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Microbiological Characteristics of Sour-Milk Feed Supplements and their Influence on Intestinal Micro-Biocenosis of Piglets

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ABSTRACT

The research paper provides the results of investigation of "Sour-milk feed supplement" for piglets based on natural association of microorganisms *Lactomyces tibeticus*. It was found that probiotic microorganisms that compose sour-milk feed supplement withstand the adverse actions of gastrointestinal tract and can be easily acclimatized in the intestinal tract of piglets. Administration of sour-milk feed supplement 5 days before the weaning and during the next 15 days after the weaning promoted formation of stable intestinal micro-biocenosis, with lacto- and bifidobacteria (10^8 – 10^9) being the main microflora, and thus had a positive effect on digestion processes in piglets.

Keywords: *Lactomyces tibeticus*, micro-biocenosis, sour-milk feed supplement, probiotics.

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INTRODUCTION

Scientific researches and practice proves that intestinal biocenosis restoration therapy by regular administration of live bacteria – representatives of normal microbial flora – plays an important role in prevention and treatment of gastrointestinal problems of young livestock. The medications of this type are also intended for restoration of intestinal microflora in post-antibiotic treatment period [1-4].

Microorganisms that form the basis of probiotics carry an important protective function in organisms of livestock and ensure their protection. Probiotics promote digestion processes in animals by activation of metabolism of carbohydrates and lipids, absorption of macro and micronutrients, encourage acid-alkaline and hydro-electrolytic balance in blood, and therefore have positive impact on growth and development of young livestock. Bacterial metabolites that compose probiotics regulate esophageal motility, considerably promote the growth rate of piglets, as well as contribute to their preservation and sound health [5-7].

Nowadays a considerable number of probiotic preparations and feed supplements with diverse composition of microorganisms for piglet breeding, especially under the conditions of rapid growth of industrial technologies, have been suggested. In fact, piglets are most vulnerable to gastrointestinal disturbances during the weaning period. Therefore active searches for the new sources which would provide for probiotic microorganisms for generation of new types of probiotics are continuously going on. For instance, a policulture of microorganisms *Oryzamyus indici* and technology of their production has been proposed in Belarus [8].

Nowadays sour-milk feed supplements for animals that are based on microbial association *Lactomyces tibeticus* arouse much interest due to a wide range of bioactive substances, relative simplicity of cultivation and possibility for a prolonged maintenance of culture in active state. [9,10,11].

Fig 1: Natural association of microorganisms *Lactomyces tibeticus*.

Lactomyces tibeticus appears in lumps of white colour, having no characteristic odour, with diameter from 3-6 mm (young fungus) to 3-5 cm (fungus before division), and sinks in water. Lumps have inhomogeneous surface and consist of 1-3 mm subcircular or oval cheese-like granules (fig. 1). According to our investigations percentage of moisture in the lump of *Lactomyces tibeticus* fluctuates within 86-89% [12], generic and species composition of microflora is represented by the following microorganisms: yeast cells of genus *Sacharomyces,* species *Candida kefir*, species *Lactobacillus fermentum* and *Leuconostoc lactis* bacteria, and acetic bacteria [13].

We have produced sour-milk feed supplement based on natural association of microorganisms *Lactomyces tibeticus* for the intestinal microflora normalization in weaned piglets (Technical specifications U 15.7-02071010-001: 2012) [16].

Sour-milk feed supplement is a beverage obtained by means of milk fermentation by microbial association of *Lactomyces tibeticus.* According to sensory characteristics it is a homogeneous viscous liquid with disrupted and non-disrupted clots, clean sour-milk odour, biting, with no specific off-flavour taste and milky-white colour.

According to physical and chemical characteristics the percentage of fat in feed supplement constitutes from 1.0 to 5.0%, protein – from 2.7 to 3.1% and titrated acidity ranges from 80 to 120^0 T.

As for microbiological characteristics the ready-to-feed beverage contains 10^8-10^9 CFU/cm^3 of viable lactic bacteria, 10^4 CFU/cm^3 of yeast. Bacteria of group *Escherichia coli, Staphylococcus aureus* in 1.0 cm^3, and pathogenic microorganisms including bacteria of genus *Salmonella* in 25.0 cm^3 were not detected.

The research objective was to determine microbiological indices for sour-milk feed supplement and its influence on the intestinal micro-biocenosis in weaned piglets.

MATERIALS AND METHODS

The research was carried out at Lviv Politechnic National University, Ukraine and at pig farm of Agro-industrial Enterprise “Medobory”, Ternopil region, Ukraine.

Sour-milk beverage “Sour-milk feed supplement” was obtained by adding of 2.5-5% starter to cow sterile milk with fat weight part of 2.5%, and further cultivation until the acidic medium value reached 85-120^0 T. Before each passage the biomass was scoured out in sterile sodium chloride physiological solution.

The acidity value of resulted lactic acid feed supplement was evaluated following the laboratory standard [14]. Antimicrobial activity of lactic acid feed supplement for test-culture growth of commensals was determined by a well method. Using a Petri dish with sterile nutrient agar of HiMedia Company, we added 1 cm^3 of test-culture suspension to 0.5% NaCl

solution, spread the suspension around the agar in Petri dish and pipetted the excessive remains of the suspension off. Then we dried Petri dishes in the incubator for 35-45 minutes at t 37^0C. Next we made wells in the medium with the well puncher №10 (well diameter 9 mm). Lactic acid feed supplement was placed into the formed wells (full well). After that cell culture dishes were incubated at temperatures that are specific for each test culture, and after the completion of incubation we evaluated the result of growth retardation of test cultures around the well with sour-milk supplement [15]. Microorganisms persistence of culture association *Lactomyces tibeticus* to unfavorable factors of gastrointestinal tract was determined by adding the *chole medicata* 10%, 20%, 30% concentrations and NaCl 2%, 4%, 6.5% concentrations to the nutrient solution.

Genus and species composition of piglets' intestinal microflora was researched on media of German company HiMedia. Isolation of staphylococci was explored on media of Baird-Parker Agar, fungi – on Sabouraud Dextrose Agar, *Escherichia coli* – on Endo Agar, enterococci – on Bile Esculin, Azide Agar, streptococci – on Streptococcus Selective Agar, lactobacteria – on M.R.S. Agar, bifid bacteria – on Bifidobacterium Agar, overall composition of microorganisms – on Mueller Hinton Agar. Identification of isolated microorganisms was carried in compliance with Bergey's Manual, 1997.

Research findings and discussion: Study of growth inhibition for pathogenic and opportunistic pathogenic microorganisms claims to be a binding condition in development of probiotic preparations. In laboratory environment we investigated the influence of sour-milk feed supplement on test-cultures of opportunistic pathogenic microorganisms. Bacteria *Escherichia coli, Staphylococcus aureus, Bacillus mesentericus, Mycobacterium luteum, Proteus vulgaris* and micromicete *Aspergillus niger* were used as test-cultures. Table 1 illustrates our research findings.

Table 1: Influence of probiotic beverage "Sour-milk feed supplement" on test cultures microorganisms growth, M±m, n=18

Name of test culture microorganisms	Well diameter, mm	Growth inhibition zone diameter, mm	Test culture susceptibility to beverage microorganisms
Escherichia coli	9	16±1	Intermediate
Staphylococcus aureus	9	22±2	Susceptible
Bacillus mesentericus	9	23±2	Susceptible
Mycobacterium luteum	9	26±2	Hypersusceptible
Proteus vulgaris	9	28±2	Hypersusceptible
Aspergillus niger	9	21±1	Susceptible

Footnote: Growth inhibition zone diameter of test cultures up to 15 mm – microorganism is not susceptible; 16-20 mm – intermediate, 21-25 mm – susceptible, more than 26 mm – hypersusceptible.

As shown in table 1, virtually all test cultures of opportunistic pathogenic microorganisms were susceptible to probiotic bacteria in lactic acid feed supplement. This indicates their considerable antagonist properties toward commensals.

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As is known, about 20-30% of lactic acid bacteria that compose sour-milk products stay alive after passing through gastrointestinal tract. Loss of microorganisms occurs due to the unfavorable gastric and intestinal environment (acidic medium of gastric fluid and presence of bilis in small intestine). Such losses decrease functional influence of sour-milk beverage in animal or human organism. Therefore we analyzed the influence of bilis and NaCl of different concentrations on acid-forming activity of *Lactomyces tibeticus.* Research results are provided in tables 2 and 3.

As shown in tables 2 and 3, active acid formation took place at NaCl concentration of 4% in culture medium and bilis – of 20%. This means that microbial association is able to withstand unfavorable conditions of gastrointestinal tract and to develop in it.

Table 2: Influence of NaCl on acid-forming activity of culture *Lactomyces tibeticus,* M±m, n=12.

Concentration of NaCl in medium, %	Acidity, %	
	Day 1	Day 2
0 (control)	58±1	134±9
2	68±4	110±16
4	59±1	78±4
6,5	58±1	56±1

Table 3: Influence of bilis on acid-forming activity of culture *Lactomyces tibeticus,* M±m, n=12.

Bilis content in medium, %	Acidity, %
0 (control)	110±0
10	124±13
20	80±6
30	65±5

For most pig farms the main reason that provokes malformation of intestinal micro-biocenosis processes in piglets after the weaning is underactive autochthonous microflora and different diet. In order to determine the influence of “Sour-milk feed supplement” on intestinal microbiocenosis in piglets, we began to feed it 5 days before the weaning and during the next 15 days after the weaning. Research results are shown in table 4.

Microbiological research of biomaterial for identification of quantitative and qualitative composition of microflora indicated that no pathogenic bacteria and fungi were detected in the intestinal tract of piglets. The main bacterial population is represented by lacto- and bifidobacteria, and their quantitative and qualitative composition is twofold larger in experimented piglets than in control ones. This indicates the positive influence of sour-milk feed supplement on piglets intestinal biocenosis, increase of antagonistic activity of gastrointestinal tract autochthonous microflora, and quantitative growth of microflora in experimented piglets. Bacteria of the genus *Proteus* – which belong to putrescent microflora and endanger normal gastrointestinal digestion in piglets and thus destroy the intestinal microbiocenosis – were detected and identified in 3 control animals. As can be seen from the

above, the application of sour-milk feed supplement before and after the weaning promoted the formation of stable intestinal micro-biocenosis with predominance of lacto- and bifidobacteria from slightly acid to neutral (6,0±0,02 – 7,0±0,4) medium response and contributed to digestion processes in piglets.

Table 4: Genus composition of intestinal microflora in weaned piglets and application of "Sour-milk feed supplement", CFU, M±m, n=20

Microflora	Groups of piglets on	
	Standard diet (control group)	Sour-milk feed supplement "*Lactomyces tibeticus*"
Total number of bacteria (large intestine)	10^8±1,1	10^{10}±0,5
Pathogenic microorganisms of the genus *Proteus*	not detected	not detected
Total number of *Escherchia coli*	0,2×10^8±0,06	1,7×10^8±0,05
Lactose negative enterobacteria	0,8×10^6±0,12	0,5×10^7±0,02
Hemolytic *Escherchia coli*	Detected in 3 piglets	not detected
Staphylococci saprophyticus	1×10^4	1×10^4
Hemolytic streptococci	not detected	not detected
Non-hemolytic streptococci	10^5	10^4
Bifid bacteria	5,6×10^7±0,5	4,8×10^9±0,5
Lactobacteria	4,2×10^6±0,6	6,3×10^8±0,4
Yeasts	0,3×10^4±0,01	0,7×10^4±0,01

Sour-milk feed supplement developed by means of milk fermentation by *Lactomyces tibeticus* microbial association demonstrates probiotic activity toward a number of testing bacteria and fungi, able to withstand considerable concentrations of bilis and sodium chloride ions, and have positive effect on the intestinal micro-biocenosis in piglets during the weaning period.

CONCLUSIONS

1. Sour-milk feed supplement for piglets based on the *Lactomyces tibeticus* microbial natural association demonstrated antimicrobial activity toward opportunistic pathogenic and pathogenic microorganisms.
2. Probiotic microorganisms that compose the sour-milk feed supplement withstand the action of unfavorable gastrointestinal factors and are easily acclimatized in the intestinal tract of piglets.
3. Administration of sour-milk feed supplement to piglets 5 days before the weaning and during the next 15 days after the weaning promoted stable intestinal micro-biocenosis, with lacto- and bifidobacteria (10^8 – 10^9) being the main microflora, and thus had a positive effect on digestion processes in piglets.

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