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Influence of various prebiotic components on the main growth indicators of probiotic bacteria

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The study aimed to select prebiotic components and determine their effect on the primary growth rates of probiotic bacteria (lactobacilli, bifidobacteria). Strains *Bifidobacterium adolectentis No 17-316* and *Lactobacillus plantarum No 7-317* were used to create the bacterial mixture. When determining the effectiveness of various sources of sugar on the intensity of growth parameters of lactic acid bacteria, experimental nutrient media were prepared with the addition of lactulose, inulin, fructose in amounts of 1.0%, 1.5%, and 2.5%. It has been experimentally proved that the use of lactulose at a concentration of 1.5% as a prebiotic component in the bacterial mixture based on lactobacilli *L. plantarum No 7-317* and bifidobacteria *B. adolectentis No 17-316* (1:2) increases the biological activity of the mixture: acid formation activity – by 10±2%, the number of live bacteria – by 15±2%. It has been established that lactulose has a higher bifidogenic and lactogenic effect than other sources of sugar (inulin, fructose). Based on the study of morphological characteristics of bacterial cultures of symbiotic types of interaction and study of the bifidogenic and lactogenic ability of some sugars, the composition of the bacterial mixture of therapeutic and prophylactic action has been substantiated: *L. plantarum No 7-317* and *B. adolectentis No 17-316* in the ratio 1:2 and prebiotic component – lactulose (1.5%). The optimal growth parameters for bifidobacteria – cultivation temperature 38.7±0.4°C and pH of the medium 6.7±0.3, and for lactobacilli – cultivation temperature 37.3±0.2°C and pH of the medium 7.2±0.1.
Keywords: lactulose, bifidobacteria, lactobacilli, concentration, colony-forming units, temperature, pH.

Introduction

Recently, diseases of the gastrointestinal tract of various etiologies in farm animals have become acute (Cave, 2003; Johnson & Meerveld, 2017). Every year, a number of factors that negatively affect the composition and activity of animal autoflora are overgrowing. As a result of reducing the number of bifidobacteria and lactobacilli in animals, digestive processes and many basic biochemical processes are disrupted. As a result, the general condition of the organism deteriorates, and its resistance to pathogenic and opportunistic microorganisms decreases (Walter, 2008; Otles, 2014). The main reasons for this are the uncontrolled use of antibiotics and poor quality biological feed additives. Issues of intestinal microecology and its violation (dysbiosis), despite this problem study's duration, constantly attract specialists' attention in various fields of science (Gaucher et al., 2019; Kasianenko et al., 2020; Paliy et al., 2020b).

To date, many biologically active additives and drugs have been developed in various countries based on products developed based on the endogenic microflora of the macroorganism (Langen et al., 2009; Hill et al., 2014; Caggianiello et al., 2016). For this purpose, various strains of bifidobacteria and lactobacilli, non-pathogenic strains of *Escherichia coli* and enterococci are usually used (Apás et al., 2010). The most well-known microorganisms used as a basis for biologicals are lactobacilli (Toghyani et al., 2011). It is known to use *Lactobacillus plantarum, L. fennentum, L. casei, L. amylovorus, L. acidophillus, L. salivarius, L. rhamnosus, L. reuleri, L. lactis* for the production of probiotics. Along with lactobacilli, bifidobacteria, in particular *Bifidobacterium animalis, B. bifidum, B. infantis, B. adolescentis, B. longum, B. thermophillus* have become widely used (Paliy et al., 2020c).

Various feed additives, premixes, prebiotics, and probiotics improve feed consumption and feed efficiency to maximize livestock productivity (Collins & Gibson, 1999; Patel & Goyal, 2012). The primary methods of prevention and treatment of diseases of the gastrointestinal tract caused by dysbacteriosis may be drugs belonging to the group of symbiotics, the use of which can improve and sometimes restore the intestinal microflora and mucous membranes of the animal organism, which in turn leads to

significant overall improving health and preventing the development of many chronic diseases (Wang, 2009; DuPont et al., 2014; Saiyed et al., 2015).

Symbiotics are substrates that have the properties of probiotics and prebiotics. Recently, for the correction of dysbiotic disorders, the prospects for the use of prebiotics have been proven – ingredients that are wholly or partially indigestible and selectively stimulate the reproduction and/or metabolic activity of one or more groups of bacteria present in human and animal intestines (Bindels et al., 2015; Rastall & Gibson, 2015; Zeng et al., 2018).

Prebiotics, for the most part, are not digested in the small intestine and enter the large intestine, where they are utilized by the microflora, ensuring its growth, stability, and activity (Gibson et al., 2010). Many carbohydrates have prebiotic properties (Grajek et al., 2005; Gibson et al., 2017). In recent decades, they are used in food, dietary supplements, and drugs to prevent and correct disorders of intestinal microbiocenosis. Among the most popular prebiotics are poly- and oligofructose, soybean oligosaccharides, galactooligosaccharides isolated from natural sources or made by biotechnological or synthetic methods (Lomax & Calder, 2009; Modesto et al., 2009; Gaggia et al., 2010).

Substances that belong to prebiotics should have two essential properties: not digested in the upper digestive tract; selectively ferment the microflora of the colon, causing the active growth of helpful microorganisms, primarily bifidobacteria and lactobacilli (Olveira & González-Molero, 2016). The positive effects of prebiotics are realized by selectively stimulating the growth of the endogenic microbiota with a simultaneous negative impact on pathogenic, opportunistic microflora and their toxic metabolites (Atia et al., 2016; Gujvinska et al., 2018).

Among prebiotics on the Ukrainian market, lactulose has the highest prebiotic index and is considered the gold standard in the class of prebiotic drugs. The range of symbiotic drugs on the Ukrainian market is quite limited. There is little information on the most optimal combinations of probiotic cultures and nutrient substrates for them in one drug. Currently, prebiotics are common in livestock and poultry farming (Toghyani et al., 2011; Gujvinska & Paliy, 2018). There is no doubt about the importance of using microbiological drugs to improve farm animals and poultry' health and increase the level of their productivity. Therefore, recently numerous studies have been conducted to study the effects of prebiotics of various origins on farm animals and poultry, to study the characteristics of their action in the industrial production of livestock products, as well as the quality of products to meet consumer requirements (Rycroft et al., 2001; Śliżewska et al., 2013; Maiorano et al., 2017). Therefore, scientific researches in this direction are relevant and timely.

Materials and methods

Experimental studies have been conducted based on the Laboratory of Veterinary Sanitation and Parasitology of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine" (Kharkiv).

To create a bacterial consortium, according to previous studies, we selected a strain of bifidobacteria *Bifidobacterium adolectentis No 17-316* and a strain of lactobacilli *Lactobacillus plantarum No 7-317* as the most promising probiotic cultures (Gujvinska & Paliy, 2018). The bifidogenic and lactogenic effects of lactulose, inulin, and fructose were studied as prebiotic components.

Lactulose is a synthetic disaccharide consisting of galactose and fructose molecules linked by a β-1,4 bond and obtained by isomerization of lactose. It is a white or almost white crystalline powder with a sweet taste, odorless, and well soluble in water (Chae et al., 2016).

Inulin is a fructan or glucofructan, the chain of which consists of 30-35 fructose residues. Easily soluble in hot water, poorly - in the cold. It has a sweet taste. Inulin-containing products have a positive effect on the regulation of metabolism in diabetes, atherosclerosis, and obesity. These products are complex and suitable for treating infectious diseases (de Souza Oliveira et al., 2012).

Fructose is a colorless crystal or a white, odorless crystalline powder with a sweet taste. It has high solubility and hygroscopicity (up to a relative moisture content of 60%). Well soluble in ethanol (95%). It has a low level of water activity and higher osmotic pressure than sucrose. Aqueous solutions of fructose are stable at pH 3.0–4.0, and temperatures from 4 to 70 °C, which can be sterilized in an autoclave (Johnson et al., 2014).

Blauroc culture medium was used to obtain the stock culture of *Bifidobacterium adolectentis No 17-316*. The composition of the medium includes: basic dry peptone – 10.0 g/L, microbiological agar – 0.75 g/L, sodium chloride – 5.0 g/L, lactose – 10.0 g/L, L-cysteine – 0.1 g/L. The basis of the nutrient medium is a hepatic extract (amine nitrogen value 90±10 mg/%). The pH of the medium – 6.5±0.1. Sterilization in an autoclave at a temperature of 121±1.0°C for 20 minutes.

To obtain the stock culture of lactobacilli *Lactobacillus plantarum No 7-317,* we used the nutrient medium MRS. The composition of the medium MRS-1 includes: manganese sulfate – 50.0 mg/L, magnesium sulfate – 200.0 mg/L, L-cysteine – 200.0 mg/L, ammonium citrate – 2.0 g/L, sodium acetate – 5.0 g/L, potassium disubstituted phosphate – 2.0 g/L, crystalline glucose – 20.0 g/L, basic dry peptone – 10.0 g/L, twin 80 – 1.0 ml/L, yeast autolysate – 50.0 g/L (amine nitrogen value 130 mg%), hepatic extract – 100.0 ml/L, purified water – up to 500 ml, hydrolyzed milk – 500 ml. pH of the medium – 6.7±0.1.

MRS-2 medium – agar-agar is added to the MRS-1 medium in the amount of 0.2% of the volume of MRS-1; the pH of the medium is 6.3±0.1. MRS-4 medium – to the MRS-1 medium agar-agar were added in the amount of 2.5% of MRS-1, pH 6.3±0.1. The MPC media were sterilized in an autoclave at a temperature of 121.0±1.0°C for 20 minutes.

Modified nutrient medium (KYNM-casein yeast nutrient medium) was used for co-cultivation of bifidobacteria and lactobacilli. The composition of the medium: manganese sulfate – 23.0 mg/L, magnesium sulfate – 90.0 mg/L, L-cysteine – 90.0 mg/L, ammonium citrate – 0.9 g/L, sodium acetate – 2.27 g/L, sodium chloride – 5.0 g/L, potassium phosphate disubstituted – 0.9 g/L, agar-agar – 0.5 g/L, yeast autolysate – 600 ml/L (value of amine nitrogen 130 mg%), pancreatic casein hydrolyzate – 200 ml/L (deep-cleaved pancreatic hydrolyzate of casein with an amine nitrogen value of 600±30 mg% is used), prebiotic component (lactitol) – 10.0 g/L, gelatin – 40.0 ml of 20% solution, purified water – up to 1.0L, the pH of the medium 7.0±0.1). The value of

amine nitrogen of the finished medium 190±15 mg%. Sterilization in an autoclave at a temperature of 121.0±1.0°C for 20 minutes.
For the experiment, the cultivation of *Bifidobacterium adolectentis No 17-316* was performed *in vitro* on media with different sources of sugars. The lyophilisate of bifidobacteria was dissolved in Blaurok medium (pH 6.5±0.1) and restored at 38.0±0.5°C for 96 hours, by two successive reseedings on Blaurok medium, lasting 48 hours each. Then the culture was placed in the prepared for cultivation modified by us control nutrient medium (KYNM-casein yeast nutrient medium) in 10%.
To determine the effectiveness of different sources of sugar on the intensity of growth parameters of bifidobacteria, several variants of media (KYNM) (pH 7.0±0.1) were prepared with the addition to the nutrient medium of fructose, inulin, lactulose in amounts of 1.0%, 1.5%, 2.5%. For comparison, we used a variant (KYNM), according to the classical technology of preparation, with a lactose content of 1.0%. According to the literature sources, the concentration of sugars was chosen for the calculation of the content of sugar sources in the nutrient media.
To determine the bifidogenic effect of different sources of sugars, we compared the biological activity of the culture by the following indicators: metabolic activity, number of live bacteria, acid formation activity, and cell morphology (Gujvinska & Paliy, 2018).
For the experiment, the cultivation of lactobacilli strain *Lactobacillus plantarum No 7-317* was performed *in vitro* on media with different sugar sources. Lactobacilli lyophilisate was dissolved in MRS-1 medium (pH 6.5±0.1) and restored at 37.0±0.5°C for 24 hours. Then subsequent generations were performed on MRS-2 (pH 7.3±0.1) and MRS-4 (pH 7.9±0.1) media. The culture was introduced into the prepared for cultivation modified nutrient medium (KYNM) in 10%. Additional sources of sugars (lactulose, inulin, fructose) in the amounts of 1.0%, 1.5%, and 2.5% were included in the nutrient medium variants. For comparison, we used a variant (KYNM), according to the classical technology of preparation, with a lactose content of 1.0%. The concentration of sugars was chosen from the calculation of the content of sugar sources in the nutrient media, according to the literature sources. To determine the lactogenic effect of these sources of sugars, a comparison was made of the biological activity of the culture by the following indicators: metabolic activity, number of live bacteria, acid formation activity, and cell morphology.
At the next stage, a comparative evaluation of the prebiotic activity of inulin, fructose, lactulose was performed in co-cultivation of the strain *Bifidobacterium adolectentis No 17-316 Lactobacillus plantarum No 7-317*. For this purpose, stock cultures of bifidobacteria, in the amount of 2.5%, and lactobacilli, in the amount of 7.5%, were restored and added to the prepared modified nutrient medium KYNM (pH 7.0±0.1), which contained 1.0±0.1% inulin and 1.7±0.1% lactulose. Cultivation was performed for 48 hours.
Tests for the growth properties of lactobacilli and bifidobacteria were determined by the number of living microbial cells by serial dilutions of the resulting suspension in saline followed by inoculation of bacterial cultures of 0.1 cm^3 from dilution 10^6 on media followed by counting the number of colony-forming units (Paliy et al., 2020a).
Determination of the acid-forming activity of the cultures was performed by the titrimetric method. In each experiment, indicators from two measurements were taken into account.
Bacterial culture with a volume of 2.5 ml was transferred into wide tubes (diameter 2 cm, height 20 cm) with 25.0 ml of Blaurok liver medium (at the rate of 1.0 ml of culture – 10.0 ml of medium), then kept for 72 hours at a temperature of 38.0±0.5°C, followed by determination of acidity in each test tube (in two parallel samples), for which each sample (10.0 ml of microbial suspension) was titrated with sodium hydroxide solution at a concentration of 0.1 M until a stable pale pink color (FF indicator – 2 drops). The pH value was monitored by potentiometry.
Titration was carried out at pH 8.5. Acidity was expressed in Turner degrees (°T) and calculated by the formula:
$T=A\times K\times 10$
where A – is the amount of 0.1 M sodium hydroxide solution, which was used for the titration, ml;
K – correction to the titer of 0.1 M sodium hydroxide solution was used;
10 – the degree of dilution of the microbial suspension.
The average value of acid formation was calculated from the values obtained for the two measurements, provided that each of them is not lower than 90°T. In the case of obtaining in one of the samples the result below 90°T, the attempt was repeated.
Probiotic bacteria acidify the growth medium by metabolizing the components of the substrate, thereby increasing the amount of biomass. Alkali or ammonia are used to adjust the pH of the culture medium. The total amount of added alkali or ammonia indicates the metabolic activity of each ratio, i.e., the greater the amount of ammonia used to stabilize the pH of the medium, the more active the metabolic processes of the bacterial culture. The metabolic activity of probiotic bacteria was determined by the total amount of alkali or ammonia added to the nutrient medium.

Results and discussion

Bifidobacteria and lactobacilli form the basis of the microflora of the gastrointestinal tract of animals and poultry. *Bifidobacterium bifidum, B. adolescentis, B. breve, B. longum* and *Lactobacillus plantarum, L. acidophilus* play the most critical role in the gastrointestinal tract of animals and birds. On the background of lacto- and bifidoflora deficiency, the pathogenic effect of staphylococci, streptococci, proteus, and fungi is the most active. We used strains of bacteria of the genus *Bifidobacterium* and *Lactobacillus* to develop a probiotic drug.
Bifidobacterium adolectentis are fixed gram-positive polymorphic rods with a bifurcation at one or both ends, 4-5 μm long, arranged in clusters or individual cells, or the form of the Roman numeral "V". Gram-colored unevenly, gram-positive, not acid-resistant. Obligate anaerobic. When growing on semiliquid media - hepatic Blaurok medium, hydrolyzed milk (HM), and casein yeast (CY) – during the first day cause uniform turbidity of the medium and within 2-3 days form brittle sediment, leaving a transparent upper part of the medium (aerobiosis zone). Individual colonies of bifidobacteria have small "cloves" or "crumbs" of white color, forming a brittle mass when shaken. Hemoorganotroph. Actively ferments carbohydrates with the formation of

acetic and lactic acid. Needs vitamins. Ferment lactose with the formation of acetic and lactic acid acidifies the growth medium to pH 4.0±0.2.

Lactobacillus plantarum microorganisms are rods from 0.7–1.1 to 3.0–8.0 μm long, arranged singly or in chains. Immobile, do not form spores, have no flagella, gram-positive. Optional anaerobes grow in an atmosphere of carbon dioxide, nitrogen, and in the presence of oxygen. MRS-1 grows in uniform turbidity and homogeneous white precipitate on a liquid medium at the bottom of the tube. In the semiliquid nutrient medium MRS-2, they form isolated colonies in the form of strands. MRS-4 forms convex, opaque, white colonies with solid edges, not pigmented on a dense nutrient medium: Chemoorganotrophs; need rich, complex nutrient media. Fermentation-type metabolism, sugar-plastic; almost half of the carbon of the final fermentation products falls to lactate. Do not restore nitrate; do not dilute gelatin; catalase-negative; do not contain cytochromes. *Lactobacillus plantarum* ferments glucose to form acid gas.

To create a bacterial consortium, according to the results of previous studies, we selected a strain of bifidobacteria, *Bifidobacterium adolectentis No 17-316* and a strain of lactobacilli *Lactobacillus plantarum No 7-317* as the most promising probiotic cultures.

We studied the number of live bacterial cells in the joint cultivation of lactobacilli and bifidobacteria in the ratio of uterine cultures in a mixture of lactobacilli: bifidobacteria – 1:1, 1:2, 2:1 (Table 1).

Table 1. The ratio of lactobacilli and bifidobacteria in the mixture

Sample No	The ratio of cultures	*B. adolectentis No 17-316*		*L. plantarum No 7-317*	
		volume, cm^3	the number of bacteria CFU/cm^3	volume, cm^3	The number of bacteria CFU/cm^3
1	1:1	5.0	10^7	5.0	10^7
2	1:2	3.33	10^7	6.67	10^7
3	2:1	6.67	10^7	3.33	10^7
4	control of bifidobacteria	10.0	10^7	–	–
5	control of lactobacilli	–	–	10.0	10^7

Regardless of the ratio of probiotic cultures among themselves, their number is 10^7 CFU/cm^3, which corresponds to the control indicators.

The intensity of bacterial growth during the cultivation period and its end is indicated by the amount of added 10.0% alkali solution, which is used to adjust the pH of the medium to the required level. Visual inspection of the samples confirms the growth and accumulation of biomass. In our experiments, in all samples over time, the formation of a white precipitate of different intensities at the bottom of the vial was observed (Table 2).

Table 2. The amount of added 10.0% alkali solution during the growth of probiotic cultures, ml (n=5)

Sample No	The ratio of cultures	Growth of probiotic cultures, hours			Total, cm^3
		24	48	72	
1	1:1	0.23	0.03	0.04	0.30
2	1:2	0.20	0.03	0.05	0.28
3	2:1	0.12	0.02	0.03	0.17
4	control of bifidobacteria	0.21	0.05	0.09	0.35
5	control of lactobacilli	0.15	0.02	0.04	0.21

The data presented in Table 2 show the level of accumulation of bacterial biomass during growth. Probiotic bacteria acidify the growth medium by metabolizing the components of the substrate, thereby increasing the amount of biomass. The total amount of alkali introduced indicates the metabolic activity of each ratio, i.e., the greater the amount of alkali used to stabilize the pH of the medium, the more active the metabolic processes in the bacterial culture. According to Table 2, the highest amount of alkali consumed in the groups of experiments using bifidobacteria and lactobacilli is observed in a ratio of 1:1 and is 0.30 cm^3. When cultivating probiotic cultures in the ratio of 1:2, the amount of alkali consumed is equal to 0.28 cm^3, which is less by 6.7%, and when the ratio of microorganisms is 2:1, this figure is lower by 43.3% compared to the sample No 1.

In addition, in control samples, when culturing only bifidobacteria, the amount of alkali consumed is 0.35 cm^3, which is 40% more than is necessary for individual cultivation of lactobacilli. It should also be noted that the amount of alkali gradually increases during the cultivation of probiotic cultures, regardless of their ratio.

The next step was to determine the number of live bacteria and control the activity of acid formation (Table 3).

Table 3. The number of live lactobacilli and bifidobacteria and the activity of acid formation (n=5)

Sample No	The ratio of cultures	*B. adolectentis No 17-316*		*L. plantarum No 7-317*	
		CFU/cm^3	acid formation activity, °T	CFU/cm^3	acid formation activity, °T
1	1:1	10^{10}–10^{11}	220±7	(4.07±0.07)×10^9	220±12
2	1:2	10^{11}–10^{12}	248±9	(5.39±0.16)×10^9*	260±17
3	2:1	10^{11}–10^{12}	228±9	(2.27±0.06)×10^9	275±12
4	control of bifidobacteria	10^{10}–10^{11}	170±9	–	–
5	control of lactobacilli	–	–	(3.75±0.12)×10^9	280±15

Note: p≤0.05

The results of the studies presented in Tables 2 & 3 indicate that control samples of monocultures of lactobacilli and bifidobacteria have high biological parameters. When cultivating the mixture, the highest rates are observed when using a ratio of 1:2.

When comparing the morphological features of cultured strains with control samples by microscopy, the preservation of the main morphological features of each strain during co-cultivation was confirmed. In samples 1–3, single or chained rods, V-shaped cells with a bifurcation at one end, or thickening at one or two ends were visually identified. In sample No 4, there are many single rods without chain formation. In sample No 5, club-shaped, V-shaped cells were recorded singly and in chains. Thus, the performed microscopy confirms the constant morphology of bifidobacteria and lactobacilli.

We established that bifidobacteria and lactobacilli grow on a specific nutrient medium and have the necessary growth indicators, which is confirmed by the results of acid formation and the number of live bacteria. Thus, we have proved the possibility of joint cultivation of bifidobacteria and lactobacilli on a specific nutrient medium; the inoculum ratio in the mixture has been determined in the amount of 1:2 – lactobacilli to bifidobacteria, respectively.

The intensity of fermentation depends on factors such as temperature and pH. The optimal growth parameters were set for bifidobacteria – cultivation temperature 38.7±0.4°C and pH of the medium 6.7±0.3, and for lactobacilli – cultivation temperature 37.3±0.2°C and pH of the environment 7.2±0.1.

Therefore, for maximum biomass accumulation during the cultivation of a mixture of bacterial cultures on a nutrient medium, we proposed the first day of growth to create conditions characteristic of bifidobacteria and then the optimal growth parameters for lactobacilli. At the initial stage during cultivation (24 hours), the pH was checked and adjusted with 10.0% alkali solution to 6.6±0.2; the next 24 hours, we performed a pH check and adjusted it to pH 7.1±0.3. The cultivation temperature during the first day was 38.9±0.3°C, and during the second day – 37.2±0.4°C (Table 4).

Table 4. Primary technological conditions for the growth of lactic acid bacteria

Cultures of microorganisms		Growth parameters of lactic acid bacteria	
		Temperature, °C	pH
L. plantarum No 7-317		37.3±0.2	7.2±0.1
B. adolectentis No 17-316		38.7±0.4	6.7±0.3
A mixture of cultures 2:1	in 24 hours	38.9±0.3	6.6±0.2
	in 48 hours	37.2±0.4	7.1±0.3

The study results showed that the proposed cultivation regime allows the simultaneous cultivation of lactobacilli and bifidobacteria in the same volume while maintaining the basic morphological and biological parameters. Changing the temperature and adjusting the pH during two days of cultivation made it possible to increase acid formation by 5-7%; the number of live bacteria remained unchanged – 10^{12} CFU/cm^3 on Blaurok medium and 5.18±0.2×109 CFU/cm^3 on MRS medium. During the experiment, the morphological characteristics of probiotic cultures corresponded to the established characteristics.

At the next stage, we studied the effect of the bifidogenic action of various carbohydrates on the leading growth indicators (number of bacteria, acid formation activity, morphology) of the strain *Bifidobacterium adolectentis No 17-316.* For the experiment, the cultivation of *Bifidobacterium adolectentis No 17-316* was performed *in vitro* on media with different sources of sugars. The bifidobacterial lyophilisate was dissolved in the Blaurok medium and restored at 38.0±0.5°C for 48 hours by two successive reseedings with the Blaurok medium. Then the culture was introduced into the culture medium prepared for cultivation in an amount of 10%. To determine the effectiveness of different sources of sugar on the intensity of growth parameters of bifidobacteria, several variants of the medium were prepared with the addition to the nutrient medium of lactulose, inulin, fructose in amounts of 1.0%, 1.5%, 2.5%. For comparison, a variant of the nutrient medium was used following the classical preparation technology, with a lactose content of 1.0%. To determine the bifidogenic effect of different sources of sugars, we compared the biological activity of the culture by the following indicators: metabolic activity, the number of live bacteria, and acid formation activity.

Different pH values control the intensity of metabolic processes of bifidobacteria during cultivation, which is the primary indicator of the transformation of sugars into organic acids, which are the end products of the metabolism of prebiotic components. The change in pH of the medium was adjusted by adding to the last 10.0% alkali solution (Table 5).

Table 5. Indicators of bifidobacteria activity, depending on the introduced source of sugars (n=3)

Sample No	Source of sugars	The amount of sugars, %	The amount of added 10.0% alkali solution, cm^3	Acid-formation activity, °T	Number of bacteria, CFU/cm^3
1		1.0	1.50±0.07	230±8	10^{10}–10^{11}
2	Lactulose	1.5	1.41±0.06	230±9	10^{10}–10^{11}
3		2.5	1.20±0.04	215±10	10^{11}–10^{12}
4		1.0	1.05±0.03	150±7	10^{8}–10^{9}
5	Fructose	1.5	1.20±0.05	165±7	10^{8}–10^{9}
6		2.5	1.21±0.04	180±8	10^{8}–10^{9}
7		1.0	0.44±0.01	115±5	10^{7}–10^{8}
8	Inulin	1.5	0.46±0.02	165±8	10^{7}–10^{8}
9		2.5	0.51±0.03	170±7	10^{7}–10^{8}
10	control without sugars	–	0.35±0.02	112±4	10^{5}–10^{6}
11	control with lactose	1.0	1.15±0.04	215±8	10^{10}–10^{11}

As shown in Table 5, the intensification of metabolic processes of bifidobacteria (the amount of added 10.0% alkali solution) was observed with the introduction of lactulose in concentrations of 1.0%, 1.5%, and 2.5%. The addition of fructose increases the metabolism of the culture but does not accumulate active biomass. The analysis of bifidobacteria control showed that the most significant number of bacteria – (10^{11}–10^{12}) CFU/cm^3 were observed when 2.5% of lactulose were added to the nutrient medium. When adding other sugars, much lower values were obtained. An indicator of the activity of accumulated biomass is the activity of acid formation. Table 5 data show that the addition of lactulose increases bifidobacteria's acidification activity and is 215°T and 230°T.

Morphological characteristics of *Bifidobacterium adolectentis No 17-316* cultures, when cultivated on all variants of the nutrient medium with the addition of sugars, were preserved. The results of bacterioscopic control confirmed their compliance with the data. According to the results of the studies presented in Table 5, we can conclude the high bifidogenic action of lactulose in concentrations of 1.0%, 1.5%, and 2.5%.

The next stage of our work was to study the level of lactogenic action of lactulose inulin fructose in different concentrations on the leading growth indicators (number of bacteria, acid formation activity, and morphology) of *Lactobacillus plantarum strain No 7-317*. During the experiment, the cultivation of lactobacilli was performed *in vitro* on media with different sources of sugars. Lyophilisate of lactobacilli was dissolved in MRS-1 medium and restored at 37.0±05°C for 24 hours. Next, sequential generations were performed on MRS-2 and MRS-4 media. Then the culture was added to the prepared nutrient medium in the amount of 10.0%. Additional sources of sugars (lactulose, inulin, and fructose) in the amounts of 1.0%, 1.5%, and 2.5% were included in the nutrient medium variants. For comparison, a variant of nutrient medium with a lactose content of 1.0% was used.

To determine the lactogenic effect of these sources of sugars, a comparison of the biological activity of the cultures was performed by the following indicators: metabolic activity, the number of live bacteria, and the activity of acid formation. The change in metabolic activity of lactobacilli was monitored by adding 10.0% alkali solution (Table 6).

Table 6. Indicators of lactobacilli activity, depending on the introduced source of sugars (n=3)

Sample No	Source of sugars	The amount of sugars, %	The amount of added 10.0% alkali solution, cm^3	Acid-formation activity, °T	Number of bacteria, 10^9 CFU/cm^3
1		1.0	1.40±0.07	320±14	3.57±0.12
2	Lactulose	1.5	1.74±0.08	350±17	4.07±0.14
3		2.5	1.87±0.07	357±16	4.24±0.18
4		1.0	0.50±0.02	200±7	2.57±0.11
5	Inulin	1.5	0.77±0.02	260±12	3.07±0.10
6		2.5	0.81±0.03	270±10	3.17±0.17
7		1.0	1.12±0.04	210±7	2.43±0.13
8	Fructose	1.5	1.25±0.05	275±9	2.74±0.15
9		2.5	1.34±0.07	280±10	2.11±0.14
10	control without sugars	–	0.43±0.05	190±11	2.00±0.10
11	control with lactose	1.0	0.75±0.03	280±5	3.01±0.12

As shown in Table 6, the intensification of metabolic processes of lactobacilli (the amount of added 10.0% alkali solution) was observed with the introduction of lactulose in concentrations of 1.0%, 1.5%, and 2.5%. Fructose also intensifies the metabolism of lactobacilli. Analysis of lactobacilli control showed that when lactulose was introduced into the nutrient medium, the number of bacteria was at a high level of 4×10^9 CFU/cm^3. When sugars (inulin and fructose) were added, the bacteria was $2–3\times10^9$ CFU/cm^3. An indicator of the activity of accumulated biomass is the activity of acid formation. As can be seen from Table 6, the

addition of lactulose increases the acid-forming activity of lactobacilli to the level of 320–357°T. Morphological features of *Lactobacillus plantarum No 7-317* bacteria during cultivation on all variants of nutrient medium with the addition of sugars were preserved, so lactulose, inulin, and fructose have the property of prebiotics. According to the results of the studies presented in Table 6, it was found that lactulose has a high lactogenic effect at a concentration of 1.5% and 2.5%; the indicators of culture activity, when using these components and concentrations, were not inferior to the option using a control medium with the addition of lactose in the amount of 1.0%.

Thus, as a result of experiments, it was found that the cultivation of *Bifidobacterium adolectentis No 17-316* and *Lactobacillus plantarum No 7-317 in vitro* on nutrient media with the addition of various sources of sugars as prebiotic components is more efficient and appropriate. In particular, lactulose has the most significant influence on the basic growth indicators of bacteria.

The next step was to determine the optimal concentration of the prebiotic component in the nutrient medium for co-cultivation of bifidobacteria strain *Bifidobacterium adolectentis No 17-316* and lactobacilli strain *Lactobacillus plantarum No 7-317.*

Since lactulose is considered the gold standard in the class of prebiotics and based on the results of previous data, a comparative evaluation of the prebiotic activity of lactulose was performed in co-cultivation of strains of *Bifidobacterium adolectentis No 17-316* and *Lactobacillus plantarum No 7-317.* For this purpose, uterine cultures of bifidobacteria, in the amount of 2.5%, and lactobacilli were restored in the amount of 7.5%. Cultures were added to the prepared modified nutrient medium KYNM (pH 7.0±0.1), containing 1.5% lactulose. Cultivation on the nutrient medium was performed for 48 hours.

To stabilize the pH during growth, a much more significant amount of alkali was used than in the cultivation of monocultures. Thus, to the sample with 1.5% lactulose – 3.2±0.12 ml of alkali. The acid formation activity of the samples was performed following the standard methods and was approximately 290°T for both samples on Blaurok medium, and differed slightly in the analysis on MRS-1 medium – 392±18°C –for sample No 1 and 380±15°C – for sample No 2. The number of live bacteria was also at a high level and was: for sample No 1 – 10^{12} CFU/cm^3 of bifidobacteria and 6.4±0.2×10^9 CFU/cm^3 of lactobacilli; for sample No 2 – 10^{12} CFU/cm^3 of bifidobacteria and 6.1±0.2×10^9 CFU/cm^3 of lactobacilli (Table 7).

Table 7. Indicators of bacterial consortium activity depending on sugar source (n=5)

Consortium activity indicator		Lactulose, 1.0%	Lactulose, 1.5%	No sugars
The amount of added 10.0% alkali solution, ml		3.4±0.12	3.1±0.10	2.6±0.3
Acid formation activity, °T	Blaurok medium	276±11	282±13	237±8
	MRS-1 medium	389±17	381±14	359±18
Number of bacteria, CFU	Blaurok medium	10^{12}	10^{12}	10^{11}
	MRS-4 medium	(5.1±0.18)×10^9	(5.9±0.17)×10^9	(4.2±0,14)×10^9

Substances that belong to prebiotics should have two essential properties: not digested in the upper digestive tract; selectively ferment the microflora of the colon, causing the active growth of beneficial microorganisms, primarily bifidobacteria and lactobacilli (Macfarlane et al., 2006).

Studies have shown that the addition to the probiotic mixture of lactulose in the amount of 1.0% and 1.5% is characterized by high levels of bacterial culture and is at a similar level. As a result of the experiments, it has been found that the cultivation of strains of bifidobacteria *B. adolectentis No 17-316* and lactobacilli *L. plantarum No 7-317 in vitro*, with the addition of 1.5% lactulose as a prebiotic component, has excellent growth rates and biomass accumulation. Therefore, we chose 1.5% lactulose as a prebiotic component. The choice of lactulose as a prebiotic component is based on a uniform bifido- and lactogenic action.

In 1957, the Austrian pediatrician F. Petuely was the first to describe the bifidogenic properties of lactulose. He proved that in artificial feeding of children with a milk mixture containing 1.2 g/100 kcal of lactulose at a ratio of lactose to protein 2.5:1, an almost pure culture of bifidobacteria is formed in the intestine, and the pH of the intestinal contents is reduced. Based on these studies, F. Petuely called lactulose "bifidus factor". The prebiotic effect of lactulose has been proven in many studies. Lactulose, by stimulating the growth of normal intestinal microflora, helps maintain the anti-infective protection of microorganisms, particularly against Shigella, Salmonella, Yersinia, and rotaviruses.

Conclusions

The dependence of the primary growth indicators (acid formation activity, number of viable bacteria) of monocultures of bifidobacteria strains *B. adolectentis No 17-316* and Lactobacilli *L. plantarum No 7-317* on the additional source of sugar added to the nutrient medium was established. It has been determined that lactulose, inulin, and fructose can be used as a prebiotic component, and they are the optimal source of sugars for probiotic cultures.

Intensification of metabolic processes of bifidobacteria (amount of added 10.0% alkali solution) were observed with the introduction of lactulose in concentrations of 1.0%, 1.5%, and 2.5%. The possibility of using lactulose as a prebiotic component in the composition of a bacterial mixture based on bifidobacteria *B. adolectentis No 17-316* and lactobacilli *L. plantarum No 7-317* has been proved. Its optimal concentration (1.5%) has been established, which increases the biological activity of the mixture: acid formation activity by 10±2%, the number of live bacteria by 15±2%.

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