry milk formulas, the content of epoxides was 25–31 mmol/kg at the time of opening the can.

It should be noted that all the studied parameters of hydrolysis and oxidation of fats are at the safest level in the “IN” mixture; the indicators of the “IX” mixture are closer to the critical values.

The research results were processed by generally accepted statistical methods using the SPSS statistical program. For the statistical analysis, the Student’s *t*-test was used.

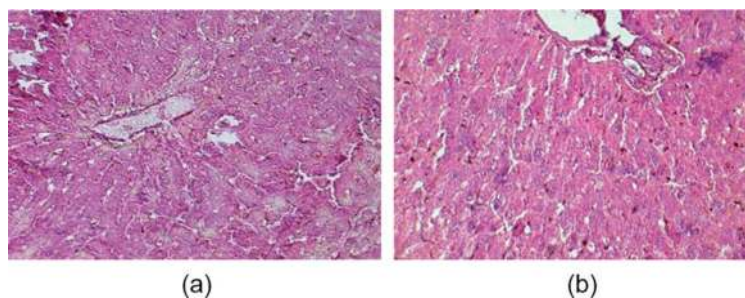


Figure 1.
Micrograph of the histological picture of the liver section of biomodels using “IN” sample (a) and the control group (b).

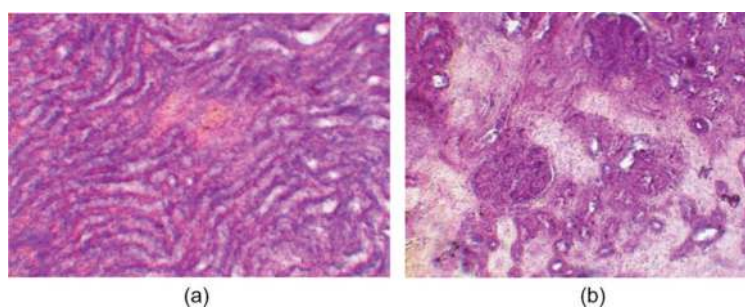


Figure 2.
Micrograph of the histological picture of a kidney picture of biomodels using “IS” (a) and “IM” (b) samples.

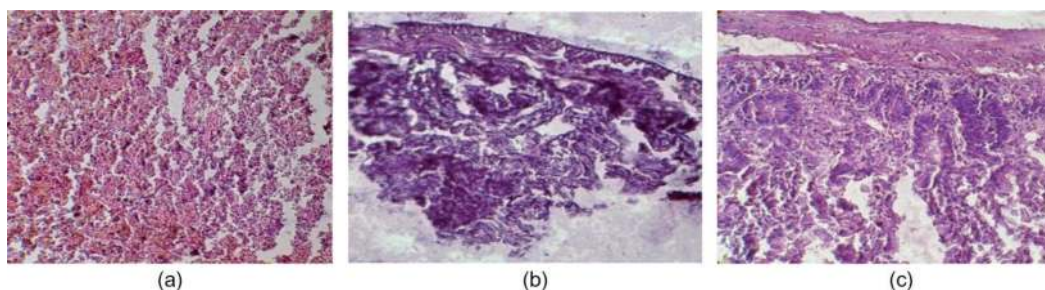


Figure 3.
Micrograph of a histological picture of a spleen section (a) and small intestine (b) of biomodels using “IL” sample, and of the large intestine of biomodels using “IX” sample (c).

In the future studies of the values of these indicators will be carried out at the end of the shelf-life period of the opened can.

3.1 Results of the second stage of research

The histological picture indicating changes occurring with a pronounced intensity is presented on micrographs (**Figures 1–3**). A detailed description of the histological picture of the tissues of the studied organs of the biomodels is presented in **Table 4**.

Structural disorders were most noticeable in Groups 4 and 5, while only in Group 1 the presence of intertubular hemorrhages was detected.

Severe suppression of lymphoid tissue in the spleen was detected in biomodels in Groups 4 and 5 and to a lesser extent in Group 1. Among all the cases studied, the structure of the wall of the small intestine was most disturbed primarily in Groups 4

Organ	Group 1IS	Group 2IN	Group 3IM	Group 4IL	Group 5IX	Control Group
Liver	Hyperemia, focal granular dystrophy, beam structure is moderately expressed.	Vessels are filled with blood, diffuse granular degeneration of hepatocytes, edema of Dissespaces, the beam structure is poorly visible.	Beam structure is poorly visible, diffuse hydroptic dystrophy in combination with granular dystrophy, edematous phenomena in the tissues.	Beam structure is absent, granular degeneration of hepatocytes, hyperemia, edema of organ tissue.	Focal hydroptic dystrophy in combination with granular degeneration of hepatocytes, the beam structure is poorly visible, hyperemia and tissue edema.	Beam structure is preserved, vessels are desolate, granular degeneration of hepatocytes.
Kidney	Focal hydroptic dystrophy in combination with granular dystrophy. Granular dystrophy of the epithelium of the tubules, the accumulation of erythrocytes between them.	Focal hydroptic dystrophy in combination with granular dystrophy of the tubular epithelium. Swelling of the tubular epithelium.	Severe hydroptic dystrophy in combination with granular dystrophy of the tubular epithelium, the vascular glomeruli are markedly enlarged.	Diffuse hydroptic dystrophy in combination with granular dystrophy of the epithelium of the tubules, the vascular glomeruli are enlarged.	Focal hydroptic dystrophy, a significant increase in vascular glomeruli with a decrease in the lumen of the Shumlyansky capsule.	Focal hydroptic dystrophy in combination with granular dystrophy of the epithelium of the tubules, the vascular glomeruli are enlarged up to the complete disappearance of the lumen of the Shumlyansky capsule.
Spleen	Thinning of the lymphoid tissue of the follicles.	Perivascular edema, moderate loss of lymphocytes in the follicles.	Some rarefaction of the lymphoid tissue of the follicles.	Decrease in the number and size of follicles with thinning of the lymphoid tissue.	Follicles are not expressed, lymphoid tissue is poorly visible.	Moderate perivascular edema and loss of lymphocytes in the follicles.
Small intestine	Slight swelling of the organ wall.	Edema of the mucous membrane and deformation of the glands.	Edema of the organ wall and deformation of the glands.	Edema of the organ wall and deformation of the glands.	Moderate edema of the mucous membrane.	Edema of the organ wall and some deformation of the glands.

Organ	Group 1IS	Group 2IN	Group 3IM	Group 4IL	Group 5IX	Control Group
Colon	Edema of the mucous membrane.	Severe edema of the mucous membrane and deformation of the glands.	Edema of the mucous membrane and deformation of the glands.	Edema and deformation of the glands.	Edem and deformation of the glands.	Edema of the mucous membrane and fragmentation of some glands.

Table 4.
Histopathological changes in the internal organs of biomodels.

and 5 and, to a lesser extent, in Groups 2 and 3. The large intestine had pronounced disturbances in the structure of the wall in the form of edema and deformation of the glands in Groups 2 and 4.

4. Conclusion

Breast milk is the natural and most physiological nutrition for a child from the first days of their life. Its composition goes beyond simple nutritional support and is the most important postnatal factor in the metabolic and immunological programming of health. A high nutritional and functional potential of breast milk has been established indicating the biological advantage and fundamental indispensability of breast-feeding for the optimum development of both healthy and sick child. However, a significant proportion of women for various reasons are unable to provide their child with natural feeding. In these cases, it is important to use optimum, high-quality, and safe bottle-feeding. As a result of the study of fat component safety of the adapted infant dry milk formulas "IM", "IS", "IN", "IL", "IX" it was found that the qualitative composition of the fat fraction corresponds to that stated on the package. None of the samples under study in terms of the average composition of prevailing fatty acids fully corresponds to the composition of human milk. According to an important indicator, that is, the ratio of polyunsaturated fatty acids $\omega 6/\omega 3$, the "IS" mixture is distinguished by the optimum value (11:1).

As for the organoleptic characteristics food systems made from dry mixtures "IS", "IN", "IM", and "IL" had a fishy smell of varying intensity whereas "IX" mixture had a more herbaceous smell. Sweet taste was most pronounced in the "IN" formula and least of all in the "IM" formula. The consistency varied slightly, the color varied from white to cream. A negative organoleptic evaluation commonly indicates an unacceptable level of oxidation of the fat component of dry milk mixtures that causes the need to adjust the shelf life of dry milk mixtures.

Safety indicators of the fat component regulated by TR CU 021/2011, TR CU 024/2011, TR CU 033/2013 are not fully respect the safety requirements for the fat component of adapted dry milk mixtures since there are no standards for the most important safety indicators of fats, that is, the content of secondary oxidation products CIPE and epoxides. According to our opinion, the studies carried out necessitate further research of the parameters of the adapted dry milk mixtures fat component during storage after the package is opened by the consumer.

Histopathological changes were most regularly observed in the studied organs of Group 5 biomodels. Slightly less intensity of pathologies was expressed in Group 4. At the same time, in almost all other groups of biomodels, pathological processes of varying severity were detected in organs, although in some cases it is possible to assume their reversibility.

Thus, the conducted biological experiment previously proved that the use of "IX" and "IL" mixtures has a certain negative impact on the internal organs of biomodels.

The revealed information requires further study using modern research methods. The data obtained indicate the need for a precise evaluation of the production technology, packaging, and storage of adapted infant dry milk formulas, as well as the advisability of improving the legal regulation of the Russian Federation and the Customs Union, in order to strengthen and control the safety of adapted infant dry milk formulas, fat component, and changes in the shelf life of these products.

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References

- [1] Gmoshinskaya MV, Surzhik AV, Ya KI, Georgieva OV, Safronova AI, Pustograev NN, et al. Indicators of physical and psychomotor development of infants in the first months of life who received an adapted milk mixture with prebiotics, nucleotides and long-chain polyunsaturated fatty acids [electronic resource], Abramova TV. [and others]. *Questions of Practical Pediatrics*. 2015;(3):24-31
- [2] Barabash NA, Melnikova UV, Pozhenko VV, Hypogalactia. Modern methods of correction and prevention: Guidelines. Tomsk: OOO "Offset Center". 2016:28
- [3] Tutelyan VA, Kon IY, editors. *Infant Food: A Guide for Doctors*. 4th ed. Moscow, MIA: Rev. and add; 2017. p. 784
- [4] Petrenko AV. Modern approaches to artificial feeding of children. In: Petrenko AV, editor. *New Science: Experience, Traditions, Innovations: International Scientific Periodical Following the Results of the International Scientific and Practical Conference (August 12, 2016, Omsk).*, Part 2. Sterlitamak: AMI; 2016. pp. 13-17
- [5] Gould JF, Roberts RM, Makrides M. The influence of Omega-3 long-chain polyunsaturated fatty acid, docosahexaenoic acid, on child behavioral functioning: A review of randomized controlled trials of DHA supplementation in pregnancy, the neonatal period and infancy. *Nutrients*. 2021;**13**(2):415. DOI: 10.3390/nu13020415
- [6] Koletzko B. Human milk lipids. *Annals of Nutrition and Metabolism*. 2016;**69**(Suppl 2):28-40. DOI: 10.1159/000452819. Epub 2017 Jan 20
- [7] Verfürden ML, Dib S, Jerrim J, Fewtrell M, Gilbert RE. Effect of long-chain polyunsaturated fatty acids in infant formula on long-term cognitive function in childhood: A systematic review and meta-analysis of randomised controlled trials. *PLoS One*. 2020;**15**(11):e0241800. DOI: 10.1371/journal.pone.0241800
- [8] Verfürden ML, Gilbert R, Lucas A, Jerrim J, Fewtrell M. Effect of nutritionally modified infant formula on academic performance: Linkage of seven dormant randomised controlled trials to national education data. *BMJ*. 2021;**375**:e065805. DOI: 10.1136/bmj-2021-065805
- [9] Shilina NM. An expert look at the role of fats in baby food. *Pediatric Pharmacology*. 2014;**11**(1):38-42
- [10] *Baby Nutrition. A Guide for Physicians* Ed. V.a. Tutel'yana, I. Ya. Konya. 4th ed. Moscow: Medical Information Agency LLC; 2017. p. 784
- [11] Martin CR, Ling PR, Blackburn GL. Review of infant feeding: Key features of breast milk and infant formula. *Nutrients*. 2016;**8**(5):279. Published 2016, May 11. DOI: 10.3390/nu8050279
- [12] Phipps R, Lynch B, Stannard DR, Gilby B, Baldwin N, Mikš MH, et al. Genotoxicity and neonatal subchronic toxicity assessment of a novel mixture of the human-identical milk oligosaccharides lacto-N-fucopentaose I and 2'-fucosyllactose. *Journal of Applied Toxicology*. 2020;**41**(4):632-649. DOI: 10.1002/jat.4071
- [13] Rodríguez-Alcalá LM, Calvo MV, Fontecha J, Alonso L. Alterations in the fatty acid composition in infant formulas and ω 3-PUFA enriched UHT Milk during storage. *Food*. 2019;**8**:163

- [14] Timby N, Domellöf M, Lönnerdal B, Hernell O. Comment on "safety and tolerance evaluation of Milk fat globule membrane-enriched infant formulas: A randomized controlled multicenter non-inferiority trial in healthy term infants". *Clinical Medical Insights Pediatrics*. 2015 Jun;**18**(9):63-64. DOI: 10.4137/CMPed.S27185
- [15] MacMahon S, Begley TH, Diachenko GW. Occurrence of 3-MCPD and glycidyl esters in edible oils in the United States. *Food Additives & Contaminants, Part A: Chemistry, Analysis, Control, Exposure & Risk Assessment*. 2013;**30**:2081-2092
- [16] Kent G. Regulating fatty acids in infant formula: Critical assessment of U.S. policies and practices. *International Breastfeeding Journal*. 2014;**9**(1):2. DOI: 10.1186/1746-4358-9-2
- [17] Makarenko MA, Malinkin AD, Bokov DO, Bessonov VV. Monochloropropanediols, glycidol and their esters in children's nutrition. *Vopr.det. dietol. (Pediatric Nutrition)*. 2019;**17**(1):38-48. (In Russian)
- [18] ISO 18363-1:2015 Animal and vegetable fats and oils. Determination of Fatty-Acid-Bound Chloropropanediols (MCPDs) and Glycidol by GC/MS —Method Using Fast Alkaline Transesterification and Measurement for 3-MCPD and Differential Measurement for Glycidol 2015. DOI: 10.20953/1727-5784-2019-1-38-48
- [19] Leigh J, MacMahon S. Occurrence of 3- monochloropropanediol esters and glycidyl esters in commercial infant formulas in the United States. *Food Addit Contam: Part A*. 2017;**34**(3):356-370
- [20] Becalski A, Feng S, Lau BP-Y, Zhao T. A pilot survey of 2- and 3-monochloropropanediol and glycidol fatty acid esters in foods on the Canadian market 2011 -2013. *Food Composition and Analysis*. 2015;**37**:58-66
- [21] Wang W, Yang J, Qi W, Yang H, Wang C, Tan B, et al. Lipidomic profiling of high-fat diet-induced obesity in mice: Importance of cytochrome P450-derived fatty acid epoxides. *Obesity (Silver Spring)*. 2017;**25**:132-140
- [22] Li P, Oh da Y, Bandyopadhyay G, et al. LTB4 promotes insulin resistance in obese mice by acting on macrophages, hepatocytes and myocytes. *Nature Medicine*. 2015;**21**:239-247
- [23] Lilly R. *Pathohistological Technique and Practical Histology.*, Eng-Rus Translation; ed. and with a Foreword by AM of AMN, V.V. Portugalova., Moscow : Mir. 1969. p. 512
- [24] Merkulov, Grigory Andreevich. *Course of pathological and histological techniques [Text]. - 5th ed., Rev. and additional - Leningrad: Medicine. Leningrad. department, 1969. - 423 p. : ill.; 22 cm*
- [25] Simakova IV. *Scientific and Applied Aspects of Safety Fast Food: Thesis. Orel: Doctor of Technical Sciences; 2015. p. 338*
- [26] Zakharova YA, Dmitrieva IN, Gordeeva EA. Membrane of fat globules of milk: Innovative discoveries already today. *Russian Bulletin of Perinatology and Pediatrics*. 2015;(6):15-21
- [27] Janssen CI, Kiliaan AJ. Long-chain polyunsaturated fatty acids (LCPUFA) from genesis to senescence: The influence of LCPUFA on neural development, aging, and neurodegeneration. *Progress in Lipid Research*. 2014;**53**:1-17. DOI: 10.1016/j.plipres.2013.10.002. Epub 2013 Oct 24
- [28] Ozcagli E, Alpertunga B, Fenga C, Berktaş M, Tsitsimpikou C, Wilks MF,

- et al. Effects of 3-monochloropropane-1,2-diol (3-MCPD) and its metabolites on DNA damage and repair under in vitro conditions. *Food and Chemical Toxicology*. 2016;**89**:1-7
- [29] Kazimirko VK, Maltsev VI. The Antioxidant System and its Functioning in the Human Body. Electronic resource., Access: <http://www.health-ua.com/>; 2004 Date of access: February 28, 2019
- [30] Kurashvili, V.A. Relief of oxidative stress using natural antioxidants, electronic resource. Access: <http://vitadoctor.com.ua>. Date of access: February 28, 2019
- [31] Simakova IV, Perkel RL, Zakrevkiy VV, Kutkina MN. Investigation of the intensity of pathogenesis depending on the oxidation state of palm oil. *Nutrition Issues*. 2016;**85**(2):36
- [32] Simakova IV, Eliseev YY, Perkel RL, Domnitsky IY, Terentyev AA, Strizhevskaya VN, et al. Assessment of the sanitary and technological safety of the fatty component of some types of snacks and flour confectionery products with a long shelf life. *Saratov Journal of Medical Scientific Research*. 2016;**12**(3):333-339
- [33] Simakova IV. Scientific and Applied Aspects of Ensuring the Safety of Fast Food Products, Thesis for a Doctor's of Eechnical Sciences Degree. Orel: Federal State Budgetary Educational Institution of Higher Education Prioksky State University; 2015. p. 462
- [34] Goicoechea E, Guillen MD. Analysis of hydroperoxydes, aldehydes and epoxydes by ¹H nuclear magnetic resonance in sunflower oil oxidized at 70 and 100°C. *Agricultural Journal of Food Chemistry*. 2010;**58**:6234-6245
- [35] Grüneis V, Fruehwirth S, Zehl M, Ortner J, Schamann A, König J, et al. Simultaneous analysis of Epoxidized and Hydroperoxidized Triacylglycerols in canola oil and margarine by LC-MS. *Journal of Agricultural and Food Chemistry*. 2019;**67**(36):10174-10184. DOI: 10.1021/acs.jafc.9b03601. Epub 2019 Aug 28