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# EFFECTS OF USING PROBIOTICS AND PREBIOTICS ON CALVES HEALTH STATUS: AN REVIEW

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***Abstract***

*The aim of the current review was to evaluate the efficacy of using microorganisms with probiotic effect and prebiotic substrate on calves' health status. Probiotics and prebiotics are currently subject to expansive research as alternative to antibiotics use in animal husbandry. This study reports the symbiotic effects on calves at weaning period (calf performance) and health applications (potential benefits in calf's health and safety use). Since preweaning period, diary calves are vulnerable due to innate immune system, not fully capable of protection the organism against pathogenic actions. Thus, in order to minimise the stress during weaning period and to encourage efficient valorisation of nutrients probiotics and prebiotics are used as natural feed supplements. The article describes interrelations of probiotics and physiological responses to weaning, given that such aspects are very important due to the detrimental balance provided from dietary and physiological mechanisms concerned weaning calves. The importance of exploiting this valuable resource rises with the demand of safer and more affordable alternatives for antibiotic growth promoters, reducing economic loss and promoting homeostasis, thus increasing farm profit.*

***Key words**: antibiotics, calves, health, probiotic, weaning.*

## INTRODUCTION

In order to archive early stage development and to avoid potential pathogenic threats, a common practice was to administrate antibiotic formulas as growth promoters in dietary feed (Dennis et al., 2019).

Antibiotics have been by far the most cost-effective measure to maintain feed efficiency and health status in intensive livestock production systems, including ruminants. Since early 2006, antibiotics administration as feed additives in animal husbandry is prohibited in the European Union. Hence, antibiotics can only be administrated to animals as a treatment or in order to prevent disease and it needs a veterinarian prescription. Given the fact that antibiotics have been reported in milk even after 2006 (Pogurschi et al., 2015), it is necessary to encourage the use of prebiotics and probiotics in dairy cows and calves diet. Therefore, replacing the use of antibiotics as health and growth promoters in animal husbandry represents a real challenge, given the needed balance between costs and effectiveness.

Diarrhoea caused by enteric pathogens in un-weaned calves represents one of the most prevalent causes of morbidity and mortality, with reported incidences of up to 75% and 57%, respectively (Muktar et al., 2015, Cho and Yoon, 2014). Important measures can be taken in order to avoid diarrhoeic diseases in young ruminants, considering their significant effects on early development and later adult productivity.

Probiotics, which are live and viable microorganisms, administrated in sufficient amounts and on a specific time scale, could have beneficial effects on the animal's organism (WHO and FAO, 2002; FAO, 2009; ISAPP, 2013). Probiotics use are supported by the EU legislation (Regulation No. 767/2009), as natural alternatives to antibiotic additives in animal feed. As pharmaceutical application, biotherapeutic drugs formulas regarding probiotics (one or more strains of the same genus or species) are specifically defined strains, tested clinically and proficient in gastrointestinal diseases (Sreeja and Prajapati, 2013). Often, biotherapeutic drug formulas may include microorganism with potential probiotic effect and other substances such as: oils, vitamins, rehydration salts and prebiotics (Markowiak et al., 2017).

Along with probiotics, prebiotics are accepted as likely substitutes for antibiotic use in animals (Gibson et al., 2017). Gibson et al. (1995) were the first to observe that prebiotics are a group of substances that interact indirectly with the hosts metabolism, in order to induce proliferation in a selective approach among host microbiome, serving as nutritive substrate for non-pathogenic bacteria.

Symbiotics are the synergistic combination of probiotics and prebiotics, which can offer to different extents beneficial effects to cattle (Radzikowski, 2017). In calves, symbiotic consumption has several proven health benefits, including an increase of intestinal beneficial bacteria, improved immune modulatory responses and reduced incidence of enteritis (Shimizu et al., 2018).

In addition, calf's intestinal microbiome has a major impact on health status, thus protecting the organism from environmental stressors. Adding physiological functions which include storage, conversion and transportation of nutrients. In neonatal calves, the gastrointestinal system (GIT) is unique, experiencing important changes between birth and the first year of life (Yeoman et al., 2018). The digestive system of new born calves in sterile, GIT colonisation starts instantly after birth. During the suckling period, calf's intestinal microbiome is represented mostly of *Lactobacillus* spp., *Lactococcus* spp., *Citrobacter* spp. and *Leuconostoc* spp. Diversity and complexity rises as a result of dietary, physiological and environmental challenges (Diao et al., 2019). At the age of three months, in the calf microbiome, beside existent bacteria an abundance of other genera are being found, including: *Bifidobacterium* spp., *Bacteriodes* spp., *Firmicutes* spp. and *Faecalibacterium* spp. (Oikonomou et al., 2013). From this age, a dynamic microbial complex expands, and it is encouraged by innate immune system to become similar as ones functioning in adults.

Enteric diarrhoea is one of the most prevalent causes of morbidity and mortality among diary calves. For this reason, it is important to prevent pathogenic actions and to encourage healthy approaches (Manyi-Loh et al., 2016).

In this context, the paper presents an evaluation of symbiotic manipulation of gut microbiome in order to optimise calf's performance and health status.

**Potential benefits in using prebiotics and probiotics in calves**

Current applications of probiotic and symbiotic on young pre ruminant animals, generally targets the gastrointestinal system, by stabilizing and enhancing lower intestine microbiome, thus minimizing the rate of gastrointestinal disorders, by supporting health and optimal nutrition. Probiotic, prebiotic and the combination between them, symbiotic claims a potential positive effect on calf health (Dar et al., 2017) and calf performance (Ysmail, 2019). Gut microbiota has a metabolic active role, having also protective and trophic functions, being positively modulated by beneficial microorganisms, in combination with or without carbohydrate substrates (Rowland et al., 2018). Previous studies have shown the beneficial health aspects and potential, throughout the manipulation of intestinal barrier microbiota, facilitating beneficial bacteria proliferation, while reducing the incidence of neonatal calf diarrhoea (Jyotimala et al., 2019), while also improving weight gains (Radzikowski, 2017). The combination of mannan oligosaccharide and *Streptococcus faecium* was investigated by Morrison et al. (2010), as addition to milk replacer on calf performance and health. Results shown a significant improvement of fed concentrate intake and calf faecal consistency, thus improving calf performance.

Lactic acid bacteria such as *Lactobacillus* spp. and *Bacillus* spp. are the most widespread probiotic supplements used and often regarded as feasible in common feeding techniques, improving average daily gains (Ballou et al., 2019; Cantor et al., 2019) and decreasing the incidence of diarrhoea (Jiang et al., 2020). Dar et al., 2017, evaluated the impact of symbiotic combination between *Lactobacillus* spp. and mannan oligosaccharide on serum biochemical profile of calves. Results shown that symbiotic supplementation had reduced the serum triglycerides and cholesterol, unaffecting serum glucose, bilirubin, creatinine and urea.

On the other hand, symbiotic administration of *Enterococcus faecium* and lactulose could improve calf performance (Fleige et al., 2007), by modulating the intestinal barrier physiology, thus decreasing the height of ileal villus, the crypts depth in the cecum and the area of lymph follicles from Peyer's patches.

**Inclusion and general use of prebiotics and probiotics in pre weaned calves**

Given the economic implications of dairy calves rearing, in order to improve calf's growth, health and future adult development, farmers give an increasing interest to calf weaning techniques. In addition, intensive dairy farming demands an accelerated weaning process (Jami et al., 2013), from pre ruminant to ruminant with minimum time and less economic resources.

Furthermore, available research results to improve calf's nutrition and development had become more available with the progress of biochemistry and biotechnologies.

The next generation of growth and health promoters are probiotic, prebiotic and symbiotic, in order to provide solutions to the antibiotic's restrictions put in place by EU Directives in all Member States since year 2006. Recent research demonstrates the multitude of biotechnological approaches applied in dairy calf's diet supplementation (Table 1.).

Colostrum administration together with the milk diet (milk quantity, quality and number of meals per day) are dependent factors that support the future development and stabilisation of calves' gastrointestinal microbiome and adaptive immune system (Neamt et al., 2019; Marin et al., 2020). For instance, probiotic, prebiotic and symbiotic administration could be included in both liquid and solid diets. During the first weeks of life, milk replacer -based diets could be improved with probiotic or symbiotic supplementation in diary calf farms (Mehdi Bahari, 2017).

Table 1. Example of biotechnological growth promoting formulations used in calf nutrition

| **Name of formula** | **Microorganism +/- Prebiotic substrates** | **Producer, recommendation of administration** |
|---|---|---|
| **Symbiotics** | | |
| Biomin©IMBO | *Enteroccocus faecium* + FOS | Biomin IMBO, mixed directly in feed |
| **Prebiotics** | | |
| BionatStart | MOS, β-glucans | 1 x 25 ml/calf/ day |
| MetSac MOS | MOS, β-glucans | - |
| PROFEED® | scFOS | 10 g/calf/day |
| **Probiotics** | | |
| Cylactin | *Enterococcus faecium* | - |
| Lactiferm | *Enterococcus faecium* | $5 \times 10^8$ - $2 \times 10^{10}$ CFU/kg feed |
| Oralin | *Enterococcus faecium* | $1 \times 10^9$ CFU/kg feed |
| BioEnterom | *Enterococcus faecium* | $1 \times 10^8$ UFC/ml/calf/day |
| Cernivet LBC ME10 | *Enterococcus faecium* | - |
| BioPlus2B® | *Bacillus subtilis, Bacillus liqueniformis* | 0.45 kg/1 ton of solid feed |
| Probios Max® | Lactic acid bacteria + vitaminic complex | Unique dose- 5 mg at calf birth |
| Probiosacc C-I | *Saccharomyces cerevisiae* | - |
| Yea Sacc | *Lactobacillus rhamnosus, Enterococcus faecium* | - |

FOS - fructooligo saccharide; MOS-mannanoligosaccharide

In addition, Ulger (2019) found that by supplementing calf diet, during the first 56 days of life (suckling period) with probiotic (*Lactobacillus acidophilus, Lactobacillus casei, Enterococcus faecium* and *Bifidobacterium bifidum*) improves calf performance and decreases blood triglyceride and iron levels. Among administration mode, diary calves' breeders often are substituting whole milk diets with artificial milk replacers. Efficacy of biotechnological application such as probiotic and symbiotics is directly affected by certain factors and circumstances. However, only an appropriate and correct dosage of administration could manifest maximum positive attributes of the probiotic strain. Other relevant factor is represented by the physical form of administration: liquid, powder, suspensions, capsules, gel, paste or pellets. In addition, dosage requirements are depending in the same time of physical form and biological potential of probiotic and symbiotic formula. Furthermore, circumstances regarding individuality and environmental conditions (e.g. diseases exposure) are criteria can be controlled at farm level throughout the implementation of biosecurity and veterinary measures.

**Effects of prebiotics and probiotics on calf's growth and feed conversion**

Future productivity and performance of dairy farms request adequate calf nutrition and care. In addition to banning antibiotic use at farm level, probiotic and prebiotic approaches represent a potential key to establish a new generation of growth promoters. Early stage development and weaning are important due to the fact that they are facilitating changes in metabolism absorption and growth (Govil et al., 2017). Furthermore, ruminal development is conditioned by solid feed administration and intake, within significant effects on the ruminal fermentation processes. The effects of probiotic are generally dependent on the specific microorganisms producing antimicrobial substances, and also on the digestive enzyme stimulation capacity. Prebiotic use showed an improvement of growth performances by improving average daily gain, feed intake and digestibility. In calf feeding, carbohydrate represent the main nutrient in producing volatile fatty acids, with potential to increase nutrient digestibility (Nagpal et al., 2015) and absorption, thus increasing feed efficiency. Ghosh and Mehla (2012) observed an increase in performance weight gain, an improvement of feed intake and feed conversion efficiency by administrating 4 g/day/calf of a mannan oligosaccharide as supplement to calf feed. Similar results were shown by Grand et al., 2013, throughout direct administration of different quantities (3 g or 6 g) of short chain fructo-oligosaccharides, enhancing the growth performance of weaned calves and decreasing feed conversion ratio, while improving carcass weight.

Prebiotic supplementation such as inulin and lactulose, in young pre-ruminant regulates the mRNA gene expression involved in intestine inflammation (Masanetz et al., 2011) and it is believed to facilitate nutritive absorption. Along with health benefits, current prebiotic mechanisms could increase macro elements absorption, for instance Cu, Ca, Mg, Fe and Zn, also $Na^+$ and colon water absorption (Singh et al., 2017). Other researchers have observed an improved capacity of prebiotics, like fructans in colon calcium transporters (Haq and Khan, 2018) increasing bone calcium formation and decreasing calcium losses (Garcia-Vieyra et al., 2014).

Probiotic lactic acid bacteria had influenced productive performance by stimulating feed intake and increasing average daily weight gains, also improving health, thus reducing the incidence of diarrhoea among young ruminants (Wallace et al., 1993; Mehdi Bahari, 2017).

Marcondes et al. (2016) research has shown that symbiotic containing mannan-oligossacharides [(MOS; prebiotic), *Lactobacillus acidophilus*, *Enterococcus faecium*, *Bacillus subtilis*, *Saccharomyces cerevisiae* and cellulase, xylanase and hemicellulose (fibro lytic enzymes)] supplementation had no effect on calves performance, instead was found that the symbiotic Bioformula Leite could represent an bio-control option in calf diarrhoea during the pre-weaning period.

**Effects of prebiotics and probiotics on calf's health**

The capacity of symbiotics on maintaining an eco-balance in the gastro-intestinal microbiome has significantly impacted calf's overall status health status (Malmuthuge et al., 2015; Pandey et al., 2015). Frequently, calf diarrhoea, scours and weight losses are results of stressors generated by husbandry practice (such as ear tagging, vaccination programs, weaning, heat or cold stress, regrouping), pathogens (*Clostridium* spp., *Escherichia* spp., *Enterococcus* spp. and *Shigella* spp.) and bacterial toxins (Taijima et al., 2001). Calf gastro-intestinal microbiome has proven to be able of modulation (Uyeno et al., 2015), as an adaptative response (Cammack et al., 2018) to environmental challenges. Opportunistic pathogens like *E. coli* are the most prevalent causes on neonatal diary calf's diarrhoea (Cho et al., 2012). In according with Muktar et al. (2015), around 80% of neonatal calf diarrhoea positively tested for at least one enteric bacterial infection, denoting that still pathogen infections are main causes. It has been accepted that the first 24 h after birth are crucial to young ruminants, within a need for adequate amount of colostrum administration (Meganck et al., 2014) starting from the first hour after calving, in order to acquire adaptive immune system (Godden et al., 2019). Calf suckling period is often associated with morbidity and mortality (Meganck et al., 2014) and represents the major economic loss factor. Mokhber-Dezfouli et al. (2007) tested a dietary probiotic mixture (*Lactobacillus, Bifidobacterium bifidum, Enterococcus faecium, Streptococcus thermophilus, Aspergillus oryzae* and *Candida pinotopesti*) added in concentrates (25 g/ head/day) and evaluated performance and health status. At the end of the experiment (90 days) body weight gain of the experimental group increased by 7 kg over the control group, and had shown lower diarrhoea incidence.

Satik and Gunal (2017), studied a natural probiotic (kefir), with the administration having a positive effect on lactic acid bacteria populations, related to pathogenic competitive exclusion, proving thus beneficial during the first eight weeks of life.

Li et al. (2012) and Loor et al. (2016) found throughout the sequencing method that calf microbiome development is age-dependent and at the age of two weeks, calf microbiome colonisation is preponderant colonised by *Pervotella* spp., compared to six weeks of age, when the vast majority of populations are *Bacterioidetes* spp.

**Safety measurements in using prebiotics and probiotics**

As the legislation enforces, in order to characterize the microorganism's probiotic action, the following indicators should be used: stress tolerance, adhesion ability, own pathogenic activity, antipathogenic activity, safety assessment and clinical trials (FAO, 2002). New approaches of the pharmaceutical industry regarding antibiotic replacement include therapies such as probiotics and symbiotics, and they are displaying them as less-aggressive methods. Pharmaceutical products impose advanced production technologies and have requirement such as isolation of bacteriologic strains with probiotic potential, description based on ecosystems, geographical region particularisation, selection and characterisation, reproduction at industrial scale. In most cases, non-selected probiotics administration to livestock had low or/and no effects on performance and/or health condition (Fuller, 1989). This might be a consequence of using probiotics which were isolated from other regions or even from other animal species.

In addition, in most publications, authors tend to discuss the efficacy of using probiotics rather than safety of usage. Therefore, in future research it would be advised to evaluate the safety of adding symbiotics in livestock nutrition. Shanahan (2012) pointed the general safety measurements of the prebiotic and symbiotic pharmaceutical formulation, as follows:

- Generalisation of safety assessments on probiotic or symbiotic formulation cannot be applied on similar probiotic or symbiotic products;
- Adverse effects and severity of effects could be specific to susceptibility by individuality;
- Raising awareness among public consumers and farmers, given the wide range of effects and influencing factors in prebiotic administration.

Microorganisms with probiotic attributes are considered generally safe, although often risk administration assessment did not have been made. Regularly, risk as gastro-intestinal or systemic infection on host, detrimental metabolic or toxic effects also immune system hyperstimulation are not taken into consideration, frequently with serious consequences.

**Effects of prebiotics and probiotics on calf's immune responses**

Immunomodulatory actions based on establishment and enhancement of innate and adapted immune system, is involved in the natural immune response mechanism and is directly influencing the animal health (Wu et al., 2019).

Intensive dairy farming, focused on productivity and product quality, requires calf artificial rearing, as a consequence, acquired microbiota and immune system of calves is often reduced and vulnerable to bacterial pathogen colonisation (Marcondes et al., 2016). In comparison with extensive production systems, the intensive calf management requires alternative solutions to support innate immune system and to improve intestinal microbiome. Roodposhti and Dabiri (2012) studied the effect of symbiotic consisting of probiotic bacteria and two types of fungi and prebiotic substances (*Saccharomyces cerevisiae* and cell wall polysaccharide) on daily growth of calves, traces of *E. coli* in calf faeces and IgG immunoglobulin content from calf blood. Research was conducted during 8 weeks, administrating to calves from the control group 1 g probiotics and 4 g probiotic, results displayed an increase on growth performance and improvement in antibody production by 0.120 mg/ml, while decreasing the number of pathogenic populations of *E. coli* by 5%.

Indart et al. (2012) studied the administration of multispecies and multi-strain probiotic (*L. helveticus, L. casei, L. fermentum, L. paracasei, L. parabuchneri, Lactobacillus gasseri* and *Lactobacillus panis, Sacchoromyces cerevisiae* and *Pichia kudravzevii*) on calf pre-weaning period, results indicating a significant increase on metabolic and microbicide activity in peripheral blood neutrophils.

Similar results were shown by Qadis et al. (2014) regarding probiotic (*Lactobacillus plantarum, Enterococcus faecium* and *Clostridium butyricum*) administration on pre-weaned calves, with positive outcomes displayed on peripheral blood leukocytes, inducing a stability among commensal microflora.

## CONCLUSIONS

Probiotic, prebiotic and symbiotic use were shown to have positive effects when administrated to claves on both performance and health. In addition, enhancing the calf development may prove an important pathway to maximise production potential as adult. Besides productive benefits, health status can be modulated throughout the immune response, protecting calves via antipathogenic action. New-born calf microbiome manipulation throughout characterisation and identification of ideal interactions between symbiotics could represent an important key in order to improve overall production. Further research is needed, considering the lack of knowledge and low understanding of the complex mechanisms of interactions, regulation and overall profitability and feasibility.

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