ปัจจัยที่มีผลต่อจุลินทรีย์ที่ย่อยสลายเยื่อใยในกระเพาะรูเมนของสัตว์เคี้ยวเอื้อง

Factors That Alter Ruminal Cellulolytic Bacteria in Ruminants

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บทคัดย่อ

สัตว์เคี้ยวเอื้องสามารถใช้ประโยชน์จากอาหารเยื่อใยที่เป็นองค์ประกอบในพืชเพื่อเป็นแหล่งพลังงานได้ เนื่องจากการทำงานร่วมกันระหว่างจุลินทรีย์ที่อาศัยอยู่ในกระเพาะรูเมน (แบคทีเรีย, เชื้อรา และ โปรโตซัว) แบคทีเรียเป็นจุลินทรีย์ที่มีบทบาทสำคัญในการย่อยสลายเยื่อใย เนื่องจากมีประชากรและกิจกรรมการทำงานมากกว่าจุลินทรีย์ชนิดอื่น โดยแบคทีเรียที่ย่อยสลายเซลลูโลสเป็นจุลินทรีย์กลุ่มหลักที่ทำหน้าที่ย่อยสลายเยื่อใยในสัตว์เคี้ยวเอื้อง ได้แก่ *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefaciens* และ*Butyrivibrio fibrisolvens* กระบวนการหมักย่อยเยื่อใยในกระเพาะรูเมนทำให้ได้ผลผลิตสุดท้ายเป็นเซลล์จุลินทรีย์และกรดไขมันที่ระเหยได้ง่าย ซึ่งเป็นแหล่งโปรตีนและพลังงานที่สำคัญของสัตว์เคี้ยวเอื้อง โดยเฉพาะอย่างยิ่งในการให้ผลผลิตเนื้อและนม เนื่องจากประสิทธิภาพการย่อยสลายเยื่อใยในกระเพาะรูเมนมีความสำคัญอย่างยิ่งต่อประสิทธิภาพการผลิตสัตว์เคี้ยวเอื้อง ดังนั้น การศึกษาปัจจัยที่มีผลต่อการทำงานของจุลินทรีย์ที่ย่อยสลายเยื่อใยในกระเพาะรูเมนจึงมีความสำคัญ เพื่อเป็นแนวทางในการเพิ่มประสิทธิภาพการการใช้ประโยชน์ของเยื่อใยและประสิทธิภาพในการให้ผลผลิตของสัตว์เคี้ยวเอื้อง

คำสำคัญ: แบคทีเรียที่ย่อยสลายเยื่อใย, จุลินทรีย์ในกระเพาะรูเมน, สัตว์เคี้ยวเอื้อง

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Abstract

Ruminant animals are able to use plant fiber as an energy source because of a symbiotic relationship with microbes in the rumen (bacteria, fungi and protozoa). Rumen bacteria play a particularly important role in the biological degradation of plant fiber because of their much larger biomass and higher activity. Among rumen bacteria, cellulolytic bacteria mostly important in fiber digestion and utilization including *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefaciens* and *Butyrivibrio fibrisolvens*. The degradation and fermentation of fiber by cellulolytic bacteria contributes to microbial yield and volatile fatty acids which are an important source of protein and energy to the host animal, especially for meat and milk production. Efficiency of fiber digestion in ruminants is critically important to the productive efficiency of the ruminant animals. Therefore, the factors regulate ruminant fiber digestion by ruminal cellulolytic bacteria should be understood in detail to maximize the functions of fiber utilization and improve productive efficiency in ruminant animals.

Keywords: cellulolytic bacteria, rumen microorganism, ruminants

Introduction

Dietary fibers are major feed resource and main source of energy for ruminants particularly in tropical and sub-tropical areas. The major components of plant cell walls are cellulose, hemicellulose, lignin (Demeyer, 1981) and pectin is a minor component of grass cell walls (Gilbert and Hazlewood, 1991). Cellulose is a main component of the plant cell wall and the most common carbohydrate on earth, its indigestible to most animals but can be hydrolyzed and fermented by rumen microorganisms (Krause et al., 2003), which consist bacteria protozoa and fungi. This is made possible by the rumen microorganisms that synthesis and secrete enzyme complex, thereby allowing hydrolysis of plant cell walls (Varga and Kolver, 1997). Bacterial species of the rumen are considered more important than protozoa and fungi in determining the extent and rate of feed degradation and utilization for the production of microbial protein and volatile fatty acids (Stewart et al., 1997). Subsequently, the host ruminant animal absorbs volatile fatty acids (mostly through the rumen wall), which form a major metabolic fuel and microbial cells that are a major source of protein and amino acids when absorbed in the small intestine to supply its maintenance needs and for the production of meat and milk (Krause et al., 2003; Miron et al., 2001).

Rumen bacteria have been the subject of intensive studies over the past 50 years, and numerous studies have described the isolation and characterization of a variety of bacterial strains from various ruminant animals (Bryant, 1959; Stewart et al., 1997). Among major rumen bacteria,

Fibrobacter succinogenes, *Ruminococcus flavefaciens*, *Ruminococcus albus*, *Butyrivibrio fibrisolvens*, *Prevotella ruminicola*, *Eubacterium cellulosolvens* and *Eubacterium ruminantium* are recognized as fibrolytic bacterial species. *F. succinogenes*, *R. albus*, *R. flavefaciens* and *B. fibrisolvens* are much higher in cellulose digestion than those of other cellulolytic ruminal species; therefore, these have been considered representative cellulolytic bacterial species in the rumen (Stewart et al., 1997; Koike and Kobayashi, 2009). Cellulolytic bacteria play an importance role in the nutrition of ruminant animals fed diets based on forage fiber, especially in digestion and utilization of dietary fiber. Thus, this paper will focus on factors effecting cellulolytic bacteria on fiber digestion in ruminants.

Predominant ruminal cellulolytic bacteria

The major cellulolytic bacteria in the rumen are *Butyrivibrio fibrisolvens*, *Ruminococcus albus*, *Ruminococcus flavefaciens*, and *Fibrobacter succinogenes*. The first three species are gram-positive but *F. succinogenes* is gram-negative (Krause et al., 1999). *B. fibrisolvens* are a group of highly xylanolytic bacteria inhabiting the rumen (Krause et al., 2003), and also produces a cellulase, but it is probably more important in the hydrolysis of hemicellulose (Varga and Kolver, 1997). *F. succinogenes*, *R. albus* and *R. flavefaciens* are three of the most abundant cellulolytic bacteria in the rumen, due to the ability of these three species to digest cellulose is much higher than that of other cellulolytic ruminal species. Therefore, *F. succinogenes, R. flavefaciens* and *R. albus* have been considered representative cellulolytic bacterial species in the rumen. In addition, all three species are able to rapidly degrade crystalline cellulose, but these species differ in their substrate utilization and fermentation end products (Russell, 2002; Table 1).

Table 1. Characteristics of predominant ruminal bacteria.

Species	Products	Primary Niches
Fibrobacter succinogenes	S, F, A	CU
Ruminococcus albus	A, F, E	CU
Rminococcus flavefaciens	S, F, A	CU
Butyrivibrio fibrisolvens	B, F, L, A	CU, HCU, ST, PC, SU

A=acetate; B=butyrate; P=propionate; F=formate; L=lactate; E=ethanol; S=succinate; CU=cellulose; HCU=hemicellulose; ST=starch; SU=sugar; PU=pectin.

Source: Adapted from Russell (2002)

However, the numbers of cellulolytic bacteria are different in rumen content. Of the cellulolytic species, *F. succinogenes* was the most dominant both in whole rumen digesta and on hay stem suspended in the rumen (Koike et al., 2003). These results were in agreement with previous reported by Koike and Kobayashi (2001), where cell numbers of *F. succinogenes* in sheep rumen were greater than those of the two *Ruminococcus* species, irrespective of dietary conditions. According to Michalet-Doreau et al. (2001; 2002) demonstrated that *F. succinogenes*

had larger rumen populations than those of *R. flavefaciens* and *R. albus* in sheep on both a roughage diet and on a high grain diet. Furthermore, Wanapat and Chredthong (2008) also found that *F. succinogenes* were the highest in population in the rumen of swamp buffalo.

Factors effecting cellulolytic bacteria in the rumen

1. Dietary concentrate and roughage

Type of diet and roughage and concentrate (R:C) ratio has been affected on fiber digestion and numbers of fibrolytic bacteria in the rumen. Feeding higher level of concentrate with excessive amount of readily fermented carbohydrates should reduce ruminal pH and fiber digestion in the rumen. Because structural carbohydrate-fermenting microbes are usually limited and unable to survive at ruminal pH less than 6.0 (Hoover, 1986). Poncet et al. (1995) reported that fiber digestibility were decreased average 8.7% with increased starch supplementation from 30-60%. In addition, Khampa and Wanapat (2006) found that cellulolytic bacteria were dramatically decreased in cattle receiving increased levels of concentrate based on high level of cassava chip (80%) in diets. Moreover, Wanapat et al. (2008) studied in Thai swamp buffalo using two source of energy in concentrate (cassava chip and corn cobs) and two ratio of roughage and concentrate (80:20 and 50:50) on rumen ecology using real-time PCR technique. This study found that cellolytic bacteria population were significant ($P<0.05$) higher when buffalos fed with con cobs than cassava chip as an energy source at R:C ratio at 50:50. In addition, population of predominant cellulolytic species were *F. succinogenes and R. flavefaciens* tented to be higher than *Ruminococcus albus* with corn cobs treatment, especially at R:C ratio at 80:20 as shown in Figure 1.

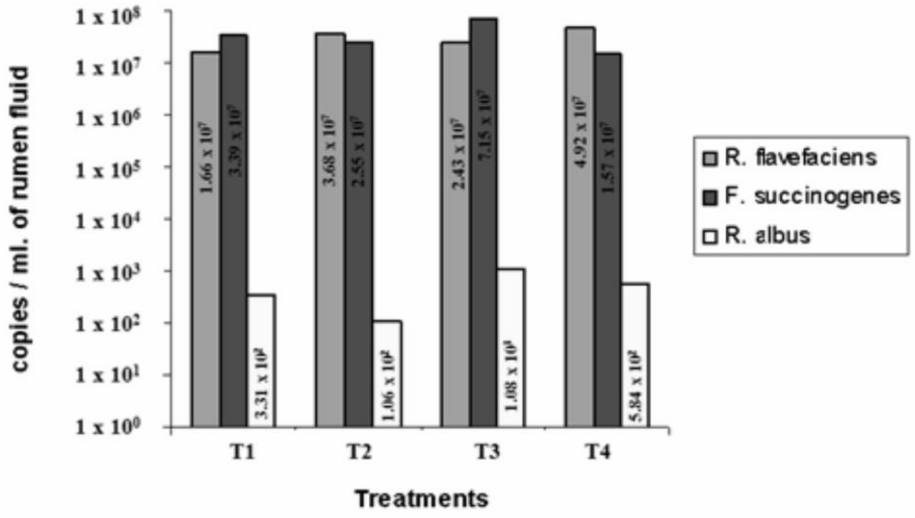

Figure 1. Effect of supplemented ration of cassava chip by corn cops on cellulolytic bacteria population using real-time PCR technique (T1=Cassava chip + R:C, 80:20; T2=Cassava chip + R:C, 50:50; T3=Corn cobs + R:C, 80:20; T4=Corn cobs + R:C, 50:50).

Source: Wanapat et al. (2008)

2. Chemical treatments

Varga and Kolver (1997) reported that chemical treatments such as sodium hydroxide, potassium hydroxide and ammonia will partially solubilize hemicellulose and lignin, as well as hydrolyze acetic, phenolic and uronic acid esters. Oxidative treatment of forage with sulfer dioxide or peroxide results in the degradation of lignin and extensive solubilization of structural carbohydrate. Chen et al. (2008) has been investigated the effects of treatment of rice straw (RS) with sodium hydroxide (SH) and ammonium bicarbonate (AB) on fermentation characteristics, fibrolytic enzyme activities and populations of liquid- and solid-associated ruminal microbes in vitro. It was found that microbial crude protein and fibrolytic enzyme activities increased with incubation time, and was higher for the treated straws than for the untreated ($P<0.05$). Both solid- and liquid-associated *R. flavefaciens* were higher in treated straws than in the untreated, with higher solid-associated *R. flavefaciens* in SH-RS than in AB-RS (Table 2).

Table 2. Effect of chemical treatment of rice straw on populations of liquid- and solid-associated microbes (% of total bacterial 16S rDNA) at 24 h of incubation *in vitro*.

Items	Rice straw treated with			S.E.M.
	None	Sodium hydroxide	Ammonium hydroxide	
Liquid-associated				
Fungi (x 10^{-5})	2.594 ^{b}	7.957 ^{a}	2.569 ^{b}	0.1925
R. flavefaciens (x 10^{-3})	0.679 ^{c}	2.606 ^{a}	0.315 ^{b}	0.0927
F. succinogenes	0.181 ^{c}	0.555 ^{a}	0.315 ^{b}	0.0145
Solid-associated				
Fungi (x 10^{-5})	0.232 ^{b}	1.345 ^{a}	0.300 ^{b}	0.0152
R. flavefaciens (x 10^{-3})	0.034 ^{b}	0.085 ^{a}	0.087 ^{a}	0.0016
F. succinogenes	5.912 ^{a}	1.126 ^{c}	5.089 ^{b}	0.1195

Means within a row with different letters (a, b, c) are significantly different ($P<0.05$).

Source: Chen et al. (2008)

3. Lipid supplementation

Dietary lipid supplements are known to cause extensive modification to digestion in the rumen. Reductions have been demonstrated in fiber and organic matter (OM) digestion. Ruminal digestion of structural carbohydrates can be reduced 50% or more by less than 10% added fat (Jenkins, 1993). Fat is usually limited to 5 to 6% of dietary DM (Pontoja et al., 1994). In addition, degree of saturation is a primary property of fats that influences whether they affect ruminal fermentation. Unsaturated fats are more inhibitory to ruminal fiber digestion than are saturated ones (Eastridge and Firkins, 1991). The mechanism of how lipids interfere with ruminal fermentation is a complex model involving partitioning of lipid into the microbial cell membrane, potency of the

lipid to disrupt membrane and cellular function, physical attachment of microbial cells to plant surfaces, and expression and activity of microbial hydrolytic enzymes. Thus, the coating theory attempts to explain reduced fermentation by a lipid layer over feed particles that inhibit digestion of cellulose. This lipid covering is proposed to cause detrimental effects by inhibiting close contact of microbial cells or their hydrolytic enzymes with feed particles. Close physical attachment of microbial matter to feed particles is necessary for cellulose digestion in the rumen (Jenkins, 1993).

4. Ruminal pH

Most ruminal bacteria prefer pH near neutrality for growth, although some species (e.g., *Streptococcus bovis* and *Prevotella ruminicola*) can grow in the pH 5 to 6 range. The predominant ruminal cellulytic bacteria are particularly sensitive to low pH. None of the three predominant cellulolytic species (*F. succinogenes, R. flavefaciens* and *R. albus*) grow at pH <6.0 (Weimer, 1993). Russell and Wilson (1996) reported that the *Ruminococci* and *F. succinogenes* could not tolerate pH 5.9, while *B. fibrisolvens* was somewhat more resistant, but even this species washed out when the pH was decreased to 5.7. Ruminal pH is one of the most important factors governing bacterial attachment to feed particle, because fibrolytic bacteria are very sensitive dependant on pH change (Sung et al., 2007). In pure culture studies, the number of adhesion cells of *F. succinogenes* to cellulose decreased when pH was reduced from 6.0 to 4.5 with the numbers being maintained between pH 6.0 to 7.0, and falling abruptly above pH 7.5 (Roger et al., 1990). According to Sung et al. (2007) found that when ruminal pH was adjusted to 5.7, 6.2 and 6.7 and three major fibrolytic bacteria attached to rice straw in mixed culture were quantified with real-time PCR. The number of *R. flavefaciens*, *F. succinogene,* and *R. albus* with pH 5.7 was significant ($P<0.05$) lower than with pH 6.7 and 6.2. Moreover, average increase in DM digestion at pH 6.7 and 6.2 than those at pH 5.7 as shown in Figure 2. In similar studies, Grant and Weidner (1992) reported that the lag in NDF digestion increased as pH fell 6.8 to 5.5 an decreasing pH of buffer <6.0 dramatically decreased NDF digestion rate for alfalfa hay and corn silage.

5. Interaction of microorganisms in the rumen

Digestion of feed in the rumen occurs by a combination of rumen microorganisms that synthesize and secrete enzyme to digest feed particle. On the other hand, the different enzyme activities that occur between types and species of organisms result in a distinct synergism and crossed feeding. This could in the end be beneficial to the animal host through increases in the digestibility and diet utilization. In general, interactions can be either positive or negative and can occur both within and between microbial types (Dehority, 1998). For example, some noncellulolytic ruminal bacteria, when combined with cellulolytic species, can enhance cellulose digestion (Miron, 1991). Similar positive effects have been reported recently with different combinations of bacteria and fungi (Marvin-Sikkema et al., 1990) Fungi may act synergistically in conjunction with the bacteria, by physically disrupting the lignified forage cells. The rumen bacteria

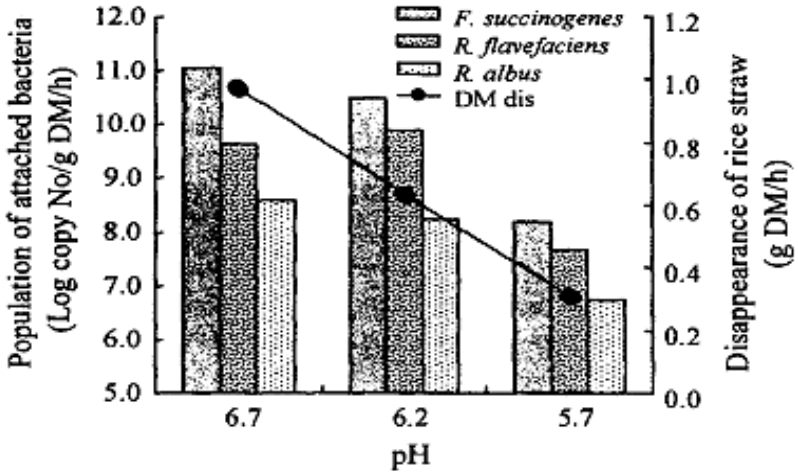

Figure 2. The relationship between initial pH, DM digestibility and bacterial attachment to rice straw after 24 h incubation.
Source: Sung et al. (2007)

Table 3. Quantification of rumen bacteria by real-time PCR.

Bacteria targeted	Log_{10} No. of 16S rRNAgene copies/mL[a]	
	Faunated	Unfaunated
Fibrobacter succinogenes	8.1 ± 6.4	7.7 ± 6.8
Ruminococcus albus	7.7 ± 6.6	8.3 ± 6.3
Ruminococcus flavefaciens	8.4 ± 6.6	8.8 ± 7.9
Prevotella ruminicola	8.9 ± 7.6	9.3 ± 7.9
Prevotella albensis	6.0 ± 5.0	ND
Prevotella bryantii	6.1 ± 5.3	ND
CUR-E cluster	9.6 ± 7.3	10.3 ± 8.6

ND=not detected.
[a]Results are shown as logarithms of 16S rRNA gene copy numbers per 1 ml of rumen fluid ± standard deviation. The values were calculated with total DNA purified from 1 ml of rumen fluid. The detection limit of the quantitative PCR was log_{10} 6.0 copies per ml of rumen fluid.
Source: Ozutsumi et al. (2006)

are thus able to enter into plant stems and tissues where the forage polysaccharide substrates are more accessible for digestion (Dehority, 1998). In contrast, a reduction in cellulose digestion has also been observed by combining certain cellulolytic bacteria. When F. succinogene and R. flavefaciens were co-cultured, cellulose digestion was reduced compared to F. succinogenes alone (Fondevila and Dehority, 1996). Russell (2002) reported that some strain of R. albus produce a bacteriocin that inhibits R. flavefaciens. Negative effects have been reported with bacteria and protozoa, which

the rapid engulfment of bacteria by the rumen protozoa leading to decreased bacteria numbers and fiber digestion in the rumen. Protozoa removal will remove a major inhitor of bacterial attachment to fiber, thus allowing more bacteria to attachment with fiber (Newblod et al., 1989). Ozutsumi et al. (2006) reported that the numbers of R. albus and R. flavefaciens were higher in the faunated rumens than in the unfaunated rumens (Table 3).

A positive interaction between other rumen bacteria has been reported, for example the combination of noncellulolytic *P. ruminicola* with either of the two cellulolytic species (*F. succinogenes* or *R. flavefaciens*) forage cellulose digestion numerically increased over that of the cellulolytic species alone (Fondevila and Dehority, 1996). Moreover, Sawanon and Kobayashi (2006) investigated the rumen bacterial interaction between cellulolytic *R. flavefaciens* and non-cellulolytic *Selenomonas ruminantium*. It was found that when *R. flavefaciens* was cocultured with one of three different strains (GA192, S137 and S150) of *S. ruminantium*, fiber digestion exceeded the value recorded by *R. flavefaciens* alone (Table 4). These results indicate that *R. flavefaciens* provides fiber hydrolysis products to *S. ruminantium* as growth substrates (succinate). In addition, *S. ruminantium* could activate *R. flavefaciens* by rapidly consuming the products. Such cross-feeding between cellulolytic and non-cellulolytic bacteria could enhance fiber digestion. Although the microbial interactions in the rumen are important on digestion and utilization of diets, but the overall rumen fermentation appears to be quite homeostatic, and is perhaps controlled to a greater extent by factors related to the feed on animal consumes rather than a specific microbial population.

Table 4. Fiber digestibility by monoculture of *R. flavefaciens* or *Selenomonas ruminantium* and by their co-cultures**.**

Bacteria strain	Dry matter digestibility			
	Avical	Orchardgrass	Rice straw	Affalfa
Monocultures				
R. flavefaciens C94	$21.4 \pm 1.3^{b,c}$	$23.0 \pm 0.4^{b,A}$	$31.8 \pm 0.3^{b,B}$	$21.5 \pm .7^{b,A}$
S. ruminantium CA192	-	-	-	0.4 ± 0.4^{a}
S. ruminantium S137	0.2 ± 0.3^{a}	0.7 ± 0.4^{a}	0.8 ± 0.4^{a}	0.5 ± 0.3^{a}
S. ruminantium S150	-	-	-	-
Cocultures				
C94 + GA192	$23.8 \pm 0.9^{c,a}$	$24.7 \pm 0.5^{c,A}$	$33.7 \pm 0.7^{c,B}$	$23.5 \pm 1.6^{c,A}$
C94 + S137	$23.6 \pm 0.9^{c,A}$	$24.9 \pm 0.7^{c,A}$	$33.9 \pm 0.7^{c,B}$	$23.5 \pm 1.6^{c,A}$
C94 + S150	$22.8 \pm 1.0^{bc,A}$	$23.6 \pm 0.3^{b,A}$	$33.3 \pm 0.7^{bc,B}$	$22.0 \pm 0.8^{bc,A}$

Values shown as mean ± SD per cent after 72 h incubation (n = 4).

$^{a–c}$ Means within the same column followed by different letters are significantly different ($P < 0.05$).

$^{A, B}$ Means within the same row followed by different letters are significantly different ($P < 0.05$).

Source: Sawanon and Kobayashi (2006)

Conclusions

Cellulolytic bacteria are the most numerous of fibrolytic microorganisms and play a major role in the biological degradation of dietary fiber. *Fibrobacter succinogenes*, *Ruminococcus albus* and *Ruminococcus flavefaciens* are presently recognized as the major cellulolytic bacterial species found in the rumen. The degradation and fermentation of fiber by cellulolytic bacteria contributes to produce protein and energy sources for the host animal. Efficiency of fiber digestion is critically important to the productive efficiency of the ruminant animals; attempts to improve rumen ecology and finding possible approaches to enhancing rumen function, especially fiber digestion by fibrolytic microorganisms would improve productive efficiency in ruminant production.

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