

# Legume and Methane Emission Reduction in Livestock

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## ABSTRACT

Legume is one of the types of high quality forages. It is available in some types of trees and shrubs. The specific thing that connotes with legume is protein content since legume can do nitrogen fixation. In regard, legumes can also be known as leaf protein sources. Legume usually also has tannin content vary around 3 – 7%. Many researchers found that protein and tannin in the legume positively impact animal production. Tannin can protect the protein from rumen degradation to become bypass protein that will be digested in the abomasum without lousy impact to the rumen ecology. Tannin also can inhibit protozoa and reduce the population of methanogenic bacteria that produce methane. Another side, a combination of high protein and tannin in the legume can be a good combination for a ruminant. For the animal, legumes with tannin content and high protein level have potency to improve animal production and reduce methane production from the rumen. Moreover, reducing methane, in this case, will impact environmental quality since methane is one of the greenhouse gas that is 21 times stronger than carbon dioxide. Feeding ruminant with legume (basal or supplementation) will have a double advantage in increasing animal production and environmental quality.

**Keywords:** Environment, forages, Methane, Ruminants, Tannin

## 1. INTRODUCTION

The livestock sector contributes to the improvement of the quality of human life. Livestock adds value to several resources that the farming family could not otherwise utilize, like the biomass (weeds, maize straw, cultivated forages, common grazing areas, surplus grain, etc.), by transforming it into valuable products (meat, milk, eggs), services (draught and pack power), or investment and biogas [1][2][3], as well as contributes to the household economy [4].

A poor quality diet leads to inefficient digestion, which leads to increased methane production and lowers animal productivity [5][6][7]. Methane production is triggered by poor quality feeds [8][7]. Methane (CH<sub>4</sub>) is considered as one of the largest sources of greenhouse gas from feedlot and dairy farms, aside from nitrous oxide (N<sub>2</sub>O) and carbon dioxide (CO<sub>2</sub>). The methane in the rumen as a product of fermentation may be considered inefficient of feed uses because CH<sub>4</sub> emissions represent an economic loss to the farmer,

where feed is converted into CH<sub>4</sub> rather than a functional product.

The livestock industry is seeking alternative additives that would improve nutrient use efficiency in ruminants. There are some strategy can be done to reduce methane emission. One of the strategies is feeding the animal with good quality feed like concentrate and legume [9]. Legume is one of good quality feed with usually high content of crude protein [10]. Legume is a protein source that is cheap and easy to find in the field that can be used. Moreover, legume is also a source of tannin, besides of protein source for the ruminant [11][12][13][9].

Tannins are water soluble polymeric phenolic that can be extracted from the plant and can be used to protect protein in the diet from microbial rumen fermentation. Tannins present in several native shrubs may inhibit the activity of rumen microorganisms [14]. Tannins can be added to the diet in a certain amount. Moderate levels of condensed tannins (2 to 4% DM) reduce rumen protein digestion and bacterial rumen activity by forming a pH-reversible bond with soluble proteins and other

macronutrients [15]. The ability of tannin to bind and protect protein and modify the rumen ecology is very promising in increasing ruminant productivity and reducing greenhouse gas (GHG) emissions.

Thus, providing cows with the best combination of pasture and concentrate feeding, improved feed and forage management and other practices to increase the digestibility and reduce residence digestion time in the rumen that will effectively reduce methane emissions from the herd [8][6][16]. The study about legume and methane emission reduction is fascinating.

## 2. LEGUME AS LEAF PROTEIN

The most expensive nutrient in animal production is protein. Protein has to be utilized by the animal efficiently. Ruminal microorganism activity is essential for using structural carbohydrates, and the synthesis of high-quality protein in ruminants [17] since microbial protein contributes about two-thirds of the amino acids absorbed by ruminants. Ruminal bacteria play a significant role in the biological degradation of dietary fiber because of their much larger biomass. Microbes cells are formed due to rumen digestion of carbohydrates under anaerobic conditions.

Supplementing poor quality roughage with fresh leaves like *Gliricidia sepium* or *Leucaena leucocephala* may also result in faster rumen outflow rates, increasing intake and providing more degradable organic matter [12]. Aside from the N provided by *Leucaena* and *Gliricidia*, another contributory factor that could have triggered higher consumption in the supplemented groups is the significantly higher outflow rate of both the liquid and solid phase of the rumen digesta [12].

The use of leaves as supplements, aside from improving feed intake and nutrient digestibility, can also provide both degradable and undegradable protein for ruminants [18]. *Leucaena leucocephala* appears to be a suitable source of protein and roughage for ruminants in the tropics when it is fed in conjunction with pasture [19][20] or molasses [21]. Small quantities of *Leucaena* can increase milk production at low cost, since it is possible to produce 10-22 tons of edible DM/ha [22]. Supplementation of *Leucaena leucocephala* on goat feeds on a basal diet of maize stover increased the organic matter and crude protein digestibility from 59.23% and 50.58% to 75.94 and 55.40%, respectively [18]. *Leucaena* forages are fed to ruminants to increase the amount of organic matter (OM) fermented in the rumen and the quantity of microorganism protein synthesized. When *Gliricidia sepium* or *Leucaena leucocephala* supplements were given, the intake of fermentable OM was highest, resulting in an inefficient synthesis of microorganism protein [23].

The *Leucaena sp.* forages probably to stimulate more efficient rumen function, giving rise to higher voluntary

intake and faster growth [24]. *Leucaena* leaf contains high nutrients especially protein [25]. In general, supplementation maize stover basal diets with *E. variegata*, *G. sepium* or *L. leucocephala* leaves improved palatability and nutrient digestibility. The significant improvement in the crude protein (CP) and other nutrients digestibility by adding *E. variegata*, *G. sepium* or *L. leucocephala* to the maize stover diets may have resulted from the high CP content of the diets [18].

Dietary ingredients significantly impact the proportion of acetate, propionate, and butyrate produced in the rumen [9]. Increasing ruminal propionate is more beneficial in increasing the generation of fermentation energy since it reduces carbons that would be lost in methane [6][16][26][27]. The maximum number of cellulose-degrading bacteria was represented by *Ruminococcus albus* and *R. flavefaciens* (59.8%), *Bacteroides succinogenes* (*Fibrobacter succinogenes*) (19.2%), *Butyrivibrio fibrisolvens* (11.1%), *Clostridium lochheadii* (3.8%) and *C. longisporum* (1.3%) [28]. The propionic acid concentrations in the rumen are greatest in *in-vivo* fermentation when the diet contains large quantities of soluble sugar or starches, and least in animals fed with poor hay.

Starch is an inexpensive source of energy that microbes can ferment in the rumen to produce microorganism protein and volatile fatty acids (VFA) used as fuels by the cow [29][27]. Fermentation of feedstuffs in the rumen yields short-chain volatile fatty acids (primarily acetic, propionic and butyric acids), carbon dioxide, methane, ammonia and occasionally lactic acid. Acetate is found in the greatest concentration in the rumen followed by propionate and butyrate [30][27]. Methane production pattern is usually linear with acetate and inversely proportional with propionic production. *F. Succinogenes* can produce succinate (S) and formate (F) that is precursor of propionate in the rumen fermentation and does not produce H<sub>2</sub> (will become methane when they meet carbon chain in the rumen). When the animal fed by legume, will trigger to enhance the population of *F. Succinogenes*, finally has potential to increase propionic production and reduce methane production [9].

Furthermore, several strategies can be used, including increased digestibility of forages and feeds, modification of bacteria in the rumen, improved feed and forage management and treatment practices to increase the digestibility, treatment of the feeds/forages to increase digestibility [9], and appropriate use of concentrated supplements. Rumen protein degradation is affected by pH and the predominant species of the microbial population [31].

### 3. LEGUME AS SOURCE OF TANNIN

A tannin is a complex group of polyphenolic compounds found in various plant species commonly consumed by ruminants [32]. Tannin is a compound that can protect protein in the diet from rumen microorganism degradation. It may alter the composition of microbial cells and reduce the extraction of microorganism cell walls from digesta [33]. Though tannins mainly exert their effects on proteins, they also affect carbohydrates, particularly hemicellulose, cellulose, starch, and pectins [32]. Tannins may also form complexes with starch and cellulose as well as protein [33]. Tannins may complex protein at the pH of the rumen and protect the protein from microbial enzymes. These complexes are unstable at the acid pH of the abomasum, and the proteins become available for digestion [33][34].

Tannin is already present in woods like oak, walnut, and mahogany. The tannins of different plant species have different physical and chemical properties, and therefore they have very diverse biological properties [32]. Tannins in high concentrations reduce intake, digestibility of protein and carbohydrates, and animal performance. Tannins have a negative effect on growth rate because it reduces intake and leads to low true digestibility of protein [33]. The tannin apparently binds to proteins and forms stable rumen complexes [14]. Moderate levels of condensed tannins (CT; 2 to 4% DM) reduce rumen protein digestion and rumen bacterial activity by forming a pH-reversible bond with soluble proteins and other macronutrients. Condensed tannins, including commercial quebracho tannin extract, have increased animal production and reduced bloat potential [15].

High concentrations of tannins reduce voluntary feed intake and nutrient digestibility. In contrast, low to moderate concentrations may improve the digestive utilization of feed mainly due to a reduction in protein degradation in the rumen and a subsequent increase in amino acid flow to the small intestine. These effects on nutrition are reflected in animal performance.

Natural plant extracts represent one of the alternatives to using antibiotic growth promoters in animal feeds [35]. Tannins lower the rate of protein degradation and amino acid deamination in the rumen [33]. The tannic acid treatment effectively decreases the rapidly soluble fraction of alfalfa and Bermuda grass silages, which could be beneficial to the animal because it would decrease the excess N in the rumen after feeding [36].

Tannin contained in legumes significantly decreased *in sacco* dry matter digestibility. It may be due to the formation of by-pass protein. In this study, *Leucaena leucocephala* is not only a better source of tannin but also a source of dietary protein for the host animal without significantly affecting rumen pH, temperature, and NH<sub>3</sub>-

N. In cattle, the amplification for *Ruminococcus albus* at 0% and 3% commercial tannin in the diet was similar. The sample with 6% commercial tannin had the finest band. These findings imply that as the level of tannin increases, the intensity of the bands decreases, which indicates that the higher level of tannin may have reduced the population of *R. albus* in the cattle [9].

On optimising PCR with a shorter cycle (20 cycles), it was shown that *Leucaena* sp (LCT) levels in the diet affected the band's intensity. The intensity of bands in the sample for *F. succinogenes* from carabao and cattle increased, indicating that LCT in the diet enhances the population of *F. succinogenes* in cattle and carabao. The population of *R. flavefaciens* from carabao and cattle decreased by 6% *Leucaena*. It indicates that *Leucaena* in the diet may inhibit the population of *R. albus* in the cattle. Data of cellulolytic amplification in carabao matched with *in sacco* degradation and feed intake. The degradation values of DM and NDF at 0% were significantly higher than 12% *Leucaena*. Total intake DM, CP, and NDF at 0% were significantly higher than 12% *Leucaena* [9].

Reducing methane production invariably increases rumen propionate [27]. Propionate has a more significant effect on methane production than acetate since propionate will be used by animals in gluconeogenesis [29]. Therefore, reducing these losses will improve the efficiency of dairy farming and potentially increase milk production [6].

### 4. LEGUME AND GREEN HOUSE GAS ISSUE

Livestock has been considered beneficial to the agro-ecosystems as it acts as an agent of nutrient recycling. In tropical countries, the ruminants are fed with agricultural by-products like cereal straws, stovers, sugarcane bagasse, and fruit pulps [28]. However, in the circumstances other than intensive animal production, livestock is often an essential part of sustainable agriculture, which integrates three main goals: environmental health, economic profitability, and social and economic equity [37].

Ruminant is always an integral part of the smallholder's farming system. Increasing its productivity would significantly alleviate their economic status. Moreover, livestock, particularly ruminant, ranks second in terms of contribution to greenhouse gas (GHG) emissions in the agriculture sector. Methane contributes 15% -20% of GHG emissions and 21 times more potent than CO<sub>2</sub> as GHG. Methane is considered one of the largest sources of GHG aside from nitrous oxide (N<sub>2</sub>O) and carbon dioxide (CO<sub>2</sub>). Methane is 21 times more potent than CO<sub>2</sub> as GHG. 1 kg of methane equals 21 kg of CO<sub>2</sub> [8].

Methane also represents energy and economic loss in the production system of ruminants. Methane is a product of ruminant's regular digestion produced in the rumen by many kinds of methane bacteria through the reduction reaction of carbon dioxide and hydrogen. It has a stable chemical character and is emitted outside through the mouth in the form of eructation [6][16]. Dairy cows typically produce 118 kg methane/ year, over twice that of non-lactating cattle. This is equivalent to 2.478 tons of CO<sub>2</sub> in inventories of GHG production [8]. Therefore, there is an interesting decrease in CH<sub>4</sub> production in the rumen for environmental and economic reasons [38]. Methane emitted from the livestock sector accounts for 38% of all agricultural GHG emissions in Canada [39] and 17.7% in Australia [6]. Around 15% - 20% of results of climate warming are due to methane [5][16]. Thus, reducing methane emissions from livestock would significantly favor the environment.

In the rumen, a group of microbes called methanogens is responsible for producing methane, utilizing surplus hydrogen in the rumen to reduce carbon dioxide to produce methane [6][27]. Methanogens are present in the rumen in large numbers, which vary from 10<sup>7</sup> to 10<sup>9</sup> cells/ml of rumen liquor depending on the type of diet given to the animals, especially the fiber content in the ration [28].

The fibrous diets promote higher acetate, resulting in more hydrogen and more methane. The methane in the rumen is produced by methanogenic bacteria [40]. Acetate is the dominant end product, but acetate production is dependent on the ability of hydrogenases to produce hydrogen gas from reduced co-factors. Hydrogen production is a thermodynamically unfavorable reaction, but methanogens scavenge hydrogen and relieve this inhibition. When carbohydrates are converted to propionate, butyrate, or lactate, dehydrogenase reactions provide alternative sinks for the reduced equivalents [41]. One of the most challenging aspects of diet formulation for lactating cows is balancing the carbohydrates. Adequate and effective fiber must be provided to stimulate chewing and secretion of salivary buffers [29]. Selected forages and concentrates, which are high in non-fiber carbohydrates, could reduce methane emissions [8]. Improving the diet's digestibility through improved pastures, concentrate feeding, and decreasing the number of cows will further reduce methane production without reducing milk production [6].

The higher dietary crude protein level increases rumen ammonia concentration [42]. Increasing rumen ammonia concentration through supplementation of protein in the diet like *Leucaena leucocephala* could increase VFA production and the cellulolytic bacteria population. Legume increases the total concentration of VFA without affecting its relative proportions and the rumen pH [43]. Feeding *Leucaena* may also stimulate the

growth of cellulolytic microorganisms. This finding was supported by PCR amplification results where *Leucaena* in the diet has enhanced the population of *F. succinogenes* in carabao and cattle [9]. Increasing population of *F. succinogenes* results in higher propionic acid. In rumen fermentation, *F. succinogenes* produces succinate, an intermediate compound for propionate production [30]. This phenomenon indicated that methanogens were inhibited by tannin. Since methane production is inversely proportional with propionate production, reduction of methane production increases rumen propionate [27][28][16][44]. The increasing rumen propionate is more beneficial in increasing the capture of fermentation energy since it reduces carbon lost in the form of methane. Ultimately reducing methane and increasing propionate will affect productivity.

Most methanogenic bacteria in cattle were affected by tannin. Thus, methane production in cattle can be reduced with dietary tannin treatment. Supplementation of *Leucaena leucocephala* in the diet increased the quality of the diet with increasing crude protein levels, and may also inhibit methanogens [9]. Decreasing the methanogens population in the rumen will have two beneficial impacts on the animal and the environment. In animals, the reduction of methanogens will decrease the amount of methane production. Supplementation *Leucaena leucocephala* can be implemented as feeding management for carabao/ buffalo and cattle [9]. The amount of methane emitted is dependent on the animals digestive system and the amount and type of feed consumed [6][39][26].

Another action that can be taken to reduce methane emission is improving feed and forage management and treatment practices time in the rumen, such as using improve feed grains and forage, the increased surface area of the feeds, the addition of fiber sources, treatment of the feeds/forages to increase digestibility, and appropriate use of concentrated supplements to increase the digestibility and reduce residence digestion [39][26]. By applying current best management practices for grazing management, balanced dairy cow nutrition and nitrogen fertilizer management, both methane and nitrous oxide emissions can be reduced to improve dairy production efficiency. As a general rule, methane emissions are reduced as digestibility and protein/energy balance is improved [6].

## 5. CONCLUSION

Feeding livestock can be addressed for environmental and economic reasons. Legumes as a leaf protein source can also source of tannin. Feed livestock with legumes will improve the quality of the diet. Thus will have a higher possibility to improve production and the same time, reduce methane emission.

In farming communities with abundant of low quality agricultural by-products as feedstuff sources like cereal straws, stovers, sugarcane bagasse, and fruit pulps, citrus pulp, and rice straw, and owing to the results of the study, it is recommended to raise ruminants and put legume in the diet to improve feed quality.

Methane emissions can be the target of livestock-specific policies, given the potential for increasing productivity through the reduction of dietary energy lost in methane. Providing more sources of some legume through planting will benefit the community and the environment. Planting legume as a fence or strip cropping will become a good investment for farmers who raise ruminants. Legumes give many benefits, e.g., increased soil quality, leaf protein source for ruminant, and tannin that will have a good impact in terms of ruminant nutrition and global environmental contribution by reducing methanogens in the rumen.

## REFERENCES

- [1] B. Suwignyo, A. Agus, and S. Padmowijoto, Cattle agroforestry production system in sandy land to alleviate poor farmer. international seminar and workshop, in *The Role of Agroforestry Education in the Revitalization Action of Agriculture, Fishery and Forestry Program and and Forestry Program and Second General Meeting of INAFE: Policy Recommendation and Campaign*, 2006, pp. 115–121.
- [2] D. F. Crawford, W. B. Anthony, and R. R. Harris, evaluation of concentrated hemicellulose extract as cattle feed, *J. Anim. Sci.*, 1978, pp. 46 32–40.
- [3] C. M. Arriaga-Jordán, A. M. Pedraza-Fuentes, E. G. Nava-Bernal, M. C. Chávez-Mejía, and O. A. Castelán-Ortega, Livestock agrodiversity of mazahua smallholder campesinosystems in the highlands of central mexico, *Hum. Ecol.*, 2005, vol. 33, pp. 821–845.
- [4] J. Hodges, Livestock, ethics, and quality of life, *J. Anim. Sci.*, 2003, vol. 81, pp. 2887–2894.
- [5] M. J. Gibbs and K. Hogan, Methane, *EPA J.*, 16 23 (1990).
- [6] R. Eckard and R. Hegarty, Best management practices for reducing greenhouse gas emissions from dairy farms, New South Wales Agric. Univ. Melbourne, [http://www.greenhouse.unimelb.edu.au/Greenhouse\\_from\\_Dairy\\_Farms.htm](http://www.greenhouse.unimelb.edu.au/Greenhouse_from_Dairy_Farms.htm) (accessed 15/02/2008), (2004).
- [7] C. F. Nicholson, R. W. Blake, R. S. Reid, and J. Schelhas, Environmental impacts of livestock in the developing world, *Environ. Sci. Policy Sustain. Dev.*, 2001, pp. 43 7–17.
- [8] F. O'Mara, Greenhouse gas production from dairying: reducing methane production, (2004).
- [9] B. Suwignyo, B. Suhartanto, N. Umami, N. Suseno, and Z. Bachruddin, Feeding strategy of ruminants and its potential effect on methane emission reduction, *J. Agric. Sci.*, 2016, vol. 8, pp. 199–204.
- [10] B. Suwignyo, F. Izzati, A. Astuti, and E. A. Rini, Nutrient content of alfalfa (*Medicago sativa* L.) regrowth I in different fertilizers and lighting,” in *IOP Conference Series: Earth and Environmental Science*, 2020, pp. 465 12035.
- [11] T. A. Atega, C. B. Alinea, A. A. Rayos, and A. Y. Robles, *Gliricidia sepium* and *Samanea saman* leaf meals as protein supplements for goats, *PCARRD Highlights 2002*, (2003).
- [12] E. A. Orden, E. M. Cruz, T. Ichinohe, and T. Fujihara, Effects of *leucaena leucocephala* and *gliricidia sepium* supplementation on outflow rate, microbial protein yield and growth of sheep fed with ammoniated rice straw, *Philipp. J. Vet. Anim. Sci.*, 2002, 28 1.
- [13] C. C. Sevilla, M. S. Billena, and M. B. Bejo, The effects of maceration of *leucaena* (*Leucaena leucocephala*) on the rumen degradation of its protein in cattle, *Philipp. J. Vet. Anim. Sci.*, 2003, 29 1.
- [14] G. Nunez-Hernandez, J. D. Wallace, J. L. Holechek, M. L. Galyean, and M. Cardenas, Condensed tannins and nutrient utilization by lambs and goats fed low-quality diets., *J. Anim. Sci.*, 1991, vol. 69, pp. 1167–1177.
- [15] B. R. Min, W. E. Pinchak, R. C. Anderson, J. D. Fulford, and R. Puchala, Effects of condensed tannins supplementation level on weight gain and in vitro and in vivo bloat precursors in steers grazing winter wheat, *J. Anim. Sci.*, 2006, vol. 84, pp. 2546–2554.
- [16] L. Y. Zhaoli, Animal husbandry production and global climate change, *Fac. Anim. Sci. Technol. Jilin Agric. Univ. Chang. Jilin*, 2002, pp. 130118.
- [17] N. R. Council, Nutrient requirements of dairy cattle: 2001. National Academies Press.
- [18] E. M. Aregheore and D. Perera, Effect of supplementation of a basal diet of maize stover with *Erythrina variegata*, *Gliricidia sepium* or *Leucaena leucocephala* on feed intake and digestibility by goats, *Trop. Anim. Health Prod.*, 2004, vol. 36, pp. 175–189.
- [19] F. J. Alvarez, A. Wilson, and T. R. Preston, *Leucaena leucocephala* as a combined source of

- protein and roughage for steers fattened on molasses/urea, *Trop. Anim. Prod.*, 1977, vol. 2, pp. 288–291.
- [20] G. Saucedo, F. J. Alvarez, N. Jimenez, and A. Arriaga, *Leucaena leucocephala* as a supplement for milk production on tropical pastures with dual purpose cattle, *Trop Anim Prod*, 1980, vol. 6, pp. 284.
- [21] B. Hulman, E. Owen, and T. R. Preston, Comparison of *Leucaena leucocephala* and groundnut cake as protein sources for beef cattle fed ad libitum molasses/urea in Mauritius, *Trop. Anim. Prod.*, 1978, vol. 3, pp. 1–8.
- [22] E. M. Hutton and W. M. Beattie, Yield characteristics in three bred lines of the legume *Leucaena leucocephala*, *Trop. Grasslands*, 1976, vol. 10, pp. 187–192.
- [23] A. Priego, R. M. Dixon, R. Elliott, and T. R. Preston, Studies on the digestion in the forestomachs of cattle of a diet based on sisal pulp I Supplementation with *Leucaena leucocephala* and rice polishings *Tropical Animal Production* 4, (1979).
- [24] F. Herrera, D. Wyllie, and T. R. Preston, Fattening steers on a basal diet of ensiled sisal pulp and molasses/urea supplemented with sunflower meal and leucaena forage, *Trop. Anim. Prod.*, 1980, vol. 5, pp. 18–24.
- [25] U. Ter Meulen, S. Struck, E. Schulke, and E. A. El Harith, A review on the nutritive value and toxic aspects of *Leucaena leucocephala*, *Trop Anim Prod*, 4 (1979).
- [26] U. S. CCTP, Agricultural Systems for Enteric Emissions Reduction. U.S. Climate Change Technology Program – Technology Options for the Near and Long Term. 2005, pp. 2–7.
- [27] V. Fellner and J. W. Spears, Effect of calcium propionate on ruminal soluble calcium and microbial fermentation, *North Carolina State Univ. Anim. Sci. Dep. Rep.*, (2004).
- [28] D. N. Kamra, Rumen microbial ecosystem, *Curr. Sci.*, 2005, pp. 124–135.
- [29] M. Allen, Forages, Fiber and the Rumen, (2003).
- [30] R. P. Lana, J. B. Russell, and M. E. Van Amburgh, The role of pH in regulating ruminal methane and ammonia production, *J. Anim. Sci.*, 1998, vol. 76, pp. 2190–2196.
- [31] A. Bach, S. Calsamiglia, and M. D. Stern, Nitrogen metabolism in the rumen, *J. Dairy Sci.*, 2005, vol. 88, pp. 9–21.
- [32] P. Frutos, G. Hervás, F. J. Giráldez, and A. R. Mantecón, Tannins and ruminant nutrition, Review, *Spanish J. Agric. Res.*, 2004, vol. 2, pp. 191–202.
- [33] J. D. Reed, Nutritional toxicology of tannins and related polyphenols in forage legumes, *J. Anim. Sci.*, 1995, vol. 73, pp. 1516–1528.
- [34] C. C. Sevilla and S. W. Purbojo, Addition of mimosa tannin protected the protein in gliricidia (*Gliricidia sepium*) from rumen microbial degradation, *Philipp. J. Vet. Anim. Sci.*, 2005, vol. 31, pp. 1.
- [35] P. W. Cardozo, S. Calsamiglia, A. Ferret, and C. Kamel, Effects of alfalfa extract, anise, capsicum, and a mixture of cinnamaldehyde and eugenol on ruminal fermentation and protein degradation in beef heifers fed a high-concentrate diet, *J. Anim. Sci.*, 2006, vol. 84, pp. 2801–2808.
- [36] G. T. Santos et al., Effect of tannic acid on composition and ruminal degradability of bermudagrass and alfalfa silages, *J. Dairy Sci.*, 2000, vol. 83, pp. 2016–2020.
- [37] M. C. Appleby, Sustainable agriculture is humane, humane agriculture is sustainable, *J. Agric. Environ. Ethics*, 2005, vol. 18, pp. 293–303.
- [38] E. M. Ungerfeld, R. A. Kohn, R. J. Wallace, and C. J. Newbold, A meta-analysis of fumarate effects on methane production in ruminal batch cultures, *J. Anim. Sci.*, 2007, vol. 85, pp. 2556–2563.
- [39] S. M. McGinn, K. A. Beauchemin, T. Coates, and D. Colombatto, Methane emissions from beef cattle: Effects of monensin, sunflower oil, enzymes, yeast, and fumaric acid, *J. Anim. Sci.*, 2004, vol. 82, pp. 3346–3356.
- [40] D. I. Demeyer and H. K. Henderickx, Methane production from glucose in vitro by mixed rumen bacteria, *Biochem. J.*, 1967, vol. 105, pp. 271–277.
- [41] J. B. Russell and J. L. Rychlik, Factors that alter rumen microbial ecology, *Science*, 2001, vol. 292, pp. 1119–1122.
- [42] D. H. Luc, N. Van Thu, and T. R. Preston, Feed intake, rumen fermentation, microbial protein synthesis and nitrogen retention in growing cattle given maize or molasses with two levels of crude protein as supplements to a basal diet of rice straw and grass, *Energy*, 150. 200 (2009).
- [43] J. H. Topps, Forage legumes as protein supplements to poor quality diets in the semi-arid tropics, (1995).
- [44] C. J. Van Nevel, D. I. Demeyer, and H. K. Henderickx, Effect of fatty acid derivatives on rumen methane and propionate in vitro, *Appl. Microbiol.*, 1971, vol. 21, pp. 365–366.