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Review

Additive Tannins in Ruminant Nutrition: An Alternative to Achieve Sustainability in Animal Production

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Abstract: Sustainable intensification involves maintaining ecosystem balance and increasing productivity per animal per unit area. Phytogenic additives can be used as an alternative to achieve sustainable intensification. Tannins are phenolic compounds present in plants that are classified according to their chemical structure into hydrolyzable and condensed compounds. When added to ruminant diets, condensed tannins exert effects on rumen fermentation, such as a reduction in rumen protein degradation and enteric methane production per unit of dry matter ingested, and may also improve weight gain. The advantage of this mechanism is that it increases dietary protein utilization, reduces nitrogen excretion in urine, and reduces nitrous oxide emissions. However, the positive effects of these compounds as nutritional additives require further investigation. Therefore, the objective of this review is to demonstrate the results hitherto known of the use of condensed tannins in ruminant nutrition. The use of tannins can result in both positive and negative effects, depending on the sources and doses administered.

Keywords: greenhouse gases; livestock; mitigation; nitrogen; rumen fermentation

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1. Introduction

In recent decades, the production of animal-based foods has become a focus of global debate, mainly for environmental and economic reasons. The search for greater productivity and efficiency in smaller spaces and deadlines has encouraged research on improving animal production capacity. Sustainable intensification is an important tool that aims to increase the productivity of the beef chain, in addition to contributing to the gradual and proportional reduction of environmental impacts, maintaining the balance of the ecosystem using management practices that respect the limits of soil and forage, and maintaining forage production with a high nutritional value [1].

Among the techniques used to increase the efficiency and productivity of the system, the management of pastures, nitrogen fertilization, and use of animals with genetic potential, in addition to nutritional management practices such as the use of food additives as a strategy to minimize undesirable nutritional and environmental impacts of the activity, as well as boost beneficial effects for the animal, still have the potential to change the ruminal environment, improve nutritional efficiency, and reduce productive losses [2].

Among the categories of existing additives, phytogenic additives are highlighted, and they include secondary plant compounds such as essential oils and tannins [3]. Tannins have long been known for their anti-nutritional effects; that is, they compromise food intake and digestibility [4]. However, these effects are associated with the ingestion of high concentrations of these compounds because of their astringency, which reduces acceptability, in addition to the formation of complexes with food compounds that can impair the digestibility of food [5]. In addition, the inclusion of tannins in cattle diets at adequate doses has the potential for sustainable intensification [6,7].

The present revision aims to critically analyze previous studies that evaluated the use of tannins as additives in ruminant nutrition and to discuss the role of tannins in the possible mitigation of greenhouse gases (GHG) in animal agriculture.

2. Inclusion of Tannins in Ruminant Feeding to Increase Sustainability

2.1. What Are Tannins?

Tannins are water-soluble phenolic polymers with the potential to form complexes with proteins and polysaccharides such as starch, cellulose, hemicellulose, and pectin, owing to the presence of hydroxyl phenolic groups [8]. They are a diverse group of compounds, usually defined as polyphenolic substances of high molecular weight, and are secondary plant compounds found in different organs and tissues of plants, such as cell walls or sheltered within vacuoles in stems, bark, leaves, flowers, and seeds [9].

The tannins were divided into two groups. Hydrolyzable tannins (HT) are easily fractionated by treatment with hot water, acids, or enzymes, which promote the release of their sugars and phenolic carboxylic acids. Non-hydrolyzable tannin compounds are called condensed tannins (CT) [8]. The chemical structures of the tannins are shown in Figure 1.

CT is comprised of polymers of flavonoids (catechin and gallocatechin), the monomeric forms of which are anthocyanidins (cyanides and delphinidins). HT is composed of esterified gallic or ellagic acid polymers (galantamines and ellagitannins) attached to a central molecule, usually a sugar or polyphenol [7]. Hydrolyzable tannins have lower molecular weight than condensed tannins and have as the central nucleus of the structure a molecule with multiple hydroxyl groups, such as glucose, glucitol, and quinin acid, partially or totally bound by ester binding to a phenolic compound, such as the caric acid, forming the galantamines and ellagitannins. Due to this structure, HT are prone to hydrolysis by acids, bases, or esterases [10].

Both HT and CT can precipitate with proteins and polysaccharides, owing to the presence of many phenolic compounds and hydroxyl groups [11]. The different structures between the two determine the potential activity of these compounds because hydrolyzable tannins are rapidly degraded into smaller phenolic groups [10].

Not all plant species produce CT, and among those that synthesize it, its concentration and chemical characteristics are highly variable. Most condensed tannin-producing plants show antinutritional effects in animals that consume them, such as decreased acceptability of the diet, lower intake of dietary CT concentrations greater than 5% in dry matter (DM), reduced nutrient digestibility (proteins, carbohydrates, and fats), and lower food efficiency [4].

Two groups of tannins can be found in the same plant and at different concentrations according to its part, but some of them may predominantly be hydrolyzable tannins, while others, condensed tannins, generate a wide variety of chemical structures [12].

Manipulation of the ruminal environment has been the target of nutritionists to improve the utilization of nutrients and reduce energy loss. One alternative for the regulation of ruminal fermentation and metabolism that has been studied is the use of tannins as food sources. These phenolic compounds have several advantages when used in ruminant feed at correct dosages [6]. Research has shown that tannins can decrease the production of enteric CH_4 and protein degradation in the rumen, increase protein flow to the duodenum, and increase microbial protein synthesis by increasing the utilization efficiency of ruminal N, resulting in lower N excretion in urine [8,13].

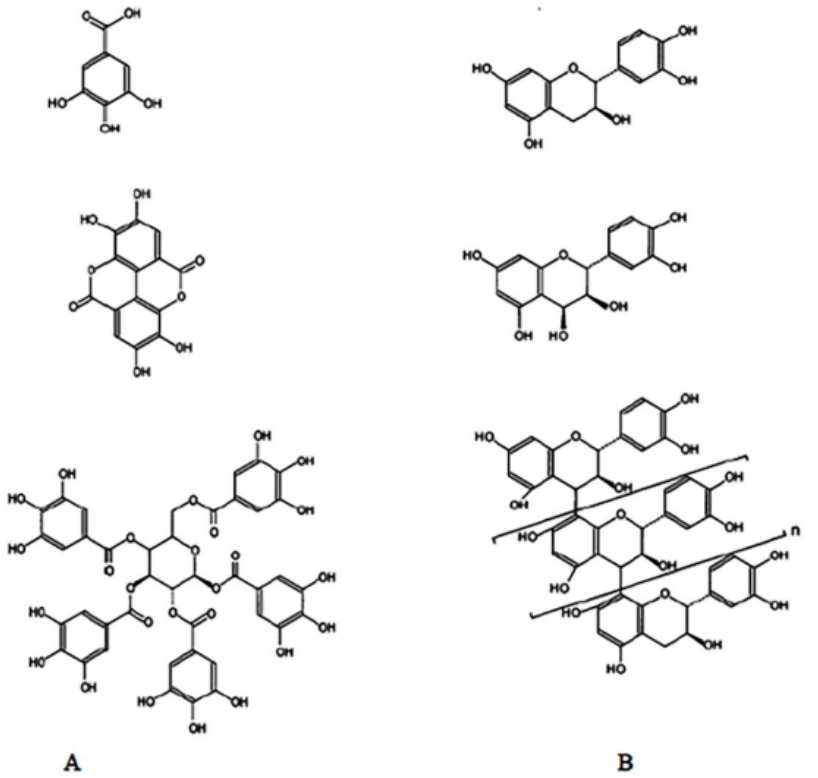

Figure 1. Included components of tannins: (**A**) hydrolyzates; and (**B**) condensates (adapted from McMahon et al. [14]).

2.2. Effects of Tannins onn Ruminants

The biological effects of tannins are influenced by the dose used, the composition of the diet, animal species, the physiological "status" of the animal, and the chemical structure of tannins, and these are determining factors for the different types of tannins to have the potential to provide both beneficial effects or adverse effects on ruminal metabolism, and consequently, animal performance [13].

Condensed tannins have antifungal and antibacterial activities and form complexes with insoluble proteins in water, causing an increase in microbial protein synthesis by reducing ruminal protein digestion due to the formation of protein–tannin complexes (protein–tannin), reducing ruminal N recycling, and inhibiting the methanogenic population due to the reduction in hydrogen production (H_2) [15].

Hydrolyzable tannins can also interact with proteins to form hydrogen bonds between the phenolic groups of tannins and the carboxyl groups of the protein chain. The strength of this binding determines the response of tannins to protein digestibility [16]. However, when the depolymerization of hydrolyzable tannins occurs in monomeric subunits of low molecular weight in the rumen, the affinity of these subunits for the protein is weaker [17], resulting in lower adsorption compared to the affinity verified by condensed tannins. Some ruminal bacteria can dissociate protein-HT complexes, indicating that these complexes are partially reversible, whereas the dissociation of protein-CT complexes is more difficult [16].

Tannins may be toxic to ruminants, as the ester bonds between proteins and phenolic compounds can be broken by ruminal microorganisms using the enzymes tanilacylhydrolases and esterases [10]. Thus, hydrolyzable tannins can be easily absorbed into the digestive tract, which provides greater potential to cause toxicity in animals, especially when high concentrations are ingested, as they can cause the release of metabolites in the rumen and, consequently, cellular damage [10]. However, when consumed at low to moderate concentrations, they can provide beneficial effects, although it was initially believed that HT would have a greater potential to cause toxicity in animals, and these toxic

effects can be avoided with gradual adaptation and supply of low concentrations (<50 g of tannin kg-1 DM 5% inclusion in DM) [18]. In a study conducted by Rivera et al. [19], supplemental tannins increased growth performance and dietary energy utilization, but differences in feedlot cattle growth performance and dietary energetics responses due to the tannin source (condensed, 5.0 g of tannin kg^{-1} DM vs. hydrolyzable 5.4 g of tannin kg^{-1} DM) were small or non-appreciable.

Tannin–protein complexes are formed through hydrogen bonds, which are stable and insoluble at pH 3.5 to 7.0, i.e., they can be formed in the ruminal environment where the pH is around 6 to 7 and dissociated in the abomasum where the pH is less than 3.5, or in the duodenum where pH values are observed around 8 during protein release [20]. Consequently, their use in ruminant diets reduces protein degradation in the rumen and increases the amount of digested protein in the small intestine [7].

Tannins form precipitates with nitrogen compounds other than proteins and peptides, such as the amino acid arginine, nitrogen bases, polyamines, chills, and chitosan. Thus, they can react with non-protein organic nitrogen compounds in a manner similar to their reaction with proteins [12]. The formation of complexes with fibers occurs at a lower intensity, unlike complexation with proteins; therefore, the impairment in digestibility is slightly affected [16].

Thus, when complexes between tannin proteins or tannin polymers (starch, cellulose, hemicellulose, and pectin) are not broken, they pass intact through the digestive tract and are excreted in the feces [21]. This can occur when tannins are administered at high doses, negatively influencing food digestion because they affect the degradation of fibrous and protein fractions of the food. The antinutritional effects of tannins are also due to their complexation with endogenous proteins and secreted enzymes [5]. Supplementation levels of tannins beyond 3.6 g of g of tannin kg^{-1} DM can negatively affect the dietary net energy utilization, decreasing feed efficiency in lambs that were fed with a high-energy corn-based diet [22].

Condensed tannins can reduce food consumption and nutrient utilization. This reduction in consumption can be attributed to the decrease in acceptance associated with increased astringency [23]. A reduction in food consumption was observed with the inclusion of more than 30 g of CT kg^{-1} DM in the total diet of Jersey steers [23]. Kahiya et al. [24] reported that values above 50 g of CT kg^{-1} DM in the total diet caused decreases in consumption and weight gain, consequently limiting the use of food in goats.

Dschaak et al. [25] reported a reduction in DM intake, but no change in digestibility, when cows were fed 30 g of CT kg^{-1} DM in the diet (Quebracho extract). The reduction effects on DM intake were verified with the supply of condensed tannins for sheep, contrary to what was verified with the supply of hydrolyzable tannins supplied at the same dose (100 g of HT kg^{-1} DM of the total diet), confirming the greater inhibitory effect on CT consumption than that of TH, which was partially associated with the lower acceptability of diets with CT extracts [17].

Paguen-Riestra et al. [26] reported a reduction in DM consumption when the CT exceed 60 g of kg^{-1} DM in steers. Martin et al. [27] reported that the appropriate dose of CT inclusion so as not to interfere with nutrient intake is 20–40 g kg^{-1} DM and can still promote beneficial effects in ruminants, such as improving the efficiency of N use.

Acceptability is often based on the astringency associated with tannin–protein complexes formed from proteins in the saliva. Thus, the higher the protein bound by the tannin–protein complex, the greater the astringency and acceptability. However, not all tannins bind to proteins equally [4]. Thus, food intake is related to acceptability. Consumption can be decreased at concentrations below 50 g kg^{-1} of DM when tannins are more effective in binding with salivary proteins. Concentrations of tannins greater than 50 g kg^{-1} of DM may not reduce consumption, which occurs when the tannin–protein complex is less effective [4].

Kardel et al. [28] evaluated the inclusion of condensed tannins at 0, 10, 20, 40, and 60 g kg^{-1} DM in steers and reported reduced protein digestibility at all inclusion levels. CT

can reduce the degradability of proteins in the rumen by binding to proteins in the diet. Tannin complexes can also be formed with microbial proteins, mucosa, and endogenous proteins [5].

The observed decreases in nutrient digestibility with the use of tannins were inconsistent. For example, Ahnert et al. [29] observed a reduction in nutrient digestibility with Quebracho extract including CT at 40 and 60 g kg^{-1} DM in the diet in heifers, but when supplemented with 10 and 20 g kg^{-1} DM, the effect was not detected. Tannin concentrations ranging from 60 to 90 g kg^{-1} DM had no negative effects on the digestibility of crude protein or DM [30]. However, Jayanegara et al. [31] observed a decrease in organic matter digestibility when tannins from different sources with different chemical structures were added to the diet. In vivo and in situ studies such as those mentioned above provide a vision and greater understanding of the complexity of the relationship between tannins and animal nutrition.

Barry and McNabb et al. [32] reported that adding CT at 30–40 g kg^{-1} DM (*Lotus corniculatus* extract) to the total diet increased abomasal flow (53%) and absorption in the small intestine (59%) without compromising the apparent digestibility of DM. The CT of *Lotus pedunculatus*, despite having increased abomasal flow (30%), reduced the apparent digestibility of DM. This could be explained by the complexation of tannins that are not present in the intestine.

Different concentrations of tannins can cause adverse effects when bound to different enzymes. They can inhibit or modify enzymatic activity. The catalytic activity of enzymes can be increased by low concentrations of tannins, which increase the spiral structures of enzymes [12]. The possible inhibition of extracellular enzymes by condensed tannins may be one of the causes of reduced digestion [33].

In cattle, when CT at 30 g kg^{-1} DM (quebracho extract) was evaluated in a diet based on DM, food intake was affected. However, the digestibility of DM and nutrients was not affected. Furthermore, the inclusion of CT decreased the acetate: propionate ratio and improved feed efficiency of the high-forage diet. The concentrations of rumen ammoniacal nitrogen (N-NH_3) were reduced in cows without decreasing milk production, indicating that less N was lost in the rumen in the form of ammonia (NH_3), owing to the decrease in protein degradation by microorganisms [25].

Food intake by animals is one of the main factors that determine the structural composition and function of ruminal microbiota. The most active microorganisms in the ruminal environment are bacteria and *Archaeas*, which are associated with important metabolic activities such as the degradation of fiber and proteins ingested by ruminants and the production of enteric CH_4 [33].

Condensed tannins can affect growth and inhibit the activity of ruminal microorganisms by binding with membrane lipoproteins, which causes a reduction in the permeability of the membrane and can cause membrane rupture when consumed at high concentrations [5].

The proteolytic activity of gram-positive bacterial cells can be inhibited by the action of CT scans that penetrate the cell walls of these bacteria and react with the structural components of the cell, binding to the coating polymers of the growing cells. The monomeric subunits of TH can also be toxic to rumen bacteria by disturbing membrane fluidity [34].

As observed by Bae et al. [35], in which the addition of condensed tannins inhibited the growth of cells, Costa et al. [16] verified a reduction in the abundance of fibrinolytic bacteria in the rumen of sheep that received CT compared with those that received HT at the same dose (100 g hi kg^{-1} DM), demonstrating the need to choose the type of tannin to be used.

Given the divergence of results found in the literature for hydrolyzable and condensed tannins, it is remarkable that the effects of tannins are dependent on the concentrations used [5].

2.3. Tannins and the Reduction in Greenhouse Gas Emissions

The primary greenhouse gases are nitrous oxide (N_2O), methane (CH_4), and carbon dioxide (CO_2), which are naturally emitted by anthropogenic activities. Among these activities, land use and agriculture are responsible for some of Brazil's emissions [36].

In pasture systems, CO_2 is emitted through the oxidation of soil organic matter, loss of stocked carbon, and decomposition of dead plant material. CH_4 is produced during ruminal fermentation and is emitted in greater proportions by eructation and smaller proportions by animal excrement. N_2O is emitted through the excretion of feces and urine in animals [37].

CO_2 is the gas with the greatest influence on climate change, accounting for approximately half of the global emissions, followed by CH_4 (20% of the total impact) and N_2O (6%), which draws attention to its heating power. CH_4 has a global warming potential 28 times greater than that of CO_2, and N_2O has a global warming potential 265 times greater than that of CO_2 [38].

With the formulation of a diet using phytogenic additives, such as tannins, better utilization of N by ruminants can reduce N losses through excretion via urine and reduce the emission of enteric and soil methane [39].

2.3.1. Tannins and the Reduction in Greenhouse Gas Emissions: N_2O from Excreta

Dietary proteins can be divided into degradable and undegradable proteins in the rumen. Part of the degradable rumen is degraded by rumen microorganisms and is used for the growth and synthesis of microbial proteins, which can be lost in the form of urea in urine when it exceeds the required amount or can be absorbed by the rumen epithelium in the form of NH_3. Thus, this compound is removed from the bloodstream by the liver and excreted in urine in the form of urea [40].

Nitrogen baling in ruminants is determined by the difference between N intake and N excretion during animal metabolism. Thus, the N provided by the diet, endogenous metabolic N from the oxidation of amino acids, and recycled N for the rumen by blood or saliva are considered N inputs, whereas nutrient outputs include NH_3, non-degraded protein (dietary or endogenous), and microbial protein excreted via urine and feces [40].

Liu et al. [41] related N excretion in feces to undigested N in the rumen, undigested microbial proteins in the small intestine, and endogenous sources. Costa-Roura et al. [42] pointed out that the reduction of N loss through urine or feces is possible through the reduction of protein degradation and by improving the capture efficiency of degraded N in the rumen through microbial protein synthesis, which is related to the availability of energy in the rumen.

Tannins can bind to true proteins and protect them from ruminal degradation by decreasing ammoniacal N. Thus, there is an increase in metabolizable protein flow to the intestine, where digestion of this fraction of food and amino acid absorption occurs more intensely [43].

These conditions result in better utilization of protein from the diet, with maximum synthesis of microbial protein in the rumen, resulting in lower excretion of N via urine [8,15]. Thus, better N utilization by the animal allows for a decrease in the release of N in the urine, and most N is directed toward fecal production if it has not been digested and absorbed.

Fecal excretion of N is influenced by the digestibility of protein and N intake [44]. With the consumption of tannins, it can be observed that the change in the nitrogen excretion route leads to a greater loss of fecal N, with a reduction in urinary N loss and, consequently, lower ammonia emissions, which reduces the possibility of production of N_2O [44] and consequently reduces environmental impacts. The use of tannins appears to be a tool to minimize N losses in animal metabolism; therefore, the use of tannins in the diet of cattle can minimize N loss and consequently reduce the emission of N_2O and CH_4 from excretes [45].

By providing tannins to ruminants, there is an improvement in the use of N by the animal via the reduction of the ruminal degradability of the protein and in the total

digestive tract, which alters the excretion pathway from urine to feces, reducing the most volatile form of N excretion into the medium [46]. Mezzomo et al. [47], when associating supplementation with condensed tannins, reported that the total N values in urine were lower, thus improving the utilization efficiency of N.

Powell et al. [48], in studies with dairy cattle, observed that tannins could reduce urea excretion and have the ability to reduce urease activity in feces [49], indicating that N excretion can be altered and that the addition of tannins in diets can improve the utilization of N in the body of animals.

Theodoridou et al. [50] observed decreased ruminal protein digestibility and a change from urinary excretion of N to fecal excretion of N in ewes fed a diet containing tannins at 2.5–3.4% of the DM. Dietary addition of tannin decreases the urinary excretion of urea, displaces N excretion from urine to feces, and reduces the excretion of N_2O in the urine of cattle [6].

2.3.2. Tannins and the Reduction in Greenhouse Gas Emissions: Enteric Methane

Tannins can also inhibit the multiplication of methanogenic *Archaeas* in the rumen, consequently reducing the emissions CH_4 via eructation [5,7]. The production of CH_4 enteric by ruminants is a fundamental process for the proper functioning of the digestive system, but it results in the loss of gross energy ingested and, consequently, reduces animal growth and development [51].

In the rumen, the population of bacteria, especially fibroblasts, degrades carbohydrates, resulting in the production of short-chain fatty acids, such as acetate, propionate, and butyrate, used by ruminants as an energy source [52]. However, in this process of fiber degradation, hydrogen is produced, which is later used by methanogenic *Archaeas* to reduce CO_2, thus causing the formation of CH_4 that will be excreted from eructation [52]. The elimination of the CH_4 produced (eructation) represents an energy loss of 2–12% of the gross energy consumed, which could be used for animal growth or productivity [53].

There are two mechanisms for the mitigation of CH_4 enteric in ruminants through the inclusion of tannins: (1) through a reduction in fermentation and (2) by inhibiting the growth of gram-positive bacteria [43]. Thus, a reduction in the production of CH_4 can occur and may be due to tannins on ruminal microorganisms or in the digestion of fiber, reducing the production of hydrogen, which is the substrate for the methanogenic *Archaeas*, and by the inhibition of protozoa associated with methane production [54]. Furthermore, tannins exert toxic effects on the methanogenic ones [31].

In the case of proteins, complexation with tannins reduces ruminal degradability and, consequently, microbial growth, thereby decreasing the production of CH_4 [3]. The ability of tannins to bind to polysaccharides and proteins depends on the plant from which they are extracted, and their molecular weight increases with increasing molecular weight [35].

In the study by Cieslak et al. [54], a change was observed in ruminal fermentation when evaluating the effect of tannins (2 g tannins kg^{-1} DM) in dairy cows. There was a reduction in methane production (8.48%), ammonia concentration (35%), and protozoan population (21%). The total concentration of short-chain fatty acids was unaffected, although there was a reduction in the acetate–propionate ratio. Supplementation with tannins at 2 g kg^{-1} DM resulted in a 45.9% reduction in ammonia concentration [55].

Several studies have shown that tannins reduce enteric CH_4 [18,31] without negatively affecting the DM [37]. A meta-analysis of 30 in vitro and in vivo experiments showed that increasing tannin levels decreased the production of CH_4 expressed with digestible organic matter [37]. Jayanegara et al. [32] concluded that an increase in the level of tannin in the diet leads to a decrease in the emission of ruminal methane (tannins at 0–135 g kg^{-1} DM) and that this reduction occurs through the inhibition of methanogenesis.

Jayanegara et al. [31] showed that anti-methanogenic activity can be obtained both with the addition of hydrolyzable and condensed tannins to the diet and that they are capable of forming complexes with fibers, which prevents the action of gram-positive bacteria and consequently reduces the formation of acetic acid. A meta-analysis by Orzuna-

Orzuna et al. [56] reported that tannins increased the concentration of propionate in the rumen, which reduced the production of CH_4.

Ramirez and Berry et al. [57] demonstrated a reduction of up to 55% in CH_4 emissions from cattle that consumed fodder rich in tannins such as Lucerne, Sulla, red clover, Chicory, and Lotus. Fagundes et al. [58] found a decrease of up to 33% in the daily emission of methane in zebrafish cattle supplemented with condensed tannins compared to animals fed a control diet. Studies developed by Patra et al. [9] also reported a 58% reduction in methanogenesis. These variations are mainly related to the different sources and types of tannins used in the experiments [55].

Theobaldo et al. [15] concluded that the inclusion of a blend of phytogenic additives containing hydrolyzable tannins at a dose of 1.5 g kg^{-1} DM ingested in cattle under grazing conditions had no potential to reduce emissions of CH_4 enteric. The response of enteric CH_4 production related to tannin feeding is highly variable, depending on the source, type, molecular weight of the tannins, and the methanogenic community present in the animal. The use of tannins is limited, and at low concentrations (<20 g kg^{-1} DM), the responses in emissions of enteric CH_4 are highly variable. In addition, a part of the decrease in CH_4 emissions due to tannins may be caused by a concomitant decline in dry matter intake and nutrient digestibility. However, the use of tannins as a potential mitigation strategy deserves further investigation to identify the types and doses of tannins that can reduce CH_4 levels without adversely affecting animal performance [45,53].

The use of tannins from ruminant nutrition should continue to be the focus of future physiological and modeling studies [59].

3. Final Remarks

The use of tannins can result in positive and negative effects when added to ruminant diets, depending mainly on the sources and doses administered. The main advantages of using tannins as additives in ruminant nutrition include improvement in nitrogen use efficiency and, consequently, reduction in enteric methane emissions and nitrous oxide from the urine of animals.

The type of tannin used and the system in which the animals meet should be considered when choosing the strategy to be followed. A relevant point that should be highlighted in the use of phytogenic additives, such as tannins, is that this is possible through the supply of tannin-rich plant extracts or compounds synthesized from natural products.

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