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Tannins and flavonoids as feed additives in the diet of ruminants to improve performance and quality of the derived products. A review

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Abstract

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Meat consumption is one of the major drivers contributing to the growth of the global animal nutrition market. Subsequently, the demand on quality of animal feed increases. Because of growing public concern about the use of pharmaceuticals in livestock production, the application of natural agents, such as tannins and flavonoids, with a potential to improve animal health and animal food product has become more desirable. The present review summarize and analyse plant derived bioactive compounds as a feed additive in the diet of cows, sheep and goats, mainly emphasizing recently published data. Particular attention has been paid to tannins and flavonoids. We concluded that animal performance and quality of the derived products can be improved by using feed additives, such as tannins and flavonoids. All the same, high doses of some compounds might reduce voluntary dry matter intake and digestion of nutrients and cause toxicity. Thus, more research is needed to fully exploit benefits of incorporating tannins and flavonoids in livestock feed and identify an optimal dose to obtain consistent beneficial effects of additives.

Keywords: animal feed; bioactive compounds; feed additives; food quality; productivity

Introduction

The livestock productions systems face multiple challenges to address the food needs of the growing population, while minimizing costs and the negative environmental impact (Thornton, 2010). Consumers desire high quality animal products, which are produced without adverse effects on animal health and welfare. Thus, more efficient and sustainable production methods in the agriculture sector are needed. Animal health and welfare are one of the critical factors in livestock production. Previously, the therapeutic and prophylactic use of antibiotics was a common strategy to reduce morbidity and mortality of animals. All the same, due to the risk of antibiotic transmission into meat and milk, and development of antibiotic resistance, the use of antibiotics for growth promotion dramatically decreased. The ban of antibiotics as feed additives in many countries has forced animal nutritionists to find alternatives to antibiotics. Therefore, the use of plant bioactive compounds, which are well accepted by consumers, has received considerable attention in the last decade. Various plant extracts and individual bioactive compounds were tested for their potential to improve animal health in general and ruminants in particular. Many plants have a wide spectrum of activities including antimicrobial and antiparasitic and might improve dry matter (DM) digestibility and reduce methane emission (Bampidis et al., 2006; Huang et al., 2018; Thornton, 2010).

Plant bioactive compounds can also be used to increase quality of meat and milk of ruminants. Due to the biohydrogenation of unsaturated fatty acids by ruminal microorganisms, ruminant fats are more saturated than non-ruminants (Jenkins et al., 2008). Dietary polyphenols might partly prevent polyunsaturated fatty acids (PUFA) from biohydrogenation in the rumen through inhibition of growth and metabolism of ruminal bacteria and, thereby, enhancing tissue deposition of PUFA and their biohydrogenation intermediates (Vahmani et al., 2020). To produce high milk yields, bioactive compounds in feed might also be important (Niderkorn & Jayanegara, 2021).

Ruminants play a valuable role in sustainable agricultural systems. Ruminants are efficient in using fibrous feeds that cannot be consumed by humans, and highly contribute to food security, especially in developing countries with growing populations (Oltjen & Beckett, 1996). Ruminants including cows and sheep are the most numerous livestock in Kazakhstan (Morgan et al., 2006). Kazakhstan's beef and sheep production reduced rapidly, following the dissolution of the Soviet Union and collapse of state-owned farms, but currently are in a gradual recovery process, although many issues remain to be solved (Tazhibaev et al., 2014).

Animal studies have been mainly focused on the effects of bioactive compounds on growth, carcass traits, microbial activity and methane production in ruminants. Flavonoids were also tested for their ability to increase drug efficiency in ruminants (Dupuy et al., 2003). Less is known about their effects on meat and milk quality. This review analyses the recent development in applications of dietary supplement with plant bioactive compounds on meat and milk quality of cattle. Particularly, we aimed to investigate potential contributions of tannins and flavonoids.

Dietary Tannins

Tannins are polyphenolic secondary plant metabolites. Tannins provide a means of defence against bacteria, fungi, viruses, environmental stress and attack by herbivores. Tannins are often considered as antinutrients, because they are able to bind to proteins, starch and digestive enzymes in the rumen and reduce the nutritive value of feeds. Moreover, tannins are astringent, which makes them less palatable.

The effect of tannins is dose dependent, and might have either beneficial or adverse effects on ruminants. High tannin concentrations reduce intake and digestibility of nutrients, while low tannin concentrations can alter rumen fermentation towards maximising microbial protein production (Frutos et al., 2020; Makkar, 2003). Type of tannins (condensed or hydrolysable) and the composition of whole diet determine the final effect. Condensed tannins, are composed by several derivatives of flavonoids, and hydrolysable tannins are composed of a core of glucose, linked to gallic acid (gallotannins), or to hexahydroxydiphenic acid (ellagitannins).

Condensed Tannins

Condensed tannins possess several biological activities, including antimicrobial (Pizzi, 2021) and anthelmintic activity (Athanasiadou et al., 2001; Hoste et al., 2006). Nevertheless, most of the beneficial effects of the consumption of condensed tannins by ruminants are thought to be related to their protein binding ability (Zeller et al., 2020). The complexes, formed with proteins reduce their accessibility to rumen degradation and thus reduce ammonia concentration in the rumen. Condensed tannins can also reduce enteric methane emission in ruminants (Patra, 2012). The impacts of tannins on enteric methane emission, rumen fermentation, and animal growth performance were recently intensively reviewed (Aboagye & Beauchemin, 2019; Min et al., 2020) and is out the scope of the present review.

The effect of condensed tannins on milk yield ranged from reduction to no effect. Some studies showed no effect of supplementing condensed tannin on either affect total milk yield, or energy-corrected milk yield (Dschaak et al., 2011; Gerlach et al., 2018). Administration of the condensed tannins (400 g/day of tannin extract from Acacia mearnsii) directly to the rumen of Holstein-Friesian cows did not affect milk yield cows (Williams et al., 2020). Neither milk fat nor protein were affected by tannins. Interestingly, Williams et al. (2020) tested a combination of cottonseed oil and tannin supplements. In this study, milk yield from cows, administrated with cottonseed oil and tannins were greater than those administrated with tannins. Grainger et al. (2009) reported a reduction in milk yield, fat and protein and a reduction in milk fat concentrations, when dairy cows were fed with 163 and 244 g/d tannins, extracted from the bark of the Black Wattle tree (*Acacia mearnsii*). Similarly to cows, dietary tannins did not affect goat milk production and composition (Nascimento et al., 2021).

Fatty acid composition in ruminant-derived foods (meat and milk) has a low content of polyunsaturated fatty acid (PUFA) and a high content of saturated fatty acids (SFA). It also contains trans fatty acids.

(Frutos et al., 2020), numerous research groups, have examined a possibility to lower the relative proportions of saturated fatty acids (SFA), and enrich n-3 PUFA relative to n-6 PUFA in intramuscular lipid. Nevertheless, the results of the attempts to improve the content of n-3 PUFA in meat and milk from ruminants were inconsistent. Supplementing peanut skin, rich in condensed tannins, modified the fatty acid profile of meat from goats (Kafle et al., 2021) and sheep (Vasta et al., 2009). Kafle et al. (2021), also concluded that condensed tannins from peanut skin positively affected quality of goat meat. Consumption of Acacia cyanophylla leaves, rich in condensed tannins, resulted in an increase of γ-linolenic acid and docosapentaenoic acid and reduction of oleic acid, but did not affect n-3 fatty acids in ewes milk (Maamouri et al., 2019). The exact mechanism, by which condensed tannins modify lipid composition is not yet well understood, but it was repeatedly suggested that dietary tannins alter the rumen microbial community and ruminal lipid metabolism (Frutos et al., 2020; Vasta et al., 2019).

Inclusion of condensed tannins to cassava silage (25 g/kg total dry matter) increased goats' use of the diet, without compromising feed conversion and milk quality (Nascimento et al., 2021). Generally, condensed tannins are found in many plants consumed by goats. It was suggested that moderate levels of condensed tannins can be tolerated by goats due to the presence of tannin-binding proteins in their saliva (Schmitt, Ward, & Shrader, 2020).

Another important property of tannins is antioxidative action. Oxidative stability is an important technological attribute of meat. Severe oxidative damage of lipids and proteins might negatively affect sensorial and nutritional meat quality, such as the generation of off-flavour, discoloration, decrease in tenderness and safety concerns (Wu et al., 2022). To improve oxidative stability, dietary supplementations with antioxidants has been used for farm animals. Traditionally, α-tocopherol was supplemented via feed. Nevertheless, dietary supply of flavonoids and tannins can also be used for the same purposes (Hässig et al., 1999). Thus, condensed tannins from Quebracho Colorado wood (Schinopsis balansae and Schinopsis lorentzii) have been shown to have a powerful scavenging activity against free radicals (Park et al., 2011). It should be all the same emphasised, that even though tannins act as antioxidants *in vitro*, they might have a pro-oxidant effect in muscle tissue.

Positive effects of condensed tannins are often explained by its effects on protein digestion and metabolism, through the formation tannin-protein complexes, which escape ruminal degradation and increase protein intestinal availability. Another explanations can be accelerated microbial protein synthesis and/or enhanced use of endogenous nitrogen in the rumen (Reed, 1995).

Attempts were also made to improve sensory quality of lamb meat by dietary tannins. Lamb meat can have a distinctive odour that causes lower consumer acceptance. Dietary supplementation with condensed tannin-rich sainfoin pellet to lambs reduced skatole and indole concentrations in fatty tissues (Priolo et al., 2009; Rivaroli et al., 2019). Inclusion of mimosa (Acacia mearnsii) extract, rich in condensed tannins caused reduction in p-cresol and 8-methylnonanoic acid levels (Del Bianco et al., 2021). Nevertheless, other off-odour related compounds were not affected by the presence of tannins in the diet. Thus, it is questionable of tannins can improve sensory quality of lamb meat.

Hydrolysable Tannins

Hydrolysable tannins are considered as potentially toxic to animals (Murdiati et al., 1991), but ruminants can adjust to dietary tannins. A recent meta-analysis of 47 studies describing in total 58 experiments on lactating dairy cows showed no effect of hydrolysable tannins on milk yield (Herremans et al., 2020). Similarly, addition of oak (*Quercus persica*) acorn with high levels of hydrolysable tannins did not affect goat milk yield and composition (Alipanahi et al., 2019). In contrast, the inclusion of hydrolyzable tannins, extracted from gallnut in lactating goat diets, decreased the percentage of C10:0 and C12:0, as well as total saturated fatty acid in goat milk (Abo-Donia et al., 2017).

Dietary inclusion (4%) of the commercially available tannin extracts from chestnut (*Castanea sativa*) and tara (*Caesalpinia spinosa*), rich in hydrolysable ellagitannis and gallotannins, respectively, did not affect the sensory properties of lamb meat as evaluated by sensory panel, but dramatically reduced the levels of p-cresol and tended to reduce aldehydes (Del Bianco et al., 2021). Both aldehydes and p-cresol contribute to off-odour in lamb meat and causes consumer dissatisfaction. Thus, their reduction in meat is desirable. Hydrolysable tannins from chestnut (80 g/kg) also reduced the concentration of several branched chain fatty acids in milk from ewes (Buccioni et al., 2015).

Reasons for Inconsistent Results among the Studies with Dietary Tannins

The discrepancies of the results on the effect of dietary tannins can be due to the different chemical structures of tannins. Additionally, the responses of tannins depends on the tannin source and the dose of dietary tannins, and the rest of dietary composition. Tannins are known to interact with other dietary ingredients, and form tannin–carbohydrate and tannin-protein complexes. Interestingly, the same dose of tannins of different origins can differently influence nutrient availability (Bueno et al., 2008). The inconsistent responses among different studies might also be related to animal raising conditions. It was also suggested that storage time of tannin-containing silages might affect the strength of tannin-protein complex, but this needs to be confirmed (Broderick et al., 2017).

More research is needed to fully exploit benefits of incorporating tannins in livestock feed and identify an optimal dose to obtain consistent beneficial effects of dietary tannins.

Dietary Flavonoids

Other feed components of ruminant's diet, which might improve animal health and welfare, as well as milk and meat production, are dietary flavonoids. Flavonoid (from Latin flavus, "yellow"), is the generic term used to identify a series of secondary plant metabolites, that are synthesized from a phenylalanine molecule and 3 of malonyl-CoA, through a pathway called the "flavonoid biosynthetic pathway". The health effect of flavonoids was discovered in 1938, by the Hungarian Nobel Prize winner Albert Szent-Györgyi, when he realized some unknown plant substances had similar effect as vitamin C in counteracting bleeding due to scurvy, a disease caused resulting from serve deficiency of this vitamin. Flavonoids are present in fruits, berries, vegetables, grain, tea, roots, bark, stem, and wine and are the largest and most abundant polyphenol subclass in the plant world. They are distributed in vascular plants in a ubiquitous way and they are recognized to exhibit a number of biological activities, such as antioxidant, anti-inflammatory, anti-haemorrhagic, vasodilators, antineoplastic, antiviral, antibacterial, ant allergic and hepatoprotective (Lee et al., 2009; Panche et al., 2016). The effects of dietary flavonoids on human health have been intensively studied, whereas studies on animals, particularly ruminants, are still limited.

Flavonoid-rich Plants

Dietary supply of flavonoids was tested for antioxidative properties (Hässig et al., 1999). Fatty acid profile, lipid peroxidation and oxidative stability in meat from lambs, infected with the gastrointestinal nematode parasite was studied with, or without dietary supplementation of two herbal mixtures (Szulc et al., 2020). In this study, the phytochemical composition of two herbal mixtures differed in the concentrations of phenolic acids (57.3 vs 22.2 g/kg dry matter (DM) of phenolic acids in the mixtures 1 and 2, respectively) and in flavonoid concentrations (41.5 vs 29.5 g/kg DM of flavonoids in the mixtures 1 and 2, respectively). Supplementation of herbal mixtures to the diets of infected-lambs decreased medium chain fatty acids and increased long chain fatty acids in meat (Szulc et al., 2020). Lipid oxidation in meat was also reduced in the meat from lambs fed herbal mixtures, probably due to the inhibitory effects of phenolic compounds on the ruminal biohydrogenation. Another study suggested that dietary supplement with camelina forage, which is rich in different phenolic compounds, reduce lipid oxidation of Semimembranosus and Vastus lateralis from sheep (Ha et al., 2022).

Hop (*Humulus lupulus* L.), a rich source of phenolic compounds and alpha and beta acids, was also tested as a dietary supplement in ruminant diet to improve animal performance and meat quality. Blanko et al. (2018) demonstrated, that meat from the lamb fed hop, were more tender, but not other important differences in meat and milk characteristics were reported. Hops (952 mg/kg DM) in a finishing diet improved ruminal fermentation in British × Charolais steers, but no effect on the growth or efficiency was observed (Wang et al., 2010). Unfortunately, neither meat no milk were investigated in that study. Hop extracts had the positive effect of inhibiting methane production in the rumen of cattle, an effect, which contributes to reduction of greenhouse gas emissions (Blaxland et al., 2021). This reduction is likely due to the presence of beta acid in the hop extracts.

Conflicting results between different studies can be due to variability of plant source and antioxidative capacity of different bioactive compounds. It might also be due to the presence of additional fermentable compounds in the diet, such as condensed tannins. Moreover, different post-slaughter conditions, such as aging and storage time of meat might contribute to the differences in the results.

Flavonoid-rich Plant Extracts

Flavonoid extracts were also tested as a feed supplement to improve animal performance and meat and milk quality. Sheep fed a diet supplemented with flavonoids extracted from Mongolia leek (*Allium mongolicum Regel*) at the concentrations of 11 to 33 mg/kg increased the daily weight gain and average daily feed intake, as well as decreased the feed conversion ratio (Muqier et al., 2017). This indicated that supplement of flavonoids extracted from Mongolia leek had potential to increase meat production of sheep, although the optimal dose and period of feeding should be determined.

Bitter orange extract of the whole fruit (*Citrus aurantium* ssp.) is currently authorised as a feed additive by EFSA (EFSA FEEDAP Panel, 2021). The EFSA Panel on Additives and Products, or Substances used in Animal Feed (FEEDAP) concluded, that this additive is safe for ruminants up to the maximum proposed use level of 400 mg/kg for veal calf, sheep, goat, and calculated maximum safe concentration in complete feed is 268 mg/kg for sow and 259 mg/kg for dairy cow (EFSA FEEDAP Panel, 2021).

Polyphenol extract from olive mill wastewaters in the diet of goat kids improved fatty acid profile (Cimmino et al., 2018). Specifically, lower proportion of saturated and higher proportion of monounsaturated fatty acids in meat were recorded. Malondialdehyde contents were also reduced in the animals fed polyphenol extract (Cimmino et al., 2018), which indicate improved oxidative stability.

Efficiency of the extract from balsam poplar (*Populus balsamifera* L.), buds was tested on fattening young bulls of Simmental breed (Balji & Knicky, 2021). In this study, bulls fed the extract (10% for 3 month) had significantly higher average daily gain and live weight at slaughter, compared to the control animals. All the same, carcass characteristics and meat parameters were not affected by balsam poplar bud extract (Balji & Knicky, 2021). These results indicate that balsam poplar-based additives can be used to improve animal growth and performance, without compromising animal welfare and meat quality.

Oregano and green tea extracts fed to Jersey cows in the last 21 days of the dry period (pre-calving) and in the first 21 days of lactation (post-calving) to evaluate a potential to improve milk productivity, milk composition and blood metabolites (Bosco Stivanin et al., 2019). Dietary supplement with oregano, but not green tea extract increased dry matter intake in the post-calving period and resulted in numerically higher corrected for energy milk production. Moreover, supplementation with oregano (10 g per cow per day) and green tea (5 g per cow per day) extracts reduced the levels of some oxidative stress biomarkers (Vizzotto et al., 2021). All the same, supplementation with green tea extract in the same study impaired feed efficiency. Nevertheless, supplementation of green tea extract might have positive health effects, because it improved intestinal microflora balance and inhibited the growth of pathogenic bacteria in calves (Ishihara et al., 2001).

Interestingly, combination of oregano and green tea extracts (0.056% of oregano extract and 0.028% of green tea extract) tended to reduce feed intake and ruminating time in lactating dairy cows (Kolling et al., 2022). Thus, oregano extract alone might enhance health of cows during the transition period and growing bulls, an effect, which should be further investigated. It should also be emphasised that enhancement, or reduction in feed intake and other parameters are highly dependent on the dose of plant extracts.

Flavonoid extract was also suggested to modify the behaviour of ruminants. Thus, supplementation with 0.04% of bitter orange extract (*Citrus aurantium*) of the whole fruit rich in naringin, reduced aggressive behaviour in bulls (Paniagua et al., 2021). Aggressive behaviour including fighting, butting, chasing and sexual behaviour induce stress and reduce some meat quality parameters. Use of dietary flavonoids to reduce aggression would be an attractive strategy to improve animal welfare and meat quality.

Individual Flavonoids

Among flavonoids, quercetin and naringin has received much attention over the past decades and a variety of beneficial effects on the productivity and health of animals, as well as rumen fermentation have been identified. Quercetin, is known to be degraded by rumen microbes with formation of 3,4-dihydroxyphenylacetic acid and 4-methylcatechol (Berger et al., 2015). Despite low bioavailability, quercetin was shown to modify animal performance.

Goats fed high grain diet with abomasal supplementation of quercetin (100 mg quercetin for 4 weeks) had a higher average daily feed intake and higher body weight, compared to goats fed without quercetin supplementation (Guo et al., 2018). The authors suggested that the improved average daily feed intake might be due to quercetin stimulation of appetite, which in turn lead to the increased body weight. The intraduodenal administration of approximately 21 g of quercetin equivalents per day increased milk protein concentration, but total milk yield was not changed (Gohlke et al., 2013a). Similarly, an acute dose of quercetin did not either change milk yield (Gohlke et al., 2013b). Interestingly, intraduodenal supplementation of quercetin during 4 weeks (36 mg of quercetin equivalents per kg of body weight) decreased the plasma concentration of glucose in cows (Gohlke et al., 2013a).

Because unsaturated fatty acids are highly susceptible to peroxidation, individual flavonoids with antioxidant properties were used to improve meat and milk quality, shelf-life and microbial safety. Dietary naringin ((1.5–3 g/kg dry matter for 3 weeks) decreased plasma TBARS in fattening lambs fed fish oil supplements, indicating suppressed lipid peroxidation (Bodas et al., 2011). Interestingly, this suppression was observed only in the unstressed animals, whereas the effect was lost following transportation stress. Nevertheless, naringin (7 or 14 g/week/animal) decreased the negative effects of thermal stress on antioxidant statue and immune function in lambs (Alhidary & Abdelrahman, 2016). In this study, naringin fed lambs had higher body weight and increased feed efficiency. Compared to quercetin, bioavailability of naringin is high in ruminants, because of bacterial deglycosylation of naringin in the rumen and the absorption of the metabolite naringenin in the small intestine.

Supplementation with hesperidin (2.5 g) also improved lamb meat antioxidant capacity, as indicated by reduction in malondialdehyde levels (Simitzis et al., 2019). To the best of our knowledge, the effect of dietary hesperidin on beef quality traits has never been studied.

Conclusion

In animal production, nutrition is one of the most important factors, which determine animal performance, as well as meat and milk quality. Dietary supplementation of ruminants with bioactive phenolic compounds, such as tannins and flavonoids might be an attractive alternative to improve growth performance, carcass traits and derived product quality. Tannins and flavonoids might beneficially affect meat antioxidant capacity and prolong meat shelf-life. The shift in fatty acid composition of meat and milk toward more PUFA might also be achieved with dietary bioactive compounds.

Even though the results on the effects of bioactive compounds in ruminant nutrition are currently conflicting, it opens up attractive possibilities to identify compounds and optimal doses to improve quality of animal-derived products without compromising animal health and welfare.

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Conflicts of Interest

Authors declare no Conflict of Interests for this article.

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