

Dietary Non-Ionic Surfactant on Rumen Fermentation and Bacterial Population in Ruminants: A Review

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Abstract: The Non-Ionic Surfactant (NIS) was evaluated for its ability to influence rumen fermentation characteristics, microbial growth, enzyme activity and digestibility in the rumen. The administration of NIS solution to the rumen increased the digestion rates, the concentrations of Volatile Fatty Acid (VFA), growth rate and increased among of rumen microorganisms. However, had not much effect on ruminal pH value and ruminal NH₃-N concentration. These results indicated that the addition of NIS could greatly stimulate the release of enzymes without decreasing cell growth rate in contrast to trends reported with aerobic microorganism. The results showed that NIS might be of use as an alternative feed additive to stimulate multiple enzyme activity and microbial growth in the rumen.

Key words: Non-Ionic Surfactant (NIS), ruminant, bacterial population, growth, cell

INTRODUCTION

It has been well recognized that rice straw and wheat straw have poor nutritional value because of their low nitrogen and high fiber content and the greater fiber makes mature plant materials less digestible. Increasing digestibility of plant for use by ruminants is desirable both in Thailand countries where excess crop residues are a disposal problem and in tropical countries where there is dry-season loss of pasture quality and optimal utilization of residues is essential for animal production. Much research has been directed towards chemical treatment of crop residues and improvements have been achieved by treatment with alkali or urea or oxidative methods (e.g., alkaline hydrogen peroxide) which increase accessibility to cell wall polysaccharides by microbial enzymes. Cong *et al.* (2009) reported that APG increased *in vitro* ruminal Dry Matter (DM) and Organic Matter (OM) digestibility of cereal straw and Volatile Fatty Acids (VFA) concentrations in a dose dependent manner. Dietary inclusion of APG increased total tract digestibility of OM and Neutral Detergent Fiber (NDF), concentrations of ruminal ammonia-N and VFA and the ratio of acetate to propionate in goats (Yuan *et al.*, 2009, 2010). In addition, researchers also found that dietary inclusion of APG affected Amino Acid (AA) composition of ruminal bacteria under conditions of different dietary F:C (Zeng *et al.*, 2011). Lee *et al.* (2003) and Hwang *et al.* (2008) reported that Tween 80 another type of NIS with similar properties as APG, stimulated the growth rate of

anaerobic microorganisms but decreased adhesion of rumen cellulolytic bacteria to forage. The increase in nutrient digestibility and changes to ruminal metabolism by APG inclusion might be due to a change in microbial populations and bacterial FA composition but there is no information available about these effects in the literature. Also, that have been devoted to manipulating the rumen environment with the aim of enhancing feedstuff utilization and improving the efficiency of ruminant production. The results of these efforts are a wide range of feed additives that are capable of influencing some component of rumen metabolism. The Non-Ionic Surfactant (NIS) is well known as an effective surfactant that stimulates the release of enzymes from a range of aerobic microbes (Deshpande *et al.*, 1987; Hung *et al.*, 1988; Yazdi *et al.*, 1990; Long and Knapp, 1991).

NON-IONIC SURFACTANTS

These surfactants do not have an electrical charge which makes them resistant to water hardness deactivation. They are excellent grease removers that are used in laundry products, household cleaners and hand dishwashing liquids. Most laundry detergents contain both non-ionic and anionic surfactants as they complement each other's cleaning action. Non-ionic surfactants contribute to making the surfactant system less hardness sensitive. The most commonly used non-ionic surfactants are ethers of fatty alcohols (Fig. 1).

Alkyl Polyglycosides (APG) are a type of mild Non-Ionic Surfactant (NIS) derived from a reaction of corn starch glucose and a natural fatty alcohol (decyl polyglucose). These surfactants are used in cosmetic lotions (Weuthen *et al.*, 1995) and cleaning agents (Nickel *et al.*, 1996) are commercially available as yellowish colored aqueous paste. Alkyl polyglycosides are non-toxic, biodegradable (Nickel *et al.*, 1996; Hill and Rhode, 1999). In addition, APG are characterised in terms of their physicochemical properties of emulsification and dispersion (Nickel *et al.*, 1996). The glycoside of APG is an efficient nonionic emulsifier and the fatty alcohol is a co-emulsifier (Weuthen *et al.*, 1995). Cong *et al.* (2009) reported that APG increased *in vitro* ruminal Dry Matter (DM) and Organic Matter (OM) digestibility of cereal straw and Volatile Fatty Acids (VFA) concentrations in a dose dependent manner. Dietary inclusion of APG increased total tract digestibility of OM and Neutral Detergent Fiber (NDF), concentrations of ruminal ammonia-N and VFA and the ratio of acetate to propionate in goats (Yuan *et al.*, 2009, 2010). Non-Ionic Surfactants (NIS) have been proposed as a feed additive with the potential to modify ruminal fermentation. The proposed mode of action of NIA is the stimulation of enzyme production by fungi and bacteria as well as the improvement in the affinity of enzymes to their substrates (Wang *et al.*, 2003; Cong *et al.*, 2009).

Alkyl Polyglycosides (APG), based on glycoside and fatty alcohol are a new category of mild nonionic surfactants. APG are characterised in terms of their emulsifying and dispersing physicochemical properties

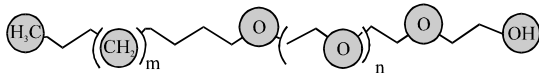


Fig. 1: Structure of non-ionic surfactants (http://www.scienceinthebox.com/en_UK/glossary/surfactants_en.html)

(Nickel *et al.*, 1996). Therefore, APG are used for cosmetic lotions and creams, detergent and cleaning agents and other medical applications. However, only few studies reported the effectiveness of APG application as a ruminant feed additive. Cong *et al.* (2009) reported that dietary APG supplementation might increase *in vitro* Dry Matter (DM) and Organic Matter (OM) disappearances of low quality roughages. In a study by Yuan *et al.* (2009), the concentrations of ruminal ammonia N (NH₃-N), total volatile fatty acids and the ratio of acetate to propionate were increased in goats fed diets containing APG. In addition, a previous study by the research group (Yuan *et al.*, 2010) demonstrated that direct dietary supplementation of APG could increase the total tract digestibility of OM, Neutral Detergent Fibre (NDF), the duodenal microbial N flow and the efficiency of microbial protein synthesis. However, there was no available data to illustrate the effect of APG or even other nonionic surfactants on the ruminal bacterial amino acid composition.

MODIFIERS OF THE VFA PROFILE

The additives are primarily aimed at increasing ruminal concentrations of propionate and thus are better suited for use in beef cattle diets. (Lee *et al.*, 2007). Total VFA concentrations at 6 h post-feeding were 90.29±2.87 and 103.05±3.92 mM/100 mL in the rumen fluids of cows on the NIS treatment and control, respectively indicating a 12.38% increase in the NIS treatment relative to the control (Table 1).

DIGESTIBILITY ENZYMES

Previous experiments on fungal cell permeability demonstrated that Non-Ionic Surfactants (NIS; surface active agents) can stimulate the release of enzymes (Reese and Maguire, 1969; Munn *et al.*, 1983). The

Table 1: Concentrations (mM) of volatile fatty acids in ruminal fluids NIS solution

Items	pH	NH ₃ -N	C2	C3	C4	References
Control ¹	6.95 ^a	64.94 ^b	38.64 ^b	12.09	7.89	Ahn <i>et al.</i> (2009)
NIS (Tween 80 (2 g day ⁻¹))	6.82 ^b	73.46 ^b	40.66 ^b	11.17	7.57	
NIS (Tween 80 (4 g day ⁻¹))	6.81 ^b	72.72 ^a	53.75 ^a	12.42	8.11	
Control ²	7.39 ^b	5.99	18.20 ^c	30.00	13.90	Yuan <i>et al.</i> (2010)
NIS (APG30) ²	8.22 ^a	5.93	21.90 ^b	22.10	13.60	
NIS (APG60) ²	8.53 ^a	5.92	25.80 ^a	27.60	12.50	
NIS (APG120) ²	8.31 ^a	6.06	22.50 ^b	26.80	14.30	
Control ³	6.24	11.90 ^a	63.35 ^c	20.03 ^c	11.10 ^c	Yong <i>et al.</i> (2011)
NIS (Tween 40) ³	6.19	11.10 ^b	66.65 ^{b,c}	21.15 ^{b,c}	11.70 ^{b,c}	
NIS (Tween 60) ³	6.16	11.18 ^b	21.86 ^{a,b}	12.06 ^b	3.13	
NIS (Tween 80) ³	6.10	10.98 ^b	71.07 ^a	22.88 ^a	12.83 ^a	
Control	-	8.96	66.50	13.50 ^b	15.10	Baah <i>et al.</i> (2005)
NIS (Tween 80.2%)	-	8.73	66.80	14.20 ^a	15.00	
Fibolytic enzyme preparation (2%)	-	8.50	68.00	14.10 ^a	14.80	
NIS (Tween 80.2%)+Fibolytic enzyme preparation (2%)	-	8.07	66.60	14.40 ^a	14.70	

^{a,b}Mean values with different superscripts in the same row differ significantly ($p \leq 0.05$); NIS = Non-Ionic Surfactants, APG = Alkyl Polyglycoside, ¹R:C 70:30; ²60:40; ³65:35

cellulase complex of aerobic ascomycetes fungus *Neurospora crassa* showed that the surfactant Tween 80 was effective in stimulating the induction and secretion of enzymes (Yazdi *et al.*, 1990), they demonstrated that the secretion of several cellulolytic enzymes of *N. crassa* is intimately linked to membrane lipid composition and the increased release of these enzymes can be explained through the alteration of membrane fluidity by the increased unsaturation of the lipids.

Effects of different NIS on the nutrient digestibilities of DM, OM, CP and cellulose were not different among treatments as compared with the control group (Table 2). Present results confirm those of present results confirm those of Baah who found that total tract digestibility coefficients of DM, nitrogen, NDF and ADF were not affected by Tween 80 treatment. Kim found that although

digestibility of crude fiber increased digestibilities of DM, CP, NDF and ADF were unaffected when compared to the control.

RUMINAL FERMENTATION CHARACTERISTICS

Ruminal pH and concentrations of total VFA and ammonia-N as influenced by the administration of NIS treatment. Rumen microorganisms produce VFA a result of their metabolic processes. In the present study, surfactant addition did change VFA concentrations and this with other *in vitro* (Wang *et al.*, 2003; Cong *et al.*, 2009) as well with some *in vivo* studies concentrations of VFA and relative concentrations of VFA were not affected by Tween 80 treatment. Kim suggested that the effects of Tween 80 on the ruminal fermentation were diet

Table 2: Effect of levels of Non-Ionic Surfactant (NIS) on nutrient digestibility

Items	OM	CP	NDF	ADF	Cellulose	References
Control ¹	-	56.1	47.4	35.0	-	Ahn <i>et al.</i> (2009)
NIS (Tween 80 (2 g day ⁻¹))	-	56.1	48.7	37.7	-	
NIS (Tween 80 (4 g day ⁻¹))	-	56.7	51.8	38.6	-	
Control ²	75.0 ^f	-	56.5 ^b	50.9	-	Yuan <i>et al.</i> (2010)
NIS (APG 30) ²	75.1 ^e	-	56.4 ^b	51.0	-	
NIS (APG 60) ²	78.1 ^a	-	61.2 ^a	54.8	-	
NIS (APG 120) ²	76.8 ^b	-	59.0 ^{ab}	52.1	-	
Control ³	61.6	63.8	-	-	55.17	Yong <i>et al.</i> (2011)
NIS (Tween 40) ³	63.7	66.4	-	-	57.75	
NIS (Tween 60) ³	63.9	63.3	-	-	57.47	
NIS (Tween 80) ³	64.4	65.8	-	-	59.63	
Sudangrass	80.6	73.9	44.0	-	-	Plascencia <i>et al.</i> (2007)
Grand	78.1	69.5	37.7	-	-	
Ground+Tween 22 g DM ⁻¹	77.2	69.5	39.8	-	-	
Macerated	79.3	71.6	48.4	-	-	
Macerated+Tween 22 g DM ⁻¹	79.6	72.0	45.4	-	-	

^{a,b}Mean values with different superscripts in the same row differ significantly (p ≤ 0.05); NIS = Non-Ionic Surfactants; APG = Alkyl Polyglycoside; ¹R:C 70:30; ²60:40; ³65:35

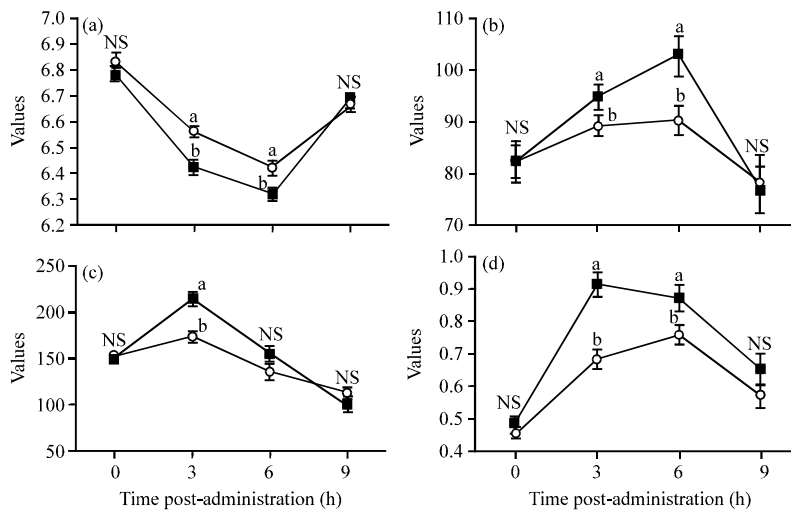


Fig. 2: Ruminal fermentation characteristics for a) pH values; b) total-VFAs; c) ammonia-N concentrations and d) microbial cell growth rates; in the rumen fluids collected from cows receiving daily intraruminal doses of 200 mL of distilled water (Control (○)) or Non-Ionic Surfactant solution (NIS treatment (■)) (Zeng *et al.*, 2011)

dependent, expressed either through altering the species composition of the rumen microbial population or through altering the interaction between the enzymes and the target substrates. Thus, the response of rotation ruminal fermentation NIS may relate to NIS dose and type and to the fermented substrate.

MICROBIAL POPULATIONS AND ENZYME ACTIVITY

The numbers of total viable and cellulolytic bacteria, protozoa and fungi were determined 6 h post-feeding in

the rumen fluids of cows (Fig. 2). When NIS solution was administered to the rumen, the total viable bacterial counts increased more than 4 fold (from 7.50 ± 1.3 to $31.0 \pm 4.6 \times 10^9$ cfu mL⁻¹ of rumen fluids) ($p < 0.01$) but cellulolytic bacteria did not affected by the administration of NIS solution with the counts of 9.5 ± 1.5 to $6.0 \pm 1.2 \times 10^7$ cells mL⁻¹. The protozoal counts decreased 2.1 fold (from 10.5 ± 1.2 to $5.0 \pm 1.8 \times 10^5$ cells mL⁻¹) when NIS solution was administered with statistical difference ($p < 0.01$) but NIS administration did not affects the fungal counts (14.0 ± 1.76 and $16.5 \pm 1.68 \times 10^4$ cfu mL⁻¹) (Fig. 3 and 4).

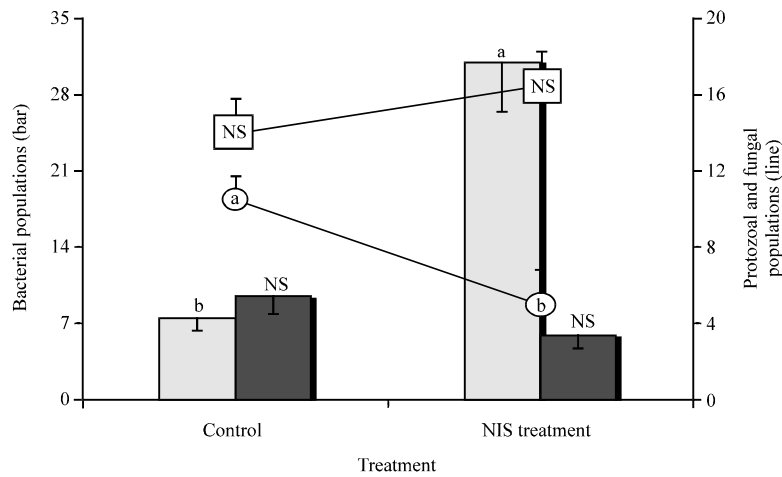


Fig. 3: Influence of administration of 200 mL of distilled water (control) or Non-Ionic Surfactant Solution (NIS treatment) on the total viable bacterial (□ bar; cfu mL⁻¹, ×10⁹), cellulolytic bacterial (■ bar; MPN mL⁻¹, ×10⁷), protozoal (○-○ line; cell mL⁻¹, ×10⁵) and fungal (■-■ line; tfu mL⁻¹, ×10⁴) counts per mL of the rumen fluids collected from cows (Zeng *et al.*, 2011)

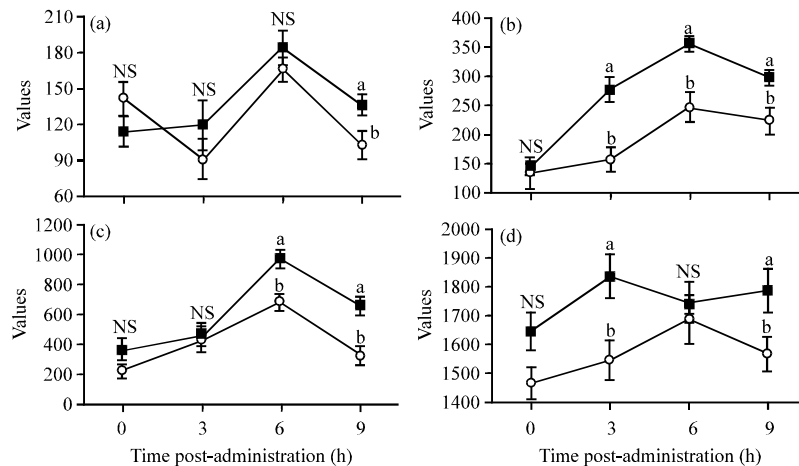


Fig. 4: The hydrolytic enzyme activities for a) protease; b) amylase; c) CMCase and d) xylanase in the rumen fluids collected from cows receiving daily intraruminal doses of 200 mL of distilled water (Control (○)) or Non-Ionic Surfactant solution (NIS treatment (■)); Zeng *et al.* (2011)

Table 3: Effect of levels of Non-Ionic Surfactant (NIS) on rumen bacterial population (Log copy number/g DM)

Items	<i>F. succinogenes</i>	<i>R. albus</i>	<i>R. flavefaciens</i>	References
Low forage¹				
None	0.006	0.004	0.003	Zeng <i>et al.</i> (2011)
APG	0.008	0.001	0.002*	
High forage¹				
None	0.011	0.005	0.011	Lee <i>et al.</i> (2007)
APG	0.013	0.002	0.003*	
Low forage²				
None	0.054	0.002	0.005	Hwang <i>et al.</i> (2008)
APG	0.055	0.001*	0.004	
High forage²				
None	0.046	0.003	0.013	Lee <i>et al.</i> (2007)
APG	0.061	0.001*	0.004	
Control	9.550 ^a	7.370 ^a	7.880 ^a	Hwang <i>et al.</i> (2008)
Tween 80	9.080 ^b	6.790 ^b	7.340 ^b	
Control	9.900	9.030	8.960	Hwang <i>et al.</i> (2008)
TE	9.800	8.650	8.990	

*Mean values with different superscripts in the same row differ significantly ($p \leq 0.05$); APG: Alkyl Polyglycoside; ¹Bacteria associated with rumen fluid; ²Bacteria associated with ruminal particles; TE: NIS (Tween 80.2%)+Fibrolytic enzyme preparation (2%)

Lee *et al.* (2007) reported that Tween 80 decreases adhesion of all three major fibrolytic bacteria. As shown in Table 3, In most studies been assumed that NIS (Tween 80 treatment) would increase cellulolytic bacterial bacteria adhesion which would be one possible cause of improved DM digestibility, VFA production, enzyme activity (Wang *et al.*, 2003; Zeng *et al.*, 2011; Lee *et al.*, 2007; Hwang *et al.*, 2008).

CONCLUSION

The results from this review papers indicated that the rumen content and ruminal bacteria but had minimal effects on ruminal pH value, ruminal NH₃-N concentration. APG probably affected rumen fermentation through reducing the ruminal pH. More studies might be needed to understand the effects of APG on rumen bacterial strains and performance of ruminants. Administration of non-ionic surfactants significantly improved the rumen fermentation characteristics (e.g., ruminal pH, ammonia-N, VFA), the ruminal microbial populations (e.g., total viable bacteria and rumen anaerobic fungi) and the hydrolytic enzyme activities in rumen fluids. These results are the effect of non-ionic surfactants obtained easily and cheaply from commercial market. Such a development is particularly significant because this study has demonstrated that administration of non-ionic surfactants into the rumen can improve nutrient utilization in ruminants. In conclusion, the NIS has a potential for industrial application as a feed additives to improve the digestion of plant material.

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