

# Effect of probiotic supplement on growing lambs of the lacon breed during the suckling period

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**Abstract:** *Dairy lambs are susceptible to many pathogens that can affect their subsequent performance. The use of probiotics in the rearing of Lacon lambs during the suckling period has been identified as a means of maintaining intestinal microbial balance. Studies have shown that probiotics can be used as an alternative therapy that prevents the use of antibiotics and, thus, can reduce the emergence and spread of antibiotic-resistant bacteria as well as antibiotic residues in dairy products, meat and milk. In addition, it increases the growth rate of the animal and its stress resistance under various conditions. In this regard, we conducted a comprehensive assessment of the impact using the therapeutic and probiotic supplement Immunobacterin-D on the growth and development of Lacon dairy lambs and their clinical parameters during the suckling period. It was found that the use of the probiotic supplement Immunobacterin-D during the suckling period of Lacon dairy lamb provided an increase in average daily gains by 16.1%, an increase in the content of total protein by 15.8% and an increase in the level of globulin proteins by 35.4%, which indicates a high resistance of the lambs' organism. Thus, the use of the probiotic Immunobacterin-D in feeding lambs had a positive effect on their growth rate, development, improved their health and adaptive capacity, as they showed greater resistance to elevated ambient temperatures compared to those that did not receive the supplement. The use of the probiotic increases the stress resistance of lambs, which can be used as one of the effective approaches in the breeding system of Lacon sheep.*

**Keywords:** *Breeding system, Gastrointestinal microbiota, Intestinal microbial balance, Sheep, Stress resistance.*

Probiotics are increasingly being used worldwide to improve animal welfare, health and productivity. The probiotics market has been growing steadily for several years and is expected to reach \$7.3 billion, growing at a CAGR of 8.8% between 2020 and 2030. Europe is one of the most important regions in the probiotics market for animal feed with a market share of 35% (Size, 2019).

The mechanism of action of probiotics is that they prevent the development of pathogenic microflora, and can also synthesize biologically active substances such as vitamins, amino acids, and enzymes, modulate the immune system, while increasing digestibility and utilization of nutrients. The addition of *Lactobacillus acidophilus*, *Lactobacillus salivarius* and *Lactobacillus plantarum* at a concentration of 10<sup>7</sup>-10<sup>8</sup> CFU/g (colony forming units) reduces the incidence of diarrhea in young calves (Signorini et al., 2012). Probiotic microorganisms create a physical barrier between the intestinal epithelial cells and their contents. Some intestinal probiotics (such as *Lactobacillus*, *Bifidobacteria*, several strains of *Escherichia coli*, and new-generation probiotics including *Bacteroides thetaiotaomicron* and *Akkermansia muciniphila*) can support intestinal epithelial homeostasis and promote health (Liu et al., 2020). In addition, probiotic bacteria produce short-chain fatty acids, which lead to a decrease in pH (Maldonado Galdeano et al., 2019). In the intestine, short-chain fatty acids are mainly produced by bacterial fermentation of cellulose. Therefore (Liao et al., 2024) suggest that safe and effective cellulolytic bacteria may become new probiotics. Recently, microbiome studies have identified a number of putative psychobiotic strains, including SCFAs. SCFA-producing bacteria have attracted particular attention from neuroscientists. Recent studies have shown that SCFA-producing bacteria, such as *Lactobacillus*, *Bifidobacterium*, and *Clostridium*, have very specific functions in various psychiatric disorders, suggesting that these bacteria may be potential new psychobiotics (Cheng et al., 2022). Probiotics increase the digestibility of the feed consumed by animals; For example, a study in buffalo calves showed that probiotic feed containing *Lactobacillus acidophilus* can provide higher dry matter absorption and nutrient digestibility compared to a group of animals whose diets lacked probiotic (Sharma et al., 2018). In another study (Boyd et al., 2011), the use of a mixture of *Lactobacillus acidophilus* NP51 and *Propionibacterium freudenreichii* NP24 as a feed additive for dairy cows improved the digestibility of fiber and crude protein by 7.6%. Similar results were obtained in poultry with regard to increased digestibility of nutrients, essential amino acids and calcium (Khalid et al., 2021; Maas et al., 2021). Recently, probiotics have also been used in sheep farming in numerous studies; Khalid et al., (2021) reported that adding probiotics to the lamb diet improves animal growth during the suckling period. In the study Shoukry et al., (2023), adding probiotics to the main diet also improved lamb growth. Studies conducted by Indian scientists in the Nellore province indicate that adding probiotics to the ram diet improves feed utilization and average daily live weight gain of animals (Khalid et al., 2021). Feeding probiotics to Barki lambs has been shown to have a positive effect on growth, nutrient absorption, and overall economic efficiency (Darwish, 2025).

An important feature of probiotics is their immunostimulatory properties (Bilal et al., 2021; Kong et al., 2020). Recently, the mechanisms of action of probiotics on the immune system have been actively studied by various scientists (Anee et al., 2021; Kober et al., 2022). It has been proven that probiotics can affect immune functions in different ways. Thus, a number of studies have been conducted on the effect of different probiotic strains on the immunological profile of the animal body (Kulkarni et al., 2022). Probiotic supplements can promote growth and development of immune systems in ruminants by regulating the structure, abundance, and fermentation levels

of the gastrointestinal microbiota (Ban and Guan, 2021; Mao et al., 2023).

Based on the reports Escobedo-Gallegos et al., (2023) there is evidence of the positive effect of probiotics on relieving stress in animals, particularly heat stress. Thus, Kotsampasi et al. (2023) indicate that the use of probiotics (direct-feeding microorganisms) by ruminants in stressful environmental conditions alleviated heat stress and increased animal productivity. As indicated by Shah et al. (2025) the addition of a probiotic preparation to the diet of bulls contributed to the avoidance of heat stress and increased nutrient digestibility, which was positively reflected in the average daily weight gains. The mechanism of how probiotics mitigate heat stress in animals can be explained by a decrease in the concentration of cortisol in the blood, which was recorded in the study by Binuni Rebez et al., (2025), where in lambs that were exposed to heat stress and did not receive a probiotic, a tendency toward lower cortisol concentration was observed.

The most promising probiotics are preparations with spore-forming bacteria of the genus *Bacillus*, as they are prominent representatives of exogenous microflora. A wide range of strains of this genus have been studied as therapeutic agent for the prevention and treatment of intestinal infections: *B. cereus*, *B. polymyxa*, *B. coagulans*, *B. brevis*, *B. megaterium*, *B. pumilus*, *B. laterosporus* etc. However, the most fully and comprehensively studied species *B. subtilis* and *B. licheniformis*. Aerobic spore-forming bacteria of the genus *Bacillus* are widely distributed in nature. Despite the fact that *Bacillus* was described more than 100 years ago, the taxonomy of the genus is still developing and improving (Bilal et al., 2021).

One of the most promising strains in the production of probiotics is *Bacillus subtilis*. The alternative name for *B. subtilis* is hay bacillus, since for the first time the cumulative culture of this organism was obtained from hay extract. In 1835, Christian Gottfried Ehrenberg first described this strain, but in his interpretation this microorganism was called *Vibrio subtilis*, later in 1872 *B. Subtilis* received its modern name (Bilal et al., 2021). Another promising culture is *Bacillus Licheniformis* or *B. Licheniformis*. It is a gram-positive, mesophilic bacterium. The principle for the action of *Bacillus Licheniformis* is its ability to regulate bacterial colonies, contributing to the formation of normal microflora in the body (Makowski et al., 2021). Thus, Onubi et al., (2015) probiotics containing *Bacillus Licheniformis* have been shown to improve the absorption of certain nutrients (calcium, zinc, and vitamin B12) and reduce the incidence of anemia, preventing infections and micronutrient deficiencies.

The new domestic probiotic Immunobacterin-D (the supplement used in this study) contains bacteria of the genus *Bacillus subtilis* and *Bacillus licheniformis*, indicating excellent potential properties in terms of impact on the animal body. Thus, according to Sidashova et al. (2022), the use of the probiotic Immunobacterin-D on bees had a positive effect, increasing the live weight of worker bees by the end of the beekeeping season by 9.15% compared to the control. This can increase the viability of bees during wintering. Also, Pogranichniy et al. (2023) reported that probiotic supplement increased average daily milk yield and milk fat content, improved cow health, and enhanced stress resistance—evidenced by a normalized AST/ALT ratio, which supports liver cell renewal. In addition, cows receiving Immunobacterin-D had a significant increase in the content of phosphorus and total bilirubin in the blood

serum. Rybachuk et al. (2020) found that adding Immunobacterin-D to German Shepherd dogs' diets normalized serum calcium levels—significantly increasing them after 14 days—and led to a marked decrease in ALT activity, indicating improved hepatocyte function likely due to reduced liver antigenic and toxic load from the symbiotic action of *B. subtilis* and *B. licheniformis*

However, based on the available literatures, no similar studies on the use of the probiotic supplement Immunobacterin-D conducted on sheep, in particular lambs during the suckling period, have not been conducted in Ukraine. Therefore, the purpose of this study was to study the impact of the use of the therapeutic and prophylactic probiotic Immunobacterin-D on the growth and development of dairy lambs of the Lacon breed and clinical indicators of their body during the suckling period.

Rules for handling animals in experiments fully comply with European legislation (Directive of the Council of the European Union No. 98/58/EC, 1998; Nalon and Stevenson, 2019). The research protocol for biochemical parameters blood of sheep was approved by the local commission on bioethics of "Ascania Nova" Institute of Animal Breeding in the Steppe Regions named after M. F. Ivanov - National Scientific Selection-Genetics Centre for Sheep Breeding, Ukraine, in accordance with Good Clinical Practice (GCP) for the protection and humane treatment of experimental animals.

A scientific experiment was conducted on dairy lambs of the Lacon breed under the conditions of the production and commercial company (PCC) "Pilgrim" of the Khmelnytskyi region to assess lamb growth resistance during the suckling period using the probiotic "Immunobacterin-D" as an immunomodulator. Two groups of ewes with lambs were formed using the group-analogue method (10 ewes and 10 lambs in each).

#### Components of probiotic supplement

1 kg of the preparation contains active ingredients: bacteria of the genus *Bacillus subtilis*, *Bacillus licheniformis*, not less than  $6 \times 10^{12}$  CFU/kg ( $6 \times 10^9$  CFU in 1 g), xylanase 300,000 units/kg, protease 5,000 units/kg, amylase 1,000 units/kg.

Newborn lambs of the experimental group, starting at 7-10 days of age, were given the prophylactic drug Immunobacterin-D at a dose of 50 g/100 kg mixed with concentrated feed. The mixture (Immunobacterin-D + concentrated feed) was prepared as follows: 25 grams of Immunobacterin-D was thoroughly mixed with 100 g of concentrate. Then, 1 kg of concentrate was added to the mixture and mixed well. Next, concentrate was added to reach 10 kg and mixed thoroughly again. Finally, more concentrate was added to bring the total mass to 100 kg, with all components thoroughly blended. The control group did not receive the probiotic. The experiment continued until the experimental lambs reached 2 months of age.

Live weight of lambs was determined by individual weighing at the start and end of the experiment. Body structure was studied at 2 months of age during weaning. Seven main measurements were taken: height at the withers; height at the sacrum; oblique body length; chest width; chest depth; chest circumference behind the shoulder blades; and pastern circumference. For a more detailed description of the animals and their development stage, the following indices were calculated from these

measurements: massiveness; compactness; chest development; stretch; boniness; long-leggedness; and deep-chestedness (Zonabend König et al., 2017). Blood for analysis was collected from the jugular vein of three lambs group before morning feeding. The following indicators were determined: AsAT, AlAT, GHT, Alkaline phosphatase, Bilirubin, Creatinine, Urea, Total protein, Albumin, Glucose, Calcium, Potassium (Polizopoulou, 2010).

Lamb adaptability was assessed by measuring body temperature and breathing rate, recorded alongside weather conditions (air temperature and relative humidity). Body temperature was measured rectally with an electronic thermometer. Breathing rate was the count of chest movements per minute (inhalation acts) while the animal was calm. Pulse rate was measured by counting heartbeats per minute on an artery near the heart. Clinical and climatic parameters were recorded over two consecutive days at 6:00 a.m. and 2:00 p.m. (Mykytyuk et al., 2021).

The coefficient of thermal sensitivity, coefficient of thermal vulnerability and heat resistance index (HRI) in young sheep were calculated as follows. The coefficient of thermal sensitivity of the body was calculated using the formula M. V. Benezra (Mykytyuk et al., 2021).

Biometric data were processed using MS Excel software and statistical functions according to S. S Kramarenko algorithms (Kramarenko et al., 2019) with the determination of the arithmetic mean, its error, as well as the statistical reliability of the studies conducted.

Studies on the effectiveness of the probiotic Immunobacterin-D were conducted on lambs of the Lacon breed. This breed belongs to the dairy production. And is considered the best dairy sheep breed in France. The live weight of breeding rams is 80-100 kg and ewes are 50-70 kg. The animals are characterized by high precocity (up to 60% of ewes can be mated at 7-10 months of age). A feature of these sheep is early weaning of lambs; afterweaning, the ewes begin milking. The average yield of marketable milk over 150 days of lactation is 155-160 (Kudryk et al., 2024).

The therapeutic and prophylactic preparation Immunobacterin-D is a white powder that dissolves well in water. The probiotic consists of *Bacillus subtilis* and *Bacillus licheniformis* at a concentration of not less than  $6 \times 10^{12}$  CFU/kg ( $6 \times 10^9$  CFU/g). The mechanism of action is that *Bacillus* bacteria inhibit the growth of opportunistic microorganisms, provide partial destruction of mycotoxins, contribute to restoration of microflora, and enhance feed digestion, thereby improving feed conversion, productivity, and animal health. It should be noted that the farm where the studies were conducted was epizootically safe regarding infectious gastrointestinal diseases.

As the results showed, use of Immunobacterin-D by the experimental group had a significant effect on live weight (Table 1). At birth, lamb live weight was  $3.5 \pm 0.11$  kg in the control group and  $3.4 \pm 0.10$  kg in the experimental group. At 2 months of age, control lambs weighed  $16.6 \pm 0.17$  kg, while experimental lambs weighed  $18.1 \pm 0.15$  kg, with average daily gains of  $218 \pm 2.1$  g and  $253 \pm 4.5$  g, respectively ( $P \leq 0.001$ ).

Thus, the advantage of the experimental group using Immunobacterin-D was 16.1%. Since absolute and average daily increases in body weight per unit of time do

not always objectively characterize growth rate, the relative growth rate is used, expressed as a percentage. It was found that relative growth of lambs in the control group was 374%, while in the experimental group it was 447%, an increase of 73 percentage points. During the experimental period, isolated gastrointestinal disorders were noted in the control group, whereas lambs in the experimental group exhibited no such disorders. Probiotic bacteria in Immunobacterin-D colonized the intestines of newborn experimental lambs with beneficial microflora, creating a barrier against opportunistic pathogens. In addition to changes in body weight, basic body measurements were taken at 2 months of age to objectively assess animal growth.

It was found that lambs in the experimental group exceeded their peers in the control group by 0.7 cm, or 1.32% in height at the withers; by 1.1 cm, or 2.04% in height at the sacrum; by 0.8 cm, or 1.42% in oblique body length; by 1.0 cm, or 5.95% in chest width; by 1.2 cm, or 5.0% in chest depth; by 1.6 cm, or 2.09% in chest circumference behind the shoulder blades. Based on the obtained mean ANOVA or, body structure indices were calculated in the control and experimental groups. Thus, lambs in the experimental group exceeded those in the control group in the indices of massiveness by 1.1%; compactness by 0.9%; chest development by 0.6% and deep chest by 1.7% ( $P \leq 0.05$ ). Body composition indices indicate that the animals developed proportionally in accordance with ontogenetic changes in body mass during growth.

It is known that the use of probiotics can improve blood parameters and the, antioxidant and immune systems in animals (Anee et al., 2021). Therefore, during the experiment, the aim was to perform a biochemical analysis of the blood of the experimental animals, as reflected in Table 4. It was found that lambs in the control group had a total serum protein content of 53.35 g/l, lambs in the experimental group had 61.80 g/L, a 15.8% increase. This suggests a sufficient amount of structural material to support weight gain. Globulins are of particular interest. This is a significant group of proteins of various structures with important biological functions. The level of globulin proteins influences young animal's future productivity and immune defenses. Thus, the total serum globulin concentration in the control group was 21.31 g/L, while in lambs receiving the probiotic Immunobacterin-D it reached 28.86 g/L, (a) 35.4 % increase, demonstrating improved resistance ( $P \leq 0.05$ ). Domestic sheep exhibit high adaptive capacity to diverse climates and feeds, enabling widespread farming across Ukraine, yet even they can suffer productivity losses under heat stress, particularly with global warming. Markers of adaptive capacity in Lacon lambs treated with Immunobacterin-D, measuring body temperature, respiratory rate, and pulse under thermoneutral and heat-stress conditions.

It was found that in the morning (21 °C) lambs of the control and experimental groups had body temperature of 38.9°C and 39.0 °C, respectively. And body temperature increased: control lambs by 0.8 °C and experimental lambs by 0.7°C. Respiration is the main vital process providing gas exchange between a living organism and the environment. In our study, an increase in air temperature to 37 °C was accompanied by an increase in respiratory rate in lambs of the control and experimental groups by 27.0% and 28.6% ( $P \leq 0.001$ ), compared to their morning rates at lower temperatures. Lambs that consumed the probiotic adapted better to elevated temperatures as their respiratory rates were higher than those of control lambs. Based

on physiological data, indices and coefficients characterizing physiological adaptation were calculated.

The heat resistance index, along with the heat sensitivity coefficient, is an objective indicators of animal responses elevated temperatures. Lambs in the experimental group receiving the probiotic Immunobacterin-D outperformed control lambs by 2.47%, which indicates their enhanced heat resistance. Animals that suffer less from heat stress have a higher growth rate. Regarding the heat sensitivity coefficient and the heat vulnerability coefficient no significant differences were observed between groups. These results warrant further study during both the suckling and intensive fattening phases. This will allow us to more accurately determine the role of probiotics in reducing heat stress in sheep. In addition, the economic efficiency of using the probiotic "Immunobacterin-D" in two-month-old lambs was evaluated.

It was established that the use of the therapeutic and prophylactic drug "Immunobacterin-D" on Lacon lambs during the suckling period (60 days) had a positive effect on their growth dynamics They achieved a 9.0 % increase in live weight and a 16.0 % increase in absolute gain, corresponding to an additional \$6.88 per kilogram of gain.

Prolonged antibiotics use leads to resistant bacteria and antibiotic residues in livestock and fish. Consequently, finding effective alternatives has become urgent. Currently, the most suitable alternative to antibiotics is probiotics. Probiotics are live microorganisms that, when consumed in optimal amounts, provide health benefits. They consist primarily of beneficial bacteria and yeasts that inhibit pathogens, enhance immunity, and restore gut microbial balance. Probiotics eliminate pathogens through multiple molecular mechanisms and modulate the host's immune response promote animal well-being (Anee et al., 2021).

Thus, Maldonado Galdeano et al. (2019) indicate that probiotics improve health by inhibiting harmful bacteria. For example, *Lactobacillus rhamnosus* and *Lactobacillus plantarum* can prevent the adhesion of *Escherichia coli* in the intestinal tract. Kawai et al. (2004) approved, that bacterias such as *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus*, *Enterococcus*, *Streptococcus*, *Bifidobacteria* can produce proteins or bacteriocins that minimize the development of pathogenic microflora. In other words, these probiotics reduce the number of harmful microorganisms in the gastrointestinal tract of animals.

In addition, probiotics enhance host immunity by modulating the immune system. Consumed probiotics stimulate the mucosal immune system (MIS) and induce signaling networks. Antigen-presenting cells identify bacterial components and trigger, primary immune responses leading to T- and B-cell development. They activate immunity via cytokines secretion that stimulates T-cell activation. Signals from dendritic cells determine T-helper polarization or T-regulatory responses, which in turn shape B-cell responses against pathogens (Van Zyl et al., 2020). Probiotics also increase digestion rates by enhancing gastrointestinal enzyme activity and improving feed digestibility (Sharma et al., 2018).

Probiotics play a significant role in ruminants. Ruminants ingest large amounts of protein and carbohydrates, which rumen microbes break down. Ruminant probiotics containing *Saccharomyces cerevisiae*, *Lactobacillus*, *Aspergillus oryzae*, *Bacillus*, and

Enterococcus confer substantial health benefits (Elghandour et al., 2020). They also boost dairy cattle milk yield: *Bacillus subtilis*, *S. cerevisiae*, and *Enterococcus faecalis* increase secretion, and *Bifidobacterium bifidum* suppresses milk allergies (Ma et al., 2020; Jing et al., 2021).

A significant positive effect of probiotics in sheep farming has also been established. Saleem, et al. (2016) indicate that probiotics improve the health of sheep and lambs. According to Kritas et al. (2006), probiotic feed components can improve the quality of sheep milk. Feed additives, including *Bacillus subtilis* and *Bacillus licheniformis*, help reduce mortality, increase the protein content in milk and enhance overall milk production in sheep.

The effect of probiotic substances on reducing stress in sheep, particularly heat stress, has also been established. As the climate in Ukraine has changed significantly in recent decades, researchers are increasingly recording abnormally high temperatures (Vozhegova, 2021). Therefore, according to Estrada-Angulo et al., (2021), the use of probiotics in lambs is recommended to mitigate heat stress in hot environmental conditions. Heat stress can lead to imbalances in the rumen and its function, an increased risk of acidosis, greater energy expenditure to maintain vital body functions, and reduced immunity (Marai et al., 2007). When exposed to high environmental temperatures, sheep that consume probiotics activate a series of physiological compensatory mechanisms such as increased rectal temperature, respiratory rate, and heart rate which help the body adapt to extreme conditions (Mc Manus, 2020).

It was found that the use of the probiotic Immunobacterin-D in the lactation period of Lacon dairy lambs provided an increase in average daily gains of 16.1%, an increase in total protein content of 15.8% and an increase in globulin proteins of 35.4%, indicating enhanced lamb resistance. Immunobacterin-D in lamb diets positively affected growth rate and development, improved health and adaptive capacity, and conferred greater heat resilience compared to unsupplemented lambs.

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