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# THE POTENTIAL EFFECT OF DIETARY TANNINS ON ENTERIC METHANE EMISSION AND RUMINANT PRODUCTION. AS AN ALTERNATIVE TO ANTIBIOTIC FEED ADDITIVES – A REVIEW

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#### Abstract

Antibiotic growth promoters in livestock nutrition cause microbial resistance which produces threats to human health. Therefore, tannins have been considered as natural alternative antibiotic feed additives which possess various biological properties including antimicrobial, anti-inflammatory, antioxidant and immunomodulatory. Additionally, these plants also have antiparasitic and anti-bloat characteristics which contribute to inhibit the enteric methane emission in order to improve nutrient digestibility, milk and meat quality, fatty acids composition and ruminant production. Antibiotic growth promoters have been practiced in animals feeding to increase feed intake, growth rate, weight gain as well as reduce metabolic disorders and energy losses in the rumen. In 2006, the European Union banned the usage of antibiotic growth promoters in the feeding of livestock. This antibiotic resistance issue has increased demand to explore the natural feed additives that might be useful for animal production system. Consequently, natural forages have been categorized as potential feed additives in animal production since it improves nutritive value, protein digestibility, increase amino acid absorption and growth rate. But, some plant materials are usually rich in tannins known as anti-nutritional factors. Therefore, the application of tanninrich plants in ruminant nutrition needs great precaution due to its possible injurious effects (dose dependent) on animal health such as metabolic disorders. Hence, there is need to give attention to the usage of tannins in ruminant nutrition as an alternative to antibiotics feed additives to investigate its effects on enteric methane emissions and ruminants production. In addition, safety and risk associated with tannins feeding have also been briefly discussed.

Key words: condensed tannins, hydrolysable tannins, feed additives, methane emission, ruminants

The world human population is increasing and it is estimated to be 9.15 billion by 2050 (Gemeda and Hassen, 2018). This might increase the demand at consumer level for organic livestock products such as milk and meat (Bunglavan and Dutta, 2013).

Regarding the above increasing population issue, the animal products consumption rate is still lower in developing countries as compared to developed countries, indicating significant need to enhance livestock production (Thornton and Gerber, 2010; Gemeda and Hassen, 2018). Antibiotic growth promoters in ruminant nutrition have been used for several years to increase the productive efficiency. However, it has been reported that antibiotic growth promoters used for long time cause microbial resistance in animals (Chattopadhyay et al., 2014). Therefore, researchers have worked to find natural alternatives to feed additives to reduce threats of drug resistance in human health.

Local plants containing tannins have been considered as potential feed additives for ruminants (Yang et al., 2015). These plants are very essential in animal feeding due to their high protein profile that is available in dry and hot season when forage resources are unavailable (Koneswaran and Nierenberg, 2008). Therefore, the Food and Agriculture Organization (FAO, 2013) has declared that there is strict need to utilize local resources in the feeding of animals to avoid the fodder scarcity in future. Browse species are usually rich in plant phenolic substances generally known as tannins (Makkar, 2003; Lee et al., 2010). Tannins are naturally growing plant secondary compounds with different molecular weight and these are present in almost all vascular plants usually fed by ruminants (Table 1) (Wang et al., 2015).

Name	Tannins
Types	Condensed tannins and hydrolysable tannins
CAS number	1401-55-4
Molecular formula	$C_{76}H_{52}O_{46}$
Molecular weight	1701.206 g/mol
Solubility in water	2850 g/L
Color	Light yellow to tan solid
Melting point	Decomposes above 200°C
Flash point	390°F
Acidity (PKa)	Ca. 10

Table 1. Chemical nature of tannic acid

Tannins have both beneficial and deleterious effects depending on its composition, concentration, animal species and physiological status (Patra and Saxena, 2011). Tannins are divided into hydrolysable (HT) and condensed tannins (CT) (Figure 1). Specifically, tannins have significant anti-bloat characteristics that makes proteins unavailable for rumen degradation and reduces urine N excretion, intestinal parasites and enteric methane emission (greenhouse gas) which, in turn, can improve the milk production, wool growth, reproductive efficiency and immune responses (Min and Hart, 2003; Ramírez-Restrepo and Barry, 2005; Waghorn, 2008; Aufrere et al., 2013; Attia et al., 2016). CT containing feed has potential effects on livestock performance (Gxasheka et al., 2015) by reducing gut parasite (Krueger et al., 2010). A study has reported that steers fed tannins based *Acacia karroo*, had improved weight gain (WG), body condition score (BCS) and carcass weight (CW) as compared to control group (Mapiye et al., 2011). Mostly, methane gas is contributed from the agriculture sector, especially from livestock. A scientific study has recorded that cattle and sheep produce methane in amounts of about 250-500 and 20–55 l/day depending on dry matter intake (DMI), respectively (Eckard et al., 2010). This not only affects the energy utilization of ruminants, but also contributes to environmental pollution (Eckard et al., 2010). Hence, the purpose of this current review was to provide the scientific insights into the use of dietary tannins to reduce enteric  $CH_4$  emission which, in turn, might minimize the effect of global warming and improve ruminant production.

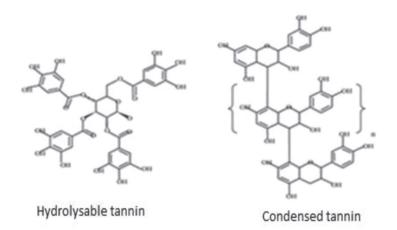


Figure 1. Chemical structure of hydrolysable and condensed tannin

## Local forages as feed additives

Several countries face the problem of fodder scarcity for livestock especially during winter season (dry season) (Gxasheka et al., 2015). Therefore, tannins containing feedstuff and crop byproduct supplementation is a common practice in rural regions of several countries including Pakistan, China and South Africa (Gxasheka et al., 2015). Generally, corn stock is a commonly fed supplement during the time of fodder scarcity (Beyene et al., 2014). Trees and bushes present in hot climatic regions are efficient feed resources for livestock (Belachew et al., 2013). But, small scale farmers use these feedstuff with poor quality fodder as feed additives to increase the palatability of feed due to its high protein content which is easily available and provides green roughages to ruminants (Yisehak et al., 2012). Some studies have used acacia species (*A. karroo, A. nilotica, A. tortilis, A. galpinii, A. sieberiana, A. hebeclada* and *A. rhemniana*) as feed supplement in farm animals (Table 2) (Ngambu et al., 2013).

All acacia species contain more than 100 g/kg DM of crude protein which is suitable to fulfill the requirements for ruminants (Jamala et al., 2013). A study observed that goat fed *A. karroo* had improved growth rate (Ngambu et al., 2013). These plant species increase feed palatability that are highly efficient for farm animals and provide good quality carcasses (Arsenos et al., 2009; Mapiye et al., 2009). However, now there is need to efficiently manage these resources without exhausting them.

	Table 2. Tallillis	70 III plant parts of A	cuciu species	
Species	% Phenolics in plant part before precipitation	% Phenolics in plant part after precipitation	% Tannins in plant part (hide-powder)	References
Acacia nilotica (Leaf)	14.00	2.20	11.80	Eldin et al. (2014)
Acacia seyal (Leaf)	7.00	0.69	6.31	
Acacia nilotica (Bark)	11.00	0.53	10.47	
Acacia seyal (Bark)	12.75	0.60	12.15	
Acacia Senegal (Bark)	4.25	0.76	3.49	
Acacia nilotica (Mature fruit)	24.75	2.60	22.15	
<i>Acacia nilotica</i> (Immature fruit)	24.50	2.40	22.10	

Table 2. Tannins % in plant parts of Acacia species

Total phenolic concentration comparative to tannic acid (TA).

#### **Tannins adaptation**

Adaptation to tannins depends on animal breeding which show various responses to tannins feeding (Papanastasis et al., 2008). Mainly, cattle and sheep ingest tannins free fodder; they do not need to produce tannin-binding salivary proteins (TBSP) (Lamy et al., 2011), because CT adaptation plays a defense mechanism in ruminants, like extracellular excretions decrease the effect of tannins on bacteria, rather than tannin degradation (Mlambo et al., 2007). Therefore, feed intake is influenced by adaptation period, primarily during first 6 days or during 6 to 24 days (Salem et al., 2005).

## Effects of tannins on animal production

Plants that contain CT have been identified as alternative resources in ruminant nutrition (Figure 2) (Min et al., 2006; Bhattarai et al., 2016; Naumann et al., 2017). Numerous tannins containing feedstuff have various effects on animals body depending on their composition, concentration dosage and intake (Huang et al., 2017). A study reported that sheep consuming a CT (H. coronarium) diet had increased body weight compared to those consuming lucerne or perennial pasture (Iqbal et al., 2002). L. corniculatus given to sheep had improved production performance as compared to control group consuming fodder treated with polyethylene glycol (PEG) (Frutos et al., 2004). In addition, voluntary feed intake and animal production is also influenced by type of CT present in feeds. A study in lambs has revealed that growth rate was reduced from 140 g to less than 50 g/day by supplementation of 26 g/kg CT containing Ceratonia siliqua (carob pulp) (Priolo et al., 2000). Moreover, sheep given CT from L. pedunculatus had decreased carcass to fat proportion as compared to lucerne diet without CT. But, CT from H. coronarium (white garland-lily) 72 g/kg of DM showed no deleterious effect on lambs weight gain (WG) (Douglas et al., 1999; Piluzza et al., 2013). Besides, the live weight gain of male lambs was increased by supplementation of 37.5 mg/kg CT containing leaves of L. acapulcensis (García-Hernández et al., 2017). These variations in consequences depend on the animal's health, environment and housing as well as tannin concentration, composition, and structural diversity.

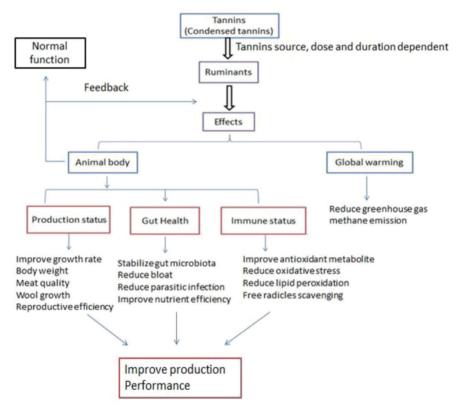


Figure 2. Effect of tannins on ruminant animal production (Carulla et al., 2005)

A feeding experiment has indicated that goats fed CT plants and Mediterranean shrubs (*Mirtus communis, Pistacia lentiscus, Quercus ilex, Arbutus unedo* etc.) had increased conjugated linoleic acid (CLA) and trans-vaccenic acid milk fat contents as compared to those animals grazed without CT feed (Vasta et al., 2008). However, some compounds other than CT can be involved in CLA production when Mediterranean shrubs were consumed by dairy sheep and goats. Another study has shown that lambs given *Hedysarum coronarium L*. (sulla) had no significant effects on meat CLA and trans-vaccenic acid contents (Priolo et al., 2005). It has proposed that *Hedysarum coronarium L*. (sulla) containing 18 g CT/kg DM diet was not sufficient to fulfill the requirements of ruminal bacteria responsible for biohydration of linoleic and linolenic acids (Priolo et al., 2005).

Moreover, lambs given 100 and 200 g/kg DM *Cistus ladanife* (grape seed extract) had improved color of fresh meat during prolonged refrigerated storage; this results was due to positive effects of dietary tannins on concentration of haem pigment and formation of metmyoglobin during storage condition (Francisco et al., 2015). But, the phenomenon in which tannins can affect myoglobin concentration is unclear. Several authors have described that it may be due to structural diversity of tannins (Table 3) (Gómez et al., 2006; Jayanegara et al., 2011; Soltan et al., 2012). Hence, tannin plants need to be given consideration because their composition, properties and chemical reactivity have significant differences and thus there is need for better understanding the conflicting results achieved by CT containing plant resources.

Species	TP	СТ	СР	NDF	ADF	References
Lespedeza cuneata	_	20.0	10.3	40.1	39.9	Puchala et al. (2005)
Guazuma ulmifolia	2.8	4.7	10.4	42.5	29.5	Gómez et al. (2006)
Acacia farneziana	10.0	4.5	24.0	42.1	26.7	Gómez et al. (2006)
Swietenia mahagoni	20.7	8.6	11.2	28.1	22.2	Jayanegara et al. (2011)
Myristica fragans	18.1	7.2	10.1	38.0	36.1	Jayanegara et al. (2011)
Prosopis juliflora	2.9	0.04	17.9	49.4	38.4	Soltan et al. (2012)
Acacia saligna	9.1	6.3	13.8	46.5	42.8	Soltan et al. (2012)
Leucaena leucocephala	5.17	2.3	23.6	78.9	51.1	Soltan et al. (2013)

Table 3. Chemical composition of tannins containing plant species (% age)

#### Effects of tannins on nutritive value of forage legumes

Forages that contain tannins are beneficial and some can be detrimental to animals health (dose dependent) (Dey and Sarathi De, 2014). For example, high concentrations of tannins can reduce feed intake, carbohydrates metabolism and protein digestibility which can ultimately cause the loss of production.

Several authors suggest that tannins concentration of 20 to 40 g/kg is considered beneficial in small ruminants (Frutos et al., 2004). A study has observed that CT concentrations less than 50 g/kg DM were nutritious for ruminants without reducing feed intake and fiber digestion (Min et al., 2003; Yuxi et al., 2015). Whereas high amounts of CT, more than 50 g/kg, were harmful for ruminants which decreased feed intake and protein digestibility (Frutos et al., 2004). Tannins have different affinity for proteins and amino acids. For example, some herbivores adapt tanniferous feed by producing proline-rich protein saliva (Mole et al., 2015). Normally, CT form complexes with proteins that are not degraded over the pH range of 3.5–7.0, but dissociate at pH below 3.5 in the abomasum and anterior duodenum (Alipour and Rouzbehan, 2010; Mokni et al., 2017). These CT-protein complexes remain undegradable in the rumen and more amino acids are absorbed postruminally (Min et al., 2003; Soltan et al., 2012; Huang et al., 2017).

A study in Yucatán (Mexico) has indicated that ruminants given CT based *L. leucocephala* in a concentration of 46 g-eq of tannic acid/kg DM had reduced rumen degradable protein from 614 to 117 g/ kg of DM and rumen undegradable protein (RUP) was increased from 386 to 888 g/kg DM as compared to a grass without tannins (Pineiro-Vázquez et al., 2015). It was reported that increase in RUP from 416 to 464 g/kg DM was due to increased excretion of fecal nitrogen. Soltan et al. (2013)

demonstrated that feed containing 8.8 g-eq TA/kg DM had increased fecal nitrogen by 70.19% and decreased nitrogen excretion in urine by 12.9% (Soltan et al., 2013). In addition, steers fed 2.4% CT based sainfoin had reduced protein digestibility by 7% (Brinkhaus et al., 2017).

Various studies suggest that carob (Ceratonia siliqua) containing 25 g/kg CT can be harmful (dose and plants species dependent), but sainfoin containing 80 g/kg CT had beneficial consequences (Mueller-Harvey, 2006). Various feeding trials have reported that CT concentrations less than 50 g/kg are beneficial for only Lotus species and it can be deleterious to other plants. Schofield et al. (2001) and Huang et al. (2011) have revealed that CT affinity for protein can be different dependent on the plant resources (Huang et al., 2011; Schofield et al., 2001). This shows that most CT-protein complexes may not fully degrade post-ruminally. Therefore, certain CT plants cannot enhance the supply of digestible protein. In vivo study has indicated that sheep fed CT extract from Acacia aneura (mulga, native forage tree of Australia) and Leucaena pallida (guaja, native forage tree of Mexico) diet had no visible effect on protein digestibility (Andrabi et al., 2005). Normally, high protein digestibility was observed which indicates the complete detachment of the tannin-protein complexes. However, CT containing L. corniculatus given to small ruminants had reduced degradation of plant methionine and cysteine in the small intestine of sheep. The proportion of digestion was less in the proximal part and was higher in the distal part of the small intestine (McNabb et al., 1993; Bunglavan and Dutta, 2013; Pathak et al., 2017). A similar study has reported that ruminants supplemented with CT containing Lotus corniculatus (birdsfoot trefoil) and Hedvsarum coronarium (sulla) had increased the absorption of essential amino acid (EAA) in the small intestine, however these outcomes were not observed in L. pedunculatus (big trefoil) and Onobtychis viclifolia (sainfoin) due to structural differences of CT (Min et al., 2003).

#### Effects of tannins on rumen microbiota

Tannins have different effects on rumen microbiota due to its diverse concentration and composition (Patra and Saxena, 2011). A study has indicated that sheep supplemented with perennial ryegrass (*Lolium perenne*), white clover (*Trifolium repens*) and *L. corniculatus* (32 g CT/kg DM), had decreased the population of proteolytic rumen bacteria (*Clostridium proteoclasticum, Eubacterium sp., Streptococcus bovis* and *Butyrivibrio fibrisolvens*) (Min et al., 2006). Tannins acceptance for microorganism depend upon the species and properties of tannin (Petek and Dikmen, 2006). Some *in vitro* studies investigated the growth and proteolytic activity of eleven ruminal samples and they suggested that steers fed *L. corniculatus* containing CT diet had transiently increased the growth of some ruminal bacteria (*C. proteoclasticum* and *Ruminococcus albus*) at lower concentration (50–100 µg/mL) but no effects were observed at higher concentrations of CT (more than 200 µg/mL), whereas nine strains of ruminal bacteria were more susceptible to CT at low concentrations (Min et al., 2005; Bunglavan and Dutta, 2013; Jonker and Yu, 2017).

Plant source	Effects	References
CT containing L. corniculatus	Decreased proteolytic rumen bacteria, <i>Clostridium proteoclasticum, Eubacterium</i> sp., <i>S. bovis</i> , and Min et al. <i>B. fibrisolvenes</i> (2005 a, t	1 Min et al. (2005 a, b)
CT containing Lotus corniculatus	Reduced populations of four proteolytic bacteria, but ruminal microbial protein were unaffected	Smith et al. (2005)
CT containing Calliandra Calothyrsus	Affect ruminal enzymes by altering the process of fermentation and by bacteriostatic and bactericidal activities; Reduced <i>Ruminococcus</i> spp. and <i>Fibrobacter</i> spp. but fungi, protozoa and proteolytic bacteria were less affected	Kumar et al. (2014) e
Hydrolysable tannins	Hydrolysable tannins are metabolized to gallic acid by ruminal microflora, no nephrotoxic or hepatotoxic effect were reported in animals	
Chestnut (HT) and quebracho (CT) extracts	Decreased Firmicutes populations and increased Bacteroidetes populations	Juan et al. (2017)
CT containing Onobrychis vicitfolia	Inhibited the growth of <i>Streptococcus bovis</i> and <i>Butyrivibrio fibrisolvens</i> ; Less effect on <i>Ruminobacter amylophilus</i> and <i>Prevotella ruminicola</i>	García-Hernández et al. (2017)
HT containing chestnut CT containing legume forages	Influenced Butyrivibrio fibrisolvens and Butyrivibrio proteoclasticum Inhibit cell growth and division of ruminal microorganisms such as Butyrivibrio fibrisolvens	Buccioni et al. (2015)
Tannins containing plants	Increased <i>Ruminobacter</i> and decreased <i>Prevotella</i> from 21.9% to 16.5% of total microbiota; Increased <i>Firmicutes/Bacteroidetes</i> ratio to affects energy metabolism and body fat accumulation in milking cows and steers; Stabilize rumen bacterial diversity; Modulate ruminal cellulolytic activity through <i>Ruminococcus</i> ; Affect <i>Treponema</i> (1.21 to 0.41%) and <i>Fibrobacter</i> (0.10 to 0.005%) population	Carrasco et al. (2016)
Grape marc (skin, stalk and seed); Altered bacterial Hydrolysable tannins (extracted from gallnut) were unaffected; with rapeseed oil; No effect on run	Altered bacterial and methanogen archaeal population; ruminal fungal and protozoan communities Moate et al. (2014); Abo were unaffected; (2014); Abo offect on rumen biohydrogenation et al. (2017)	: Moate et al. (2014); Abo-Donia et al. (2017)
Chestnut ( <i>Castanea sativa</i> ) and quebracho tannins ( <i>Schinopsis lorentzii</i> )	Reduced Prevotella and Fibrobacter; Enhanced Ruminococcaceae and Firmicutes; Negative effect on ureolytic genera, Butyrivibrio and Treponema	Juan et al. (2017)

#### Effects of tannins on ruminant species

Tannins-containing diet improves the efficiency of protein digestion, especially rumen escape protein and increases daily weight gain (DWG) (Barry and Mcnabb, 1999; Min et al., 2003). Min et al. (2003 and 2005) described that steers consuming wheat forage with addition of 1.5% CT (quebracho) increased average daily gain (ADG) by 15% (Min et al., 2003 and 2005) and cattle consuming wheat forage and plant tannins had improved body weight gain due to modification of rumen metabolites and microbial populations (Table 4) (Min et al., 2003 and 2005). Generally, sheep consuming CT-containing diets reduced proteolysis of high quality forage, but increased rumen by-pass protein (Min et al., 2003). A study observed that feedlot heifers given corn and CT supplemented feed had increased feed intake, average daily gain and total weight gain (Rivera-Mendez et al., 2016).

Tannins have potential to increase protein supply and reduce parasitic infection in domestic animals. Hervás et al. (2003) found that in sheep, CT extract diet (quebracho) fed at less than 1.5 g/kg LW (live weight) or less than 83 g/kg DM/day was not toxic for digestive tract and plasma profile (Hervás et al., 2003). However, feeding sheep CT up to 3 g/kg LW or 188 g/kg/DM/day was harmful for plasma metabolites and digestive track (lesions) (Hervás et al., 2003). Plasma urea concentration was suppressed in dairy cows by consumption of mimosa tannins (3.5 g/kg DM) but no negative effects appeared in milk production (Min et al., 2015). On the other side, no negative effect was observed in lambs by consumption of HT containing chestnut tannins (20.8 g/kg/daily DM) or (15–25 kg LW) (Petek and Dikmen, 2006). A similar study has revealed that heifers had increased ADG about 8% to 19% by addition of mimosa and chestnut tannins diet respectively, and no detrimental effect were observed on esophagus and blood profile. The ADG was increased due to reduction of parasitic infections such as *Cooperia* and *O. ostertagi* fecal parasites (Min et al., 2015).

In cattle, the addition of 1.5% mimosa and chestnut tannins per kg/DMI had no adverse effect on blood profile except plasma cholesterol level. Cattle consumed 1.5% chestnut tannins had increased plasma cholesterol level after 70 days trial, but these outcomes were not associated with mimosa tannins, indicating that HT may affect lipid metabolism by unidentified mechanisms. The above findings also indicated that feeding low level tannins (less than 1.5% tannins/DMI) to ruminants had no disadvantageous effects. Tannins protect the gut mucosa from pathogens and oxidative damage. Furthermore, tannins reduce the peristaltic movement during indigestion which subsequently prevent the incidence of diarrhea in young calf (Gai et al., 2011).

#### Effects of tannins on intestinal absorption

Generally, several studies mentioned that CT reduces rumen degradability of protein and enhances the availability of protein absorption in the small intestine (Iqbal et al., 2007; Getachew et al., 2008; Pathak et al., 2017). In high producing animals, low concentration of CT increases supply of essential amino acids (EAA) in the intestines without influencing the feed intake, milk let-down and feed conversion ratio (Wanapat, 2003; Soltan et al., 2012). However, the absorption of EAA was reduced at higher CT concentrations. The limited concentration of tannins enhance protein metabolism inside the abomasum and small intestine, and improve the absorption of amino acids (Min et al., 2003; Wanapat, 2003). In addition, microbial protein flow to small intestines does not decrease by tannins; thereby CT-protein complexes make protein unavailable for rumen degradation and provide protein supply to small intestine for maximum absorption (McSweeney et al., 2001; Pathak et al., 2017), which is essential for lactating cows.

#### Effects of tannins on wool growth

Several authors have observed that sheep consuming CT feed had increased weight and fleece growth due to CT effect on the absorption of sulfur containing amino acids (SAA) (Min et al., 2001; Mueller-Harvey, 2006; Bunglavan and Dutta, 2013). Protein plays a key role in clean wool production, with a high cysteine quantity but amino acids containing sulfur have detrimental effect on wool production (Reed et al., 1990). CT containing diet can increase the loss rate of cystine in the blood plasma, mainly due to reducing losses of SAA in the rumen. CT supplemented diet can increase fleece growth due to increasing the absorption of SAA and various other EAA (Waghorn, 2008; Pathak et al., 2017). However, CT from L. corniculatus had beneficial effects on wool production at 22-38 g CT/kg DM (Min et al., 2003). Whereas, CT containing sulla and L. pedunculatus had deleterious effects at > 50 g/kg DM. On the other hand, the response of wool growth was variable when CT quantity was less than 22 g CT/kg DM. According to Priolo et al. (2000), wool growth was increased in lambs by supplemented CT extract from L. corniculatus at dose of 35 g/kg DM. The results variation was due to differences in chemical composition, concentration and dosage of CT, which can affect the biological activity (Pathak et al., 2017).

#### Effects of tannins on milk secretion

Tannins have diverse effects on dairy animals. In dairy cattle, the addition of L. corniculatus had increased milk production by 60% and protein quantity by 10% compared to control group (without CT) (Table 5). Min et al. (2003) has reported that CT based L. corniculatus given to milking ewes had no effect on milk let-down in early lactation, but secretion rates of whole milk, protein and lactose were enhanced by 21, 14 and 12% during mid and late lactation, respectively (Min et al., 2003). In lactating animals, CT had increased milk quantity but there was no effect on feed intake (Min et al., 2003).

Furthermore, CT at 35 g/kg DM purified from *L. corniculatus* had increased milk production in lactating ewes and wool growth in lambs without causing injurious effects on feed intake, milk protein or lactose and fat percentage (Berard et al., 2011). A study has exposed that quebracho (QT) and chestnut tannins and/or grape seeds containing dietary phenols with soybean oil supplemented to small ruminants had increased linoleic, vaccenic and rumenic acids concentration and reduced the concentration of saturated fatty acids in ewes milk (Buccioni et al., 2015). Several reports have investigated that CT had increased milk yield in small ruminants as compared to control group (Vasta et al., 2008; Salem, 2010). In goats, the milk yield/day was increased by supplementation of 20% grape seed skin extract (GSSE) after 20 days of treatments (Mokni et al., 2017). In the above study, GSSE was composed of flavonoids (25.42%), non-flavonoids (74.57%) and tannins (5.25%) (Mokni et al., 2017). Specifically, grape

seed extract (GSE) increased the milk production in dairy cows, due to modification of ruminal metabolism (Gessner et al., 2015). Dey and Sarathi De (2014) also described that dairy cows given CT had improved milk yield. Some authors explored that CT based *Acacia mearnsii* (bark of black wattle tree) had no effects on milk production in dairy cows (Gerlach et al., 2018 a, b). Furthermore, decreased milk yield was observed in ewes supplemented with QT, whereas acacia species consumed by ruminants had also no effects on milk production. Several other studies claim that protein protection from microbial degradation results in improved milk production in dairy cows, sheep and goats (Attia et al., 2016). These various consequences were expected due to different chemical composition, properties and chemical reactivity of tannins which in turn deviate the rumen microbial ecosystem and volatile fatty acids concentration (Minieri et al., 2014; Carreno et al., 2015).

#### Tannins as an alternative to antibiotics growth promoters

Antibiotic growth promoters cause microbial resistance in food animals which produce threats to human health (drug resistance). Therefore, plant secondary compounds (tannins) have been classified as an alternative to antibiotic growth promoters which might prevent diseases and enhance production in ruminants (Huang et al., 2017). Ideal antibiotic feed alternatives should have the same beneficial effects of antibiotics growth promoters (AGPs), such as positive effects on animal performance (average daily gain), antibacterial activity, antioxidant property, improvement of the nutrient availability, gut microbiota and immunity (Huang et al., 2018).

The European Medicines Agency (EMA) and the European Food Safety Authority (EFSA) have suggested that these compounds have antimicrobial and growth promoting characteristics. They have potential to promote animal growth depending on plants efficacy, dosage, composition and concentration. In cattle, some studies showed that due to lack of sufficient data it is difficult to reach a final decision regarding tannin plants as growth promoters (EFSA, 2014). But, several other studies revealed that tanniferous plants can reduce the incidence of diarrhea and improve the gut health in young calves (Hook et al., 2010). These feedstuffs are required at higher concentrations in feed (to achieve antimicrobial effects) to increase body weight gains. However, higher concentration negatively affects meat quality.

The possible mechanisms proposed to understand antimicrobial activity of tannin is due to direct action on microbial metabolism, inhibition of microbial enzymes, formation of complexes with bacterial cell membrane, deprivation of metal ions or deficiency of the substrates essential for microorganism (Liu et al., 2013). Various evidences have revealed that tannins possess antimicrobial ability due to its inhibitory action on microbial cell membrane (McAllister et al., 2005; Liu et al., 2013) through cell aggregation and/or disruption of cell membranes. However, protein precipitation is a universal property for all dietary tannins, but anti-microbial ability of tannins is microbe speciesspecific that is closely associated to the composition and chemical structure of tannins. Normally, dietary tannins have greater anti-microbial activity against Gram-positive bacteria as compared to Gram-negative bacteria (Smith and Mackie, 2004) because of an outer membrane of Gram-negative bacteria that possess a lipid bilayer structure (lipopolysaccharide and proteins) and an inner layer composed of phospholipids.

Plant source	Species	Effects	References
	Sheep	Reduced protein degradation in the rumen and increased milk production	Min et al. (2003)
CT containing leaves of Ficus bengalensis	Cows	Positive impact on milk production	Dey and Sarathi De (2014)
Tannin containing plants	Ruminant	Negative effect on milk fatty acid composition, depending on the concentration of condensed tannins	Kumar et al. (2014)
CT containing chestnut	Dairy ewes	Slightly increased milk production; Increased concentration of linoleic (LA) and $\alpha$ -linolenic acid ( $\alpha$ -LNA) in milk; Increased in total solids and casein fraction profile were unaffected	Buccioni et al. (2015)
Water-soluble quebracho (CT) extract from bark of Dairy cows Shimopsis spp	Dairy cows	Increased concentration of milk true protein at 0.45% CT extract; Minor effects on milk composition by supplementation of CT extract at 3% DM	Dschaak et al. (2011); Aguerre et al. (2016)
HT extract (gallnut) with rapeseed oil	Ewes	Increased production of <i>trans</i> -9, <i>trans</i> -11 CLA isomer. Chemical composition of milk were unaffected in ewes, milk fatty acid composition was not affected, milk composition depends on concentration of CT	Abo-Donia et al. (2017)
HT containing castalagin and vescalagin	Dairy cows	Improved milk quality, milk yield, and udder health	Ali et al. (2017)
Grape seed and skin supplement (GSSE)	Dairy ewes	Improved milk yield after 20 days of treatment	Mokni et al. (2017)
CT containing bark of A. mearnsti	Dairy cows	Reduced milk protein yield; Milk yield was unaffected with supple- Katrin et al. (2018) mentation of CT during 63 day trial	Katrin et al. (2018)
CT containing grape marc (by-product of skin, stalk, Dairy cows stem and seed)	Dairy cows	Milk production was unaffected; Reduced milk fat yields, Decreased Moate et al. (2014) saturated fatty acids concentrations; Improved concentrations of mono- and polyunsaturated fatty acids, especially cis-9,trans-11 linoleic acid	d Moate et al. (2014)

		Table 6. Antimicro	Table 6. Antimicrobial effects of tannins containing plants	containing plants		
	Tannins based plant species ( <i>Terminalia arjuna</i> )	plant species <i>a arjuna</i> )	Tannins based (Catharant	Tannins based plant species (Catharanthus roseus)	Tannins based plant species ( <i>Piper betel</i> )	plant species betel)
Bacterial strains	Antimicrobial effects	ial effects	Antimicrol	Antimicrobial effects	Antimicrob	Antimicrobial effects
	Aqueous extract	Acetone extract	Aqueous extract	Acetone extract	Aqueous extract	Acetone extract
B. subtilis	Negative	Positive	Negative	Negative	Negative	Positive
S. aureus	Positive	Positive	Negative	Negative	Positive	Negative
Ent. fecalis	Negative	Positive	Positive	Negative	Negative	Negative
M. luteus	Positive	Positive	Negative	Negative	Negative	Negative
E. coli	Negative	Positive	Positive	Negative	Negative	Negative
K. pneumoniae	Negative	Positive	Positive	Negative	Negative	Negative
Sal. typhi	Negative	Positive	Negative	Negative	Negative	Negative
Sal. paratyphi B	Negative	Positive	Negative	Negative	Positive	Positive
Sh. flexneri	Ι	Positive	Negative	Negative	Positive	Positive
Ps. aeruginosa	I	Ι	Negative	Negative	Negative	Negative
P. vulgaris	I	Negative	Negative	Negative	Positive	Positive
Asp. niger	I	Negative	Negative	Negative	Negative	Negative
C. albicans	I	Negative	Positive	Negative	Positive	Negative
Ser. marsescens	I	Negative	Negative	Negative	Positive	Negative
Reference: (Kurhekar, 2016)	r, 2016).					

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Conversely, some species of plants containing CT have strong activity against Gram-negative bacteria. Very clearly, some authors have investigated that all pathogenic bacteria including *Salmonella*, *Escherichia coli* O157:H7, *Shigella*, *Pseudomonas*, *Helicobacter pylori* and *Staphylococcus* were sensitive to tannins supplementation (Doss et al., 2009; Banso and Adeyemo, 2007; Liu et al., 2013). Wang et al. (2013) focused 12 tannins species and observed that only CT based purple prairie clover (*Dalea purpurea* Vent) and phlorotannins (PT) from brown algae (*Ascophyllum nodosum*) possessed strong anti-*E. coli* and anti-*E. coli* O157:H7 activity. Another study has exposed that PT have greater antimicrobial activity compared with CT and HT (Wang et al., 2009). It has been proposed that antimicrobial property of tannins depend on the number of hydroxyl groups and liberation of hydrogen peroxide during oxidation of tannins (Mueller-Harvey, 2006).

A similar study has found that HT extracted from *Rhizophora apiculata* can be used as potential agent against yeast (Lim et al., 2006). The antimicrobial properties of HT is linked with the hydrolysis of ester linkage between gallic acid, usually as multiple esters with D-glucose, which affects the synthesis of cell wall and cell membrane. Therefore, taken together, it has been concluded that screening, identification and source of tannins results in great diversity in their antimicrobial activities that can be effective and specific to target microbes depending on tannins species (Table 6).

#### Antiparasitic effects of tannins

CT from forages as alternatives to antiparasitic drugs provide beneficial effects (Carvalho et al., 2012). CT rich plants have beneficial effects on animal health due to its direct antiparasitic effects towards worm egg count, worm fecundity (no. of eggs) and egg hatchability (Figure 3) (Athanasiadou et al., 2001; Nguyen et al., 2005; Githiori et al., 2006; Alonso-Diaz et al., 2011; Mejia-Hernandez et al., 2014).

CT extract from sainfoin has mild anthelmintic (antiparasitic) effect on ruminant parasites (Table 7) (Heckendorn et al., 2007; Bhattarai et al., 2016). A study has reported that sainfoin had reduced egg hatchability of Trichostrongylus colubriformis and inhibited the egg development of nematodes and lungworm (Strongyloides spp., Ostertagia spp., Cooperia spp., Oesophagostomum spp. and Trichostrongylus spp.) (Molan et al., 2000; Yuxi et al., 2015). These consequences were dose dependent. Azuhnwi et al. (2013) observed that sainfoin reduced the shedding of Haemonchus eggs on pasture which can reduce chances of pasture contamination and prevent the gut parasitic infection (Azuhnwi et al., 2013). Another study exposed that heifers fed mimosa (Acacia mearnsii), and chestnut (Castanea sativa) tannins diet had reduced Cooperia and Ostertagia fecal egg count but H. contortus were unaffected (Min et al., 2015). Moreover, worm egg count and worm fecundity for Nematodirus battus and Trichostrongylus colubriformis (intestinal species) was reduced by feed application of CT plants in sheep, however there was no difference for Teladorsagia circumcincta and H. contortus (stomach and abomasum species) (Luque et al., 2000). It is possible that the efficacy of plant purified tannin against H. contortus was due to short residence time of larvae in gut ecosystem at less than 30 min (Pathak et al.,

2013), signifying that direct contact between tannins and nematodes might require to reduce worm load or larval development in host animals.

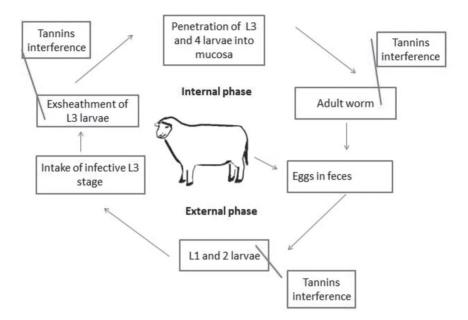


Figure 3. Effect of tannins on parasitic infection (Paolini et al., 2005)

	10010 /.	Effect of taining on gut parasite	
Plant source	Species	Effects	References
1	2	3	4
CT containing quebracho (Schinopsis sp.) bark extract	Goat	Decreased in the development of third larval stage of <i>Haemonchus contortus</i> ; Reduced worm fertility and egg count	
CT extract from legume tanniferous forages	In vitro	Reduced egg hatching and larval develop- ment of <i>Trichostrongylus colubriformis</i>	Molan et al. (2002)
CT containing plants	Sheep	Reduced worm numbers and worm fecun- dity for the intestinal parasite ( <i>T. colubrifor- mis</i> and <i>Nematodirus battus</i> ); No effect for the abomasum species ( <i>Haemonchus con- tortus</i> and <i>Teladorsagia circumcincta</i> )	
CT containing feedstuffs	Goat	Reduced adult worm fecundity of <i>T. colubriformis</i> and <i>H. contortus; T. circumcincta</i> were unaffected	

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Table /	Effect	ot	tanning	on	out	parasite

		Table 7 – contd.	
1	2	3	4
CT containing chicory (Cichorium intybus)	Sheep	Decreased number of <i>T. circumcincta</i> but <i>Trichostrongylus</i> sp were unaffected;	Molan et al. (2003)
	Young deer	Hindered the motility of <i>Dictyocaulus</i> sp (lungworm) first larvae (L1); No effects on the egg hatching and development of nematode larvae; Reduced the viability of lungworm and intestinal larvae; Reduced lungworm infections	
CT with a crude extract containing sesquiterpene lactones from chicory leaves	Deer	Reduced the motility of first (L1) and third (L3) larvae of lungworm; Hindered third larval (L3) mixed species of gastrointestinal	Jackson et al. (2006)
CT containing sulla ( <i>He-dysarum coronarium</i> )	Lamb	Decreased egg counts of Trichostrongylus colubriformis and Ostertagia circumcincta	Niezen et al. (2002)
CT containing extract from plants	Sheep	Decreased levels of parasitism; <i>In vivo</i> reduced infection of <i>T. colubriformis</i>	Athanasiadou et al. (2005)
Forage containing chicory ( <i>Cichorium intybus</i> ), sulla ( <i>Hedysarum coronarium</i> ) and lotus ( <i>Lotus pedun-</i> <i>culatus</i> )	Sheep	Reduced worm load of <i>T. circumcincta</i> and larval development	Tzamaloukas et al. (2005)
CT containing sainfoin	Goat	Reduced infections of nematode and in- creased resistance against nematode	Paolini et al. (2005)
Mimosa ( <i>Acacia mearn- sii</i> ) and chestnut ( <i>Casta- nea sativa</i> )	Heifers	Decreased <i>Cooperia</i> and <i>Ostertagia</i> fecal egg count; <i>H. contortus</i> were unaffected	Min et al. (2015 a, b)
CT containing <i>L. cornicu-</i> <i>latus</i>	Lamb	Strongyloid nematode parasites in lambs grazing <i>L. corniculatus</i> suggest a decrease in the degree of parasite control from the abomasum to the rectum	Piluzza et al. (2013)
CT-containing forages ( <i>H. coronarium</i> and <i>L. pedunculatus</i> )		Reduced worm burden of strongyloid nem- atode from the abomasum to the rectum; Enhanced resistant to parasite infection and reduced gut worm load	
CT containing leaves of Lysiloma acapulcensis	Lamb	Decreased the parasitic infection and fecal egg counts at dose of 37.5 mg/kg BW	García-Hernán- dez et al. (2017)

Table 7 – contd.

Ruminant fed mimosa and chestnut tannins diet had decreased *Cooperia* fecal eggs count by 8 to 13%, respectively. However, heifers fed chestnut tannins had 57% reduced *Ostertagia* fecal eggs count at day 41 when compared with control group (without CT feed) (Min et al., 2015). A trial in sheep has observed that plant extracts containing *C. quadrangularis* had reduced egg hatchability 88% at concentration of 1 mg/ml. Whereas, ruminants given *Schinus molle* caused 95% mortality of adult parasites at concentration of 10 mg/ml and reduced by 96% egg hatchability for *H. contortus* at concentration of 1 mg/ml (Zenebe et al., 2017). The mechanism of

action how CT produce anthelmintic effect is still unknown, but there is a possibility that CT cause paralysis, worms mortality and/or hindrance in worms (larval stage) motility in gastrointestinal tract.

#### Anti-bloat effects of tannins

CT extract from legume forages (*Lotus corniculatus, Coronilla varia, Onobrychis vicciifolia* or *Astragalus cicer* L.) fed sole diet or mixed with bloat forming fodder play a key role in the prevention of ruminal bloat by making protein-complexes in the rumen (Mueller-Harvey, 2006; Rochfort et al., 2008; Yuxi et al., 2012). Tanniferous fodder such as sainfoin, *Lotus* spp. does not cause bloat at low concentration of 1–5 g/kg (Abeynay-ake et al., 2011). The exact mechanism how tannins prevent bloat is still unclear. But, it is proposed that CT prevent bloat by inhibition of slime-producing bacteria or disrupting the reaction of proteinaceous frothy foam (Ehsan et al., 2013; Addisu, 2016). Therefore, tannin-containing plants have been considered in ruminants production.

## Enteric methane emission

Methane production is a normal and important process in ruminants (Figure 4). Hydrogen (H<sub>2</sub>) is produced in the rumen during fermentation and methanogens use H<sub>2</sub> as an energy source (Janssen, 2010), which results in CH<sub>4</sub> (methane) formation. The microorganisms that produce methane as a byproduct of their respiration are called methanogens and the whole process of CH<sub>4</sub> formation is called methanogenesis (Jafari et al., 2019). CH<sub>4</sub> is produced as a result of feed fermentation in the rumen. However, about 89–90% of CH<sub>4</sub> is produced in the rumen and exhaled by the mouth and nose (Kempton et al., 1976; Kumar et al., 2014 a, b). A study has reported that in young calf, the process of CH<sub>4</sub> production and expulsion begins at the age of 4 weeks when the reticulorumen starts to develop (Jafari et al., 2019). CH<sub>4</sub> is more hazardous than CO<sub>2</sub> which causes climatic changes and affects the global warming (Bodas et al., 2012). Hence, there is need to find alternative feed additives to mitigate enteric CH<sub>4</sub> emission in order to secure green environment and livestock production.

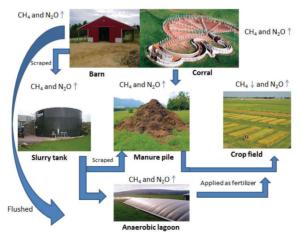


Figure 4. Mechanism of methane production and emission (Jafari et al., 2019)

#### Dietary tannins application to reduce methane emission

Mitigation opportunities to reduce  $CH_4$  production is a big challenge. Several techniques of  $CH_4$  inhibition have been used to mitigate the enteric  $CH_4$  (Patra and Yu, 2014). But, some of them have harmful effects on ruminant microbiology and fermentation especially at high concentration (Patra and Yu, 2013). Moreover, various  $CH_4$  inhibitors are toxic to ruminants (Patra and Yu, 2012). In the meantime, modern consumer demands to use natural products like phytochemicals to modify rumen ecosystem. More than 200,000 plant secondary metabolites (PSM) structures have been identified (Hartmann, 2007), but most of PSM have been categorized into three main classes: tannins, essential oils and saponins (Bhatta et al., 2014). Especially tannins such as HT and CT are complex class of PSM that have complex structures and biological activities (Bhatta et al., 2009).

From the last few decades, the utilization of tannins in animal nutrition has been addressed in the previous in vitro and in vivo studies. A study has recorded that about 25% of the global anthropogenic methane (CH<sub>4</sub>) emission is produced by enteric fermentation in the animals, and this proportion has increased almost 50% in the rural societies, which contribute more than 90% by rumen fermentation (Abberton et al., 2008; Kumar et al., 2014 a, b; Jafari et al., 2019). Greenhouse gases such as methane (CH<sub>4</sub>), carbon dioxide (CO<sub>2</sub>), nitrous oxide (N<sub>2</sub>O), and ozone (O<sub>2</sub>) cause environmental variation and affect the global warming by infrared radiation in the atmosphere (Lashof and Ahuja, 1990). Methane is classified as a trace gas that is expected to have a worldwide concentration of  $1774 \pm 1.8$  parts per billion (ppb), with overall increase of 11 ppb since 1998 (Hook et al., 2010). Specially, it is an intoxicating trace gas due to its global warming action, 25 times higher than CO<sub>2</sub> with 12 year atmospheric lifetime (Ellis et al., 2007; Hook et al., 2010). Thus, CH<sub>4</sub> is considered as the second largest anthropogenic greenhouse gas after CO<sub>2</sub> (Hook et al., 2010). It has been reported that 50–60% of CH<sub>4</sub> emissions is produced from agricultural areas, particularly from livestock and ruminants are categorized as the primary source of CH<sub>4</sub> production (Ellis et al., 2007; Hook et al., 2010).

Numerous studies have indicated that 80 million tons  $CH_4$  are produced annually worldwide (Eckard et al., 2010), of which almost 47% are contributed from agricultural areas and 39% from livestock (Ellis et al., 2010; Hook et al., 2010; Gerber et al., 2013). Comparatively, ruminants are higher producers of  $CH_4$  as compared to monogastric animals (Gunun et al., 2017). Farm animals such as ruminant species (cattle, sheep, and goats) produce almost 86 million metric tons (Tg)  $CH_4$ /year (McMichael et al., 2007). Another similar study has observed that almost 18.9 Tg, 55.9 Tg and 9.5 Tg  $CH_4$  is produced from dairy cattle, beef cattle, and small ruminants (sheep and goats), respectively (McMichael et al., 2007). Johnson and Johnson (1995) have provided data of annual global  $CH_4$  production. The above authors have exposed that 6.2-8.1 Tg and 0.9-1.1 Tg  $CH_4$  are produced from buffalo and camels, respectively.

 $CH_4$  emissions undergo a loss of 2–12% of gross energy intake in ruminants that contributes to the global warming (Wanapat et al., 2015). Another study has reported that cattle produce 60-160 kg  $CH_4$ /year and small ruminants (sheep and goats) produce 10–16 kg  $CH_4$ /year, depending on feed types, particle size and dry mater intake (DMI) (Hristov et al., 2013). Mueller-Harvey (2006) examined that

ruminants consuming highly nutritive diet increase the greenhouse gases (Mueller-Harvey, 2006). Puchala et al. (2005) reported that ruminants fed CT extract from *L. cuneata* had 180 g/kg reduced  $CH_4$  as compared to those plants containing *Digitaria ischaemum* (Schreb.) and *Festuca arundinacea* (Schreb). A study has revealed that tannin containing *L. pedunculatus* and *Salix caprea* (goat willow) diet decreased  $CH_4$  emissions from the rumen (16 to 20% per unit intake) (Waghorn et al., 2002; Wallace et al., 2002). However, *L. corniculatus* based silage also reduced  $CH_4$  by 23% per unit intake (g  $CH_4$ /kg DM intake) and by 13% per unit production (g  $CH_4$ /kg milk solids) (Woodward et al., 2001). Conversely, some studies have explained that tannin containing diets can reduce methane emissions from ruminants.

Several authors have discussed that CT and saponins have ability to reduce enteric  $CH_4$  emission (Figure 5) (Wanapat et al., 2014; Anantasook et al., 2016; Gunun et al., 2017). A trial in ruminants has observed that dietary rambutan peel (RP) had significant effect on protozoa and methanogens via modification in the rumen ecosystem (Gunun et al., 2018). In the above study, RP (*Nephelium lappaceum* L.) was composed of CT and saponins (Gunun et al., 2018). Ruminants supplemented with 16 mg RP increased the rumen metabolism and reduced methane emission. Furthermore, addition of CT containing mangosteen peel in ruminants fed at dose of 2–6% DM had decreased total gas production (Paengkoum et al., 2015). *In vitro* study has observed that 8% addition of CT plants extracts in feed reduced CH<sub>4</sub> by 30% (Denek et al., 2017). CH<sub>4</sub> and protozoal population was reduced by addition of RP with 0.2–0.6% saponins (Gunun et al., 2018).

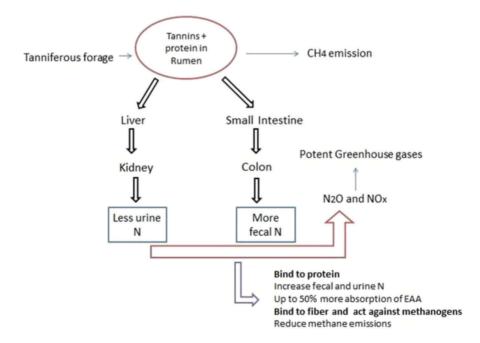


Figure 5. Effect of tannins on methane emission (Patra et al., 2016)

However, it is very important to note that methanogenesis can be reduced by hindering the production of  $H_2$  and methanogens by the application of CT (Tavendale et al., 2005; Pathak et al., 2017). Bhatta et al. (2009) observed that methanogenesis in rumen can be inhibited primarily by decreasing the methanogen or the protozoal population by supplementation of tannins rich plants. Furthermore, it has been observed that both tannins supplementation (HT and CT) have potential to inhibit methanogenesis as compared to those diets without tannins supplementation. In another study, Guglielmelli et al. (2011) reported that plant phenoloic forage (sainfoin) given to ruminants had decreased *in vitro* CH<sub>4</sub> production. While gallotannins feed containing HT caused 50% hindrance of CH<sub>4</sub> production with less injurious effect on ruminants as compared to monomers vs. polymers plants (Bhat et al., 1998).

*In vitro* study investigated that CT based *Acacia cyanophylla* supplemented at 60% and 30% reduced  $CH_4$  production by 37.5% and 56.25%, respectively (Rira et al., 2015). The results were attributed to high CT concentration in *Acacia cyanophylla* which was recorded toxic for rumen microbes, particularly methanogens, ciliate protozoa and fiber degrading microbes (Kamra et al., 2006). Moreover,  $CH_4$  production was inhibited by *Acacia cyanophylla* supplementation due to modification in the profile of volatile fatty acid and increases in the concentration of propionate (Kamra et al., 2006). Jayanegara et al. (2015) described that tannins containing plants such as sumac, chestnut, quebracho and mimosa given at concentration of 1 mg/mL of rumen liquid decreased ruminal  $CH_4$  emissions, which was in accordance with previous results which stated that tannins have inhibitory effect on rumen methanogenesis (Jayanegara et al., 2012). Thus, both *in vitro* and *in vivo* studies concluded that increased tannin concentration (0 to 177 g/kg) significantly decreased  $CH_4$  production (Jayanegara et al., 2012).

Interestingly, all studies have confirmed that both HT plus CT plants were effective in reducing in vitro CH<sub>4</sub> production as compared to only HT based diet (Bhatta et al., 2012). But, tannin extracts containing phenolic fractions were more effective compared with plant leaves comprising tannins (Bhatta et al., 2009). A study by Bhatta et al. (2014) exposed that tannins have direct inhibitory effects on methanogenesis by affecting rumen specific microorganisms named archaea because protozoa provides H, to methanogens as a source of electrons and therefore, tannins reduce CH, production by its antiprotozoal activity against methanogens. The effect of CT and HT was different on ruminal protozoa due to less inhibitory action of HT against protozoa as compared to CT (Śliwiński et al., 2002; Pinski et al., 2015). However, CT concentration at 50 g/kg of DM did not affect the parameters of ruminal fermentation. Beauchemin and McGinn (2006) also reported that addition of quebracho tannin extract at 2% (1.8% CT) of DM was not suitable to reduce  $CH_4$ production. However, another in vitro and in vivo study by Jayanegara et al. (2012) revealed that higher tannin concentrations at 177 g/kg reduced CH<sub>4</sub> production. It is proposed that the difference in results was due to tannin supplement sources, concentration, composition, dosage and the period of tannins adaptation.

Very specifically, another study showed that lambs supplemented with tannins based plants leaf mixture (*Ficus infectoria* and *Psidium guajava*) at concentration of 1% to 2% had significantly reduced  $CH_4$  emission (Dubey et al., 2011). It has

been proposed that the positive effect of tannins was probably due to the following three reasons i) direct effect on methanogens growth in the rumen (Williams et al., 2011) ii) indirect effect on methanogens by less availability of hydrogen for rumen microorganisms (Patra, 2010) iii) inhibitory effect of CT on methanogenesis that can reduce the ratio of acetate to propionate (Hatew et al., 2016), which may increase transfer of hydrogen to propionate. They concluded that tannins could affect rumen methanogenesis without affecting the other fermentation parameters and their impact on rumen fermentation was different according to their specific type, source and concentration. Therefore, it has been now confirmed by several studies that  $CH_4$  production can be reduced by inhibiting the population of protozoa and methanogenes through supplementation of tanniferous forages (Table 8), which can be helpful to improve livestock production and control climatic changes in the future (Anantasook et al., 2015; Pathak et al., 2017).

Plant source		Effects	References
1	2	3	4
CT containing Acacia mearnsii	Sheep	13% reduced methanogensisis	Carulla et al. (2005)
Condensed tannin extract ( <i>Schinopsis</i> <i>quebracho-colorado</i> ) and tannin containing sorghum silage	Cattle	No effect on methanogenesis	Beauchemin et al. (2007) De Oliveira et al. (2007)
CT based L. peduncula- tus or L. corniculatus	In vivo (ruminants)	Reduced methane (20–30%) per unit of digestible dry matter intake (DDMI); Reported HTs and CTs toxicity towards methanogens in methanogenic digesters	
CT containing Callinada calothyrsus and Fleminga macro- phylla	Lambs	24% reduced methane emission	Tiemann et al. (2008)
CT containing mimosa and quebracho tannin HT containing chestnut	Sheep	Affect all microbes but less effect on $N_2O$ and methane; Less effect on $N_2O$ and no effect on methane and microbes	Deaville et al. (2010)
Condensed tannin containing Lespedeza cuneata	Goats	Reduced methane about 57%	Hook et al. (2010 a)
CT containing sainfoin	Sheep	Affect methanogens but less effect on $N_2O$ and methane	Theodoridou et al. (2010)
Hydrolysable tannin supplementation	Ruminants	Reduce methane emission	Menezes et al. (2011)
CT containing grape- seed HT containing valonea HT containing my- rabolan	In vitro	Affect methanogens and all microbes but less effect on methane	Pellikaan et al. (2011)

Table 8. Effect of tannins on methane emission

1	2	3 4	
Condensed tannins (pine bark)	Goat	Decreased <i>Methanobrevibacter</i> popula- Min et al. (2014) tion	)
Condensed tannins in vitro	Bovine	Decreased <i>Methanobrevibacter</i> popula- Saminathan et al tion (2016)	l.
HT containing chestnut; CT containing quebra- cho	Steers	Reduce <i>Methanosphaera</i> and methano- Juan et al. (2017) genic archaea; Methanogens (phylum Euryarchaeota) were less abundant in steers (1.37 ver- sus 2.03%)	)
Grape marc (skin, stalk, stem and seed)	Dairy cows	20% reduced $CH_4$ emissions Moate et al. (201	(4)
HT containing chestnut and valonea extract	In vitro	Decreased methane emission (14 to Wischer et al. 17%) (2013)	

Table 8 - contd.

## Safety and risk associated with dietary tannins

Tannins as a natural alternative feed additive are safe (dose dependent) for all animals and have not reported any environmental hazards. On the other hand, in vitro trial has investigated genotoxicity in rats, but in vivo genotoxicity and oral exposure carcinogenicity has not been recognized. The European Food Safety Authority (EFSA) Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) also did not categorize tannins based reproductive toxicity. However, FEEDAP panel have reported that tannins cause hazardous effects in workers via inhalation or exposure through direct contact with the skin, eye and mucous membrane (EFSA, 2014). Several findings demonstrated that high concentration of chestnut tannins caused liver and kidney toxicity in ruminants, but no adverse effects were found on the liver, kidney, stomach, and the small intestine in neonatal pigs and rats during 28 days trial (Min et al., 2015). The European Food Safety Authority has suggested that tannins high concentrations as compared to recommended concentration cause toxicity such as toxic level of tannins supplementation in adult ruminants and young calf is >15000 and >1500 mg/kg feed, respectively, while safe level of tannins application in adult ruminants and young calf is 15000 and 1500 mg/kg feed, respectively. Taken together, various studies showed that 15 mg tannic acid/kg diet is safe for all animals (EFSA, 2014). Therefore, it is recommended that caution should be taken before the applications of tannins in animal ration in order to achieve the positive consequences.

## **Conclusion and recommendation**

Livestock production has significant role to eliminate poverty of millions of people and build a healthy society. Recently, the world is facing several challenges including food security, greenhouse effects, global warming and increasing global population. Antibiotic growth promoters cause microbial resistance in food animals and create food safety issues (drug resistance) in humans. Therefore, it is necessary to investigate natural alternatives to antibiotic feed additives. Regarding the above issue, tannins have been classified as an alternative to antibiotic growth promoters. Tanniferous plants have potential to improve ruminant production and minimize the problem of global warming. Tannins containing forages have antimicrobial, antiparasitic and anti-bloat characteristics which, in turn, may improve rumen fermentation, protein absorption, energy efficiency (reducing methane emission), milk yield and fatty acid composition which, in turn, enhance ruminant production. In addition, methane mitigation strategies may not only improve ruminant production but also reduce the contribution of global methane emission from livestock. Hence, it has been clearly reported that tannin associated beneficial and deleterious effects are dose, duration, source, concentration and composition dependent. Thus, preventive measures should be used before the applications of tannins in ruminant nutrition in order to achieve the optimum production.

#### Gaps in the literature and recommendation for future study

However, due to a lack of sufficient studies on the presence of hydrolysable tannins in leaves of trees and shrubs, further studies are needed to exploit the full potential of this approach.

Quantification *in vitro* of the tannin-induced protection of proteins from degradation in the rumen in combination with measurement of gas and microbial mass may predict effects of tannins in the rumen. Hence, an *in vitro* approach simulating the absorptive phase should be developed to study the effects of tannins on the absorption of nutrients.

Comparison of tannin levels (by using an array of methods) in a plant species grown under well-defined environmental conditions in a glasshouse will also advance knowledge on the factors controlling tannin biosynthesis. Thereby, comparison of accessions of the same types of trees/shrubs planted at different locations should be investigated to identify unequivocally the factors influencing tannin biosynthesis.

Tannins have a strong anti-oxidant activity but very little is known on the significance of tannins in ruminants with respect to their anti-oxidation property. Therefore, studies should be conducted to know the 'threshold level' of tannins in a feedstuff below which these have beneficial effects on livestock.

Furthermore, molecular biology may help to explore the structural diversity relationships of tannin forages. Such finding might be helpful for better understanding the nutritional properties of tannin-containing fodder in future.

## Abbreviation

ADG, average daily gain;  $\alpha$ -LNA,  $\alpha$ -linolenic acid; BCS, body condition score; CH<sub>4</sub>, methane condition score; CLA, conjugated linoleic acid; CO<sub>2</sub>, carbon dioxide; CT, condensed tannins; DM, dry matter; DMI, dry matter intake; EAA, essential amino acid; EFSA, European Food Safety Authority; FAO, Food and Agriculture Organization; FEEDAP, EFSA Panel on Additives and Products or Substances used in Animal Feed; GSE, grape seed extract; GSSE, grape seed skin extract; H<sub>2</sub>, hydrogen; HT, hydrolysable tannins; IPCC, Intergovernmental Panel on Climate Change; LA, linoleic acid; LW, live weight; QT, quebracho tannins; RP, rambutan peel; **RUP**, rumen undegradable protein; **TBSP**, tannin-binding salivary proteins; **WG**, weight gain.

# Declarations

## Ethics approval and consent to participate

According to rules and regulation of Animal Care Committee of Guangdong Ocean University (Guangzhou, People's Republic of China)

Consent for publication
Not applicable.
Availability of data and materials
Data sharing is not applicable to this article.
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Authors' contributions
AN wrote the main text of manuscript. GL and YN collected the data. LA have

AN wrote the main text of manuscript. GL and YN collected the data. LA have contributed to double checking grammar. YZ and MX participated in its design and coordination. ST and CS contributed to drafting and the revision of the paper. All authors read and approved the final manuscript.

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