



## ***In vitro* assessment of the relationships between the digestion of different types of rice straw in the rumen.**

Wali, A<sup>1</sup>; Nishino, N<sup>2</sup>

<sup>1</sup>Agriculture Faculty, Sheikh Zayed University, Khost, Afghanistan

<sup>2</sup>Graduate School of Environmental and Life Science, Okayama University, Japan

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### **Abstract**

The aims of this study were to examine bacterial communities in relation to the rumen digestion of rice straw and to understand how concentrate supplements affect gut bacteria involving the digestion of a rice straw-based diet. The substrates were rice straw (RS) alone (experiment 1) and RS with 25% concentrates (barley and kidney beans) (experiment 2). The genomic DNA was collected to determine bacterial diversity by conducting denaturing gradient gel electrophoresis (DGGE). V6–V8 region group-specific (Clostridium and Bacteroides) primers were employed in the analyses. The DGGE band pattern was subjected to cluster analysis to demonstrate the similarity and difference between dietary treatments and solid-liquid fractions. Fiber digestibility, gas production, and volatile fatty acid (VFA) concentration were increased with incubation time. The differences between solid and liquid fractions were significant in total bacteria, Clostridium, and Bacteroides communities. These results indicate a stable community structure of Clostridium and Bacteroides groups involved in the digestion of rice straw-based diets in the rumen.

### **Introduction**

Ruminant animals rely on forage as a primary nutritional component, but forages like rice straw are high in fiber, low in protein, and poorly digestible, which presents a challenge for Afghan farmers who primarily feed their cattle such roughage during the dry season (Van Soest 1994). Afghanistan's livestock population includes approximately 3.7 million cattle and 18 million sheep and goats, many of which are fed low-quality, high-fiber feed (Fitzherbert 2007). To improve meat and milk production, diets need higher nutritional density through increased concentrates, but this affects the gut bacterial community (Chesson 1988). This study focuses on understanding the relationship between fiber digestion, rumen bacterial communities, and the effects of concentrate supplements on the digestion of rice straw in cattle (Beever and Mould 2000).

### **Methods**

In this study, a randomized factorial design was used with two experiments. In experiment 1, 12 rice straw samples from four locations in Afghanistan (Nangarhar and Khost provinces) were collected, and in experiment 2, one rice straw type was selected with barley (25%) and kidney beans (25%) used as supplements. All samples were analyzed in triplicates. The chemical composition was analyzed in the laboratory at Kabul University, and rumen fluid was collected from Holstein dairy cows in Japan (McManus et al. 1976). The *in vitro* gas production technique

was employed using 87 glass vials, with samples incubated for various time points (Pell and Schofield 1993). Gas production and fermentation parameters like pH, VFA, and bacterial communities were measured. Microbial DNA was extracted and amplified using PCR, and microbial community profiling was done using DGGE (Yu and Morrison 2004). Scanning electron microscopy (SEM) was used to analyze rice straw morphology, and bacterial populations were monitored through nested PCR techniques (Hatfield et al. 1999).

Table.1. VFA concentration (mmol/l) of four types rice straw in different incubation of periods

Incubation period	Ingredient				P Value
	RS-1	RS-2	RS-3	RS-4	
24 h Incubation period					
Acetic acid	31.38± 3.78	19.78±1.58	21.59±7.92	17.58±1.04	*
Propionic acid	12.65±1.75	8.02±1.58	11.49±4.94	6.30±0.21	*
Butyric acid	3.81±0.56	2.54±1.03	2.05±0.05	2.08±0.62	NS
Total VFA	49.075	20.6	35.12	26.62	*
48 h Incubation period					
Acetic acid	39.90±0.59	30.15±4.09	35.42±7.94	28.34±1.44	*
Propionic acid	17.64±4.65	16.73±1.42	18.55±0.13	16.44±1.65	NS
Butyric acid	3.41±0.16	1.94±0.26	3.48±1.53	2.13±0.19	*
Total VFA	60.95	48.81	57.44	48.9	*
120 h incubation period					
Acetic acid	73.24±8.00	61.40±13.29	63.44±9.21	58.14±6.56	*
Propionic acid	22.79±7.53	22.20±0.20	20.94±0.04	21.24±0.47	NS
Butyric acid	5.07±0.41	4.09±1.40	4.68±2.38	4.96±1.70	NS
Total VFA	105.34	87.92	89.17	84.49	*

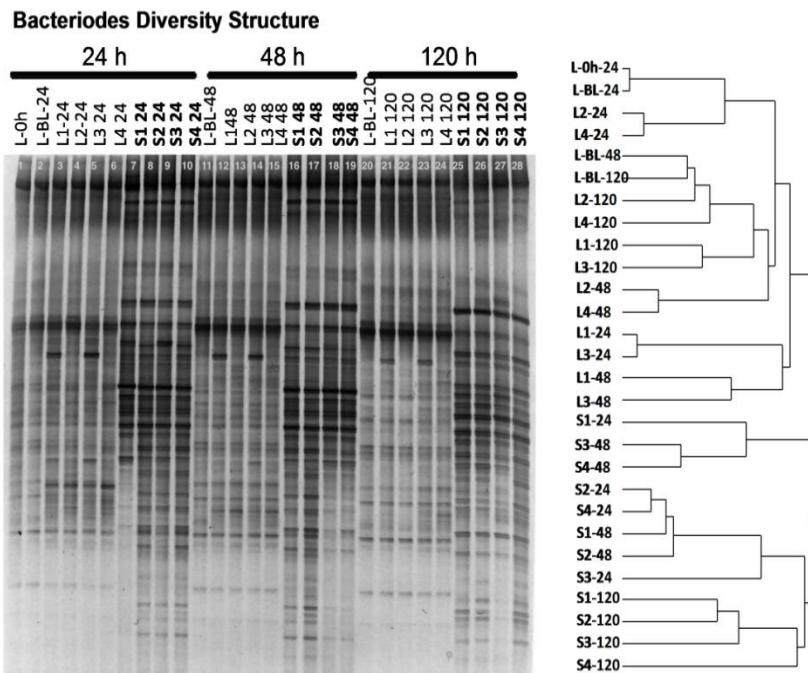
The four types of rice straw from different provinces of Afghanistan, RS-1; rice straw of Ningarhar sorkhrood, RS-2; rice straw of Ningarhar behsood, RS-3; rice straw of Khost mandozai, RS-4; rice straw of Khost shamal. Data are mean ± SD. \*P<0.05, NS; not significant.

## Results

### Fermentation Parameters

The fermentation parameters for rice straw alone showed that cumulative gas production increased over the incubation period, with significant differences observed at 72, 96, and 120 hours. RS-3 produced the most gas, followed by RS-1, and RS-2 produced the least. NDF digestibility increased over time, but no significant differences were found between the straw types. The total VFA concentration increased with incubation, with RS-

1 achieving the highest concentration, followed by RS-3. RS-1 and RS-3 also exhibited higher acetate concentrations throughout the incubation, while propionic acid was higher only at 24 hours. When rice straw was supplemented with concentrates (barley and kidney beans), cumulative gas production increased significantly, especially with barley (RS-BR) and kidney beans (RS-KB) after 48 hours. The pH decreased with supplementation but not significantly. RS-KB showed a significant increase in total VFA concentration, followed by RS-BR, with propionic and butyric acids significantly higher in RS-KB. Acetic acid, however, was unaffected by concentrate supplementation.



**Figure 1.** Liquid- and solid-associated bacterial communities determined by DGGE employing group specific primers for *Bacteroides-Prevotella*. The first letter stands for liquid (L) and solid (S)-associated bacteria, the second letter for 1,2,3,4 is the four types of rice straw, and the following description in parenthesis for 24, 48 and 120 hours of

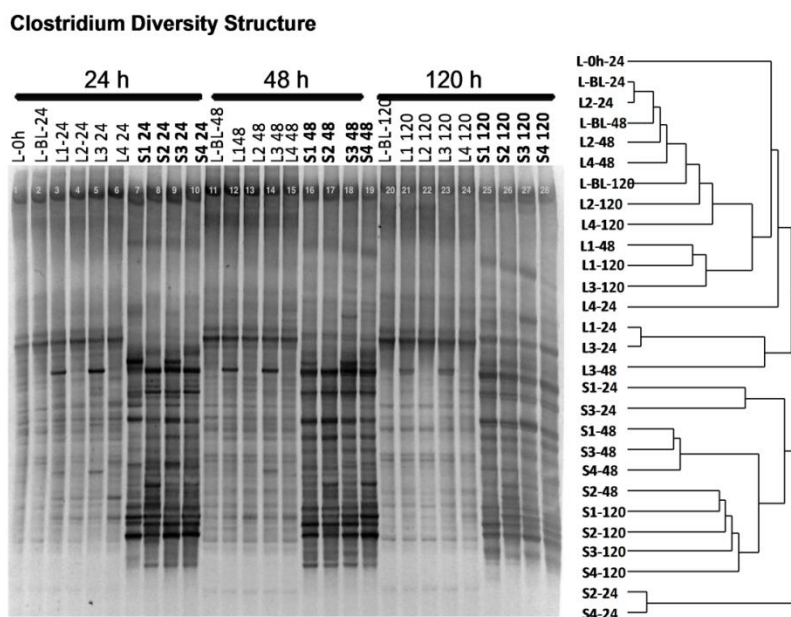
**Scanning electron microscopy (SEM) & PCR-DGGE**

Scanning electron microscopy analysis revealed that rice straw had smooth hyphae at the beginning of the incubation, but over time, the hyphae became increasingly degenerated and damaged, with the damage size growing at 24, 48, and 120 hours. PCR-DGGE analysis of the bacterial communities showed that, for rice straw alone, there was no significant change in the Clostridium and Bacteroides-Prevotella groups throughout incubation. Solid-associated bacterial fractions showed a higher density of dominant bands, with RS-1 and RS-3 showing distinct band patterns in the liquid fraction compared to RS-2 and RS-4. In the rice straw and concentrate treatments, the bacterial community profiles showed clear differentiation between solid and liquid fractions, with Clostridium groups in the liquid fraction affected by both barley and kidney bean supplementation, and the Bacteroides-Prevotella group showing changes only in the liquid fraction with RS-KB. However, there was no significant impact on the solid-associated bacterial community by either concentrate.

**Discussion**

The cumulative gas production varied significantly between the types of rice straw, with differences linked to the chemical composition, especially fiber content (Hatfield et al. 1999). Straw with lower lignin and higher

hemicellulose content (RS-3 and RS-1) showed better fermentation parameters, including higher NDF digestibility, which increased over the incubation period. This slow digestion is attributed to the complex interactions between lignin and structural carbohydrates, which are challenging for microorganisms to break down (Chesson 1988). The increased VFA concentration also reflected the improved fermentation rate, which was consistent with higher gas production (Plaizier et al. 2008). The rice straw varieties exhibited variability in chemical composition, with differences in NDF and ADF content affecting gas production and VFA concentration (Beever and Mould 2000). The degradation of rice straw cell walls increased over time, with visible differences in degradable tissue zones correlating with fermentation parameters and gas production (Van Soest 1994).



**Figure 2.** Liquid- and solid-associated bacterial communities determined by DGGE employing group specific primers for *Clostridium*. The first letter stands for liquid (L) and solid (S)-associated bacteria, the second letter (1,2,3,4) is the four types of rice straw, and the following description in parenthesis for 24, 48 and 120 hours of

The bacterial communities in the solid and liquid fractions showed distinct profiles, primarily due to the attachment of fibrolytic bacteria to solid particles, which are predominant in rumen fiber digestion (Silva et al. 1987). Solid-associated microbes were more abundant throughout the incubation periods, aligning with findings that fiber digestion is mainly driven by these microbes (Jung and Varel 1988). The addition of concentrates (barley and kidney beans) led to increased gas production and propionic acid concentration, while acetic acid was unaffected, suggesting that starch-enriched diets favor propionate production (Pell and Schofield 1993). The lack of significant changes in the solid-associated bacterial community may be due to the low concentrate proportion in the diet, as evidenced by the relatively stable pH (Nagaraja and Titgemeyer 2007). However, the liquid-associated bacterial communities, particularly in the *Clostridium* and *Bacteroides-Prevotella* groups, were affected by the concentrate treatments, likely due to the presence of undigested solid particles in the liquid fraction (Yu and Morrison 2004).

The results indicate that although the digestibility of rice straw was not notably improved by the concentrate supplementation, the changes in microbial communities and fermentation parameters underscore the role of microbial dynamics in fiber and starch digestion (Chen et al. 2008).

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