Rumen defaunation for increased ruminant productivity: Strategies and Effects

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Abstract: The rumen, to ruminant animals is very important because it inhabits diverse and complex microbial ecosystem. These microbs help in ruminant digestion and assimilation of nutrients from forage and different types of diet. Defaunation is the elimination of microfauna, and ciliated protozoa from the rumen. Many attempts by researchers to achieve this have been made to justify the role and mechanisms of these microbs. There are several methods of achieving this, such as chemical, isolation of new born animals, dietary manipulations and use of plant extract as defauning agents, this review therefore, tries to summarize different strategies used and effect of defaunation on the rumen ecology and animal productivity. The discussed concepts and advances concerning defaunation will be useful for the researchers, animal producers, feed formulators and other stakeholders in order to boost their economic gain and profit through feed modification.

Keywords: Rumen, Defaunation, tannin, plant extracts, saponin

Introduction

Many factors have caused some restrictions to the use of tropical forages and some feeding material as livestock feed, some of which include low levels of good quality protein or nitrogen, less fermentable carbohydrates and lipids, and low digestibility because of high fiber content (Diaz et al., 1993; Wanapat, 2000; Ngamsaeng et al., 2006 and Ngamsaeng et al., undated). These in turns, have led to poor animal performance because of decreased feed intake (VFI) and an imbalance in the absorbed nutrients (protein/energy ratio), causing slow growth, and low reproductive rate and milk production performance (Diaz et al., 1993; Ngamsaeng et al., undated). Such limitations are aggravated by the presence of anti-nutritional factors which may also affect the voluntary intake of the animals as well as feed digestibility so that some plants are less accepted or even rejected (Ba and Ngoan, 2003). These anti-nutritional compounds such as tannins, lignins, saponins, mimosine, and others are assumed to be synthesized in plants to protect them against invading microbes and insects. When consumed by the animals, these compounds limit the growth of different types of microbes (useful and undesirable) in the rumen as quality of roughage exhibits close relationship with rumen ecology, microbes, and fermentation patterns (Wanapat, 2000; Kamra, 2005).

Rumen manipulation by way of removing protozoa (defaunation) is an alternative way of increasing absorbed nutrients (Gebeyehu and Mekasha, 2013). Though protozoa contribute to fiber digestion, it also posts negative effects on bacteria as protozoa prey on bacteria when dietary protein is lacking (USDA, 2007). This is disadvantageous to the host animal fed with low-protein, highly fibrous diets, so that eliminating or reducing the number of protozoa increases the amount of microbial protein and dietary protein available to the host animal (Leng et al., 2011). This review therefore, tries to summarize different strategies used and effect of defaunation on the rumen ecology which in turns leads to improved animal productivity.

Defaunation defined and techniques

Defaunation is the process of making the rumen of animals free of rumen protozoa and the animal is called defaunated animal. There are several ways to defaunate the animals in order to obtain a ruminant animal free from rumen ciliate protozoa. One of the methods of producing defaunated animals is the separation of newborn animals from their dams after birth and preventing them from any contact with the adult ruminant animals. The newborn animals should be separated 2 to 3 days after birth (Jouany, 1978). During this time the newborn animals gets contaminated with the native bacterial population but do not get rumen ciliate protozoa (Fonty et al., 1984). However, once the animal is separated, proper care should be taken so that the isolated animals do not come in contact with any adult animals as well as any contamination from the handlers who look after faunated and defaunated animals

Another method of defaunation is by use of chemicals. Majority of researchers has used this method for obtaining animals free from rumen ciliate protozoa. The chemicals which have been widely used to defaunate the animals are copper sulphate (Ramprasad and Raghavan, 1981), manoxol (Chaudhary et al., 1995) and sodium lauryl sulphate (Santra et al., 1994a; Santra and Karim, 1999). Chemicals which are used as defaunating agent are introduced in the rumen of animals either orally by a stomach tube or through rumen fistula. However, these chemicals are not only toxic to the rumen protozoa but also kill the other rumen microorganism like bacteria.

Dietary manipulation can also be used to defaunate ruminants. The ciliate protozoa are very much sensitive to change in rumen pH, so activity of ciliate protozoa is adversely affected when the pH of the rumen falls below 5.8 and if the rumen pH falls below 5.0, the ciliate protozoa may be completely eliminated. Therefore, offering high energy feed (especially cereal grains like barley, maize etc) to the starved (for 24 hours) animals creates acidic condition in the rumen and rumen pH fall below 5.0. This fall in rumen pH eliminates the ciliate protozoa completely and the animal becomes defaunated. However one serious disadvantage of this method is that chances of developing acidosis in treated animal is more. Once rumen acidosis develops, the animals will suffer from various secondary complications. The drenching of vegetable oils can also eliminate ciliate protozoa and hence can be used as a defaunating agent (Newbold and Chamberlain, 1988; Nhan et al., 2001).

Potential of plant extracts as defaunating agent

Although, secondary metabolites such as tannin and saponine, may appear detrimental to animal feeding and nutrition, but they can also be used to manipulate rumen function and fermentation (Ba and Ngoan, 2003; Fulgueira et al., 2007 and Leng et al., 2011). Since rumen microorganisms play a great role in the digestion process and in the production of volatile fatty acids needed by the ruminant, it must then be noted that nutrient requirements of the ruminant animal depends on them. However, as reported to be prevalent in many tropical fodder plants, saponins and tannins are known to act as defaunating agents (Makkar et al., 1995; Pell et al., 2001 and Leng et al., 2011). Many studies have also validated the use of tannins and saponins being toxic to protozoa (Diaz et al., 1993; Monforte-Briceno et al., 2005), and the use of some tree leaves or plant extracts reduced the number of rumen protozoa which in turn has led to improved intake, fiber digestion, and weight gain (Eugene et al., 2004; Ozdemir et al., 2006; Patra and Saxena, 2009 as cited by Lopez -Camarena, et al., 2010).

Furthermore, forages of Centrosema pubescens, Desmodium intortum, Fern leaf, Leucaena leucocephala, Vigna parteri, Desmodium uncinatum as defaunating agents were tested in vitro but Enterolobium cyclocarpum was found to be effective in vitro and in vivo (Leng et al., 2011). Williams (1991) present positive effect of reducing the concentration of rumen protozoa as it could increase microbial protein production and amino acids available for absorption, and a decline in protozoal numbers is linked with increasing propionate Poungchompu et al. (2009) stated that populations of protozoa and fungi dramatically reduced when dairy heifers were fed with plants containing condensed tannins and saponins. According to Moss et al., (2000), all rumen microbes including protozoa are vulnerable to saponin-induced changes in cell membrane properties.

Effect of defaunation on the rumen ecosystem rumen microbes

Defaunation causes both qualitative and quantitative change in rumen bacterial population. For instance, it has led to bacterial population increase (Chaudhary et al., 1995) since rumen protozoa feed on the rumen bacteria to meet our their nitrogen requirement. A total of 4 to 45 g bacterial dry matter is engulfed by rumen protozoa per day per sheep (Coleman, 1975). Defaunation increase the number of amylolytic (Fig. 2) bacteria due to elimination of nutritional competition between bacteria and protozoa for using starch (Kurihara et al., 1978) whereas the cellulolytic bacterial population become decreased (Jouany et al., 1988). Fungal population in the rumen also increase due to defaunaton (Smet et al., 1992). Orpin and Letcher (1983/84) reported a predation of fungal zoospore by rumen protozoa but till today it is unclear whether the increase in fungal population following defaunation is a consequence of reduction in the predation by the protozoa or increased availability of nutrients for fungal growth in the absence of protozoa.

Rumen pH: The buffering capacity of the rumen seems to be better in presence of protozoa on a wide variety of diets. The rumen pH starts falling immediately after ingestion of feed, both in faunated and defaunated animals whereas, the drop in pH was much higher in defaunated than in faunated animals (Jouany et al, 1988; Nagaraja et al., 1992; Mendoza et al., 1993; Santra et al., 1996; Santra and Karim, 2002a). Rumen protozoa engulf the readily fermentable carbohydrate (starch) which is stored in their body as amylopectin (Williams and Coleman, 1992; Santra

et al., 1996; Hristova et al., 2001; Santra and Karim, 2002a) and thus decrease the rate of carbohydrate (starch degradation) fermentation (Williams and Coleman, 1992), resulting in a lower pH in the rumen of defaunated compared to faunated animals.

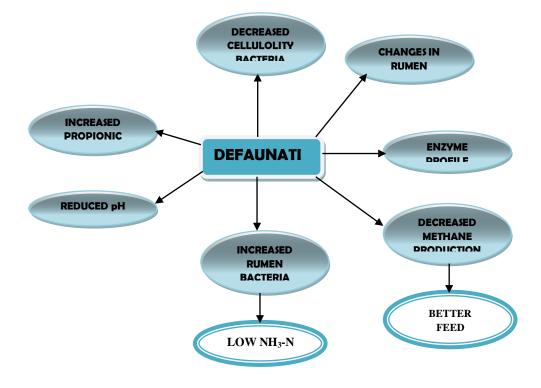


Fig. 1: Schematic representation of the effects of defaunation on rumen ecosystem

Volatile fatty acid (VFA) production: The effect of defaunation on the production and composition of VFA is variable. The VFA production rate and its composition are greatly influenced by experimental diet. Increase in TVFA concentration in defaunated animals was reported by Punia et al. (1987), Santra et al. (1996) and Santra and Karim (2002a) while non-significant effect was recorded by Itabashi et al. (1982), and Ivan et al. (1992). However, lower VFA production in the rumen of defaunated animals reported by several workers because of diet effect (Jouany et al., 1981; Kayouli et al., 1983; Hegarty et al., 1991; Chaudhary et al., 1995). Higher VFA conecntration in the rumen of faunated animals may be due to higher hydrolytic enzyme activity in the rumen protozoa because about 40- 60% of hydrolytic enzyme activity is found in the rumen protozoa (Agarwal et al., 1991) and also due to stimulatory effect of protozoa over bacteria (Onodera et al., 1988). The ciliate protozoa engulfed the feed particle and degrade it to acetic acid and butyric acid during carbohydrate metabolism (Demeyer and Van Nevel, 1979; Ushida et al., 1986a,b). Defaunation should, therefore, increase the molar proportion of propionic acid (Williams and Withers, 1991; Mendoza et al., 1993; Chaudhary et al., 1995). Moreover higher propionate production in the rumen of defaunated animals may be due to increase and shift in ruminal bacterial population. It has been reported that in defaunated animal' s number of acetate producing species such as Ruminococcus spp. are not predominant while succinate producing bacteria such as Bacteroides spp. are predominant (Kurihara et al., 1978). These changes in ruminal bacterial population may stimulate more propionate production in the rumen of ciliate free animals. However, the reported effects of defaunation on VFA composition are variable. Demeyer et al. (1982), Itabashi et al. (1984), Rowe et al. (1985) and Ushida and Jouany (1990) reported that the molar proportion of propionate in defaunated and fauntaed animals was similar whereas, Demeyer et al. (1982) and Ivan et al. (1992) reported lower proportion of propionate production in the rumen of defaunated animals.

Ammonia nitrogen concentration: Significant reduction in ammonia-N concentration (Fig. 1) in the rumen of defaunated animals was reported by several workers (Itabashi et al., 1984; Chaudhary et al., 1995; Santra et al., 1996; Nhan et al., 2001; Santra and Karim, 2002a). Ammonia is utilized by bacteria to meet their nitrogen requirement for body protein synthesis while ciliate protozoa do not use it. In defaunated animals, the number as well as activity of

rumen bacteria increases (Eadie and Gill, 1971) resulting in more uptake/utilization of ammonia by bacteria and as a result, ruminal ammonia concentration is reduced. Further, low production of free amino acid from the degradation of protein or peptide in absence of ciliates and/or lower rate of recycling of microbial nitrogen in the rumen of defaunated animals (Demeyer and Van Nevel, 1979), could have contributed to lower ruminal ammonia nitrogen concentration. The recycling of bacterial nitrogen in the rumen is higher in presence of ciliate protozoa (Hristova et al., 2001) and the number of ruminal bacteria capable to utilize ammonia decrease with increased ruminal break down of dietary protein (Leng, 1982).

Microbial protein synthesis: Microbial protein synthesis in the rumen of defaunated animals was higher than faunated animals (Bird et al., 1994). It is now generally accepted that in absence of rumen ciliate protozoa, the efficiency of rumen bacterial growth is enhanced and more microbial protein flows from reticulo-rumen to duodenum (Bird and Leng, 1985). Although bacteria and protozoa are active in synthesis of microbial protein, outflow of microbial protein in to duodenum is primarily of rumen bacterial origin. About half of the microbial protein in the rumen can be of protozoal origin while as a proportion of the microbial protein leaving the rumen, protozoal protein is usually under 10% because of higher rate of bacterial was out from reticulo-rumen (Owens and Zinn, 1988). Additionally, the absence of rumen protozoa is known to increase the efficiency of net bacterial growth due to elimination of protozoal protein and increasing rumen bacterial turn over (Demeyer and Van Nevel, 1979). This could have resulted in more microbial protein flow to the duodenum in the defaunated animals.

Enzyme profile: Rumen ciliate protozoa secrete various hydrolytic enzyme which are responsible for breakdown of the plant cell wall poly saccharides (Williams and Coleman, 1988; Agarwal et al., 1991). The ciliate protozoa and fungi are most important microbial groups of the rumen organisms required for the ruminal digestion of plant fibre (Amos and Akin, 1978). Carboxymethyl cellulase enzyme activity was lower in the rumen of defaunated than faunated animals (Williams and Withers, 1991; Santra et al., 1996; Santra and Karim, 2002). About 62% of the total rumen cellulase enzyme activity is associated with rumen protozoal population (Coleman, 1986). Hence elimination of ciliate protozoa decreases ruminal cellulase enzyme activity (Fig. 1). The activity of other carbohydrate degrading enzymes like amylase, xylanase and β -glucosidase are not affected by the presence or absence of ciliate protozoa in the rumen (Santra et al., 1996; Santra and Karim, 2002). Protease enzyme activity was lower in the rumen of faunated than defaunated animals (Ushida and Jouany, 1985; Santra et al., 1996). The specific activity of protease enzyme from bacterial fraction is 6-10 times higher than that from protozoal fraction (Brock et al., 1982). Defaunation, increases the number of ruminal bacterial population, resulting in higher ruminal protease enzyme activity. Bacteria are the only source of ruminal urease enzyme (Cook, 1976) while ciliate protozoa have no urease enzyme activity (Onodera et al., 1977) and hence ciliates cannot utilize urea for their body protein synthesis. However, observed similar urease enzyme activity in the rumen of faunated and defaunated animals (Santra et al., 1996; Santra and Karim, 2002) needs further studies.

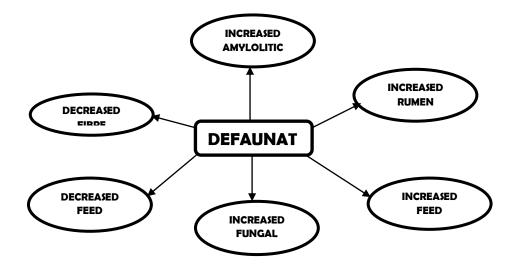


Fig. 2: Schematic representation of the effects of defaunation on nutrient digestibility and animal performance

Methane production: Defaunation as seen in Fig 1, is reported to considerably decrease the methane production compared with the normal faunated animals (Jouany et al., 1988; Williams and Coleman, 1992; Santra et al., 1994). The reduction in methane production in absence of rumen protozoa has been attributed to various reasons. Rumen protozoa contribute hydrogen moiety for the production of methane by the methanogenic bacteria (Prins and Van Hoven, 1977; Van Hoven and Prins, 1977). Further, ectosymbiotic attachment methanogens have with ciliate protozoa and elimination of their symbiotic partner on defaunation results in reduced methane production. Reviewing the published literatures on the topic, Kreuzer (1986) calculated that defaunation decreased energy losses through methanogenesis by 5.5 to 7.9% of gross energy intake.

Conclusion

Rumen is a natural fermentative anaerobic system which should be manipulated essentially by altering the composition of rumen microflora. There is ample scope to manipulate the rumen by feeding local plants or tree leaves or agro industrial by products to defaunate the animals for improving its productivity. Different strategies are involved in defaunation like separation of newborn animals from their dams after birth, use of chemicals (copper sulphate, manoxol and sodium lauryl sulphate) for obtaining animals free from rumen ciliate protozoa and use of plant extracts such as secondary metabolites. Defaunation therefore, increases the number of amylolytic bacteria due to elimination of nutritional competition between bacteria and protozoa for using starch, whereas the cellulolytic bacterial population becomes decreased; fungal populations in the rumen also increase. Defaunation can also significantly decrease the methane production compared with the normal faunated animals since protozoa contribute hydrogen moiety to produce methane by the methanogenic bacteria. However, protozoa may be non-essential for ruminant, still they have significant role to play in the rumen metabolism specially to stabilize the rumen because rumen protozoa contribute 40-50% of the total microbial biomass and enzyme activities in the rumen.

References

- 1. Agarwal, Neeta., N. Kewalramani, D. N. Kamra, D. K. Agrawal and K. Nath. (1991). Hydrolytic enzymes of buffalo rumen: Comparison of cell free rumen fluid, bacterial and protozoal fractions. Buff. J. 2:203-207.
- 2. Amos, H. E. and D. E. Akin. (1978). Rumen protozoal degradation of structurally intact forage tissue. Appl. Environ. Microbiol. 36:513-522.
- Ba, N. B. and L. D. Ngoan. (2003). Evaluation of some unconventional trees/plants as ruminant feeds in Central Vietnam Hue University of Agriculture and Forestry.LivestockResearch forRuralDevelopment 15(6)
- 4. Bird, S. H. and R. A. Leng. (1985). Productivity response to eliminating protozoa from the rumen of sheep. Rev. Rural Sci. 6:109-117.
- Bird, S. H., B. Rommulo and R. A. Leng. (1994). Effects of Lucerne supplementation and defaunation on feed intake, digestibility, N retention and productivity of sheep fed straw based diets. Anim. Feed Sci. Technol. 45:119-129
- 6. Brock, F. M., C. W. Forsberg and S. J. G. Buchman. (1982). Proteolytic activity of rumen micro-organisms and effect of proteinase inhibitors. Appl. Environ. Microbiol. 44:561-569.
- Chaudhary, L. C., A. Srivastava and K. K. Singh. (1995). Rumen fermentation pattern and digestion of structural carbohydrate in buffalo (Bubalus bubalis) calves as affected by ciliate protozoa. Anim. Feed. Sci. Technol. 56:111-117.
- 8. Coleman, G. S. (1975). The inter relationship between rumen ciliate protozoa and bacteria. In: Digestion and Metabolism in the Ruminant (Ed. W. Mc Donald and A. C. I. Warner). The University of New England Publishing Unit, pp. 149-164.
- 9. Cook, R. A. (1976). Urease activity in the rumen of sheep and distribution of ureolytic bacteria. J. Gen. Microbiol. 92:32-49.
- 10. Demeyer, D. I. and C. J. Van Nevel. (1979). Effect of defaunation on the metabolism of rumen microorganisms. Br. J. Nutr. 42:515-524.
- 11. Diaz, A., M. Avendano and A. Escobar. (1993). Evaluation of Sapindus saponaria as a defaunating agent and its effects on different ruminal digestion parameters. LivestockResearch forRuralDevelopment. 5(2): 1-5
- 12. Eadie, J. M. and J. C. Gill. (1971). The effect of the absence of rumen ciliate protozoa on growing lambs fed on roughage and concentrate diet. Br. J. Nutr. 26:155-167.

- Eugene, M., H. Archimede, B. Michalet-Doreau, G. Fonty. (2004). Effects of Defaunation on Microbial Activities in the Rumen of Rams Consuming a Mixed diet (fresh Digitaria decumbens grass and concentrate). 53:187 – 2000
- 14. Faichney, G. J. and D. A. Griffiths. (1978). Behaviour of solute and particle markers in the stomach of sheep given a concentrate diet. Br. J. Nutr. 40:71-82.
- 15. Fonty, G., J. P. Jouany, J. Senaud, Ph. Gouet and J. Grain. (1984). The evolution of microflora, microfauna and digestion in the rumen of lambs from birth to four months. Can. J. Anim. Sci. Supp.:165-169.
- 16. Fulgueira, C.L., S.L., Amigot, M. Gaggiotti, L.A. Romero, J.C. Basilico. (2007). Forage quality: Techniques for testing. Fresh produce Global Science Books
- 17. Gebeyehu, A. and Y. Mekasha. (2013). Defaunation: Effects on Feed Intake, Digestion, Rumen Metabolism and Weight Gain. Wudpecker Journal of AgriculturalResearch 2(5):134-141
- 18. Hegarty, R. S., J. V. Nolan and R. A. Leng. (1991). Sulphur availability and microbial fermentation in fauna free rumen. Arch. Anim. Nutr. 41:725-736.
- 19. Hristova, A. N., M. Ivan, L. M. Rode and T. A. McAllister. (2001). Fermentation characteristics and rumen ciliate protozoal populations in cattle fed medium or high barley based diet. J. Anim. Sci. 79:515-524.
- Itabashi, H., T. Kobayashi, R. Morii and S. Okamoto. (1982). Effect of ciliate protozoa on the concentration of rumen and duodenal volatile fatty acid and plasma glucose and insulin after feeding. Bull. Nat. Inst. Anim. Ind. (Japan), 39:21-32.
- 21. Ivan, M., D. des. Dayrell, S. Mahadevan and M. Hiderolou. (1992). Effect of bentonite on wool growth and nitrogen metabolism in fauna free and faunated sheep. J. Anim. Sci. 70:3192-3202
- Jouany, J. P. (1978). Contribution a l'etude des protozoaries cilies de rumen: leur dynamique, leur role dans la digestion et leur interct pour le ruminant. These de Doctorat, Universite de Clermont II, no d'Ordre 256, Vol. 2, pp. 195.
- Jouany, J. P., B. Zainab, J. Senaud, C. A. Groliere, J. Grain and P. Thivend. (1981). Role of the rumen ciliate protozoa Polyplastron multivesiculatum, Entodinium spp. and Isotricha prostoma in the digestion of mixed diet in sheep. Reprod. Nutr. Dev. 21:871-884.
- 24. Jouany, J. P., D. I. Demeyer and J. Grain. (1988). Effect of defaunating the rumen. Anim. Feed Sci. Technol. 21:229-265.
- 25. Kamra, D.M. (2005).RumenMicrobialEcosystem.Current Science 89(1):124-135
- Kayouli, C., C. J. Van Nevel and D. I. Demeyer. (1983). Effect de la defaunation du rumen sur la degradibilite des proteins de saja mesure in sacco. In: IVth Interantional Symposium on Protein Metabolism and Nutrition, Clermont-Ferrand, France. Vol. II. Lee Colloques de l'INRA16, INRA edn., pp. 251-254.
- 27. Kurihara, Y., T. Takechi and F. Shibata. (1978). Relationship between bacteria and ciliate protozoa in the rumen of sheep fed purified diet. J. Agric. Sci. 90:373-381
- 28. Kurihara, Y., T. Takechi and F. Shibata. (1978). Relationship between bacteria and ciliate protozoa in the rumen of sheep fed purified diet. J. Agric. Sci. 90:373-381.
- Leng, R.A., S.H. Bird, A. Klieve, B.S. Choo, F.M. Ball, G. Asefa, P. Brumby, V.D. Mudgal, U.B. Chaudhry, S.U. Haryono and N. Hedratno. (2011). The Potential For Tree Forage Supplements To Manipulate Rumen Protozoa To Enhance Protein To Energy Ratios In Ruminants Fed On Poor Quality Forages. FAO pp 1-15. Accessed last January 10, 2014. D:/cd3wddvd/NoExe/.../meister11.htm.
- 30. Leng, R.A., S.H. Bird, A. Klieve, B.S. Choo, F.M. Ball, G. Asefa, P. Brumby, V.D. Mudgal, U.B. Chaudhry, S.U. Haryono and N. Hedratno. (2011). The Potential For Tree Forage Supplements To Manipulate Rumen Protozoa To Enhance Protein To Energy Ratios In Ruminants Fed On Poor Quality Forages. FAO pp 1-15. Accessed last January 10, 2014. D:/cd3wddvd/NoExe/.../meister11.htm.
- 31. Lindsay, J. R. and J. P. Hogan. (1972). Digestion of two legumes and rumen bacterial growth in defaunated sheep. Aust. J. Agric. Res. 23:321-330.
- Lopez- Camarena, J., J.R. Orozco Hernandez, J.H. Medina Villareal, J.J. Uribe Gomez, H. Verdin Sanchez and V.O. Fuentes – Hernandez. (2010). Evaluation Of Robenidine To Defaunate Pelibuey Lambs And The Production And Digestibility. Journal of Animal andVeterinaryAdvances 9(1):44-46
- Makkar H.P.S., K. Becker, H. Abel and C. Szegletti . (1995). Degradation of condensed tannins by rumen microbes exposed to Querbracho tannin (QT) in rumen Simulation Technique (RUSITEC) and effects of QT on fermentative processes in the RUSITEC. J. Sci. FoodAgric. 69: 495-500.
- 34. Mendoza, M. G. D., R. A. Britton and R. A. Stock. (1993). Influence of ruminal protozoa on site and extent of starch digestion and ruminal fermentation. J. Anim. Sci. 71:1572-1578.

- 35. Mendoza, M. G. D., R. A. Britton and R. A. Stock. (1993). Influence of ruminal protozoa on site and extent of starch digestion and ruminal fermentation. J. Anim. Sci. 71:1572-1578.
- 36. Monforte Briceno, G., C. SandovaL, L. Ramirez, and C. Capetillo. (2005). Defaunating capacity of tropical fodder trees: Effects of polyethylene glycol and its relationship to in vitro gas production. Elsevier B.V.
- 37. Moss, A.R., J. Pjouany and J. Newbold. (2000). Methane production by ruminants: its contribution to globalwarming. Ann. Zootech. **49**: 231–253.
- 38. Nagaraja, T. G., G. Towne and A. A. Beharka. (1992). Moderation of ruminal fermentation by ciliate protozoa in cattle fed a high grain diet. Appl. Env. Microbiol. 58:2410-2414.
- 39. Ngamsaeng, A. (Undated). Effects of supplementing local plants on rumen fermentation, microbial protein synthesis, digestibility and voluntary feed intake in beef cattle steers.
- Ngamsaeng, A., M. Wanapat and S. Khampa. (2006). Effects of Mangosteen Peel (Garcinia mangostana) Supplementation on Rumen Ecology, Microbial Protein Synthesis, Digestibility and Voluntary Feed Intake in Cattle. Pakistan Journal of Nutrition 5(5): 445 – 452
- 41. Nhan, N. T. H., N. V. Hon, M. T. Ngu, N. T. Von, T. R. Preston and R. A. Leng. (2001). Practical application of defaunation of cattle on farms in Vietnam: Response of young cattle fed rice straw and grass to a single drench of ground nut oil. Aisan-Aus. J. Anim. Sci. 14:485-490.
- Onodera, R., N. Yamasaki and K. Murakami. (1988). Effect of inhabitation by ciliate protozoa on the digestion of fibrous materials in vivo in the rumen of goats and in vitro rumen microbial ecosystem. Agric. Biol. Chem. 52:2635-2637.
- 43. Onodera, R., Y. Nakagawa and M. Kandatsu. (1977). Ureolytic activity of the washed suspension of rumen ciliate protozoa. Agric. Biol. Chem. 41:2177-2182.
- 44. Owens, N. F. and R. Zinn. (1988). Protein metabolism of ruminant animals. In: The Ruminant Animal Digestive Physiology and Nutrition (Ed. D. C. Chruch), Prentice-Hall, Englewood Cliffs, NJ, pp. 227-249.
- 45. Ozdemir, M., M. Cinar, S. Haliloglu and A. Eryavuz. (2006). Effects of Defaunation and Dietary Nitrogen Source on Sodium, Potassium, Iron and Zinc in the Rumen Fluid, Plasma and Wool of Lambs. Turkish Journal of Veterinary and Animal Science 30:367-373
- Patra, A.K., D.N. Kamra and N. Agarwal. (2006). Effect of plant extracts on in vitro methanogenesis, enzyme activities and fermentation of feed in rumen liquor of buffalo. In:Anim. Feed Sci. Technol., 128:276-291.
- 47. Pell, A.N., R.I. Mackie, I. Mueller-Harvey, L.R. Ndlovu. (2001). Tannins: analysis and biological effects in ruminant feeds (special issue). Anim Feed Sci Technol **91**:112-113
- Poungchompu, O., M. Wanapat, C. Wachirapakorn, S. Wanapat and A. Cherdthong. (2009). Manipulation of ruminal fermentation and methane production by dietary saponins from mangosteen peel and soapberry fruit. J.Agricul. Sci. 63: 389-400.
- 49. Prins, R. A. and W. Van Hoven. (1977). Carbohydrate fermentation by the rumen cilia Isotricha prostoma. Protistologica. 13:549-556.
- 50. Punia, B. S., J. Leibholtz and G. J. Faichney. (1987). The role of rumen protozoa in the utilization of paspalum hay by cattle. Br. J. Nutr. 57:395-406.
- 51. Ramprasad, J. and G. V. Raghavan. (1981). Note on the growth rate and body composition of faunated and defaunated lambs. Indian J. Anim. Sci. 51:570-572.
- 52. Rowe, J. B., A. Davies and A. W. J. Broome. (1985). Quantitative effect of defaunation on rumen fermentation and digestion in sheep. Br. J. Nutr. 54:105-119.
- 53. Santra, A. and S. A. Karim. (2000). Growth performance of faunated and defaunated Malpura weaner lambs. Animal Feed Science and Technology. **86**:251-260.
- 54. Santra, A. and S. A. Karim. (1999). Efficacy of sodium laurel sulfate as defaunating agent in sheep and goats. International J. Anim. Sci. 14:167-171.
- 55. Santra, A., D. N. Kamra and N. N. Pathak. (1996). Influence of ciliate protozoa on biochemical changes and hydrolytic enzymes in the rumen of Murrah buffalo (Bubalus bubalis). Buffalo J. 1:95-100.
- 56. Santra, A., Kamra, D. N. and Pathak, N. N. (1994). Effect of defaunation on nutrient digestibility and growth of Murrah buffalo (Bubalus bubalis) calves. International J. Anim. Sci. 9:185-187.
- 57. Santra, A., Kamra, D. N. and Pathak, N. N. (1994). Effect of defaunation on nutrient digestibility and growth of Murrah buffalo (Bubalus bubalis) calves. International J. Anim. Sci. 9:185-187.
- 58. Smet, De. S., D. I. Demeyer and C. J. Van Nevel. (1992). Effect of defaunation and hay:concentrate ratio on fermentation, fibre digestion and passage in the rumen of sheep. Anim. Feed Sci. Technol. 37:333-344.

- 59. Ushida, K., J. P. Jouany and P. Thivend. (1986). Role of rumen protozoa on nitrogen digestion in sheep given two isonitrogenous diets. Br. J. Nutr. 56:407-419.
- 60. Van Hoven, W. and R. A. Prins. (1977). Carbohydrate fermentation by the rumen ciliate Dasytricha ruminantium. Protistologiaca. 13:599-606.
- 61. Veira, D. M., M. Ivan and P. Y. Jui. (1983). Rumen ciliate protozoa: effects on digestion in the stomach of sheep. J. Dairy Sci. 66:1015-1022.
- 62. Wanapat, M. (2000). Rumen Manipulation to ncrease the Efficient Use of Local Feed and Productivity of Ruminants in the Tropics. Asian-Australian. Journal of Animal Science 13
- 63. Williams, A and G. Coleman. (1991). The Rumen Protozoa. New York, NY, USA: Springer-Verlag.
- 64. Williams, A. G. and E. Susan. Withers. (1991). Effect of ciliate protozoa on the activity of poysaccharidedegrading enzymes and fibre breakdown in the rumen ecosystem. J. Appl. Bacteriol. 70:144-145.
- 65. Williams, A. G. and G. S. Coleman. (1992). The rumen protozoa. New York, Springer-Verlag.