

## Antagonistic Activity of a Probiotic Prolam in Point of Bacterial Pathogens and its Influence on an Intestines Microbiocenosis, the Immune and Clinical Status of Calfs

<sup>1</sup>Irina V. Cheremushkina, <sup>2</sup>Alexey G. Shakhov, <sup>2</sup>Larisa Yu. Sashnina,  
<sup>2</sup>Anton E. Chernitsky and <sup>2</sup>Tatyana A. Yerina

<sup>1</sup>Federal State Budgetary Educational Establishment of Higher Professional Education “Voronezh State University of Engineering Technologies”, Voronezh, Russian Federation

<sup>2</sup>State Scientific Institution All-Russian Research Veterinary Institute of Pathology, Pharmacology and Therapy of the Russian Agricultural Academy, Voronezh, Russian Federation

---

**Abstract:** Application of intensive technologies in animal industry leads to increase a stress sensitivity of animals to decrease in their immune status and development of pathological states. For increase in safety of young growth including due to decrease in its incidence and a loss of cattle from diseases, it is developed the feed additives including the probiotics, prebiotics and (or) other components stimulating immunological resistance of an organism of animals, their growth and efficiency. In the present study, it is studied the possibility of inclusion of the probiotic prolam containing viable strains of lactobacilli, lactic acid streptococcus and bifidus bacteria in structure of the feed additive, developed for increase of efficiency of calf breeding. It is investigated that a probiotic prolam *in vitro* shows the expressed antagonistic activity concerning escherichias and the salmonellas which are one of the main bacterial causative agents of gastrointestinal infections of calfs during the colostric and suckle seasons of breeding.

**Key words:** Calfs, probiotic prolam, antagonistic activity, microbiocenosis, natural resistance, cytokines, gastrointestinal diseases

---

### INTRODUCTION

One of the problems of veterinary science is development for animal industry of ways of increase of safety of young growth and its efficiency including due to decrease in incidence and a loss of cattle from diseases of various etiology. Sharpness of this problem is connected with that application of intensive technologies in modern farms conducts to increase a stress sensitivity of animals to decrease in their immune status and development of pathological states (Pilipenko, 2008; Chervinets, 2006; Anufriyeva, 2010; Gorlov, 2015; Nader-Macias *et al.*, 2008; Langel *et al.*, 2015; Magalhaes *et al.*, 2008; Masmanian, 2008; Al-Mawly *et al.*, 2015; Tao *et al.*, 2012; Windeyer *et al.*, 2014).

The greatest relevance during the colostric and suckle seasons of breeding of calfs, especially from group of risk (animals with signs of morphological and functional insufficiency) has the gastrointestinal diseases. In their etiology along with causative agents of virus (rota-, corona-, parvo-, Viral Diarrhea Diseases of Mucous

Membranes (VD-DMM), Virus of Infectious Rhinotracheitis (VIR), etc.) and bacterial (colibacillosis, salmonellosis, clostridiosis, etc.) infections the opportunistic microflora and disbacteriosis is played a large role which are characterized by permanent quantitative and quality changes of the bacteria which are the parts of normal microflora (Tarkanov, 2007; Ovod, 2005; Grigorieva, 2005; Mishchenko, 2008; Oleynik, 2009; Gorkovenko *et al.*, 2009; Nikolaev, 2010; Arbuzova, 2010; Motorygin, 2011; Kapustin, 2013; Yankovsky, 2005; Coura *et al.*, 2015; Damman *et al.*, 2015; Fulton *et al.*, 2015; Lukas *et al.*, 2007; Millemann, 2007; Navarre *et al.*, 2000).

In this regard to their prevention, it is applied the means of specific protection and also preparations pro-and prebiotic action.

Probiotics in modern meaning is the bacterial preparations from live germ cultures, intended for correction of microflora of the person and animals and treatment of diseases (Shakhov, 2008; Sukhina, 2012; Sisyagin, 2015; Panin, 2012; Blum *et al.*, 1999; Bunesova *et al.*, 2012).

The mechanism of action of probiotics is based on compulsory invasion of intestines by competitive strains the indigenous bacteria, exercising nonspecific control of level of opportunistic microorganisms by their replacement from structure of intestinal population and control of development at them of factors of pathogenicity (Solovyova, 2010; Novick, 2006; Kuchumova, 2011; Lomax, 2009).

Possessing the expressed antagonistic properties to concrete pathogens and opportunistic bacteria, thanks to the ability to produce antimicrobial and antibiotic substances (lactic acid, hydrogen peroxide, lysozyme, bacteriocins, etc.), the probiotics thereby protect a gastrointestinal tract from inflammatory processes (Shakhov, 2008; Ermolenko, 2007; Kramarev, 2008; Petrakov, 2014; Menard *et al.*, 2004; Viera *et al.*, 2013). Besides, the indigenous microflora which is a part of probiotics belongs to factors of nonspecific resistance of a human body and animals and takes part in development of antibodies (Haptseva, 2011; Ovod, 2006; Sisyagin, 2015; Krapivina, 2011; Lifanova, 2013; Gill *et al.*, 2000; Herich and Levcut, 2002).

In recent years for increase of efficiency of breeding of calfs the searches are conducted, directed on use of the cheap, harmless and suitable for mass application feed additives, including the probiotics, prebiotics and (or) other components increasing immunological resistance of animal organisms, stimulating their growth and efficiency (Feklisova, 2005; Korneeva, 2012; Krapivina, 2011; Arushanyan, 2013, 2015; Morozova, 2015; Ewaschuk *et al.*, 2004; Maldonado, *et al.*, 2012; Mokhber-Dezfouli *et al.*, 2007; Ripamonti *et al.*, 2011).

Due to the development of complex feed additive for young growth of farm animals (calfs) it is necessary to prove a choice of pro-biotic cultures for inclusion in its structure.

The purpose of researches to study antagonistic activity of a probiotic prolam concerning bacterial pathogens, its influence on process of formation of a microbiocenosis of intestines, the immune and clinical status of calfs.

## MATERIALS AND METHODS

A pro-biotic preparation prolam which is widely applied in pig-breeding and poultry farming, represents a suspension, which contains viable strains of lactic bacteria of *Lactobacillus delbrueckii* ssp. bulgaricus (B-5788), *Lactobacillus acidophilus* 43c (B-3235) in quantity not less  $-5 \times 10^7$  CFU  $\text{nm}^{-3}$ , lactic streptococcus of *Lactococcus lactis* ssp. lactis 57<sub>4</sub> (B-3145), *Lactococcus lactis* ssp. lactis 170<sub>4</sub>-5

(B-3192)- $5 \times 10^7$  CFU  $\text{nm}^{-3}$  (colony forming unit), Bifidobacterium animal 83 (AC-1248)- $1 \times 10^7$  CFU  $\text{nm}^{-3}$  and adjuvants water, molasses beet, lactoserum.

For determination of antagonistic activity of a probiotic in the relation the test-cultures of *E. coli*, *S. dublin* and *S. aureus* were used a Disco-Diffusion Method. The 1 mL of daily cultures brought in sterile petri dish (with a titer  $10^5$  bacteria  $\text{mL}^{-1}$  according to the standard of turbidity) pathogenic strains of the specified bacteria and then on 10 mL melted and refrigerated to 45-40°C meat-peptone agar. Contents of petri dishes were mixed carefully rotary motions for homogeneous distribution of material. In the fixed agar covering of petri dishes with a metal stamp cut out holes with a diameter of 10 mm and brought in them on 100 mL of a probiotics.

After 20 min holding at the room temperature the petri dishes placed in the thermostat (37°C) on 24-48 h. Then there was determined a diameter of zones of a growth inhibition of microorganisms round a hole including its diameter. For control of pathogenic microorganisms it was sowed in petri's dishes with meat-peptone agar without pro-biotic cultures (Netrusov, 2005).

Studying of influence of a probiotic on process of formation of a microbiocenosis of intestines, the immune and clinical status is carried out on 32 newborn calfs which were divided into 2 groups. To calfs of experimental group (n = 16) applied a probiotic "prolam" in a dose  $5-7 \text{ nm}^{-3}$  with colostrum (milk) and daily during the first 7 days of life, the preparation did not apply for a control group (n = 16).

Calfs after the birth keeping in individual indusiums, within 3 days to them was given colostrum (milk) of mother and then the milk, made by ripening (formic acid). Animals were not submitted to drug treatment.

Within 10 days for calfs it was conducted the clinical observation. At animals of experimental group before and after of termination of application of a preparation and at the same time, at intact calfs investigated a microbial view of thick part of bowel and the immune status. Excrements were served as material for research, whole blood and blood serum of calfs.

For definition of quantitative and qualitative structure of intestinal microflora from excrements prepared tenfold dilutions from 1:10-1:10<sup>10</sup> in the phosphatic buffer (pH 7,0). Inoculation of medium made from the received dilutions: meat-peptone agar, Endo, a salt agar, a blood agar (meat-peptone agar from 5% content of erythrocytes of a ram), Kitta-Tarozzi, Wilson-Bler, Blaurock and small cattle (for excretion of lactobacilli). After incubation within 18-24 h there was counted colonization of microorganisms of each type. Recalculation was conducted on 1 g

excrements taking into account extent of dilution (Gorkovenko *et al.*, 2009). Studying of cultural and morphological and biochemical properties of the excretion microorganisms was done by the standard methods (Sidorov *et al.*, 1995).

Bactericidal, lysozyme and complementary activity of serum of blood was identified by the modified techniques (Shakhov *et al.*, 2007). The 1 mL of serum and 0.1 mL of daily bouillon culture of *Escherichia coli* were added for research BCAS in 4.5 mL of Hottinger bouillon, only culture of a microorganism brought in control tests. Contents of test tubes were mixed carefully, in 2 mL of blend measured the Optical Density ( $OD_{490}$ ). Blend, remained in test tubes was incubated in the thermostat at 37°C within 3 h and repeatedly measured optical density. Activity was identified in units of inhibition of growth of optical density in experimental tests in comparison with control.

At determination of LCAS to 0.1 mL of serum of blood there was added 0.4 mL 0.06 M phosphatic buffer (pH 7.2-7.4) and 2 mL of a microbial suspension of *Micrococcus lysodeiaticus* with an optical density 0.215  $OD_{540}$ . Control was contained 0.5 mL of the phosphatic buffer and 2 mL of a suspension of a microorganism, standard samples were included 0.4 mL of the phosphatic buffer, 0.1 mL of solution of lysozyme with known activity and 2 mL of a suspension of a micrococcus. Tests were incubated 30 min at 37°C and measured the Optical Density ( $OD_{540}$ ). Activity of lysozyme was evaluated in microgram/mL.

For definition of KCAS the 0.1 mL of serum of blood was added to 5.9 mL 0.89% of NaCl solution and control samples contained 5.9 mL of bi-distilled water. Tests incubated 30 min at 37°C, control at 56°C for an inactivation of system of a complement. The haemolytic mixture added to all test tube on 4 mL of prepared and warmed at 37°C within 30 min after an incubation which contained of 2.5% solution of erythrocytes of a ram and rabbit haemolytic serum. Tests placed for 30 min in a water bath at 37°C, centrifuged at 3000 rpm within 10 min. The optical density of a supernatant was measured at  $OD_{520}$ . Activity was evaluated as a percentage by ratios of optical density of experimental tests to the control.

For identification of Phagocytic Activity Leukocytes (PAL), Phagocytic Index (PI), Phagocytic Number (PN) to 0.5 mL of the blood, stabilized by heparin (5000 Pieces  $mL^{-1}$ ), it was added 0.5 mL *Staphylococcus aureus* suspension (1.5 billion  $mk mL^{-1}$ ), inactivated on a water bath at 100°C within 60 min. Tests were incubated 30 min. at 37°C, swabs were made on the fat-free glasses three times and then it was fixed by methyl alcohol and painted Giemsa staining according to Romanovskiy-Gimze.

Phagocytic activity leukocytes was evaluated in percent of active leukocytes (phagocytes) in 100 counted the neutrophilic leukocytes.

The phagocytic index was identified by division of number of the phagocytic bacteria on number of active neutrophils and phagocytic number accordingly on total quantity of the counted neutrophils.

Concentration of pro-inflammatory (IL-1 $\beta$ , TNF- $\alpha$  (Tumor Necrosis Factor), IL-8), immune-regulatory (IL-2, IL-4, IFN- $\gamma$  (interferon)) and anti-inflammatory (IL-10) of cytokines in serum of blood of calfs was identified by method of the immune-enzymatic analysis according to the approved methodics to the corresponding sets of instruments for diagnosis ("Vektor-Best" Russia).

## RESULTS

The probiotic prolam had high antagonistic activity concerning *Escherichia coli* (a zone of a delay of 26.0 $\pm$ 0.82 mm) and *Salmonella* (23.0 $\pm$ 0.62 mm). The preparation showed smaller activity concerning golden *Staphylococcus aureus* (17.0 $\pm$ 0.63 mm).

At animals of both groups during the colostric period it was registered a diarrheic syndrome but number of the diseased, weight and duration of clinical course were various.

In control group, the incidence of gastrointestinal diseases was made 100% with an average duration of 6.4 days. The Entero-toxic form of colibacteriosis was registered at 8 calfs (50.0%). The rotavirus genome was found at 4 animals (25.0%), their disease was in a heavy form. Coronavirus genome was found at all calfs on 7-8 days.

At experimental group, the gastrointestinal diseases were registered in 62.5% cases with an average duration of 5 days. Entero-toxic form of colibacteriosis was noted at 4 animals (25.0%). In the first day, the rotavirus genome was found at 2 calfs (12.5%) and their disease was in a heavy form.

The analysis of a condition of the microbial view of thick part of bowel of intestines and indicators of nonspecific resistance of calfs in the 1st days is testified that at animals of experimental and control groups they had no essential distinctions.

Formation of the microbial view of intestines of intact calfs was shown by increase on the 7th day of level of indigenous microflora: *Lactobacilli* in 25.4 times; *Bifidobacteria* in 12.6 and the lactose-positive *Escherichia coli* in 306.6 times and also the content of opportunistic microorganisms: the lactose-negative *Escherichia coli* in 223.9; *Enterococcus* in 67.9 and 172.8; *Citrobacter* in 15.3; *Enterobacter* in 57.3; golden *Staphylococcus aureus* in

**Table 1: Microbial view of large department of intestines of calfs**

Name of microorganisms	Quantity of microorganisms lg CFU firm <sup>-3</sup> (colony forming unit) in excrements of calfs			
	Intact		Got "prolam"	
	Ist day	7th day	Ist day	7th day
<i>Lactobacillus</i> spp.	8.24±0.71	9.64±0.88	8.54±0.94	1065±078*
<i>Bifidobacterium</i> spp.	9.48±0.81	10.58±0.83*	975±0.32	11.67±0.62*
<i>E. coli</i> (lact.+)	6.17±0.3	8.66±0.21*	6.54±0.1	7.68±0.84*
<i>E. coli</i> (lact.-)	5.36±0.091	7.71±0.34*	5.71±0.7	6.59±0.01*
<i>Enteroc. faecium</i>	4.74±0.087 (75%)	6.58±0.12*	4.65±0.65	5.73±0.38*
<i>Enteroc. faecalis</i>	4.37±0.75 (75%)	6.61±0.51*	4.58±0.37	5.44±0.4*
<i>Citrobacter</i> spp.	3.43±0.27 (50%)	4.61±0.64*	3.41±0.29 (50%)	3.38±0.54 (75%)
<i>Enterobacter</i> spp.	3.03±0.17 (50%)	4.78±0.80*	3.71±0.69 (50%)	3.43±0.2 (75%)
<i>Staph. aureus</i>	3.47±0.1 (50%)	3.60±0.31 (75%)	3.85±0.03 (25%)	n/d
<i>Proteus</i> spp.	50%	25%	n/d	n/d
<i>Staph. (saprophyt.; apidermidis)</i>	3.47±0.12 (75%)	3.59±0.74 (75%)	3.43±0.2	4.43±0.2*

\* Microorganisms were identified at 100% of animals; (%). The frequency of their excretion; n/d Didn't drop

1.3 times at increase of frequency of its excretion for 25.0%, decrease in frequency of isolation a proteus in 2 times. It is also noted that they had insignificant increase of the contents of the saprophytic staphylococcus in 1.3 times (Table 1).

Prolam's application favorably affected on process of formation of a microbiocenosis of intestines. At calfs of experimental group on the 7th day in comparison with a background (1 day), it was registered more essential (than at intact animals) increase of content of lactobacilli (in 130.5 times), bifidobacterium (in 83.0 times) and less substantial increase of level of opportunistic microflora: the lactose-negative escherichias in 7.6 times, enterococcus in 12.0 and 7.3; decrease in quantity of citrobacters in 7.8%, enterobacters in 1.9 times and increase of level of saprophytic microflora in 10 times.

It should be noted that at the animals got prolam and comparing indicators of a microbial view of intestines of calfs of both groups on the 7th day, the content of lactobacilli in 10.2 times, bifidobacterium in 12.3 and the saprophytic staphylococcus in 6.9 times was higher in comparison with intact animals and the quantity of potentially pathogenic microorganisms was lower: Enterococcus faecalis in 13.6 times, the lactose-negative escherichias in 13.2, bacteria of the genus *Citrobacter* spp. in 26.7 and *Enterobacter* spp. in 28.4 times and the frequency of excrement of the last ones on 25.0%. Besides, the *S. aureus* and a bacterium of the genus *Proteus* were not defined from them (Table 1).

Thus, populations of bifido bacterium and lactobacilli were prevailed in an intestinal biocenosis by 7th day of life at the calfs which got prolam and isolation frequency from excrements of potentially pathogenic microflora and its population level were low that testifies to beneficial effect of a probiotic on process of formation of a microbial view at calfs during the colostric period. At animals of control group the intestinal microflora was characterized by rather low content of indigenous microflora (a

*bifidobacterium* and a *lactobacillus*), high frequency of excretion and the content of potentially pathogenic microorganisms.

At research of the immune status it is determined that under the influence of the changed habitat at animals of control group on the 7th days had a decrease the BCAS on 12.4, PAL on 6.1, PN on 22.6, PI on 20.6% and KCAS in 14 times.

It is noted that there was increase of indicators cellular (PAL on 22.6%, PN on 23.7 and PI on 10.8%) and humoral (BCAS on 7.7%) links of nonspecific protection at calfs after application of "prolam" in the specified time, also there was decrease in KCAS in 2.2 times. Increase of PAL, PN, PI and the BCAS at less expressed than in control decrease of KCAS was testified to activation of humoral and cellular links of nonspecific resistance (Table 2).

Thus, prolam's application favorably affected the level of natural resistance during adaptation of an organism of newborn calfs to new conditions. At the animals, receiving a probiotic, indicators of nonspecific protection were higher, than at calfs of control group (BCAS on 13.3%, KCAS in 4.9 times, PAL on 22.1%, PN on 52.1%, PI on 30.2%).

At calfs of intact group on the 7th day there was determined the increase in serum of blood of level of pro-inflammatory cytokines of IL-1β, TNF-α and IL-8 and also IFN-γ, having antiviral action, on 44.9; 35.3; 16.2% and in 2.2 times, respectively. Concentration of cytokines of IL-2, stimulating the cellular immune reaction also was increased on 58.3% as well as an anti-inflammatory mediator of IL-10 on 72.7% and the level of interleukin of IL-4, stimulating the humoral reaction by the end of the colostric period was decreased in 1.7 times (Table 3). The low content of IL-4 conducts to insufficient synthesis of antibodies with B-cages to the increased egestion of cytokines of an inflammation and prostaglandins and it is promoted to long inflammatory reaction, the exhaustion of

Table 2: Indicators of nonspecific resistance of calfs

Indicators	Groups of calfs and terms of researches (days)			
	Intact		Got "prolam"	
	Ist day	7th day	Ist day	7th day
BCAS (%)	77.3±2.19	67.6±2.3***	71.1±6.08	76.6±6.17
KCAS (% haem)	15.4±0.3	1.1±0.13***	10.8±3.40	4.9±1.11
LCAS (mg mL <sup>-1</sup> )	1.6±0.05	1.6±0.15	1.6±0.03	1.5±0.04*
PAL (%)	78.6±3.81	72.5±1.25	77.2±1.85	88.5±1.25***
PN	6.2±0.75	4.8±0.25*	5.9±0.64	7.3±0.71
PI	7.8±0.64	6.2±0.33*	7.4±0.6	8.2±0.81

Table 3: The content of cytokines in blood serum of calfs of intact group and animals before and after of application of a probiotic (pc/mL)

Indicators	Intact calfs		Got "prolam" calfs	
	Ist day	7th day	Ist day	7th day
	IL-1β	48.0±0.44	69.4±0.85***	47.0±0.56
IL-2	28.3±1.11	44.8±4.19***	31.3±0.94	34.0±0.44*
IL-4	1.9± 0.25	1.1±0.08	2.0±0.52	5.9±0.88***
IL-8	15.7±1.96	19.4±1.58	15.1±0.92	6.7±0.53***
IL-10	3.3±0.15	5.7± 1.34*	3.4±0.67	10.8±0.58***
IFN-γ	24.7±1.32	54.8±4.18***	26.2±1.78	34.7±1.34***
TNF-α	24.9±0.54	33.7±0.88***	22.7±0.65	2.8±0.82***

\*, \*\*, \*\*\*p ≤ 0.05, 0.01, 0.001

immune-competent cages and immune system in general (Ketlinsky, 2008). Increase of the contents at calfs of control group of cytokines of IL-10 having strong anti-inflammatory and immune-modulate effect, testifies to the compensatory reaction, directed on inhibition of excess synthesis of pro-inflammatory cytokines of IL-1β and TNF-α.

Under the influence of "prolam" there was an essential increase in concentration of IL-4 in 3.0 times, directing development of the humoral immune reaction in Th<sub>2</sub> way, the level of the anti-inflammatory IL-10 in 3.2 times, conducted to considerable decrease in the content of pro-inflammatory cytokines of IL-1β in 2.1; IL-8 in 2.3 and TNF-α in 8.1 times and stimulating secretion of immune-globulins with B-cages. Also an application of "prolam" was followed by insignificant increase of concentration of IL-2 on 6.5%, regulating the cellular-mediated reaction of animals during adaptation to new conditions and also IFN-γ on 32.4% (Table 3) having antiviral action and strengthening of the cytokines reactions, mediated by T-lymphocytes and NK-cages (Groot *et al.*, 2005).

## DISCUSSION

Transition of the born calf from conditions of sterile prenatal isolation in habitat, seeded of various microorganisms where majority from which is potentially dangerous to immunological unripe organism is made on it powerful stressful impact. Normal microflora of various biotopes and first of all, a gastrointestinal tract plays the leading role in protection of newborns against potentially pathogenic microorganisms.

It is determined by the conducted researches on optimization of process of formation of a microbiocenosis of intestines and immune system at calfs during the colostric period that application of a probiotic prolam promoted the best adaptation of newborns to change of environment, the increased antigenic load and formation of the adequate immune reaction that favorably affected their clinical status.

At the calfs receiving prolam the content of lactobacilli and bifidobacterium was higher, than at animals of intact group and the lactose-negative escherichias, enterococcus, bacteria of the genus *Citrobacter* and *Enterobacter* and the frequency of excretion of the last ones. *Staph aureus* and bacteria of the genus *Proteus* were not isolated from them.

It is known that the role of probiotics consists first of all in maintenance of colonizational resistance mucous intestines to a contamination of opportunistic microorganisms and in decrease in risk of development of the disbacteriosis, provoking and complicating gastrointestinal diseases at young growth of animals (Andreyeva, 2012; Nugumanov, 2013; Frizzo *et al.*, 2008; Nader-Macias *et al.*, 2008; Vlkova *et al.*, 2006).

Also, it is determined that the bifidobacterium which are a part of probiotics and a lactobacillus have antagonistic properties concerning pathogenic microorganisms: they force out pathogens by creation of low values pH milieu, suppressing growth of the pathogenic and opportunistic microorganisms and synthesize the antibacterial substances including organic acids, peroxide of hydrogen, bacteriocins, various low-molecular peptides and proteins with fungicide action

(Fedorov, 2014; Sisyagin, 2015; David, 2014). Lactobacilli, having ability to adhesion, suppress growth and reproduction of the representatives of foreign microflora coming from the outside; prevent a resuscitation of the last, blocking receptors of cages of mucous membranes from adhesives of potentially pathogenic bacteria (Belmer, 2006; Petrakov, 2014; Ohland *et al.*, 2010; Qadis *et al.*, 2014a-c; Signorini *et al.*, 2012).

Prolam's application was promoted to increase of quantity of active neutrophiles in the bloodstream to increase in their absorbing ability and also bactericidal action of serum of blood that it is pointed to activation of nonspecific resistance. Increase of phagocytic activity of neutrophiles under the influence of a preparation was followed by reduction in comparison with control, an expenditure of a complement, it means that there is necessary more participation of proteins of complement system for a lysis of bacterial cages for non-stimulated phagocytes.

It is known that the probiotics, containing in the composition a complex from different types of indigenous microflora (a lactobacillus, a lactococcus and bifidobacterium), promotes not only to maintenance of a normal microbiocenosis of open antrums but also have the expressed immune-stimulating action. Also, it is determined that separate strains of these microorganisms can considerably increase phagocytic ability of macrophages to potentiate production of interleukines, interferon and other mediators, it means to increase nonspecific immune-resistance (Yankovsky, 2005; Fleige *et al.*, 2009; Qadis *et al.*, 2014a-c). It is proved the role of lactobacilli in decrease in inflammatory processes in response to infection with viruses and protection of tissues and cages of an epithelium of intestines and the upper air passages from their damaging action (Herich and Levcut, 2002; Mortazavian, 2007).

Thanks to researches of A.Yu. Mironov with co-author (Mironov, 2011), it was determined the high ability of lactobacilli to the organization of biofilms on mucous, their interaction with immune system of intestines. Pro-biotic bacteria actively participate in formation of early protection against infections, increasing concentration of secretory IgA, phagocytic activity of immune-competent cages, bactericidal, lysozyme and complementary activity of serum of blood (Panin, 2012; Blum *et al.*, 1999; Gabryszewski, 2011; Klaenhammer *et al.*, 2012).

Thus, "prolam", containing in the composition of lactobacillus, lactococcus and bifidobacterium, has the expressed immune-stimulatory action. The microorganisms which are a part of "prolam", participating in formation of an intestinal biocenosis at early stages of

formation of an immune system, are primary antigens for native leukocytes, stimulatory of their proliferation and activation that it is promoted to faster development of the humoral immune response at contact with alien bacteria mainly on Th<sub>2</sub> way. In addition, it is determined a high efficiency of lactobacilli in oppression of virus-induced inflammation and uncontrollable synthesis of interleukins ("cytokine storm") due to decrease of fluxing of granulocytes in the infected tissues (Gabryszewski, 2011; Qadis *et al.*, 2014a-c).

Thus, prolam's application to newborn calfs during the colostric period was promoted of the optimization of process of formation of a microbiocenosis of intestines to the increase of natural resistance and the adaptive immune response to antigenetic influence, accompanied with decrease of the incidences of animals, duration and weight of a course of gastrointestinal pathology.

The obtained data on antagonistic activity of prolam concerning escherichias and salmonellas, its influence on an intestines microbiocenosis, the immune status of calfs testify to possibility of inclusion of a probiotic in composition of the developed complex feed additive for young growth of farm animals (calfs).

## CONCLUSION

Prolam's application to calfs in the period of a neonatality promotes optimization of process of formation of an intestinal microbiocenosis, increase of natural resistance of an organism and the adaptive immune reaction to anti-genetic influence which had the reduction of disease incidence of animals, duration and severity of gastrointestinal pathology. The obtained data are the basis for inclusion of a probiotic prolam in the developed feed additive for young growth of farm animals (calfs).

## ACKNOWLEDGEMENTS

Research is performed within the Federal Target Program "Researches and Development in the Priority Directions of Development of a Scientific and Technological Complex of Russia for 2014-2020" according to the Agreement No. 14.577.21.0139 on granting of a subsidy (the unique identifier of scientific researches and experimental development of RFMEFI57714X0139).

## REFERENCES

- Andreyeva, A.V., 2012. Normal microflora of intestines of shoats, stress at weaning age. Andreyeva, A.V. and E.T. Muratova (Eds.). Scientific notes of the Kazan State Academy of Veterinary Medicine named after N.E. Bauman. Kazan, T. 203, pp: 15-19.

- Anufriyeva, T.A., 2010. The mixed infections of animals: review. Anufriyeva, T.A., O.A. Borisova, T.V. Zhanbano, I.A. Borisova, Vladimir: VNRIHA, pp: 123.
- Arbuzova, A.A., 2010. Etiological aspects of nascency of gastrointestinal diseases of calfs of the early post-natal period. Arbuzova, A.A. (Ed.). Scientific notes of the Kazan State Academy of Veterinary Medicine named after N.E. Bauman, T200, pp: 11-18.
- Arushanyan, A.Ya., 2013. Prevention of sharp intestinal diseases of the newborn calfs of a bacterial etiology with use of the metabolic prebiotics: Abstract of diss.... candidate of veterinary sciences. Krasknodar, pp: 22.
- Arushanyan, I.F., 2015. Population health and efficiency of animals depending on genetic and paratypical factors. Gorlov, I.F. and E.A. Kuznetsova (Eds.). Collection scientific works of International scientific and practical conference. Population health of animals and emergent infections in modern conditions N. Novgorod, P. 1, pp: 43-55.
- Al-Mawly, J., A. Grinberg, D. Prattley, J. Moffat, J. Marshall and N. French, 2015. Risk factors for neonatal calf diarrhoea and enteropathogen shedding in New Zealand dairy farms. *Vet. J.*, 203 (2): 155-160. DOI: 10.1016/j.tvjl.2015.01.010.
- Belmer, S.V., 2006. Intestinal microflora and value of prebiotics for its functioning. Belmer, S.V. and A.V. Malkoch (Eds.). *Attending Medical Doctor*, No. 4, pp: 60-65.
- Blum, S., S. Blum, S. Alvarez, D. Haller *et al.*, 1999. Intestinal microflora and the interaction with immunocompetent cells. *Ant. Leewen*, 76: 199-205.
- Bunesova, V., K.J. Domig, J. Killer, E. Vlkova, J. Kopeeny, J. Mrazek, S. Roekova and V. Rada, 2012. Characterization of bifidobacteria suitable for probiotic use in calves. *Anaerobe*, 18 (1): 166-168. DOI: 10.1016/j.anaerobe.2011.09.008.
- Coura, F.M., M.D. Freitas, J. Ribeiro, R.A. de Leme, C. de Souza, A.A. Alfieri, E.J. Facury Filho, A.Ú. de Carvalho, M.X. Silva, A.P. Lage and M.B. Heinemann, 2015. Longitudinal study of Salmonella spp., diarrheagenic *Escherichia coli*, Rotavirus and Coronavirus isolated from healthy and diarrheic calves in a Brazilian dairy herd. *Trop. Anim. Health Prod.*, 47 (1): 3-11. DOI: 10.1007/s11250-014-0675-5.
- Chervinets, Yu.V., 2006. Bacteriocinogenicity of high-antagonistic strains of lactobacilli. Chervinets, Yu.V., V.M. Bondarenko, N.A. Shabanova, A.M. Samoukina and V.M. Chervinets (Eds.). *Journal of Microbiology, Epidemiology and Immunobiology*, No. 7, pp: 78-82.
- Damman, A., A.F. Viet, S. Arnoux, M.C. Guerrier-Chatellet, E. Petit and P. Ezanno, 2015. Modelling the spread of Bovine Viral Diarrhea Virus (BVDV) in a beef cattle herd and its impact on herd productivity. *Vet. Res.*, 46 (1): 12. DOI: 10.1186/s13567-015-0145-8.
- David, W., 2014. Probiotics as regulators of inflammation: a review. *Funcional Foods un Health and Disease*, 4 (7): 299-311.
- Ewaschuk, J.B., J.M. Naylor, M. Chirino-Trejo and G.A. Zello, 2004. Lactobacillus rhamnosus strain GG is a potential probiotic for calves. *Can. J. Vet. Res.*, 68 (4): 249-53.
- Ermolenko, E.I., 2007. Antimicrobial action of lactobacilli. Ermolenko, E.I. and O.V. Rybalchenko (Eds.). *Medicine XXI century*, No. 6, pp: 41-48.
- Fedorov, Yu.N., 2014. Primary immunodeficiencies of animals: immunogenetic and clinic-immunological characteristic (review). Fedorov, Yu.N., V.I. Klyukina and M.N. Romanenko (Eds.). *Agricultural Biology*, No. 4, pp: 3-15.
- Feklisova, L.V., 2005. Optimization of results of treatment of the children sick with sharp intestinal infections, at using of domestic biological microbic preparations. Feklisova, L.V. (Ed.). *Bulletin of the Russian Academy of Sciences*, No. 12, pp: 17-24.
- Fleige, S., W. Preissinger, H.H. Meyer and M.W. Pfaffl, 2009. The immunomodulatory effect of lactulose on Enterococcus faecium fed preruminant calves. *J. Anim. Sci.*, 87 (5): 1731-1738. DOI: 10.2527/jas.2007-0494.
- Frizzo, L.S., E. Bertozzi, L.P. Soto, M.V. Zbrun, G. Sequeira, R. Dalla Santina, R. Rodriguez Armesto and M.R. Rosmini, 2008. The effect of supplementation with three lactic acid bacteria from bovine origin on growth performance and health status of young calves. *J. Anim. Vet. Adv.*, 7: 400-408.
- Fulton, R.W., H.R. Herd, N.J. Sorensen, A.W. Confer, J.W. Ritchey, J.F. Ridpath and L.J. Burge, 2015. Enteric disease in postweaned beef calves associated with Bovine coronavirus clade 2. *J. Vet. Diagn. Invest.*, 27 (1): 97-101. DOI: 10.1177/1040638714559026.
- Gabryszewski, S.J., 2011. Lactobacillus-mediated priming of the respiratory mucosa protects against lethal pneumovirus infection. Gabryszewski, S.J., O. Bachar, K.D. Dyer *et al.* (Eds.). *J. Immunol.*, 186 (2): 1151-1161. PubMed: 21169550.
- Gill, H.S., J. Rutherford, J. Prasad and P.K. Gopal *et al.*, 2000. Enhancement of natural and acquired immunity by Lactobacillus rhamnosus (HN001), Lactobacillus acidophilus (YN017) and Bifidobacterium lactis (HN019). *Brit. J. Nutr.*, 83 (2): 167-176.

- Groot, J., L.W. de Kruijt, W.J.A. Boersma, W.G. Buist and C.G. van Reenen, 2005. Age, gender and litter-related variation in T-lymphocyte cytokine production in young pigs. *Immunology*, 114: 495-505.
- Gorkovenko, N.E., Yu.A. Makarov, A.M. Kuzmenko and V.A. Serebryakova, 2009. Severe indigestion of intestines of newborn calves of a bacterial etiology. *Works RRIEV*, T. 75, pp: 179-181.
- Grigorieva, G.I., 2005. Role of microorganisms (bacteria and viruses) at developing of gastrointestinal diseases of newborn calves. Grigorieva, G.I., A.A. Arbizova M.A. Kulchinskaya and M.A. Panitkov (Eds.). *Veterinary Pathology*, No. 4, pp: 108-113.
- Haptseva, O.Zh., 2011. Influence of a acidate on microflora of a digestive tract of calves. Haptseva, O.Zh. (Ed.). *Vestnik of Veterinary*, No. 4, pp: 35-36.
- Herich, R. and M. Levcut, 2002. Lactic acid bacteria, probiotics and immune system. *Vet. Med.*, 47 (6): 169-180.
- Kapustin, A.V., 2013. Specific structure of clostridium of cattle. Kakpustin, A.V., A.V. Motorygin and N.K. Bukova (Eds.). *Vestnik Vet. Sci.*, 1 (64): 71-73.
- Ketlinsky, S.A., 2008. Cytokines. Ketlinsky, S.A. and A.S. Simbirtsev (Eds.). SPb: JSC Publishing House Foliant, pp: 552.
- Klaenhammer, T.R. *et al.*, 2012. The impact of probiotics and prebiotics on the immune system. *Nat. Rev. Immunol.*, 12: 728-734.
- Korneeva, O.S., 2012. Prebiotic property of a mannoza and its influence on specific resistance. Korneeva, O.S., I.V. Cheremushkin, A.S. Glushchenko, N.A. Mikhaylov, A.P. Baturo, E.E. Romanenko and S.A. Zlygostev (Eds.). *Journal of microbiology, Epidemiology and Immunobiology*, No. 5, pp: 51-57.
- Kramarev, S.A., 2008. Protective functions of intestinal microflora. Krakmarev, S.A. and O.V. Vygovskaya (Eds.). National Medical University named after A.A. Bogomoltsa, D.S. Yankovsky, G.S. Dymant, JSC "Firm", "OD" Prolisok. *Health of the Child*, 2 (11): 83-90.
- Krapivina, E.V., 2011. Level of natural resistance and immune status at calves at application of a pro-biotic medicine on the basis of lactobacilli. Krapivina, E.V., B.V. Tarakanov, E.A. Maslennaya, D.V. Ivanov, A.V. Polyakov and O.V. Poty. *Problems of Biology of Productive Animals*, No. 1, pp: 78-84.
- Kuchumova, S.Yu., 2011. Physiological value of intestinal microflora. Kuchumova, S.Yu., E.A. Poluektov, A.A. Sheptulin and V.T. Ivashkin (Eds.). *Gastroenterology, Annex to the CONSILIUM MEDICUM J.*, No. 2, pp: 75-78.
- Lifanova, Ya.V., 2013. Influence of a pro-biotic preparation of lactobacilli on the immune status of the calfs keeping in a zone of the increased pollution Cs<sup>137</sup>. Lifanova, Ya.V., E.S. Petrakov, Yu.N. Fedorov and E.V. Krapivina (Eds.). *Problems of Biology of Productive Animals*, pp: 91-98.
- Langel, S.N., W.A. Wark, S.N. Garst, R.E. James, M.L. McGilliard, C.S. Petersson-Wolfe and I. Kanevsky-Mullarky, 2015. Effect of feeding whole compared with cell-free colostrum on calf immune status: The neonatal period. *J. Dairy Science*, DOI: 10.3168/jds.2014-8422.
- Lomax, A.R., 2009. Probiotics, immune function, infection and inflammation a review of the evidence from studies conducted in humans. Lomax, A.R. and P.C. Calder (Eds.). *Curr. Pharm Des.*, 15 (13): 1428-1518. PubMed: 19442167.
- Lukas, F., I. Koppova, V. Kudrna and J. Kopecny, 2007. Postnatal development of bacterial population in the gastrointestinal tract of calves. *Folia Microbiologica*, 52: 99-104.
- Magalhaes, V.J., F. Susca, F.S. Lima, A.F. Branco, I. Yoon and J.E. Santos, 2008. Effect of feeding yeast culture on performance, health and immunocompetence of dairy calves. *J. Dairy Sci.*, 91 (4): 1497-509. DOI: 10.3168/jds.2007-0582.
- Maldonado, N.C., C.S. de Ruiz, M.C. Otero, F. Sesma and M.E. Nader-Macias, 2012. Lactic acid bacteria isolated from young calves-characterization and potential as probiotics. *Res. Vet. Sci.* 92 (2): 342-349. DOI: 10.1016/j.rvsc.2011.03.017.
- Masmanian, S.K., 2008. A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature*, 453: 620-625.
- Menard, S., C. Candalh, G.C. Bambou, K. Terped, N. Cerf-Bensussan and M. Heyman, 2004. Lactic acid bacterial secrete metabolites anti-inflammatory properties after intestinal transport. *Gut*, 53: 821-828.
- Millemann, Y., 2007. Diagnosis of neonatal calf diarrhoea. *Revue de Medecine Vet.*, 160: 404-409.
- Mokhber-Dezfouli M.R., P. Tajik, M. Bolourchi and H. Mahmoudzadeh, 2007. Effects of probiotics supplementation in daily milk intake of newborn calves on body weight gain, body height, diarrhea occurrence and health condition. *Pak. J. Biol. Sci.*, 10 (18): 3136-40.
- Mortazavian, A., 2007. Principles and methods of microencapsulation of probiotic microorganisms. Mortazavian, A., S.N. Razavi, M.R. Ehsani and S. Sohrabvandi (Eds.). *Iranian J. Biotechnol.*, 1 (5): 3-22.



- Mironov, A.Yu., 2011. Genetic certification and studying of ability to formation of biofilms the lactobacilli allocated from an oral cavity of healthy people. Mironov, A.Yu. and V.M. Gavrilova (Eds.), Chervinets, etc. Clinical Laboratory Diagnostics, No. 2, pp: 44-46.
- Mishchenko, V.A., 2008. Structure of diseases of a gastrointestinal tract of newborn calfs. Mishchenko, V.A., D.K. Pavlok, V.V. Dumova, T.B. Nikeshina, A.P. Ponomarev, A.V. Kononov and S.V. Levchenko (Eds.). Vet. Sci. Kuban, No. 5, pp: 22-23.
- Morozova, L.A., 2015. Hematologic indicators and a microbiocenosis of a digestive tract of calfs during feeding "Laktur" feed additive. Morozova, L.A., I.N. Mikolaychik and E.V. Dostovalov (Eds.). Vestnik of SyuSU. "Food and Biotechnologies" Series, 1 (3): 76-82.
- Motorygin, A.V., 2011. Definition of qualitative and quantitative structure of microorganisms at an intestinal dysbiosis at calfs. Motorygin, A.V. and E.M. Levchenko (Eds.). Agricultural Biology, No. 2, pp: 103-107.
- Nader-Macias, M.E., M.C. Otero, M.C. Espeche and N.C. Maldonado, 2008. Advances in the design of probiotic products for the prevention of major diseases in dairy cattle. J. Ind. Microbiol. Biotechnol., 35 (11): 1387-1395. DOI: 10.1007/s10295-008-0438-2.
- Navarre, C.B., E.B. Belknap and S.E. Rowe, 2000. Differentiation of gastrointestinal diseases of calves. Vet. Clin. North Am. Food Anim. Pract., 16 (1): 37-57.
- Netrusov, A.I., 2005. Workshop on microbiology. Netrusov, A.I. (Ed.). M.: "ACADEMA", pp: 603.
- Nikolaev, O.N., 2010. Etiology and prevention of gastrointestinal diseases of calfs. Practician, No. 1, pp: 26-31.
- Novick, G.I., 2006. Biological activity of microorganisms-probiotics. Novick, G.I., A.A. Samartsev and N.I. Astapovich (Eds.). Applied Biochemistry Microbiol., 2 (42): 187-194.
- Nugumanov, O.V., 2013. Influence of a probiotic "Vitafort" and "Vetom" on structure of intestinal microflora of shoats. Nugumanov, O.V., F.S. Khaziakhmetov and A.V. Andreyeva (Eds.). Basic Researches, No. 6, pp: 606-610.
- Ohland, C.L. and W.K. MacNaughton, 2010. Probiotic bacterial and intestinal barrier function. Am. J. Physiol. Gastrointest Liver Physiol., 298: 807-819.
- Ovod, A.S., 2005. Prevention of an intestinal dysbiosis of young growth agricultural animals. Vestnik of Veterinary Science, 4 (35): 35-38.
- Ovod, A.S., 2006. Importance of probiotics in prevention of diarrhea of calfs. Ovod, A.S. and L.Ya. Setrakova (Eds.). Materials of International scientific and practical conference "Actual Problems of Infectious Pathology and Immunology of Animals". M.: "Izograf", pp: 317-318.
- Oleynik, A., 2009. Neonatal diarrheas of calfs. A. Oleynik (Ed.). Dairy and Meat Cattle Breeding, No. 2, pp: 26-28.
- Panin, A.N., 2012. Probiotics in animal husbandry, state and prospect. Panin, A.N., N.I. Malik and O.S. Ilayev (Eds.). Veterinary Science, No. 3, pp: 3-8.
- Petrakov, E.S., 2014. Biological properties of lactobacilli of intestinal microflora and their value in normalization of physiological functions at farm animals (review). Petrakov, E.S. and N.S. Petrakova (Eds.). Problems of Biology of Productive Animals, No. 2, pp: 5-31.
- Pilipenko, V.I., 2008. Probiotics as signal molecules: *Saccharomyces boulardii*. Pilipenko, V.I. (Ed.). Clinical Gastroenterology and Hepatology, T.1, No. 6, pp: 456-462.
- Qadis, A.Q., S. Goya, M. Yatsu, Y.U. Yoshida, T. Ichijo and S. Sato, 2014a. Effects of a bacteria-based probiotic on subpopulations of peripheral leukocytes and their cytokine mRNA expression in calves. J. Vet. Med. Sci., 76 (2): 189-195.
- Qadis, A.Q., S. Goya, M. Yatsu, A. Kimura, T. Ichijo and S. Sato, 2014b. Immune-stimulatory effects of a bacteria-based probiotic on peripheral leukocyte subpopulations and cytokine mRNA expression levels in scouring holstein calves. J. Vet. Med. Sci., 76 (5): 677-684.
- Qadis, A.Q., S. Goya, K. Ikuta, M. Yatsu, A. Kimura, S. Nakanishi and S. Sato, 2014c. Effects of a bacteria-based probiotic on ruminal pH, volatile fatty acids and bacterial flora of Holstein calves. J. Vet. Med. Sci., 76 (6): 877-885.
- Ripamonti, B., A. Agazzi, C. Bersani, P. De Dea, C. Pecorini, S. Pirani, R. Rebutti, G. Savoini, S. Stella, A. Stenico, E. Tirloni and C. Domeneghini, 2011. Screening of species-specific lactic acid bacteria for veal calves multi-strain probiotic adjuncts. Anaerobe, 3: 97-105. DOI: 10.1016/j.anaerobe.2011.05.001.
- Shakhov, A.G., 2008. Achievements and main directions of researches on studying of diseases. Shakhov, A.G. (Ed.). Actual problems of diseases of young growth in modern conditions. Materials of the International Scientific and Practical Conference. Voronezh, pp: 3-12.

- Shakhov, A.G., Yu.N. Fedorov, A.N. Panin *et al.*, 2007. Methodical recommendations about an assessment and correction of nonspecific resistance of animals. In collection: New methods of researches on problems of veterinary medicine. M.: Russian Academy of Agrarian Sciences, Ch. III, pp: 174-215.
- Signorini, M.L., L.P. Soto, M.V. Zbrun, G.J. Sequeira, M.R. Rosmini and L.S. Frizzo, 2012. Impact of probiotic administration on the health and fecal microbiota of young calves: a meta-analysis of randomized controlled trials of lactic acid bacteria. *Res. Vet. Sci.*, 1: 250-258. DOI: 10.1016/j.rvsc.2011.05.001.
- Sidorov, M.A., D.I. Skorodumov and V.B. Fedotov, 1995. Determiner of zoopathogenic microorganisms. M.: Kolos, pp: 320.
- Sisyagin, P.N., 2015. Associative respiratory diseases of calfs and correction of the immune status of their organism. Sisyagin, P.N., G.R. Redzhopova and E.P. Sisyagina (Eds.). Collection scientific works International scientific and practical conference "Population health of animals and emerge infections in modern conditions". N. Novgorod P.1., pp: 62-69.
- Solovyova, N.V., 2010. Correction the disbiotic violations at diseases of a digestive tract and liver dietary supplements with pro-biotic action. Solovyova, N.V., S.N. Leykhter, T.A. Bazhukova, A.G. Solovyov and O.V. Lebedeva (Eds.). *Reviews on Clinical Pharmacology and Medicinal Therapy*, T. 8, No. 3, pp: 48-57.
- Sukhina, M.A., 2012. Antagonist activity of lactobacilli of a thick gut. Sukhina, M.A., O.A. Burgasov, V.G. Zhukhovitsky and N.D. Yushchuk (Eds.). *Journal of Microbiology of Epidemiology and Immunology*, No. 1, pp: 41-49.
- Tarkanov, B.V., 2007. Biological prerequisites of a probiotic therapy and efficiency of application of Laktoamilovarin in animal husbandry. Tarkanov, B.V. (Ed.). *Problems of Biology of Productive Animals*, No. 1, pp: 89-101.
- Tao, S., A.P. Monteiro, I.M. Thompson, M.J. Hayen and G.E. Dahl, 2012. Effect of late-gestation maternal heat stress on growth and immune function of dairy calves. *J. Dairy Sci.*, 12: 7128-71236. DOI: 10.3168/jds.2012-5697.
- Viera, A.T., M.M. Teixeira and F.S. Martins, 2013. The role probiotics and prebiotics in inducing gut immunity. *Frontiers Immunol.*, 445 (4): 1-12.
- Vlkova, E., I. Trojanová and V. Rada, 2006. Distribution of Bifidobacteria in the gastrointestinal tract of calves. *Folia Microbiologica*, 51: 325-328.
- Windeyer, M.C., K.E. Leslie, S.M. Godden, D.C. Hodgins, K.D. Lissemore and S.J. LeBlanc, 2014. Factors associated with morbidity, mortality and growth of dairy heifer calves up to 3 months of age. *Prev. Vet. Med.*, 113 (2): 231-240. DOI: 10.1016/j.prevetmed.2013.10.019.
- Yankovsky, D.S., 2005. Microbial ecology of the person: modern opportunities of its maintenance and Restoration. Yankovsky, D.S. (Ed.). Kiev: Expert LTD., pp: 362.