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Asian Journal of Animal and Veterinary Advances

Academic
Journals Inc.

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OPEN ACCESS Asian Journal of Animal and Veterinary Advances

ISSN 1683-9919
DOI: 10.3923/ajava.2018.201.209

Review Article
Utilization of Polyethylene Glycol and Tannase Enzyme to Reduce the Negative Effect of Tannins on Digestibility, Milk Production and Animal Performance

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Abstract
Tannin compounds are widely distributed in many species of plants commonly consumed by ruminants, where these compounds play a role in protection from predation and perhaps as pesticides and in plant growth. Tannins are divided into two major groups and condensed hydrolysable tannins. One of problems of feeding agro-industries and agricultural by-products to farm animals are regarded to high content of anti-nutrients factors such as tannins and alkaloids. Tannins had a negative or positive effect on ruminants depending on the type of tannin consumed, amount ingested, chemical structure and molecular weight and the animal species involved. Low tannins concentrations may improve the digestive utilization of feed mainly due to a reduction in protein degradation in the rumen and a subsequent increase in amino acid flow to the small intestine. While, increase amounts of tannin concentrations in diets animals reduce feed intake, nutrient digestibility, milk production and animal performance. Addition of polyethylene glycol had beneficial effects in monogastrics and both beneficial and adverse effects in ruminants depend upon amount of tannins in the diet. Also, tannase enzyme can hydrolyze tannins substrates releasing gallic acid and glucose.

Key words: Tannins, polyethylene glycol, tannase enzyme, milk production, digestibility, rumen, blood parameters

Received: October 15, 2017 Accepted: December 26, 2017 Published: April 15, 2018

Citation: Ahmed Mahmoud Abd El Tawab and Mostafa Sayed Abdellatif Khattab, 2018. Utilization of polyethylene glycol and tannase enzyme to reduce the negative effect of tannins on digestibility, milk production and animal performance. Asian J. Anim. Vet. Adv., 13: 201-209.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

There are many limiting factors for using diets in animals feed such as, low protein content, high crude fiber content, low available energy, minerals and vitamins. Low digestibility mean a poor quality and low palatability in addition to presence of some anti-nutritional factors such as anti-protein, anti-vitamin and anti-mineral etc[1-3].

In all over the world many researches have been devoted to reduce harmful of anti-nutritional factors (ANFs) in animals' diets. In treatment with mechanical and physical methods (heating, chopping, cutting, grinding and milling, moistening, soaking in water and steaming under pressure, ensiling etc.) were employed[4]. Chemical methods include acids (sulfuric and hydrochloric acid), alkaloids (sodium hydroxide), ammonia, urea and polyethylene glycol etc. and biological methods with (fungi, enzymes, ionophores, antibiotic, bacteria and yeasts etc.) were investigated[5-8].

The ANFs are natural or synthetic substance and their presence in human foods and animals feed, reduce growth and interfere with the absorption of nutrients[9]. Examples of these ANFs are tannins, phytate, protease and excessive dietary fiber. The objective of this review was to investigate the effect of polyethylene glycol and tannase enzyme to reduce the negative effect of tannins on feed intake, digestibility, rumen fermentation, milk production, blood chemistry and animal performance.

Definition and classification of tannins: Tannins are group of plant secondary compounds which have been known and used by humans for centuries[10]. The tannin compounds are widely distributed in many species of plants, where they play a role in protection from predation and perhaps as pesticides and in plant growth regulation[11].

Tannins are defined as a phenolic compounds with high molecular weight ranging from 500 to over 3000 of gallic acid esters[12] and up to 20000 of proanthocyanidins[13] containing sufficient hydroxyls and carboxyl groups to form effectively strong complexes with proteins (mainly), carbohydrates (cellulose, hemicelluloses, pectin, starch etc.), alkaloids, nucleic acids and minerals[14-20].

Tannins are traditionally classified into two major groups: The condensed and the hydrolysable tannins. Hydrolysable tannins (HT) are made up of a carbohydrate core whose hydroxyl groups are esterified with phenolic acids (mainly gallic and hexahydroxydiphenic acid). The condensed tannins (CT) or proanthocyanidins are non-branched polymers of flavonoids units (flavan-3-ol, flavan-3, 4-diol) and usually have a higher molecular weight than the HT (1000-20000 Da compared to 500-3000 Da)[14,17,21]. Although, this division of the tannins is the most widely accepted, many authors believe it does not fully reflect their chemical complexity[14,17].

Chemical and physical properties of tannins: The tannins of different plant species have different physical and chemical properties[15] and therefore, they have very diverse biological properties[22]. Tannins are non-crystallizable, amorphous compounds. They are soluble in water, ethyl alcohol, glycerol and acetone and dilute alkalies.

Tannins bind with proteins to form tannins-protein complexes, the degree of affinity between the participating molecules residing in the chemical characteristics of each[14,16,22,23]. The high affinity of tannins for proteins lies in the formers great number of phenolic groups. These provide many points at which bonding may occur with the carbonyl groups of peptides[14,23-25].

The complexes formed between tannins and proteins or other compounds are generally unstable. The bonds uniting them continually break and re-form[10]. Kumar and Singh[26] suggested that complexes could come about through four types of bond:

- Hydrogen bonds (reversible and dependent on pH) between the hydroxyl radicals of the phenolic groups and the oxygen of the amide groups in the peptide bonds of proteins
- By hydrophobic interactions (reversible and dependent of pH) between the aromatic ring of the phenolic compounds and the hydrophobic regions of the protein
- By ionic bonds (reversible) between the phenolate ion and the cationic site of the protein (exclusive to HT)
- By covalent bonding (irreversible) through the oxidation of polyphenols to quinones and their subsequent condensation with nucleophilic groups of the protein. For a long time it was believed that the formation of tannin-protein complexes was owed mainly to hydrogen bonds. However, it is now known that hydrophobic interactions are important

Tannins in feedstuffs: Several agro-industries and agriculture by-products are of considerable importance as livestock feeds in less developed countries contain tannins. Also tannins present in a wide variety of plants used as foods and feeds including sorghum and barley[27], beans [28], millet[29], *Acacias*, *Albizias*, *Ficus sp.*, *Calliandra calothrysus*, *Gliricidia sepium*, *Leucaena leucocephala*, *various* species of *Prosopis* and *Quercus*[1]. McLeod[14], Rittner and Reed[30], Perevolotsky[31] and Wood *et al.*[32] reported that tannin compounds are widely distributed throughout the plant kingdom, especially trees, shrubs, herbal and leguminous plants. Kumar and D'Mello[33] found that hydrolysable tannins were abundant in fruits, leaves, galls and pods of dicotyledons such as chestnut, oak and other species. However, condensed tannins are even more widespread. Some species contain both types of tannins. Tannins are not restricted to tropical feeds, lotus, sainfoin and other temperate species have been found to contain condensed tannins[34]. While not all tree and shrub fodders and browses contain tannins, most certainly do. The occurrence of tannins is not restricted to particular limited classes of plants or climatic zones. Tannins are, therefore, highly likely to be consumed in all agricultural systems where trees and shrubs are used as livestock feed.

Makkar[1] listed 28 agro-industrial by-products which might be useful as feeds. Nineteen of these by-products, including various seeds and seed cakes, have been shown to contain polyphenols. Cassava leaves, potentially useful forage, contain condensed tannins[35]. Sorghum grain and stover can also contain tannins[36].

Decrease the negative effects of tannins on the animals

Inactivation of tannins by supplementing polyethylene glycol (PEG): Polyethylene glycol (PEG), with a molecular weight of 20000 g mol^{-1}, is a nonionic detergent which forms complexes with hydrolysable and condensed tannins over a wide pH range (2-8.5)[6]. Furthermore, protein may be released from the protein-tannin complex by exchange reaction with polyethylene glycol[37].

The complex comprised of PEG and tannins is insoluble in boiling water, most organic solvents and neutral and acidic detergent solutions. Such complexes do not respond to most colorimetric methods for determination of tannins[37,38]. Hydrogen bonding between oxygen (ether) of the PEG chain and the phenolic hydroxyl group on the tannin moiety might explain the precipitation phenomena. It suggests that there is a considerable analogy between PEG-tannin and protein-tannin complexation[39]. Thus, the information obtained from the amount of PEG binding (PEG-b) to a plant sample might be analogous to that obtained from protein precipitation capacity. Furthermore, because of the solubility of PEG in water and most organic solutions, PEG-b can be measured *in situ* without a need to pre-extract the tannins from the sample. Polyethylene glycol may react *in situ* with tannins that cannot be extracted with conventional organic solvents because these tannins are bound to proteins and cell-wall components. Determination of tannins which are bound to cell components is possible in some cases[40], but this method is too complicated for routine use or for screening large numbers of samples.

Supplementation of PEG has been shown to have beneficial effects in monogastrics and both beneficial and adverse effects in ruminants depend upon amount of tannin compounds in the diets. Addition of PEG had beneficial effects for feedstuffs (high tannins) such as *Quercus calliprinos*, *Pistacia lentiscus*, *Ceratonia siliqua*[41,42], *Hedysarum coronarium*[43], *Zizyphus nummularia*[44], *Acacia aneura*[45,46], *Desmodium ovalifolium* and *Flamingia macrophylla*[47], *Lotus penduncula tus*[48,49] and *Acacia saligna*[50], which are rich in tannins. Inactivation of tannins through PEG increased the availability of nutrients and decreased microbial inhibition, which in turn increased degradability of nutrients leading to better animal performance. While, Addition of PEG to *Lotus corniculatus* diets (contain low condensed tannins) decreased wool growth, weight gain[51,52] and milk yield[53]. These decreases are attributed to a substantially lower absorption of amino acids from the intestine resulting from increased digestion of proteins in the rumen[54].

Degradation of tannins by tannase enzyme: Tannase enzyme is composed by two enzymes: A depsidase that hydrolyzes the depside bonds and an esterase that catalyzes the cleavage of ester. Haslam and Stangroom[55] and Murad *et al.*[56] found that the esterase/depsidase ratio of *Aspergillus niger* tannase modified by cultural methods and isolation procedures, suggesting the presence of two different enzymes. However further analysis indicated that esterase and depsidase are isoenzymes with low specificities capable of hydrolyzing both esters and depsides of gallic acid with different relative specificity for each substrate. Beverini and Metche[57] isolated two separate isoenzymes, tannase I and II from *Aspergillus oryzae*, with esterase and depsidase activity, respectively. Barthomeuf *et al.*[58] and Curiel *et al.*[59] purified tannases with both esterase and depsidase activity.

Tannase catalyzes the hydrolysis of ester and depside bonds present in gallotannins, complex tannins and gallic acid esters[60,61]. However, the enzyme does not affect the carbon-carbon bonds, thus tannase is unable to hydrolyze condensed tannins[55].

Tannase completely hydrolyzes tannic acid to gallic acid and glucose[62]. Iibuchi *et al.*[63] studied the intermediary compounds formed during this hydrolysis by thin layer chromatography. Formation of 2, 3, 4, 6-Tetragalloylglucose, two kinds of monogalloylglucose and free gallic acid was noted. The same products in the hydrolysate of 1,2,3,4,6,-Pentagalloylglucose was detected.

Tannase hydrolyzes other substrates such as methyl gallate, propyl gallate, digallic acid, epicatechin gallate and epigallocatechin gallate-releasing gallic acid[59,64]. Tannase also acts on ellagitannins such as rosacyanin or phyllanemblinin. In those cases, tannase selectively hydrolyses the galloyl moieties, yielding gallic acid and degalloylatedellagitannins[65,66].

Effect of tannins on nutrients digestibility and nutritive value: Tannins can be beneficial or harmful to ruminants, these depending on amount consumed by animals, the type of structure and molecular weight and on the physiology of the consuming species[10,23]. It is important to remember that all the quantities mentioned in this revision should be taken with great caution since different analytical methods and especially different standards (e.g., quebracho, tannic acid, catequin, cyanidin, delphinidin or internal standards from the plant itself etc.) can provide very different and therefore, ambiguous results[19].

Tannins can also shift site of protein digestion (increase ruminal escape protein) and nitrogen excretion (from urine to feces) in ruminants[67,68], due to their ability to bind and precipitate protein[20]. Tannins also bind carbohydrates to some extent, which also have effect on fermentation and nutritional value of some diets[20].

Perez-Maldonado and Norton[69] used tropical forages for feeding sheep and goats. They found that with increasing levels of condensed tannins (CT) in the diet (0-2.3%) N digestibility decreased but insignificant effect on either feed intake, organic matter (OM) or neutral detergent fiber (NDF) digestibility. Norton and Ahn[70] have shown that drying calliandra significantly decreased CT content (3.7-2.5%) and when provided as a supplement (30% diet) to pangola grass hay for sheep, fresh (frozen) calliandra depressed the feed intake of hay, decreased N, OM, NDF and acid detergent fiber (ADF) digestibility. Similarly, increasing CT contents of leucaena leaf (2-14%) decreased *in vitro* dry matter digestibility (IVDMD) of leaf material[71].

Tannins probably have greatest effect on the nutrition of animals in arid and semi-arid environments where *Acacia* sp., are a significant source of supplemental and reserve feed. Mulga (*Acacia aneura*) contains such high levels of tannins that the availability of N and S from protein digestion in the rumen is so restricted that sheep suffer N and S deficiencies in the rumen, which limits feed intake and productivity[72,73]. While some relief from these effects can be afforded by additional supplements of N, P and S. The daily application of 24 g PEG alone increased DM intake by 78%, converted live-weight loss (-64 g/day) to gain (36 g/day) and resulted in an almost 3-fold increase in the volumetric growth of wool. It is of interest that while PEG supplementation increased N digestibility (36.6-58.4%) there was no effect on DM digestibility (49.7-48.8%) which suggests that the tannins of mulga are having a specific effect on intake, unrelated to the rates of feed digestion and removal from the rumen. Similar effects of tannins have been reported for a wide range of tropical forages[74]. Biological treatment (tannase enzymes or nitrogen fixing bacteria) to diets containing tannin lead to the digestibility coefficient, was high and more efficient and nutritive values DCP and TDN[6-8,75]. Ensiling the agro-industrial by-product mixture which was composed from grape pulp, radicell, olive pulp and date seeds drastically decreased its tannins and alkaloids content to low level from (0.66 and 0.35) to (0.15 and 0.15), respectively. There was significant increase in all nutrients digestibilities (DM, OM, CP, CF and NFE) and TDN[3]. Also, treated sunflower meal with heating, formaldehyde, polyethylene glycol lead to increase digestible coefficient and nutritive values (DCP and TDN)[7,76].Used polyethylene glycol (PEG 6000), to reduce the adverse effect of tannins. It was concluded that tannins in browse species (with high tannin content) had inhibitory effects on rumen microbial fermentation as indicated by the gas production. It has observed that addition of PEG increased organic matter digestibility and metabolizable energy (ME). Rubanza *et al.*[77] stated that the *in vitro* digestibility of browse leaves declines with increase in tannin content. Moreover, Elahi and Rouzbehan[78] used three species of oak trees leaves (*Quercus persica*, *Q. infectoria* and *Q. libani*) for *in vitro* study and the chemical analysis phenols of compound (78, 115, 104 total phenols, 73, 109, 100 total tannins, 14, 15, 12 condensed tannin and 46, 87, 62 hydrolysable tannin, respectively). Researchers indicated that all species had higher values ($p<0.05$) in gas production, OMD, DMD and ME with addition of PEG compared with control.

Xu *et al.*[79] found that digestibility of DM, organic matter, crude protein and energy were slightly decreased with increased wet green tea grounds in diets. Also, Perez-Maldonado and Norton[69] reported that celluloytic activity was inhibited in the rumen of sheep fed diets of different CT content and decreased of digestibilities OM, NDF and ADF was noted.

Effect of tannins on some rumen liquor parameters: Tannin compounds reduce the degradation of dietary protein in the rumen and decrease ruminal ammonia concentrations, which may suggest an inhibition of proteolytic enzymes in the rumen[71]. The reduction in protein degradation is associated with a lower production of ammonia nitrogen and a greater non-ammonia nitrogen flow to the duodenum[80-82].

Yanez Ruiz *et al.*[83] found that ruminal VFA concentration in goats and wethers increased ($p<0.05$) and pH and ammonia-N concentration decreased ($p<0.05$) in animal fed diet containing olive cake (high tannins). While, Xu *et al.*[79] stated that no differences among treatments were observed in pH value and total volatile fatty acids concentrations. However, the rumen ammonia-N contents tended to decrease in animal fed diets containing included wet green tea grounds.

Hassan[5] reported that ruminal ammonia-N, VFA concentration, rumen volume and microbial protein synthesis were significantly ($p<0.05$) higher in animals fed *Acacia silage* with or without polyethylene glycol than those fed fresh *Acacia*. However, treated sunflower meal with heating, formaldehyde, polyethylene glycol lead to lower value of ruminal ammonia-N. However, insignificant differences among the diets were detected for rumen pH and TVFA's concentrations[76]. Moreover, biological treatment (tannase enzymes or nitrogen fixing bacteria) to diets containing tannin lead to increase ruminal total volatile fatty acids (TVFA's) and ammonia nitrogen (NH3-N)and decrease of rumen pH[6]. While, there were no difference observed for ruminal pH and TVFA's[75].

Effect of tannins on some blood parameters: There are insignificant differences in blood parameters (total protein, albumin, globulin, glucose and urea) when the animals were fed on diets containing tannins such as date seeds or olive pulp or grape pulp[3,7,8]. While, Ben Salem *et al.*[84] reported that goats fed high levels of phenolic compounds particularly lignin and tannins (34.8 g as tannic acid kg^{-1} DM) had blood total proteins, albumin and blood urea in normal range.

Silanikove *et al.*[42] observed lower serum urea concentration in goats fed shrubs containing tannins that attributed to low nitrogen availability. Also, Abdel-Halim[85] pointed that there were a decrease in blood total proteins and urea level in goats fed tannins containing diets than control (6.48 vs 7.0) but A/G ratio was lower (0.86 vs 1.03). Moreover, Kholif *et al.*[2] observed decreasing in blood content of total proteins, albumin, A/G ratio and urea in the ration of tannins contained by-products compared with ensiled ration.

Effect of tannins on milk production and composition: Tannins have varied effect on milk production and milk components yield depending on concentration of tannins. The low concentration increased milk yield and milk protein percent via increasing protein protection which allow more amino acid pass and absorbed in small intestine[52,86,87]. However, the high concentration of tannins decreased milk production and negatively affects milk components[7,8]. Gilboa *et al.*[88], Decandia *et al.*[89] and Abd El Tawab *et al.*[7] reported the high concentrations of tannins caused a decrease in milk yield and composition and digestibility coefficients, but this negative effect disappeared when polyethylene glycol supplemented todiets. Also, Wang *et al.*[53] reported that milk production was increased by 21% during mid and late lactation in ewes fed *L. corniculatus* (44.5 g CT kg^{-1} DM) with added polyethylene glycol. It is also reported significant increase in the efficiency of milk production, increase protein (14%) and lactose production (12%) and a decrease in the fat content of the milk. This increase of protein might be explained by the greater availability of intestinal amino acids, especially of methionine and lysine, which are thought to limit milk production. The authors attributed greater concentration of lactose to the greater glucose supply since most lactose synthesis in the mammary gland relies directly from 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. The latter authors indicated that the increase in lactose concentration occurred without modification of the molar proportions of volatile fatty acids, which attributed due to the action of tannins. The reduction in the concentration of fat was attributed to a simple dilution effect as the concentrations of lactose and protein increased.

CONCLUSION

Summarizing the literature data presented in this review paper, it can be concluded that tannase enzyme and polyethylene glycol were used for animal nutrition and had positive effect on animal performance and productivity.

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