Fermented camel milk as a probiotics source for poultry farming

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Abstract
Currently, antibiotics are increasingly becoming the object of close attention due to the potential development of antibiotic-resistant pathogens after prolonged use, the use of antimicrobials also seriously pollutes the environment and destroys the ecological balance of nature. Feed antibiotics have been banned in the EU since 2006, and there is an active discussion of draft laws in this area in Russia. The poultry industry ranks second in the world in terms of output, so it is important to ensure the health of livestock and product safety. Since the purpose of this study is to investigate the effectiveness of the use of lactic acid bacteria (LAB) strains isolated from shubat (fermented camel milk) for the tasks and problems of the poultry industry, the theoretical basis of the study includes the analysis of research papers and statistical reports on the problems of the industry, the use of antibiotics in animal husbandry, and lactic acid bacteria as possible alternatives. The final part is devoted to the analysis of the practical studies results comparing different types of milk, their microbiological composition, and the advantages of shubat for the probiotics production for poultry. Due to the peculiarities of camel milk and the "ethnicity" of shubat, quantitative and qualitative data of isolated microorganisms from these products have advantages over other species.

Keywords: poultry industry; probiotics; lactic acid bacteria; shubat; camel milk; food safety.

Practical Application: Based on microorganisms, it is possible to develop a multi-strain probiotic preparation that includes various types of LAB. Their creation, testing, and release as goods produced in Kazakhstan can have a positive impact on the dynamics and quality of the economic growth of the republic, and have a beneficial effect on the quality and safety of poultry products.

1 Introduction
Over the past 50 years, animal husbandry and poultry farming, in particular, have developed in many areas to achieve maximum efficiency of growth, meat yield, and to reduce economic costs. Today, agricultural industries need to pay more attention to how animal husbandry affects the environment and food safety (Maślanka et al., 2015). As in many other industries, the global paradigm is shifting from an emphasis on production efficiency to an emphasis on public food safety. Issues related to the use of growth-stimulating antibiotics demonstrate this paradigm shift more than clearly. Over the past four decades, antibiotics have been used in animal husbandry to improve growth rates and protect animals from the negative effects of pathogenic microorganisms. Currently, antibiotics are increasingly becoming the object of close attention due to the potential development of antibiotic-resistant pathogenic bacteria after prolonged use (Ferket, 2007). Bacterial diseases of birds caused by antibiotic-resistant strains of pathogenic microorganisms have become widespread (Fisinin, 2012). The Infectious Diseases Society of America (ISDA) writes that due to the resistance of bacteria to antibiotics, about USD 26 billion is spent annually on medicine. It is noted that about 280 people in the UK and 1.519 across Europe die every year due to antibiotic-resistant forms of E. coli contained in poultry meat (Pranita et al., 2020). The microbiota of the gastrointestinal tract (GIT) of poultry is normally colonized by a huge number of different microorganisms. Of particular interest are lactic acid microorganisms, most of which have an enormously positive effect on the body and productivity of poultry, the properties of lactic acid microorganisms indicate the prospects of using them as probiotic additives in poultry farming (Gindullin, 2013).

Lactic acid bacteria can replace antibiotics in poultry farming (Stoyanova, 2017; Abbas Hilm, 2010) and play a role in the prevention and treatment of certain diseases, which would lead to a gradual decrease in the number of antibiotics and other antimicrobials, which is extremely important for the development of environmentally friendly food (Dikhanbayeva et al., 2019). In addition, the colonization of the gastrointestinal tract of animals and birds with beneficial microflora would allow for a high growth rate, reduce morbidity, and increase the economic efficiency of the industry. To create the safest, most effective probiotic drugs with a rational cost, research and comparative
2 Materials and methods

Since the purpose of this study is to investigate the effectiveness of the use of strains of lactic acid bacteria isolated from shubat for the tasks and problems of the poultry industry, the theoretical basis of the study is logically divided into three main blocks, based on the list of tasks identified by the authors. Despite the fact that the subject of the study is limited to the use of shubat probiotic microorganisms in poultry farming. In the furtherance of this goal, the following tasks are highlighted: to investigate the state and prospects of the poultry industry in the world and the Republic of Kazakhstan, in particular; to carry out a general assessment of the effectiveness of probiotics for poultry; to analyse the possibility to develop probiotics production based on lactic acid microorganisms of shubat in the Republic of Kazakhstan.

3 Results and discussion

3.1 Development and prospects of the poultry industry

Maintaining a balance between the growing demand for food from the world’s population and global agricultural production is necessary to ensure food security, which means physical and economic access to adequate and safe nutrition that meets consumer preferences. According to the Food and Agricultural Organization (FAO), between 1960 and 2010, the world’s food production almost tripled, and the population almost doubled. This has led to an increase in food production per capita by more than 30%. It is estimated that to meet the growing demand for food, agricultural production should increase by 60% in the next 40 years (Ovcharova & Petrakov, 2018).

The dominant livestock industries in the world are pig farming (112.33 million tonnes), poultry farming (109.02 million tonnes) and cattle breeding, which includes cow and buffalo meat (67.99 million tonnes), which accounts for 91.80% of meat production in the world (Food and Agriculture Organization, 2013). Evidently, poultry farming, being in second place, practically does not lag behind the leader and makes up a significant part in providing the population with animal protein (meat and eggs). The level of consumption for the majority of respondents is in the range of 36-60 kg/person/year, which indicates a huge dependence on poultry meat in the diet, which resembles the global annual consumption of an average of 42.9 kg per person (Ishak et al., 2018). By country, the leaders of poultry farming are the USA, China, and Russia. Animal husbandry in the world is moving from small-scale households to larger and more commercialized enterprises. Most of China’s growing demand for livestock products will be provided by domestic producers. However, these farms will increasingly rely on imported fodder to feed the growing livestock. Unlike China, the United States is more provided with agricultural land. While China’s population is four times more than in the United States, the United States has about one-third more arable land than China. China came in second place after the United States in terms of total poultry
meat production. The total meat consumption per capita in China, however, is still lower than in the USA (Gale, 2002). As for the Republic of Kazakhstan, poultry farming there is one of the most effective branches of the agro-industrial complex, which provides the greatest return on production per unit of material resources spent (Shubat, 2015). The poultry industry is characterized by a lack of seasonality of production, which ensures a monotonous movement of income and the use of labour resources. From an economic standpoint, the most important factor in the development of poultry farming is the presence of a balanced market. To increase production and provide markets with high-quality and competitive products against the background of increased attention to the health of the population, it is necessary to ensure the health of livestock (Mohamed et al., 2022).

Worldwide, poultry diseases will continue to be a major problem for the poultry industry and its strategic future. An outbreak of any disease can turn into an epidemic and have a significant negative impact on the world trade in poultry products. An increase in the cost of feed and raw material prices, and their availability, will negatively affect the growth of industry and consumer purchasing power, especially after the COVID-19 pandemic. In addition, an increase in the production of biogas and biofuels will lead to a reduction in the areas available for the production of grain and animal feed (Gale, 2002). Many foodborne diseases can be transmitted along the food chain. In the available literature, salmonella and campylobacteria are poultry bacteria that are most often responsible for human foodborne diseases (Stoyanova, 2017; Zhanabayeva et al., 2021). Not only chicken-like, but also goose-like domestic birds are susceptible to diseases. Among the three most frequently diagnosed diseases of waterfowl is mycoplasmosis of bacterial aetiology. Viral and bacterial diseases of waterfowl cause significant damage to reproductive herds due to the easy way of spreading these infections. The fight against zoonotic diseases and foodborne pathogens involves a deep understanding of how microbial pathogens invade and colonize, and the circumstances that stimulate or stop the growth of each strain of the organism. Appropriate biotechnical measures on farms, and preventive measures, can significantly reduce the likelihood of diseases and avoid the consequences that they entail (Drabik et al., 2020).

3.2 Antibiotics and their alternatives in poultry farming

Antibiotics as feed additives have played a role in history, and their use has significantly contributed to the development of animal husbandry. With the constant increase in the level of science and technology, their shortcomings are increasingly becoming apparent. Blind use of antibiotics in feed will harm livestock and poultry and endanger human health, thereby polluting the environment. Specific manifestations are: increased resistance of pathogens due to their colonization and exclusivity, destruction of the ecological balance of normal flora and decreased immunity of the body; since the remnants of drugs directly threaten human health, the amount of antibiotics has increased exponentially since people became ill, and has even developed to the point where antimicrobials became ineffective; widespread use of antimicrobials seriously pollutes the environment and destroys the ecological balance of nature. In the large Republic of Sudan, studies show high rates of resistance to antibiotics such as trimoxazol (95%), noroxacin (89%), cephalixin (81%), tetracycline (75%), peoxacin (69%), nalidixic acid (65%), ciprofloxacin (59%) and oxacin (56%). The importance of these antibiotics is conditioned by their use both in veterinary medicine and in medicine (with the exception of peoxacin, which is still structurally related). There is a high correlation between the use of veterinary antimicrobials and resistance to them in pigs, poultry, and cattle, as “producers” of food (Ishak et al., 2018).

Considering the experience gained in some European countries, antibiotics have been banned as “growth-promoting” for productive livestock and poultry since January 2006. Practical conclusions obtained based on experience gained in Europe revealed several problems after the prohibition of antibiotics in poultry nutrition, namely: growth was disrupted and feed conversion was reduced. In addition, this affected the health of birds: the level of ammonia and the humidity of the litter were greatly increased with developing dermatitis of the pads of the paws and, consequently, the overall welfare of the animals decreased significantly. In addition, the proportion of health problems has increased, intestinal disorders caused by clostridial infections and dysbiosis have become more frequent (Gale, 2002).

Due to the ban on the use of feed antibiotics in the EU in 2006, there was a need to expand the use of probiotics and feed additives as an alternative to feed antibiotics (Ovcharova & Petrakov, 2018). In 2020–2021, there is an active discussion about banning the addition of antimicrobials to animal feed without a prescription. The Government of the Russian Federation supports relevant amendments to the laws “On veterinary medicine” and “On the circulation of medicines” prepared by the Rosselkhoznadzor (Vlasova, 2021). Rosselkhoznadzor has detected antibiotics in animal products, especially often – in milk. In 2013, coccidiosis was found in commercial eggs. Such violations, and the view in the EU, where a ban on feed antibiotics has been active since 2006, become an incentive for the application of similar measures in Russia, and the CIS countries. Various antimicrobials have been used in Europe, but Regulation (EC) No. 1831/2003 states that “Antibiotics other than coccidostats or histomonostats should not be allowed as feed additives”. Antibiotic resistance is a serious problem for humanity. To reduce this phenomenon, the EU ban on antibiotics as growth promoters should be globalised worldwide (Vieco-Saiz et al., 2019).

The history of research and the use of lactic acid bacteria is very long, but the first studies on probiotics of lactic acid bacteria began only in the late 1970s. Currently, the positive effect of lactic acid bacteria and probiotics has been widely recognised worldwide. People have recognised the physiological function of lactic acid bacteria, and LAB are widely used in food, medicine, animal and poultry feed, and other aspects (Zendeboodi et al., 2020). In China, already in 1995, a test was conducted on the effect of adding lactic acid bacteria to the diet on the growth and development of chickens. Compared with the control group (the group of antibiotics), the chickens in the experimental group, to which lactic acid bacteria were added, grew faster, their feathers after moulting were smoother and developed more evenly, their average live weight (LW) increased by 8.4%–11.5%,
the feed conversion rate increased by 13.8%-21.3%. At the same time, lactic acid bacteria and antibiotics had the same effect in preventing common diseases of chickens, especially dysentery. In Europe, permission for the use of microorganisms as animal feed additives is subject to strict regulation (Damién et al., 2015). An important criterion for evaluating the effectiveness of strains is the spectrum of its antimicrobial action, that is, activity against different groups of microorganisms (Stoyanova, 2017).

New or improved products containing lactic acid bacteria are appearing on the market. An example is the growing supply of probiotics. Probiotics are defined as “live strains of strictly selected microorganisms that, when administered in sufficient quantities, benefit the health of the body” (Hernandez-Patlan et al., 2019). It is worth noting that many types of lactic acid bacteria have the GRAS (Generally Recognised as Safe) status established by the US Food and Drug Administration (FDA), and the Qualified Presumption of Safety (QPS) status established by the European Food Safety Authority EFSA (Ovcharova & Petrakov, 2018). LAB can inhibit the adhesion of conditionally pathogenic bacteria. In addition, they are able to produce lysozyme, amino acids, and substances similar in action to antibiotics, having an antagonistic effect against conditionally pathogenic microorganisms, protozoa, and putrefactive microflora. LAB reduce the penetration of toxins into the blood, having a detoxifying effect on the body. In addition, LAB stimulate the body’s defence mechanisms based on an increase in the rate of regeneration of mucous membranes and the effect on the production of antibodies (Gindullin, 2013; Dikhanbayeva et al., 2021b).

The most common microorganisms used as probiotics in animal husbandry are lactic acid bacteria (LAB) from the genera Lactobacillus, Pediococcus, Lactococcus, Enterococcus, Streptococcus, and Leuconostoc. However, only representatives of the genera Lactobacillus, Streptococcus, Pediococcus, Enterococcus and Weissella are most commonly used in poultry farming. Although the effectiveness of probiotics that reduce the level of enteral pathogens is obvious, one of their disadvantages is that they require cooling or lyophilisation for long-term storage or can be encapsulated to increase their stability/viability when included in feed, which increases the cost of production at the industrial level, making it unprofitable (Hernandez-Patlan et al., 2019; Tellez et al., 2012).

Lactic acid bacteria-bacteriocins improve the growth indicators of chickens with C. perfringens problems, allowing them to restore weight at a similar level to healthy birds (Vieco-Saiz et al., 2019). Thus, to optimize the system of diagnostic, therapeutic, and preventive veterinary measures and obtain safe and high-quality products, it is recommended to introduce a probiotic composition from strains selected in accordance with the epizootic situation at poultry enterprises at the early stages of poultry production. Reducing the economic costs of veterinary measures is associated with a significant restriction of the use of antibacterial drugs and the absence of the need for their use at the middle and late stages of broiler growth. Its effectiveness is achieved due to the parallel colonization of beneficial microorganisms in the gastrointestinal tract of poultry and the inhibition of pathogenic and conditionally pathogenic bacteria by an antibiotic (Animal Health, 2017).

### 3.3 Investigation of the effectiveness of shubat probiotics

Probiotics based on lactic acid bacteria are considered a potential alternative to the use of antibiotics in poultry farming, since they do not leave residues in meat and eggs, considering their action, the diversity of microorganisms by species, and even between strains of the same species, and differences in their metabolic activity can affect their effectiveness.

Shubat is a fermented milk drink obtained from camel milk. This fermented milk product is obtained in the result of spontaneous fermentation of camel milk under the influence of local microflora. Its fat content reaches 8%. It is well preserved and does not lose its qualities (Tyurina, 2010). Pathogenic microorganisms such as Salmonella, Shigella and others were not detected during the studies of shubat (Nadtochii et al., 2018), despite the fact that shubat is formed as a result of spontaneous fermentation and has lower hygienic characteristics compared to pasteurized milk. However, despite this, the state requirements of standards for raw materials and technology are applied to the production of shubat (General technical conditions, 2015). Unpasteurized milk is used for the preparation of shubat, 10-40% of the finished product is added to it, which serves as a starter. The starter culture contains lactic acid rods (streptobacteria), lactic acid streptococi, and yeast fermenting lactose. In turn, camel milk and shubat are rather underinvestigated due to the relatively low popularity of camel breeding in the world (Gammoh et al., 2022). Camel milk contains a lot of nutrients and minerals, and also, having high bactericidal properties, it can be stored longer than the milk of other farm animals (Worldgonesour, 2014). Thus, based on the qualities of camel milk, its advantages over other products, and also considering the fact that the activity of microorganisms of different species and even strains of the same species can have significant differences, the study in this area is promising (Amelia et al., 2021). For the Republic of Kazakhstan, this is quite an urgent issue, since the number of productive camels in the republic is quite large, and there are also milk research centers. Available studies show differences in microbiota composition between camel milk from different countries (Table 1) (Kudaibergenova et al., 2020a).

There are publications where it is said that camels of different regions of the republic have milk of different qualitative composition (Novitsky, 2016; Worldgonesour, 2014). All this allows making several different samples with further assessment of their effectiveness. In addition, the effectiveness of probiotics

<table>
<thead>
<tr>
<th>Geography and year</th>
<th>Species composition of microflora and its percentage</th>
</tr>
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<tbody>
<tr>
<td>Kuwait (2019)</td>
<td>Enterococcus spp. (24.2%), Lactococcus spp. (22.4%), Pediococcus spp. (20.7%), Weissella spp. (10.3%)</td>
</tr>
<tr>
<td>China (2009)</td>
<td>Lactobacillus spp. (44%), Enterococcus spp. (19%), Leuconostoc spp. (10%), Weissella spp. (3%)</td>
</tr>
<tr>
<td>Egypt (2013)</td>
<td>Enterococcus spp. (81.6%), Lactococcus spp. (9%), Lactobacillus spp. (9%), Aerococcus viridans spp. (9%)</td>
</tr>
<tr>
<td>Kazakhstan (2015)</td>
<td>Enterococcus spp. (51.3%), Lactococcus spp. (10.9%), Lactobacillus spp. (29.8%), Leuconostoc spp. (8%)</td>
</tr>
</tbody>
</table>

for poultry is influenced by other factors: the type of origin, the method of preparation of probiotics, the survival of colonizing microorganisms in the gastrointestinal tract, the environment in which poultry is raised, the time and method of application of probiotics, the immunological condition, the pedigree of poultry, age and concomitant use of antibiotics (Akhmetsadykova et al., 2014; Otutumi et al., 2012).

Studies conducted in Kazakhstan show a good prospect in the use of shubat as a source of probiotics (Akhmetsadykova et al., 2014). Microorganisms from shubat were isolated in a sufficiently large amount (Table 1), which indicates that it is a source of valuable probiotic strains. The shubat strains identified by the study (Harimurti & Hadisaputro, 2015), such as Enterococcus faecium, Lactobacillus reuteri, Lactobacillus paracasei, Lactococcus lactis, Lactobacillus plantarum, are used as probiotic microorganisms capable of normalising the gastrointestinal tract of farm animals and poultry. In another study, 8 strains of LAB isolated from the natural symbiotic starter culture of shubat were found in samples of fresh camel milk from the Ile district of Almaty region: Lactococcus paracasei, Lactococcus plantarum, Streptococcus diacetylactis, Lactococcus acidophilis, and Streptococcus lactis (Kudaibergenova et al., 2020b). An important criterion for evaluating the effectiveness of strains is the spectrum of its antimicrobial action, that is, activity against different groups of microorganisms.

Thus, lactococci isolated from the milk of Buryat and Iranian national lactic acid drinks of mixed lactic acid and alcoholic fermentation (similar to shubat) were distinguished by a high level of inhibitory activity, had a wider spectrum of antibacterial action relative to industrial products (Zouari et al., 2020). They effectively suppressed the growth of both gram-positive and gram-negative bacteria, and showed a fungicidal effect – suppressed the growth of mycelial fungi and yeast (Stoyanova, 2017). It follows from the results of these studies that the diversity of strains having different geographical origin and having similar functional capabilities is a consequence of the specific conditions that are formed in the ecological environment of their habitat. Such authentic lactococci have improved mechanisms of adaptation to stressful environmental conditions and played an important role in evolutionary processes (Raiesi Gahroui et al., 2022). Notably, these strains of LAB are of scientific and practical interest in terms of expanding biological diversity, their possible use as bioconservatives, and for the preparation of complex probiotics (Stoyanova, 2017). Each strain of LAB has its own corresponding bacteriocin. All isolated strains of LAB have valuable probiotic properties. Based on these microorganisms, it is possible to build a fourth-generation combinative preparation, including various types of LAB and yeast, since the use of probiotic preparations consisting of various types of microorganisms is optimal for maintaining the health of young birds, and the development of the general immunity in poultry (Harimurti & Hadisaputro, 2015). Another study clearly reflects the advantages of the quantitative composition of the microflora of collected camel milk and shubat over koumiss (Table 2).

Shubat of the Atyrau and Kyzylorda regions showed low results of quantitative analysis (4 and 13, respectively). The results obtained can be interpreted as wide biodiversity of the microflora of national products, which confirms their stability over time. Despite the emergence of other fermented milk products, especially those obtained with the help of artificial industrial fermenters, this did not cause the disappearance of “endemic” species of microorganisms (Baubekova et al., 2011, 2014).

In addition to the possibility and prospects of using these strains for the industrial production of probiotic preparations, it is necessary to conduct a full assessment of the probiotic properties of the isolated LAB strains. The complexity of identifying and classifying strains complicates research, since useful properties can only be inherent in certain strains (Barati et al., 2022).

There are very few developments in the field of probiotic prospects and effectiveness of the LAB of camel milk and dairy products based on it. It is important to further study camel milk, from the standpoint of the potential probiotic properties of LAB, in comparison with the milk of other productive animals in various regions of Kazakhstan as a unique state with a widely variable climate. The situation is similar for European countries, in particular, for Poland (Orazov et al., 2018).

### 4 Conclusions

Antibiotics have played a big role in the development of animal husbandry, but have also introduced a number of problems. In addition to antimicrobial resistance, another problem is the presence of their residues in animal products and their transmission with food to humans. The modern intensive livestock industry must adapt to the production of livestock products without antibiotics that stimulate growth. This is a very topical issue for poultry farming, as this industry is in second place in terms of production and is constantly developing to provide the growing population with animal protein. The prohibition of antibiotics in animal feed has an economically negative impact on the livestock sector due to various and uncontrolled bacterial diseases. Innovative alternatives for the production of feed and supplements for productive animals are required to help control the growing antibiotic resistance.

In this study, lactic acid bacteria were analysed as an alternative to antibiotics, with an emphasis on LAB isolated from shubat. LAB have many properties that positively affect the

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Table 2. Quantitative data of isolated cultures from the microflora of fermented dairy products of camel and mare milk.

<table>
<thead>
<tr>
<th>No.</th>
<th>Microorganisms</th>
<th>South Kazakhstan Region (5 farms)</th>
<th>Almaty Region (Sarzhaylau company)</th>
<th>Number of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Camel milk</td>
<td>Shubat</td>
<td>Koumiss</td>
</tr>
<tr>
<td>1.</td>
<td>Bacteria</td>
<td>26</td>
<td>29</td>
<td>13</td>
</tr>
<tr>
<td>2.</td>
<td>Yeasts</td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>

Source: Baubekova et al. (2011).
gastrointestinal microflora of birds, their growth and development, and reduce the incidence rate without the use of antibiotics. The microflora of camel milk and shubat is more diverse and voluminous relative to products from cow and mare milk, which in combination with the properties of camel milk itself represents a huge potential for further study. In addition, the composition of the microflora differs between milk of camels from different countries and between the herds of different regions of the Republic of Kazakhstan. Shubat, as an “ethnic” drink, has an authentic microflora, whose LAB have improved adaptation mechanisms. Based on these microorganisms, it is possible to build a combinative probiotic preparation that includes various types of LAB. At the moment, a combination of antibiotics and probiotic complexes can be used to reduce the volume of antibiotics and improve growth indicators, until studies of LAB and their effectiveness are completed.

Further study will be a very important step in the creation of complex probiotics based on local strains of lactic acid bacteria of shubat. Their creation, testing and release as goods produced in Kazakhstan can have a positive impact on the dynamics and quality of the economic growth of the republic.

References


