

# Changes in calf productivity and resistance as a result of using the lactulose-based feed additive

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**Abstract.** Objective: the aim of the research is to make a comprehensive assessment of the prebiotic feed additive effect on calves. The experiment was carried out in work conditions on 10 black-and-white Holstein-cross calves at the age of 2 to 32 days. To conduct the experiment, an experimental group and a test group have been formed. Each group has included five milk-fed calves at the age of 2 days and older. All the calves have been given colostrum in their first 2–4 hours after birth and then they have been fed three times a day, at regular intervals. The following methods are used: clinical, microbiological, immunological and statistical. The article describes the prebiotic lactulose-based additive effect on the intestinal microbiocenosis development in one-month old calves and presents an assessment of humoral and cellular components of natural resistance in calves. The research results show a positive effect of the lactulose-based additive on the symbiotic microflora of the gastrointestinal tract that improves the natural resistance of the body and the physiological status of animals, reduces the disease duration and contributes to an increase in weight gain. After feeding the calves with the lactulose-containing preparation during their first month of life, the weight gain of each calf in the experimental group has been 21.8 kg, or + 51 % of the initial weight and in the test group 19.0 kg, or + 41 % of the initial weight. Feeding the additive has an impact on the two components of natural resistance: serum bactericidal activity in the experimental group calves has been higher by 17.8 % and the phagocytosis activity has been higher by 30.5 %, compared to the test group calves. Feeding calves with the lactulose-containing additive helps reduce the illness duration, stimulates the increase in live weight and affects the natural resistance level of newborn animals positively.

## 1 Introduction

Despite the great advances in practical biology, many aspects of the dynamics of viability indicators during ontogenesis remain insufficiently studied [1]. It becomes clear that in the course of life, very often there can be changes in various parameters of the organism, which are far from favorable in all cases. In this regard, it is of great importance to identify the age characteristics of various indicators related to the regulatory systems of body [2, 3].

The elucidation of the specific features of the dynamics of functionally significant indicators in organisms caught in adverse environmental conditions is also of great interest [4, 5]. One of these very frequent factors in productive animal factors is various gastrointestinal diseases [6].

The etiology of these diseases in calves is complex and diverse. The diseases can be caused by both viral, bacterial agents, protozoa, and the combined course of infection and invasion when the natural resistance is reduced [7, 8]. Numerous research works have revealed that the own microflora of humans and animals has a direct impact on the body state [9–11].

Some researchers call the microbiota as “a microbial organ”, which has an immune stimulating effect as well

as participates in the synthesis of vitamins, short-chain fatty acids, proteins and gases involved in regulating gastrointestinal peristalsis [12].

There are a lot of investigations focused on using probiotics for restoring the beneficial intestinal flora [13–16]. There is evidence that probiotic microorganisms introduced from outside are not able to be fixed in the gastrointestinal tract. They present only in the plankton form and are excreted within a short time [17]. Under these conditions, the optimal solution to the problem is using prebiotic preparations that develop optimal conditions for their own symbiotic microflora growth [18, 19].

Various substances, such as biotin, pantothenic acid, cysteine, riboflavin, amino sugar, oligosaccharides, etc., have a stimulating effect on the native microflora growth [20, 21]. Lactulose is considered to be a prebiotic with the highest prebiotic index among those known [22, 23]. Lactulose does not break down in the upper part of the gastrointestinal tract and passes immediately into the large intestine, after which it is used by bifid bacterium as a nutrient medium [24, 25].

**Objective:** the aim of the research is to make a comprehensive assessment of the prebiotic feed additive effect on calves.

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## 2 Materials and methods

The study was conducted in strict accordance with the ethical principles established by the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes (adopted in Strasbourg on March 18, 1986 and confirmed in Strasbourg on June 15, 2006).

The research has been carried out in November-December, on a non-contagious farm in one of the agricultural enterprises in the Vologda region. The experiment has been carried out in work conditions on 10 black-and-white Holstein-cross calves at the age of 2 to 32 days. To conduct the experiment, an experimental group and a test group have been formed. Each group has included five milk-fed calves at the age of 2 days and older. All the calves have been given colostrum in their first 2–4 hours after birth and then they have been fed three times a day, at regular intervals.

All the animals have been kept according to the farm technology. The calves in the experimental group have been given 0.4 ml of the additive per 1 kg of live weight in addition to the main diet for 30 days. The preparation has been given individually, using a dispenser.

The calves took into account body temperature, heart rate and respiratory movements. They also took into account the incidence of gastrointestinal diseases, the nature of feces, the safety, the duration of the disease, the effectiveness of treatment. Evaluation of live weight was carried out three times: during the formation of groups, in the middle and at the end of the experiment.

The intestinal microflora assessment has been carried out three times (at the age of 2, 16 and 32 days) by the microbiological method. The sample collection and study, identification of microorganisms have been carried out according to the existing methods [26]. The obtained results have been given concerning all microorganisms, in case of a comparative analysis in lg CFU/g, and in determining the limits of regulatory indicators – in CFU / g, that is colony-forming units per one gram of the test material.

The natural resistance level has been estimated by the state of the cellular component of protection (phagocytic activity of blood neutrophils) and by the state of the humoral component (serum bactericidal activity and serum lysozyme activity). The sample collection and study have been carried out according to the existing methods [27].

The results obtained are processed with a standard package of statistical programs.

## 3 Results

### 3.1 The additive effect on the clinical state of the animals and their weight gain

The health status of the animals has been under total control during the experiment. The experimental group animals as well as the test group animals have shown gastrointestinal disorder signs on the 4th-5th day of life. In the course of the treatment (rehydration therapy,

antibiotics treatment), the diarrhea syndrome periodically worsened twice in the experimental group and three times in the test group. Fatal cases have not been registered. In the experimental group, the gastrointestinal tract activity has been stabilized faster – on the 12th day in comparison to 20 day-long disease in the test group.

The average calf weight in the experimental group has been  $42 \pm 1.52$  kg, and in the test group  $46.2 \pm 3.7$  kg, the difference being out of statistical control. In the first 14 days of the experiment, the average daily weight gain of calves in the test group has been 10.3 % higher compared to the experimental group ( $0.457 \pm 0.141$  kg and  $0.41 \pm 0.22$  kg, respectively), the difference is insignificant and it is out of statistical control.

Two weeks later, the daily weight gain of calves in the experimental group has been 27 % more compared to the test group ( $940 \pm 0.21$  kg and  $0.74 \pm 0.18$  kg, respectively). During the 30-day monitoring period, the average daily weight gain in the experimental group has been  $0.703 \pm 0.1$  kg, compared to  $0.61 \pm 0.084$  kg in the test group, that is, the calves have gained weight by 15 % faster (figure 1).

### 3.2 The additive effect on the intestinal microbiocenosis development in calves

The intestinal microflora examination in two-day calves has revealed the number of typical *Escherichia*: in the test group it has been 5.77 lg CFU/g and in the experimental group – 6.08 lg CFU/g; no significant differences have been found. Yeast microflora has been also present.

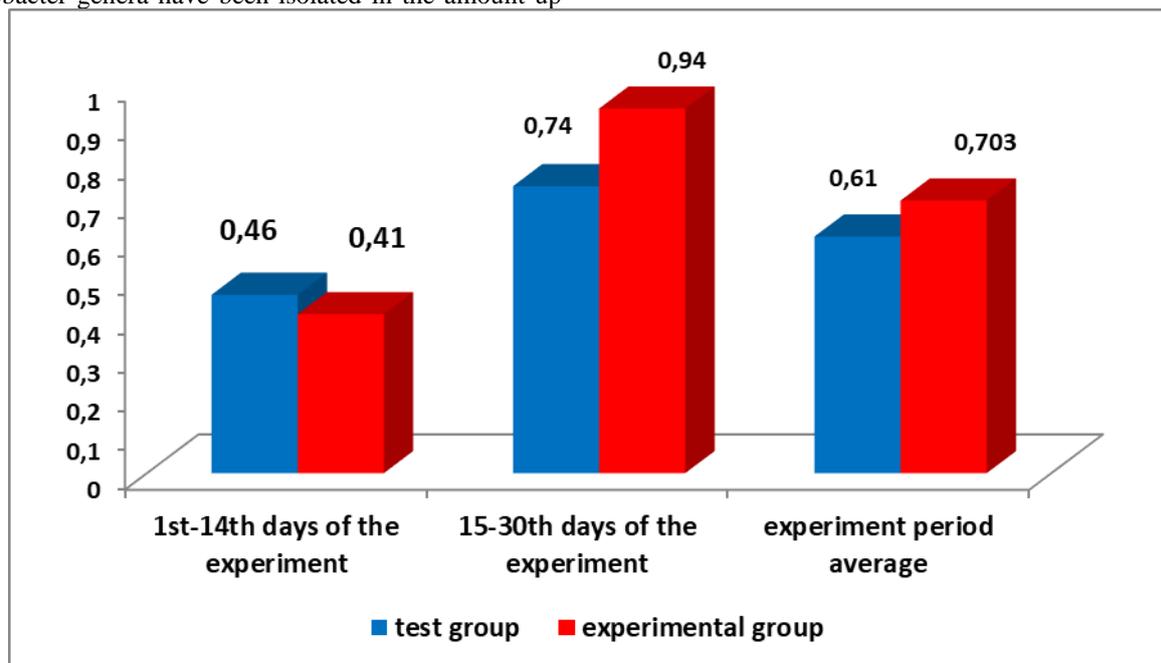
The number of bifid bacterium has been  $2.6 \times 10^5$  CFU/g and  $1.6 \times 10^5$  CFU/g and no significant differences have been found: 6.41 lg CFU/g and 6.20 lg CFU/g in the test group and in the experimental group, respectively. Enterococcus index has not been different in the groups and has amounted to 7.0 lg CFU/g in all animals under the experiment. Lactobacilli growth has not been registered.

The number of bifid bacterium in the 16-day old calves of the test group has increased by 16 % compared to the initial level (to 7.0 lg CFU/g), in one calf their number has decreased by 34 % (to 4.0 lg CFU/g). In the experimental group, an increase in the number of bifid bacterium has averaged 38.5 % (up to 8.2–9.1 g CFU/g). The number of lactobacilli in the experimental group calves has been 7.6 lg CFU/g, which is 52 % more than the number of lactobacilli in the test group calves (5.0 lg CFU/g).

The number of enterococci in the test group has decreased from 7.0 lg CFU/g to 4.39 lg CFU/g and amounted 62 % of the initial level. In the experimental group, the number of enterococci has decreased by 33 % that is to 4.69 lg CFU/g.

In the test group calves, an increase in *Proteus* number has been recorded up to  $10^4$  CFU/g. One calf of the experimental group also revealed the presence of *Proteus* in inoculation, but in a smaller amount of  $10^1$  CFU/g. In the calves of the test group, conditionally

pathogenic microorganisms of the Enterobacter and Citrobacter genera have been isolated in the amount up to 3.0 lg CFU/g.



**Fig. 1.** Dynamics of weight gain in young animals during the experiment according to the observation periods.

Yeast-like and mold fungi have been found in all samples from test group calves in the amount up to 2.14 lg CFU/g; in the experimental group, only one calf have revealed them in the amount not exceeding 2.0 lg CFU/g, which is 7 % lower than in the test group. The number of E. coli (typical) in the test group increased by 35 % – from 5.77 lg CFU/g to 7.7 lg CFU / g and in the experimental group their number decreased by 16 % – from 6.08 lg CFU/g to 5.14 lg CFU/g.

The third study at the age of 32 days has shown that in all samples from the test group calves, the number of bifid bacterium increased by 11 % that is to 7.14 lg CFU/g. In the experimental group, an increase in the number of bifid bacterium averaged 50 %, or to 9.32 lg CFU/g. The number of lactobacilli in the experimental group calves has significantly increased to 9.3 lg CFU/g, which has been by 86 % more compared to the test group – 5.0 lg CFU/g.

The number of enterococci in the test group has decreased to 5.0 lg CFU/g, which has accounted for 71 % in comparison to the initial level. In the experimental group, the number of enterococci has also decreased by 53.8 % or to 3.77 lg CFU/g.

### 3.3 Estimation of natural resistance in animals

The experimental group animals as well as the test group animals have undergone the nonspecific resistance estimation twice during the experiment according to the following items: serum bactericidal activity, serum lysozyme activity and phagocytic activity of blood neutrophils.

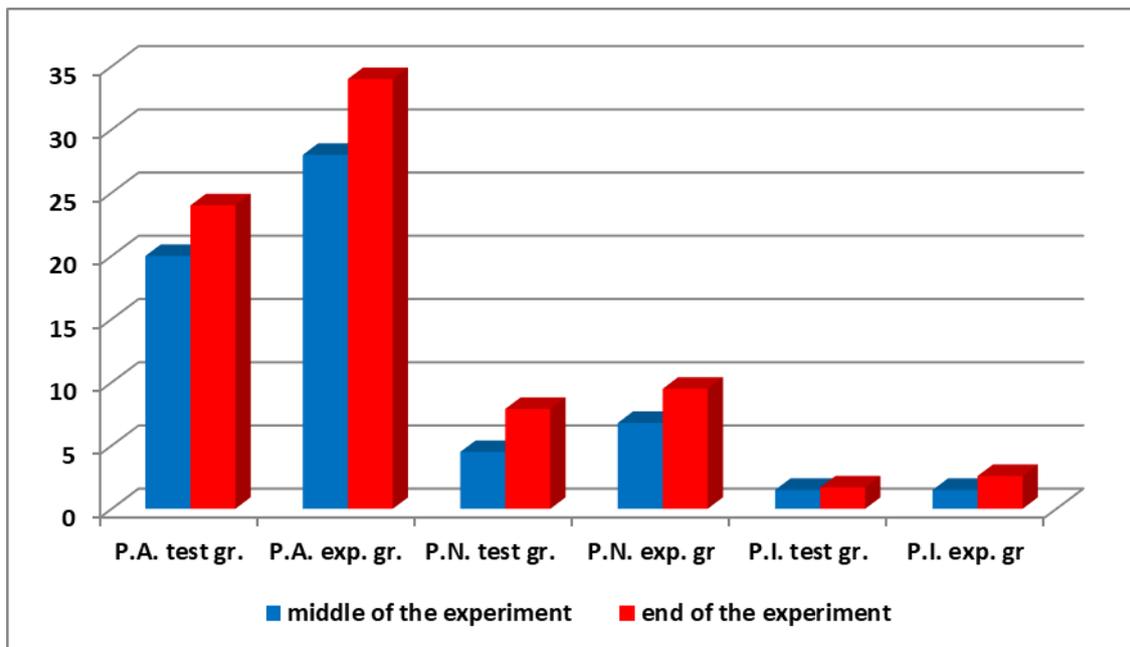
In the middle of the experiment (16 day-old calves) no significant difference in the serum bactericidal activity between the test group animals and the

experimental group animals has been found ( $69,6 \pm 7,9$  % и  $66,2 \pm 11,0$  %, respectively). At the end of the repetitive experiment (32 day-old calves), the serum bactericidal activity indicator has been higher by 17.8 % in the experimental group calves than in the test group and has reached  $91,27 \pm 7,16$  % in comparison to  $77,4 \pm 4,08$  (the revealed difference is considerable and statistically significant,  $P > 0,99$ ). The serum lysozyme activity indicator has been changing slightly in the test and experimental groups of animals and the difference between the groups has not been statistically significant.

Taking into account the changes in cellular protection of the calves, it is possible to note a significant increase in phagocytic activity (FA, %) in the experimental group by 18.05 %, relative to the initial level. As for the test group, at the end of the experiment the phagocytic activity level of neutrophils has been higher by 30.5 % (the difference is statistically significant  $P > 0,99$ ) (Fig. 2).

By the end of the experiment, the phagocytic number (FN of microbial bodies) has increased in the both groups in comparison to the initial indicator: in the test group by 75 %, and in the experimental group by 39.6 %. During the entire experiment period, the phagocytosis intensity in the experimental group has been higher than in the test group by 51 % in the middle of the experiment and by 20 % at the end of the experiment (the difference has been significant,  $P > 0,95$ ).

The phagocytic index (PI of microbial bodies) in the middle of the experiment has not been different in the groups. In the repetitive testing the experimental group calves have revealed an increase in the absorption capacity of neutrophils by 82 %, and the test group calves by 12 %. The difference between the groups at the end of the experiment has been 58.3 %.



P.A. test gr. – phagocytic activity test group; P.A. exp. gr. – phagocytic activity experimental group  
 P.N. test gr. – phagocytic number test group; P.N. exp. gr. – phagocytic number experimental group  
 P.I. test gr. – phagocytic index test group; P.I. exp. gr. – phagocytic index experimental group

**Fig. 2.** Indicators of the cellular component protection

## 4 Discussion

Taking into account general physiological parameters, such as body temperature, pulse and respiration rates, the results of monitoring the clinical animal state have shown their compliance with the average optimal value for the young animals of this age [28].

By the end of the experiment, the experimental group calves have exceeded the test group calves in the average daily weight gain by 15 %; during the observation period, the increase in live weight of one calf in the experimental group was + 51 %, and in the test group + 41 % compared to the initial weight.

The difference in the weight gain is evidently connected with the duration of the gastrointestinal disturbances – the period of disturbances in the experimental group animals has been shorter and, consequently, the digestibility of feed nutrients has been better.

Periodic exacerbation of the diarrhea syndrome may be the result of a long-term treatment with antibiotics, which have suppressed the pathogenic microflora and prevented the development of a complete microbiocenosis of the gastrointestinal tract. When using antibiotics, lactulose has provided a better survival of symbiotic microorganisms [5].

This fact is confirmed by the result of a microbiological examination of the calf intestine contents. The test group calves have revealed the coactant microcenosis imbalance (decrease of bifid bacterium and lactobacterium number) and, as a result, the overgrowth of conditionally pathogenic microflora and yeast-like fungi. Microorganisms of the *Peptostreptococcus* genus have been found in the test group calves. These germs are conditionally pathogenic and, according to some authors, can provoke gastrointestinal disorders [38]. Under conditions of an

unformed microbiocenosis, the overgrowth of these microorganisms can be the reason for periodic tympany in the 1.0–1.5 month-old calves on this farm.

The microbiota examination has showed that the additive feeding contributes to a faster development of the balanced intestinal microbiocenosis in young animals. The symbiotic microflora development (lacto- and bifid bacterium) inhibits the colonization of the intestine by *Escherichia*, yeast and mold fungi, as well as other conditionally pathogenic microorganisms [29]. The calves with a balanced intestinal microbiocenosis assimilate feed components better and, consequently, gain in weight faster. The experimental group calves have caught up with the test group calves after two weeks of the experiment, and later they have surpassed them in the growth rate [30].

Quite a few sources emphasize that the symbiotic intestine microflora is able to influence the natural resistance level positively [14]. An estimation of the nonspecific resistance factors: humoral level (serum bactericidal activity, humoral serum lysozyme activity) and phagocytic level (phagocytic activity, phagocytic number and phagocytic index) have shown that the experimental group calves have had a higher level of natural nonspecific resistance [31, 32]. Thus, the introduction of the prebiotic additive into the ration, indirectly through the formation of the gastrointestinal tract microbiota, has a positive effect on the natural resistance level of animals.

On the basis of the research results, it is possible to confirm a complex positive effect of the lactulose-containing additive on young cattle in their first month of life [33, 34].

The positive effect of lactulose on the symbiotic microflora results in higher natural resistance, the physiological status of animals, reduces the disease duration and helps increase the weight gain [35].

## 5 Conclusion

After feeding the calves with the lactulose-containing preparation during their first month of life, the weight gain of each calf in the experimental group has been 21.8 kg, or + 51 % of the initial weight and in the test group 19.0 kg, or + 41 % of the initial weight. The calves fed with the lactulose-containing additive during their first month of life have had 22.5 % more bifid bacterium than the calves in the test group. The experimental group calves have had 86 % more lactobacilli compared to the test group calves. In general, the microbiota has been richer and the number of conditionally pathogenic microorganisms has been insignificant. In the background of antibiotic treatment, lactulose has ensured a better survival of symbiotic microorganisms. The Bifidobacterium flora activity growth in the experimental group has been 39 % higher than in the test group. The Peptostreptococcus genus microorganisms, which are conditionally pathogenic, have been found in the test group calves. Feeding the additive has an impact on the two components of natural resistance: serum bactericidal activity in the experimental group calves has been higher by 17.8 % and the phagocytosis activity has been higher by 30.5 %, compared to the test group calves. Feeding calves with the lactulose-containing additive helps reduce the illness duration, stimulates the increase in live weight and affects the natural resistance level of newborn animals positively.

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