PROBIOTIC MICROORGANISMS AND HERBS IN RUMINANT NUTRITION AS NATURAL MODULATORS OF HEALTH AND PRODUCTION EFFICIENCY – A REVIEW

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Abstract

Probiotics, prebiotics, synbiotics, direct-fed microbials, and herbs may improve the production efficiency in ruminants. The beneficial effect of selected specific microbes on animal health is reflected in protection against pathogens, stimulation of immunological response, increased production capacity, and mitigation of stress effects. Phytobiotic plants used in the nutrition of ruminant animals increase feed palatability. This in turn has a positive effect on feed intake and, consequently, increases production performance. Pectins, terpenes, phenols, saponins, and antibiotic-like substances contained in phytobiotics prevent irritation, diarrhea, and increase the activity of digestive enzymes. Thanks to the abundance of biologically active substances such as flavonoids, glycosides, coumarins, carotenoids, polyphenols, etc., phytobiotics exhibit immunostimulatory and antioxidant properties as well. Given such a wide range of effects on health status and production parameters in animals, an attempt was made in this review to compile the current knowledge on the possible application of these natural growth stimulants in ruminant nutrition and to demonstrate their potential benefits and/or risks for breeding these animals.

Key words: ruminants, probiotics, herbs, health status, production efficiency

Feed additives have been supplemented as growth stimulants in animal production for several decades. New nutritional solutions have been sought to replace feed antibiotics, which were withdrawn from animal production in the European Union since 2006. The role of the antibiotic additives was mainly limited to regulation of the microflora in the animal gastrointestinal tract through control of the growth of unfavorable microorganisms and their toxins (Biernasiuk et al., 2010). The interest in natural growth stimulants that can be used in both prophylaxis and treatment of animals has largely increased (Kiczorowska et al., 2017 b; Windisch et al., 2008). Animal production is closely associated with nutrition and consumer's health. Animal intestinal pathogens, such as *Campylobacter*, *Salmonella*, *Listeria*, and *Yersinia*,

are a direct source of food contamination and a cause of zoonoses. One of the methods for natural stimulation of animal production is the addition of probiotics, i.e. live microorganisms that are symbionts of normal intestinal microflora, and direct-fed microbials (DFM) to animal nutrition.

Probiotics, prebiotics, and synbiotics have proved effective especially in young animals or in the early neonatal period. They exert a beneficial impact on the stability and protection of the intestinal ecosystem. Bacteria colonizing the digestive tract have a double function: they exert a positive effect on the mucosa and activate defense mechanisms to stimulate the immune response and maintain homeostasis. They also influence digestive and metabolic processes as well as immune response (Patel et al., 2015).

In recent years, many animal nutrition scientists and practitioners have attached great importance to phytobiotics containing many biologically active substances (Skoufos et al., 2020; Waszkiewicz-Robak et al., 2017; Windisch et al., 2008) or bioactive compounds extracted from plants, e.g. extract fractions (alkaloids, saponins, terpenes, glycosides), semi-synthetic phytochemicals (phosphorylated alkaloids and glycosides, triterpene derivatives), and synthetic substances identical to their natural counterparts (e.g. thymol, carvacrol, cinnamaldehyde, capsaicin, eugenol) (Kiczorowska et al., 2019; Radkowska, 2013). The bioactive compounds contained therein increase feed palatability and stimulate appetite. Additionally, as regulators of digestive functions, they influence gastrointestinal motility and secretion of digestive juices, reduce the occurrence of diarrhea, and regulate the pH value in the gastrointestinal tract (Kulakova et al., 2019). They can also act as protective agents and regulators of metabolism (Oh et al., 2017). Some herbal raw materials exhibit anabolic, anti-stress, and analeptic properties and can even mitigate the negative effects of anti-nutritional substances contained in feed (Oh and Hristov, 2016).

Consumers are becoming increasingly aware of the importance of the origin and quality of food. The present society exhibits greater awareness of the dangers associated with the use of chemical agents in agriculture and the harmful effects of their residues on food products; hence, natural feed additives are gaining importance (Grela et al., 2017). The growing profitability of food production and animal and human welfare represent a worldwide trend determining the relevance of the development of highly effective phytobiotics and their use in animal nutrition (Kiczorowska et al., 2017 a).

The aim of the review is to compile the present knowledge of the possible use of natural growth stimulators, e.g. probiotics, direct-fed microbials, prebiotics, synbiotics, and some phytobiotics, e.g. some herbs, in the ruminant nutrition and to show potential benefits and possible risks.

The role of intestinal microflora in ruminant health status

Health status in animals is associated with the health of the intestines (Sahu et al., 2019; McCann et al., 2017; Zhang et al., 2016; Kiczorowska et al., 2018). Under normal circumstances, commensal bacteria are an essential health asset with a nutritional function and a protective effect on the intestinal structure and homeostasis. Bacteria colonizing the digestive tract reduce the tension of the intestinal walls via

inhibition of bacterial fermentation, which may lead to formation of intestinal gas. They protect the gastrointestinal tract from the growth of pathogenic microorganisms (viruses, bacteria, and fungi) by stimulation of the growth of beneficial microflora and reduction of the level of toxic metabolism products. The intestinal microflora, its metabolism, and protective functions maintain the integrity of the intestinal barrier, the loss of which may lead to a gradual increase in intestinal permeability, causing transition of physiological state into pathological inflammation (Bagno et al., 2018). Owing to the production of organic acids, microorganisms introduced into the animal gastrointestinal tract reduce the intestinal content pH and improve digestion and absorption of nutrients, e.g. by increasing the pool of digestive enzymes. They also enhance the activity of certain intestinal enzymes, i.e. lactase, sucrose, and maltase, which consequently improves the feed digestibility. Probiotics intensively stimulate the animal immune system by activation of gut-associated lymphoid tissue (GALT) and positively modulate the activity of the immune system by reduction of the activity of proallergic lymphocytes (allergy prophylaxis) (Anadón et al., 2019). In polygastric animals, the rumen is the most important microbial ecosystem with the predominance of fiber-degrading groups representing *Ruminococcus*, *Bacteroides*, *Butyrivibrio*, and *Fibrobacteres* together with major groups such as *Lactobacillus*, *Megasphaera*, *Streptococcus*, *Prevotella*, and *Selenomonas*. Some anaerobic fungi, ciliate protozoa, and a large number of methanogens are present in the rumen as well (Francesca et al., 2010).

The microbiome of ruminant animals, especially the early one present in newborn calves, plays an important role in long-term development of their health status, influences the development, maturation, and activation of gut-associated lymphoid tissue, strengthens the selective transport function and epithelial tissue continuity in gastrointestinal walls (Niwińska et al., 2019). The intestinal microbiota is very abundant in microbial species, with the best-known role of *Bifidobacterium* and *Lactobacillus* strains. As confirmed in many studies, these strains very effectively protect the animal organism against enteric infections (Sheikh et al., 2019; Graham et al., 2016).

Appropriate conditions for the development of young animal-specific bacterial flora are crucial in cattle breeding. In the first weeks of life, the calf drinks milk, which enters the distal sections of the gastrointestinal tract via the esophagus gutter but does not use the digestive possibilities of the rumen. Nevertheless, it is important that the gastrointestinal tract should be colonized by bacteria and protozoa as soon as possible. The final products of microbiota metabolism stimulate the morphological and physiological development of the rumen, which becomes able to fulfill its physiological role in digestion and metabolism during the transition to solid feed (Ellingsen et al., 2016).

The stability of microbiota plays an important role in the processes occurring in the rumen of adult animals as well, although their microbiological ecosystem is extremely diverse. This is especially emphasized in the first period of lactation when the rumen content may undergo microbiological imbalance. This is often associated with a change in the cow's diet. In this period, the amount of roughage is limited in favor of forage that is rich in easily digestible carbohydrates (concentrated feeds). In such conditions, a substantial part of the organic matter contained in the feed is

rapidly fermented in the rumen, causing a rapid increase in the content of volatile fatty acids. The pH decline below 6.0 is accompanied by a decrease in the activity of cellulolytic bacteria and the number of protozoa, which promotes the occurrence of subclinical rumen acidosis. Reduced feed intake and, consequently, other metabolic diseases are observed then (Bravo and Wall, 2016). As indicated in investigations, supplementation of the diet for dairy cows with probiotic preparations containing bacteria characteristic for ruminant microbiota can stabilize rumen pH and, in the case of diagnosed acidosis, reduced the duration of this disease (Julien et al., 2018; Radzikowski, 2017; McCann et al., 2017). *Saccharomyces cerevisiae* yeast are highly effective in modulation of fermentation in the rumen in adult ruminants. Stabilization of rumen pH within the range of values that do not promote the development of acidosis (Vyas et al., 2014), reduction of lactic acid concentration, and improvement of cellulose digestibility have been observed as well (Marden et al., 2008; Malekkhahi et al., 2016). It is particularly important to identify the gut microbiota specific for the different animals.

Direct-fed microbials

The description and mode of action

Direct-fed microbials (DFM) are used in breeding ruminant animals as well. DFM production is based on spore-forming bacteria that are effective in delivering these strains to target organs. The microorganisms used in DFM for ruminants include lactic acid-producing bacteria (LAB), lactic acid-utilizing bacteria (LUB), and other microorganisms, e.g. *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Streptococcus*, *Bacillus*, and *Propionibacterium* species, *Megasphaera elsdenii* and *Prevotella bryantii* strains, and yeast products containing *Saccharomyces* and *Aspergillus* (Elghandour et al., 2015). Their role in feeding ruminants is to provide an optimal microbiological environment in the animal gastrointestinal tract. The activity of DFM is multifaceted, as some bacteria influence the rumen, while others have an impact on the gastrointestinal tract (Puniya et al., 2015). LUB communities mainly reduce the concentration of lactic acid and maintain a normal level of pH, as e.g. *Megasphaera elsdenii*, which utilize lactic acid in the rumen. Both LAB and LUB optimize the conditions prevailing in the rumen and improve the efficiency of intake of feed nutrients (Seo et al., 2010).

DFM used in cattle production are dominated by lactic acid-producing bacteria, e.g. *Enterococcus* spp., *Streptococcus* spp., *Lactobacillus* spp., and *Pediococcus* spp. These bacteria also produce antibacterial substances such as bacteriocin, mevalonolactone, benzoic acid, diacetyl, reuterin, or methylhydantoin, which protect the animal gastrointestinal tract against the growth of pathogenic bacteria (McAllister et al., 2011). Bacteriocin and hydrogen peroxide produced by LAB do not allow binding of substrates to ribonucleotide reductase, thus interfering with the synthesis of the deoxyribonucleic acid (DNA) of pathogenic microorganisms (Dicks and Botes, 2010).

The effect of DFM on ruminants performance and health

Streptococcus and *Lactobacillus* bacteria are used most commonly in young calves. The bacteria play an important role via modulation of the microbial balance in the gastrointestinal tract and inhibition of pathogen growth by e.g. reduction of

the concentration of Gram-positive bacteria (Elghandour et al., 2015). Dicks and Botes (2010) found that *Bifidobacteria* produce acetic and lactic acids in a 3:2 ratio, which may also be beneficial for controlling Gram-negative bacteria and other microorganisms such as molds and yeasts. This was confirmed by Frizzo et al. (2010), who found a significant reduction in the frequency of diarrhea after supplementation with DFM. Yeast DFM preparations can prevent excessive lactate production, improve fermentation in the rumen, and increase feed digestibility. DFM can also compete and inhibit the growth of pathogens (Varankovich et al., 2015). In addition to the regulation of the development of diarrhea and improvement of body weight gain, LAB can influence nutrition efficiency, especially by improvement of dry matter intake and the yield and quality of milk with higher fat content (Galyean et al., 2000; Elghandour et al., 2014). In dairy cows, DFM improves blood sugar and insulin levels (Oetzel et al., 2007), whereas the main function of DFM in fattening cattle is to reduce pathogenic bacteria such as *Escherichia coli* (Elghandour et al., 2015).

In addition, DFM can modulate the host immune system via stimulation and activation of dendritic cells, natural killer cells, macrophages, T- and B-cells, and intestinal neutrophils (Krehbiel et al., 2003). As reported by Matsuguchi et al. (2003), *Lactobacillus casei* Shirota and *Lactobacillus rhamnosus* are also involved in the stimulation of macrophages to produce cytokines (tumor necrosis factor α – TNF- α).

Probiotics

Probiotics definition and role in the digestive tract

Probiotics are naturally occurring microbes, which upon administration improve the health status of animals by competing with pathogenic microbes and nutrient utilization through their positive influence on gut microflora (Bayatkouhsar et al., 2013; Uyeno et al., 2015). In recent years, the technical definition of probiotics has been broadened to include products containing microbes or their end products (i.e. fermented dairy products, etc.). Currently, the definition of probiotics refers to a product containing viable defined micro-organisms, which alter the microbiota (by implantation or colonization) in the host digestive tract and by that exert beneficial health effects (Hutkins et al., 2016). In cattle husbandry, probiotics have been primarily utilized to increase the growth rate, milk production, or production efficiency (Lehloeny et al., 2008); however, in recent years, probiotic preparations have been developed to address other health concerns in cattle. The presence of rumen bacteria promotes intestinal development, thus protecting these animals from pathogens and preventing diarrhea (Sheikh et al., 2019). This reduces the weaning time and maintains the balance in rumen microbiota, thereby increasing the production of such enzymes as amylase, urease, protease, and cellulase, consequently increasing the utilization of fibrous foods. Probiotic bacteria also produce a number of substances that inhibit or eliminate pathogenic microorganisms, e.g. organic acids, hydrogen peroxide, and bacteriocins. They reduce the pH value thus creating adverse environmental conditions for pathogenic bacteria and inhibiting their growth. They also produce short chain fatty acids (including acetic, propionic, and butyric acids), which

are energy material for the colon epithelium. Probiotics have a cytoprotective effect, stimulate the processes of intestinal epithelium regeneration thanks to the production of arginine, cysteine, and glutamine, and stimulate intestinal peristalsis (Sanchez et al., 2017).

Impact of probiotics on calf rearing

Administration of probiotics to calves early in life is highly beneficial. A stable microbial load of *Lactobacillus acidophilus* and *Lactobacillus plantarum* species has been observed to improve weight gain and immunocompetence in young calves (Al-Saiady, 2010). It has been evidenced that supplementation of feed for calves with *Lactobacillus acidophilus* and *Streptococcus faecium* cultures between birth and 5 days of age not only reduced the prevalence of diarrhea cases by over 70% (and calf falls by 99%), but also contributed to enhanced feed conversion (Sahu et al., 2019). Zhang et al. (2016) demonstrated beneficial effects of dietary supplementation with *Lactobacillus plantarum* and *Bacillus subtilis* on growth efficiency, apparent nutrient digestibility, and stress-related indicators in Holstein calves in the first 12 weeks of life. The authors reported that the oral administration of *Lactobacillus plantarum* improved the rate of T-cell transformation ($P>0.05$), and the addition of a mixture of *Lactobacillus plantarum* and *Bacillus subtilis* increased the rate of T-cell transformation ($P>0.05$) and reduced cortisol levels. Moreover, the *Lactobacillus plantarum* supplementation improved growth efficiency and nutrient digestibility but reduced weaning stress in the calves.

Effect of probiotics on ruminants performance

In dairy ruminants, live yeasts (*Saccharomyces cerevisiae*) have been indicated to improve performance, the most consistent effects being an increase in dry matter intake and milk production (Poppy et al., 2012).

In beef cattle, probiotics were found to increase some production parameters. The probiotic treatments positively influenced the growth performance and promoted the feed efficiency of the animals (Francesca et al., 2010). Some of these benefits were related to increased ruminal pH, stimulated growth, and fiber-degrading activities of cellulolytic microorganisms leading to increased fiber digestibility. Probiotics enhancing immunoglobulin levels were found to exert positive effects on growth performance, production, and disease resistance (Guedes et al., 2008). Similar positive effects of the use of probiotics were observed in the case of other ruminants (sheep, camels) (Mosoni et al., 2007). Probiotics should work synergistically with ruminal microbes (McCann et al., 2017).

Clearly improved production parameters were observed when a probiotic *Saccharomyces cerevisiae* yeast strain was used (Poppy et al., 2012). The supplementation not only improved dry matter intake, but also increased the production of milk and improved its chemical composition in terms of the protein and fat concentration. Leicester et al. (2016) confirmed these observations demonstrating a beneficial effect of the probiotic on better absorption of nutrients in the distal segments of the gastrointestinal tract in cows. The authors emphasized that the beneficial production effects were related to the maintenance of rumen pH stability above 5.6. This was achieved by sustenance of the activity of lactic acid-producing bacteria at a constant level and/or increasing the activity of cellulose-hydrolyzing bacteria, with simultaneous

production of antibacterial substances protecting the gastrointestinal tract against colonization by pathogenic bacteria (Kenney et al., 2015). Furthermore, probiotics in ruminants promote an increase in weight gain and milk production (Graham et al., 2016). In this context, probiotic additives have been developed to improve productivity and animal health (Allen et al., 2013).

Prebiotics

Prebiotics definition and mode of action

Prebiotics are non-digestible nutrients exerting a beneficial effect on the health of the host via selective stimulation of the growth and/or activity of colon-colonizing bacteria. The main examples of prebiotics are dietary fibers and oligosaccharides. Like prebiotics, organic acids in feed can be inherent or added. They function by decreasing the pH of an environment, limiting feed spoilage, and contributing to lower pathogen survival in the gut (Castro et al., 2009).

The use of prebiotics in cattle has been limited due to the ability of ruminants to degrade most of these compounds. However, progress in rumen protective technologies may allow these compounds to be used in feedlots and dairy cattle. These include biotechnological treatments yielding products that protect nutrients, mainly proteins and fats, from transformations and degradation in the rumen (Singh et al., 2019). The biotechnological processes yield anaerobically fermented yeast cultures to produce the desired metabolites. Modification of rumen metabolism through prebiotic supplementation primarily influences the rate and degree of ruminal fermentation and the growth of a beneficial bacterial population (including cellulolytic bacteria). This is accompanied by an increase in colony forming units and populations of cellulolytic, proteolytic, and general anaerobic bacteria as well as lactic acid bacteria (Garcia-Mazcorro et al., 2019). Consequently, the amount of energy available for microbial protein synthesis increases. This is associated with an increase in the synthesis of microbial protein and the flow rate of various forms of nitrogen (specific, bacterial, and nutrient) into the duodenum and further intestinal segments and stabilization of the metabolism of easily soluble carbohydrates, which prevents rumen acidosis (Sari et al., 2019).

Effect of prebiotics on performance and functionalities in calves

Concurrently, there is an increase in the retention and utilization of nitrogen and an increase in the supply of amino acids to the intestines. This usually results in a higher dry matter intake from the feed ration as well as stimulation of milk production and the efficiency of its ingredients (Callaway et al., 2008). The positive effects of mannan oligosaccharides (MOS) supplementation on organic matter digestibility increased with the percentage of fiber in the diet, suggesting improvement in rumen fermentation by yeast supplementation (Desnoyers et al., 2009). In beef cattle, growth parameters (average daily gain, final weight, intake, and feed-to-gain ratio) were improved by continuous MOS supplementation (Chaucheyras-Durand and Durand, 2010). The examples and results of the use of prebiotics and probiotics in animal production are shown in Table 1.

Table 1. Probiotics and prebiotics in ruminant nutrition as modulators of production efficiency

Animals	Length of the study	Dietary product	Quantities and the mode of supplementation	Major findings	Reference
Holstein calves (female)	–	MO	4 g/d on milk replacer	Starter intake ↑, BW → (during the pre-weaning period), BW ↑ (the week after weaning), ADG →, FCR ↑, <i>E. coli</i> fecal counts →	Terré et al., 2007
Blond d' Aquitaine calves	–	BIO-MOS®, Alltech, Inc	0.4% in pellet feed of	ADG ↑, Alpha globulins ↑, beta globulins ↑ NEFA ↑, harmful intestinal bacteria ↓	Tassinari et al., 2007
Cows	Fistulated non-lactating	<i>Saccharomyces cerevisiae</i> yeast	0.3 (SC0.3) and 1 g (SC1.0) of SC/day	Ruminal pH ↑, ammonia concentration →, VFA ↑, fibrolytic activity of rumen bacteria ↑	Guedes et al., 2008
Heifers	8.0±1.2 months	Yeast culture	1 g/kg as fed basis	ADG→, FCR ↑, H →	Lascano et al., 2009
Calves	3–4 d of age	<i>L. acidophilus</i> , <i>L. plantarum</i> , <i>L. acidophilus</i> 27sc	1.25 g/100 kg of milk	BW ↑, serum Ig G concentration, Hb ↑, PCV ↑	Al-Saiady, 2010
Calves	Weaning to 90 d	Cellooligosaccharide (CE) or <i>Saccharomyces cerevisiae</i>	–	ADG ↑, FCR ↑, Plasma insulin concentration↑, Total fecal score ↓	Hasunuma et al., 2011
Calves (crossbreed)	5 days – 2 months of age	MOS	–	ADG ↑, FCR ↑, Total fecal score ↓, fecal coliform count ↓	Ghosh and Mehla, 2012
Calves	–	FOS	0.5% to 1% of total mixed ration	organic matter digestibility ↑, dry matter digestibility ↑	Samanta et al., 2012
Calves	Immediately after birth	MSPB	2 g/d/calf	BW ↑, FCR →, H →, Fecal population of LAB ↑	Bayatkouhsar et al., 2013

Veal calves	8/10 d – 168 day of age	scFOS	Calf milk replacer with and without 3 or 6 g	ADG ↑, FCR ↓, Carcass quality ↑ Fecal conc. of SCFAs ↑, Microbial fermentation ↑	Grand et al., 2013
Holstein calves	–	<i>L. plantarum</i> GF103, <i>B. subtilis</i> B27	1.7×10^{10} CFU/h.d, 1.7×10^8 CFU/h.d	Dry matter intake and ADG →, FCR ↑, digestibility of crude protein and total phosphorus ↑, T-lymphocyte transformation rate ↑, cortisol ↓	Zhang et al., 2016
Steers crossbred (Charolais × Beefmaster)	–	<i>Lactobacillus acidophilus</i> , <i>Bifidobacterium thermophilum</i> , <i>Bifidobacterium longum</i> , <i>Enterococcus faecium</i> , <i>Saccharomyces cerevisiae</i>	3.6×10^7 CFU of bacteria, 8×10^6 CFU of <i>Saccharomyces cerevisiae</i>	Digestibility of dry matter, crude protein and neutral detergent fiber →	Encinas et al., 2018
Surti buffalo calves	–	Prebiotic: mannan-oligosaccharides; Probiotic: <i>Lactobacillus acidophilus</i> , Prebiotic + Probiotic	10 g/calf/day, 3 g/calf/day, 10 g/calf/day + 3 g/calf/day	BW ↑, ADG ↑, FCR ↑, heart girth ↑, height at withers ↑ body length ↑	Ratre et al., 2019

ADG, average daily gain; BW, body weight; FCR, feed conversion ratio; H, health; Hb, hemoglobin; NEFA, non esterified fatty acids; PCV, packed cell volume; LAB, lactic acid bacteria; VFA, volatile fatty acids; MSPB, multi-species probiotic; CSPB, calf-specific probiotic; sc FOS, short chain fructooligosaccharide; MOS, mannanoligosaccharide ↑ increase; → no significant differences; ↓, decrease; $P < 0.05$.

Several types of oligosaccharides have been suggested to have specific functionalities in calves. Mannan oligosaccharides (MOS) are complex mannose sugars that are believed to block colonization of pathogens in the digestive tract. They are an excellent medium for probiotics, stimulating the growth of their population, which leads to increased production of organic acids, reduction of the pH of intestinal contents, and inhibition of the activity of harmful intestinal microflora (Uyeno et al., 2015). Fructooligosaccharides prevent adhesion of Enterobacteriaceae, mainly *Escherichia coli* and *Salmonella* species, to the intestinal epithelium (Benyacoub et al., 2008). Galactosylactose (GOS) can protect against intestinal pathogens via its anti-adhesive activity towards enteric pathogens present on the surface of gastrointestinal epithelial cells. Therefore, supplementation with MOS, fructooligosaccharides (FOS), and GOS may improve the growth performance of calves in the pre- or post-weaning stages (Heinrichs et al., 2009). The supplementation of calves with 4 g/d MOS in milk replacer led to stimulated starter intake after weaning (Terré et al., 2007). A diet for calves supplemented with FOS at a concentration from 0.5% to 1% of the total mixed ration (w/w) significantly improved the organic matter and dry matter digestibility of the total mixed ration through optimization of the rumen metabolic profile aimed at an increase in the amount of energy available for microbial protein synthesis and enhancement of its intensity (Samanta et al., 2012).

Effect of prebiotics on ruminants health status

Cows treated with MOS exhibited increased serum rotavirus neutralization titers, while MOS-consuming calves showed increasing serum protein concentrations, IgA concentrations, and thus enhancement of humoral immune. The highest levels of gammaglobulins and better immune response were found in the blood of calves that have been given MOS in their diets (Terré et al., 2007). Cows treated with MOS during the dry period enhanced their immune response to rotavirus and tended to enhance the subsequent transfer of rotavirus antibodies to calves. Similarly, calves treated with the highest dose (1000 ppm) of MOS given via milk replacer were characterized by improved immune status (Swartz, 2018). There have been attempts to elucidate the mechanism of acquisition of antiviral immunity in animals based on cellular and molecular interactions between pathogenic and probiotic microorganisms in appropriate *in vitro* system models. The beneficial and detrimental roles of TLR3 (toll-like receptor 4) in innate antiviral immune responses in mucosa tissues were mainly analyzed. The ability of beneficial microorganisms to modulate differentially cell responses to TLR3 (toll-like receptor 3) stimulation was assessed using several strains of *Lactobacilli* and *Bifidobacteria* (Chiba et al., 2012). It was found that some strains, e.g. *L. rhamnosus*, *S. thermophilus*, *B. infantis*, and *B. breve*, were able to increase IFN- β (interferon- β) levels (Kobayashi et al., 2017). Increased IFN- β production after activation of TLR3 induced by probiotic strains may exert a significant effect on protection against intestinal viruses. Furthermore, after interaction with the IFN- α/β receptor, IFN I increases the expression of antiviral proteins capable of reduction or inhibition of viral replication. The authors confirm that, as immunobiotics, probiotics can play a significant role in enhancement of innate and adaptive immune responses against the bovine intestinal virus (Bunešová et al., 2012; Villena and Kitazawa, 2014).

Table 2. Direct-fed microbials (DFM) in ruminant nutrition as modulators of production efficiency

Animals	Length of the study	Dietary product	Quantities and the mode of supplementation	Major findings	Reference
1	2	3	4	5	6
Holstein cows.	Starting 10 d prepartum and continued until about 23 d in milk	<i>Enterococcus faecium</i> , <i>Saccharomyces cerevisiae</i>	2 g DFM/cow per d	Fat milk percentage in first lactation ↑, milk protein percentage in second lactation ↑,	Oetzel et al., 2007
Holstein bull calves	–	<i>Propionibacterium jensenii</i> 702	1.1 × 10 ⁸ and 1.2 × 10 ⁹ CFU/kg day	ADG ↑, FCR ↑	Adams et al., 2008
Dairy cows periparturient and early-lactation	Starting 2 wk before anticipated calving until 119 d in milk	<i>Propionibacterium strain P169</i>	6 × 10 ¹¹ CFU/d	In rumen fluids: acetate ↓, propionate, butyrate ↑; In plasma: glucose, β-hydroxybutyrate →, nonesterified fatty acid ↑; energetic efficiency ↑	Weiss et al., 2008
Dairy cows	From wk –6 to –3 relative to parturition	<i>Prevotella bryantii</i>	2 to 29 × 10 ¹⁰ CFU/dose	Feed digestion ↑, milk fat ↑, acetate and butyrate concentrations in the rumen ↑, lactate ↓, NH ₃ -N ↑,	Chiquette et al., 2008
Young calf	35 days	<i>Lactobacillus casei</i> DSPV 318T, <i>Lactobacillus salivarius</i> DSPV 315T and <i>Pediococcus acidilactici</i> DSPV 006T	The inoculum with three microorganisms suspended in 0.15 M NaCl was orally administered on a 10 ⁹ CFU/kg live weight daily dose.	Growth performance ↑, digestion of lactose ↑, CHOL ↑, 2010	Frizzo et al., 2010
Lactating primiparous cows	–	<i>Propionibacterium freudenreichii</i> 53-W, <i>Lactobacillus pentosus</i> D31, <i>Lactobacillus bulgaricus</i> D1	2.9 × 10 ¹⁰ colony forming units (CFU)/cow/d, 3.6 × 10 ¹¹ CFU/cow/d, 4.6 × 10 ¹⁰ CFU/cow/d	Body weight gain ↑; CH ₄ intensity ↑; fermentation parameters, bacterial, archaeal and protozoal numbers, milk fatty acid composition →	Jeyanathan et al., 2019

Table 2 – contd.

1	2	3	4	5	6
Lactating primiparous cows	–	<i>Propionibacterium freudenreichii</i> 53-W, <i>Lactobacillus pentosus</i> D31, <i>Lactobacillus bulgaricus</i> D1	2.9×10^{10} colony forming units (CFU)/cow/d, 3.6×10^{11} CFU/cow/d, 4.6×10^{10} CFU/cow/d	Body weight gain ↑; CH ₄ intensity↑; fermentation parameters, bacterial, archaeal and protozoal numbers, milk fatty acid composition →	Jeyanathan et al., 2019
Norwegian Red dairy cows	35 ± 18 days in milk	<i>Propionibacterium thoenii</i> strain T159	5×10^{11} CFU	Methane emissions per unit DM <i>in vitro</i> ↓; total <i>Propionibacterium</i> in the rumen ↑	Chen et al., 2020

ADG, average daily gain; BW, body weight, DFM, direct-fed microbials; FCR, feed conversion ratio; CHOL, cholesterol, ↑ increase; → no significant differences; ↓, decrease; P<0.05.

Synbiotics

Synbiotics are products containing a mix of probiotics and prebiotics. They act in synergy and are an optimal combination of properly selected probiotic bacteria and a prebiotic, which is an energy source and stimulator for beneficial bacteria (Malik et al., 2019). Synbiotics are also referred to as antibacterial, anti-cancer, anti-allergic, and immune-stimulating agents. Through modification of the overall bacterial profile in the intestines, synbiotics have an indirect beneficial effect on the host organism (Sharma et al., 2018).

Previous studies on synbiotics specify the appropriate selection of bacterial species and prebiotics that support their growth and development (Table 2). Morrison et al. (2010) observed that the administration of a combination of a probiotic (*Streptococcus faecium*) and a prebiotic (MOS) had a positive effect on the increase of feed intake and improvement of the structure of feces in calves. An equally positive effect of a synbiotic (probiotic bacteria and two types of fungi) and a prebiotic substance (*Saccharomyces cerevisiae* cell wall polysaccharide) in calf rearing was reported by Roodposhti and Dabiri (2012). The study consisted in 8-week administration of a synbiotic mixture containing 1 g of probiotics and 4 g of prebiotic to calves. The study results demonstrated improved average daily gain and a reduced count of *E. coli* in feces, but no statistically significant cellular immune response was observed. Similarly, Marcondes et al. (2016) used a symbiotic complex containing mannan oligosaccharides (MOS; prebiotic), *Lactobacillus acidophilus*, *Enterococcus faecium*, *Bacillus subtilis*, *Saccharomyces cerevisiae* (probiotic), and cellulase, hemicellulase, and xylanase (fibrolytic enzymes) in calves and reported improvement in the digestibility of feed components and better control of diarrhea. In turn, Gorlov et al. (2020) reported that enrichment of a synbiotic with a mineral complex improved not only the parameters of growth and digestion of nutrients but also hematolytic indicators of blood and the chemical composition of milk in terms of the content of proteins (α -lactalbumin and β -lactoglobulin) and fat (unsaturated fatty acids).

Herbs

Herbs as feed additives

Herb plants are characterized by a number of medicinal properties that cannot be completely replaced by synthetic additives. These are mainly secondary plant metabolites such as terpenes, phenols, and nitrogen-containing compounds. These extracts are used as possible enhancers of productivity for farm animals and alternatives to veterinary drugs, due to their broad antimicrobial, antiparasitic, antioxidant, anti-inflammatory, and immunity stimulating properties (Al-Yasiry and Kiczorowska, 2016). Bioactive substances contained in plants are absorbed more efficiently by the animal's organism and have a more favorable effect than the same synthetic substance (Faniyi et al., 2016; Klebaniuk et al., 2017; Skoufos et al., 2020). The therapeutic effect depends mainly on the type, quality, physical form, and time of application of herbs (Table 3). Chemical compounds accumulate in various plant parts and at specific stages of plant development; therefore, the value of herbs, which depends on the concentration of bioactive compounds, is largely determined by the date of harvest and often even by the time of day. The quality of herbal raw materials

may vary, which results from e.g. the cultivation method, different soil abundance, or even atmospheric conditions (Meena et al., 2017). To establish constant parameters of the quality of plant raw materials, especially in relation to biologically active substances, to ensure an appropriate quality of the product, standardization must be carried out. Consequently, the level of active substances in phytobiotic formulations available on the market is maintained at a constant and reproducible level, which facilitates investigations in this field. Such standardized phytobiotic formulations for livestock, including complex phytobiotics, have been the focus of interest for the last two or three decades. Their broad spectrum of activity includes improvement of animal health and the quality of animal products (Saparova and Zubova, 2019).

Effect of herbs on nutrients digestibility

Biologically active substances present in herbs such as flavonoids, phenols, glycosides, coumarins, saponins, terpenes, alkaloids, and anthracene exert a multidirectional effect on the organism. Herbs can improve the palatability of feed (Kiczorowska et al., 2015). They offer a possibility to modulate digestive processes, stimulate appetite, and improve animal immunity and health (Faehnrich et al., 2017). Herbal feed additives improve nutrient utilization and absorption in ruminants by stimulation of the secretion of saliva (Waszkiewicz-Robak et al., 2017). For instance, garlic, cayenne pepper, ginger, anise, and cumin enhance the synthesis of bile acids in the liver and excretion thereof in bile, which is beneficial for the digestion and absorption of lipids. Most herbs stimulate the function of pancreatic enzymes (lipases, amylases, and proteases) and increase the activity of digestive enzymes of gastric mucosa. In addition to the effect on bile synthesis and enzyme activity, herbs accelerate the digestion in the gastrointestinal tract in ruminants (Frankic et al., 2009).

Essential oils extracted from herbs as used as natural feed additives improve rumen fermentation processes via stimulation of the production of volatile fatty acids. Volatile fatty acids are important energy substrates for ruminants and end products of rumen microbial fermentation. Supplementation with essential oils extracted from herbs has been shown to increase the total concentration of volatile fatty acids and lead to improved feed digestion (Christaki et al., 2012). They can also prevent methanogenesis, improve protein metabolism and feed efficiency, and increase the amount of conjugated linolenic acid in milk (Amlan, 2011). Since plant extracts can act at different levels in carbohydrate and protein degradation pathways, careful selection and combination thereof can be a useful tool for effective modulation of bacterial fermentation in the rumen (Calsamiglia et al., 2007).

Effect of herbs on ruminants performance

The best result can be obtained using properly selected mixtures. Herbs are a very efficient way to improve the health of cattle and the quality of animal products – meat and milk. The desired effect is evident especially during prolonged and regular use. Their share in the total ration is low and usually ranges from 0.2 to 2% of dry matter (Caroprese et al., 2020). With their therapeutic properties, herbs are currently included in the list of alternative and safe feed additives replacing antibiotic growth stimulants that were banned in the EU countries.

Table 3. Effects of phytobiotics in ruminant nutrition

Animals	Length of the study	Dietary product	Quantities and the mode of supplementation	Major findings	Reference
1	2	3	4	5	6
Holstein bull calves	< 7 d old, 40 ± 1 kg BW initially, 48/trial	Apex plant botanicals in the milk replacer (MR), starter feed or both.	0.05% (18% CP)	ADG ↑, FCR ↑	Hill et al., 2007
Dairy cows	90–120 d of lactation	2% of herbal mixture (common chamomile (<i>Matricaria chamomilla</i>), common yarrow (<i>Achillea millefolium</i>), common agrimony (<i>Agrimonia eupatoria</i>), nettle (<i>Urtica dioica</i>), narrowleaf plantain (<i>Plantago lanceolata</i>), St. John's wort (<i>Hypericum perforatum</i>), and lady's mantle (<i>Alchemilla pastoralis</i>) as an additive to concentrated feed	–	Somatic cells ↓, Total bacterial count (including thermophilic bacteria)	Kraszewski et al., 2008
Holstein dairy heifers -		<i>Fructus Ligustri Lucidi</i> , <i>Radix Astragali</i> and <i>Radix Codonopsis</i>	10 g per heifer per day	ADG ↑, BW ↑, FCR ↑ dry matter intake →, immune function ↑, blood antioxidant function ↑	Qiao et al., 2013
Cows: Limousine and Hereford	Young females and males of beef cattle	Herbal mixture 1: thyme, purple coneflower, oregano, cinnamon; herbal mixture 2: thyme, purple coneflower, garlic, licorice, caraway	3% of the predicted daily dry matter (35, 20, 15, 15, and 15%, respectively)	Quality of colostrum ↑, diarrhea ↓, ADG ↑, FCR ↑, plasma IgG ↑	Klebanuk et al., 2014

Table 3 – contd.

1	2	3	4	5	6
Holstein dairy cows	–	Cumin seeds	0, 100, 200, and 300 g/d	Nutrient intake ↑, milk production ↑	Ghafaria et al., 2015
Beef cattle (Limousine, 100-650 kg)	–	A mixture of dried chopped herbs: 3% of the predicted daily dry matter (35, 20, 15, 15, and 15%, respectively) <i>Echinacea purpurea</i> Moench), garlic (<i>Allium sativum</i> L.), thyme (<i>Thymus vulgaris</i>), caraway (<i>Carum carvi</i>), and licorice (<i>Glycyrrhiza glabra</i>)		ADG ↑, FCR ↑, protein and fat digestibility ↑	Klebanuk et al., 2016
Dallagh sheep	–	<i>Mentha piperita</i> essential oil <i>Mentha pulegium</i> essential oil + basal diet	110 mg/d, 110 mg/d	Effect on performance, blood parameters, pH, ammonia, protozoa, and total viable bacterial count in the rumen →, <i>E. coli</i> in the rumen ↓, <i>Lactobacillus</i> in the rumen ↑	Mohamadi et al., 2017
Brown Swiss neonatal calves	Initial body weight 43.2±3.8 kg	<i>Trachyspermum ammi</i> oil, <i>Thymus vulgaris</i> essential oils mixed	1 and 2 mL/d, 1 and 2 mL/d with milk per calf	nutrient digestibility and blood biochemical and hematological parameters ↑	Ebrahimi et al., 2018
Male Dallagh sheep	Initial body weight 35±2 kg	<i>Glycyrrhiza glabra</i> (licorice) and <i>Urtica dioica</i> (nettle) powder in mixtures	10% dry matter	FCR ↑, % <i>Lactobacillus</i> in the rumen ↑, somatic cells ↑; BW, <i>E. coli</i> and pH in the rumen →; glucose, TP, TG, CHOL, albumin in blood →	Rahchamani et al., 2019

TP, total protein; ADG, average daily gain; BW, body weight; FCR, feed conversion ratio; TG, triglycerides; CHOL, cholesterol; ↑, increase; →, no significant differences; ↓, decrease; P<0.05.

Herbs stimulate appetite, thus increasing feed intake and contributing to a higher feed conversion rate per 1 kg of weight gain. Investigations conducted by Kraszewski et al. (2008) have confirmed that addition of 1.0% and 2.0% of herbs: mint (*Mentha*), nettle (*Urtica*), chamomile (*Matricaria chamomilla*), thyme (*Thymus vulgaris*), sage (*Salvia officinalis*), fennel (*Foeniculum vulgare*), pansy (*Viola tricolor* L.), and fenugreek (*Trigonella*) to concentrated feed contributes to increased solid feed intake in all feed rations, significantly higher final body weight, higher daily weight gains, and improved feed conversion rates per 1 kg of gain.

Lemon grass (*Cymbopogon Citrullus*) and peppermint (*Mentha piperita*) are interesting herbs used for improvement of the health status and productivity of animals. Applied as feed additives, they have been shown to improve production performance in beef and dairy cattle (Wanapat et al., 2008; Zmora et al., 2012). Similarly, Chinese herbs (*Fructus Ligustri Lucidi*) have been reported to help in maintenance of good health status of cows and increase the efficiency of dairy production. These herbs supplemented at a dose of 10 g/cow/day to diet increased tract digestibility of diet's dry matter and organic matter, average daily gain, and feed efficiency (Qiao et al., 2013). In turn, cumin seeds (*Cuminum cyminum*) added at a level of up to 200 g/day to cow diets have been found to improve performance, decrease cholesterol concentrations, and influence milk yields (Ghafaria et al., 2015).

As shown by Faniyi et al. (2016), some herbs, e.g. thyme (*Thymus vulgaris*), coriander (*Coriandrum*), turmeric (*Curcuma longa*), cumin (*Carum carvi*), clove (*Dianthus*), and cinnamon (*Cinnamomum cassia*), can also significantly reduce gas production in the rumen by controlling fermentation in this organ. Chaudhry and Khan (2012) and Kongmun et al. (2011) examined *in vitro* fermentation in the rumen with the use of coriander, turmeric, cumin, clove, and cinnamon and demonstrated the activity of the herbs as natural antibiotics. They were found to contribute to elimination of methane-producing intestinal bacteria, which led to up to 40% reduction of the production of the gas. Herbs also function as natural growth stimulators in the nutrition of ruminant animals. Many herbs also increase sheep milk yields, e.g. nettle (*Urtica dioica* L.), caraway (*Carum carvi* L.), corn spurry (*Spergula arvensis*), meadow salsify (*Tragopogon pratensis*), and bristly hawkbit (*Leontodon hispidus* L.) (Siminska et al., 2009). Essential oil (EO) preparation (containing a mixture of natural and nature-identical EO) supplemented at levels of 50, 100, and 150 mg/kg of concentrated feed has been reported to improve feed utilization and performance of high-yielding dairy Chios ewes (Giannenas et al., 2011). However, the activity of some essential oils, e.g. garlic oil, cinnamaldehyde, eugenol, or capsaicin depends on pH and diet, and their application may not always be beneficial. An example is capsaicin, which exerts a slight effect in high-forage diets, but may have an adverse impact on the acetate:propionate ratio and N-ammonia concentration.

Not all meadow and pasture plants are eagerly consumed by animals, and some of them may even be harmful. The most poisonous plants include buttercups (*Ranunculus* L.), cypress spurge (*Euphorbia cyparissias* L.), marsh horsetail (*Equisetum palustre* L.), and autumn crocus (*Colchicum autumnale* L.). Meadow and pasture weeds can exert a negative effect on the quality of animal products. Although they often do not harm animals, these plants produce various chemical compounds in

their tissues, which have a negative impact on animal products. Plants containing large amounts of tannins, terpenes, or polymerized phenols, e.g. the mugwort (*Artemisia*) or knotweed (*Polygonum bistorta* L.), may contribute to bitter aftertaste of milk and meat (Siminska et al., 2009). Therefore, seasonal quality control of pasture sward is recommended in order to eliminate unfavorable plants from its composition.

The antioxidant properties of herbs and their effect on health status

The discussion of the health-enhancing properties of herbs should not ignore their strong antioxidant properties. The antioxidant bioactive substances contained in herbs include mainly carotenoids (xanthophylls and carotenes) and polyphenols (flavonoids, anthocyanins, phenolic acids, stilbenes, and lignans), alkaloids, terpenes, saponins, essential oils, flavonoids, nucleotides, polysaccharides, glycoproteins, tannins, and mucilage (Bichra et al., 2020; Reddy et al., 2020). Herbs with the strongest antioxidant properties include cinnamon (*Cinnamomum cassia*), oregano (*Origanum vulgare*), turmeric (*Curcuma longa*), cumin (*Carum carvi*), basil (*Ocimum basilicum*), ginger (*Zingiber officinale*), black pepper (*Piper nigrum*), rosemary (*Salvia rosmarinus*), thyme (*Thymus vulgaris*), marjoram (*Origanum majorana*), chili pepper (*Capsicum*), garlic (*Allium sativum*), and cardamom (*Elettaria cardamomum*) (ranked according to the decreasing antioxidant potential) and purple coneflower (*Echinacea purpurea*), garlic (*Allium sativum*), aloe (*Aloe vera* L.), mountain arnica (*Arnica montana* L.), oregano (*Origanum vulgare*), nettle (*Urtica dioica*), ginseng (*Panax ginseng*) (Sotek et al., 2018). Their presence even at low concentrations in feed mixes helps to neutralize free radicals in the animal body generated under cellular stress. Reactive radicals can damage cell membranes and even DNA, which impairs the overall state of the immune system and other functions of the organism (Ingale et al., 2017). In the feed mix herbs addition can lead to an increase in the level of immunoglobulins (IgA, IgG, and IgM), enhancement of the phagocytic activity of macrophages, an increase in the number of stimulated B and T lymphocytes, and even inhibition of the growth of cancer cells (Duvvu et al., 2018; Seckin et al., 2018; Ozkaya et al., 2018). Investigations on sheep conducted by Gladine et al. (2007) confirmed that herbal extracts, e.g. from rosemary, entering the rumen environment, still maintained their antioxidant potential, which makes it possible to be used at the cellular level.

A formulation from cat's claw or vilcacora (*Uncaria tomentosa*) has been reported to improve cellular immunity in calves (Tamminen et al., 2018). A beneficial effect of the complex composition of alcoholic infusions of seven medicinal plants: the root of palmate rhubarb of Tungut (*Rheum palmatum* var. *Tanguticum*), dill (*Anethum graveens*, L.), root of wild sunflower (*Inula helenium* L.), the root of German iris (*Iris germanis* L.), hemlock herb (*Conium maculatum* L.), the leaves of the bogbean (*Menvathes trifoliata* L.) and sage (*Salvia officinalis*) on the value of health status parameters in the blood of calves was observed. In their blood, the total protein content increased by 17%, the level of gamma globulins increased by 18%, and the IgG, IgM, and IgA concentrations were higher on average by 15%. Additionally, elevation of T-lymphocytes by 46% and B-lymphocytes by 24% ($P < 0.01$) was observed, compared with the control group (Kozyr et al., 2019). In turn, Seckin et al. (2018) reported that the addition of dried purple coneflower to the diet of cows in

the perinatal period did not influence the content of immunoglobulins in the blood of their offspring. *Moringa oleifera* is characterized by high immunity-enhancing potential, which is associated with its high content of β -sitosterol (90 mg/g) and other phenolic (8 μ g/ml) and flavonoid (27 μ g/ml) compounds. *Moringa oleifera* leaves also exhibit potent antibacterial properties, which is associated with the presence of 4-(α -L-rhamnosyloxy) benzyl isothiocyanate (A) and 4-(α -L-rhamnosyloxy) phenylacetonitrile (Rajanandh and Kavitha, 2010). The beneficial effect of *Moringa oleifera* in the ruminant breeding was confirmed by Sayed-Ahmed et al. (2018) in their studies on goats. In animals fed mixtures supplemented with *Moringa oleifera* leaves, the authors noted better digestibility of feed nutrients, optimal rumen fermentation levels, and even higher milk yield and quality in terms of fat, protein, and lactose content. The significant improvement of production parameters was associated with an increase in the activity of the animal immune system and lower susceptibility to harmful bacterial infections.

Summary

Probiotics, DFM, prebiotics, synbiotics, and herbs can improve the production efficiency. It has been found in many studies that the inclusion rate and the concentrate level in total feed significantly influence the ecology of the rumen and thus nutrient digestion and nutrient availability in ruminants. Various scientific reports confirm the beneficial effect of these natural feed additives on animal health, particularly in terms of protection against pathogens, stimulation of immunological response, increased production capacity, and mitigation of the effects of stress. Prebiotics and synbiotics can be used alternatively or support the effect of probiotics. The use of combinations of these components demonstrating a synergistic effect may be even more efficient in the stimulation of intestinal microbiota and protection of animal health.

Herbal additives can be also a good dietary supplement in ruminant production. Phytobiotic compounds exert a positive effect already during storage of feed. With their high antioxidant potential, they protect fats from oxidation. The enhanced palatability of the feed associated with the presence of aromatic oils has a positive effect on feed intake and, consequently, increases production performance. Biologically active substances stimulate digestion and stabilize the gastrointestinal environment, which improves the utilization of feed and even improves the quality of animal products, e.g. milk. The content of e.g. flavonoids, terpenes, tannins, or carotenoids is responsible for the immunostimulatory and antioxidant properties of phytobiotics. They beneficially model the health status of ruminants, which allows them to use their production potential fully. With their wide range of positive effects on health status and production parameters in animals, these natural stimulators are highly recommended for large-scale breeding of ruminant animals.

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