

Review / Derleme

Overview of plant extracts and plant secondary metabolites as alternatives to antibiotics for modification of ruminal fermentation

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Summary: Ionophore antibiotics in sub-therapeutic doses have been used since 1970s to improve ruminal fermentation. But use of antibiotics as feed additives was banned in the European Union and Turkey in 2006 since they leave residues in animal products and develop resistance in bacteria. This has shifted the focus of the studies to safer alternatives such as plant extracts and their secondary metabolites to alter ruminal fermentation in order to improve ruminant productivity. In this review, general and recent knowledge about plant extracts and plant secondary metabolites as modifiers of ruminal fermentation are summarized. Furthermore, potential efficacies and possible disadvantages of these substances are also discussed.

Keywords: Plant extracts, plant secondary metabolites, rumen fermentation.

Ruminal fermentasyonun modifikasyonunda antibiyotiklerin alternatifi olarak bitki ekstraktları ve sekonder bitki metabolitlerine genel bir bakış

Özet: İyonofor grubu antibiyotiklerin subterapötik dozları ruminal fermentasyonun verimliliğini artırmak için 1970'li yıllardan itibaren kullanılmaya başlanmıştır. Fakat hayvansal ürünlere kalıntı bırakma ve bakterilerde direnç oluşturma riskleri nedeniyle yem katkı maddesi olarak antibiyotiklerin kullanımı 2006 yılından itibaren Avrupa Birliği ülkelerinde ve Türkiye'de yasaklanmıştır. Bu yasak ruminal fermentasyonu değiştirerek ruminant verimliliğini yükseltmek amacıyla antibiyotiklere alternatif olabilecek bitki ekstraktları ve bunların sekonder metabolitleri gibi daha güvenli maddeler üzerine yoğun araştırmalar yapılmasına neden olmuştur. Bu derlemede, ruminal fermentasyonun modifikasyonunda kullanılan bitki ekstraktları ve ikincil bitki metabolitleri ile ilgili genel ve güncel bilgiler özetlenmiştir. Ayrıca bu maddelerin potansiyel faydaları ve olası dezavantajları da tartışılmıştır.

Anahtar sözcükler: Bitki ekstraktları, ikincil bitki metabolitleri, rumen fermentasyonu.

Introduction

Gram positive rumen bacteria produce more ammonia, hydrogen, and lactate than Gram negative species. Therefore, substances which selectively inhibit Gram positive bacteria such as ionophore antibiotics including monensin, lasalosis, and salinomycin improve animal productivity via increasing propionate production and decreasing methane production, proteolysis of dietary protein and accumulation of lactate (36). However, the use of antibiotics in animal nutrition has been prohibited in the European Union (27) as well as in Turkey (26) in 2006, due to the risk of residues in animal products as well as to the concern about the appearance of resistant strains of bacteria. After this regulation, the idea of "natural is better" has spread among consumers and producers. The most common natural feed additives which can be offered as alternatives to antibiotics are plant extracts and bioactive plant secondary metabolites. Secondary plant

metabolites which are derived from the primary metabolisms such as photosynthesis, glycolysis and citric acid cycle do not have any nutritive value and direct contribution to growth, reproduction and development. However, they have a broad range antimicrobial activity and serve to protect plants against pathogens, parasites, herbivores, predators, inter-plant competition and abiotic stresses as desiccation and uv. radiation (15). Plants usually localize secondary compounds in specialized vacuoles, glands, cell walls or plant part surfaces to protect their own tissues. The compounds are then released when the plant part is crushed or punctured (22). Many studies have revealed that defensive secondary metabolites are often synthesized in response to stressor. For example, after herbivory simulation by repeat cutting, lucerna (*Medicago sativa*) has been shown to increase its saponin content (22). So, secondary plant metabolites can be considered as survival and defense mechanisms of plants

instead of immune system in animals (41). Moreover, these substances give plant specific colors and odors (15). Phenolic compounds (carotenoids, alkaloids, phenolic acids, flavonoids and tannins), saponins and essential oils are the best known representatives of plant secondary metabolites. Recent research has been greatly focused to exploit these plant metabolites as natural alternatives to antibiotic feed additives for modification of rumen fermentation hereby mitigating methane emission, improving protein utilization and improving animal productivity (6, 21, 32). This review will focus primarily on general properties, mode of actions, potential efficacies and possible limitations of plant secondary metabolites for modification of ruminal fermentation.

Phenolic compounds

Phenolic compounds, i.e. flavonoids, phenolic acids and tannins, are the most common phytochemical groups found in plants which exhibit several bioactivities such as antimicrobial, antioxidant, antiviral, anti-inflammatory. Other less common phenolic compounds include coumarins, lignans, quinones and stilbens (19).

Flavonoids and phenolic acids: Among phenolic compounds, flavonoids are the most studied group. Flavonoids consist of several subclasses; flavanols (quercetin, kaempferol, myricetin), flavons (luteolin and apigenin), flavanons (naringenin), anthocyanins and isoflavonoids (genistein). Another important class of phenolic compounds is phenolic acids which consist of two major groups; hydroxybenzoic acids (gallic acid, p-hydroxybenzoic acid, protocatechuic acid, vanillic acid, etc.) and hydroxycinnamic acids (ferulic acid, caffeic acid, coumaric acid, chlorogenic acid and cinnamic acid, etc.) (19).

Mirzoeva et al. (23) reported that flavonoids and phenolic acids have antimicrobial effects, in particular, on Gram positive rather than Gram negative bacteria. Therefore, these substances might modify ruminal fermentation similar to ionophore antibiotics. An *in vitro* study showed that flavonoid-rich plant extracts (*Punica*, *Betula*, *Ginkgo*, *Camellia* and *Cudrania*) reduced methane accumulation, number of ciliate and Gram positive bacteria populations such as *Ruminococcus albus* and *R. flavefaciens* (21). Mulberry leaf flavonoid and resveratrol were also found to improve the digestibility and utilization of nutrients and reduce ruminal methane emission in sheep (9). Furthermore, *Rosmarinus officinalis* (rosemary) and *Salvia officinalis* (sage) extracts decreased *in vitro* acetate production and acetate to propionate ratio (A:P) (14). These effects could be attributed to probable inhibitory effects of phenolic compounds especially on Gram positive bacteria. On the other hand, flavonoids can stimulate the fermentative activity of rumen bacteria (6, 13). Dry extracts of *Lavandula officinalis* (lavender) and

Solidago virgaurea (goldenrod) with high flavonoid content have improved the ruminal fermentation via increasing production of total volatile fatty acids (VFA) (6) and, *Achillea millefolium* (yarrow) increased both degradabilities of crude protein and cell-wall constituents and yield of biomass production (7). Cashew nut shell liquid (40) and *Olea europaea* (olive leaf) extract (28) containing antibacterial phenolic compounds have increased the total VFA and propionate production. In another study, phenolic compounds extracted from honey bees' propolis increased production of acetate and total VFA (33). Demirtaş and Pişkin (13) also reported that *Urtica dioica* (stinging nettle), *Matricaria chamomilla* (chamomile) and *Vitex agnus-castus* (chasteberry) extracts with phenolic compounds have stimulated the fermentative activity of rumen microorganisms, increased the production of VFA and dry matter digestibility (except *U. dioica*). However, the lack of decrease in A:P suggests that they affect microbial fermentation by using a mechanism different from monensin. These effects might be a result of hydrolyzation of phenolic compounds to more bioactive forms by rumen bacteria. These products can stimulate the synthesis of aromatic amino acids and enhance the enzymatic activity of some groups of bacteria (3, 7). Cellulolytic bacteria were reported to protect themselves against toxic effects of phenolic compounds in this way, and also able to use hydrolyzation end products as a carbon source (10). So, phenolic compounds can interact rumen microorganisms in a positive as well as negative way (6). It should also be considered that resulting effects might be formed by other plant metabolites even at very small quantities in the extract (7). Plant extracts are a complex mixture of several different biochemical substances and, amount of seconder metabolites in the extract can be varied depending on the used part of plants, harvest time, storage conditions and extraction method. Thus, to determine the fraction of phytochemicals responsible from the effect is so difficult (37). This seems to be the most important factor which limits obtaining tangible results from the studies with plant extracts on ruminal fermentation.

Tannins: Tannins, also known as polyphenols, are water-soluble polyphenolic compounds in the range of 500-5000 molecular weight units. They can be classified into two subtypes, condensed and hydrolysable types, which can form potent complexes with proteins, sugars, and starches stable at pH 3.5 to 7 (38). Tannins are widespread in plants, especially in legumes, cereals, and fruits and restrict nutritional value and digestibility of plants significantly when their amount is higher than 5% (2). The activity of tannins in rumen is not fully understood. Many authors have reported that tannin supplementations have strong effects on inhibiting methane production. On the other hand, tannins indirectly inhibit fiber degradation (5).

Tannins were suggested to bind to feed proteins and protect them from microbial digestion in rumen. Tannin-protein complexes are unable to protect their stability at low pH of abomasum and are dissociated in small intestine. Accordingly, the passage rate of proteins to the small intestine increase in the presence of tannins. Aguerre et al. (1) reported that increasing tannin extract levels from quebracho and chestnut in the diet protected dietary protein from rumen degradation. Authors confirmed these results considering the lower rumen ammonia, branched-chain VFA and blood urea nitrogen concentrations. On the other hand, tannins have detrimental effects on dry matter intake, milk protein content, milk protein yield, and nutrient digestibility. Authors have suggested that undissociated tannin-bacterial protein or tannin-dietary protein complexes may reduce access of intestinal enzymes. The other possibility according to authors is that, tannin may bind to feed protein and endogenous enzyme in the intestine, and decrease overall protein availability even if tannin-protein complexes were completely reversible in abomasum. Supplementing tannic acid in the ration of beef cattle also has been reported to decrease digestibility of crude protein in all received doses as well as of methane production (42). Although reducing effects of tannins on ruminal protein degradation and methane emission seem to be an advantage for ruminant nutrition, their adverse effects on feed digestibility and productivity restrict the use of them as feed additive.

Saponins

The word "saponin" means "soap" in Greek and saponin containing plants were used for washing in ancient times (39). Saponins constitute primarily sapogenins and glycosides found generally in angiospermous plants are divided into two groups as steroidal and triterpenoid saponins. They protect plants against bacterial and fungal diseases (38). Lucerne and soybeans are the main examples of saponin-rich plants which are widely used in ruminant diets. *Yucca shidigera* (yucca), *Quillaya saponaria* (soapbark) and *Sapindus sp.* (soapberries) are also the most common sources of saponins.

Saponins act on rumen fermentation mainly by increasing the flow of amino acids to the small intestine via reducing protein degradation and thereby ammonia and urea concentrations in rumen. The effects of saponins on nitrogen metabolism in the rumen were attributed mainly to their toxic effect on protozoa which are largely responsible from nitrogen retention in the rumen because of their proteolytic activity on both dietary and microbial proteins (30). However, some studies showed that protozoa count unchanged (20) or even increased (35) in the presence of saponins. Newbold et al. (24) reported that

some rumen bacteria can hydrolyse saponins to their free glycoside fractions, and, thus, saponins lose their toxic effects on protozoa. Ivy fruit saponins have also reported to reduce methane emissions through direct inhibition on methanogen population rather than elimination of rumen protozoa (4). For instance, Patra (29) reported that the reduction of methane production could be related to direct effect on archaea activity and/or indirect effect on protozoa abundance. However, in long-term studies saponins activity seems to be inconsistent and even decreasing (18) probably due to microbial adaptation (29). Furthermore, saponins can increase propionate concentration at the expense of acetate and butyrate (18). Effects of saponins on feed digestibility, on the other hand, were closely related to inclusion levels of saponins. Accordingly, Jayanegara et al. (17) reported that saponins decrease methane emissions at both low and high levels. However, low levels of saponins increased nutrient digestibility while high levels decreased this parameter. On the other hand, effects of saponins are more pronounced when they are directly added to the rumen rather than mixed with the diet (25). The reason of reduced efficiency of saponins when mixed with the diet might be that saponins are degraded or 'inactivated' by some still unidentified salivary components (25). This situation can be a restrictive factor for their potential use as feed additives.

Essential oils

Essential oils are volatile essences which can be derived from leaves, flowers, barks, seeds and roots of various plant species with steam distillation or solvent extraction methods. They were divided into two groups according to their chemical structures as terpenoids and phenylpropanoids (8).

Essential oils can exist at different amounts in different parts of the plants and, defense the plant with the antimicrobial activity of bioactive substances such as carvacrol, thymol, eugenol, anethole, geraniol, capsaicin, limonene etc. Essential oils can penetrate and diffuse to cell walls of Gram positive bacteria because of their hydrophobic and lipophilic structures. Thus, they cause structural changes in the cell membrane and disrupt the ionic balance between inside and outside of the bacterial cell similar to antibiotics (16). Origanum, garlic, clove, peppermint and eucalyptus oils were reported to reduce methane production and decrease the abundance of archaea, protozoa as well as of major cellulolytic bacteria (i.e., *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *R. albus*) (31). Essential oils from rosemary, cinnamon, dill seeds (11) and vanillin (32) reduced the production of ammonia and methane. Studies have shown that the effects of essential oils on ruminal fermentation were closely associated with their received

doses. Pawar et al. (34) reported that the lowest level of garlic oil (167 µl/l) was the most appropriate level of inclusion while higher doses (333, 500, 667 and 833 µl/l) were detrimental for feed digestibility and fermentation. On the other hand, cinnamon bark oil adversely affected the ruminal fermentation by decreasing the production of total VFA even at the lowest concentration (167 µl/l). This is attributed to strong antimicrobial activity of essential oil on both Gram-positive and -negative bacteria. Thus, they inhibited rumen microbial fermentation in general. Also, the effects of essential oils on ruminal fermentation can vary depending on the chemical nature of essential oils which changes their antimicrobial spectrum. Essential oils were reported to have antimicrobial activity mostly on Gram positive bacteria. At the same time, some small molecules of essential oils can diffuse to membrane of Gram negative bacteria (18). Therefore, toxic effects of some essential oils to rumen microbes would preclude their potential use in ruminant diets. Investigation of the minimal inhibitor concentrations (MIC) of active compounds on rumen microorganisms can help to determine antimicrobial spectrum and suitable doses of essential oils.

According to Cobellis et al. (12), the use of essential oils as feed additives in ruminants seems to be difficult and limited for several reasons. Variable compositions and purity levels of even the same essential oil depend on plant species, growth environment of the plants such as; soil composition, temperature and moisture, growth stage of plant, parts of the plants used and extraction method restrict the standardization of essential oils. High volatility of essential oils that can change their chemical stability and antimicrobial activity is the other disadvantage of essential oils. Essential oils can also negatively affect taste and smell of feed hence, they can restrict consumption of feed by ruminant.

Conclusions

Plant extracts and secondary plant metabolites have potential to modify ruminal fermentation and improve animal productivity. Effects of plant secondary metabolites on ruminal fermentation are favorable if they increase or do not change VFA production (or with a desirable change in molar proportions of VFA) and feed digestibility while they decrease ammonia concentration and methane production. However, these products use varied, complex and usually more than one mechanism unlike antibiotics which act against to certain targets. The effects of plant extracts and secondary metabolites on rumen fermentation and feed digestibility on the other hand, vary greatly according to their dose, antimicrobial spectrum and the amount of active metabolites of the plant. Defining of minimal inhibitor concentrations of single active compounds individually on pure cultures of

rumen bacteria will be useful to determine antimicrobial spectrum and effective dose. After then, investigating the effects of chosen compounds in continuous culture systems would be beneficial to determine persistency of the effects in mixed rumen bacterial populations and over time. Molecular profiling to detect microbial changes would provide more apparent and tangible information to the literature. Verification of results with *in vivo* trials is also essential to define the true value of plant metabolites for altering rumen fermentation.

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