Effects of Non-ionic Surfactants on Enzyme Distributions of Rumen Contents, Anaerobic Growth of Rumen Microbes, Rumen Fermentation Characteristics and Performances of Lactating Cows**

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ABSTRACT : A series of experiments was carried out to determine the possibility for the non-ionic surfactant (NIS) as a feed additive for ruminant animals. The effect of the NIS on ① the enzyme distribution in the rumen fluids of Hereford bulls, ② the growth of pure culture of rumen bacteria and ③ rumen anaerobic fungi, ④ the ruminal fermentation characteristics of Korean native cattle (Hanwoo), and ⑤ the performances of Holstein dairy cows were investigated. When NIS was added to rumen fluid at the level of 0.05 and 0.1% (v/v), the total and specific activities of cell-free enzymes were significantly ($p<0.01$) increased, but those of cell-bound enzymes were slightly decreased, but not statistically significant. The growth rates of ruminal noncellulolytic species (*Ruminobacter amylophilus*, *Megasphaera elsdenii*, *Prevotella ruminicola* and *Selenomonas ruminantium*) were significantly ($p<0.01$) increased by the addition of NIS at both concentrations tested. However, the growth rate of ruminal cellulolytic bacteria (*Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefaciens* and *Butyrivibrio fibrisolvens*) were slightly increased or not affected by the NIS. In general, NIS appears to effect Gram-negative bacteria more than Gram-positive bacteria; and non-cellulolytic bacteria more than cellulolytic bacteria. The growth rates of ruminal monocentric fungi (*Neocallimastix patriciarum* and *Piromyces communis*) and polycentric fungi (*Orpinomyces joyonii* and *Anaeromyces mucronatus*) were also significantly ($p<0.01$) increased by the addition of NIS at all concentrations tested. When NIS was administrated to the rumen of Hanwoo, Total VFA and ammonia-N concentrations, the microbial cell growth rate, CMCase and xylanase activities in the rumen increased with statistical difference ($p<0.01$), but NIS administration did not affect at the time of 0 and 9 h post-feeding. Addition of NIS to TMR resulted in increased TMR intake and increased milk production by Holstein cows and decreased body condition scores. The NEFA and corticoid concentrations in the blood were lowered by the addition of NIS. These results indicated that the addition of NIS may greatly stimulate the release of some kinds of enzymes from microbial cells, and stimulate the growth rates of a range of anaerobic ruminal microorganisms, and also stimulate the rumen fermentation characteristics and animal performances. Our data indicates potential uses of the NIS as a feed additive for ruminant animals. ***(Asian-Aust. J. Anim. Sci. 2003. Vol. 16, No. 1 : 104-115)***

Key Words : Non-ionic Surfactants, Enzyme Activity, Microbial Growth, Rumen Microorganism, Ruminal Fermentation, Lactation Performances, Hanwoo, Holstein

INTRODUCTION

Previous experiments on fungal cell permeability demonstrated that non-ionic surfactants (NIS, surface active agents) can stimulate the release of enzymes (Munn et al., 1983; Reese and Macguire, 1969). Experiments conducted on the celluase 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). Yazdi et al. (1990) have 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. The effect of surfactants have been attributed to at least three causes: i) action on the cell membrane causing increased permeability (Reese and Macguire, 1969), ii) promotion of the release of bound enzymes (Reese and Macguire, 1969), and iii) decrease in growth rate due to reduced oxygen supply (Hulme and Stranks, 1970). In preliminary experiments, we observed that the NIS dramatically increased the digestion rates of cereal grain and orchard grass hay, succinate dehydrogenase, lactate dehydrogenase, and polysaccharide-degrading enzyme activities in the culture medium grown on mixed rumen anaerobic microorganisms (unpublished data). These results indicated that NIS might be of use as an alternative feed additive to stimulate multiple enzyme activities in the rumen. In general, however, surfactants decrease the growth rate of aerobic microorganisms due to a reduced oxygen supply (Hulme and Stranks, 1970), but there is no such information available with anaerobes.

Therefore, a series of experiments was carried out to determine the possibility for the NIS as a feed additive for ruminant animals. The effect of the NIS on the enzyme distribution in the rumen fluids, the growth of pure culture

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of rumen anaerobic bacteria and fungi, the ruminal fermentation characteristics, and the performances of Holstein dairy cows were investigated.

EFFECTS OF NIS ON THE ENZYME DISTRIBUTIONS

To study the effect of NIS on the ruminal enzyme distribution, the rumen contents were obtained from ruminally fistulated Hereford bulls that were fed twice a day (06:00 and 16:00 h) a ration consisting of 100% alfalfa hay and trace minerals and vitamins. Ruminal contents were collected from the bottom of the rumen 4 h after the morning feeding, squeezed through four layers of cheese cloth and poured into a separating funnel that had been gassed with oxygen-free CO_2. The filtrate was anaerobically incubated at 39°C for up to 60 min to allow feed particles to buoy up. All feed particles that had risen to the surface were removed by aspiration, and the liquid portion was anaerobically filtered using nylon cloth (25 μm pore size) to eliminate any remaining small feed particles. The filtered liquid was used as rumen mixed microorganism.

To check the effect of the NIS on the enzyme distribution, Tween 80 [polyoxyethylene (20) sorbitan monooleic acid, obtained from Sigma] was added to a final concentration of 0.00, 0.05 and 0.10% (v/v) to 500 mL of mixed rumen microorganisms. The sample was incubated for 3 h (without shaking) in a 39°C incubator, and then centrifuged (10,000×g for 15 min at 4°C). The supernatant was retained and used as cell free enzyme solution. The microbial pellet was resuspended in the same volume (final volume 500 mL) of sodium phosphate buffer, sonicated by ultrasonication for 2 min (maximum output) with 3 second intervals on ice using a Vibra Cell™ sonicator under anaerobic condition to disrupt microbial cells. The sample was then centrifuged (10,000×g for 15 min) and the supernatant containing the released soluble protein was used as the cell bound enzyme solution. Cellulase, xylanase, pectinase, amylase and barley glucanase were assayed with substrates of carboxymethyl cellulose (CMC), oat spelts xylan, pectin, starch and barley glucan, respectively. The reducing sugars, which had been released into the supernatants, were assayed colorimetrically (Miller, 1959). One unit of enzyme activity was defined as the amount of enzyme that produced 1 μmol of glucose or xylose equivalent of reducing sugar per minute. Protease was assayed using azocasein. Total protein concentrations were determined using the Bio-Rad (Bio-Rad Laboratories, Richmond, California, USA) protein reagent with bovine gamma-globulin as a standard.

The effects of NIS on the production of individual enzyme activities in the supernatant (cell-free enzyme or extracellular enzyme) and microbial cell-bound (cell-associated enzyme and intercellular enzyme) fractions of the rumen contents are shown in Table 1.

When NIS was added to rumen fluid, after a 3 h incubation, the total enzyme production of cell-free cellulase (CMCase) was significantly ($p<0.01$) increased, but that of cell-bound cellulase was significantly ($p<0.01$) decreased at all concentrations tested. The total xylanase activity was found to follow the same pattern as the CMCase activity. The cell free enzyme activities of protease, amylase and barley glucanase were also greatly increased, the best being a 223% (502 vs 1,115 IU) increase in barley glucanase with 0.05% NIS, and a 221% (463 vs 1,024 IU) increase in xylanase with 0.1% NIS, but the presence of pectinase in the fractions was not affected by the addition of NIS. The protein contents of cell-bound and cell-free fractions were 1,330.97 and 1,812.93, 972.27 and 2,069.33, and 1,816.53 and 2,522.17 μg·mL of crude enzyme solutions for 0.00%, 0.05% and 0.10% NIS, respectively. Protein contents of cell-free fraction were also significantly ($p<0.01$) increased by the addition of NIS, however, the of cell-bound fraction were significantly decreased by the addition of NIS when added at a concentration of 0.05%, but not at 0.10%. However, specific enzyme activities in the cell-bound fraction were increased when 0.05% NIS was added, but decreased when NIS was present at a 0.10%. Specific enzyme activities of xylanase, protease, amylase and glucanase in the cell-free fraction were significantly ($p<0.01$) increased when NIS was added at both concentrations.

Although NIS is well known to be the most effective surfactant in stimulating the release of enzymes from the cellulase complexes of a range of aerobic fungi (Deshpande et al., 1987; Wittenberger et al., 1987), its effects on anaerobic rumen microorganisms have not previously been reported. Our results indicated that the NIS stimulated release of enzymes from mixed anaerobic rumen microorganisms. A similar trend has been previously observed for aerobic microorganisms. In our results, NIS not only dramatically stimulated individual cell-degrading and other enzyme activities but also dramatically promoted the release of microbial cell-bound enzymes into the ruminal fluids and/or digesta. Yazdi et al. (1990) 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 an alteration of membrane fluidity due to an increase in the proportion of unsaturated lipids. Although pectinase secretion was not stimulated by NIS in anaerobic mixed rumen

Table 1. Effect of NIS(non-ionic surfactants) on the enzyme activities located in supernatant and cell-bound fractions of rumen fluids

Enzymes	Enzyme activity distributed in[a] 0.00 % NIS		0.05 % NIS		0.10 % NIS	
	cell-bound	cell-free	cell-bound	cell-free	cell-bound	cell-free
Total enzyme activity (IU)[b]						
CMCellulase	846.61±22.22^{a*}	352.69±7.64^{e}	626.18±15.19^{c}	456.76±3.65^{d}	744.70±32.63^{b}	473.54±18.67^{d}
Xylanase	1691.88±49.91^{b}	463.42±18.24^{e}	1427.47±35.05^{c}	874.72±24.88^{d}	1924.39±40.21^{a}	1023.61±57.99^{d}
Pectinase	460.36±12.07^{a}	586.96±4.31^{a}	531.14±49.65^{a}	607.24±52.76^{a}	565.29±61.69^{a}	471.10±164.48^{a}
Protease	45.49±4.74^{cd}	90.69±1.97^{b}	40.09±1.59^{d}	141.85±10.81^{a}	64.38±2.24^{c}	147.72±3.28^{a}
Amylase	114.43±4.96^{d}	377.63±15.46^{c}	52.32±8.24^{d}	584.14±32.51^{b}	67.57±1.35^{d}	706.14±22.90^{a}
Barley glucanase	1279.53±501.73^{a}	501.73±7.59^{c}	1124.81±45.02^{b}	1115.34±38.75^{b}	1211.15±56.09^{ab}	1101.47±14.61^{b}
Specific enzyme activity (IU·mg protein^{-1})[c]						
CMCellulase	636.04±15.99^{a}	194.59±4.29^{c}	643.92±9.70^{a}	220.88±4.61^{c}	410.67±5.71^{b}	187.59±5.35^{c}
Xylanase	1271.70±42.69^{b}	255.71±10.31^{e}	1470.23±51.95^{a}	423.31±16.67^{d}	1070.20±70.21^{c}	405.50±20.66^{ed}
Pectinase	345.95±9.77^{b}	323.86±3.26^{b}	548.79±59.73^{a}	294.47±28.53^{b}	320.08±54.09^{b}	186.13±64.74^{b}
Protease[d]	34.16±3.48^{d}	50.08±1.69^{bc}	41.23±1.43^{dc}	68.72±5.92^{a}	35.86±2.81^{d}	58.55±0.65^{ab}
Amylase	86.01±3.97^{c}	208.67±10.78^{b}	53.80±8.36^{cd}	282.87±18.30^{a}	37.61±2.66^{d}	280.04±9.25^{a}
Barley glucanase	961.05±24.39^{b}	276.89±5.64^{e}	1158.83±56.65^{a}	539.59±23.08^{dc}	675.01±59.38^{c}	437.08±10.67^{d}

[a] After a 3 h incubation with NIS (0.00, 0.05 and 0.10%), rumenal fluid contents were centrifuged and the supernatant was assayed (cell-free), rumen microbial cell fraction was separated by centrifugation, suspended in an equal volume of buffer, sonicated, centrifuged, and the supernatant was assayed (cell-bound).

[b] Enzyme activities (IU) are expressed as μmol reducing sugars released by 1 mL of crude enzymes in min, except protease activity.

[c] IU·mg protein^{-1}, specific activities (μmol reducing sugars released mg^{-1}·protein min^{-1}).

[d] Protease activity are expressed as μg azocasein hydrolyzed h^{-1}·mL of crude enzymes^{-1}.

* Each value represents Mean±standard error. When present in the same row, Means with different superscript letters are significantly different ($p<0.01$).

microorganisms as we reported herein, it is clear that the levels of cellulase, xylanase, protease, amylase and glucanase levels in the cell free fraction were significantly increased by this surfactant. The effects of NIS such as Tween 80 may be due to an increase in the permeability of the anaerobic microbial cell membrane, thus permitting more of the enzymes to be released, as postulated for aerobic fungal species (Reese and Maguire, 1969; Yazdi et al., 1990).

Our results indicated that cellulase, xylanase and glucanase are mainly cell-bound type, whereas protease and amylase distributed in rumen fluids (cell-free). Extracellular (cell-free) enzymes are more important than intercellular (cell-bound) enzymes in rumen forage digestion, in terms of microbial mass as well as enzymatic activity (Weimer et al., 1990). However, we observed that cell-free enzymes which are the key enzyme for degradation of forages (i.e. cellulase and xylanase) were much lower than cell-bound enzyme in the rumen contents. Thus, to manipulate ruminal forage fermentation, it is essential to know how to release enzymes associated to microbial cell wall (cell-bound) toward rumen fluid (cell-free). To increase them, NIS might be of use as a novel potential tool for this purpose based on our results.

EFFECTS OF NIS ON THE GROWTH OF RUMEN BACTERIA

To study the effects of NIS on the growth rate of pure strains of rumen bacteria, cellulolytics including *Fibrobacter succinogenes* strain S85, *Ruminococcus albus* strain 8, *R. flavefaciens* strain FD1 and *Butyrivibrio fibrisolvens* strain A46, and noncellulolytics including *Ruminobacter amylophilus* strain H-18, *Megasphaera elsdenii* strain B159, *Prevotella ruminicola* strain 23 and *Selenomonas ruminantium* strain S23 were used. All strains were obtained from the Lethbridge Research Centre Culture Collection. All measurements reported herein are averages of five replicates. The anaerobic technique of Bryant and Burkey (1953) was used throughout the experiment. All of the bacteria were grown in Hungate tubes containing a 10mL of modified Dehority's medium {Scott and Deholity (1965) modified by Weimer et al. (1990)} without resazurine, but with glucose, cellobiose and starch as carbon sources. NIS was added to a final concentration of 0.00, 0.05 and 0.10% (v/v) to the tube. Incubations were performed anaerobically in batch culture at 39°C without shaking for 4, 8, 12, 16 and 20 h. Growth was determined turbidimetrically by measuring the absorbance at 650 nm in a spectrophotometer, after washing the cell pellets three times with 100 mM sodium phosphate buffer (pH 6.5).

The growth rates of four pure strains of non-cellulolytic rumen bacteria at different treatment times are shown in Figure 1. In general, the growth rate was significantly increased by NIS at all concentrations tested, however, 0.05% NIS was most effective in stimulating the growth rate. *R. amylophilus* is thought to be the predominant starch digester although it is not always detectable in the rumen contents, and *M. elsdenii* is a known lipid utilizing species which is believed to play a major role in producing branched-chain volatile fatty acids in the rumen (Allison, 1978). The growth rate of *R. amylophilus* was significantly ($p<0.01$) increased by NIS after 12 and 16 h incubations whereas the growth rates of *M. elsdenii*, *P. ruminicola* and *S. ruminantium* were dramatically increased with the statistical difference ($p<0.01$) by the addition of NIS at all concentrations and incubation times tested.

P. ruminicola is found in the highest numbers in the rumen of animals fed virtually all diets (Russell and Wison, 1988) and it is presumed to play a role in proteolysis (Wallace and Brammall, 1985), and in the uptake and fermentation of peptides (Munn et al., 1983). The growth rate of this organism was also strongly increased by the addition of NIS at the both concentration at all incubation times tested.

S. ruminantium constitutes 22-51% of the total viable count of rumen bacteria isolated from animals fed cereal grains (Caldwell and Bryant, 1966). The growth rate of this organism was only very slightly increased compared to the other non-cellulolytics tested by the addition of NIS, at both concentrations tested, although the increase was statistically significant.

The growth rates of four pure strains of cellulolytic bacteria are shown in Figure 2. The rumen is a complex microbial ecosystem, but few ruminal bacteria are cellulolytics. Early work by Hungate (1950) demonstrated that *F. succinogenes*, *R. albs* and *flavefaciens* were the predominant cellulolytic bacteria in the rumen. Some strains of *B. fibrisolvens* are also considered cellulolytic, but their capacity to digest cellulose is limited (Halliwell and Bryant, 1963). When measured after 8 h of incubation, NIS added to the growth medium at a concentration 0.05% stimulated the growth rate of *F. succinogenes*, which is considered to be the most effective of the rumen bacteria in utilizing cellulose from plant tissues (Miller, 1959). Addition of 0.10% NIS stimulated the growth of *F. succinogenes* to a greater extent than did 0.05%, although there was no significant difference between these two conditions after 8 and 12 h incubations. In general, the growth rate of the cellulolytics tested was not greatly influenced by the addition of NIS, in contrast to what was observed for the non-cellulolytic bacteria.

Together with *F. succinogenes*, *R. albus* and *flavefaciens*

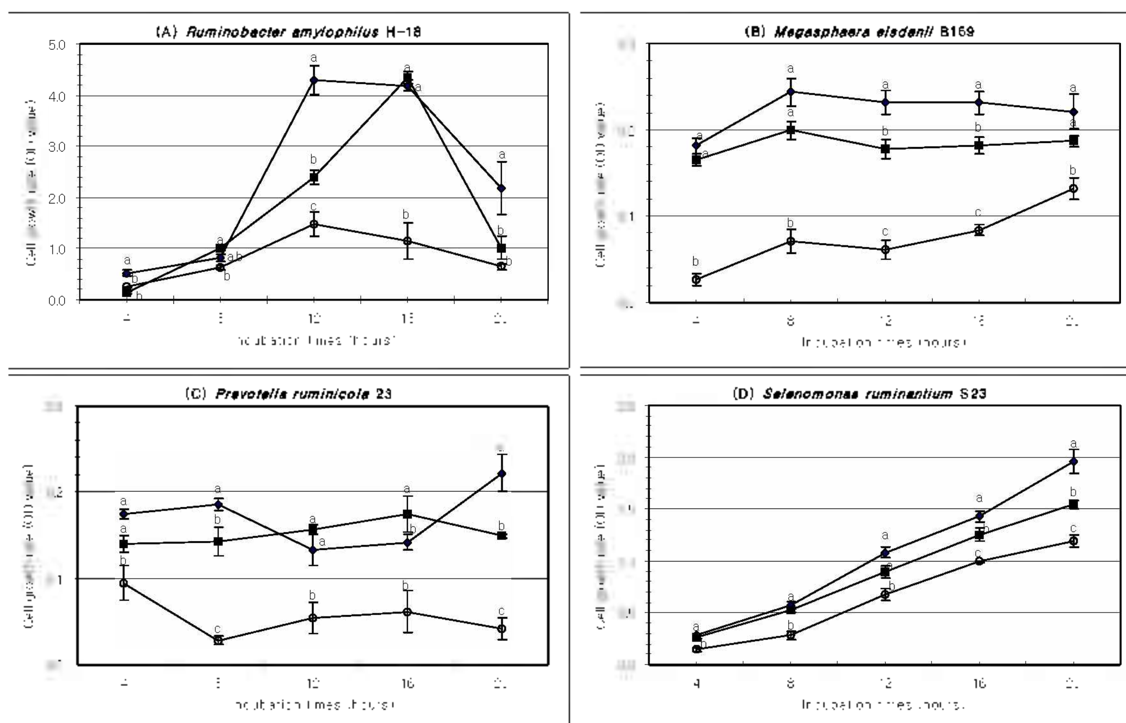

Figure 1. The influence of non-ionic surfactants (NIS) on the growth rates of pure strains of non-cellulolytic rumen anaerobic bacteria. NIS was added to a final concentration of 0.00% (○-○), 0.05% (●-●) and 0.10% (■-■). Bars represent standard errors, where no bars are shown the error was smaller than the symbol. The letters at the tops of the bars indicate statistical significance; means with different letters are significantly different ($p<0.01$).

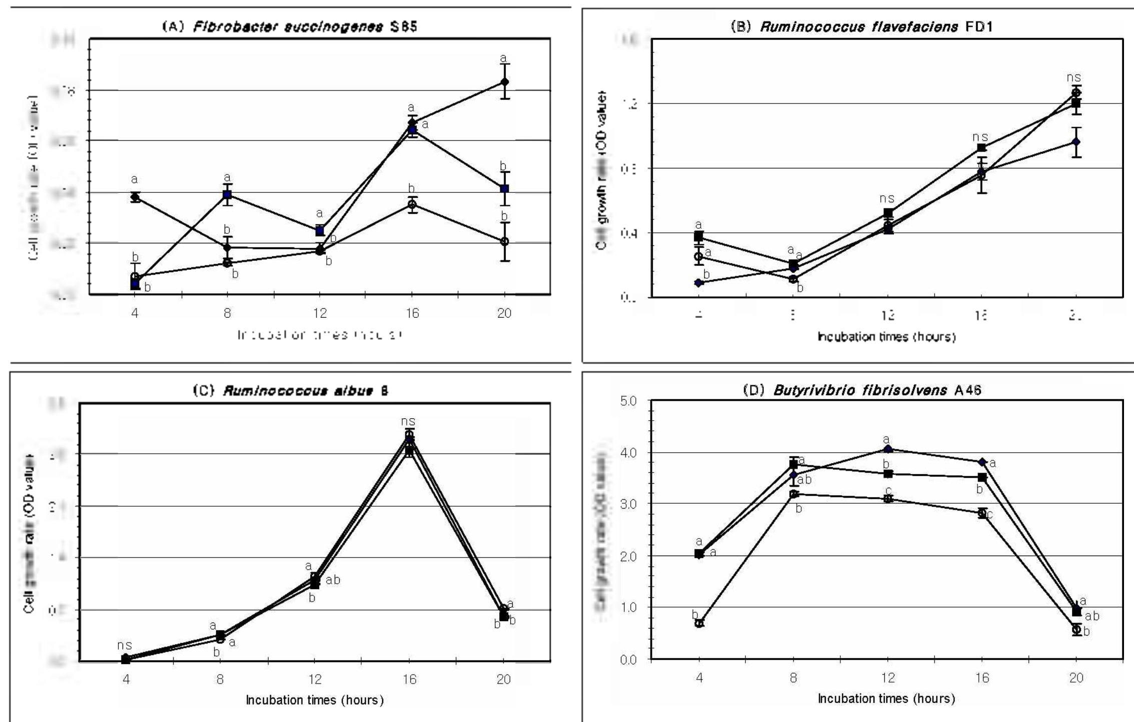

Figure 2. The influence of non-ionic surfactants(NIS)on the growth rates of pure strains of cellulolytic rumen anaerobic bacteria. NIS was added to a final concentration of 0.00% (○-○), 0.05% (●-●) and 0.10% (■-■). Bars represent standard errors, where no bars are shown the error was smaller than the symbol. The letters at the tops of the bars indicate statistical significance: means with different letters are significantly different (p<0.01).

are the major species involved in cellulose degradation in the rumen, however. *R. albus* and *flavefaciens* were not affected by the addition of NIS. The growth rate of *R. albus* increased linearly up to 16 h and then quickly decreased, however, the growth rate of *R. flavefacience* increased linearly up to 30 h and subsequently decreased. In all 8 strains of bacteria tested. only *Ruminococcus* genus (*R. flavefaciens* and. *albus*) whithout NIS effect, was positive organism, the others. 6 pure species of bacteria were negative organisms in Gram reaction. *B. fibrisolvens* is also considered to play a minor role in cellulose degradation in the rumen (Heinrichova et al., 1985). The growth rate of *B. fibrisolvens* increased rapidly up to 8 h, remained constant for more than 8 h (stationary phase). and then rapidly decreased beyond the 16 h incubation time point. In general. NIS appears to effect Gram-negative bacteria more than Gram-positive bacteria: and non-cellulolytic bacteria more than cellulolytic bacteria.

In general. the addition of the surfactant NIS caused a decrease in aerobic microbial growth rate due to a reduced oxygen supply (Hulme and Stranks. 1970). If the effect (decrease of cell growth rate) is detected in anaerobic rumen microorganisms as with aerobic microorganisms, it would be impossible to apply NIS as a feed additive for ruminant animals. However, the addition of surfactant NIS at the levels of 0.05 and 0.10% did not have any negative effect on the microbial cell growth rate based on our results. The growth rates of all strains of rumen anaerobic bacteria tested except *Ruminococcus* genus were dramatically increased by the addition of NIS.

Our results indicated that the addition of 0.05% NIS could greatly stimulate the release of some of enzymes without decreasing cell growth rate in contrast to trends reported with aerobic microorganism. These experiments also suggested that the influence of NIS on some bacteria may be genus specific. In general. NIS appears to have a greater effect on Gram-negative than Gram-positive bacteria, and non-cellulolytic bacteria than cellulolytic bacteria. It is interesting that NIS has only negligible effect on the growth of *Ruminococcus* species (*albus* and *flavefaciens*) which belong to Gram-positive. whereas a dramatic increase in growth rates was observed in all the Gram-negative tested. This phenomenon can be explained by the fact that NIS is intimately linked to membrane lipid composition of Gram negative microbial cell. and the increased cell growth can be explained by effects on membrane fluidity caused by the increased unsaturation of the lipids (Yazdi et al., 1990). The insensivity of Gram-negative bacteria from the toxicity of fatty acids (Tween 80 used as NIS in this experiment is mainly composed of oleic acid) may be attributed to the prevention of fatty acid penetration by the lipopolysaccharide layer of their outer membrane.

EFFECTS OF NIS ON THE GROWTH OF RUMEN FUNGI

To study the effects of NIS on the growth rate of pure strains of rumen anaerobic fungi, monocentric rumen fungi including *Neocallimastix patriciarum* strain 27 and *Piromyces communis* strain 22, and polycentric rumen fungi including *Orpiomyces joyonii* strain 19-2 and *Anaeromyces mucronatus* strain 543 were used. All strains were also obtained from the Lethbridge Research Centre Culture Collection. All of the fungi were grown in Hungate tubes containing a 10 mL of semi-defined medium B (Lowe et al., 1987) without no nitrogen source, but with glucose, cellobiose and starch as carbon sources. Incubations were performed anaerobically in batch culture at 39°C without shaking for 1, 2, 3, 4 and 5 d. Growth rates were estimated by measuring the cell protein contents using the Bio-Rad method after sonicating the cells. After 3 and 5 day incubation, liquid cultures were harvested and the fungi were enumerated by the thallus-forming units (TFU) method (Theodorou et al., 1990), with five replicates per dilution. For the TFU assay, filter sterilized anaerobic solution of streptomycin sulfate, ampicillin (sodium salt) and chloramphenicol (sodium succinate salt) were added to attain a final concentration of 0.1 mg·ml^{-1} of each antibiotic.

Based on cell protein content, the growth rate of the monocentric rumen anaerobic fungi, *N. patriciarum* and *P. communis* was significantly ($p<0.01$) increased by the addition of 0.05% NIS. In contrast, the growth of the monocentric rumen fungi tested was suppressed by the addition of 0.10% NIS, although the inhibition was only statistically significant in the case of *P. communis* (Figure 3).

The growth rates of polycentric rumen anaerobic fungi, *O. joyonii* and *A. mucronatus* were significantly ($p<0.01$) increased by the addition of NIS at all concentrations. When present at 0.05%, NIS was found to stimulate the growth of the monocentric fungi, but growth was inhibited by 0.1% NIS. These results indicated that NIS had not only a nutritional effect on the growth of rumen anaerobic fungi, but also toxic effect on the growth when present at higher levels. When enumerated fungi using thallus-forming units by roll-tube method after 3 and 5 days incubation, we observed that the growth rate of fungi was also significantly increased by the addition of NIS (Figure 4), although the growth of *A. mucronatus* was suppressed by NIS after a 5 day incubation.

As observed with the pure strains of rumen bacteria,

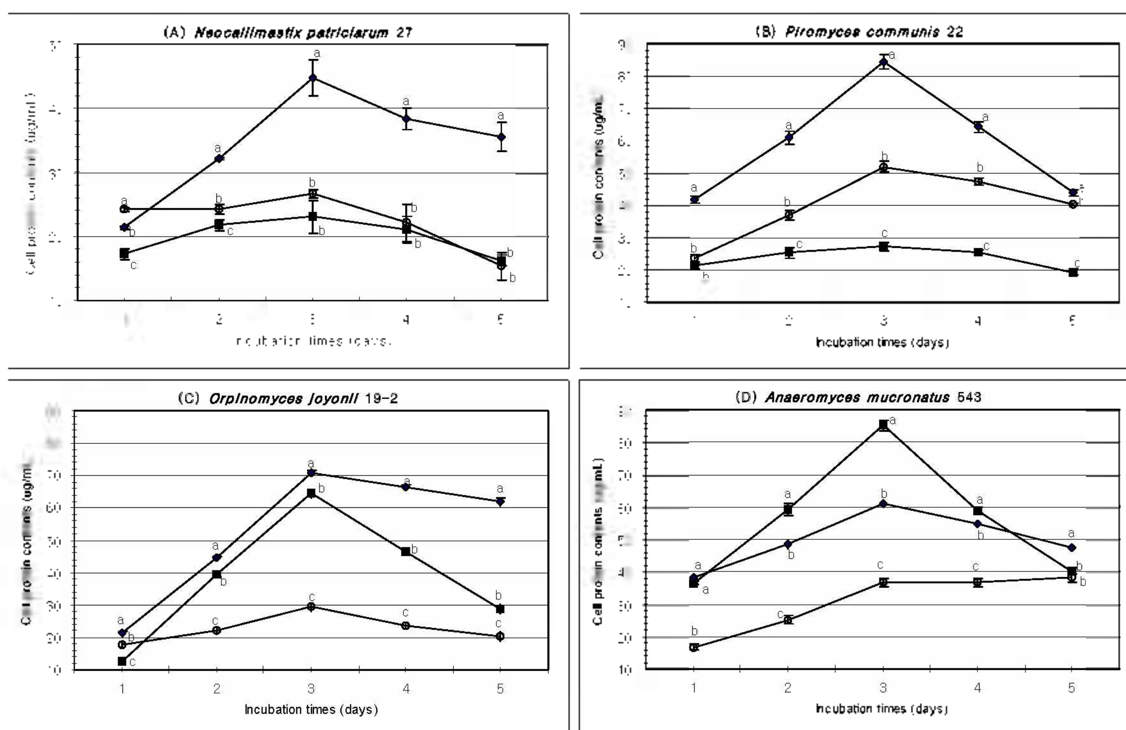

Figure. 3. The effects of non-ionic surfactants (NIS) on the growth rates (as fungal cell protein contents) of pure strains of mono- and poly-centric rumen anaerobic fungi. NIS was added to a final concentration of 0.00% (○-○), 0.05% (●-●) and 0.10% (■-■). Bars represent standard errors, where no bars are shown the error was smaller than the symbol. The letters at the tops of the bars indicate statistical significance, means with different letters are significantly different ($p<0.01$).

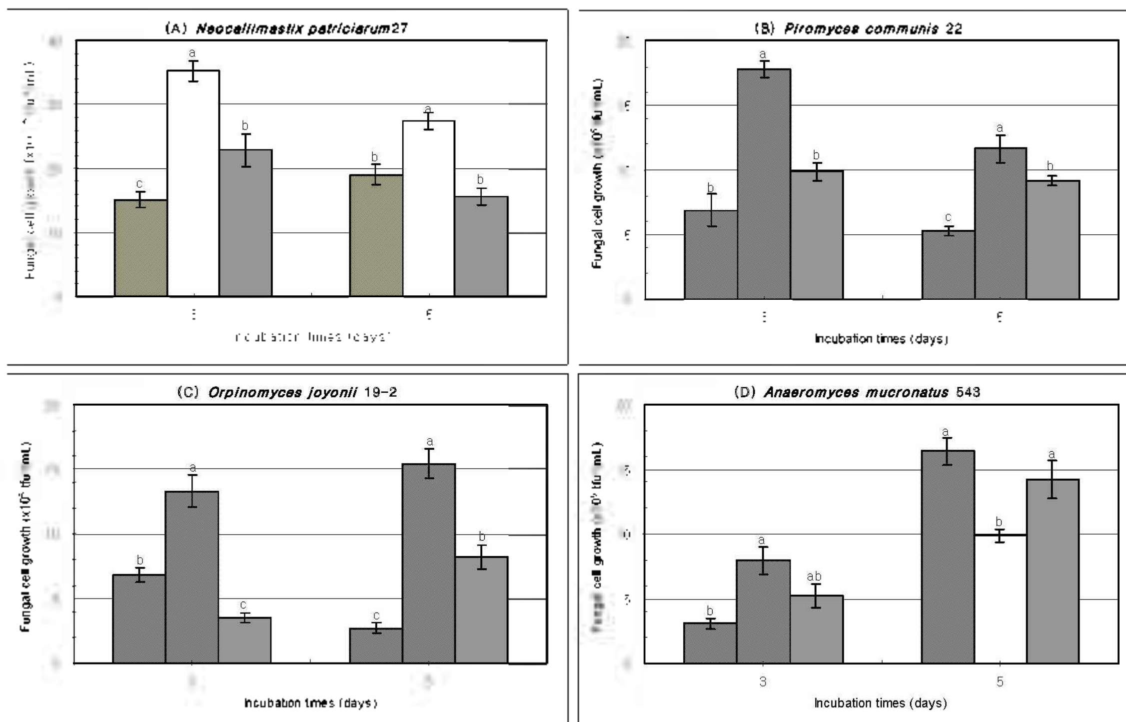

Figure 4. The effects of non-ionic surfactants(NIS) on the growth rates (as thallus forming unit per mL culture) of pure strains of mono- and poly- centric rumen anaerobic fungi. NIS was added to a final concentration of 0.00% (□), 0.05 % (□) and 0.10% (□). Bars represent standard errors. The letters at the tops of the bars indicate statistical significance: means with different letters are significantly different ($p<0.01$).

NIS had no effect on the anaerobic fungal growth, but significantly increased the fungal cell protein content and TFU. Inclusion of 0.05% NIS in the growth medium was stimulatory to all rumen fungi tested. Non-ionic surfactant has previously been reported to increase the yield of a number of extracellular enzymes but not intracellular enzymes such as glucosidase and xyloxidase. This surfactant appears to affect cell permeability of certain microorganisms (Paunescu et al., 1964) as it promotes both uptake and exit of compounds through modification of plasma membrane permeability (Reese and Maguire, 1969). Furthermore, NIS is known to have an inhibitory effect on the growth of aerobic microorganisms by reducing oxygen supply (Hulme and Stranks, 1970). However, we have found that the growth rates of anaerobic and aerobic rumen microorganisms were not inhibited by the presence of NIS. To our knowledge, this is the first study that rigorously examines the effects NIS on anaerobic microbial growth. These aspects should be considered in the commercial applications of this surfactant for use as in feed additives aimed at increasing the digestibility of feedstuffs.

EFFECTS OF NIS ON THE RUMINAL FERMENTATION CHARACTERISTICS

To study the effect of administration of NIS on ruminal fermentation, microbial cell growth rate and hydrolytic enzyme activities in the rumen of Korean native cows (Hanwoo). Eight mature Hanwoo were randomly assigned to two different treatments with 4 cows per treatment.

Control animals received 100 mL of distilled water through rumen cannulae at 08:00 and 16:00 each day. NIS treated animals were given 100 mL of NIS solutions (Mixed solutions composed of 70 mL of distilled water and 30 mL of NIS) as same manner of control treatment. All animals were exposed to each treatment for 10 days before rumen collection. Samples of ruminal contents were collected via the rumen cannulae at 0, 3, 6 and 9 h post morning feeding. Samples were blended for 1 min, then immediately strained through four layers of cheese cloth under the flushing of oxygen free CO_2 gas, and pH was measured. Strained rumen fluids were used for the analysis of ammonia-N and VFA, the determination of cellulase and xylanase activities, and for microbial cell growrh. Ammonia-N content was determined by the method of Chaney and Marbach (1962). The concentration of volatile fatty acid (VFA) was determined by gas liquid chromatography (Packard, 439-GLC) according to the method of Erwin et al. (1961). Activities of cellulase and xylanase were assayed as same manner for the previous study (Effects of NIS on the enzymes distributions).

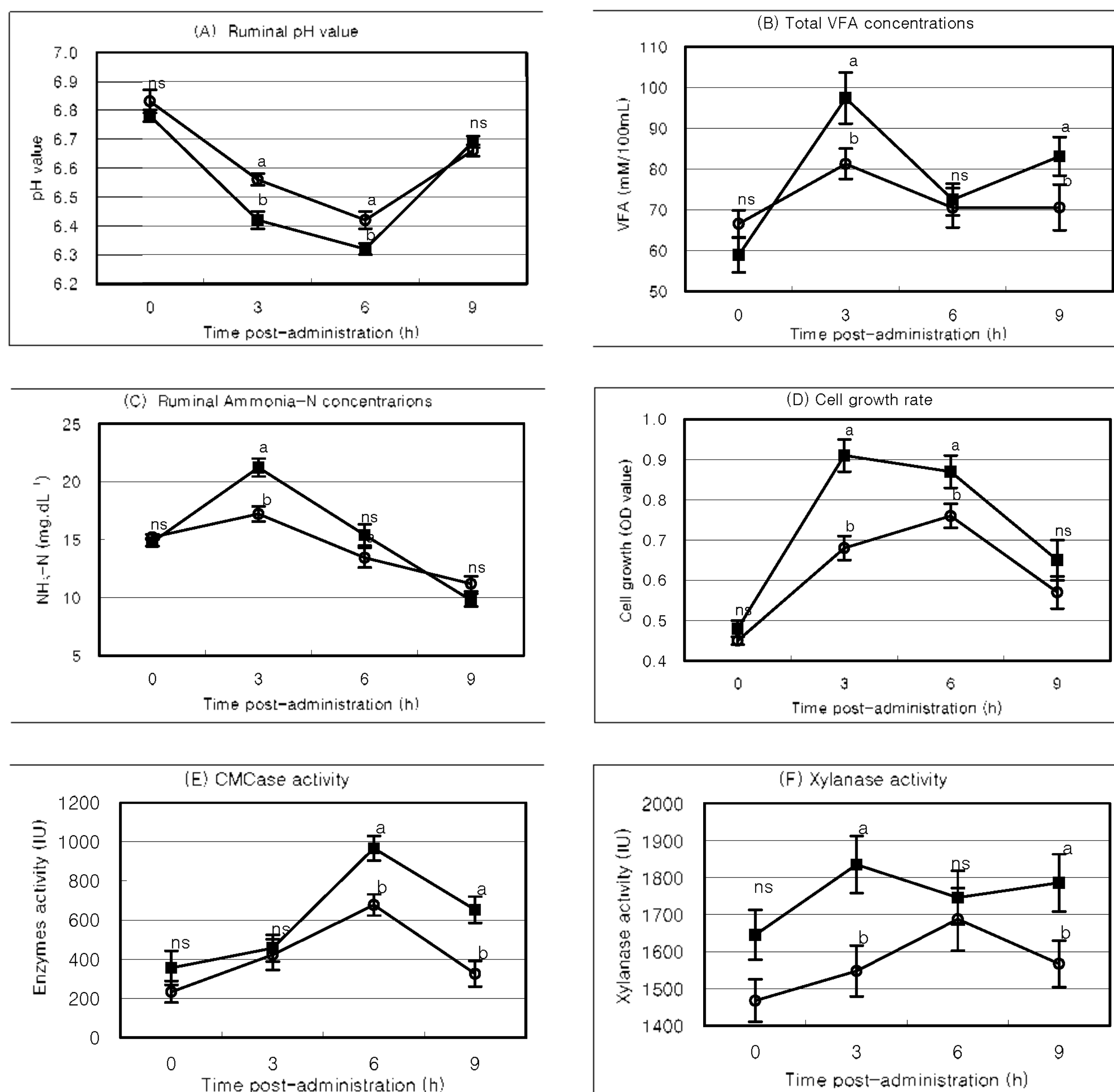

Figure 5. The effects of non-ionic surfactants(NIS) on ruminal fermentation characteristics of the Korean native cattle (Hanwoo). NIS was administrated to a final concentration of 0.05% for the rumenvolume (about 130 L) of Hanwoo through rumen cannulae (○-○, control ; ■-■, 0.05% NIS administration). Bars represent standard errors. The letters at the tops of the bars indicate statistical significance; means with different letters are significantly differrent ($p<0.01$).

Ruminal fermentation characteristics, cell growth rate and hydrolytic enzyme activities of the Hanwoo as influenced by the administration of NIS treatments are presented graphically in Figure 6. The NIS administrated Hanwoo had lower ruminal pH compared to those that were treated with distilled water (Control). Ruminal pH was significantly ($p<0.01$) affected by the NIS administration except at 9 h post-feeding and tended to increase as the post-feeding time increased. pH values (6.32±0.02~6.83±0.04) checked in the experiment were considered to be within the optimum range for both proteolysis and cellulolysis as reviewed by Tamminga (1979).

Ammonia-N concentration at 6 h post-feeding was 13.45 and 15.39 mg·dl^{-1} in rumen fluids of Hanwoo on the control and NIS treatment, respectively, indicating a 14.4% increase in NIS treatment. Regardless of treatment ruminal NH_3-N concentrations (ranging from 9.78±0.56 to 21.21±0.77 mg·L^{-1}) were above the level (5 mg·dL^{-1})

indicated by Satter and Slyter (1974) as being adequate for microbial growth and microbial protein synthesis. The total VFA concentration at 3 h post-feeding in the rumen of Hanwoo administrated NIS increased by 20% compared to control treatment. The elevated concentrations of total VFA by NIS treatment was primarily due to the increased concentrations of acetate and propionate. Appreciable amounts of isoacids were not detected. The acetic and propionic acids were higher in NIS treatment and the lower in control treatment (Data not shown).

When NIS was administrated to the rumen of Hanwoo, the microbial cell growth rate in the rumen increased 34% (from 0.68 to 0.91 OD value) with statistical difference ($p<0.01$), but NIS administration did not affect at the time of 0 and 9 h post-feeding.

CMCase and xylanase activities in the rumen of Hanwoo are also shown in the bottom plate of Figure 6. The higher xylanase activity ($p<0.01$) was found in the rumen of

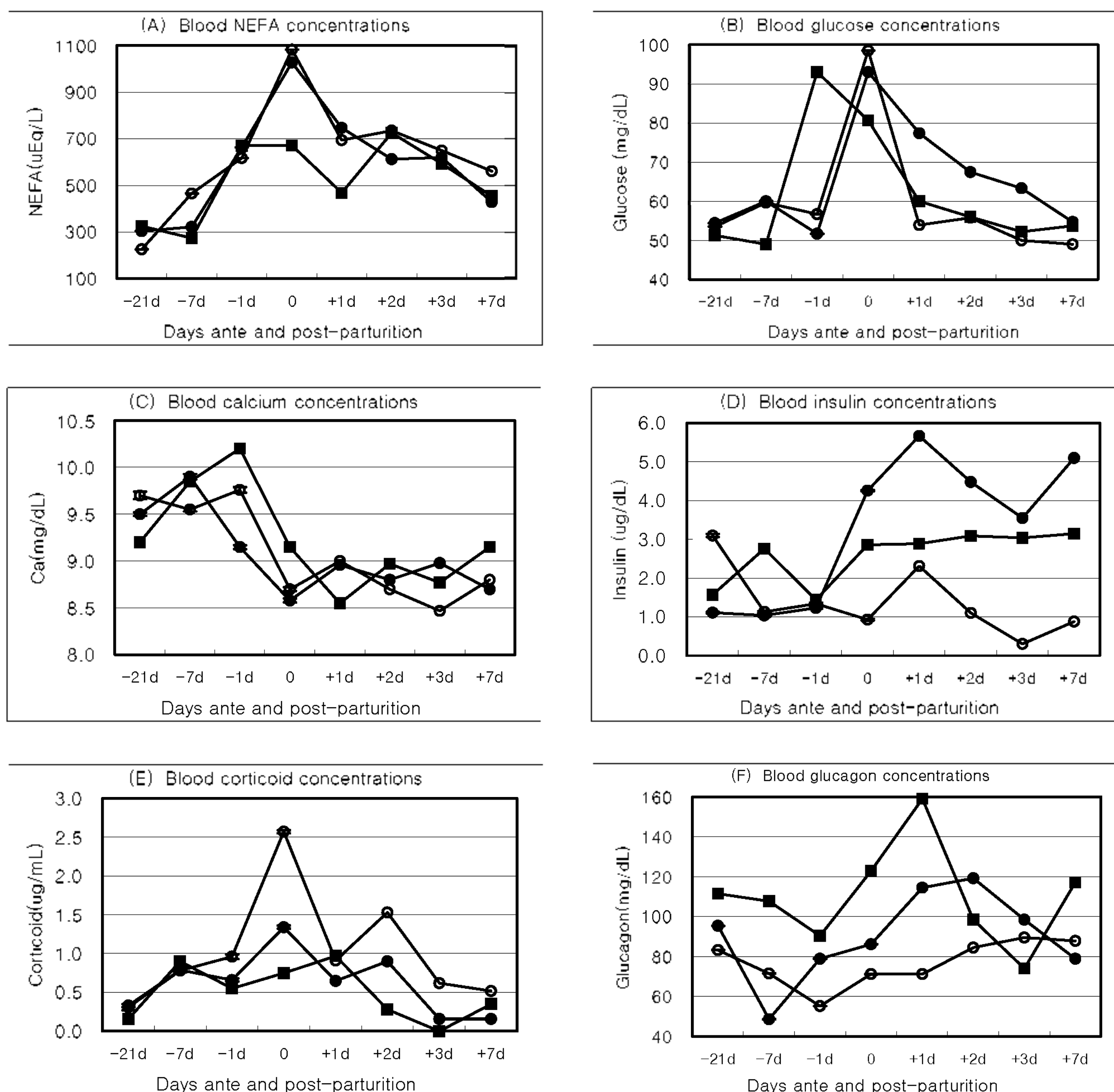

Figure. 6. The effects of non-ionic surfactants (NIS) and direct fed microbes (DFM) on the concentrations of blood metabolites in gestating and lactating Holstein cows fed NIS-(■-■) and DFM-(●-●) treated and untreated (○-○) total mixed rations (TMR). NIS and DFM were added rationally 50 mL and 20 g to a TMR, respectively. (day 0=date of calving).

Hanwoo administrated NIS, especially at 3 and 9 h post-feeding. A similar trend toward increased CMCase activity after the same treatment was also observed in rumen samples collected after 6 and 9 h post-feeding with more effects being obtained after NIS administration.

EFFECTS OF NIS ON THE PERFORMANCE OF LACTATING COWS

To study the effects of NIS on the feed intake, concentrations of blood metabolites and performance of lactating cows, 22 pregnant Holstein dairy cows were divided into 3 groups by parity and milk production from their previous lactation, lactation number, live weight and body condition score. Within each group, cows were randomly allocated to the control, DFM or NIS treated TMR (total mixed ration). Cows in each group were housed in a free-stall. The free stalls were bedded with sawdust, which were changed weekly. Cows were group-fed total mixed rations (TMR) containing DFM or NIS, or untreated TMR (as a control). Animals were fed once a day starting at 08:00 h, and the stalls and alleys were cleaned two times a day at 13:00 h and 19:00 h. Direct fed microbes (DFM) were purchased from Korean markets, They reported that the manufacturer composed of yeast cultures, *Clostridium butyricum* and some active enzyme (amylase, proteases, peroxidase, phosphatase). NIS and DFM were added rationally 50 mL and 20 g to a TMR, (about 1,200 mL and 500 g per ton of TMR, respectively) respectively. Dry matter intake was determined on a daily basis. Cows were milked twice daily at 08:00 and 15:30 h. Milk yields of individual cows were recorded daily. Milk fat, protein and lactose were determined using a Milko-Scan analyser (Foss Electric, Hillerod, Denmark). Blood samples were collected from all experimental animals just before cows were offered TMR. Blood samples from the coccygeal vessels were collected into two evacuated collection tubes containing potassium ethylene diamine tetra-acetic acid and placed on ice. Once collected, blood samples were centrifuged (15 min at 900×g at 4°C) and plasma was stored at -80°C pending analysis for glucose and non-esterfied fatty acid (NEFA). Insulin was determined in samples pooled across sampling times on an equivolume basis. Plasma glucose and NEFA concentrations were determined using a selective clinical chemistry analyser. Concentrations of glucose were measured as ionic hydrogen (test kit no. 981285; Kone Instruments Corporation) and NEFA as hydrogen peroxide (test kit no. 994-75409; Wako Chemicals, Ltd., Neuss, Germany). Plasma insulin concentrations were measured by radioimmunoassay according to the recommended procedures supplied by the kit manufacturer (test kit no. 10-9169-01, Pharmacia and Upjohn Diagnostics Ltd., Uppsala, Sweden). The body condition score of cows was determined (scale 1-5) according to the method of Wildman et al. (1982) at the start and end of the experimental period. Body condition scores were based on a five-point scale with 0.25-unit intervals, where 1, emaciated and 5, obese. Lameness scores were also based on a five-point scale, where 1, normal gait; 2, mild lameness; 3, moderate lameness; 4, lame and 5, severely lame.

The dry matter intake, milk yields and body codition score (BCS) for Holstein dairy cows are presented in Table 2. From the beginning to the end of the experiment, the DMI were higher in the DFM and NIS groups than in the control group; values fluctuated slightly over the time. As expected, Milk production of cows fed the DFM and NIS treated TMR was higher than that of cows receiving the control TMR, consistent with a higher intake of treated TMR. The BCS did not differ among the three groups at the starting spot of experimental periods and, however,

Table 2. Effect of non-ionic surfactants (NIS) and direct fed microbes (DFM) on dry matter intake, lactation performance and body condition scores of Holstein cows

Items	Treatments		
	Control	DFM	NIS
Animals (heads/treatment)	8	7	7
Dry matter intake (kg/head)			
1-21days before parturition	11.6	12.2	12.0
Parturition day (0 day)	9.2	10.1	9.9
1-21days after parturition	16.0	17.9	17.7
1-42days after parturition	18.5	19.6	19.6
Milk yields (kg/day)			
1-21days after parturition	28.6	29.2	30.8
1-42days after parturition	31.9	33.5	33.9
General	30.6 (100)	31.8 (104)	32.7 (107)
Milk fat (%)	3.86	4.01	4.02
4% Fat-corrected milk	29.6 (100)	31.8 (107)	32.8 (110)
Body condition score			
Start of the experimental period	3.4	3.5	3.4
End of the experimental period	3.8	2.8	2.9

decreased greatly 42 days after calving (Control: 3.8, DFM: 2.8, NIS: 2.9) by the addition of DFM or NIS to TMR .

Concentrations of blood metabolites in gestating and lactating Holstein cows are given in Figure 6. Blood NEFA(non-esterfied fatty acid) concentration decreased by the addition of NIS, the value quickly increased at the end of gestation and the value slightly decreased after calving; DFM treatment did not differ from control group. Blood glucose values were not also affected by the addition of DFM and NIS; values greatly increased at parturition day and decreased thereafter. Plasma insulin values fluctuated over the time of the experiment with a trend to increase during lactation and were greatly influenced by the addition of DFM and NIS.

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