International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 7, Issue 5 -2020

Review Article

DOI: http://dx.doi.org/10.22192/ijarbs.2020.07.05.002

Surfactants as Feed Additives in Ruminants: A Review

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Abstract

Surfactants are amphiphilic compounds, which act as surface active agents. Amphiphilic compound means, they possesses an equal ratio between the polar and non polar portions of each molecule. Surfactants play an important role in detergent formulations. The food and beverage industry is a great detergent consumer, due to the extreme cleanliness demanded by this kind of industrial activity. Surfactants play an important role in detergent formulations. There is increasing interest on this topic in ruminant nutrition, because of the unique ability of nonionic surfactants in enhancing the fibre digestion which opens up the possibility of their use as feed additives in ruminants, wherein enhanced fibre digestion and subsequent productive benefits like increased milk yield and growth rate, with resultant economic benefits to the farmers. In addition, surfactant properties such as, low toxicity, functionality under extreme conditions, based on renewable substances and biologically degradable nature, are added advantages. The diversity of these molecules supports their potential application in the field of animal nutrition.

This paper reviews the various aspects of surfactants as feed additives, such as basic mechanism of action and classification, are discussed. The structure of nonionic surfactants, is initially discussed clearly, followed by detailed description of the processes by which they bring about manipulation of rumen fermentation. In this, the mechanism of enhanced fibre digestion, results of various investigations in ruminants, synergistic effects with other beneficial compounds like enzymes, dose rate and method of administration are also discussed.

Keywords: Surfactants, feed additives, rumen fermentation, cows

1. Introduction

Since feed is a major cost in ruminant production, enhancing digestive efficiency remains a driving objective in the industry. Almost 75 per cent of the total cost of rearing a cow is constituted by fed cost alone. Among the various feeds for ruminants, cereals are increasingly being used for human food and also for production of bio-fuels, nowadays. Therefore, roughages are the major food for ruminants and hence, increasing the digestive efficiency of roughages is a major concern in the food industry.

Although forages remain the major feed source, it is widely believed that the efficiency of feed utilisation by ruminants has remained relatively unchanged during the last two decades. It is high time that new innovations that enhance the digestive efficiency in dairying and feedlot cattle are explored and experimented.

Feed additives are not nutrients, but certain compounds which act basically by stimulating the growth of rumen microbes, thereby altering the animal metabolism. Their utility is in improving growth rate, productivity and efficiency of feed utilization in healthy livestock on an optimum plane of nutrition (Icchponani, 1991).

The conventionally used feed additives for altering rumen microbes and thereby the fermentation, are ionophore antibiotics, enzymes, methane production inhibitors, inhibitors of proteolysis or deamination, and buffers. The increased digestive efficiency realised through the use of these compounds is the result of major shifts in microbial fermentation pathways. For example, the selective use of antibiotics can alter the rumen microbial population and ultimately influence the end products of digestion.

Antibiotics are, however, used only in meat producing animals because of the risk of antibiotic, transfer to milk (Chattopadhyay, 2014). Production responses of ruminants fed on forage or concentrate-based diets plus exogenous enzymes have been inconsistent (Sujani and Seresinhe, 2015).

With different types of diets, long-chain fatty acids and halogenated homologues of methane have been found to reduce methane production in the rumen. The main limitation with the use of these additives is that rumen microbes are able to adapt and degrade them after about one month of treatment. Another disadvantage is that the beneficial effect appears to be consistent only in forage-based diets that favour methane production (Patra *et al*., 2017).

Buffers are mainly used under conditions where the feeding of high levels of grains can induce an active fermentation and cause excess production of acids within the rumen. They act by regulating and maintaining the pH at levels at which the cellulolytic microorganisms can be of maximum effectiveness (pH = 6-7). However, at pH levels, such as these, the digestion of starch and proteins are generally decreased, as result of which the increased digestibility of cell wall contents brought about, when buffers are fed, is nullified (Scholl *et al*., 2018).

This has necessitaitated a situation wherein, the ruminant nutritionists are forced to look for a novel feed additive with enhanced fibre digestion properties and that is where, surfactants, fits the bill. Surfactants, which play an important role in detergent formulations, as cleaning agents, have also been used in the food processing industry as emulsifiers and extenders. The most well known physicochemical property of surfactants is their interfacial activity, when placed in solution, which means their ability to align at the interfaces is a relection of their tendency to assume the most energetically stable orientation.

One type of nonionic surfactant (NIS), the polyoxyethylene sorbitan esters, are synthesised by the addition, via polymerisation, of ethylene oxide to sorbitan fatty acid esters. These NIS, widely known as polysorbates (Eg:- Tween 60 and Tween 80) were reported to have the ability to hydrolyse, even newspaper (Castanon *et al*., 1981).

However, the effects of NIS such as these on ruminant fermentation and digestion have not been contemplated to date. The present review paper attempts to discuss the structure, mechanism of enhanced fibre digestion, results of various investigations in ruminants, synergistic effects with other beneficial compounds like enzymes, dose rate and methods of administration, would help in utilising NIS to optimise the digestive process in ruminants, with subsequent enhancement in productivity and profitability.

2. Review of Literature

2.1. Surfactants

Surfactants include all surface active agents that are organic or organic-metal molecules that exhibit polar and solubility behaviour that result in the phenomenon known as surface activity. The most commonly recognised phenomenon in this respect is the reduction of the boundary between two immiscible fluids (Shelford and Kamande, 2001).

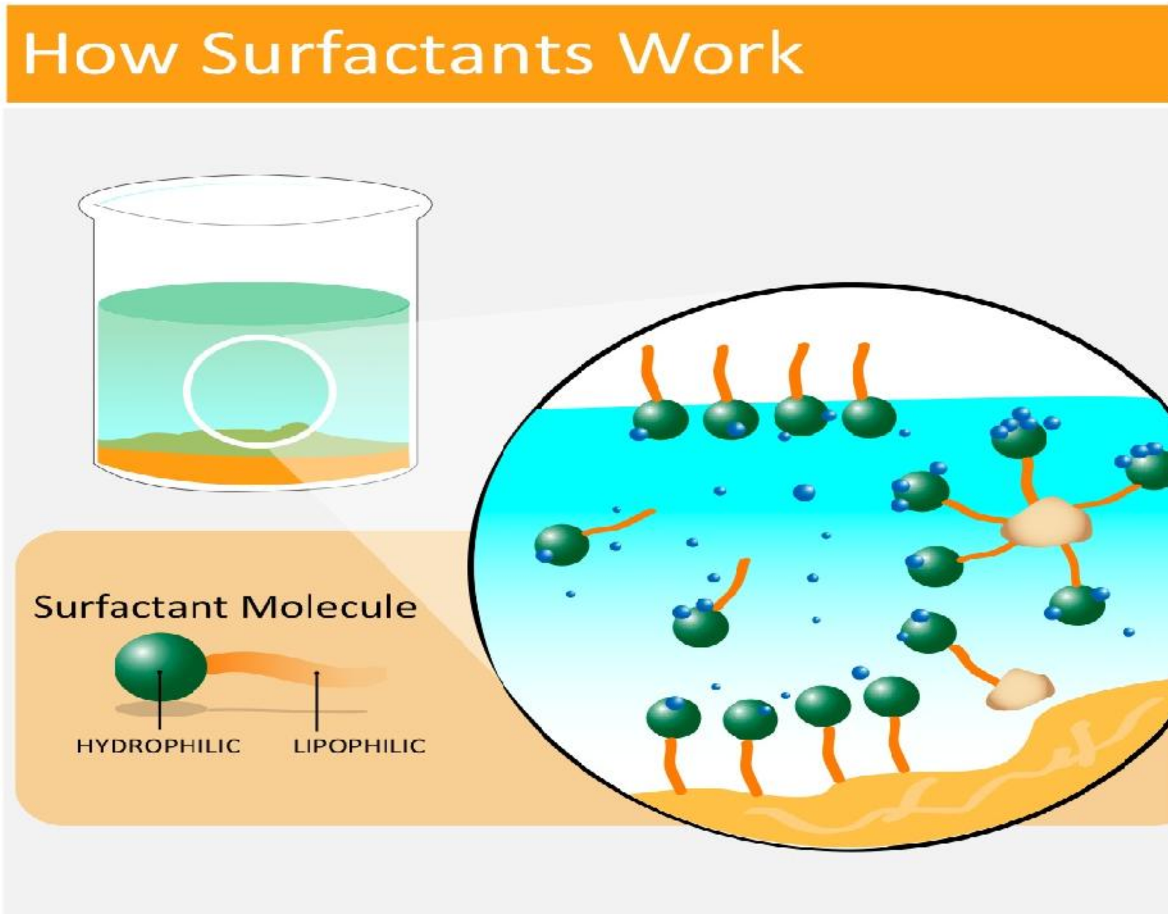
A surfactant is a surface active agent. It is an amphiphilic compound, which possesses an equal ratio between the polar and non polar portions of each molecule. When placed in an oil in water system, the polar groups are attracted to orient towards water – hydrophilic and the non polar groups are oriented towards the oil – lipophilic. Thereby, the surfactant molecule lowers the interfacial/ surface tension between the oil and water phases (Singh *et al*., 2006).

2.1.1. Commonly used surfactants

The most commonly used surfactants are soaps and detergents. They are surfactants with detergent properties. They alter the interfacial properties, so as to remove a phase (dirt phase) from solid particles.

2.1.2. Classification of surfactants

Surfactants are classified into four groups:
1) Anionic
2) Cationic
3) Nonionic
4) Zwitter ionic



(Annual Report on Progresses in Chemistry, 2003)

2.2. Anionic surfactants

They account for nearly 50 per cent of the world's total surfactants. Hydrophilic part is an anion (a negatively charged ion like Cl-). They are dissociated in water. Examples are the alkali soaps and detergents like sodium lauryl sulphate.

2.3. Cationic surfactants

They account for only 4 per cent of the world's total surfactants. Hydrophilic part is an cation (a positively charged ion like Na+ or K+). They are also dissociated in water. Examples are the quaternary ammonium compounds like benzalkonium chloride.

2.4. Zwitter ionic surfactants

They are also called as amphoteric surfactants. That is, a single surfactant molecule exhibits both anionic and cationic dissociations. They account for only 1 per cent of the world's production. Examples are:

1) Amino acids and phospholipids (natural)
2) Betaines and sulphobetaines (synthetic)

2.5. Non Ionic Surfactants (NIS)

They do not ionize in aqueous solution. Their hydrophilic group is a non dissociable type. A large number of these are made hydrophilic by the presence of poly ethylene glycol chain obtained by the polycondensation of ethylene oxide and hence they are also known as 'polyethoxylated nonionics'.

2.5.1. Polyethoxylated nonionics

They account for almost 40 per cent of the world's surfactants. Atleast four to five ethylene groups are needed to ensure a good solubility in water. In some cases, the ethoxylation degree can be 20 or even 40.

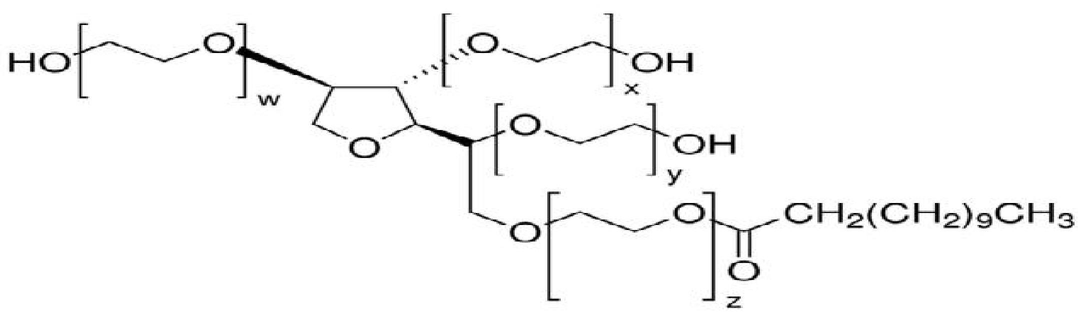
Hexitols are alcohols produced by the reduction of hexoses. Eg:- From glucose, the alcohol sorbitol is produced. Sorbitol, when heated at an acid pH, two hydroxyl (OH -) groups merge through an ether link to form a five to six carbon atom cycle called sorbitan. Sorbitan ring has 4 OH – groups which can react either with

1) fatty acids to form sorbitan esters, whereby lipophilicity is enhanced, or
2) the polyethylene oxide condensate whereby hydrophilicity is enhanced (Annual Report on Progresses in Chemistry, 2003).

2.5.1.1. Polysorbates

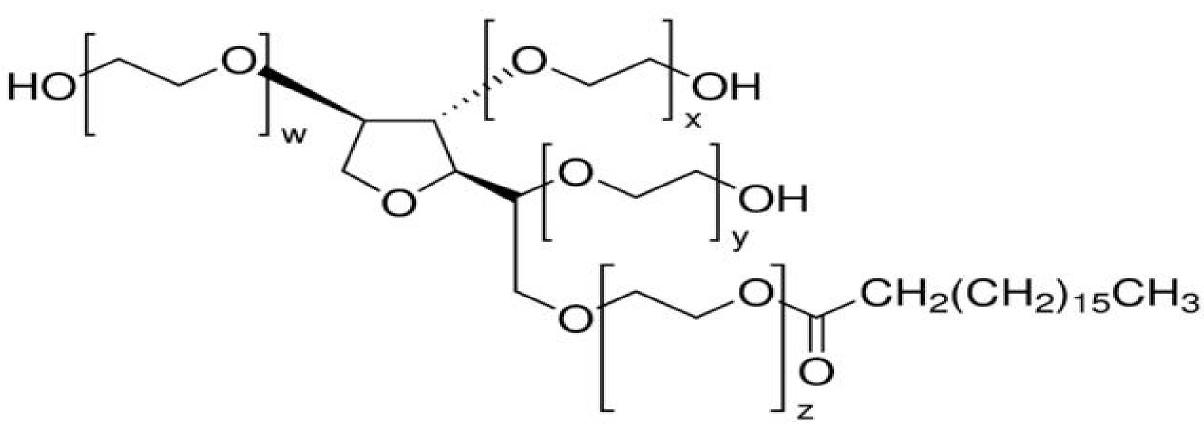
The polyoxyethylene sorbitan esters, are synthesised by the addition, via polymerisation, of ethylene oxide to sorbitan fatty acid esters. They are widely known as polysorbates. The polysorbate surfactants most widely used are, polyoxyethylene sorbitan monolaurate (Tween 20), where one lauric acid molecule is attached; polyoxyethylene sorbitan monostearate (Tween 60) where one stearic acid molecule is attached and polyoxyethylene sorbitan monooleate (Tween 80), where one oleic acid molecule is attached; whose structures are depicted below:

Structure of Tween 20



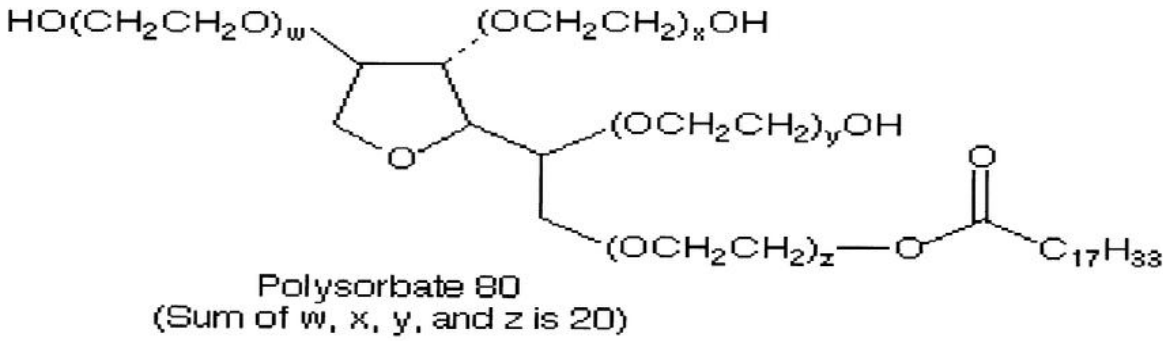
(Annual Report on Progresses in Chemistry, 2003)

Structure of Tween 60



(Annual Report on Progresses in Chemistry, 2003)

Structure of Tween 80



(Annual Report on Progresses in Chemistry, 2003)

2.2. Manipulation of Rumen Fermentation

The rumen is a large fermentation vat which accounts for nearly 10 to 20 per cent of the live weight of the animal. The large size of the rumen allows food to accumulate and ensures that sufficient time is allowed for the rather slow breakdown of nutrients present in food. In addition to these, the movements of the reticulo-rumen and also the act of rumination help in breaking up the food and exposing it to the attack of micro organisms.

2.2.1. Basic rumen physiology

The normal rumen pH is in the range of 5.5 to 6.5. The temperature of the rumen is 38-42 degree centigrade. The rumen ammonia concentration ranges from 85 to 300 mg per litre. The microbial protein synthesised is 200g per kg of organic matter digested.

The bacterial count in the rumen ranges from 10^9 to 10^{10} per ml of rumen content. The protozoal number is 10^6 per ml of rumen content. The fungi account for 10^3 to 10^5 per ml of rumen content.

The total volatile fatty acid (TVFA) content of the rumen is 70-150 milli moles per litre and the volatile fatty acid (VFA) profile in percentage is, acetate 61: propionate 18: butyrate 13, on normal diets given to cattle.

The principal rumen gases are carbon dioxide and methane. While carbon dioxide accounts for 40 per cent of the total gas produced, methane contributes 30 to 40 per cent of the total gases. The hydrogen content is 5 per cent, while the nitrogen and oxygen content varies. Approximately 4.50 g of methane is produced for every 100g of carbohydrate digested. About 7 per cent of the gross energy in the feed is lost as methane (Hoover and Miller, 1992; MacDonald *et al*., 1995).

2.2.2. Mechanism of enhanced fibre digestion

The aim of adding NIS, such as Tweens in the diet of ruminants is to enhance fibre digestion in the digestive tract. There are three main factors which facilitate the enhanced fibre digestion:

2.2.2.1. Plant factors

The cell wall of plants consists of lignin bonds, which cannot be broken by the mammalian enzymes. NIS surrounds the lignin parts of the substrates via strong hydrophobic interaction, whereby the lignin bonds are broken. This enables easier degradation of cellulose or hemicellulose by the enzymes secreted by the cellulolytic bacteria and fungi (Shelford and Kamande, 2001; Lee *et al*., 2007).

NIS enhances the hydration of feed particles, which result in the loosening of the structure of cell walls with resultant increase in the enzyme holding capacity of the cells. This brings the cellulolytic enzymes into quick and intimate contact with the accessible and inaccessible substrate sites, which ultimately results in increased dry matter digestibility (Goto *et al.*, 2003; Wang *et al*., 2004; Singh *et al*., 2006).

2.2.2.2. Microbial factors

The enzyme substrate interaction is increased by enhancing the adhesion of rumen microbes to feed particles. This increases the accessibility and thereby ensures the direct contact of enzymes secreted by the microbes, to the accessible and inaccessible substrate sites. NIS also brings about a significant increase in the number of viable cellulolytic bacteria and anaerobic fungi, which ultimately enhances the digestibility (Lee *et al*., 2004). A fourfold increase in total viable bacterial counts was observed, while the protozoal counts decreased by 2.10 fold, with the anaerobic fungal counts being unaffected (Liu *et al*., 2013).

2.2.2.3. Feed processing factors

Feed processing techniques like grinding, decrease the particle size whereby more number of enzyme binding sites in the substrate are exposed and enzyme substrate interaction is enhanced (Shelford and Kamande, 2001).

2.2.3. Mechanisms by which cellulolytic enzyme activity is increased

Once a conducive environment is ensured in the rumen, by the fulfilling of the above three factors, the NIS acts and releases the fibre digesting enzymes. There are basically two mechanisms by which the fibrolytic enzyme activity is increased:

2.2.3.1. Stimulates the release of enzymes from the cellulase enzyme complex

2.2.3.1.1. NIS increases the permeability of microbial cell membrane, whereby, several cellulolytic enzymes, associated with *Fibrobacter succinogenes* (Singh and Kewalramani 2009a), the most effective celluloytic rumen bacteria and the aerobic fungus (*Neurospora crassa*) are released (Lee *et al*., 2007). These cellulolytic enzymes are linked to the membrane lipids of the microbes. NIS brings about increased unsaturation of these lipids and thereby alters membrane fluidity with resultant increase in permeability of the microbial cell (Lee *et al*., 2004; Singh and Kewalramani, 2009a and b).

2.2.3.1.2. NIS also ensures release of cellulolytic and hemicellulolytic enzymes from the cellulolytic bacteria and aerobic fungi. NIS ensures the release of cellulose enzyme complex, which is usually a bound enzyme associated with the microbial cell wall. As a result, the enzymes in the cellulose complex, viz., carboxy methyl cellulase, β- glucosidase and cellobiohydrolase are released. These enzymes attack cellulose at different positions to break the cellulose chain and cellulose is digested. NIS also ensures the release of the hemicellulose digesting enzyme xylanase from the bound form, so that hemicellulose is also digested (Lee *et al*., 2007).

The net result of all these is that fibre digestion is increased, with resultant increase of acetate in ruminal VFA and subsequent increase in milk fat (Liu *et al*., 2013).

2.2.3. Influence of addition of NIS on the digestibility of nutrients

Addition of the NIS, Tween 80 at 0.10 per cent in total mixed ration (TMR) significantly increased the digestibility coefficients of Dry Matter (DM), Crude Protein (CP), Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF) in lactating dairy cows, than those fed on the unsupplemented control (Shelford and Kamande, 2001). Narayanan *et al.* (2016) reported that the crude fibre (CF), NDF and ADF digestibilities of heifers fed on complete rations supplemented with Tween 80 at 0.10 level were significantly higher than those fed on the unsupplemented control.

Goto *et al.* (2003) observed that the *in vitro* dry matter digestibility (IVDMD) of barley grain and orchard grass hay was significantly higher at 0.20 level of supplementation of Tween 80 than the control. Singh and Kewalramani (2009b) reported that Tween 80 supplementation at the rate of 0.10 per cent level on a substrate comprising of concentrate mixture and wheat straw in 50:50 ratio, significantly increased the IVDMD, when compared to the unsupplemented control.

Significant increase in the digestibility of dry matter and other nutrients on supplementation of Tween 80 in rations, at 0.01 per cent level, when compared to the unsupplemented control, were also reported by Wang *et al*. (2004) in beef cattle and Chen *et al*. (2011) in Merino sheep.

2.2.4. Influence of addition of NIS on the microbial protein synthesis

Kamande *et al.* (2000) reported that both Tween 60 and Tween 80 increased rumen bacterial protease activity. Addition of NIS increased the proteolytic activity in the rumen, whereby more of dietary protein was degraded in the rumen. This resulted in an increase in the production of rumen ammonia nitrogen which was taken up by the microbes, thereby bringing about an increase in the synthesis of microbial protein

(Singh and Kewalramani, 2009a and b). Tween supplementation was found to significantly increase the duodenal microbial nitrogen flow and efficiency of microbial protein synthesis (Yuanklang *et al*., 2010). Ahn *et al*. (2009) observed that beef steers fed high roughage diets, *viz.,* corn and barley as grain substrate and rice straw and timothy hay, as roughage, supplemented with Tween 80 at the rate of 0.05, 0.10 and 0.20 per cent, showed significantly higher rumen ammonia level, DMI, average daily gain, and better feed efficiency than the unsupplemented control, suggesting that NIS enhanced ruminal fermentation of fibrous parts of feeds, which consequently resulted in better and efficient rumen fermentation, ultimately resulting in better performance of steers fed on high roughage diet.

2.2.5. Influence on rumen pH

Kim *et al*. (2004) reported that the rumen pH, at 6 h after feeding, of Hanwoo steers fed on a basal diet; basal diet supplemented with Tween 80, at the rate of 5.00 and 10.00 g per day were similar, with the pH values in both the treatment groups being 6.65, while the unsupplemented control group had a pH of 6.72. Chen *et al*. (2011) observed that the rumen pH of Chinese Merino sheep supplemented with Tween 40, 60 and 80, at the rate of 10 g per day each, were similar, with the values being in the range of 6.10 to 6.24 and normal, indicating that rumen pH was generally unaffected on NIS supplementation.

2.2.5. Influence on TVFA

Goto *et al.* (2003) reported that supplementation of Tween 80 at the rate of 0.05 and 0.10 per cent on a substrate consisting of barley grain and orchard grass hay and Singh and Kewalramani (2009b) at the rate of 0.20 per cent on a substrate comprising of concentrate mixture and wheat straw, in a concentrate to roughage ratio of 60:40; both in the rumen liquor of cows, *in vitro*, significantly increased the TVFA concentration in the respective treatment groups, as compared to the unsupplemented controls.

A significant increase in the TVFA concentration of Tween supplemented animals was reported by in dairy cows. The increased TVFA must have resulted from an increase in the growth of rumen microbes and increased fibre digestion, with the result that energy availability to the animal for milk production is increased as reported by Chen *et al*. (2011).

2.2.6. Influence on volatile fatty acid (VFA) profile

Wan-Jae *et al.* (2005) reported that supplementation of Tween 80 at 0.10 and 0.20 per cent in total mixed ration (TMR), significantly increased the molar percentage of propionate, *in vitro*. These workers as well as Singh and Kewalramani (2009a) also reported that growth rate of non cellulolytic bacteria which are propionate producers was increased, *viz*., *Prevotella ruminicola, Selenomonas ruminantium, Bacteroides amilophilus and Megasphaera elsdeni*. Adhesion of such bacteria to the feed is increased. As a result, there is increased propionate and decreased acetate. Since propionate yields more energy than acetate, more milk is produced, as observed by Liu *et al.* (2013).

2.2.7. Influence on methane production

In vitro rumen methane production from a substrate comprising of TMR pre-treated with Tween 80 at 0.10 and 0.20 per cent level, were significantly lower than those fed on an unsupplemented control (Kim *et al*., 2005). As mentioned earlier, reduced methane production, will result in increased energetic efficiency and subsequently increased productive response by way of milk and meat.

2.2.8. Influence on milk production

Milk production was found to be increased by 0.76 kg per cow per day in Holstein cows fed on a TMR supplemented with Tween 80, at the rate of 0.20 per cent when compared to the unsupplemented control. The total milk production per cow increased by 64 kg in the Tween supplemented animals over a 12 week period, when compared to the controls (Shelford and Kamande, 2001). Significant increase in milk yield and milk fat of cows fed on Tween 80 supplemented rations was also reported by Lee *et al.* (2003) and Kim *et al*. (2006).

2.2.9. Influence on growth

Baah and Shelford (1999) reported that Red Angus steers fed on TMRs supplemented with Tween 80 at 0.10 per cent had a significantly higher bodyweight gain and a better feed conversion efficiency than those fed on the unsupplemented control. Ahn *et al*. (2009) observed that beef steers fed on high-roughage diets, supplemented with Tween 80 at 0.15 per cent had a significantly higher bodyweight gain, than those fed on the unsupplemented control. Narayanan *et al.*

(2016) reported a significantly higher body weight gain and better feed conversion efficiency in heifers fed on complete rations supplemented with Tween 80 at 0.10 level as compared to those fed on the unsupplemented control.

2.2.10. Synergistic effects

2.2.10.1. With ionophore antibiotics like monensin: Wang *et al*. (2004) conducted *in vitro* incubations of barley grain and barley silage based TMRs, to study the effects of Monensin and Tween 80, alone and in combination, on ruminal fermentation. These researchers reported that supplementing Tween 80 with Monensin synergistically brought about a reduction in acetate: propionate ratio in the incubation mixtures. This effect of increased availability of propionate may have potential to improve energy efficiency in feedlot cattle, as observed by Liu *et al.* (2013).

2.2.10.2. With cellulolytic enzymes like xylanase and cellulase: NIS has got synergistic effect when supplemented with cellulolytic enzymes also. Baah *et al.* (2005) reported that Holstein cows fed on TMRs supplemented with Tween 80 and a mixture of fibrolytic enzymes, enhanced ruminal concentrations of propionate, but do not affect total tract digestibility and microbial nitrogen production. Hwang *et al.* (2008) conducted an *in vitro* experiment using timothy hay as substrate to investigate the effects of the mixture of Tween 80 and cellulolytic enzymes (xylanase and cellulase) and reported that the combination resulted in a significantly higher IVDMD, than the unsupplemented control. They also found that feeding the same timothy hay to Holstein steers also significantly increased *in vivo* nutrient digesibilities of DM, CP, CF, NDF and ADF; TVFA; propionic acid and decreased the acetate to propionate ratio compared to those fed on non-treated hay, suggesting that increased energetic efficiency, better body weight gain and feed conversion efficiency could be obtained in beef cattle, by using NIS-enzyme combination.

2.3. Optimum dose rate

0.10 per cent is the optimum dose rate of Tween and the dose should not exceed 0.20 per cent of the ration (Kamnade *et al*., 2000; Lee *et al*., 2003; Lee *et al*., 2004; Liu *et al*., 2013).

2.4. Methods of administration

Surfactants are available in either a liquid or powdered form.

2.4.1. Liquid form: If a liquid form is used, the surfactant may be sprayed onto the feed material and simultaneously mixed, or preferably diluted in the same or separate aqueous solutions, such as a buffer solution with a pH range from 4.50 to 7.00 before application (Shelford and Kamande, 2001; Liu *et al*. 2013).

2.4.2. Powder form: In case of powder form, the surfactant is incorporated into an inert carrier such as celite, diatomaceous earth or silica and then mixed with the feed. When provided as an NIS coated on a solid, the surfactant preferably may constitute at least 50 per cent of the dry weight of the product. If provided as a solid it may be applied to the feed material directly and then evenly mixed with the feed material. In case of solid form of NIS, the resulting feed can either be fed immediately to livestock or stored and fed at a later time. (Shelford and Kamande, 2001; Liu *et al*., 2013).

2.5. Toxicity of surfactants

Most anionic and non-ionic surfactants are nontoxic, having LD50 comparable to sodium chloride. At low concentrations, within the recommended doses, surfactant application is unlikely to have a significant effect on man, animal and environment (Rosen and Kunjappu, 2012).

3. Conclusion

Rumen fermentation can be altered by the supplementation of non ionic surfactants with economic benefits in the form of more milk and more meat and enhanced utilization of more fibre containing feeds, thereby improving the productivity of livestock. However, in light of the facts mentioned above, it should be borne in mind that the use of surfactants to improve the quality of ruminant diets, without compromising on productive performance and economy is a big challenge because of the complex nature of the digestive tract of ruminants.

4. Acknowledgments

The authors express their gratitude to the Kerala Veterinary and Animal Sciences University, Pookode, Wayanad for providing the necessary fund and facilities required for the study.

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DOI:10.22192/ijarbs.2020.07.05.002

How to cite this article:

Biju Chacko. (2020). Surfactants as Feed Additives in Ruminants: A Review. Int. J. Adv. Res. Biol. Sci. 7(5): 8-17.
DOI: http://dx.doi.org/10.22192/ijarbs.2020.07.05.002