UDC 619:616.98:579:591.434:636.22/.28.082.35 **DOI 10.36016/JVMBBS-2021-7-4-3**

PECULIARITIES OF FORMATION OF INTESTINAL BACTERIOCENOSES IN CALVES IN DIFFERENT TECHNOLOGICAL PERIODS OF RAISING

Hadzevych O. V., Hadzevych D. V., Stegniy B. T., Dunaiev Yu. K.

National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: olgagadzevych@gmail.com

Summary. The article presents data on the peculiarities of the bacteriocenoses formation in calves in different technological periods of raising and different animal housing systems. It has been shown that in calves up to three days of age the quantitative content of microorganisms is minimal. The amount of bifidobacteria and lactobacilli does not exceed 3 log CFU/g. In calves older than three days of age, their level increased, and depending on the method of housing, ranged from 5 log CFU/g (in calves with group housing) to 8 log CFU/g (in calves with individual housing). The microbiocenosis in calves with an individual housing system was characterized by a more constant and less variable composition of *Escherichia coli* ($4 \pm 0.8\times10^2$ CFU/g) and bacillary spore microflora ($3 \pm 1.3\times10^1$ CFU/g). In case of the group housing of calves, the content of bifidobacteria was lower ($20.9 \pm 5.5\times10^6$ CFU/g), and the content of *E. coli* and saprophytic microorganisms of the genus *Bacillus* was high and more variable ($30 \pm 20.4\times10^5$ CFU/g and $31 \pm 11.3\times10^2$ CFU/g respectively). In calves older than fifteen days of age, the amount of lactobacilli ranged from 6 to 8 log CFU/g, and the number of bifidobacteria ranged from 7 to 10 log CFU/g. Thus, to exclude dysbiotic disorders, it is necessary to maintain the optimal composition and quantitative level of the main microflora of the intestinal tract, in particular the number of lactobacilli should be at least 6 log CFU/g, bifidobacteria at least 7 log CFU/g, *E. coli* no more than 7 log CFU/g (except for calves under three days of age)

Keywords: dysbiosis, normal intestinal microflora

Introduction. The body of animals is exposed to a range of adverse factors that alter the normal functioning of main vital systems and cause the development of dysbiotic disorders, including the gastrointestinal tract. This and a number of other factors are associated with an increase in the incidence of enteritis in livestock farms (Batrakov et al., 2021; Trofimov, 2019; Pudovkin et al., 2019; Efimova and Udalova, 2011; Shakhov, Sashnina and Erina, 2016; Avdeeva et al., 2016; Usachev, 2010; Burova and Blokhin, 2017; Andreeva et al., 2015; Maslianko et al., 2013; Lapinska, 2013; Kalinichenko, Korotkykh and Tishchenko, 2016; Basova, et al., 2016).

In most farms, the disease of newborn calves reaches 90–120%, i. e. calves are mostly sick on the 2^{nd}–3^{rd} days of life and they get sick again on the 5^{th}–7^{th} days after birth (Batrakov et al., 2021). Complications after antibiotic therapy are equally important in the development of intestinal dysbiosis. During treatment, large doses of antibiotics are often prescribed, after which the occurrence or deepening of pre-existing dysbacteriosis occurs in almost each case (Burova and Blokhin, 2017).

Feeding calves with milk and colostrum containing antibiotics promotes the spread of resistant microorganisms and disrupts the formation of adequate specific and nonspecific response of the organism. Even microdoses of antimicrobial drugs disrupt the formation of a normal intestinal microbiocenosis (Pudovkin et al., 2019).

Normoflora competes for pathogens, and the mechanisms of inhibition of their growth are quite diverse: selective binding of surface receptors of cells, especially epithelial; pronounced antagonism against pathogenic species (Efimova and Udalova, 2011).

Thus, the in-depth study of bacteriocenoses in animals is a topical issue that will enable to recommend in practice more effective means and measures to combat and prevent infectious diseases.

Knowledge of the qualitative composition of the normoflora and the dynamics of quantitative changes in bacteriocenoses can predict the clinical manifestations of dysbiotic disorders, the development of enteritis, timely take measures to maintain a stable intestinal bacteriocenosis, or its correction.

Therefore, the **aim of the research** was to study and analyze the taxonomic composition of bacteriocenoses of the gastrointestinal tract in calves in different technological periods of raising.

Materials and methods. The research was conducted in the Laboratory for the Study of Bacterial Diseases of Animals of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine) in 2021. To determine the normocenosis, fecal samples and smears from the intestinal tract of calves were studied in different technological periods of raising.

Samples were taken from calves up to three days of age, in colostrum and milk periods under different housing systems. There were no differences in the parameters of the microclimate, rations, except for the system of housing (group and individual) for calves. Animal studies have been conducted taking into account the basic principles of bioethics. Housing, caring for animals and feeding them was carried out following the norms and rations. Quantitative and qualitative diet in animals with different systems of housing did not differ.

The material for studying the microbiocenosis of the colon cavity was its content. The contents of the intestine were collected from calves with sterile spatulas in sterile containers. The material was delivered to the laboratory and examined no later than 2 hours after selection. To determine the quantitative value of microorganisms we made serial ten-fold dilutions of homogenized material in sterile isotonic sodium chloride solution from 10^{-1} to 10^{-10}.

From each tube of the titration series, 1 cm^3 of homogenate was cultured on optimal nutrient media for each species of microorganisms and incubated under optimal temperature conditions and periods. Simple and selective nutrient media produced by Farmaktiv LLC (Ukraine) and HiMedia Laboratories Prv. Ltd. (India) were used. Endo, Ploskirev, Levin, MacConkey mediums, bismuth-sulfite agar, Olkenitsky medium, selenite broth (for the accumulation of salmonella), Simons medium (for the differentiation of enterobacteria by their property to use sodium citrate as the only source of carbon) were used to isolate enterobacteria. For staphylococci we used yolk-salt agar, Chistovich medium; for streptococci — media containing glucose (1%), blood (5–10%) and serum (10–20%); for fungi and yeast — Saburo and Suslo-agar; for anaerobes — Kitt-Tarotzi, Wilson-Blair agar, L.D. agar with esculin (for anaerobes), Voget-Fredett agar. Blaurock medium was used to isolate bifidobacteria, and LactoBacagar was used to isolate lactobacilli. To determine the hemolytic activity of microorganisms we used 5% blood agar, coagulase activity — dry citrate rabbit plasma produced by PJSC 'Pharmstandard-Biolik' (Ukraine).

After incubation of the cultures on the media under optimal conditions, the colonies grown from each dilution were counted. The population level of microorganisms was indicated in the decimal logarithm of the indicator — log CFU/g (colony forming units in 1 g of feces).

To determine the number of microorganisms we took into account the degree of dilution, the number of colonies that grew, the inoculation dose. The number of CFU/g was calculated by the formula:

$$M = \frac{N}{V} \times 10^{n+1},$$

where: M — the number of microorganisms in 1 g of feces;
N — the average number of colonies in 1 bacteriological cup;
V — the volume of suspension applied during plating on the agar surface;
10^{n+1} — dilution, from which the plating was carried out.

Bacteria were identified according to Bergey's Manual of Systematics Bacteriology (Goodfellow et al., 2012).

The obtained results were processed by methods of variation statistics using Microsoft Excel for Windows 2010. To compare mean values Student's *t*-test was used (Van Emden, 2019).

Research results. It was found that in calves up to three days of age the quantitative content of microorganisms is minimal. The number of bifidobacteria and lactobacilli did not exceed $3 \pm 1.8 \times 10^3$ CFU/g, *E. coli* and enterococci — $4 \pm 0.8 \times 10^2$ CFU/g, saprophytic microorganisms of the genus *Bacillus* and yeast-like fungi of the genus *Candida* — $7 \pm 2.1 \times 10^2$ CFU/g.

The level of lactobacilli on the 4^{th}–14^{th} days of age increased to $190 \pm 14.5 \times 10^6$ CFU/g under the group housing, and $11 \pm 2.8 \times 10^7$ CFU/g under the individual housing. The level of bifidobacteria on the 4^{th}–14^{th} days of life increased to $2 \pm 1.5 \times 10^7$ CFU/g under the group housing, and $4 \pm 0.7 \times 10^8$ CFU/g under the individual housing. In the feces of animals aged 15–30 days, the content of bifidobacteria increased to $77 \pm 3.2 \times 10^7$ CFU/g under the group housing, and $141 \pm 41.8 \times 10^8$ CFU/g under the individual housing.

E. coli was detected during the entire raising period in 100% of cases, from $2 \pm 1.0 \times 10^2$ CFU/g up to $19.6 \pm 1.5 \times 10^6$ CFU/g. The content of *E. coli* in animals older than three days of age increased from $2 \pm 1.0 \times 10^2$ CFU/g to $30 \pm 20.4 \times 10^5$ CFU/g. The level of enterococci in the feces of animals older than three days of age ranged from $6 \pm 0.3 \times 10^2$ CFU/g to $41 \pm 1.0 \times 10^2$ CFU/g.

The highest content of enterococci was in the body of calves of 15–30 days of age, their number was $129 \pm 43 \times 10^3$ CFU/g under the group housing, and $4 \pm 1.0 \times 10^5$ CFU/g under the individual housing. It was observed that under the group housing of animals, the number of enterococci was more variable.

Representatives of the genus *Bacillus* and yeast-like fungi of the genus *Candida*, in comparison with other bacteria, were present in the studied material in smaller quantities. During the colostrum period, the number of microorganisms of the genus *Bacillus* did not exceed $310 \pm 41.31 \times 10^1$ CFU/g, in animals of 15–30 days of age — $17 \pm 8.54 \times 10^3$ CFU/g. The content of yeast-like fungi of the genus *Candida* did not exceed $23 \pm 17.1 \times 10^2$ CFU/g.

Staphylococci were not isolated in all technological groups. Their content was minimal (from 0 to $6 \pm 2.1 \times 10^3$ CFU/g) in calves up to three days of age.

Sulfite-reducing clostridia (*Clostridium* spp.) were not detected in the intestinal contents in 100% of cases. Their minimum content (from 0 to $5 \pm 2.3 \times 10^3$ CFU/g) was in calves of the colostrum period. The maximum content of sulfite-reducing clostridia was in calves of 15–30 days of age, but their number did not exceed $30 \pm 12.3 \times 10^3$ CFU/g.

The content of bifidobacteria and lactobacilli was more stable in calves kept individually in boxes. Calves kept in groups had higher levels of saprophytic microorganisms of the genus *Bacillus*. Their content in calves under the group housing was from 2–4 log CFU/g, and under the individual housing did not exceed 3 log CFU/g. The concentration of yeast-like fungi of the genus *Candida* in both groups did not exceed 3 log CFU/g.

Thus, the microbiocenosis in calves under the individual system of their housing is characterized by a high level of bifidobacteria, lactobacilli and a more

constant and less variable composition of *E. coli* and bacillary spore microflora. Under the group housing of calves, the content of lactobacilli was lower and the content of *E. coli* was high. It was observed that the range of quantitative indicators of *E. coli*, clostridia, staphylococci, enterococci, and saprophytic spore bacteria was more variable. Under the group housing, low levels of bifidobacteria were observed, the concentration of which did not exceed 8 log CFU/g. According to the results of research, the dynamics is observed that the higher the number of anaerobic spore-forming microorganisms, the lower the number of lactobacilli, the lower the number of bifidobacteria, the higher the number of *E. coli*. Thus, there is a correlation between the quantitative indicators of bifidobacteria and *E. coli*, between the high content of *E. coli* and low content of lactobacilli, between the high content of clostridia and low content of lactobacilli.

The results of bacteriological studies on the peculiarities of the formation of bacteriocenoses in calves in different technological periods of raising and for different systems of housing are shown in Table 1.

Table 1 — Dynamics of the quantitative composition of the microflora of the large intestine of calves in different technological periods of raising

Indicators, CFU/g		**Age of calves, day**					
		1–3		**4–14**		**15–30**	
Form of housing		**Individual (n = 10)**	**Group (n = 10)**	**Individual (n = 10)**	**Group (n = 10)**	**Individual (n = 10)**	**Group (n = 10)**
Lactobacilli *Lactobacillus*	log	2	2	7–8	6–8	6–8	6–7
	M ± m	$5 \pm 1.2\times10^{2}$	$7 \pm 2.2\times10^{2}$	$11 \pm 2.8\times10^{7}$*	$190 \pm 14.5\times10^{6}$	$192 \pm 93.6\times10^{6}$*	$82 \pm 11.5\times10^{6}$
Bifidobacteria *Bifidobacterium*	log	3	3	8	7	8–10	7–8
	M ± m	$3 \pm 1.8\times10^{3}$	$1 \pm 0.8\times10^{3}$	$4 \pm 0.7\times10^{8}$*	$2 \pm 1.5\times10^{7}$	$141 \pm 41.8\times10^{8}$*	$77 \pm 3.2\times10^{7}$
E. coli	log	2	2	5	5–6	5–7	6–7
	M ± m	$4 \pm 0.8\times10^{2}$	$2 \pm 1.0\times10^{2}$	$6 \pm 1.2\times10^{5}$*	$30 \pm 20.4\times10^{5}$	$113 \pm 52\times10^{5}$*	$19.6 \pm 1.5\times10^{6}$
Staphylococci *Staphylococcus*	log	0–3	0–3	0–3	2–4	2–5	3–5
	M ± m	$2 \pm 1.4\times10^{3}$	$6 \pm 2.1\times10^{3}$	$6 \pm 2.2\times10^{3}$*	$15 \pm 4.6\times10^{3}$	$196 \pm 96.6\times10^{3}$	$191 \pm 36.7\times10^{3}$
Sulfite-reducing clostridia (*Clostridium*)	log	0–2	0–2	0–2	0–3	0–4	0–4
	M ± m	$3 \pm 1.0\times10^{2}$	$6 \pm 2.1\times10^{2}$	$6 \pm 1.8\times10^{2}$*	$5 \pm 2.3\times10^{3}$	$16 \pm 8.7\times10^{3}$*	$30 \pm 12.3\times10^{3}$
Enterococci (*Enterococcus*)	log	2	2	2	2–3	5	3–5
	M ± m	$4 \pm 0.7\times10^{2}$	$1 \pm 0.8\times10^{2}$	$6 \pm 0.3\times10^{2}$*	$41 \pm 1.0\times10^{2}$	$4 \pm 1.0\times10^{5}$*	$129 \pm 43\times10^{3}$
Saprophytic microorganisms of the genus *Bacillus*	log	0–1	0–1	1–2	1–3	2–3	3–4
	M ± m	$3 \pm 1.3\times10^{2}$	$7 \pm 2.1\times10^{2}$	$61 \pm 2.1\times10^{1}$*	$310 \pm 41.3\times10^{1}$	$17 \pm 6.7\times10^{2}$*	$17 \pm 8.54\times10^{3}$
Yeast-like fungi of the genus *Candida*	log	0–2	0–2	0–2	2–3	2	2–3
	M ± m	$1.6 \pm 0.5\times10^{2}$	$2.2 \pm 1.0\times10^{2}$	$2.3 \pm 1.3\times10^{2}$*	$24 \pm 15.7\times10^{2}$	$7 \pm 9.7\times10^{2}$*	$23 \pm 7.1\times10^{2}$

Note. * — $p \leq 0.05$ in relation to the group method of animal housing.

Knowledge of the qualitative composition of the normoflora and the dynamics of quantitative changes in bacteriocenoses can predict the clinical manifestations of dysbiotic disorders, the development of enteritis, timely take measures to maintain a stable intestinal bacteriocenosis, or its correction.

According to the results of these studies it can be noted that to exclude dysbiotic disorders it is necessary to maintain the optimal composition and quantitative level of the main microflora of the intestinal tract, in particular the number of lactobacilli should be at least 6 log CFU/g, bifidobacteria at least 7 log CFU/g, the amount of *E. coli* not more than 7 log CFU/g (except for calves up to three days of age).

Discussion. According to the analysis of literature data, no uniform normative indicators on the composition of the normal flora of the intestinal tract in calves in different technological periods of raising and under different housing systems were found (Batrakov et al., 2021; Trofimov, 2019; Pudovkin et al., 2019; Efimova and Udalova, 2011; Shakhov, Sashnina and Erina, 2016; Avdeeva et al., 2016; Usachev, 2010; Burova and Blokhin, 2017; Andreeva et al., 2015; Maslianko et al., 2013; Lapinska, 2013; Kalinichenko, Korotkykh and Tishchenko, 2016; Basova, et al., 2016). Of course, individual housing of animals in all respects is better than group one, in particular the feed is better normalized and dosed, no competition and negative impact between weak and strong calves, minimal stress (Trofimov, 2019; Shakhov, Sashnina and Erina, 2016).

According to the results of our research, it was concluded that the content of bifidobacteria and

lactobacilli in calves kept individually in boxes was more stable and high. In calves kept in groups, higher and variable levels of saprophytic microorganisms of the genus *Bacillus*, yeast-like fungi of the genus *Candida*, enterococci, *E. coli* and clostridia were observed.

Batrakov et al. (2021) in their studies noted that a particularly important factor in the individual housing of calves is significant deterioration of conditions for bacterial and viral contamination of the body of calves and the environment. With this technology, calves have the best specific resistance, and each calf acquires a specific microflora, with which it functions and develops normally.

Data of Basova et al. (2016) show that in the early postnatal period intestinal microorganisms predominate, the content of which reached 9–10 log CFU/g, the content of enterococci and enterobacteria was compared with the number of symbiotic microorganisms (bifidobacteria and lactobacilli) and was within 3–4 log CFU/g. Fungi of the genus *Candida*, clostridia and hemolytic forms of bacteria were isolated from the feces of calves, the number of which ranged 1–2 log CFU/g. By the 30th day of life in the feces of calves of all groups there was a decrease in lactose-positive escherichia, increased number of lactobacilli to 5 log CFU/g and saprophytic staphylococci up to 4 log CFU/g. Clostridia were not isolated from feces of one-month-old calves.

In contrast, Efimova and Udalova (2011) in their monograph noted that the normal composition of bifidobacteria for animals is from 7 to 10 log CFU/g, lactobacilli — from 5 to 7 log CFU/g, clostridia — from 4 to 5 log CFU/g, escherichia — up to 7 log CFU/g, enterococci — from 6 to 7 log CFU/g, staphylococci — from 3 to 4 log CFU/g, microorganisms of the genus *Bacillus* — from 3 to 4 log CFU/g, fungi — up to 3 log CFU/g, enterobacteria — from 0 to 5 log CFU/g.

According to the results of our research, it was found that in calves up to three days of age the quantitative content of microorganisms is minimal. The number of bifidobacteria and lactobacilli did not exceed 3 log CFU/g, clostridia — from 0 to 2 log CFU/g, escherichia — 2 log CFU/g, enterococci — 2 log CFU/g, staphylococci — from 0 to 3 log CFU/g, microorganisms of the genus *Bacillus* — from 0 to 1 log CFU/g, fungi — from 0 to 2 log CFU/g.

Usachev (2010) in his work noted that the enteric microbiocenosis in lambs is formed by the 10th day of age. Thus, the composition of bifidobacteria for day-old animals was 2–4 log CFU/g, lactobacilli — 2–3 log CFU/g, escherichia — 2 log CFU/g, enterococci — 2–3 log CFU/g, microorganisms of the genus *Bacillus* — 1 log CFU/g, fungi — 1 log CFU/g. The composition of bifidobacteria for animals of 10 days of age was 9–10 log CFU/g, lactobacilli — 7–8 log CFU/g, *E. coli* — 7 log CFU/g, enterococci — 5–6 log CFU/g, microorganisms of the genus *Bacillus* — 5 log CFU/g, fungi — 3 log CFU/g.

Andreeva et al. (2015) in newborn calves isolated bifidobacteria in the amount from 5.8 ± 0.1 log CFU/g to 6.0 ± 0.1 log CFU/g, lactobacilli — from 3.6 ± 0.1 log CFU/g to 3.8 ± 0.05 log CFU/g. In calves, the number of bifidobacteria reached maximum values at three months of age (7.9 ± 0.1 log CFU/g); the number of lactobacilli — at 30 days of age (7.0 ± 0.1 log CFU/g).

There are scientific studies that show that the intestinal microflora in newborns is formed in 43% of mothers, including the vagina, and 28% of the environment, including air and floor units (Diao, Zhang and Fu, 2019; Bi et al., 2019). In addition, it is noted that feeding regimens affect the transmission of bacteria to newborns from mothers and the environment (Bi et al., 2019). It has been reported that microbial colonization of mammalian intestines begins to form before birth, but these observations are contradictory due to problems with reliable sampling and analysis of low-content microbiota (Alipour et al., 2018; Mayer et al., 2012). The microbial composition of the intestinal microflora resembles an oral microbiocenosis, not fecal or vaginal. During the first day after birth, microorganisms of the genus *Escherichia*, *Shigella*, and *Clostridum* settle in the rectum. The microflora changes within seven days, after that its composition already resembles the microbiocenosis of the rectum of adult animals (Alipour et al., 2018). In general, the intestinal microbiocenosis of calves undergoes dynamic changes during the first twelve weeks of life and this is due to the peculiarities of the digestive system of ruminants (Uyeno, Sekiguchi and Kamagata, 2010).

In our research, we studied changes in the normal flora of the intestine over 30 days. It was found that the composition of the microflora depended on the method of housing. *E. coli* was detected in 100% of cases during the entire period, starting from the first day of life. The content of *E. coli* in animals older than three days of age increased from $2 \pm 1.0\times10^{2}$ CFU/g to $30 \pm 20.4\times10^{5}$ CFU/g, clostridia — from $3 \pm 1.4\times10^{2}$ CFU/g to $30 \pm 12.3\times10^{3}$ CFU/g.

Conclusions. 1. It was found that in calves up to three days of age the quantitative content of microorganisms is minimal. The amount of bifidobacteria and lactobacilli did not exceed 3 log CFU/g. In calves older than three days of age, their level increased, and depending on the method of housing, ranged from 6–8 log CFU/g in calves under the group housing, 7–8 log CFU/g — in calves under the individual housing.

2. The microbiocenosis in calves of 4–14 days of age under the individual housing system was characterized by a high level of bifidobacteria ($4.2 \pm 0.7\times10^{8}$ CFU/g), lactobacilli ($11.1 \pm 2.8\times10^{7}$ CFU/g), the more constant and less variable composition of *E. coli* ($6 \pm 1.2\times10^{5}$ CFU/g) and bacillary spore microflora ($61 \pm 2.1\times10^{1}$ CFU/g).

3. Under the group housing of calves of 4–14 days of age, the content of bifidobacteria was lower ($2 \pm 1.5\times10^{7}$ CFU/g), and the content of *E. coli* and saprophytic microorganisms of the genus *Bacillus* was high and more variable ($30 \pm 20.4\times10^{5}$ CFU/g and $310 \pm 41.3\times10^{1}$ CFU/g respectively) than in the individual housing system.

References

Alipour, M. J., Jalanka, J., Pessa-Morikawa, T., Kokkonen, T., Satokari, R., Hynönen, U., Iivanainen, A. and Niku, M. (2018) 'The composition of the perinatal intestinal microbiota in cattle', *Scientific Reports*, 8(1), p. 10437. doi: 10.1038/s41598-018-28733-y.

Andreeva, A. V., Nikolayeva, O. N., Kadyrova, D. V. and Altynbekov, O. M. (2015) 'Correction microbiocenosis of intestines of newborn calves' [Korrektsiya mikrobiotsenoza kishechnika novorozhdennykh telyat], *Scientific Notes of the Kazan State Academy of Veterinary Medicine named after N. E. Bauman [Uchenye zapiski Kazanskoy gosudarstvennoy akademii veterinarnoy meditsiny imeni N. E. Baumana]*, 222(2), pp. 16–18. Available at: https://elibrary.ru/item.asp?id=23766615. [in Russian].

Avdeeva, Y. A., Medvedeva, O. A., Korolev, V. A. and Kalutskii, P. A. (2016) 'Influence of probiotics bactistatin and normobact on the composition of the microbiocenosis of the large intestine in experimental dysbiosis' [Vliyanie probiotikov baktistatin i normobakt na sostav mikrobiotsenoza tolstogo kishechnika pri eksperimental'nom disbioze], *Bulletin of the Orenburg State University [Vestnik Orenburgskogo gosudarstvennogo universiteta]*, 6, pp. 45–48. Available at: https://elibrary.ru/item.asp?id=26533969. [in Russian].

Basova, N. Y., Staroselov, M. A., Skhatum, A. K., Fedorov, Y. E., Pachina, V. V. and Markov, A. N. (2016) 'Influence of imactin on development of intestinal microbiocenosis in newborn calves' [Vliyanie imaktina na stanovlenie mikrobiotsenoza kishechnika telyat], *International Research Journal [Mezhdunarodnyy nauchno-issledovatel'skiy zhurnal]*, 3(3), pp. 36–37. doi: 10.18454/IRJ.2016.45.147. [in Russian].

Batrakov, A. Ya., Plemyashov, K. V., Videnin, V. N. and Yashin, A. V. (2021) *Prevention and Treatment of Dyspepsia in Newborn Calves [Profilaktika i lechenie dispepsii u novorozhdennykh telyat]*. Saint Petersburg: Kvadro. ISBN 9785906371711. Available at: https://veterinary.lenobl.ru/media/content/docs/13297/%D0%94%D0%B8%D1%81%D0%BF%D0%B5%D0%BF%D1%81%D0%B8%D1%8F.pdf. [in Russian].

Bi, Y., Cox, M. S., Zhang, F., Suen, G., Zhang, N., Tu, Y. and Diao, Q. (2019) 'Feeding modes shape the acquisition and structure of the initial gut microbiota in newborn lambs', *Environmental Microbiology*, 21(7), pp. 2333–2346. doi: 10.1111/1462-2920.14614.

Burova, O. A. and Blokhin, A. A. (2017) 'Systematic approach to the development of methods of prevention of gastro-intestinal diseases in newborn calves' [Sistemnyy podkhod k razrabotke metodov profilaktiki zheludochno-kishechnykh bolezney novorozhdennykh telyat], *Agricultural Science Euro-North-East [Agrarnaya nauka Evro-Severo-Vostoka]*, 2, pp. 46–50. Available at: https://elibrary.ru/item.asp?id=28862005. [in Russian].

Diao, Q., Zhang, R. and Fu, T. (2019) 'Review of strategies to promote rumen development in calves', *Animals*, 9(8), p. 490. doi: 10.3390/ani9080490.

Efimova, L. V. and Udalova, T. A. (2011) *Effective Microorganisms in Cattle and Pig Nutrition [Effektivnye mikroorganizmy v kormlenii krupnogo rogatogo skota i sviney]*. Krasnoyarsk: Krasnoyarsk Research Institute of Animal Husbandry of the Russian Academy of Agricultural Sciences. ISBN 9785904896294. Available at: https://elibrary.ru/item.asp?id=23987218. [in Russian].

Goodfellow, M., Kämpfer, P., Busse, H.-J., Trujillo, M. E., Suzuki, K., Ludwig, W. and Whitman, W. B. (eds) (2012) *Bergey's Manual of Systematic Bacteriology, Vol. 5: The Actinobacteria, Part A and B*. 2nd ed. New York, NY: Springer. doi: 10.1007/978-0-387-68233-4.

Kalinichenko, S. V., Korotkykh, O. O. and Tishchenko, I. Yu. (2016) 'The topical areas of creation and improvement of probiotics' [Suchasni napriamky stvorennia ta udoskonalennia probiotykiv], *Ukrainian Biopharmaceutical Journal [Ukrainskyi biofarmatsevtychnyi zhurnal]*, 1, pp. 4–10. doi: 10.24959/ubphj.16.1. [in Ukrainian].

Lapinska, A. P. (2013) 'Formation of the microbiocenosis of farm animals and poultry, problems and perspectives' [Formuvannia mikrobiotsenozu silskohospodarskykh tvaryn i ptytsi, problemy ta perspektyvy], *Grain Products and Mixed Fodder's [Zernovi produkty i kombikormy]*, 2, pp. 29–34. Available at: http://nbuv.gov.ua/UJRN/Zpik_2013_2_11. [in Ukrainian].

Maslianko, R. P., Bozhyk, L. Ya., Romanovych, M. S. and Fliunt, R. B. (2013) 'Peculiarities of the development of intestinal infections in calves caused by opportunistic microorganisms' [Osoblyvosti rozvytku kyshkovykh infektsii u teliat, vyklykanykh umovno-patohennymy mikroorhanizmamy], *Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnologies named after S. Z. Gzhytskyj. Series: Veterinary Sciences [Naukovyi visnyk Lvivskoho natsionalnoho universytetu veterynarnoi medytsyny ta biotekhnolohii imeni S. Z. Gzhytskoho. Seriia: Veterynarni nauky]*, 15(1_1), pp. 137–141. Available at: http://nbuv.gov.ua/UJRN/nvlnu_2013_15_1(1)__25. [in Ukrainian].

Mayer, M., Abenthum, A., Matthes, J. M., Kleeberger, D., Ege, M. J., Hölzel, C., Bauer, J. and Schwaiger, K. (2012) 'Development and genetic influence of the rectal bacterial flora of newborn calves', *Veterinary Microbiology*, 161(1–2), pp. 179–185. doi: 10.1016/j.vetmic.2012.07.023.

Pudovkin, D. N., Shchepetkina, S. V., Karpenko, L. Yu. and Rishko, O. A. (2019) *Diseases of Young Cattle: Practical Recommendations [Bolezni molodnyaka krupnogo rogatogo skota: prakticheskie rekomendatsii]*. 2nd ed. Saint Petersburg: Saint Petersburg State Academy of Veterinary Medicine. ISBN 9785869839213. [in Russian].

Shakhov, A., Sashnina, L. and Erina, T. (2016) *Intestinal Microbiocenosis and Immune Status of Calves and Their Correction: Dysbacterioses and Immunodeficiencies in Hypotrophy and Asphyxia [Mikrobiotsenoz kishechnika i immunnyy status telyat i ikh korrektsiya: disbakteriozy i immunodefitsity pri gipotrofii i asfiksii]*. Saarbrücken: Palmarium Academic Publishing. ISBN 9783659604102. [in Russian].

Trofimov, A. F. (2019) 'Creating optimal conditions for calves' [Sozdanie optimal'nykh usloviy dlya telyat], *Our Farming. Veterinary and Livestock. [Nashe sel'skoe khozyaystvo. Veterinariya i zhivotnovodstvo]*, 2, pp. 24–29. [in Russian].

Usachev, I. I. (2010) 'Bacteriocenosis of the gastrointestinal tract of newborn lambs during its natural and experimental formation' [Bakteriotsenoz zheludochno-kishechnogo trakta novorozhdennykh yagnyat pri estestvennom i eksperimental'nom ego formirovanii], *Sheep, Goats, Wool Industry [Ovtsy, kozy, sherstyanoe delo]*, 4, pp. 76–78. Available at: https://elibrary.ru/item.asp?id=36259421. [in Russian].

Uyeno, Y., Sekiguchi, Y. and Kamagata, Y. (2010) 'rRNA-based analysis to monitor succession of faecal bacterial communities in Holstein calves: Calf faecal community succession', *Letters in Applied Microbiology*, 51(5), pp. 570–577. doi: 10.1111/j.1472-765X.2010.02937.x.

Van Emden, H. F. (2019) *Statistics for Terrified Biologists*. 2nd ed. Hoboken, NJ: John Wiley & Sons. ISBN 9781119563679.