*Himachal Journal of Agricultural Research 49(2): 141-154 (2023)*

# The potential use of tea seed and tea seed saponins in ruminants and poultry nutrition - A review

**Manjeet Kumar***

Department of Animal Husbandry
Veterinary Hospital, Thill, District Kangra-176 055, India.

*Corresponding author: dr.manjeet.13@gmail.com
Manuscript Received: 22.09.2023; Accepted: 31.10.2023

## Abstract

**The use of antibiotics as a feed additive in livestock feeding is controversial due to emergence of antibiotic-resistant strains that pose threat to both animal and human health. Phytochemicals such as saponins, have been tried as potential alternatives to conventional antibiotics for use as growth promoters in livestock and poultry feeding. Tea saponins are triterpenoids distributed in the roots, stems, leaves and seeds of the Camellia plant. Recent studies indicate that saponins derived from tea seed have the potential to increase feed digestibility and reduce methane emissions in ruminants. Tea seed saponins (TSS) improve rumen fermentation by killing protozoa and modulate rumen metabolism by influencing ruminal bacteria and fungi. Supplementation of tea seed saponins in animals feeding have shown to decrease the total blood cholesterol and trigyceride, increase the HDL–Cholesterol and improve the meat quality. Tea seed have been safely used in animal feeding in the form of tea seed meal (also known as tea seed cake) with variable results. This review article enriches our knowledge about tea seed and tea seed saponins from various aspects and encourages more future studies on rumen fermentation pattern and meat quality for enhancing animal production and contributing to environmental protection.**

**Key words:** Camellia, meat quality, methane emission, rumen fermentation, tea saponin and tea seed

Nowadays food safety, animal health, environmental protection, economical feeding are the key issues in front of commercial livestock sector. Feed additives such as antibiotics, have been frequently used in food animal to enhance the performance of growth and feed efficiency due to a rise in the global demand for animal protein (Menkem *et al*. 2019, Nadeem *et al.* 2020). Because of emergence of antimicrobial drugs resistance, there is an urgent need to develop strategies to replace antibiotics for food-producing animals, especially livestock and poultry (Lillehoj *et al*. 2018, Torres *et al*. 2021, Iqbal and Ashraf 2020). One of the possible alternatives toreplace antibiotics as a growth promoter and to decrease enteric methane emmission in animal production is the use of phytogenic feed additives (Bajagai *et al*. 2020, Candinegara 2020). Phytogenics such as saponins, tannins, & essential oils are plant-derived substances in the diet that are directed to positively affect feed quality, animal health and animal products by means of their specifically efficacious substances (Karásková *et al*. 2015, Singh and Gaikwad 2020, Caipang *et al*. 2021). Saponins, a naturally occurring terpenoid, is an important plant secondary metabolite found in many plants (El Aziz *et al*. 2019, Gunun *et al*. 2019, Khejornsart *et al*. 2021). Many researches demonstrated that various plant parts of the genus *Camellia* (Tea) for example; seeds, flowers, leaves and root contain saponin (Yu and He 2018b). Tea plant belonging to genus Camellia and family Theaceae, is cultivated all across the world (Parmar *et al*. 2012, Li *et al*. 2023). India ranks $2^{nd}$ after China as one of the leading producers of tea in the world and contribute to 23% to the world tea production (Basu *et al.* 2010). Studies have reported that tea seed extract (Yang *et al*. 2015, Gaurav 2015, Kumar *et al*. 2017, Jadhav *et al*. 2016) and tea seed meal (Kumar 2016) are rich source of saponins. Tea saponins have been reported to suppress methane production, reduce rumen protozoa counts, and modulate rumen fermentation patterns (Kumar 2015, Jadhav *et al*. 2016, Liu *et al*. 2019, Qu *et al*. 2023). Supplementationwith tea saponin improves nutrient intake, nutrient digestibility and increased microbial N yield (Zhou *et al*. 2011, Kumar *et al*. 2017, Jadhav *et al.* 2017). Tea seed saponin supplementation in poultry has shown beneficial effects on growth, meat quality, and blood cholesterol (Gaurav 2015). However, there is lack of research regarding utilization of either tea seed or tea seed saponin of Indian origin in Livestock and poultry feeding. Therefore exploration of tea seed as feed additive or as a meal in animal feeding is an area of future research.

**Tea seed saponins**

Crude tea saponin extracted from tea plant (seeds, leaves or roots) are pentacyclic triterpene (Vincken *et al.* 2007; Fan *et al*. 2021; Liu *et al*. 2022). Saponin powder obtained from tea seed is light brown in colour and easily soluble in water (Jadhav *et al*. 2016). There are many methods of saponin extraction like water extraction, organic solvent extraction, ultrasonic-assisted extraction & supercritical extraction from tea seed but alcohol extraction, especially ethanol extraction, appears to provide a higher yield (Yu and He 2018a).

**Tea seed cake**

The feed constitute the major production cost in livestock and poultry (Jones *et al*. 2020, Neethirajan 2020). Low cost locally available feed constituents like tea seed can be safely used to replace the costly feed ingredients up to certain levels because of its nutritional value (Kumar 2016, Kumar *et al*. 2017). Traditionally, defatted tea seed cake is used for animal feeds, detergent and organic fertilizers. The cake contains 14–20% crude protein and 17 types of amino acids, eight of which are essential (Yao *et al*. 2019). Tea seed cake is rich source of omega fatty acids (ω-3, ω-6, and ω-9) (Rawdkuen *et al.* 2016). The major contents of tea seed cake are sugar (37.6%), tea saponin (10%), proteins (8.8%) lipid (7.3%) and Ash (2.8%) (Zheng *et al*. 2016). Tea seed meal can be safely added up to 9.8% of concentrate mixture (2.88% of diet) in the diet of adult Gaddi goats (Kumar 2016).

**Table 1. Saponin content in tea seed & tea seed cake**

| Part of tea plant | Saponin content (%) | Reference |
|---|---|---|
| Tea seed meal | 13.1 and 21.1% | Chaicharoenpong and Petsom, 2009 |
| Tea seed pomace | 8% | Chen *et al.* 2010 |
| *Camellia sinensis* L. seed | 19% | Chen *et al*. 2022 |
| *Camellia sinensis* L. flower buds | 7% | Chen *et al*. 2022 |
| Seeds of *C. sinensis* | 6.5-25.1 | Jadhav *et al*. 2016 |
| Seeds of *C. sinensis* | 15.35 | Kumar *et al*. 2017 |
| Camellia seed shell | 8% | Li *et al.* 2012 |
| Seed cake of *Camellia oleifera* | 15-20% | Liu *et al*. 2016 |
| Tea seed oil cake | 2.41-8.08 | Sarmah *et al.* 2018 |
| Tea seed | 10-13% | Yamauchi *et al*. 2001 |
| Camellia cake extract | 11.8% | Yang *et al.* 2015 |
| *Camelia oleifera* seed | 22.79 | Zhang *et al*. 2022 |
| Seeds of *C. sinensis* | 12% | Zhao *et al.* 2015 |
| *Camelia oleifera* seed | 10-15 | Zhao *et al.* 2020 |
| Tea seed cake | 10-17% | Zheng *et al*. 2016 |

**Table 2. Chemical composition of tea seed (Kumar *et al.* 2017)**

| Sr. No. | Constituents | Percentage |
|---|---|---|
| 1 | Organic Matter (OM) | 97.50 |
| 2 | Crude Protein (CP) | 8.45 |
| 3 | Ether Extract (EE) | 21.80 |
| 4 | Neutral Detergent Fibre (NDF) | 44.40 |
| 5 | Acid Detergent Fibre (ADF) | 36.40 |
| 6 | Saponin | 15.35 |

**Table 3. Levels of elements in the tea seed oil cake in comparison to Soybean meal and Sunflower meal (Njuguna *et al.* 2013)**

| Elements | Assam Variety | Chinese Variety | Soybean meal | Sunflower meal |
|---|---|---|---|---|
| Sodium (%) | 2.3 | 2.4 | 8.3 | 4.9 |
| Calcium (%) | 0.66 | 0.60 | 0.37 | 0.38 |
| Magnesium (%) | 0.25 | 0.20 | 0.25 | 0.38 |
| Manganese (%) | 0.16 | 0.19 | 1.13 | 1.36 |
| Phosphorus (ìg/g) | 3.8 | 1.1 | 5.5 | 6.0 |
| Zinc (ìg/g) | 18.0 | 15.0 | 12.0 | 17.0 |
| Copper (ìg/g) | 4.7 | 5.1 | 18.0 | 32.0 |

Values expressed in dry matter basis of sample

### 1) Effects of tea seed & tea saponins on rumen microbial population

#### a) Effect on rumen protozoa and fungi

Rumen protozoa and ruminal fungi contributing up to 50% & 8%, respectively of the bio-mass in the rumen (Sylvester *et al*. 2004, Kameshwar and Qin 2018). The primary effect of saponins in the rumen appears to be to inhibit the population of ruminal protozoa (defaunation), which might enhance the efficiency of microbial protein synthesis (MCP) and protein flow to the intestine (Patra and Saxena 2009). Hu *et al.* (2005a) reported that the protozoal counts were reduced by 19, 25, 45 and 79% when increasing doses of tea saponin were added (10, 20, 30 and 40 g/kg) to the substrate *in-vitro*. Similarly, when tea seed saponins (TSS) were added at levels of 0.0%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% and 1.0% of substrate (varying concentrate to roughage ratio) the protozoal count decreases significantly with increasing concentration of TSS (Jadhav *et al*. 2016). The protozoal population ($10^4$/ml) decreased by 54.8% at 1.0% level of TSS after 24-h incubation. Kumar (2015) also reported reduction in protozoal count by 38% & 14% with the addition of tea seed saponins (0.6%) and tea seed (containing 0.6% saponins), respectively. Another study conducted by Guo *et al.* (2008) showed that at 0.4 mg tea Saponin/ml rumen fluid decreased rumen protozoa and fungi count by 50% and 79%, respectively. Guyader *et al*. (2017) studied the effect of 7 tea saponin doses (from 0 to 0.50 g/L) *in-vitro* on protozoa concentrations and found that the protozoa concentration reduced by 51% at 0.50 g/L dose. Similarly when 0.4 or 0.8 mg tea saponins were added to the substrate the protozoan count decreases significantly *in vitro* (Hu *et al*. 2006). There was 15% reduction in the population of protozoan reported when Dorper × thin-tailed Han crossbred ewes were supplemented with 2.0 g tea saponin (TS)/head/day) (Liu *et al*. 2019). Similar findings were also reported in an *in-vivo* study when healthy Holstein cows drenched with 0 (control), 20, 30 and 40 g/d of tea saponin (Yan *et al*. 2016). He observed significant decrease in rumen fluid protozoa and no effect on fungal population. In an another study, When tea seed saponin was added in the basal diet of beef steers, it decreased the protozoal count of genus *Entodinium* and increased *Polyplastron* and *Eudiplodinium* genera (Tan *et al.* 2020). Saponins form complexes with sterols of protozoal cell membrane and the membrane become impaired and finally disintegrate, leads to lysis of protozoal cell (Wallace *et al*. 2002, Wina *et al*. 2005). Supplementation with low levels (10 g/cattle per day) of tea saponin can significantly increase rumen fungal population (*Saccharomyces* and *Aspergillus*) in cattle (Qu *et al.* 2023). Tea saponin acts by destroying the cell membrane structure, leading to the leakage of cell contents and inhibit the growth of mycelium, down-regulate the expression of several hyphae and biofilm related genes in fungi (Yu *et al.* 2022). Zhou *et al.* (2011) & Mao *et al*. (2010) reported no significant effects on rumen fungi on long term feeding of tea saponins which may be because of enzyme carbohydrases released by rumen fungi that degrades saponins.

**b) Effect on rumen methanogen and bacteria:**

The methanogen population richness in rumen ecosystem is a hundred fold lower than that of the bacterial communities (De Mulder *et al.* 2017). Methanogens live in close association with rumen protozoa & utilize the $H_2$ liberated by protozoa in metabolic processes to produce $CH_4$, filling an important function in the rumen ecosystem (Hook *et al*. 2010, Morgavi *et al*. 2010). Few *in-vitro* and *in-vivo* studies have been conducted to explore the effect of saponins on rumen bacteria and methanogens with variable results. Supplementation of 2.0 g tea saponin (TS)/head/day) in ewes had no significant effect on the population of methanogens, *Ruminococcus flavefaciens*, *Ruminococcus albus*, *Butyrivibrio fibrisolvens* and increased population of *Fibrobacter succinogenes* observed (Liu *et al.* 2019). Similarly, Tan *et al.* (2020) reported no significant effect on methanogen population by tea seed saponins (TSS) supplementation (6, 10, 15, 20, 25 and 30 g/d of TSS powder) in steers. Mao *et al.* (2010) also reported no effect on the populations of methanogens, *Ruminococcus flavefaciens* and *Fibrobacter succinogenes* with 3 g/day of tea saponins in sheep diets. Guo *et al.* (2008), in an *in vitro* study reported decrease methanogenesis using tea saponins due to decreased activity of the mcrA gene (an indicator of the methanogenic activity of the rumen methanogen) and increased the relative abundance of *Fibrobacter succinogenes* but no effect on the relative abundance of *Ruminococcus flavefaciens*. When healthy Holstein cows drenched with tea saponin (0, 20, 30 and 40 g/d) in different groups, the population of *Butyrivibrio fibrisolvens* of tea saponin groups were significantly decreased, but no significant changes on methanogens population observed (Yan *et al.* 2016). Significant increase ($P \leq 0.05$) in the number of total, cellulolytic and lactic acid bacteria in saponin fed groups were reported when Awassi lambs 3-4 months of age supplemented with tea leaves saponins (Alaidi and Al-Galbi 2021). Klita *et al.* (1996) found that the susceptibility of rumen protozoa and lack of susceptibility of rumen bacteria to saponins is due to the presence of cholesterol in eukaryotic cell membrane (including protozoa) and not in prokaryotic cells (bacteria).

**2) Effects of tea seed and tea saponin on rumen fermentation**

**a) Effects on ammonia production**

Jadhav *et al.* (2016) in an *in vitro* experiment reported 35% reduction in Ammonia-N concentration at 0.9% level of tea seed saponins. Kumar, (2015) observed decrease in ammonia-N by 45% at 0.6% of tea seed saponin and up to 2 % with tea seed (0.6% of tea seed saponin) using different roughage to concentrate ratio in an *in-vitro* study (Kumar 2015). However, there was no significant difference observed in rumen Ammonia N nitrogen when tea seed meal was supplemented in goats with tea seed meal containing @0.4% and 0.6% saponin of dry matter intake (Kumar 2016). Ammonia- N concentration *in vitro* decreased with increasing level of tea saponin and was 8, 18, 21 and 27% lower at 24 h incubation when the tea saponin were added at levels of 10, 20, 30 and 40 g/kg, respectively (Hu *et al.* 2005a). Similarly, 19% reduction in ammonia concentration was observed for 0.4 mg/ml tea saponin in defaunated rumen fluid, a little higher than the effect on defaunated medium (Hu *et al.* 2005b). Feeding of tea saponin at 3 g/day to defaunated animal reduced ammonia concentration in the rumen fluid by 4%, which was much less than the reduction in $NH_3$ concentration observed by only defaunation (31%) (Zhou *et al*. 2011). Decreased ammonia production observed from 10.7 to 8.3 mmol/L ($p < 0.001$) when ewes were supplemented with tea seed saponin at 2.0 g/head/day with no effect on ruminal pH (Liu *et al.* 2019). Similar findings were also reported using tea leaves saponins (60 or 120 or 180 mg/ kg feed) in lambs, the concentration of rumen ammonia decreased significantly ($P \leq 0.05$) with increasing concentration of tea leaves saponins (Alaidi & Al-Galbi 2021). Similarly, when four different levels of tea saponins were fed to the Qinchuan cattle as treatments (0, 10, 20 & 30 g/cattle per day), results indicate that there was decrease in ammonia N production in tea saponins supplemented groups. Zhou *et al.* (2012) reported that ruminal pH and ammonia N concentrations were not affected when goats where supplemented with 400, 600, 800 mg tea saponin/kg of DM. Wei *et al.* (2012) did not find any change in ammonia N and ruminal pH in invitro study using tea saponin 1.6 mg/g. Wina *et al.* (2005) suggested that the decrease in rumen $NH_3$ concentration caused by the addition of saponins in the diet was due to an indirect result of the reduced protozoa count.

**b) Effects on rumen Methane production**

The $CH_4$ production in the rumen is significantly influenced by the protozoa population because of inter-species transfer of $H_2$ from the protozoa to methanogens (Li *et al.* 2018). Saponins decreases ruminal methane production because of defaunation and/or directly by decreasing the activities (i.e. rate of methanogenesis or expression of methane-producing genes) and numbers of methanogens (Patra and Saxena 2009). Few studies have reported that tea seed saponins decrease methane production because of its inhibitory effect on ciliated rumen protozoa (Mao *et al.* 2010, Zhou *et al*. 2011, Wang *et al*. 2012). According to Mao *et al.* (2010), 3 g/day of tea saponins supplementation in lambs diet reduces the methane production by 27.7%. In an *in vivo* study, feeding of tea saponin at 3 g/day to defaunated animal decreased methane production in the rumen fluid by 10.6%, (Zhou *et al*. 2011). He reported that decreased $CH_4$ production was due mainly to effects of tea saponins on reducing numbers of rumen protozoa, and thus lowering methanogenic activity of the associated methanogens. In an *in vitro* study, Methane production (ml/gm Organic Matter degraded) decreased by 32.5% when 0.8 % of tea seed saponin was added to a substrate of varying roughage to concentrate ratio (Jadhav *et al*. 2016). Similar findings were also reported by Kumar (2015) with tea seed saponins in an *in-vitro* study. He reported that Methane production was decreased up to 35 % with the addition of tea saponin at 0.6% level (in terms of digestible Dry Matter) and no significant difference observed with tea seeds powder. Similarly, in the faunated rumen fluid, addition of 0.2 and 0.4 mg/ml tea saponin decreased methane production by 13.3 and 14.3%, respectively (Hu *et al*. (2005b). Wei *et al*. (2012) also reported numerical decrease in methane production in *in-vitro* study using tea saponin 1.6 mg/g substrate. In an *in-vivo* study, supplementation of 2.0 g tea saponin (TS)/head/day) in ewes resulted in an 8.8% decrease in the daily methane emissions (L/kg $BW^{0.75}$) (Liu *et al.* 2019). Guyader *et al*. (2015) reported that supplementation 0.5 % tea saponin alone did not affected methane emission but significantly affected methane emission by 28 % when supplemented with 0.5% tea saponin & 2.3% of nitrate in dairy cows. No significant difference in methane production observed between the treatments when lambs 3-4 months of age supplemented with tea leaves saponins (60 or 120 or 180 mg/ kg feed) (Alaidi and Al-Galbi 2021). When Cannulated and non-cannulated steers fed the tea seed saponins (6, 10, 15, 20, 25 and 30 g of TSS powder) and basal diet, compared with cannulated (8.0 ± 1.20 ng/mL) animals, the addition of 30 g of TSS in the basal diet increased ($P \leq 0.01$) blood $CH_4$ concentration in non-cannulated (15.6 ± 1.74 ng/mL) animals (Ramírez-Restrepo *et al*. 2016). Inclusion of tea saponin significantly reduced methane production in the faunated rumen fluid, but not in the defaunated rumen fluid, suggesting that inhibition of methanogenesis by tea saponin might be due to their antiprotozoal activity, eventually decreases amount of hydrogen available for the process of methanogenesis in the rumen (Szumacher-Strabel and Cieslak 2010).

**c) Effects on Volatile fatty acids (VFA)**

Variable responses on rumen VFAs have been reported by different researcher when using tea saponins. In an *in-vitro* study when tea seed saponins (TSS) were added (0.0%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% and 1.0%) to the substrate, the short chain fatty acids production increased linearly with increasing level of TSS (Jadhav *et al*. 2016).Liu *et al*. (2019) reported increase in molar proportion of propionate and decrease in the acetate:propionate ratio when 2.0 g tea saponin (TS)/head/day) supplemented in ewes indicating a transformation of the rumen to propionate fermentation from acetate fermentation. In an *in vitro* study, addition of 30–40 g tea saponin /kg diet tended to increase the propionate, but had little effect on acetate and butyrate (Hu *et al.* 2005a). Alaidi and Al-Galbi (2021) reported significant increase ($P \leq 0.05$) in both acetic, propionic and butyric acids in lambs supplemented with tea leaves saponins. Wei *et al*. (2012) reported decrease in the molar proportion of butyrate and minor acids (valerate, iso butyrate and isovalerate) but no change in acetate and propionate in *in-vitro* using tea saponin 1.6 mg/g substrate. Kumar (2016) reported no significant difference in rumen pH and TVFA when tea seed meal was supplemented in goats with tea seed meal containing @0.4% and 0.6% saponin of DMI. Significant increase in propionic acid and butyric acid concentrations and no effect on total volatile fatty acid observed when healthy dairy cows drenched with 0 (control), 20, 30 and 40 g/d of tea saponin (Yan *et al.* 2016). Similarly, no effect on ruminal total volatile fatty acid concentrations was observed when goats were supplemented with 400, 600, 800 mg tea saponin/kg of DM (Zhou *et al.* 2012).Qu *et al.* (2023) reported supplementation with low levels (10 g/cattle per day) of tea saponin in cattle, the acetate to propionate (A:P) ratio decreases significantly. When Brahman steers were supplemented with 6, 10, 15, 20, 25 and 30 g of tea seed saponin powder, reduced total volatile fatty acid (VFA) concentration ($P \leq 0.05$) and modified pattern of individual molar VFA concentrations ($P \leq 0.05$) was observed in tea seed saponins groups (Ramírez-Restrepo *et al*. 2016). In the rumen, the formation of acetate and butyrate results in production of $H_2$ gas, a substrate that methanogenic archaea use to reduce carbon dioxide resulting in the production of methane (Moss *et al.* 2000).

**3) Effect of tea seed and tea seed saponins on Microbial protein synthesis**

Microbial protein supply to the duodenum should be increased for efficient use of dietary protein and energy. Kumar *et al.* (2017) in an *in-vivo* study observed that during short term & long term feeding of tea seed saponins (0.4% of DMI) and tea seed (2.6% of DMI) there was significant increase in microbial protein synthesis (18%) in Gaddi kids as compare to control estimated in terms of purine derivatives (allantoin, uric acid, xanthine and hypoxanthine). In a similar study, Microbial N supply was 33.33% and 22.22% higher in 0.4% & 0.8% level of tea seed saponin fed groups of goats as compared to control in Gaddi goats (Jadhav *et al.* 2017). During a metabolism trial conducted by Kumar (2016) in goats observed that the purine derivatives excretion and absorption (mmol/day) and microbial protein supply were higher for when tea seed meal was supplemented containing @0.4% and 0.6% saponin of DMI. Mao *et al.* (2010) also observed higher microbial protein synthesis in lambs fed diet with tea saponin (3 g/day of tea saponins) as compared to control. Significant increase in microbial protein synthesis by 20.20% observed when 30 g/d tea saponin supplemented in healthy Holstein cows as compare to control (Yan *et al*. 2016). Similar findings were also reported by Liu *et al*. (2003). He observed that an increase in microbial protein synthesis of 49% occurred in the presence of 0.8% of tea saponins in an *in vitro* fermentation. Newbold *et al.* (2015) observed that the elimination of protozoa from the rumen could increase microbial protein supply to the host by up to 30%. Jouany (1996) reported that ciliated rumen protozoa contributed significantly to intra-ruminal cycling of microbial N and reduced the efficiency of microbial protein synthesis, thus reducing population of ruminal protozoa may improve N utilization and increase the flow of microbial protein to duodenum.

**4) Effect of tea seed and tea seed saponins on growth and feed intake**

Kumar *et al*. (2017) did not find any effect of saponin supplementation on Dry Matter Intake when male Gaddi kids were supplemented with tea saponins and tea seed @0.4% of dry matter intake. They also reported total BW gain (kg) and ADG (g/d) in tea seed saponin group were 33.5% & 33% higher than control group and similar results also seen with tea seed supplemented group. In another study, Jadhav *et al.* (2017) observed no significant difference in intake of DM but average daily gain was 22.3% higher in 0.4 % tea seed saponin supplemented group of goats. Hu *et al.* (2006) reported that supplementation of tea saponin (3 g/day) improved growth performance in goats compared to group supplemented with 6 g/day as well as control group, but the feed conversion ratio was improved in both saponin fed groups. There was no significant effect in overall dry matter intake and change in body weight when tea seed meal was supplemented in goats with tea seed meal containing @0.4% and 0.6% saponin of DMI (Kumar 2016). When tea saponin powder (TSP) was supplemented in Holstein dairy cows at the rate of 0 (control), 20, 30, or 40 g/d per head, lowest amount of DMI observed in cows fed 40 g/d TSP (Wang *et al.* 2017). Similar results noted when Brahman steers supplemented with 6, 10, 15, 20, 25 and 30 g of tea seed powder, Overall, DMI was not affected, but relative to all diets, administration 30 g of the supplement was associated with significantly ($P$< 0.001) reduced DMI, scours and bloat disorders (Ramírez-Restrepo *et al*. 2016). Gaurav (2015) reported a higher growth rate in chicken when tea seed saponin was supplemented (@ 600 mg/kg). Dry matter intake & Weekly body weight change and FCR in birds fed different diets were statistically non significant. Chi *et al*. (2017) reported no significant difference for change in body weight was found between the birds orally administered TS in drinking water for seven days at 5 mg/kg BW. When chicks were supplemented with tea saponins in the diet they responded quickly to the presence of tea saponin in the diet by decreasing feed intake due to bitter taste of saponin in the diet (Ueda 2001). Dietary saponins reduced atmospheric ammonia in poultry due to inhibition in the release of gaseous ammonia through the inhibition of the enzyme (urease) which catalyse the reaction resulting in the release of gaseous ammonia and increased growth rate (Cheeke and Nakuae 1993). Rumen protozoa cause protein turnover by engulfing bacteria, defaunation enhances the nitrogen utilization and lead to an increase in growth and production (Wina *et al.* 2005). The negative effect of saponins on feed intake is because of reduced palatability, digestibility of protein and suppression of nutrient transport (Francis *et al.* 2002).

**5) Effect of tea seed and tea seed saponin on digestibility**

Jadhav *et al.* (2017) reported that dry matter, organic matter, NDF, ADF and cellulose digestibilities were significantly lower when goats were supplemented with 0.8% of tea seed saponins. Similarly, *in vitro* true dry matter and organic matter digestibility significantly decreased with increasing concentration of tea seed saponins (at levels of 0.0%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% and 1.0% of substrate) (Jadhav *et al.* 2016). Digestibility of DM, OM, CP, and fibre fractions during both short term as well as long term feeding of tea seed saponins in Gaddi goats were not affected (Kumar *et al*. 2017). Dry matter, organic matter, crude protein, ether extract and crude fibre metabolizability were not affected in poultry when supplemented with tea seed saponin (600mg/kg) (Gaurav 2015). With addition of tea seed saponin to the basal substrate, true dry matter as well as true organic matter digestibility were decreased significantly with increasing levels of tea seed saponins (0.2% to 0.6%) in an *in-vitro* study (Kumar 2015). There was no significant difference observed in the digestibility of Dry Matter, Crude protein, ADF, NDF when tea seed meal was supplemented in goats with tea seed meal containing @0.4% and 0.6%

saponin of DMI (Kumar 2016). Decrease in apparent digestibility of dry matter reported in calves supplemented with soybean meal and 5% tea seed meal (Fan *et al.* 2018). Dietary tea Saponin levels did not affect the intake, apparent disappearance in the forestomach and apparent whole tract digestibility of DM, N, NDF and ADF, or apparent digestibility in the small intestine when goats where supplemented with 400, 600, 800 mg tea saponin/kg of DM (Zhou *et al.* 2012). Supplementation of 2.0 g tea saponin (TS)/head/day) in ewes increased the apparent digestibility of organic matter (OM), nitrogen (N), neutral detergent fibre (NDF), and acid detergent fibre (ADF) ($p < 0.001$) (Liu *et al*. 2019). Guyader *et al.* (2015) observed a numerical increase in NDF and ADF digestibility in dairy cows supplemented with 0.5 % of DMI tea saponin. Wei *et al*. (2012) reported in *in-vitro* study reported numerically higher organic matter digestibility for tea saponin substrate using tea saponin 1.6 mg/g.

**6) Effect of tea seed and tea seed saponins on Nitrogen, Ca and P balance**

Kumar *et al*. (2017) reported significant Increased N retention in short term and long term feeding of tea seed saponin (0.4% of DMI). He also reported significant increased N retention in tea seed supplemented groups during long term indicates that tea seed saponin in the diet has some role in improved N retention. In tea seed fed group though the N retention was numerically higher during short term, however, only during long term the effect was significant indicating that tea seed supplementation needed more time for getting the beneficial effect as compared to TSS supplementation. Similar findings were also reported by Jadhav *et al.* (2017) in which N balance was improved in 0.4% and 0.8% supplementation of tea saponin than the control group. Gaurav (2015) observed higher nitrogen retention in poultry fed with tea seed saponin (0.06%). Supplementation of 2.0 g tea saponin (TS)/head/day) in ewes decreased fecal N and urinary N outputs, resulting in a significant N retention (Liu *et al*. 2019). However, supplementation of 0.5% of tea saponin had no effect on N balanace & purine urinary excretion in dairy cows (Guyader *et al.* 2015). Similarly, Kumar (2016) reported no significant difference in the excretion of N through faeces as well as through urine and N balance in all the groups when tea seed meal was supplemented in goats containing @0.4% and 0.6% saponin of DMI. Santoso *et al*. (2007) reported that N-balance was improved by saponins supplementation due to reduced degradation of bacterial cells in saponin fed groups due to the effect of defaunation and thereby increased supply of microbial proteins. Jadhav (2014), Kumar (2015) &Kumar (2016) reported that there was no significant difference on Ca intake, Ca balance, P intake and P balance in tea seed saponin and tea seed meal fed goats group. However, Gaurav (2015) observed Ca intake, Ca retention, P intake and P retention were significantly higher ($p<0.01$) in tea seed fed group in broiler.

**7) Effect of tea seed and tea seed saponins on blood biochemical parameters**

The tea seed saponin (Jadhav *et al.* 2017, Kumar *et al.* 2017, Gaurav 2015) and tea seed meal (Kumar 2016) supplementation in goats and poultry did not affect the levels of Hb, PCV, total protein, albumin, globulin, blood urea nitrogen, bilirubin and creatinine and the levels were within the normal range Normal hemtocrit readings, blood biochemical parameters and serum enzymes in the study indicated that no hemolytic effect of saponin occurred when it was fed to goats and poultry and there also no occurrence of liver or kidney dysfunction. Diets supplemented with 30 g of TSS in Brahman steers were associated with higher chloride ($P< 0.01$) and alkaline phosphatase ($P< 0.05$) blood concentrations, and lower serum concentrations of potassium and urea nitrogen ($P< 0.01$), iron and total lipase ($P< 0.05$) (Ramírez-Restrepo *et al.* 2016). Wu and Zhong (1999) reported that serum ALT and AST in saponin fed group were significantly lower in tea seed saponin supplemented groups although in normal range may be due to hepatoprotective effect of saponins. Concentrations of total protein and albumin in the goats receiving 3 g of tea saponin /day were higher than those receiving 0 and 6 g of tea saponin/day (Hu *et al.* 2006). He observed that serum cholesterol level was decreased and the high density lipoprotein cholesterol increased in the tea saponin fed animals. He also reported that the concentration of glucose and activities of glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase were not affected by the addition of tea saponin, suggesting that tea saponin have no adverse effect on hepatic metabolism. Fan *et al.* (2018) reported that serum total protein concentration and serum glucose concentration was significantly higher in 5% tea seed meal supplemented group in weaned male calves than that of control.

**8) Effect of tea seed saponins on cholesterol, total lipid and meat quality**

Kumar *et al.* (2017) observed decreased levels of triglyceride and low density lipoprotein (LDL) cholesterol ($p<0.05$) and increased levels of high density lipoprotein (HDL)-Cholesterol (12.5%) in tea seed saponin supplemented groups in goats. Jadhav *et al.* (2016) reported decreased in blood triglyceride levels, significant increase in HDL and no effect on total cholesterol on 0.4 and 0.8% supplementation of tea saponin in goats as compared to control. Kumar (2016) reported lower blood triglyceride & LDL levels, significantly higher HDL level and no effect on total cholesterol in goats supplemented with tea seed meal containing 0.6% saponin of DMI. Blood triacylglycerol level declined linearly ($P<0.01$) when goats were supplemented with 400, 600, 800 mg tea saponin/kg of DM (Zhou *et al.* 2012). Gaurav (2015) observed decrease in total blood cholesterol and trigyceride and increase in HDL–Cholestrol in starter and finisher phase in broiler supplemented with tea seed saponin. He also reported that the average live weight of birds and dressed weight (%) were numerically higher but not significant in tea seed supplemented groups. He observed no significance difference ($p>0.05$), in the appearance of cooked chicken meat but significance difference observed in flavor among groups supplemented with tea seed saponins. Hu *et al.* (2006) reported that serum cholesterol level was decreased and the high density lipoprotein (HDL) cholesterol increased in the tea saponin fed animals. Oakenfull and Sidhu (1990) reported that saponins act either directly, by inhibiting absorption of cholesterol from the small intestine, or indirectly, by inhibiting reabsorption of bile acids. Where direct inhibition of cholesterol absorption occurs, saponins prevented absorption of not only a high proportion of dietary cholesterol, but also of a high proportion of the cholesterol derived from bile and desquamation of mucosal cells.

**9) Effect of tea seed saponins on the immune system**

There are limited studies on effect of tea seed saponins and saponins from other sources on immune response of animals. Supplementation of tea seed or of tea seed saponin (@ 0.4 % of DMI supplementation did not affect humoral immune response (using 20% Sheep RBCs suspension by Haemagglutination test) and cell mediated immune response (using by delayed type hypersensitivity (DTH) reaction) in goats (Kumar *et al.* 2017) Similarly, no significant difference was observed in antibodies titer on day 0, 7, 14, 21 and 28 when tea seed meal was supplemented in goats with tea seed meal containing @0.4% and 0.6% saponin of DMI (Kumar 2016). In an another study when 4 rumen fistulated cows were infused with tea saponins at the dose rate of 0, 15, 30 & 45 g/day, the serum immunoglobulin content and serum IL-1 were significantly higher in 30g/day & 15g/day tea saponins group respectively than control (Chang *et al.* 2017). When tea saponin powder (TSP) was supplemented in Holstein dairy cows at the rate of 0 (control), 20, 30, or 40 g/d per head, the plasma concentration of IL-2 linearly increased as the supplemental value of TSP increased and plasma concentration of TNF-á linearly increased as the supplemental value of TSP increased ($P< 0.01$) (Wang *et al.* 2017). Enhanced immune responses, such as lymphocyte proliferation induced by concanavalin A and lipopolysaccharides, and serum Newcastle disease virus- and infectious bronchitis virus-specific antibodies were also observed in chickens when supplemented with tea saponins orally (5mg/kg BW) (Chi *et al.* 2017).

**10) Antioxidant effect of tea seed saponin**

Kumar (2016) reported that there was no

significant difference in SOD, Catalase and Glutathione peroxidase activity between groups when tea seed meal was supplemented in goats with tea seed meal containing @0.4% and 0.6% saponin of DMI. Similar results also were reported by Zhou *et al.* (2012) who found that plasma concentrations of glutathione peroxidase and malondialdehyde were not affected when goats were supplemented with 400, 600, 800 mg tea saponin/kg of DM. Gaurav (2015) observed that the antioxidant activity of tea seed increases with increase in temperature estimated by DPPH assay. The concentration of SOD linearly increased as the supplemental value of tea saponins (TS) increased ($P < 0.01$), with the greatest value observed for cows fed 40 g/d of TS and no significant difference was found for the concentration of GSH-Px in cows fed with TS (Wang *et al.* 2017). Tea seed saponin supplementation in mice (140 mg/kg·day) reduced oxidative stress in mice indicated by the increased serum and liver levels of SOD, GSH, and T-AOC and decreased ROS and MDA levels (Cao *et al.* 2022). A study was conducted to see the effect of in drinking water on oxidative stress induced by cyclophosphamide in chickens. Significantly increase in total antioxidant capacity, total superoxide dismutase, catalase, glutathione peroxidase, glutathione, ascorbic acid, and á-tocopherol, and decreased malondialdehyde activity observed on oral supplementation of tea saponins in chicken (Chi *et al*. 2017).

## Conclusion

Tea saponins have the ability to manipulate the rumen microbial community and reduce the enteric $CH_4$ emission. Tea saponins also have the ability to lower blood cholesterol and improve meat quality. However, the effect of tea saponins seems to be dependent on the composition of the diet. More future studies are needed to explore the active structural components and exact mechanism of action of tea saponins.

## References


Alaidi RH and Al-Galbi HA. 2021. Impact of tea leaves saponins on some rumen parameters of Awassi lambs. Plant Archives **21** (1): 2068-2071.

Bajagai YS, Alsemgeest J, Moore RJ, Van TT and Stanley D. 2020. Phytogenic products, used as alternatives to antibiotic growth promoters, modify the intestinal microbiota derived from a range of production systems: an in vitro model. Applied Microbiology and Biotechnology **104**: 10631-10640.

Basu MA, Bera B and Rajan A. 2010. Tea Statistics: Global Scenario. Journal of Tea Science **8**: 121-124.

Caipang CMA, Suharman I, Avillanosa AL and Gonzales-Plasus MM. 2021. Influence of phytogenic feed additives on the health status in the gut and disease resistance of cultured fish. In *IOP* Conference Series: Earth and Environmental Science **695** (1): 012024.

Candinegara T. 2020. Challenges of using feed additives in Indonesia. In *IOP* Conference Series: Earth and Environmental Science **492** (1): 012001.

Cao W, Wang K, Liang C, Su Y, Liu S, Li J, Qing H, Zeng Z, Dai L and Song JL. 2022. Dietary tea seed saponin combined with aerobic exercise attenuated lipid metabolism and oxidative stress in mice fed a high fat diet (HFD). Journal of Food Biochemistry **46** (12): 14461.

Chaicharoenpong C and Pestom A. 2009. Quantitative thin layer chromatographic analysis of the saponins in tea seed meal. Phytochemical Analysis **20**: 253-255.

Chang X, Zhang Y, Zhao S, Jiang Q, Wang B, Fang L, Xiong B and Jiang L. 2017. Effects of tea saponin on immune function of dairy cows. Chinese Journal of Animal Nutrition **29** (3): 1039-1045.

Cheeke PR and Nakuae HS. 1993. The effect of Yucca schidigera extract (De- Odorase) on the air quality with the performance of chickens and rabbits. Proceedings, Western Section. American Society of Animal Science **44**: 106-108.

Chen C, Zhu H, Kang J, Warusawitharana H K, Chen S, Wang K, Yu F, Wu Y, He P, Tu Y and Li B. 2022. Comparative transcriptome and phytochemical analysis provides insight into triterpene saponin biosynthesis in seeds and flowers of the tea plant (*Camellia sinensis*). Metabolites**12**(3): 204.

Chen YF, Yang CH, Chang MS, Ciou Y P, Huang YC. 2010. Foam properties and detergent abilities of the saponins from *Camellia oleifera*. International Journal of

Molecular Sciences **11**: 4417–4425.

Chi X, Bi S, Xu W, Zhang Y, Liang S and Hu S. 2017. Oral administration of tea saponins to relive oxidative stress and immune suppression in chickens. Poultry Science **96** (9): 3058-3067.

De Mulder T, Goossens K, Peiren N, Vandaele L, Haegeman A, De Tender C, Ruttink T, de Wiele T V and De Campeneere S. 2017. Exploring the methanogen and bacterial communities of rumen environments: solid adherent, fluid and epimural. FEMS Microbiology Ecology **93** (3): 251.

El Aziz M, Ashour A and Melad A.2019. A review on saponins from medicinal plants: chemistry, isolation, and determination. Journal of Nanomedicine Research **8**: 6–12.

Fan L, He Y, Xu Y, Li P, Zhang J and Zhao J. 2021. Triterpenoid saponins in tea (*Camellia sinensis*) plants: biosynthetic gene expression, content variations, chemical identification and cytotoxicity. International Journal of Food Sciences and Nutrition **72** (3): 308-323.

Fan Q, Bi Y, Diao Q, Cheng S, Fu T and Tu Y. 2018. Effects of diets containing palm kernel meal, oil tea seed meal and tea seed meal on digestion, metabolism and serum biochemical indexes of weaned calves. Chinese Journal of Animal Nutrition **30** (2): 743-754.

Francis G, Kerem Z, Makkar HP and Becker K. 2002. The biological action of saponins in animal systems: a review. British journal of Nutrition **88** (6): 587-605.

Gaurav AK. 2015. Studies on supplementation of *Chlorophytum* root and *Camellia* seed as feed additives in broiler ration. M V Sc Thesis, Deemed University, Indian Veterinary Research Institute, Izatnagar, India: 34-56.

Gunun P, Gunun N, Khejornsart P, Ouppamong T, Cherdthong A, Wanapat M, Sirilaophaisan S, Yuangklang C, Polyorach S, Kenchaiwong W and Kang S. 2019. Effects of Antidesma thwaitesianum Muell. Arg. pomace as a source of plant secondary compounds on digestibility, rumen environment, hematology, and milk production in dairy cows. Animal Science Journal **90** (3): 372–381.

Guo YQ, Liu JX, Lu Y, Zhu WY, Denman SE, McSweeney CS. 2008. Effect of tea saponin on methanogenesis, microbial community structure and expression of mcrA gene, in cultures of rumen micro organisms. Letters in Applied Microbiology **47** (5):421-426.

Guyader J, Eugène M, Doreau M, Morgavi DP, Gérard C and Martin C. 2017. Tea saponin reduced methanogenesis in vitro but increased methane yield in lactating dairy cows. Journal of Dairy Science **100** (3):1845-1855.

Guyader J, Eugène M, Doreau M, Morgavi DP, Gérard C, Loncke C, Martin C. 2015. Nitrate but not tea saponin feed additives decreased enteric methane emissions in nonlactating cows. Journal of Animal Science **93** (11): 5367-5377.

Hook SE, Wright ADG and McBride BW. 2010. Methanogens: methane producers of the rumen and mitigation strategies. Archaea **2010**: 945785.

Hu WL, Liu JX, Ye JA, Wu YM. and Guo YQ. 2005a. Effect of tea saponin on rumen fermentation *in vitro*. Animal Feed Science and Technology **120**: 333–339.

Hu WL, Wu YM, Liu JX, Guo YQ and Ye JA. 2005b. Tea saponins affect *in vitro* fermentation and methanogenesis in faunated and defaunated rumen fluid. Journal of Zhejiang University-Science **6** (B): 787–792.

Hu W, Liu J, Wu Y, Guo Y and Ye J. 2006. Effects of tea saponins on in vitro ruminal fermentation and growth performance in growing Boer goat. Archives of Animal Nutrition **60** (1): 89-97.

Iqbal MN and Ashraf A. 2020. Poultry Feed Supplements (Vinegar and Yogurt) as Antibiotic Alternatives for Maximizing Growth Performance and Feed Efficiency. PSM Microbiology **5** (2): 51-53.

Jadhav RV, Kannan A, Bhar, R, Sharma OP, Bhat TK, Gulati A, Rajkumar K, Sharma R., Mal G, Singh B and Sharma VK. 2017. Effect of tea (*Camellia sinensis*) seed saponin supplementation on growth performance, nutrient utilization, microbial protein synthesis and hemato biochemical attributes of *Gaddi* goats. Animal Nutrition and Feed Technology **17**: 255-268.

Jadhav RV, Kannan A, Bhar R, Sharma OP, Gulati A, Rajkumar K, Mal G, Singh B and Verma MR. 2016. Effect of tea (*Camellia sinensis*) seed saponins on *in vitro* rumen fermentation, methane production and true digestibility at different forage to concentrate ratios. Journal of Applied Animal Research **46** (1): 118–124.

Jadhav VR. 2014. Studies on effect of supplementation of tea seed saponin on growth performance and nutrient utilization in goats. M V Sc Thesis, Deemed University, Indian Veterinary Research Institute, Izatnagar, India, 55-62.

Jones SW, Karpol A, Friedman S, Maru BT and Tracy B P. 2020. Recent advances in single cell protein use as a feed ingredient in aquaculture. Current opinion in biotechnology **61**: 189-197.

Jouany JP. 1996. Effect of rumen protozoa on nitrogen utilization by ruminants. Journal of Nutrition **126**: 1335-1346.

Kameshwar AKS and Qin W. 2018. Genome wide analysis reveals the extrinsic cellulolytic and biohydrogen generating abilities of neocallimastigomycota fungi. Journal of Genomics **6**: 74–87.

Karásková K, Suchý P and Straková E. 2015. Current use of phytogenic feed additives in animal nutrition: A review. Czech Journal of Animal Science **60**: 521-530.

Khejornsart P, Cherdthong A and Wanapat M.2021. In vitro screening of plant materials to reduce ruminal protozoal population and mitigate ammonia and methane emissions. Fermentation **7**(3):166.

Klita PT, Mathison GW, Fenton TW and Hardin RT. 1996. Effects of alfalfa root saponins on digestive function in sheep. Journal of Animal Science **74**: 1144–1156.

Kumar M, Kannan A, Bhar R, Gulati A, Gaurav AK and Sharma VK. 2017. Nutrient intake, digestibility and performance of Gaddi kids supplemented with tea seed or tea seed saponin extract. Asian-Australasian Journal of Animal Sciences **30** (4): 486-494

Kumar M. 2015. Effect of supplementation of tea seed and tea seed saponin on the performance of male *Gaddi* goats. M V Sc Thesis, Deemed University, Indian Veterinary Research Institute, Izatnagar, India. 41-81.

Kumar V. 2016. Effect of supplementation of tea seed meal on performance of male *Gaddi* goats. M V Sc Thesis, Deemed University, Indian Veterinary Research Institute, Izatnagar, India. 33-60.

Ku-Vera JC, Castelán-Ortega OA, Galindo-Maldonado FA, Arango J, Chirinda N, Jiménez-Ocampo R, Valencia-Salazar SS, Flores-Santiago EJ, Montoya-Flores MD, Molina-Botero IC, Piñeiro-Vázquez AT. 2020. Strategies for enteric methane mitigation in cattle fed tropical forages. Animal **14** (S3): 453-s463.

Li JW, Li H, Liu ZW, Wang YX, Chen Y, Yang N, HuZ H, Li T, Zhuang J. 2023. Molecular markers in tea plant (*Camellia sinensis*): Applications to evolution, genetic identification, and molecular breeding. Plant Physiology and Biochemistry **198**:107704.

Li M., Haiyan Z and Yongzhong C. 2012. Study on tea saponin extraction from shell of oil-tea Camellia seeds. In first International Conference on Mechanical Engineering and Material Science (MEMS 2012) (pp. 267-272). Atlantis Press.

Li Z, Deng Q, Liu Y, Yan T, Li F, Cao Y, Yao J. 2018. Dynamics of methanogenesis, ruminal fermentation and fiber digestibility in ruminants following elimination of protozoa: a meta-analysis. Journal of Animal Science and Biotechnology **9** (1): 1-9.

Lillehoj H, Liu Y, Calsamiglia S, Fernandez-Miyakawa ME, Chi F, Cravens RL, Oh S, Gay CG.2018. Phytochemicals as antibiotic alternatives to promote growth and enhance host health. Veterinary Research **49** (1): 1-8.

Liu JY, Yuan WZ, Ye J and Wu Y. 2003. Effect of tea (*Camellia sinensis*) saponin addition on rumen fermentation in vitro. In Matching herbivore nutrition to ecosystems biodiversity. Tropical and subtropical agrosystems. Proceedings of the Sixth International Symposium on the Nutrition of Herbivore; Camacho JH, Castro CAS, Eds.; Merida, Mexico, 3:561-564.

Liu Y, Geng Y, Zhang S, Hu B, Wang J and He J. 2022. Quantitative analysis and screening for key genes related to tea saponin in *Camellia oleifera* Abel seeds. Food Bioscience **49**: 101901.

Liu Y, Li Z, Xu H and Han, Y. 2016. Extraction of saponin from *Camellia oleifera* Abel cake by a combination method of alkali solution and acid isolation. Journal of Chemistry **2016**: 6903524.

Liu Y, Ma T, Chen D, Zhang N, Si B, Deng K, Tu Y, Diao Q. 2019. Effects of tea saponin supplementation on nutrient digestibility, methanogenesis, and ruminal microbial flora in Dorper crossbred ewe. Animals **9** (1): 29.

Mao HL, Wang JK, Zhou YY and Liu JX. 2010. Effects of addition of tea saponins and soybean oil on methane production, fermentation and microbial population in the rumen of growing lambs. Livestock Science **129**: 56-62.

Menkem ZE, Ngangom BL, Tamunjoh SSA and Boyom FF. 2019. Antibiotic residues in food animals: public health concern. Acta Ecologica Sinica **39** (5): 411-415.

Morgavi DP, Forano E, Martin C and Newbold CJ. 2010. Microbial ecosystem and methanogenesis in ruminants. Animal **4** (7): 1024-1036.

Moss AR, Jouany JP and Newbold CJ. 2000. Methane production by ruminants: its contribution to global warming. Annalse de Zootechnie **49**: 231–235.

Nadeem SF, Gohar UF, Tahir S F, Mukhtar H, Pornpukdeewattana S, Nukthamna P, Moula Ali AM, Bavisetty SC and Massa S. 2020. Antimicrobial resistance: more than 70 years of war between humans and bacteria. Critical Reviews in Microbiology **46** (5): 578-99.

Neethirajan S. 2020. The role of sensors, big data and machine learning in modern animal farming. Sensing and Bio-Sensing Research **29**: 100367.

Newbold CJ, De La Fuente G, Belanche A, Ramos-Morales E, McEwan NR. 2015. The role of ciliate protozoa in the rumen. Frontiers in Microbiology **6**: 1313.

Njuguna DG, Wanyoko JK, Kinyanjui T and Wachira FN. 2013. Mineral elements in the Kenyan tea seed oil cake. International Journal of Research in Chemistry and Environment **3** (1): 253-61.

Oakenfull D and Sidhu G. 1990. Could saponins be a useful treatment for hypercholesterolemia? European Journal of Clinical Nutrition **44**: 79–88.

Parmar N, Rawat M and Kumar JV. 2012. *Camellia sinensis* (Green tea): A Review. Global Journal of Pharmacology **6** (2): 52-59.

Patra AK and Saxena J. 2009. The effect and mode of action of saponins on the microbial populations and fermentation in the rumen and ruminant production. Nutrition Research Reviews **22** (2): 204-219.

Qu X, Raza SH, Zhao Y, Deng J, Ma J, Wang J, Alkhorayef N, Alkhalil SS, Pant SD, Lei H and Zan L. 2023. Effect of tea saponins on rumen microbiota and rumen function in Qinchuan beef cattle. Microorganisms **11**(2): 374.

Ramírez-Restrepo CA, O'Neill CJ, López-Villalobos N, Padmanabha J, Wang JK, McSweeney C. 2016. Effects of tea seed saponin supplementation on physiological changes associated with blood methane concentration in tropical Brahman cattle. Animal Production Science **56**(3):457-465.

Rawdkuen S, Murdayanti D, Ketnawa S and Phongthai S. 2016. Chemical properties and nutritional factors of pressed-cake from tea and sacha inchi seeds. Food Bioscience **15**: 64-71.

Santoso B, Kilmaskossu A and Sambodoc P. 2007. Effects of saponin from *Biophytum petersianum* Klotzsch on ruminal fermentation, microbial protein synthesis andnitrogen utilization in goats. Animal Feed Science and Technology **137**: 58–68.

Sarmah K, Das P, Saikia GK and Sarmah TC. 2018. Biochemical characterization of tea (*Camellia* spp.) seed oil cake. Bulletin of Environment, Pharmacology and Life Sciences **7** (8): 98-102.

Singh J and Gaikwad DS. 2020. Phytogenic feed additives in animal nutrition. Natural Bioactive Products in Sustainable Agriculture 273-289.

Sylvester JT, Karnati SKR, Yu Z, Morrison M, Firkins JL. 2004. Development of an assay to quantify rumen ciliate protozoal biomass in cows using real-time PCR. The Journal of Nutrition **12**: 3378-3384.

Szumacher-Strabel M and Cieslak A. 2010. Potential of phytofactors to mitigate rumen ammonia and methane production. Journal of Animal and Feed Sciences **19**: 319–337.

Tan C, Ramírez-Restrepo CA, Shah AM, Hu R, Bell M, Wang Z, McSweeney C. 2020. The community structure and microbial linkage of rumen protozoa and methanogens in response to the addition of tea seed saponins in the diet of beef cattle. Journal of Animal Science and Biotechnology **11**: 1-10.

Torres RT, Carvalho J, Fernandes J, Palmeira JD, Cunha MV and Fonseca C. 2021. Mapping the scientific knowledge of antimicrobial resistance in food-producing animals. One Health **13**: 100324.

Ueda H. 2001. Short-term feeding response in chicks to tea saponin. The Journal of Poultry Science **38** (4): 282-288.

Vincken JP, Heng L, de Groot A and Gruppen H. 2007. Saponins, classification and occurrence in the plant kingdom. Phytochemistry **68**: 275–297.

Wallace RJ, McEwan NR, McIntosh FM, Teferedegne B and Newbold CJ. 2002. Natural products as manipulators of rumen fermentation. Asian-Australasian Journal of Animal Sciences **15**: 1458–1468.

Wang B, Tu Y, Zhao SP, Hao YH, Liu JX, Liu FH, Xiong BH and Jiang LS. 2017. Effect of tea saponins on milk performance, milk fatty acids, and immune function in dairy cow. Journal of Dairy Science **100** (10): 8043-8052.

Wang JK, Ye JA and Liu JX. 2012. Effects of tea saponins on rumen microbiota, rumen fermentation, methane production and growth performance—a review. Tropical Animal Health and Production **44**:697-706.

Wei ML, Ren LP, Zhou ZM and Meng QX. 2012. Effect of addition of three plant extracts on gas production, ruminal fermentation, methane production and ruminal digestibility based on an in vitro technique. Journal of Animal Veterinary Advances **11**: 4304-4309.

Wina E, Muetzel S and Becker K. 2005. The impact of saponins or saponin-containing plant materials on ruminant production-a review. Journal of Agricultural and Food Chemistry **53**: 8093– 8105.

Wu J and Zhong. 1999. Production of ginseng and its bioactive components in plant cell culture: Current technological and applied aspects. Journal of Biotechnology **68**: 89-99.

Yamauchi Y, Azuma K, Tomita M, Horie H and Kohata K. 2001. Development of a simple preparation method for tea-seed saponins and investigation on their anti yeast activity. Japan Agricultural Research Quarterly **35** (3): 185-188.

Yan S, Zhao S, Jiang Q, Fang L, Zhou M, Min W and Jiang L. 2016. Effects of tea saponin on rumen fermentation and rumen microflora of dairy cows. Chinese Journal of Animal Nutrition **28** (8): 2485-2496.

Yang P, Zhou M, Zhou C, Wang Q, Zhang F and Chen J. 2015. Separation and purification of both tea seed polysaccharide and saponin from camellia cake extract using macroporous resin. Journal of Separation Science **38** (4): 656-662.

Yao GL, He W, Wu YG, Chen J, Hu XW and Yu J. 2019. Structure and functional properties of protein from defatted *Camellia oleifera* seed cake: Effect of hydrogen peroxide decolorization. International Journal of Food Properties **22**(1): 1283-1295.

Yu XL and He Y. 2018a. Development of a rapid and simple method for preparing tea-leaf saponins and investigation on their surface tension differences compared with tea-seed saponins. Molecules **23** (7): 1796.

Yu XL and He Y. 2018b. Tea saponins: Effective natural surfactants beneficial for soil remediation, from preparation to application. RSC Advances **80**: 24312–24321.

Yu Z, Wu X and He J. 2022. Study on the antifungal activity and mechanism of tea saponin from *Camellia oleifera* cake. European Food Research and Technology **248**: 783–795.

Zhang X, Ma H, Quaisie J, Gu C, Guo L, Liu D, Chen Y, Zhang T, 2022. Tea saponin extracted from seed pomace of *Camellia oleifera* Abel ameliorates DNCB-induced atopic dermatitis-like symptoms in BALB/c mice. Journal of Functional Foods **91**: 105001.

Zhao W, Li N, Zhang X, Wang W, Li J and Si Y. 2015. Cancer chemopreventive theasaponin derivatives from the total tea seed saponin of *Camellia sinensis*. Journal of Functional Foods **12**: 192-198.

Zhao Y, Su R, Zhang W, Yao GL and Chen J. 2020. Antibacterial activity of tea saponin from *Camellia oleifera* shell by novel extraction method. Industrial Crops and Products **53**: 112604.

Zheng T, Xing Y, Zhou Z, Cristhian C and Jiang J. 2016. Enhancement of bioethanol production using a blend of furfural production residue and tea-seed cake. Bio Resources **11** (3): 7451-7461.

Zhou XCS, Tan WJ, Salem ZL, Geng AZM, Tang MM, Wang SX, Han M and Kang XF. 2012. Effects of dietary supplementation of tea saponin (Ilex kudingcha CJ Tseng) on ruminal fermentation, digestibility and plasma antioxidant parameters in goats. Animal Feed Science and Technology **176**: 163-169.

Zhou YY, Mao HL, Jiang F, Wang JK, Liu JX and McSweeney CS. 2011. Inhibition of rumen methanogenesis by tea saponins with reference to fermentation pattern and microbial communities in Hu sheep. Animal Feed Science and Technology **166–167**: 93–100.