IMPORTANCE OF PREBIOTIC, PROBIOTIC AND PHYTOBIOTIC FEED SUPPLEMENTS IN CALF NUTRITION

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ABSTRACT

This study is aimed at evaluating the effects of feed additives on health status and live weight gain in calves.

In the experiment, calves were divided into two groups. The first group, the experimental group, was administered RumiForm Digest and RumiForm Digest Yeast+. The second group, the control group was not exposed to any feed additives.

The birth weight of calves in both groups was at the same average values 38 kg. The average weight of the calves at 7 days of age was 46 kg in the control group and the average weight of the experimental group was measured to be 48 kg. A more significant difference in average weights was observed at 90 days after birth. In the control group, the average weight was 137 kg, while in the experimental group the average weight was 152 kg.

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Diarrhoea occurred in both the control and experimental groups. The highest incidence of diarrhoea was recorded in the control group. Diarrhoea disease was recorded in 11 % of the 202 calves. Mortality occurred in both the experimental and control groups. The overall mortality rate was 3.5 %.

In this study, a beneficial effect of probiotic feed supplements on growth was demonstrated. At 7 days after birth and on the 90th day after birth, the p-value < 0.01. The effect of probiotic feed supplements on health status was not demonstrated in this experiment. The p-value was > 0.05 for the incidence of diarrhoea and for the comparison of mortality.

From the data obtained, it can be confirmed that the administration of probiotic feed supplements has a positive effect on weight gain. But a positive effect on health status could not be demonstrated.

Keywords: calves; nutrition; probiotics; prebiotics; phytobiotics; health status

INTRODUCTION

Nutrition is a complex process of interrelated activities (Gaislerová et al., 2019). Adherence to the principles of this complex process is crucial to ensure healthy and resilient individuals who are able to withstand the pathogenic microorganisms that constantly surround them (Rincker et al., 2011; Gaislerová et al., 2019).

In calf rearing, diarrheal diseases are the most common and serious health problem (Cho et al., 2014; Klein-Jobstl et al., 2014). These diseases are the leading cause of mortality in newborn calves (Maier et al., 2022). It is a multifactorial disease caused by a combination of infectious and non-infectious factors (Sung-Hwan et al., 2019). Diarrhoeal diseases are caused by a variety of factors. From dietary and husbandry to infections caused by different types of enteric pathogens (Illek, 2018a). Diarrhoeal diseases cause significant economic losses in calf rearing (Cho et al., 2014).

Feed additives are used in livestock nutrition for many different reasons. Some additives supplement the needs of essential nutrients, while others affect feed intake or increase nutrient conversion (Wenk, 2000). For this reason, new, effective and gentle feed additives are constantly being sought to promote healthy development of calves (Gaisler et al., 2019).

Many microorganisms are used in livestock nutrition. These microorganisms are referred to as probiotics and prebiotics (Wenk, 2000). Live microorganisms that have a beneficial effect on improving the intestinal microbial balance of the host are referred to as probiotics (Gibson et al., 1995). The most commonly used probiotics include lactic acid bacteria. In particular, the genera *Lactobacillus*, *Streptoccocus*, *Enterococcus* and *Lactoccocus* (Ouwehand et al., 2002). Other bacteria used are representatives of the genera *Bifidobacterium* or the yeast *Saccharomyces cerevisiae*. Probiotic cultures improve resistance to infectious diseases, increase growth performance and production, improve feed conversion, and promote food digestion and nutrient absorption. Effective probiotics in the gastrointestinal tract stimulate health-promoting microorganisms while suppressing pathogenic microorganisms by competitive exclusion (Dawson et al., 1990).

Non-digestible feed ingredients that promote the growth or activity of a particular bacterium or group of bacteria already resident in the digestive tract and improve the health status of the host are referred to as prebiotics (Gibson et al, 1995). Prebiotics serve as a specific source of nutrients for health-promoting microorganisms that are commonly found in the gastrointestinal tract (Wenk, 2000). The main advantage of feeding prebiotics in animal nutrition, especially in the diet of young animals, is the beneficial effect on the competitive exclusion of pathogenic microorganisms (Savage et al., 1995).

Preparations that are a mixture of probiotics and prebiotics are called synbiotics (Jonova et al., 2021). This term refers to products whose prebiotic component selectively supports the probiotic component. Due to this synergistic effect, probiotics and prebiotics are administered together to restore gut microbial balance (Trafalska et al., 2004). The mixture of probiotics and prebiotics has a positive effect on feed intake, weight gain, milk digestibility and faecal consistency (Marcondes et al, 2016). Roodposhti et al. (2012) add that feeding synbiotics has a positive effect on reducing the incidence of coliforms and to increase IgG levels in newborn calves. In addition, synbiotics are also produced that can reduce greenhouse gas emissions (Jonova et al., 2020). According to Uyeno et al. (2015), the reason for their positive effect on the organism is to provide energy and carbohydrates for rumen microorganisms, which are essential in the fermentation process. In ruminants, products combining oligosaccharides with bacterial cultures are used (Hamala et al., 2012).

Plant extracts, also known as phytobiotics, are natural bioactive substances naturally occurring in plants. These substances affect feed intake, taste and product quality through their pigmentation, digestion, antimicrobial effect and by reducing unsaturated fatty acids in the digestive tract (Wenk, 2000). Phytobiotics exhibit probiotic effects (Smulski et al., 2020), positively affect animal performance (Nowak et al., 2017) and in dry cow nutrition have a positive effect on colostrum composition and quality. Higher serum IgG concentrations were measured in calves born to cows fed these herbal supplements. Furthermore, a significant reduction in diarrhoeal disease was observed. Phenolic compounds, terpenes, alkaloids, saponins, essential oils, polysaccharides and many other substances have the effect of increasing the phagocytic activity of macrophages, the number of T and B lymphocytes and stimulating interferon synthesis. These substances are found in plants such as *Echinacea*, garlic (*Allium sativa*), *Aloe vera*, mountain arnica (*Arnica montana*), oregano (*Origanum vulgare*) and nettle (*Urtica*) (Frankič et al., 2009). Thyme (*Thymus vulgaris*), oregano (*Origanum vulgare*) or sage (*Salvia officinalis*) have the strongest microbial activity (Windisch et al., 2008). Herbal extracts of oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*) reduce intestinal pathogenic microorganisms responsible for diarrhoeal diseases in calves (Stefanska et al., 2020). Garlic (*Allium sativa*) can also be added to feed in the treatment of diarrhoeal disease in the form of an extract (Kekana, 2014). Another herb used in the treatment of diarrhoeal disease is chestnut (*Castanea sativa*) (Bonelli et al., 2018). According to Smulski et al. (2020), the use of high concentrations of extracts and essences is required to achieve antibiotic-like therapeutic effects. For this reason, phytotherapy is recommended mainly as a preventive measure.

MATERIAL AND METHODS

The experiment took place on a farm of Holstein-Friesian dairy cattle in the southern Pilsen region. Calves were placed immediately after birth in individual outdoor cubicles, where they remained until one month of age. They were then moved, in groups of 10 to 15, to group housing in a calf pen on feeders. The calves were housed there until about 90 days of age and then weaned in the same groups and transferred to a plant-based diet. Finally, at 6 to 7 months of age, they were transferred to a large-capacity calf house.

The calves were fed twice daily with 4 litres of colostrum for the first 4 days after birth. From day 5 after birth, they were also fed twice daily with 4 to 5 litres of native milk, with access to water and starter ad-libitum. They were fed milk formula (MKS) from day 30 of age with gradual weaning until about 3 months of age using automatic feeders. Also with access to water and ad-libitum starter.

Only heifers were included in the study. Feed additives were administered from day 1 after birth until about 1 year of age. During the experiment, the animals were divided into two groups. Individuals in the first group were fed RumiForm Digest (yeast, sorbitol, sodium chloride) - Calf Feed Supplement twice daily at a dose of 10 ml for the first 30 days after birth. Then, from 3 months of age, they were fed RumiForm Yeast+ (calcium carbonate, inactive yeast) at a dose of 50 g until about 1 year of age. The second group of calves was the control group and no feed supplement was given to these calves during the experiment.

Feed consumption (colostrum, native milk, MKS, starter, water) was monitored during the experiment. All calves were sampled for blood (from the jugular vein) using a haemoscope (HEMOS H-02 for cattle with needle) between the second and fifth day of age. The collected blood was allowed to precipitate for 24 hours at room temperature and the resulting blood serum was used to assess the total protein level using an optical refractometer. The refractometer was calibrated before each use. Further collections were made around day 60 after birth and the total protein level in the blood serum was also determined.

The course of the experiment involved monitoring and recording the health status and weight gains of all calves. Particular attention was paid to diarrhoeal diseases, for which a detailed overview (duration, course, colour and consistency of faeces) was recorded. The course of treatment and drugs used were also recorded.

Calves with any signs of disease were treated according to the standard farm protocol, as established by the farm veterinarian. The course of treatment was guided by the current health status of the individual.

For diarrhoeal disease, the most commonly used rehydration preparation was Glucosol plv.sol. - preparation for oral administration, containing transport and energy components (glucose, glycine), substitutes for ion losses (sodium, potassium), components promoting stimulation (citrate, acetate) and alkalizing components (citrate, acetate, bicarbonate). In addition, Duphalyte was applied - a rehydration solution with a complex composition, containing vitamins, electrolytes, amino acids and nutritional components. This preparation was applied subcutaneously (s.c.). In justified cases, antimicrobials were applied.

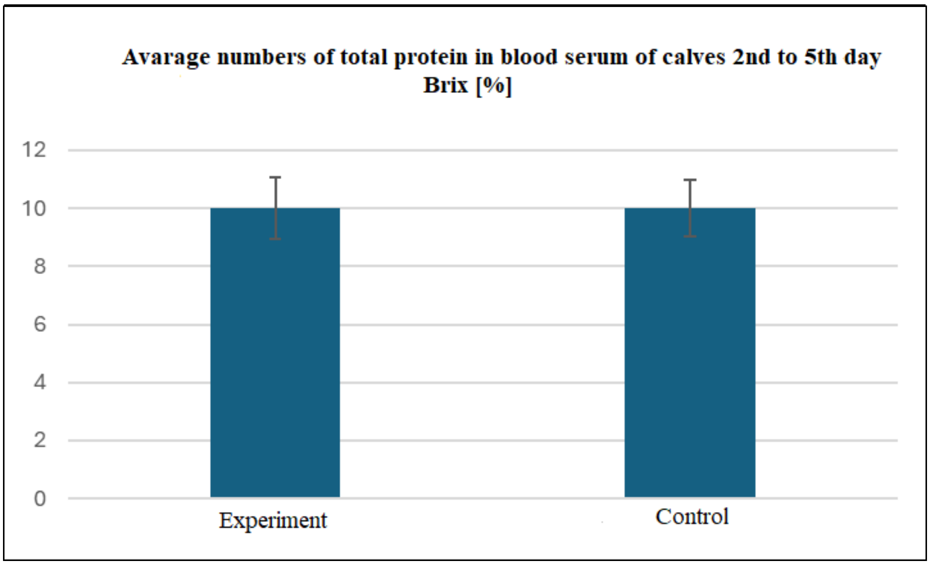
Data were processed using STATISTICA 12 software. Among the tests, chi-square test and t-test were used.

RESULTS

In the study, calves were divided into one experimental group and one control group.

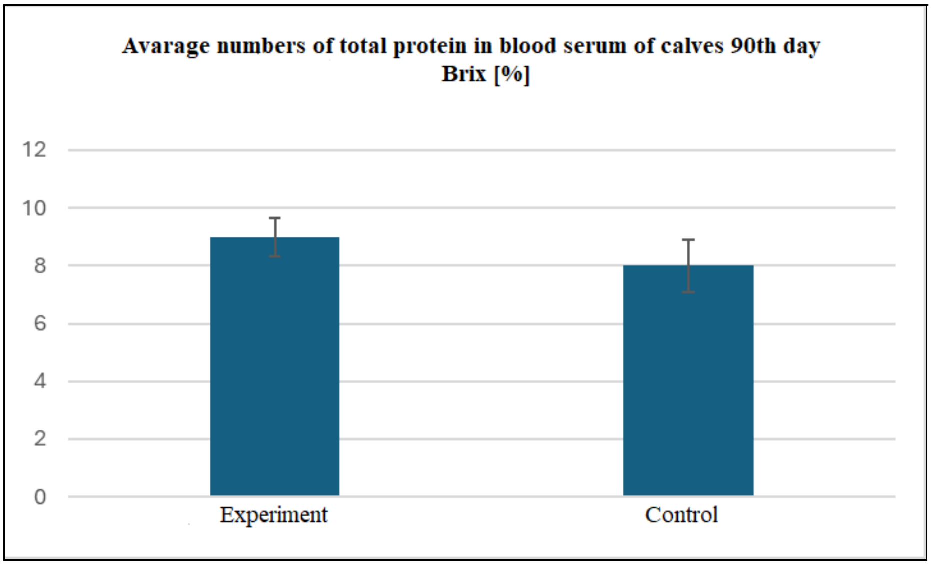
Evaluation of the level of total protein in blood serum

During the study, blood was collected from all animals between days 2 and 5 after birth and then on day 90 after birth. The level of total protein was then determined from the blood serum using an optical refractometer.



Graph 1. Avarage numbers of total protein in blood serum of calves 2nd to 5th day

The mean level of total protein in blood serum (Graph 1) was at the same value of 10.0 Brix [%] between the second and fifth day in both groups. The minimum value found was 5.4 Brix [%], and the maximum value was 12.3 Brix [%]. Based on the parametric Student's t-test, there was no significant difference between the groups (N = 101; t-test = 0.9204; p = 0.1792).

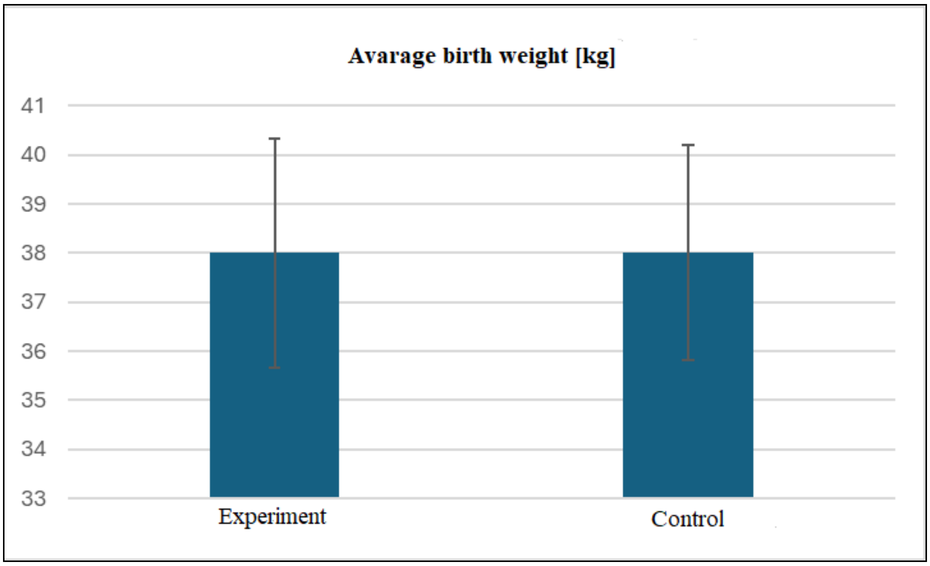


Graph 2. Avarage numbers of total protein in blood serum of calves 90th day

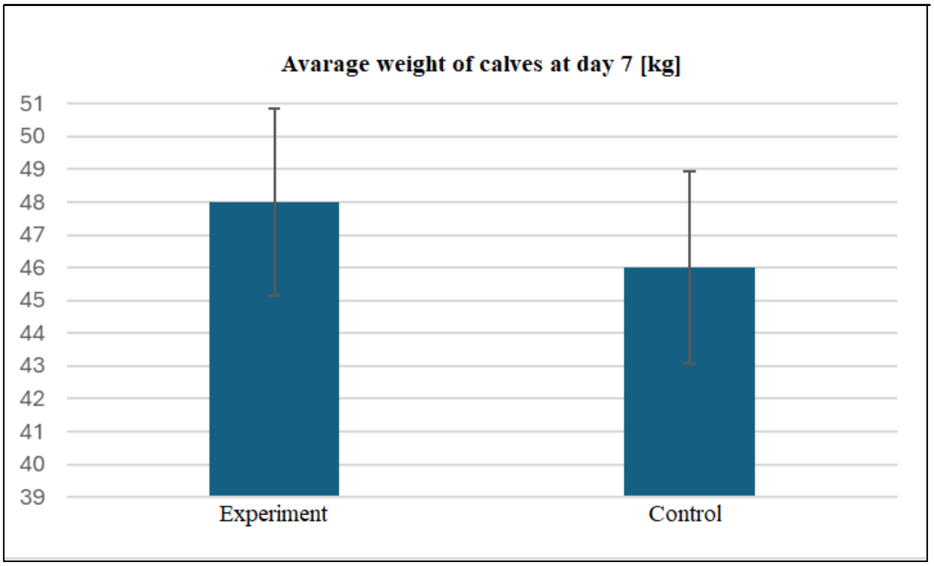
The mean total serum protein counts at 90 days of age (Graph 2) ranged from 8.0 Brix [%] in the control group to 9.0 Brix [%] in the experimental group. The minimum value observed was 7.0 Brix [%], the maximum value measured was 10.7 Brix [%]. Based on the parametric Student's t-test, there was a significant difference between the groups (N1 = 77, N2 = 78; t-test = 2.0692; p = 0.0202).

Growth assessment

Birth weight (Graph 3) was the same in both groups. The mean weight of both experimental and control groups was 38 kg. The lowest weight measured was 30 kg, while the maximum weight recorded was 45 kg. Based on the parametric Student's t-test, it was verified that there was no significant difference between the groups at the beginning of weighing (N = 101; t-test = 0.9927; p = 0.1610).The experiment included weight at birth, at seven days of age and at 90 days of age.

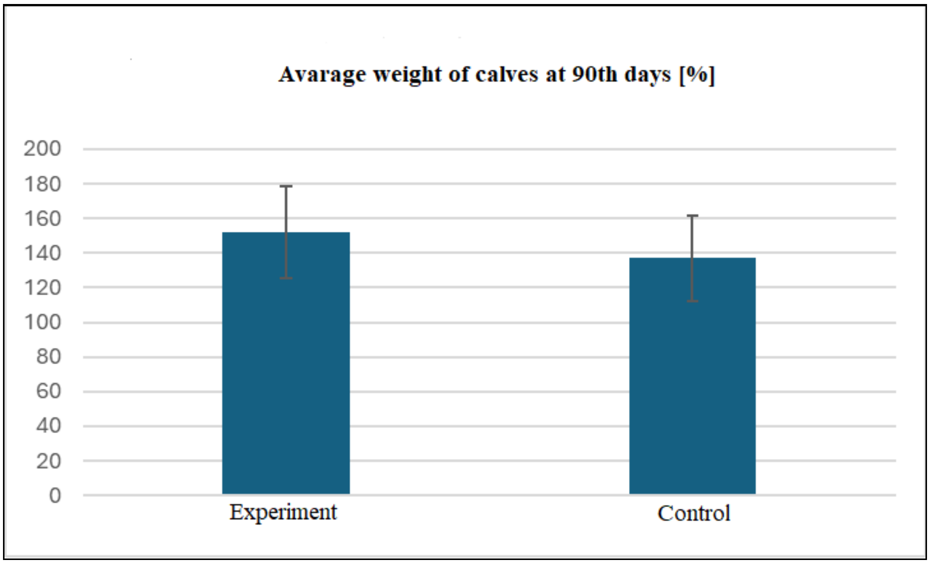


Graph 3. Avarage birth weight of calves



Graph 4. Avarage weight of calves at day 7

The average weight at 7 days of age (Graph 4) was 48 kg in the experimental group, while the average weight of the control group was 46 kg. The lowest recorded value was 36 kg, while the highest was 55 kg. Based on the parametric Student's t-test, there was a highly significant difference between the groups (N1 = 99, N2 = 101; t-test = 4.8793; p = 0.00000109).

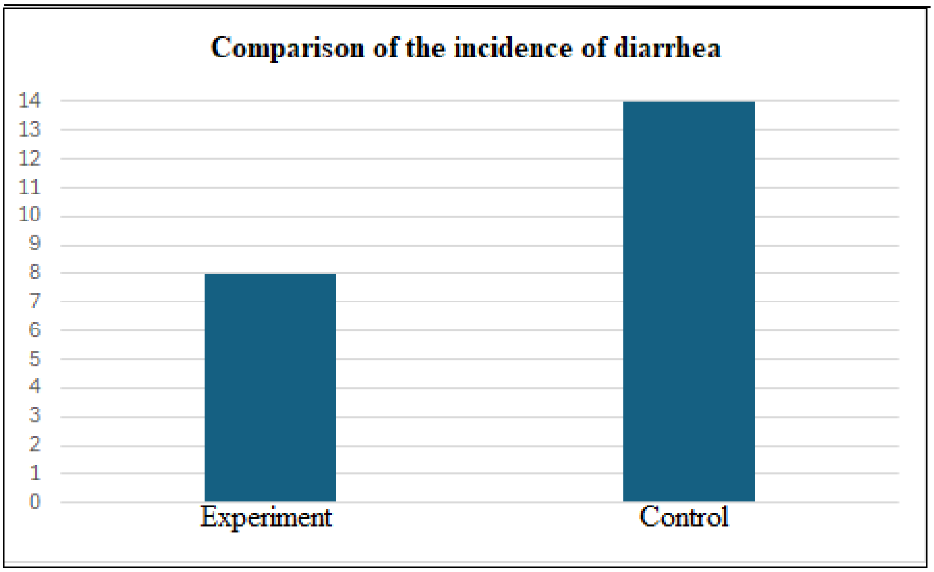


Graph 5. Avarage weight of calves at 90th days

A more significant difference between the groups was observed at 90 days of age (Graph 5), when the average weight of the experimental group was 152 kg, whereas the average weight of the control group was 137 kg. The lowest value recorded was 85 kg and the highest 215 kg. Using a parametric Student's t-test, there was a highly significant difference between the groups (N = 78; t-test = 3.5723; p = 0.0002).

Health assessment

Throughout the experiment, all subjects were monitored and recorded for their health status and treatment progress. In particular, the course and treatment of diarrhoeal diseases were recorded in detail.



Graph 6. Comparison of the incidence of diarrhea between the experimental and control group Diarrhoeal diseases (Graph 6) occurred in both groups. The highest incidence of diarrhoea was recorded in the control group, with 14 individuals suffering from diarrhoea. In the experimental group, diarrhoea occurred in eight individuals. When comparing the experimental group with the control group, no significant difference was found using chi-square test (N = 101; x2 = 1.84; p = 0,1749).

In the experiment, diarrhea in calves (Table 1) was observed from day 6 after birth to calves at 18 days of age. Diarrhoeal disease was most common in animals around day 7 of age. Diarrhoea lasting 1 day was the shortest with the most severe course. Diarrhoea lasted the longest at 10 days. The colour of the faeces ranged from light yellow to brown, with 4 animals showing diarrhoea with blood.

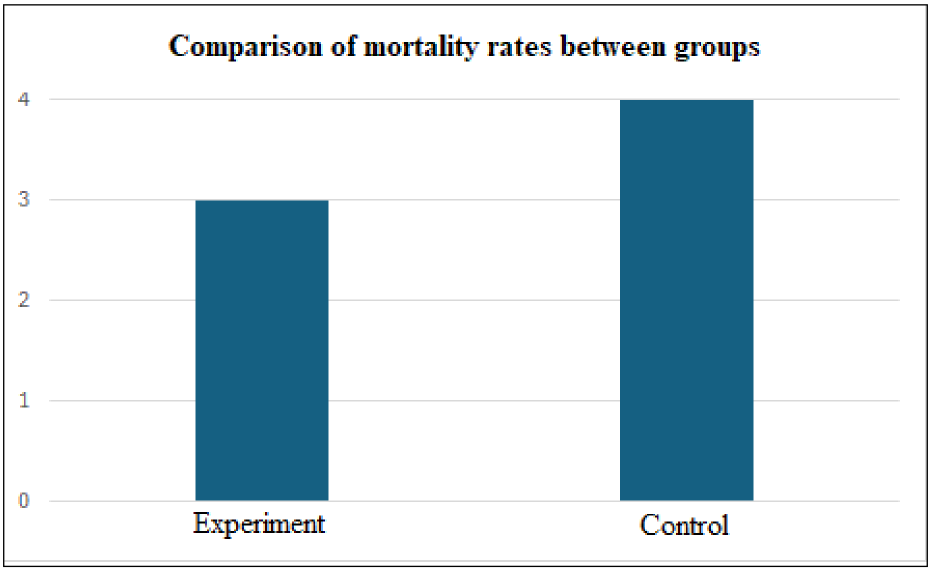
Table 1. Course of diarrhoeal diseases in calves

Group in the experiment	When diarrhea appeared	Number of animals	Duration	Colour, cocsistency
Control	6th day after birth	1	7 days	light brown colour, very watery consistency
	7th day after birth	2	2-5 days	brown colour, with admixture of blood, watery consistency yellow colour, mushy consistency
	8th day after birth	1	10 days	brown colour, with admixture of blood, watery consistency
	9th day after birth	1	5 days	yellow colour, mushy consistency
	10th after birth	3	2-3 days	yellow colour, mushy consistency yellow colour, thin consistency light brown colour, watery consistency
	11th after birth	3	2 days	yellow colour, watery consistency light brown colour, mushy consistency
	13th after birth	1	2 days	light yellow colour, mushy consistency
	18th after birth	2	1-7 days	light yellow colour, thin consistency, with admixture of mucus light yellow colour, very watery consistency, with admixture of blood
Experiment	7th after birth	3	4-8 days	light yellow colour, watery consistency, with admixture of blood yellow colour, mushy consisteny
	8th after birth	1	3 days	light yellow colour, watery consistency
	11th after birth	1	3 days	light brown colour, mushy consistency
	12th after birth	1	9 days	light brown colour, thin consistency
	13th after birth	1	3 days	yellow colour, mushy consistency, with admixture of mucus
	15th after birth	1	6 days	light brown colour, watery consistency, with admixture of mucus

Mortality

The mortalities that occurred during the study were each due to a different cause. In the experimental group, the death of a seven day old heifer occurred with a total serum protein level of 9.1 Brix [%] between days 2 and 5. Mortality occurred unexpectedly without any symptoms. A similar case was the death of a 15 day old calf. In this individual, the serum total protein level between days 2 and 5 was 8.9 Brix [%]. The last mortality within the experimental group was recorded in an individual suffering from diarrhoea.

Within the control group, two deaths were due to injury. Another reason was very severe diarrhoea with a rapid course, which ended in death after the first day. The last mortality occurred in a 53 day old individual with pneumonia.



Graph 7. Comparison of mortality rates between groups

Mortality (Graph 7) was recorded in both experimental and control groups. There were 3 mortalities in the experimental group while 4 mortalities occurred in the control group. Based on statistical analysis chi-square test, there was no significant difference in this case either (N = 101; x2 = 0.07; p = 0.7899).

DISCUSSION

Diarrhoeal diseases are most commonly addressed during the calf rearing period (Cho et al., 2014; Katsoulos et al., 2020). Diarrhoea most commonly occurs around days five and seven of calf age (Urban, 1997). This statement was confirmed in this study. In this experiment, diarrheal disease was the most common problem in calves, and was observed most frequently around day seven of calf age. Diarrhoea was more commonly observed in the control group. A higher number of deaths also occurred in this group.

From the data obtained, there was no evidence of more frequent health problems or deaths in calves with serum total protein levels below 8.6 Brix [%]. This value is considered satisfactory to ensure passive immunity (Bielmann et al., 2010).

From the experiment conducted in this study, a beneficial effect of probiotic feed supplements on weight gain can be demonstrated, but a beneficial effect on calf health cannot be shown. These conclusions are also consistent with the studies by Zábranský et al. (2022) and Zábranský et al. (2023). The study conducted by Zábranský et al. (2022) also found different live weight gains for the different types of probiotics tested. However, this claim is disagreed with by Simon et al. (2001) who claim that the improvements in weight gain and feed conversion are only sporadic. Authors Uyeno et al. (2015) and Renaud et al. (2019) add that studies focusing on health status are insufficient.

CONCLUSION

The calf rearing period is the most important part of a calf's life. Poor management of rearing (nutrition, housing, zoohygiene, etc.) has a negative impact not only on the viability of the individual but also on its future production. Proper management of rearing will result in a healthy and strong calf and a quality animal that will perform well.

In the study, the average level of total protein in blood serum between day 2 and day 5 was measured to be 10.0 Brix [%]. Total serum protein levels above 8.6 Brix [%] were found in 86% of the animals. Levels below 8.6 Brix [%] were also measured among calves. The minimum value measured was 5.4 Brix [%]. There was insufficient transfer of passive immunity in these calves. The average total serum protein counts at 90 days of age ranged from 8.0 Brix [%] to 9.0 Brix [%]. Total serum protein values above 8.6 Brix [%] were recorded in 75% of calves. The lowest recorded serum total protein level was 7.0 Brix [%] and the maximum was 10.7 Brix [%]. These values are not indicative of the transmission of passive immunity but are indicative of the current quality of the diet and the current health status of the animal. Values below 8.6 Brix [%] indicate a lack of protein in the feed, while values above 10.7 Brix [%] indicate dehydrated calves with overly concentrated blood.

The data obtained in this study showed that the average weight of newborn calves was 38 kg. After seven days, the average weights for both groups remained at approximately the same values. In the experimental group, the average weight was 48 kg and in the control group, a value of 46 kg was measured. The lowest weight was measured in a calf weighing 36 kg. On the other hand, the highest value was obtained in an individual weighing 55 kg. When weighed at 90 days of age, the experimental group had an average weight of 152 kg and the control group 137 kg.

Diarrhoeal diseases were observed in both experimental and control groups. A higher incidence of diarrhoeal disease was observed in calves in the control group. In this group, 14 calves out of a total of 101 calves developed diarrhoea. Whereas in the experimental group, diarrhoea occurred in eight out of 101 calves. Calves suffered from diarrhoea most frequently around day 7 after birth. The course of treatment was guided by the current health status of the animals. The most severe course of the disease was in the 18-day-old calf. This diarrhoea resulted in the death of the calf.

Mortality occurred within both groups. Among the calves that died were individuals suffering from diarrhea, pneumonia or with trauma. A mortality of 3.5% occurred during the experimental period.

In this study, a positive effect of probiotic feed supplements on weight gain can be statistically demonstrated, but a positive effect on health status cannot be statistically demonstrated.

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