

Perspectives in the use of tannins in animal production & health: a review

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Abstract

Tannins are a group of polyphenolic compounds that are widely present in plant region and possess various biological activities including antimicrobial, anti-parasitic, anti-viral, antioxidant, anti-inflammatory, immunomodulation, etc. Tannins have traditionally been regarded as “anti-nutritional factor” for monogastric animals and poultry, but recent researches have revealed some of them, when applied in appropriate manner, improved intestinal microbial ecosystem, enhanced gut health and hence increased productive performance. Therefore, tannins are the major research subject in developing natural alternative to in-feed antibiotics. Strong protein affinity is the well-recognized property of plant tannins, which has successfully been applied to ruminant nutrition to decrease protein degradation in the rumen, and thereby improve protein utilization and animal production efficiency. Incorporations of tannin-containing forage in ruminant diets to control animal pasture bloat, intestinal parasite and pathogenic bacteria load are another 3 important applications of tannins in ruminant animals. In conclusion, use of tannins in appropriate manner may help to improve animal performance and health.

Keywords: Tannins; anti-nutritional factor; performance; Animal health & production

Introduction

Animal husbandry is one of the important activities of farmers in the developing countries including India and to improve the performance of animals and poultry antibiotic have been used for several decades. However, it is widely believed that use of antibiotics as growth promoters promotes evolution and/or selection of antibiotic-resistant microorganisms in farm animals (Chattopadhyay, 2014). Extensive researches have been done over the last couple decades to search for natural alternatives to in-feed antibiotics and plant compounds (or phytochemical compounds) have been identified to have great potentials (Yang *et al.*, 2015).

Naturally occurring plant compounds including tannins, saponins and essential oils are extensively assessed as natural alternatives to in-feed antibiotics. Among them, tannins are the major research subject in developing natural alternative to in-feed antibiotics (Redondo *et al.*, 2014).

Tannins are a group of polyphenolic compounds that are widely present in plant kingdom and possess various biological activities including antimicrobial, anti-parasitic, anti-viral, antioxidant, anti-inflammatory, immunomodulation etc. Strong protein affinity is the well-recognized property of plant tannins and has successfully been applied to ruminant nutrition to decrease protein degradation in the rumen, thereby improve protein utilization and animal production efficiency. Incorporations of tannin-containing forage in ruminant diets to control animal pasture bloat, intestinal parasite and pathogenic bacteria load are other important applications of tannins in ruminant animals (Huang *et al.*, 2018).

Tannins have traditionally been regarded as “anti-nutritional factor” for monogastric animals and poultry, but recent researches have revealed some of them, when applied in appropriate manner, improved intestinal microbial ecosystem, enhanced gut health and hence increased productive performance. The applicability of plant tannins as an alternative to in-feed antibiotics depends on many factors that contribute to the great variability in their observed efficacies.

Chemical structure of tannins

Tannins are naturally occurring heterogeneous group of phenolic compounds with diverse structures that share their abilities to bind and precipitate proteins. Tannins are primarily classified into 3 major groups: hydrolyzable tannins (HT), condensed tannins (CT) and phlorotannins (PT). The first 2 groups are found in terrestrial plants while PT occurs only in marine brown algae.

Hydrolyzable tannins (HT)

Hydrolyzable tannins are made up of a polyol core (commonly D-glucose), which is esterified with phenolic acids (mainly gallic or hexahydroxydiphenic acid). The molecular weight of HT ranges from 500 to 3,000 Da. They are susceptible to hydrolysis by acids, bases or esterases, thus can be easily degraded and absorbed in the digestive tract and may cause potential toxic effects in herbivores.

Condensed tannins (CT)

Condensed tannins are oligomeric or polymeric flavonoids consisting of flavan-3-ol units that include catechin, epicatechin, gallic catechin and epigallocatechin. Compared to HT, CT has more complex structures and higher molecular weight ranging from 1,000 to 20,000 Da. Unlike HT, only strong oxidative and acidic hydrolysis can depolymerize the CT structures that are also not susceptible to anaerobic enzyme degradation.

Phlorotannins (PT)

The PT, which are structurally less complex than terrestrial tannins (HT and CT), is formed as a result of the polymerization of phloroglucinol (1,3,5-trihydroxybenzene).

Occurrence of tannins

Tannins are widely distributed in plant kingdom, especially abundant in nutritionally important forages, shrubs, cereals and medicinal herbs. The CT are the most common type of tannin in forage legumes, trees and shrubs while HT are often present in leaves of trees and browse shrubs in tropical areas. Generally, tannins are more abundant in vulnerable parts of the plants, e.g., new leaves and flowers. The PT is concentrated in the phloem located in the cytoplasm of cells within the outer cortical layers of the thalli. Chemical structures and concentrations of tannins vary greatly among plant species, growth stages and growing conditions such as temperature, light intensity, nutrient stress and exposure to herbivores.

Tannins: older concept

In the past some researchers often described tannins as anti-nutritional factors because it interferes with nutrient utilization like dietary protein as well as enzymes and also interferes with structural carbohydrate polymers like cellulose and hemicellulose. It forms chelate with some minerals like iron and to form the ferrous-tannates insoluble complex and decrease the availability of the iron. It also hampers fibre digestion due to the cellulase inactivation and reduces feed intake due to their astringent effect (Mueller-Harvey, 2001).

Tannins: New perspectives

Tannins in high concentrations reduce intake, digestibility of protein, carbohydrates and animal performance. Tannins in low to moderate concentrations prevent bloat and increase the flow of non-ammonia nitrogen and essential amino acids from the rumen (McNabb *et al.*, 1993). Condensed tannins are expected to bind proteins with a high affinity, providing protection from degradation by rumen microbes. Forage containing CT has been reported to minimize the detrimental effects associated with a heavy load of internal parasites (Athanasidou *et al.*, 2001), prevents production of free radicals and may support their scavenging (Cerda *et al.*, 2005). Tannins possess various biological activities including antimicrobial, anti-parasitic, anti-viral, antioxidant, anti-inflammatory, immunomodulation etc. (Huang *et al.*, 2018).

Biological activity of tannins

Tannins are plant secondary metabolites that serve as a part of plant chemical defense system against invasion by pathogens and attack by insects. Tannins have shown numerous biological activities and some of them, which are most important to the modern food animal production, are as follows:-

Antimicrobial property

The antimicrobial activities of tannins have long been recognized and the toxicity of tannins to bacteria, fungi and yeasts has been reviewed. The mechanisms proposed to explain tannin antimicrobial activity include inhibition of extracellular microbial enzymes, deprivation of the substrates required for microbial growth, direct action on microbial metabolism through inhibition of oxidative phosphorylation, metal ions deprivation or formation of complexes with the cell membrane of bacteria causing morphological changes of the cell wall and increasing membrane permeability (Liu *et al.*, 2013). Evidences have shown that the microbial cell membrane is the primary site of inhibitory action by tannins (Liu *et al.*, 2013) through cell aggregation and disruption of cell membranes and functions. Although protein precipitation is a universal property for all tannins, anti-microbial activity of tannins is microbial species-specific and is closely related to the chemical composition and structure of tannins.

Generally, antimicrobial activity of tannins against Gram-positive bacteria has been reported to be greater than against Gram-negative bacteria (Smith and Mackie, 2004), because Gram negative bacteria possess an outer membrane that consists of a lipid bilayer structure which is composed of an outer layer of lipopolysaccharide and proteins and an inner layer composed of phospholipids. However, tannins especially CT isolated from several plants have been shown to possess strong activity against Gram-negative bacteria. Phlorotannins also have greater antimicrobial activity than CT and HT. The antimicrobial property of tannins depends on number of hydroxyl groups and liberation of hydrogen peroxide upon oxidation of tannins. Pathogenic bacteria such as *Escherichia coli* O157:H7, *Salmonella*, *Shigella*, *Staphylococcus*, *Pseudomonas* and *Helicobacter pylori* were all sensitive to tannins (Liu *et al.*, 2013). Because of the vast sources of tannins, which results in great diversity in their antimicrobial activities, screening and identification of tannins that are effective and specific to target microbes would continuously be a research endeavor.

Anti-parasitic property

Anti-parasitic properties of tannins have been demonstrated by both *in vitro* and *in vivo* studies. The anthelmintic mechanisms of plant tannins have been suggested through “direct” action of tannins on parasite cells by 1) reducing establishment of the infective third-stage larvae in the host thereby reducing the host invasion, 2) reducing excretion of nematodes eggs by the adult worms and 3) reducing development of eggs to third-stage larvae and through “indirect” action by improving the host's resistance to nematodes. Condensed tannins extracted from legume tanniniferous forages such as sainfoin (*Onobrychis viciifolia*), big trefoil (*Lotus pedunculatus*), birds foot trefoil (*Lotus corniculatus*) and sulla (*Hedysarum coronarium*) reduced the proportion of *Trichostrongylus colubriformis* hatched eggs and inhibited egg development of lungworm and gastrointestinal nematodes (mixed species of *Ostertagia*, *Oesophagostomum*, *Cooperia*, *Trichostrongylus*, and *Strongyloides*) in a dose-dependent manner (Molanet *et al.*, 2002). However, similar to their antimicrobial activities, the anthelmintic effects of tannins vary greatly depending on chemical composition and structure of tannins, the parasite species or growth stages and the host species.

Antioxidant property

Naturally occurring phenolic compounds have long been recognized as effective antioxidants. The antioxidant property of tannins has wide application in food industry and medical field to prevent oxidative stress related diseases such as cardiovascular disease, cancer or osteoporosis. It has been shown that CT and HT of relatively high molecular weight exhibited greater antioxidant activities than simple phenolics (Hagerman *et al.*, 1998).

The number of hydroxyl groups and the degree of polymerization of tannins are considered to be correlated with their abilities to scavenge free radicals (Ariga and Hamano, 1990). Tannins with the most hydroxyl groups are most easily oxidized and therefore possess greatest antioxidant activity. It has been speculated that dietary tannins may spare other nutritive antioxidants during digestive process or they may protect proteins, carbohydrates and lipids in the digestive tract from oxidative damage during digestion (Marshall and Roberts, 1990).

The potential of tannins as biological antioxidants has been indicated in many *in vitro* and *in vivo* studies. However,

the antioxidant mechanism of tannins in animal tissues is unknown. Further research in this area is needed, especially because enhancing antioxidant status is suggested to be one of the most benefits of feeding tannins to animal wellbeing and performance.

Anti-inflammatory property

Phenolic compounds such as flavonoids, condensed tannins and gallotannins having anti-inflammatory properties. These phenolic compounds such as flavonoids, condensed tannins and gallotannins are known to inhibit some molecular targets of pro-inflammatory mediators in inflammatory responses.

CT is antagonists of particular hormone receptors or inhibitors of particular enzymes such as COX enzymes. E.g. Proanthocyanidins from grape seed, leucoanthocyanidins from the hot water bark extract of the black spruce showed a strong anti-inflammatory activity.

Gallotannins exert various biological effects ranging from anti-inflammatory to anticancer and antiviral properties. The mechanisms underlying the anti-inflammatory effect of tannins includes the scavenging of radicals and inhibition of the expression of inflammatory mediators, such as some cytokines, inducible nitric-oxide synthase (iNOS) and COX-2 (Mohammed *et al.*, 2014).

It needs to be pointed out that most of the studies in this area were conducted using *in vitro* models. The efficacy of the anti-inflammatory action of tannins in animal body after digestion needs to be evaluated further in *in-vivo* model.

Anti-virus property

Tannin show antiviral activity by affecting different stages of viral replication, including the extracellular virions themselves, their attachment to the cell, their penetration into the cell and the replication process in the host cell, as well as the assembling of new viral particles, transport proteins, polysaccharides and viral enzymes. In almost all of the above mentioned stages, the tannin activity is due to their ability to bind permanently to the proteins of the capsid or supercapsid, either to specific viral enzymes required for viral replication or to newly synthesized viral proteins evolved in the composition of the new viral particles.

Tannins have been shown to have significant activity against some virus, e.g., human immunodeficiency virus (HIV), bovine adeno-associated virus and noroviruses. Yang *et al.* (2013) found that a HT (chebulagic acid) had considerable anti-enterovirus 71 activities *in vitro* and efficiently reduced mortality and relieved clinical symptoms through the inhibition of viral replication in mice model. Phlorotannins isolated from *E. cava* have been demonstrated to possess strong activity against influenza virus neuraminidase, porcine epidemic diarrhea virus (PEDV) by inhibiting viral entry and viral replication and HIV-1. Many studies have been conducted on tannins effects against the replication of human immunodeficiency virus (HIV), and the results of the various teams indicate that tannins have several targets of action in the HIV replicative cycle. Ellagitannins isolated from *Tuberarialignosa* inhibited HIV's entry into MT-2 cells (Bedoya *et al.*, 2010). Other authors have reported on ellagitannins (geraniin and corilagin) that reduced HIV replication by inhibiting the HIV-1 protease and HIV-1 integrase enzymes (Notka *et al.*, 2004).

All the above information demonstrated that tannins possess varying anti-virus activities depending on chemical compositions and structures. *In vivo* studies are needed to explore the potential of tannins as natural anti-virus agents to be used in animal and poultry industries.

Use of tannins in ruminants

Tannins especially CT are widely distributed in nutritionally important forages, trees, shrubs and legumes, which are commonly consumed by ruminants. Therefore, the effects of CT on ruminant nutrition, health and production have been extensively studied and reviewed.

Ruminal fermentation

Condensed tannins can have beneficial or detrimental effects on ruminants, depending on their amount consumed by animals, their type and chemical structure as well as the composition of the rest of the diet, especially CP concentration of the diet (Mueller-Harvey, 2006). It is generally believed that CT in forage in low to medium (<50 g/kg DM) concentration benefit ruminants in terms of improving protein utilization without negatively affecting feed intake and nutrient digestion depending on CT source and analytical method/standard used to determine concentration. Likewise, Fagundes *et al.* (2020) have stated that condensed tannins from *L. procumbens*, *D. paniculatum*, *L. leucocephala*, *D. ovalifolium*, and *F. macrophylla* were effective in modifying ruminal fermentation, which indicates a promising alternative to ionophores for methane reduction in beef cattle. Similarly, Orzuna-Orzuna *et al.* (2021) reported that supplementation of tannins in beef cattle had improved ruminal fermentation characteristics by reducing ruminal NH₃-N concentration and increasing rumen propionate and butyrate concentration. Likewise, Orzuna-Orzuna *et al.* (2021) had stated that the addition of tannins in sheep diets improves productive performance, antioxidant status in blood serum, oxidative stability of meat and some other characteristics related to meat and carcass quality.

Growth

Various researchers have shown that tannin supplementation under low to moderate concentration (10-40 g/kg DM) improves animal growth by improving digestive utilization of feed by ruminants, mainly because of reduction in ruminal protein degradation and as a consequence, a greater availability of (mainly essential) amino acids for absorption in the small intestine. Dey et al. (2008) studied the effect of graded levels of CT on growth rate in lambs. Lambs fed on CT-1.5% recorded significantly higher ($p < 0.05$) average final body weight (kg) compared to those given supplement CT-0 and CT-2.0%, while body weight of animals under CT-1.0% was intermediate. The positive response of ADG to 1.5% level of CT in the supplement gives an indication that the binding effect of tannins was pronounced only at this level by supplying protein to the lower gut and subsequently its more efficient use for tissue growth. In a meta-analysis, Orzuna-Orzuna et al. (2021) had demonstrated that tannin supplementation does not affect weight gain, feed intake, or feed efficiency in beef cattle.

Milk production

Tannin supplementation may result in increased milk production, increased protein and lactose production. This increased concentration of protein is due to the greater availability of intestinal amino acids, especially of methionine and lysine, which are thought to limit milk production. Tannins mainly exert this effect on proteins, but they also affect other feed components to different degrees. Their main effect on proteins is based on their ability to form hydrogen bonds that are stable between pH 3.5 and 8.0 (approximately). These complexes stable at rumen pH and dissociate when the pH falls below 3.5 (such as in the abomasum, pH 2.5-3) or is greater than 8 (for example in the duodenum, pH 8), which explains much about the activity of tannins in the digestive tract (Hagerman *et al.*, 1992). Evidently, the modifications of the digestibility caused by tannin ingestion are mainly associated with changes in the ruminal fermentation pattern, along with changes in intestinal digestibility. The greater concentration of lactose can be explained by greater glucose supply; most lactose synthesis in the mammary gland relies directly on blood glucose, and in ruminants gluconeogenesis mainly involves propionic acid and amino acids. Thus, a greater availability of amino acids would contribute to greater synthesis of glucose, resulting in increase milk production.

Dey et al. (2014) conducted an experiment to see the effect of condensed tannin supplementation through *Ficus bengalensis* leaves on milk production in crossbred cows. They concluded that the daily milk yield was significantly higher ($p < 0.05$) in group received supplemented diet. The 4% fat corrected milk yield was also significantly ($p < 0.01$) higher in group received FBLM diet. Likewise, Menci et al. (2021) had stated that the dietary supplementation of tannin extract at the dose of 150 g/day in dairy cows showed no effect on milk quality, whereas in dry season the milk from cows eating tannins showed lower BCFA concentration, C18:1 t10 to C18:1 t11 ratio, and rumenic to linoleic acid ratio.

Wool production

Clean wool is mainly protein, with high cystine content and the availability of sulphur-containing amino acids (SAA) has significantly affected wool production (Reis, 1963). Similarly, Dey et al. (2008) studied the effect of graded levels of condensed tannin through *Ficus infectoria* on wool yield in lambs and found that the total wool yield (g) and yield per day (g) were significantly higher (both linear and quadratic $P < 0.01$) for the treatment CT-1.5 % compared to similar wool yield by lambs in CT-0 and CT- 2.0% treatments.

Role of tannin in bloat prevention

Probably the most successful application of tannins in ruminant production is to reduce frothy bloat. Bloat or tympany is a common digestive disorder in ruminants, caused by the formation of stable protein broth in the rumen of animals fed with high nutritive value legumes including white clover or Lucerne. The condition is characterized by an accumulation of gas in the rumen and reticulum that can impair both digestive and respiratory function. Tannins by precipitating protein during chewing and rumination reduce protein solubility in the rumen thereby decrease bloat occurrence. Therefore, moderate concentration of tannins in the food of animals destabilizes the protein foams which refers them bloat safe.

Li et al. (1996) has estimated that as little as 1.0 mg CT/g DM is needed to prevent pasture bloat. Incorporation of CT-containing forage such as sainfoin into alfalfa has been proved an effective method in controlling alfalfa pasture bloat. Similarly, Min et al. (2012) conducted an experiment to see the effect of plant tannin supplementation on bloat frequency. Twenty-six heifers were allocated to 3 treatments that included a control (non-tannin group) and 2 types of tannins (mimosa and chestnut tannins). Plant tannins (1.5% of DMI) were supplemented once daily mixed with a textured feed (500 g/animal). They found that daily supplementing mimosa and chestnut tannins to heifers grazing wheat forage minimized bloat frequency.

Tannins as anthelmintic

Another major application of tannins in ruminants especially in grazing ruminants is to control digestive parasites. In all grazing ruminants, gastrointestinal nematodes (GIN) are often implicated as a main cause for substantial production losses in extensive farming operations worldwide. Fecal excretion of nematode eggs into the environment during grazing or browsing is a major route for a wide spread contamination and feco-oral infestation of host animals. Repeated use of chemically produced anthelmintics, recommended and prescribed by veterinarians, represents an

effective treatment/control program for GIN. However, development of anthelmintic resistance in nematodes, together with the current trend for organic farming, has increased the demand for alternatives to chemoprophylaxis in order to reduce or exclude the use of anthelmintic drugs to control parasites.

Hoste et al. (2012) reported that tannin-rich plants show an anthelmintic effect on various gastrointestinal nematodes by affecting different stages of the parasite life cycle. Tannins from mimosa (HT), chestnut (HT) and quebracho (CT) are effective against various intestinal parasites in ruminant. It seems that dietary concentration below 20 g/kg DM of tannins is ineffective in controlling ruminant intestinal parasites. Lopes *et al.* (2016) conducted an experiment to see the effect of tanniniferous food from *Bauhinia pulchella* on pasture contaminated with gastrointestinal nematodes from goats. Sixteen cross bred goats that were naturally infected with gastrointestinal nematodes were fed tanniniferous concentrate from the leaves of *B. pulchella* and compared to a separate paddock of control animals without condensed tannin supplementation. They concluded that condensed tannin from *B. pulchella* showed anthelmintic activity, affected egg viability and reduced pasture contamination.

Tannins as an antioxidant

Another application of tannin in ruminants is to reduce oxidative stress. Gulcin *et al.* (2009) reported that tannic acid is the effective natural antioxidant component that can be used as food preservative agents or nutraceutical. Chaurasiya *et al.* (2018) conducted an experiment to see the effect of feeding tannin rich Oak (*Quercusleucotrichophora*) leaves on antioxidant status of parasitic infected goats in Kumaon hills. They concluded that the antioxidant property from GSH, SOD and catalase was significantly increased in oak fed groups than grass fed groups. Hematological values of GSH, SOD and catalase are the representative of antioxidant status of body (Han *et al.*, 2004).

Tannin as antimicrobial agent

The gastrointestinal tract (GIT) of ruminants is the main reservoir of enterohemorrhagic *Escherichia coli* O157:H7, which is responsible for food-borne infections in humans that can lead to severe kidney disease. Recent researches have shown that incorporation of tannins or tannin-containing forage into diets reduces food borne pathogens in ruminant digestivetract.

Supplementation of chestnut tannin at the concentration of 15 g/kg DM decreased fecal shedding of *E. coli* for cattle fed hay diets (Min *et al.*, 2007). Huang *et al.* (2015) also found that lambs challenged with *E. coli* O157:H7 fed diets containing 36 g of purple prairie clover CT/kg DM shed significant less *E. coli* O157:H7 than lambs fed diets without CT.

Use of tannins in monogastric animals

Unlike for ruminants, tannins have traditionally been considered as ‘anti-nutritional’ factors in monogastric nutrition with negative effects on feed intake, nutrient digestibility and production performance. Therefore, it is almost a common practice in feed industry to minimize the use of tannin-containing feed in swine and poultry diets or to take measures to reduce their dietary concentrations if such feed are used. However, several recent reports showed that low concentrations of several tannin sources improved health status, nutrition and animal performance in monogastric farm animals (Brus *et al.*, 2013).

Compared with other domestic animals, pigs seem to be relatively resistant to tannins in the diets and they are able to consume relatively high quantities of tannin- rich feedstuffs without presenting any toxic symptoms. This is likely due to parotid gland hypertrophy and secretion in the saliva of proline-rich proteins that bind and neutralize the toxic effects of tannins. Brus *et al.* (2013) investigated the effect of chestnut wood tannins and organic acids on growth performance and faecal microbiota of pigs from 23 to 127 days of age. The result indicated that the supplementation of chestnut wood tannins and organic acids can improve the growth performance in period from 82-127 days mainly by reducing harmful *E. coli* counts and by increasing counts of beneficial Lactic acid bacteria.

The mechanisms of growth promoting effects of tannins in monogastric animal are much less understood compared with those in ruminants. The growth promoting action of tannins in monogastric animal relies on the balance between their negative effects on feed palatability and nutrient digestion through protein and enzyme complexation and positive effects on promoting the health status of intestinal ecosystem through their anti-microbial, anti-oxidant and anti-inflammatory activities. Compared to the vast sources of tannins for ruminants, sources of tannins used for monogastric animals are rather limited and so far only few have been studied and showed potential as feed additive. The final impact of tannins on animal performance depends on the type of animals and their physiological status, feed, type of tannins and their concentrations in the diets.

Use of tannins in poultry

Wang *et al.* (2008) conducted a study to see the effect of grape seed proanthocyanidin extract supplementation on growth performance of broilers infected with *Eimeriatenella* and they concluded that the lowest mortality and the greatest growth gains were recorded in the group of birds fed with GSPE between 10 to 20 mg/kg. In the second experiment they concluded that GSPE supplementation at the level of 12mg/kg of diet significantly reduced the mortality, lesion scores and improve antioxidant status in birds infected with oocysts of *E. tenella*.

Conclusion

Apart from the nutritional attributes, their anthelmintic, anti-bloat, anti-oxidative, anti-inflammatory, anti-microbial, anti-methanogenic roles have been well acclaimed under several studies. Thus, the use of tannins as feed additives confirms that tannins are a valuable alternative to complement or replace the use of AGPs in industrial livestock production.

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