Rumen Microflora and its Significance to Ruminant Feeding in the Tropics.

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Abstract

Presently feed used for ruminants consists of forages as well as agricultural and industrial by-products, etc. Ruminants possess a special ability to degrade many substances including toxic substances due to their unique rumen micro-organisms which are not present in monogastric animals. The ability of ruminal microorganisms to modify toxic and non-toxic substances is well established. This ability which is mostly favourable for the host animals may sometimes be detrimental. Tropical countries are known for their large diversity in flora and fauna, including plants with toxic properties and microorganisms with a wide adaptability. The emphasis of this review will be placed on the microbial degradation of forages with low digestibility in tropical countries. The discussion will be focused on the possible improvement of the nutrition of ruminants in tropical countries.

Introduction

Presently, ruminants receive a variety of feed. In extreme case farmers feed ruminants like monogastric animals, ignoring the fact that they are ruminants, due to the availability of feed and the adaptability of the animals. As the human population increases, we produce a greater variety and quantity of waste products which can be consumed by animals but consequently the land available for both housing and agriculture is reduced. Nutrient energy in most of these by-products is not readily available and is not sufficient, even for the ruminant digestive system. This is especially the case for cellulosic materials which have slower rates of digestion because the cellulose is in a highly organized and complex form, and because this kind of feed generally lacks the nitrogen necessary to balance the large amounts of available carbon. We are now trying to manipulate the rate of digestion of various feeds to generate conditions suitable for both humans and ruminants. We are trying to accelerate those rates that are slow, while delaying those with a high initial rate of nutrient release which can cause digestive disturbances, such as acidosis, bloat, and liver abscesses (Cheng *et al.*, 1991). The former objective is more relevant and more important to tropical countries. Theoretically it is conceivable that up to 90% of ruminant feed could consist of by-products which have been modified to improve the digestibility. In practical terms, the benefits from the successful attainment of the objectives would be 1) to make under-utilized by-products available as ruminant feed, 2) to improve the digestibility and efficiency of utilization of these by-products for animal feed, and 3) to reduce the requirement for hay and animal feed production thus freeing land for more valuable crops. In Asia, ruminants are kept by smallholders on a small scale and the production systems are closely integrated with farming systems. One of the limiting factors for the expansion of production is the lack of availability

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of feed resources, as under such farming systems the farmers do not have adequate feed resources or the capacity to increase the amount of available feed. Usually agricultural by-products are utilized for feeding domestic ruminants. As seen in the present decline in the area available for forage production, ruminants will have to increasingly depend on fibrous residues and by-products for their energy sources. Although there are still problems to be solved for the utilization of these by-products and many of them can not be used without some detoxifying treatment, the quantities of these by-products together with the characteristics of tropical ruminants which are able to convert low quality feed into milk and meat (Abdullah, 1987), suggest that these by-products are likely to play an important role as potential feed sources in ruminant nutrition.

Characteristics of the forage material

Forages differ greatly in their composition and structure. Temperate and tropical forages are covered on the outer surface by a thin waxy cuticle, which contains a hard basal polyester layer. Immediately below the cuticle lie the plant cells, in which the cell walls account for the bulk of the aerial portion of the plant. The cell wall is composed primarly of cellulose and hemicellulose, with a small proportion of lignin (Van Soest, 1982).

The cuticular surface layer presents a strong barrier to invasion by rumen microorganisms. The cutin layer appears to be totally resistant to microbial digestion within the rumen, except for some rumen fungi which may penetrate it (Ho *et al*., 1988). Cutin forms the structural component of the plant cuticle and is a polyester with C-16 and C-18 hydroxy- and hyroxyepoxy fatty acids. The cutin fraction accounts for 0.2% of the cell wall of wheat straw to 2.4% of that for mature alfalfa (Van Soest, 1982). The ester linkage of cutin is hydrolyzed by some pathogenic fungi and some aerobic bacteria; however, there are no reports on rumen bacteria exhibiting cutinase activity (Cheng *et al*., 1990b). Most of the usable plant nutrients are internal. External plant tissues are only poorly colonized by rumen microorganisms while the inner tissues are heavily colonized. However physical disruption (eg., chewing) is necessary to allow optimal microbial access to the inner tissues which are then actively colonized (Cheng *et al*., 1990a).

Characteristics of ruminants

Ruminants possess a complex stomach system, in which the stomach is divided into three or four compartments, the first and the largest of which is the rumen. It is here where continuous anaerobic fermentation takes place by a complex community of microorganisms consisting of protozoa, bacteria, fungi and probably other unknown microorganisms. When ruminants are born their rumen is in a germ-free condition and the unique flora and fauna start to be established after birth. Once established, the rumen microbial community is very stable and changes only when the nutrients are changed (Cheng and Costerton, 1980) which may account for the fact that the feed efficiency in ruminants has remained the same in the past decades with the exception of some success using feed additives with ionophore (rumensin or monensin and lasalocid) while that of pigs and chickens has greatly improved. Although the definite cause remains unknown, it is well known that some ruminants, notably the buffalo and the yak can utilize poor quality feed more efficiently. Our experimental results indicated that the cellulolytic bacteria, *Fibrobacter succinogenes* (formerly called *Bacteroides succinogenes*) and *Ruminococcus flavefaciens* isolated from water buffalo possess a more active cellulase activity compared with strains isolated from cattle (Tables 1 and 2). This is probably because these animals have been kept on a low-quality of roughage feed for a long time. Our preliminary study indicated that unusually active cellulolytic bacteria are present in the rumen of the lesser mouse deer (*Tragulus javanicus*). This is not surprising as

Table 1 Fermentation products produced by strains of *F. succinogenes* isolated from the rumen of a steer and a water buffalo

Strain S (WB)	Fermentation products (mM)[z]			
	Acetate S (WB)	Butyrate S (WB)	Succinate S (WB)	Lactate S (WB)
a (112)	5.0 (10.1)	− (1.3)	9.2 (11.5)	−
Z (116)	5.2 (7.3)	− (0.6)	8.7 (12.7)	− (1.2)
b (123)	4.9 (6.1)	− (1.2)	8.8 (12.6)	− (1.1)
M (127)	5.5 (7.1)	− (0.6)	7.9 (11.9)	− (1.2)
C (126)	5.5 (5.6)	− (−)	7.7 (12.7)	− (1.7)
A (114)	5.0 (8.0)	− (−)	7.9 (10.2)	− (1.7)
D (104)	5.2 (8.1)	− (−)	7.5 (11.5)	− (−)
o (121)	3.9 (7.4)	− (1.2)	8.7 (10.1)	− (0.8)
O (128)	4.8 (4.8)	− (−)	7.5 (11.1)	− (2.8)
d (120)	4.6 (4.4)	− (−)	7.6 (12.3)	− (1.8)
V (107)	4.2 (7.0)	− (−)	7.9 (11.5)	− (−)

[z] Cultures were incubated for 5 days at 39℃ in 10 ml of Scott and Dehority's medium with 30 mg of Whatman No. 1 cellulose filter paper.
Note : S : Steer. WB : Water buffalo.

Table 2 Fermentation products produced by strains of *R. flavefaciens* isolated from the rumen of a steer

Strain S (WB)	Fermentation products (mM)[z]		
	Acetate S (WB)	Lactate S (WB)	Succinate S (WB)
1 (102)	2.9 (6.5)	2.2 (−)	4.5 (8.5)
2 (106)	3.4 (6.9)	2.2 (−)	4.4 (6.8)
3 (115)	2.8 (6.2)	2.0 (2.2)	4.4 (7.8)
4 (136)	3.3 (8.7)	2.3 (0.6)	4.1 (9.4)

[z] Cultures were incubated for 5 days in 10ml of Scott and Dehority's medium containing 30 mg of Whatman No. 1 cellulose filter paper. None of the strains produced ethanol.
Note : S : Steer. WB : Water buffalo.

highly cellulolytic microorganisms are essential for the breakdown of highly fibrous jungle forages and tree leaves which serve as the natural diet of the mouse deer.

Plant toxins and rumen microorganisms

Leucaena is widely grown in the tropical and subtropical countries. The plant is potentially an excellent source of crude protein. However, its use as feed has been limited because it contains mimosine, a toxic amino acid that causes low weight gains, poor health conditions and hair loss in both ruminants and non-ruminants. Mimosine toxicity is more acute in non-ruminants owing to the absence of endogenous microorganisms capable of enzymatic detoxification. Mimosine is hydrolyzed in the rumen by microbial enzymes to DHP, a potent goitrogen and sometimes to 2-hydroxy-3-(1H)-pyridone, a structural isomer of DHP which is probably as goitrogenic as DHP.

Previous studies carried out in Canada (Kudo *et al.*, 1984; 1986) and Malaysia (Kudo *et*

al., 1989a) on the *in vitro* metabolism of mimosine showed that degradation occurred in the microorganism fraction and that concentrate diets increased the microorganism population that detoxifies mimosine, but probably not sufficiently to completely escape from mimosine poisoning. When we examined the rate of mimosine degradation in the rumen fluid of cattle fed five different diets, significant ($P<0.01$) differences were detected between the highest rate on a blue grass-molasses diet and the lowest rate on corn silage. The highest rate obtained with bluegrass (*Poa pratensis*)-molasses suggests that high rates of metabolism can also be associated with inocula from diets other than concentrate feed. Intermediate rates were obtained with alfalfa hay, fresh alfalfa herbage and orchard grass hay (*Dactylis glomerata*) but these rates were not significantly different. *In vitro* rates for mimosine and DHP degradation using ruminal contents were analysed in Malaysia during 1988 (Kudo *et al.*, 1989a). All the rumen samples from cattle, sheep and water buffalo failed to disrupt the heterocyclic ring, suggesting the complete absence of DHP detoxification. In 1989, the active rumen fluid from Indonesian goats fed and adapted on *Leucaena* was perfused into one of these cattle. Not only the perfused animal but also the untreated animals were able to degrade DHP (Kudo *et al.*, 1989b; 1990c). During this study, we demonstrated that the ability to detoxify DHP is very rapidly passed from treated to untreated neighboring animals. To date, only the rumen microorganisms in Hawaii, Indonesia and the Bahamas are able to detoxify mimosine *in vivo* and protect the animals from any deleterious effects caused by the plants. We should note that in tropical countries the quality of feed supplied, including that supplied to laboratory animals is unsatisfactory, varying from batch to batch. Some feeds contain toxic substances, which can not be degraded even with the special capabilities of rumen microorganisms. As there are too many toxic plants, especially in tropical countries, we can not describe all of them. For a general review on the ruminal metabolism of other natural toxins and synthetic substances, the reader is referred to reviews by James *et al.* (1975) and Allison (1978).

Factors affecting feed degradation

1 Microbial attachment

Recent studies have demonstrated that for the digestion of cellulose (Fig. 1 and 2) and starch, attachment to these insoluble substrates is a pre-condition for both pure cultures and natural mixed population if digestion is to proceed. Cellulolytic species differ in the nature of their attachment to insoluble substrates and in the nature of their enzymatic attack (Minato and Suto, 1978; Kudo *et al.*, 1987b; McAllister *et al.*, 1990). When these substrates are placed in the rumen, they become attached to each substrate very rapidly (<15 min). Among the major cellulolytic bacteria in the rumen, *F. succinogenes* is very closely attached to the substrate while *R. flavefaciens* is attached over a small distance and *Ruminococcus albus* over a much greater distance. This spatial distribution appears to be controlled by the width of the glycocalyx structure used by these species to become attached to cellulose. In these three bacterial species, the cellulases reach and digest the colonized cellulose substrate. Similarly, the digestion of starch is affected by the structure of the glycocalyx of the amylolytic organisms (Cheng *et al.*, 1990a). Amylase producers adhere to starch but not to cellulose, while cellulose decomposers adhere to cellulose but not to starch (Minato and Suto, 1978). Cheng (1980) postulated that rumen microorganisms can be classified into three groups (I) Microorganisms attached to the rumen wall, (II) Microorganisms living freely in the rumen and (III) Microorganisms attached to feed particles. In the rumen, as much as 75% of these microorganisms are attached to feed particles. Microorganisms in the rumen have a variety of surfaces to which they may become attached, and a distinct population of microorganisms adheres to each different surface. From an ecological viewpoint, bacteria with the ability to become attached to feed particles have a great advantage over non-

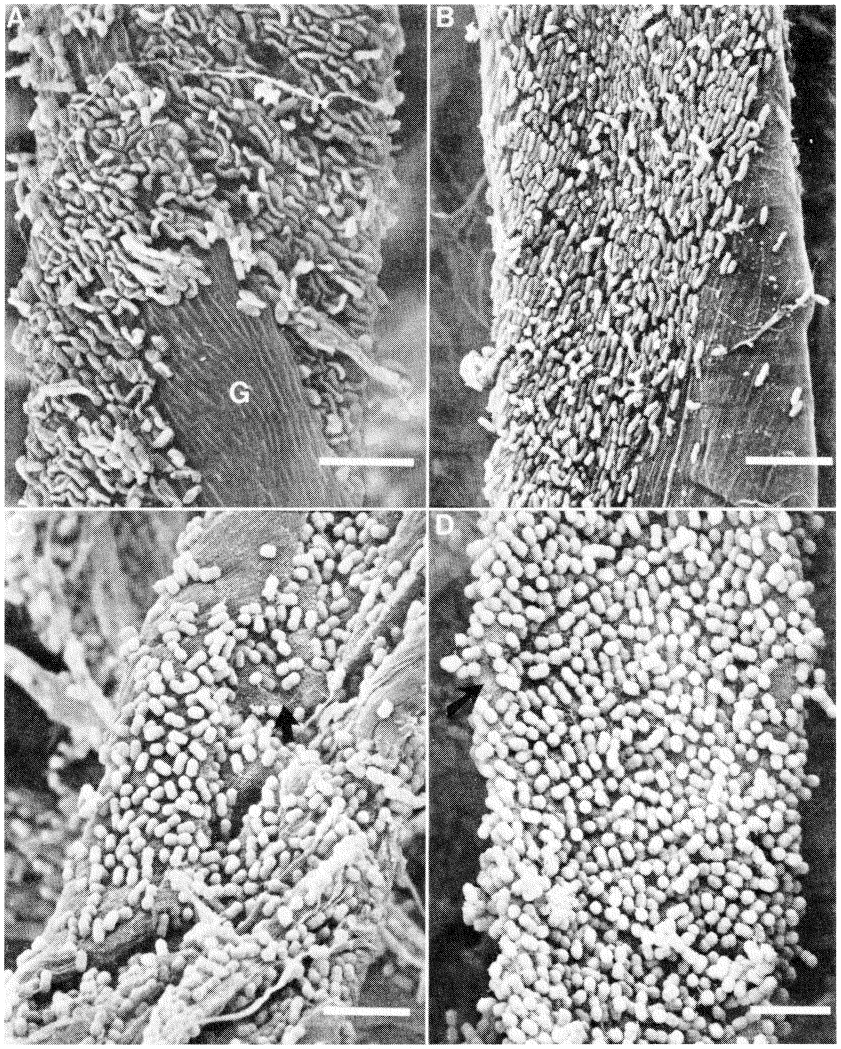

Fig. 1 **SEM of Whatman no. 1 filter paper incubated with cellulolytic bacteria at 39℃ for 20h showing extensive bacterial adhesion to cellulose fibers. (A) *Fibrobacter succinogenes* strain BL 2. Note that grooves (G) have formed as a result of cellulose digestion and that some deformation of cells is evident. (B) *F. succinogenes* strain E. Note that the bacterial cells attach avidly to the fiber and are oriented in the same direction as the cellulose sub-fibers. (C) *Ruminococcus flavefaciens* 2. Note that the extensive secretion of exopolysaccharide glycocalyx material by these organisms has produced a fibrous network (C and D), seen here after dehydration during preparation of SEM. Bars=5 μm.**
Source : Kudo *et al.*, 1987b. Figures are reproduced with permission from Canadian Journal of Microbiology.

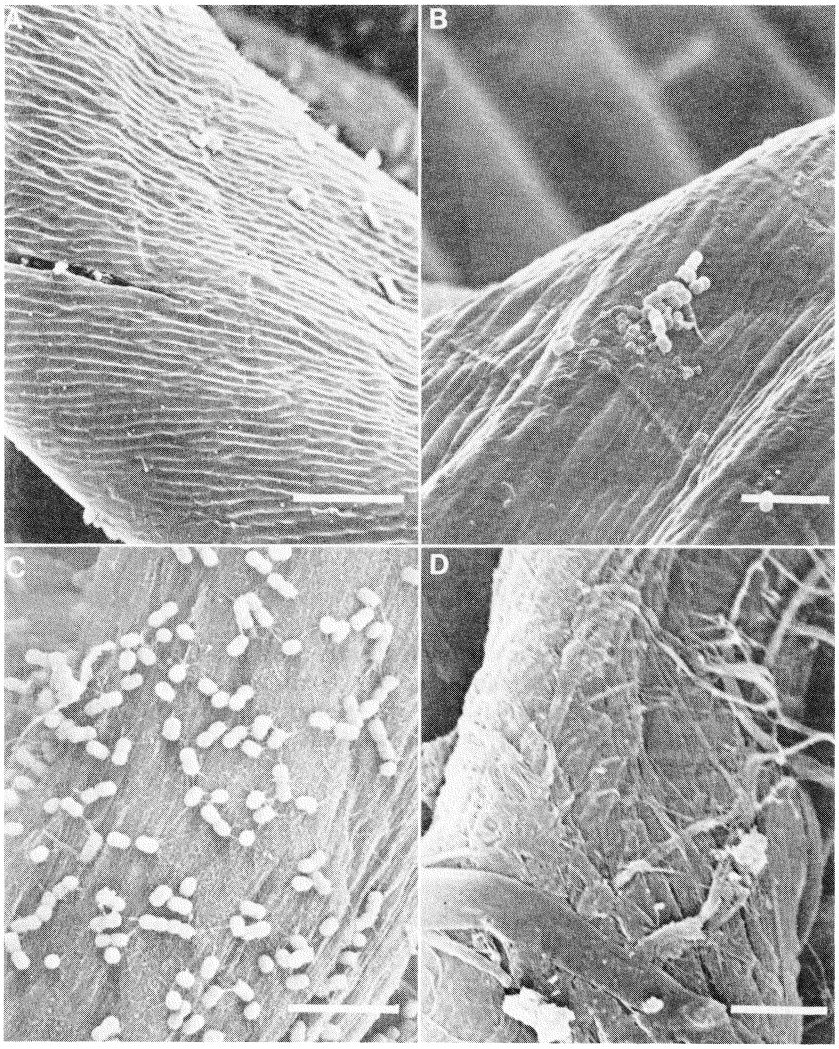

Fig. 2 **SEM of bacterial detachment from Whatman no. 1 filter paper (A-C). Filter paper was first incubated with cellulolytic bacteria at 39℃ for 20h and then treated with 0.1% methylcellulose at 39℃ for 1h. These SEM show the very effective detachment of cellulolytic bacteria from their cellulosic substrata that is caused by methylcellulose. (A) *F. succinogenes* strain BL2. Note very regularly formed parallel grooves, as evidence of fiber degradation, and the complete absence of adherent bacteria. (B) *Ruminococcus albus* strain SY3. There is some irregular evidence of digestion, in the form of grooves or pits, and only a few adherent cells remained. (c) *R. flavefaciens* strain 1. Note that some cells are still attached to the fiber by means of fibrous extracellular material that formed a network over the cellulose surface and that no grooves or pits are formed as a result of digestion. (D) Filter paper was incubated with cells of *F. succinogenes* at 39℃ for 20h, in the presence of 0.1% methylcellulose, and no bacterial adhesion or evidence of digestion can be seen on the cellulose fiber surface. Bars=5μm.**
Source : Kudo *et al.*, 1987b. Figures are reproduced with permission from Canadian Journal of Microbiology.

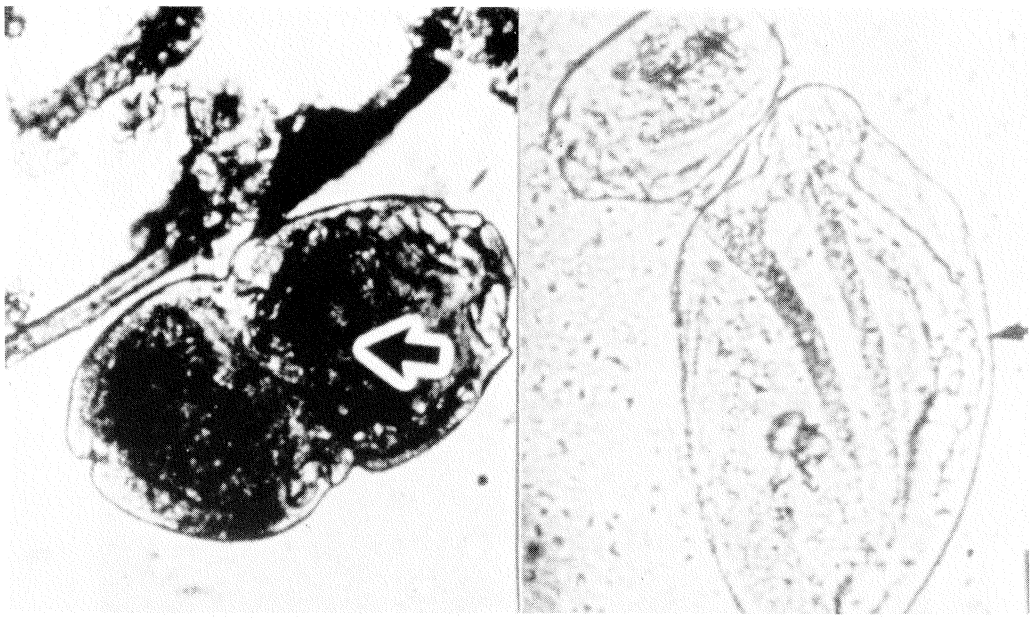

Fig. 3 Light photomicrographs show rumen ciliates that are found in cattle fed barley straw. All contain fragments of barley straw (arrows) inside the cell. (A) *Polysplastron multivesiculatum*, in binary fission (x 240). (B) *P. multivesiculatum*. Note the transformation of the shape of the cell outward due to the plant material ingested (x 310).

attaching microorganisms, which can be removed more quickly from the rumen environment by flow. In addition to adhesion, the ingestion of feed particles was observed in protozoa (Fig. 3). The importance of the adhesion of microorganisms to substrates suggests that better feed efficiency could be attained if the attachment could be promoted.

2 Digestive consortia

Pure cultures of cellulolytic bacteria and fungi digest cellulose *in vitro* but the digestion does not proceed at a similar rate to that seen in the rumen unless consortia are formed with non-cellulolytic *Treponema bryantii, Butyrivibrio fibrisolvens* and methanogenic bacteria. Electron microscopy of partly digested plant material shows that consortia of *F. succinogenes, R. flavefaciens* and *R. albus* are in direct contact with the cellulose fibers, while cells of *Treponema* species, *Butyrivibrio* species, and methanogenic bacteria are more loosely associated. Morphological examination, by electron microscopy, showed that the cells of *T. bryantii* are associated with the plant cell wall materials in straw, but that cellulose digestion occurs only when these organisms are present with cellulolytic species such as *F. succinogenes*. These results show that cellulolytic bacteria interact with non-cellulolytic *Treponema* to promote the digestion of cellulosic materials (Kudo *et al.*, 1987a). Recent studies have shown that both protozoa and fungi may be active members of consortia (Imai *et al.*, 1989; Kudo *et al.*, 1990a; 1900b; Ho *et al.*, 1988). We have recently discovered that mycoplasms tend to be associated with most rumen fungi cultures (Kudo *et al.*, 1990b). We have confirmed that the rumen fungal cultures, reportedly pure, were also contaminated with these cell wall-deficient bacteria. Thus, the microbial ecology of the rumen is obviously very complex, including many interactions of microbial species and even though primary cel-

lulolytic organisms may be present in a system, many other factors may be required to facilitate actual cellulose digestion.

Practical applications for animal production

As data on recent ruminant production indicate that immediate improvements in feed efficiency in ruminants may not be expected, however, there is some potential. Some of the forages and by-products, for example *Leucaena* and palm oil by-products, may require manipulations to reduce the toxicity. Palm kernel cake (PKC) is widely used as animal feed but its high concentration of copper can not be overlooked and we observed that protozoa and fungi were almost completely destroyed due to the copper content. Although the rate of growth was not significantly affected, in the faunated cattle the belly girth significantly increased and the body length tended to decrease during the 36 week period after birth (Itabashi *et al.*, 1990). Since some of the other palm oil by-products are known to contain copper, before they can be used practically, copper toxicity must be reduced through plant breeding programs or other methods. Alfalfa (*Medicago sativa L.*) is a valuable leguminous forage crop with a high yield and an excellent nutritive value. However, the risk of bloat caused by the high initial rates of microbial digestion and nutrient release from the more digestible leaves often limits its use. We (Kudo *et al.*, 1985) have shown that through plant breeding programs this adverse effect can be limited in order to reduce the high initial rate of digestion and provide an engineered forage that is equivalent to the original plant, but does

Table 3 Comparison of two strains of alfalfa, selected for slow and fast initial rates of digestion (IRD). Values are the average of all observations across the three cuts for each of the two strains

Variable	Time (h) after feeding	Slow-IRD strain	Fast-IRD strain	Pooled standard error	Difference between strains[z]
Alfalfa					
Partial digestibility (%)[y]	-	46.5	52.3	0.6	**
Dry matter (%)	-	20.1	20.1	0.2	NS
Rumen fluid	0	110	130	5	**
Chlorophyll	2	137	162	5	**
(μg/ml)	4	140	172	5	**
Soluble protein	0	7.62	8.40	0.20	**
(mg/ml)	2	8.81	10.27	0.19	**
	4	8.60	9.89	0.20	**
Soluble carbohydrates	0	5.81	6.88	0.20	**
(mg/ml)	2	7.48	8.83	0.22	**
	4	6.74	7.96	0.20	**
Volatile fatty acids	0	3.04	3.60	0.10	**
(mMol/dl)	2	7.49	8.65	0.11	**
	4	7.11	8.30	0.11	**
pH	0	7.32	7.27	0.03	*
	2	6.54	6.35	0.03	*
	4	6.63	6.34	0.02	*

[z] NS=not significant, $P>0.05$. *, ** =Significant at $P<0.05$ and $P<0.01$, respectively.
[y] Percent dry matter disappearance from nylon bags *in situ*, 6-h digestion.
Source : Kudo *et al.*, 1985.

not have an adverse effect on the ruminants (Table 3). This concept could be applied to low-quality forages in tropical countries by similar plant breeding programs to increase the initial rate of digestion. Tropical ruminants are known to be better converters of poor quality feed. Therefore, breeding based on this fact and the better chewing characteristics of certain individual animals may enable to improve animal production in tropical countries.

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Discussion

Haryanto, B. (Indonesia) : The digestibility of feed in tropical countries is usually low. In relation to the rumen microorganisms, do you have any information on the energy requirement of rumen microorganisms?

Answer : We have not carried out such studies.

Pradhan, K. (India) : Have you been able to isolate bacterial species (cellulolytic and proteolytic) showing marked differences in their activities between cattle and buffalo? If

so, please name those present in the rumen of cattle and buffalo.

Answer : We isolated *Fibrobacter succinogenes* and *Ruminococcus albus* as cellulolytic bacteria. We did not study proteolytic bacteria.

Ku Vera, J. C. (Mexico) : Is there any evidence for the increase of dry matter degradability in the rumen with the inclusion of phenylpropanoic acid (attachment promoter of bacteria) in the ration?

Answer : The results are inconsistent.