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Scope of utilization of tannin & saponin to improve animal performance

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Abstract
Optimum animal production depends on the type of feedstuffs available for the whole year. Plant materials are usually rich in anti-nutritional factors/ phytochemical known as tannins and saponins. Tannins are generally classified into two groups: hydrolysable tannins (HT) and condensed tannins (CT). Tannins have both harmful and beneficial effects depending on their nature, concentration, animal species, animal health and feed composition. Tannin based forages have anti-bloat and anthelmintic characteristics which improve the quality of meat and milk products, fatty acids composition and ruminant antioxidant status **(**Blackmon *et al.*, 2016) [9]. Tannin and energy only had interaction effect on the crude protein intake and had a tendency to reduce rumen gas production (Pobletea *et al.*, 2020) [30]. Condensed tannins have positive impact on milk production and antioxidant status in lactating cows. (Dey and De. 2014).The saponins have showed potential to modulate rumen fermentation patterns and mitigate *in vitro* CH4 production (Goel *et al.* 2008). Use of saponins rich forages may have synergistic effect on the rumen fermentation pattern and improves DM intake on palatability, nutrient digestibility, rumen metabolites and carcass quality of male goat (Hundal *et al.* 2019) [29]. Tannins and saponins if used at correct concentration to alter rumen fermentation pattern and increased microbial protein synthesis which may be utilized for promoting animal production without any adverse effects.

Keywords: tannins, saponins, production performance, nutrients utilization, rumen fermentation pattern

Introduction
The term tannin was first applied by Seguin in 1796 to denote substances present in plant extracts, which were able to combine with protein of animal hides; prevent their putrefication and convert them into leather. Tannins are astringent, bitter-tasting plant polyphenols that bind and precipitate proteins. The term tannin refers to the source of tannins used in tanning animal hides into leather; however, the term is widely applied to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups (such as carboxyls) to form strong complexes with proteins and other macromolecules. Tannins have molecular weights ranging from 500 to over 3,000 (Handa, S.S. and Kapoor. V.K., 2003) [24].

According Horvath (1981) [28]: "Any phenolic compound of sufficiently high molecular weight containing sufficient hydroxyls and other suitable groups (i.e. carboxyls) to form effectively strong complexes with protein and other macromolecules under the particular environmental conditions being studied" Tannins have both harmful and beneficial effects depending on their nature, concentration, animal species, animal health and feed composition. Tannin based forages have anti-bloat and anthelmintic characteristics which improve the quality of meat and milk products, fatty acids composition and ruminant antioxidant status (Blackmon, T. K., *et al.*, 2016) [9]. In addition, these tannins containing plants also contribute to inhibit the enteric methane emission (Anantasook *et al*., 2016; Gunun *et al.*, 2017) [2, 23]. Tannins have both harmful and beneficial effects depending on their nature, concentration, animal species, animal health and feed composition. Some tannin Containing Plants induce the toxicity in ruminants and monogastric animals which in turn, decrease the feed intake, nutrient digestibility, protein availability and production performances (Bernes *et al*., 2000, Huang *et al.*, 2018) It is needed to explore the different strategies to control the injurious effect of dietary tannins on animals health. Tannins have the ability to form complexes with protein, amino acids and polysaccharides. CT binds with protein and makes tannin-protein complexes unavailable for degradation by ruminal bacteria and these complexes 60% increase the absorption of amino acid in the small intestine (Jonker & Yu, 2017) [31].

Physical Properties
- Color: Reddish brown and Dark brown
- Solubility: Soluble in water, acetone, glycerols, and alcohol.
- State: non-crystalline
- Taste: Bitter and Puckering taste

Classification of tannins
Tannins are generally classified into two groups: hydrolysable tannins (HT) and condensed tannins (CT).

Hydrolysable tannins
These tannins are hydrolyzed by acids, or enzyme and produce gallic acid and ellagic acid. Chemically, these are esters of phenolic acid like gallic acid and ellagic acid. The tannins derived from gallic acid are known as gallitannins and from that of ellagic acid are known as gallitannins. The gallic acid is found in rhubarb, clove and ellagic acid is found in eucalyptus leave and myrobalans and pomegranate bark. These tannins treated with ferric chloride to produced blue or black colour.

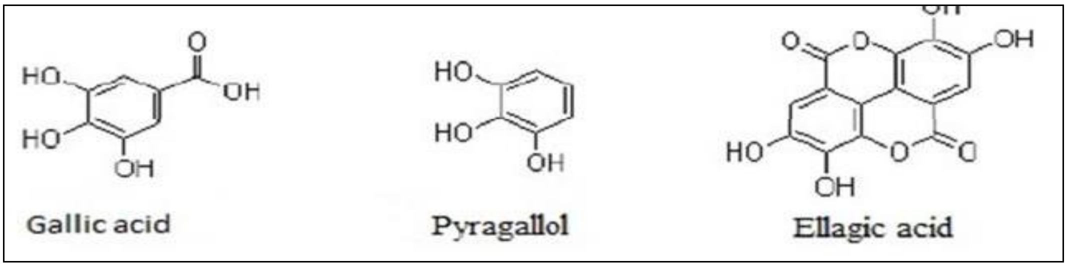
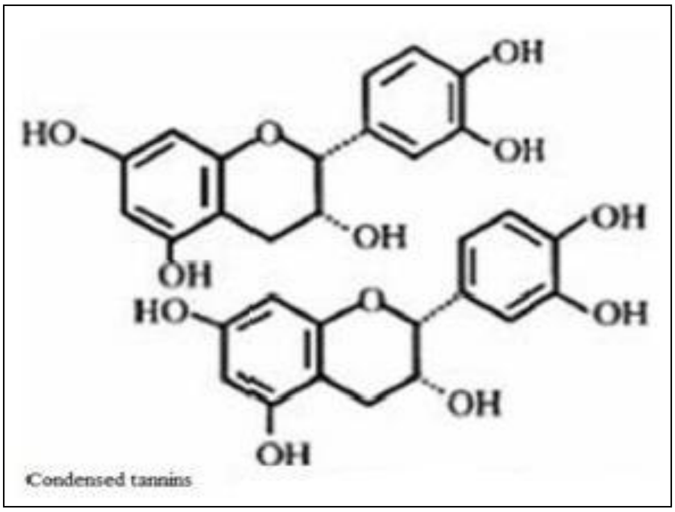


Fig 1: Chemical Structure of Hydroysable tannins (HT) (Huang *et al.*, 2018)

Condensed tannins
These tannins are resistant to hydrolysis and they derived from the flavonols, catechins and flavan-3, 4-diols. On treatment with acids or enzymes they are decomposed into phlobaphenes. On dry distillation condensed tannin produce catechol. These tannins are called as catechol tannins. These tannins are found in cinchona bark, male fern, areca seeds, tea leaves and wild cherry bark, bahera fruits, Amla, etc. they produce green colour with ferric chlorides.



Mechanism of action of Condensed Tannin
Condenses tannin and plant protein present in the plant and during the process of mastication cell's will rupture and least to formation of insoluble condenses- tannin protein complex which on reaching of rumen protein degradation by rumen microbes at ph 5.7 result in separation of condenses tannin protein complex which take place abomasums ph 2.5 to 3.5 and this free CT and protein reaches' duodenum at ph 7 protein in digested and absorbed.

Biological activities of tannins
Tannin-rich foods have potential antioxidant, cancer-fighting activity, and reduce the risk of developing cardiovascular disease as well, although their consumption inhibit non heme iron bioavailability (Kitunen *et al.,* 2017). The oxidation reduction reaction that occurs during the interaction of phenolic hydroxyls with metal ions reduces the absorption of metal ions such as calcium and iron. Yet an outside of tissues interaction between calcium and tannins has a blood-pressure-lowering effect (He *et al.*, 2015) [26]. Moreover, tannin-collagen interaction forms complexes that are expected to be stabilized mainly through hydrogen bonding between the protein amide carbonyl and the phenolic hydroxyl as well as the covalent and hydrophobic bonds. Such complexes are substantially important in dental application, particularly the polyhydroxylated PACs. This is mainly because the polyhydroxylated PACs can form an insoluble matrix with carbohydrates and proteins (Bedran-Russo *et al*., 2014) [8]. On the other hand, the interaction of tannins with saliva proteins decreases saliva lubricity results in feeling of dryness by causing contraction of tongue epithelial tissue. This phenomenon is referred to as convergence or astringency. The study of fruit astringency has obtained considerable attention since astringent substances have appreciable biological activity such as antibacterial, antiviral, antiinflammatory, antioxidant, anticarcinogenic, antiallergenic, hepatoprotective, vasodilating, and antithrombotic activities (He *et al*., 2015) [26].

Tannins are implicated in the antiulcer properties of several herbal products. Particularly, in peptic ulcer disease, tannins act by precipitating the microproteins and thus form a protective pellicle that prevents absorption of toxic substances, and boosts resistance to the proteolytic enzymes, a related activity against H. pylori. Moreover, in experimental models, such compounds that are isolated from secondary tannic metabolites showed both in vivo and in vitro activity against peptic ulcer disease (De Jesus *et al.*, 2012) [17]. In addition, the anticancer activities of tannins against a range of cancer cells have been investigated. In most of the cell lines such as hepatocellular carcinoma (HCC), breast cancer, lymphocytic leukemia, lung cancer, and epidermoid carcinoma, tannins demonstrated cytotoxic activity in a dose-dependent fashion.

The structural activity relationship studies pointed out the number of galloyl moieties in tannins have direct relation to higher anticancer efficacy (Cai *et al*., 2017). Besides, as the degree of polymerization of tannins increases, which results in increase in number of hydroxyl groups, the free radical inhibitory activity of the resulting compound will be stronger. Furthermore, tannins can readily react with oxygen radicals and release a large number of free hydrogen due to the phenolic hydroxyl groups present in their structure. Being strong free radical scavengers, tannins can reduce the risk of aging and related diseases (cardiovascular disease, aging, cataracts) (He *et al*., 2015) [26].

Tannins have strongly displayed cardioprotective activity through inhibiting elastin-degrading enzymes, inducing stabilization of pericardial tissue, and decreasing calcification of the glutaraldehyde-fixed aortic wall (Sieniawska and Baj, 2017b) [46]. Furthermore, in vitro studies depicted that procyanidin-containing cocoa inhibits platelet aggregation, which is a common pathology to cardiovascular diseases. It was also reported that tannins from Arbutus unedo (or “Strawberry Tree”) leaves inhibited thrombin induced platelet aggregation. Leaves of this tree are traditionally utilized as a remedy for high blood pressure (Maca´kova´ *et al.*, 2015).

The antibacterial activities of polyphenols such as tannins, some flavonoids, and xanthones revealed that they are effective against Methicillin-resistant Staphylococcus aureus (MRSA) (Hatano *et al.*, 2005) [25].

Several plants-based phenolic antimicrobial agents are available in certain food known for their tannin, essential oils, resins, and glycosides content. For instance, the bioactive component in cacao and tea is catechin, gallic acid in berries, allicin in garlic, eugenol in cloves, thymol and carvacrol in oregano, eugenol and thymol in sage, and allyl isothiocyanate in mustard (Anon, 2001) [4]. Moreover, antimicrobial hydrolysable tannins and proanthocyanidins exist in different medicinal plants, vegetables, and fruits. These secondary plant metabolites act as antibacterial agent through different mechanisms, including disintegration of bacterial cell wall, interfering bacterial metabolism, and by inhibiting extracellular enzymes.

Their antiviral activity is also reported to be related to the basic tannin structure. Mechanistically, the tannins have been demonstrated to interfere with the viral adsorption of HSV and HIV, which is an action attributed to the tannins’ ability to bind to viral surface proteins.

In addition, most active ellagitannins and several proanthocyanidins have been reported to be potent reverse transcriptase inhibitors (Smeriglio *et al*., 2017) [48].

Processes for Reducing Anti nutritional Factors

There are several methods of treating seeds including soaking, germination, fermentation, extrusion, pre-cooking with steam, etc.

Soaking

Traditionally, this operation was aimed at reducing the cooking time. This is an operation to hydrate the seeds by facilitating the diffusion and dissolution anti nutrients to the water phase. Soaking facilitates digestion and improves the absorption of contain nutrients by body. The effect of soaking on ANFs content depends on its duration. Normal soaking requires an average of 8 to 12 hours at room temperature gives some dipping processes carried out in different types of grains. A soak of 24 hours allows a significant reduction of its phytate content. The reduction rates are 17% for rice grains, 21% for maize grains, 23% for soybeans and 28% for millet. According to the results of a study on the evolution of anti nutritional factors of two cowpea seed varieties, soking in 24 hours resulted in a loss of 8.4% in phytates. This loss of phytate levels has been confirmed or even exceeded by the studies of. The latter obtained a reduction in the phytate content of 22.4 and 23.7% on two varieties of cowpea after 24 hours of soaking. The simple soaking of cowpea reduces the stachyose content by 30%. These FAN reductions, which vary differently, can be attributed to their difference in water solubility. However, other dipping solutions are experimented such as the use of sodium bicarbonate. Addition of sodium bicarbonate ($NaHCO_3$) to the soaking water can lead to significant reductions in ANF levels. Indeed, a 12 hours soaking with addition of sodium bicarbonate resulted in reductions of 41, 45 and 40% in total alpha-galactoside (stachyose and raffinose) in three varieties of red beans respectively. These results show that there is a difference in the contents of reduced ANFs according to the soaking solution used. The addition of sodium bicarbonate caused a greater reduction compared to soaking with simple water. Thus, the sharp reduction observed during the soaking with addition of sodium bicarbonate is due to the tenderness of the seeds facilitating the rapid dissolution of these factors in the soaking solution. These studies show that soaking is a good way to reduce ANFs in legumes such as cowpea. Thus, the loss of these ANFs during soaking of the seeds can be explained differently. Regarding tannins, classified as polyphenols, are located at the level of the teguments and their loss during soaking of the seeds is attributed to its effect of creating an ionic environment. The modified ionic environment could in turn modify the permeability of the integument, thus allowing larger and faster losses.

In addition, the decrease in phytic acid during this soaking process is attributed to its phytase catalyzed hydrolysis. Thus this hydrolysis of the phytate molecule causes a release of minerals that become available for intestinal absorption.

Table 1: Different soaking processes for reducing anti-nutrition factors

Seeds Types	Soaking Solution Types	Ratio (Weigh : Volume)	Soaking Times / Soaking Temperature	Reduction Rate	References
Chick Pea	Solution (water + 0,01N NaOH + 0,01N HCl)	1 : 5	-----/-------	-----/------	(Haileslassie and al., 2016) [49]
Beans	Tap water	1 : 5	12 hours /----	13.04 %, 20.82 % for tannins	(Khandelwal and al., 2010) [50]
Cereal Grains (Millet, Sorghum And Maize, Rice)	Mineral water (Evian)	1 : 3	24 hours/ 30°C	28 % of phytates	(Lestienne and al., 2005) [24]
Bean	Distilled water	1 : 3	----/----	------/-------	(Piecyk and al., 2012) [51]
Red Bean	water (pH= 6,9) 0,05% Na_2CO_3, PH= 8,2 solution	1 : 3	12hours/ ambient	40, 41 and 45 % of alpha-galactoside	(Shimelis and Rakshit, 2007) [52]
Cowpea	Mineral water (Evian)	1 : 5	24 hours/ 30°C	Increase (-11.62%)	(Lestienne and al., 2005) [24]
Cowpea	Distilled water Solution of 0,02% Na_2CO_3; pH= 8,3)	1 : 10	2 , 4 et 6 hours/-------	10.81%, 19.60% for phytates and 22.22%, 32.92% for tannins	(Avanza and al., 2013) [14]

Sprouting

By definition, germination is a process that incorporates events beginning with the absorption of water by the mature dry seed and ending with the protrusion of the radical or more generally of a portion of the embryo through the envelopes of the seed. Germination is often used to improve the concentration and bioavailability of nutrients in foods. It improves the nutritional value of cereals and legumes by increasing protein digestibility, essential amino acid content and vitamins, while decreasing some anti nutritional factors.

Thus, during germination, important changes occur between the seeds and the culture medium. Changes include among others to the secretion of ions, oxygen and enzymes and a wide range of primary and secondary carbon-containing metabolites.

Germination can cause phytate hydrolysis and decrease their inhibitory effects on mineral uptake. It lasts a few days and can be initiated by a few simple steps namely rinsing the seeds to remove all the impurities, soaking the seeds in the water. Table 2 gives a summary of the conditions used for germination for different types of cereals and pulses. Germination results in 37 to 81% reduction of phytate in different types of cereals and legumes.

Table 2: conditions used for germination of different types of karnels

Seeds Types	Ratio Seed : Soaking Solution(W/V)	Times /Temperatures/ Nature Of Soaking Solutions	Duration Of Germination	Reduction Rate	References
Millet, Sorghum	-----	24 hours/25°C/-----	7 days	------	(Ochanda and al., 2010) [61]
Amaranth	-----	Night/----/ Distilled water + 0,2% formaldehyde solution	72 hours	34,66% of tannins	(Olawoye and Gbadamosi, 2017) [62]
Beans (Green Beans / Mung Beans)	1 :5	12hours/ambiente/ Tap water	24 hours	43% of tannins	(Khandelwal and al., 2010) [50]
Red Beans	1 : 5	12 hours/25°C/ Distilled water + 0.01% (w/v) d'eau de javel	4 days	76% of tannin ; 92% of phytic acid	(Shimelis and Rakshit, 2007) [52]

Steam Pre-Cooking

Steam pre - cooking is a unit operation used for the processing of certain food products, in this case rice and millet. It is applied to enhance the nutritional quality of rice. This process reduces FAN levels in legume and cereal seeds.

In fact, steam pre – cooking resulted in a 52% reduction in phytate content of beans, 56.5% in sunflower and 47.9% in rice grains. In the production of "moin-moin" (steamed cowpea paste containing seasonings), there was a decrease in phytate contents between 7.8-14.0% and tannins of 19.6-24.7%. This decrease in phytate during heat treatment would be the result of their degradation by heat. This is due to the thermo labile nature of phytic acid.

For tannins, they are localized at the level of grain teguments and are destroyed during the heat treatment process. In this thermal process, the temperature and the treatment time are good optimization parameters. Reductions in average phytic acid content from 38.14 mg/g for untreated cereal brans at 18.72 mg/g for cereal brans subjected to steam pre-cooking at 100 ° C for 25 minutes, 18.08 mg / g at 110 ° C for 25 minutes and 18.74 mg / g at 115 ° C for 25 minutes were recorded. These recordings showed that the maximum reduction (52.60%) of the phytic acid content was obtained at 110 ° C for 25 minutes. Thus, in the case of cowpea, similar losses in ANFs could be considered because of the heat labile nature of some of them or their location in teguments whose heat facilitates their destruction. A combination of this treatment technique with the other processes could give better reductions in ANFs while retaining nutritional and organoleptic qualities of the final product.

Extrusion Cooking

Extruder whose use for food processing for the first time dates back to 1869 and has led to a widespread development of extrusion cooking. The extrusion cooking technique is a process that combines thermal and mechanical processes to produce a precooked product. This technique reduces ANFs by increasing the bioavailability of minerals. It thus makes it possible to reduce phytates by approximately 30%. It was found by reductions in phytic acid of 26.73% and 20.75% in extruded seeds of beans (Vicia faba L.) and green beans (Phaseolus vulgaris) respectively. Studies have shown that during the extrusion cooking process some phytic acid molecules are hydrolysed to inositol penta-, tetra- and triphosphates.

However, the extrusion temperature and the moisture of the product to be extruded play an important role in the reduction of ANFs. Thus, a 55.83% reduction in phytic acid was observed during extrusion cooking of rice bran at a temperature of 140 ° C and 20% humidity. Lower moisture content during extrusion results in a lower degradation of the phytic acid of grain brans. According to at 115°C the average values of phytic acid in extruded rice brans were 19.92, 18.63 and 17.35 mg/g at 14, 17 and 20% humidity respectively. The observation of these results makes it possible to remember that the extrusion. Temperature is a factor that can significantly influence the ANFs content compared to the moisture of the extruded product. Thus, 140°C temperature and 20% humidity would be the most appropriate parameter pair for reducing ANFs in a food product. In addition, the technique of extrusion cooking compared to the other processes studied above would be preferable. Indeed, it makes available minerals without destroying them while other processes such as soaking and germination cause a diffusion of these minerals in the soaking water. (Gupta *et al.*, 2017)

Fermentation

Fermentation is one of the effective, inexpensive and nutritionally beneficial domestic processes. Fermentation not only improves food safety and shelf life, naturally preserving food and also improves nutritional value.This is a treatment technique that starts with the hydrolysis of starch by the action of enzymes. In general, lactic acid bacteria are essential in fermentations for the production of metabolites, degradation of cyanogenic glucosides, production of enzymes, probiotic properties and the production of many other molecules. The reduction of FAN levels during fermentation is attributed to the activity of fermentative microorganisms. Fermentation leads to a decrease in alpha - galactosides and phytates and has a positive effect on the availability of iron and other minerals. It promotes optimal pH for the enzymatic degradation of phytates. It reduces the levels of some anti nutritional factors, particularly phytates and α-galactosides, in cereals and legumes, for millet, it reduces phytate and raffinose content by 75% and 83% respectively. Fermentation is therefore a microbial and enzymatic method of treatment which not only extends the shelf life of foods but leads to a significant reduction in FAN levels. This reduction role is indeed made possible by the lowering of the pH. Compared with soaking, the reduction rate obtained by fermentation is more significant; this leads to the conclusion that the use of fermentation for the treatment of seeds legumes and particularly cowpea would be a good asset of the nutritional point.

Role of Tannins in Animal Husbandry

Bacterial and fungal infection is also a threat to the poultry, livestock, and animal husbandry which is responsible for high level of mortality. To overcome this problem, several antibiotics have been used for decades that proved to be very effective; consequently, it improves animal and poultry production in the world. But it is well known that extreme application of antibiotics promotes the antibiotic resistant among the microorganisms in cattle (Rdondo *et al.*,2014, Liu XL *et al.*,2013 & Chattopadhgay *et al.*,2014) [11]. Therefore, in-feed antibiotics and plant-based antibacterial agents, such as phytogenic compounds (e.g., tannin), have been discovered and promoted, which have great promises in future. In recent past, great attention has been given to antibacterial activities of tannins and their effects as dietary source in animal (Rdondo *et al.*,2014). It has been concluded that tannins with saponins and essential oils can be used as in-feed antibiotics against bacteria, fungi, and yeasts. Because, tannins are toxic to bacteria and potentially inhibit growth of *Salmonella*, *Shigella*, *Staphylococcus*, *Pseudomonas*, and *Helicobacter pylori*, but it would be noteworthy that they show species specific antibacterial activity. Moreover, tannin-containing forage in cattle diets helps to control animal pasture bloating, intestinal parasite, and disease causing bacteria in ruins of animals. Tannins can hinder microbial growth by using several mechanisms including lack of nutrient to bacterial cell, inactivate vital extracellular enzymes, inhibition of oxidative phosphorylation, chelation of metal ions, and complex formation with membrane andproteins (Liu XL *et al.*, 2013). It has been seen that condensed type of tannins are mainly present in forage legumes, trees, shrubs, tree leaves, and browse shrubs, but their concentration vary from species to species that influenced by environmental conditions also

(Huang Q *et al.*,2017). Tannins from mimosa (HT), chestnut (HT), and quebracho (CT) have been used as in-feed antibiotics in animals (Min BR *et al.*,2015) [6]. But the major challenge for tannins as antibiotic is the lack of systematic and comprehensive studies on the various aspects such as doses, side effects on digestions simultaneously prolong use can develop resistance against the in-feed antibiotics as in case of normal antibiotics (Huang Q *et al*.,2017). Moreover, tannins are anti-nutrient factors for monogastric animals and poultry. Tannins can also act as the anti-nutrients in rumens of livestock due to their binding to vital biomolecules in biological systems. Several adverse effects such as availability of nutrients, metal ions chelation, binding with proteins and hinder.

The growth of beneficial microflora have been observed in the cattle gut. To test the adverse effect of tannin as diet component on lamb gut microflora and fermentation was studied. Both types of tannins, that is, hydrolysable and condensed with 4% extract of chestnut (*Castanea sativa*, *Caesalpinia spinosa*), *mimosa* (*Acacia negra*), and *gambier* (*Uncaria gambir*) feed to lamb. The results show that tannins meagerly affect gut microflora including fungi in the lamb gut for 45 days. Simultaneously, it also shows that high level of tannin inclusion in diet proved as antimicrobial agent against the harmful methanogens and protozoa without affecting ruminal fermentation (Salani *et.al,* 2010).

Research Findings of Scope of Utilization tannin to improve animal performance

Effects of Growth performance

A total of eighteen (18) healthy, Holstein x Sahiwal crossbred growing bulls (initial body weight of 162.8±12.7 kg at 15±0.80 months) from the Dairy Training and Research Institute, University of the Philippines-Los Baños were used in this study. The experimental animals were arranged in a 2x2 factorial set-up using unbalanced Randomized Complete Block Design with four to five replicate animals per treatment. The bodyweight of the experimental animal was used as the blocking factor. The four treatments were low energy concentrate without tannin (LENT), low energy concentrate with tannin (LEWT), high energy concentrate without tannin (HENT), and high energy concentrate with tannin (HEWT).

The animals were subjected to 14 weeks (98 days) of feeding including the 14-days adaptation period. After 14 days, the animals were weighed again when their feed intakes were stable. The forage was offered *ad libitum* daily with the concentrate supplemented twice a day (0800 and 1600h) at 3% of their BW on a DM basis and 75:25 forage to concentrate ratio. The forage was composed of available improved grasses and legumes. The growing bulls were kept in individual cages (0.75 m x 1.2 m) with separate feed bunk where clean water was made available at all times. The individual cages were regularly cleaned and maintained throughout the experiment. (Pobletea, *et al.* 2020) [30].

Table 3: Effects of Growth performance and daily nutrient intake of growing Holstein-Friesian x Sahiwal bulls fed with low and high energy rations with or without dietary tannin

	Groups				
Variables	**LENT**	**LEWT**	**HENT**	**HEWT**	**SEM**
Initial wt., kg	157.38	166.50	163.60	162.75	6.49
Final wt., kg	189.50	204.30	202.80	207.63	9.05
Total body wt. gain, kg	32.13	37.80	39.20	44.88	4.61
Average daily gain, kg/d	0.33	0.39	0.40	0.46	0.05
Feed conversion ratio	19.23	16.28	16.04	13.84	1.93
Total DM intake, kg/d	6.00	6.01	6.08	6.17	0.08
Concentrate intake	1.50	1.50	1.52	1.54	0.02
OM intake, kg/d	5.13	5.26	5.24	5.40	0.07
ME intake, Mcal/d	16.17	16.36	16.59	17.12	0.41
CP intake, kg/d	0.69^{ab}	0.70^{ab}	0.72^{a}	0.68^{b}	0.01
NDF intake, kg/d	3.58	3.62	3.52	3.51	0.05

**Note: Means in the sme row with different superscripts differ significantly (p<0.05); SEM= standard error of mean; LE= low energy; HE= high energy; NT= no tannin; WT=w ith tannin; T= tannin; E= energy.

Dietary tannin and feed energy levels have no effect in improving growth performance and rumen volatile fatty acids. Tannin and energy only had interaction effect on the CP intake. Increasing the energy of the diet improved only DM and NDF digestibilities. (Pobletea, *et al.* 2020) [30].

Effect of Milk production and composition from Holstein cows fed dififfrent levels of condensed tannins from Acacia mearnsii.

Five multiparous Holstein cows were used. Cows were distributed in a 5 × 5 Latin square, balanced for carryover effects, with an experimental period of 20 days (14 for adaptation and six days for collection). Treatments were inclusion levels of extract of CT from black wattle (*Acacia mearnsii*) at 0, 6.1, 12.2, 18.4, and 24.6 g/kg of diet dry matter (DM) of diet, which was mixed with the other ingredients in the concentrate. The extract was obtained in a very fine powder form, it was well mixed and there were no visual signs of sorting. According to the manufacturer, the product contained 80.52% of CT, 18.04% non-tannins, 1.44% insolubles, 2.11% ash, 59.80 ppm of iron, pH 4.9% and 5.65% humidity. Thus, considering the presumed tannin concentration in the commercial product, the doses chosen were 0, 5, 10, 15, and 20 g/kg CT in dietary DM. (Andre S. Avila *et. al* 2020) [3].

Table 4: Effect of Milk production and composition from Holstein cows fed dififfrent levels of condensed tannins from Acacia mearnsii.
Condensed Tannin Inclusion (g/kg of DM P- Value

Variable	0	5	10	15	20	SEM	CT	L	Q
MP (kg/d)	28.63	28.57	29.07	27.90	27.90	1.03	0.75	-	-
ECMP (kg/d)	26.59	26.58	27.15	24.82	24.43	0.85	0.03	0.01	-
ECMP/DMI	1.26	1.28	1.31	1.27	1.23	0.05	0.68	-	-
Fat (g/kg)	32.73	32.42	33.24	30.62	30.18	1.33	0.15	-	-
Protein (g/kg)	31.07	31.69	30.72	29.42	28.86	0.93	0.05	0.01	-
Lactose (g/kg)	46.03	45.23	46.30	45.04	45.23	1.09	0.51	-	-
Casein (g/kg)	24.36	24.90	24.20	22.79	22.47	0.81	0.05	0.01	-
TS (g/kg)	116.7	117.7	121.8	114.2	114.1	3.30	0.19	-	-
MUN (mg/dL)	14.91	16.27	16.09	15.29	14.92	0.65	0.16	-	-

Milk production, energy-corrected milk yield, fat, lactose, total solids and milk urea nitrogen did not differ (p>0.10) with increasing CT levels. Energy corrected milk and casein was reduced linearly (p<0.05) with inclusion of this additive, with a reduction in casein concentration in milk (g/kg) of 7.76% with the maximum inclusion of CT compared to the control treatment. There was a trend for a reduction in milk protein content (p<0.10) with a reduction of 7.11% in the treatment with greatest CT inclusion; however, it adversely affected the animals' performance by reducing the milk protein concentration. Therefore, based on these findings, the use of CT from *Acacia mearnsii* would be recommended for dry animals, or perhaps for growing animals due to its negative effects on milk protein concentration. (Andre S. Avila *et. al* 2020) [3].

Saponin

Saponins are secondary metabolites synthesized by many different plant species and marine animals. They derive their name from the Latin word "sapo" meaning soap, due to their surfactant properties, which allows forming stable soap-like foam when shaken in aqueous solution. They are large molecules and contain a hydrophobic part, composed of a triterpenoid (30 carbon atoms) or steroid backbone (27 carbon atoms with a 6-ring spirostane or a 5-ring furostane skeleton) and a hydrophobic part consisting of several saccharide residues linked to the hydrophobic scaffold through glycose bonds. (Augustin *et al*., 2011) [5].

Saponins are glucosides with foaming characteristics. Saponins consist of a polycyclic aglycones attached to one or more sugar side chains. The aglycone part, which is also called sapogenin, is either steroid (C27) or a triterpene (C30). The foaming ability of saponins is caused by the combination of a hydrophobic (fat-soluble) sapogenin and a hydrophilic (water-soluble) sugar part. Saponins have a bitter taste. Some saponins are toxic and are known as sapotoxin.

The physiochemical and biological properties of saponins have led to a number of traditional and industrial applications. They have traditionally been used as natural detergents. The combination of a hydrophobic aglycone backbone and hydrophilic sugar molecules confers foaming and emulsifying properties of saponins (Moses T el al 2014) [45]. The name 'saponin' is derived from the Latin word 'sapo,' meaning soap, as a soapy lather forms when plants containing saponins are agitated in water. They also exhibit a variety of biological activities. Plant-derived triterpenoid and steroidal saponins have been used in the production of steroid hormones in the pharmaceutical industry, as food additives, fire extinguishers and in other industrial applications. Other interesting biological applications include their use in anti-inflammatory, hypocholesterolemic and immune-stimulating remedies (Cheeke PR *et al* 2006 & Liu *et.al* 2016) [12, 42].

Mechanism of action of saponins

The main function of secondary metabolites of the plants was providing defense against many pathogens and herbivores. In other words, plants secrete secondary metabolites as their defensive system. As saponins are one of the categories of secondary metabolites, their main function is to provide protection to the plants against many pathogens and herbivores. The various activities of saponins such as antimicrobial, antifungal, antiviral, antihelminthic, insecticidal, larvicidal and molluscicidal activities were very well documented. But the molecular and biochemical mechanisms of various activities of different saponins were not well elucidated (Dourmashkin *et al.*, 2005). First reported based on their experiments, saponins cause membrane perturbation by the formation of pores on the membrane. Based on their observation on formation of pores or pits on the membrane, (Bangham and Horne 1962 and Glauert *et al.*,1962) [7, 22] concurrently reported that the presence of cholesterol on the target membrane is essential for the saponins to induce pore formation. According to their reports, saponins and cholesterol associated spontaneously into a micelle-like complex and the hydrophilic sugar moieties are thought to be located in the central of the complex and leads to the development of aqueous pores. Such pores can increase the permeability of membrane and enabling the macromolecules and ions to pass through the membrane bilayer. There after many scientists elucidated the molecular basis of the membrane penetration activity by saponins and confirmed the impact of membrane composition on the ability of saponins to cause membrane perturbation. These results supported directly or indirectly by many reports on the membrane composition as several researchers found that cholesterol is a major lipid of membranes and cholesterol is known as membrane moderator/membrane plasticizer. (Kruijff 1992 & 1995) studies on cholesterol as a target for toxins reported that the specific orientation of cholesterol within the membrane facilitates channel formation by polyene antibiotics, bacterial protein toxins and various sterols, inspired the many researchers to further elucidate the molecular mechanism of saponins action. (Kenji 2000) [33] and (augustin 2011) [5] reported the molecular basis of the saponins activity which supported and expand the initial hypothesis and reported that saponins incorporation into the membranes occurs spontaneously and it is due to the lipophilic/hydrophobic character of aglycone portion and interactions between the sugar chains of the incorporated saponins responsible for the phase-separation phenomena. These accumulations finally leads to the membrane curvature, which may be due to either formation of pores within these plaqus/matrices or due to hemitubular alterations as protuberances leads to vesiculation, or they caused membrane domain disruption.The pores provide the explanation for the changes in ion conductivity and the movement of macromolecules upto proteins through the membrane. (Keukens et al. 1992 & 1995) [34, 35] and (Dourmashkin et al. 1962) were also reported the absence of pore-like structures when some steroidal saponins targeted the membranes. (Dourmashkin et al. 1962)also revealed that these steroid saponins even prevent the formation of pores on the subsequent exposure by pore-forming saponins. Finally computational studies by Lin and Wang revealed that both pore formation and hemitubular vesiculation may exist parallel and the chemical properties(type of aglycone portion and sugar chains) of the saponins determines the predominate perturbation type. (Krawczyk et al., 2010) [36] demonstrated the diversity of the different saponins on their ability to cause membrane perturbation. Lin and (Wang 2010)developed an alternative model based on molecular dynamic simulations, according to this model saponins migrate into lipid rafts (membrane domains enriched with cholesterol and sphingomyelin) and forms complexes with cholesterols and leads to lipid raft disruption (Brown DA, London E 2000) [10] and leads to membrane alterations in terms of their structural and permeable properties.

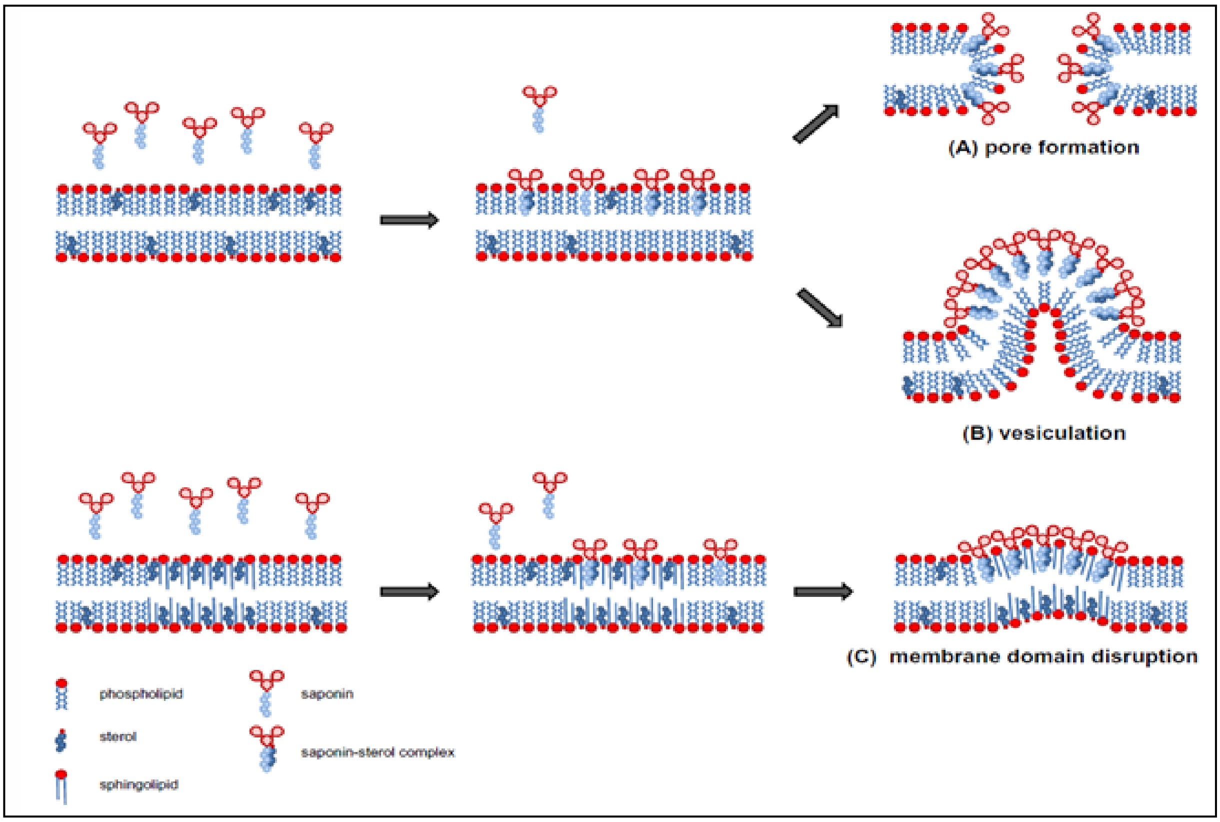


Fig 2: Mechanism of action of Saponins

Role of Saponin
Molluscicidal activity
Some saponins have shown molluscicidal activity against the snail *Biomphalaria glabrata*, intermediate host of *Schistosoma mansoni* parasite that causes schistosomiasis that affects millions of people in Asia, Africa and South America. The monodesmosidic saponins containing the sapogenin oleanolic acid are those with the highest activity, because these saponins interact with greater intensity with the cholesterol present in cell membrane altering their permeability, promoting the formation of pores and allowing leakage of liquids. This same mechanism of action can be used to explain the activities and ictiotoxic spermicides presented by some saponins (Lacaille-Duboi SMA, W Ragner H 1962) [37] The eradication of the intermediate hosts and chemotherapy are the only ways to control schistosomiasis. Currently, the main molluscicide used is niclosamide. However, the high cost makes their use impractical for populations where schistosomiasis is endemic, besides being toxic to humans. Thus, due to the high toxicity of saponins for molluscs and their low toxicity for humans when orally ingested, the substances produce good candidates for development of an alternative chemotherapy to combat the causative vector of schistosomiasis (Lanzotti V *et al.*, 2012) [39].

Anti-hypercholesterolemic activity
Hypercholesterolemia is a risk factor that contributes to the development and progression of atherosclerosis and subsequent cardiovascular disease. Epidemiological and clinical data have shown that high concentrations of LDL cholesterol in the bloodstream is the large pivot of these diseases (Choti HJ *et.al.*,2010) [14] Bioactive compounds with hypocholesterolemic activity have been conducted, among the most studied are soluble fibers, phytosterols, phospholipids, soy protein, stearic acid and saponins (Cohn JS. *et.al.*,2010) [15].

There are several mechanisms which siphoning may reduce cholesterol levels:

1) Formation of an insoluble complex where it is added to the beta-hydroxysteroid, thereby decreasing intestinal cholesterol absorption, producing an increase of sterols which are excreted along with feces;
2) Adsorption of bile acids in the diet of the fibers is increased in the presence of saponins because they form micelles with large molecular weights, which prevent bile acids that are reabsorbed. Thereby the increase occurs in the liver through the conversion of cholesterol into bile acids;
3) Interaction with cells of the intestinal mucosa promoting a higher permeability of these cells and subsequently a rapid loss of cell function by increasing proliferation promoting exfoliation and loss of this function. Thus, it contributes to a further increase in the excretion of cholesterol;
4) The presence of sugars, β-1, 4 connected enhances the absorption of soluble fiber and promotes the reduction of fatty acids, resulting in a decrease of liver cholesterol (Milgate J *et.al* 1995) [44]ss

Anti-inflammatory and antiallergic activity
The evaluation of anti-inflammatory activity of saponins have been performed using models of inflammation with carrageenan (Levy L.,1969) [40] In general, the oleanane and ursan sapogenins are those with higher activity. The mechanisms considered for this activity include corticomimeticactivity inhibiting the degradation of the glucocorticoid of release of mediators of inflammation, inhibition of enzyme formation and inhibition of increased vascular permeability (Matsuda H *et.al.*,1995) [43].

Cytotoxic and antitumor activity

There have been numerous reports of scientific papers in relation to the cytotoxic properties of saponins, however saponins do not always have high cytotoxic antitumor properties. The cytotoxic mechanism of saponins occurring via inhibition of DNA synthesis induces a reverse phenotypic transformation into tumor cells. When the antitumor mechanism occurs through the inhibition of vessels around the tumor, there is an inhibition of tumor growth. Inhibition of metastasis, as well as immunostimulation and observed chemoprevention mechanisms are important in antitumor compounds.As almost all saponins induce apoptosis in tumor cells, they become the preferred drug in treating cancer because they eliminate tumor cells with low side effects for the patient, avoiding mainly necrosis (Lacaille-Dubois MA *et.al* 2000) [38].

Antiviral activity

Some saponins may inhibit DNA synthesis of the herpes simplex virus, such as those with sapogenin of the oleanane type, whereas sapogenins type ursan inhibit the synthesis of viral capsid protein of the same virus. Other saponins may also inhibit virus type II polio through inhibition of the attack of the virus to the host cell. These compounds have been shown to reduce experimental keratitis caused by herpes simplex virus in rabbits. Saponin obtained from *Glychyrrhiza glabra*, glycyrrhizin, is the main representative of biological activity. It promotes the inhibition of virus comprising DNA and RNA, including HIV-1 in vitro. This process occurs via inhibition of the protein kinase C, which works to link these viruses to CD4+ receptors present on T cells and to inhibit the enzyme reverse transcriptase (Lacaille-Dubois MA *et.al* 2000) [38].

Anti-diabetic activity

Many natural products are used in folk medicine as hypoglycemic. Some of them have been isolated, identified and their activities have been evaluated by in vivo testing in animals with induced diabetes. There are several mechanisms by which saponins exert their effects, among the most important we can mention the inhibition of adrenocorticotropin-induced, suppression of lipogenesis activated by insulin which leads to their increase, increased glycolytic activity, decreased gluconeogenesis and lipolysis synthesis r-RNA and m-RNA, which act on the metabolic regulation in diabetic rats (Lacaille-Dubois MA *et.al* 2000) [38].

Antifungal, antiparasitic and antibacterial activity

The interest in new antimicrobial agents from plants has been restored during the last 20 years, as traditional antibiotics (mainly those derived from microorganisms) are ineffective produce several side effects and favor the development of resistant strains (Escalante AM *et.al*.,2002) [21] The antifungal, antiparasitic and antimicrobial activity of saponins can be observed by the ability to form complexes with cholesterol membrane resulting in an increase in permeability and consequent leakage of cytoplasmic contents (Lanzotti V *et.al*.,2012) [39].

Immunomodulating activity

Saponins can greatly impact the immune system due to their ability to act as adjuvant by stimulating immunological response against antigen and their oral administration facilitates the absorption of large complex molecules (Cheeke PR.,2000) [13]. Saponin based adjuvants and immunomodulatory potential via cytokine interplay were reported by many researchers Saponins as vaccine adjuvants were also reported (Kensil CR.,1996) Due to their structural complexity and toxicity, saponins have been limited their use in human vaccines, but the evolution of new processing and purification techniques yields different fractions with optimal immunological adjuvant activity and with minimal toxicity and hemolytic activity (Cox *et.al.,* 2002) [16] consequently, there is a significant progress in the development of saponins as new generation vaccines.

Research Findings of Scope of Utilization tannin to improve animal performance

Effect of dietary addition of tea saponins for 6 wk on DMI, milk yield, and milk composition in dairy cows

A total of 8 primiparous and 12 multiparous lactating Holstein dairy cows were blocked based on parity and milk production and were randomly assigned to 1 of 4 dietary groups, with 5 cows in each group (2 primiparous and 3 multiparous cows). The cows were fed the same basal diet with TSP supplementation of 0 (control), 20, 30, or 40 g/d per head. Feeding and milking occurred 3 times per day (07:30, 14:30, and 21:30 h). The experiment lasted for 8 wk, with a 2-wk adaption period and a 6-wk sampling period. (B. Wang *el al*.,2017).

Table 5: Effect of dietary addition of tea saponins for 6 wk on DMI, milk yield, and milk composition in dairy cows

Item	Tea saponins, g/d per head				SEM	P-value	
	0	20	30	40		Linear	Quadratic
DMI, kg/d	25.3	25.3	25.2	22.5	0.45	<0.01	0.01
Yield, kg/d							
Milk	36.9	37.1	36.6	31.5	1.08	<0.01	0.04
ECM	37.1	37.1	37.8	32.3	0.90	0.01	0.01
Fat	1.22	1.23	1.28	1.13	0.029	0.12	0.02
Protein	1.15	1.13	1.15	0.92	0.040	<0.01	0.03
Lactose	1.89	1.85	1.81	1.47	0.063	<0.01	0.04
Total solids	4.52	4.23	4.38	3.67	0.206	0.03	0.32
Milk composition, %							
Fat	3.35	3.32	3.50	3.56	0.050	<0.01	0.39
Protein	3.05	3.06	3.16	2.95	0.099	0.67	0.31
Lactose	5.09	4.99	4.95	4.76	0.065	<0.01	0.52
Total solids	11.8	11.4	12.0	11.8	0.35	0.84	0.75
MUN, mg/dL	16.6	15.9	13.1	16.0	0.48	0.05	<0.01
SCC, $\times 10^3$/mL	47.2	44.0	42.0	45.2	0.65	0.02	<0.01
Feed efficiency	1.47	1.46	1.46	1.42	0.050	0.52	0.82

Note:-ECM (kg/d) = 0.3246 × milk yield (kg/d) + 13.86 × fat yield (kg/d) + 7.04 protein yield (kg/d) (Orth, 1992)., Feed efficiency = milk yield (kg/d)/DMI (kg/d).

Intermediate dose of TSP (20 and 30 g/d) had no significant effect onfeed intake, but the supplementation of 40 g/d TSP decreased dairy cows' DMI and resulted in lower milk yield. The ECM of cows showed a quadratic response when the supplementation dose of TSP increased after 6 wk of feeding. The ECM of cows fed 40 g/d TSP first declined but rose after 3 wk of feeding. The milk UFA in cows fed 40 g/d TSP was greater during the first 4 wk of feeding but later tended to be similar to that of cows receiving the other treatments, indicating potential adaption to high doses of TSP supplements in dairy cows. Regardless of dosage, TSP may reduce oxidative stress and improve immunity in cows. In total, the supplementation of TSP at an appropriate dosage is potentially good for the health of dairy cows and can help dairy cows generate more milk UFA with short term feeding (B. Wang *el al.*,2017)

Effect of herbal feed additive containing saponins on caracass & carcass parameters of Beetal kids

A 90 days growth trial was conducted on male Beetal goat kids (8; 5 months old; average live weight 14.05±0.41 kg) randomly distributed into 2 equal groups. The animals in control group were fed TMR containing concentrate mixture (maize 32%, barley 20%, soybean meal 15%, groundnut cake 15%, wheat bran 15%, mineral mixure 2% and salt 1%) and green oats in 50:50 ratios on DM basis as per NRC (2007) feeding standard. Those in experimental group were fed control TMR supplemented with *M. uniflorum* dry extract @ 2% of DM intake. The animals were stall fed individually at 9:00 AM daily and had free access to fresh water twice a day and were taken out in the yard for 1 h exercise daily (hundal *et al*.,2019) [29].

Table 6: Effect of supplementing *M. uniflorum* to TMR on the carcass of Beetal kids

Parameter	Weight (kg		PSE	P value	As percent of live weight		PSE P value	
	Control	*M. uniflorum*			Control	*M. uniflorum*		
Slaughter weight	15.51	16.48	0.72	0.546				
Dressed carcass	6.48	7.13	0.34	0.372	38.85	40.11	0.48	0.209
RTC carcass	6.25	7.09	0.50	0.272	37.36	39.89b	0.53	0.003
Edible offal (g)	0.73	0.78	0.077	0.711	4.36	4.34	0.14	0.949
Inedible offals	3.41	3.78	0.25	0.346	20.38	21.28	0.26	0.083

(Hundal *et al*., 2019) [29] supplementing the TMR with *M. uniflorum* (kulthi) at 2% of DM intake resulted in higher weight of most of the primal cuts expressed as per cent of dressed weight can be achieved in Beetal goat kids.

Table 7: Effect of herbal feed additive containing saponins on carcass parameters of Beetal kids

Parameter (kg)	Control	M.Uniflorum	PSE	P-Value
Meat Color (Hunter Color Values)				
Lightness	24.55	23.09	0.71	0.335
Redness	6.53	5.83	0.26	0.181
Yellowness	8.25	7.38	0.32	0.183
Chemicial Composition (%) of Meat				
Dry matter	22.6^a	23.9^b	0.32	0.016
Total nitrogen	13.01	12.87	0.04	0.104
Fat	8.13^a	11.30^b	0.72	0.000

No significant differences were recorded for lightness (L*), redness (a*) or yellowness (b*) as analogus colour values between control and M. uniflorum supplemented group, which indicated no adverse effect of feeding test herb on meat colour of goat kids (Hundal *et al.*, 2019) [29].

5. Conclusion

Tannins and saponins are generally regarded as anti nutritional factors, certain types of tannins and saponins at low concentrations alter rumen fermentation pattern and microbial protein synthesis for the improvement in the production from the ruminants. These are widely distributed in feedstuffs and these feedstuffs as such can be used in small amounts to get their beneficial effects.It is important to determine the correct dosage of tannins and saponins for promoting optimal ruminal health and production without adverse effects.

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