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Effects of microbial synergism on fibre digestion in the rumen

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The primary sources of energy found in forages are the structural polysaccharides, cellulose, hemicellulose and pectin. These three components genérally account for about 400–600 g/kg forage dry matter (Lagowski *et al.* 1958; Waite & Garrod, 1959; Chesson & Forsberg, 1988). The inability of most animals to digest these structural polysaccharides has resulted in some of them adopting a microbial population which can. In essence the animal provides an environmentally suitable area for growth of these micro-organisms, which in turn digest the forage structural carbohydrates and thereby supply energy to the host (Hungate, 1972; Dehority, 1986). Since most of these digestive tract micro-organisms have complex nutritional requirements and can only utilize one or two of the major polysaccharides, synergism between the various organisms can be important for the efficient use of forages by the ruminant animal.

Microbial synergism is defined as increased growth or productivity resulting from the combination of two or more micro-organisms, which exceeds the additive effects of their separate activities. In general, this occurs through crossfeeding of hydrolysis products, utilization of end-products or production of an essential nutrient. One of the best examples of crossfeeding of hydrolysis products in the rumen is probably the utilization of cellodextrins by non-cellulolytic rumen bacteria (Scheifinger & Wolin, 1973; Bryant & Wolin, 1975; Russell, 1985). End-product utilization is best exemplified by the rumen methanogens, which use hydrogen and carbon dioxide to generate energy through production of methane (Stumm *et al.* 1982; Russell & Wallace, 1988; Wolin & Miller, 1988). Conversion of succinate, a normal end-product of many rumen bacteria, to propionate would be another example of this type of synergism (Scheifinger & Wolin, 1973; Russell & Wallace, 1988; Wolin & Miller, 1988). Nutritional interdependence, production of a nutrient by one species which is essential for a second species, generally involves the vitamins, amino acids and branched-chain fatty acids (Miura *et al.* 1980; Wallace, 1985; Wolin & Miller, 1988).

PLANT STRUCTURE AND DIGESTIBILITY

In contrast to the classical types of synergism described previously, microbial synergism as related to forage digestion appears to depend on removal of so-called 'masking' constituents. The isolated plant structural carbohydrates, i.e., cellulose, hemicellulose and pectin, are readily digested by rumen micro-organisms; however, their availability in the intact plant can be limited and varies both with plant species and maturity (Dehority & Johnson, 1961; Dehority *et al.* 1962; Dehority & Scott, 1967). For example, Kamstra *et al.* (1958) compared in vitro mixed-culture cellulose digestion using the intact plant and the cellulose and holocellulose fractions isolated from the same forage as substrates. Their results suggested that the cellulose was shielded or protected from digestion by 'encrusting substances' indigenous to the whole plant. Since both the extent of digestion decreased and lignin content increased with plant maturity, they speculated that lignin may be deposited as an encrusting substance around the cellulose during growth of the plant.

Additional support for the 'encrustation' theory was obtained from studies where physical reduction of forage particle size (ball-milling) drastically increased the extent of cellulose digestion. The increase became larger as the forage matured (Dehority & Johnson, 1961). Almost identical results were obtained when simple physical solubility of the forage cellulose in cupriethylene diamine (a cellulose solvent) was measured. Recent studies would indicate that the hemicelluloses form a matrix in the cell wall which surrounds the cellulose fibrils (Akin, 1986). Lignin, phenolic and acetic residues are chemically bound to this hemicellulose matrix by ester and possibly glycosidic links (Van Soest, 1982; Chesson & Forsberg, 1988). On the other hand, lignin does not appear to be directly bound to cellulose itself (Chesson & Forsberg, 1988). The lignified cell walls apparently restrict access of the rumen micro-organisms and their associated enzymes to the structural polysaccharides in forage, thereby reducing their digestibility in the intact plant.

CELLULOSE DIGESTION

Bacteria. The extent of cellulose digestion from eleven forages, as determined with two pure cultures of cellulolytic rumen bacteria, a mixed rumen bacterial fermentation and from in vivo digestion trials with sheep is presented in Table 1 (unpublished results from a study reported by Dehority *et al.* 1967). For almost every forage, the extent of cellulose digestion was slightly higher for *Fibrobacter succinogenes* A3c; however, the overall mean was not different from the mixed-culture fermentation. In contrast, mean cellulose digestibilities were significantly lower ($P<0{\cdot}05$) with both *Ruminococcus flavefaciens* B34b and in vivo. These findings clearly point out that differences exist in the ability of single cultures to digest forage cellulose and that in vivo digestibilities are probably influenced by rate of passage. In a separate study, Dehority & Scott (1967) measured the ability of eight cellulolytic and one non-cellulolytic strain to digest cellulose from twelve forages (eight grasses and four lucerne (*Medicago sativa*)). In general, their results followed the same pattern as shown in Table 1. Similar results have subsequently been reported from other laboratories, using a number of different strains and species of cellulolytic rumen bacteria (Kock & Kistner, 1969; Morris & van Gylswyk, 1980; Chesson *et al.* 1986).

Table 1. *Extent of forage cellulose digestion by two pure cultures of rumen cellulolytic bacteria, by a mixed rumen culture and by sheep in vivo**

	Cellulose digestion (%)			
Forage†	A3c	B34b	Mixed culture	In vivo
Orchardgrass (*Dactylis glomerata*): 1	77·1	51·1	72·4	69·7
2	63·7	39·7	59·4	59·8
Bromegrass (*Bromus inermis*): 1	82·5	52·6	79·4	72·1
2	51·8	19·8	50·6	53·2
Reed canary-grass (*Phalaris arundinacea*): 1	78·2	44·4	76·1	67·6
2	66·0	30·9	66·3	60·6
Timothy (*Phleum pratense*): 1	85·4	55·1	83·1	74·0
2	59·6	32·5	58·3	58·8
Lucerne (*Medicago sativa*): 1	61·5	58·9	59·9	60·5
2	51·1	42·4	54·0	50·9
3	44·9	41·0	51·4	51·8
Mean	65·6[a]	42·6[b]	64·6[a]	61·7[c]

[a,b,c] Means with unlike superscript letters were significantly different ($P<0·05$).

* A3c, *Fibrobacter succinogenes* A3c; B34b, *Ruminococcus flavefaciens* B34b. Mixed culture, a 48 h in vitro fermentation; in vivo, values determined in sheep digestion trials.

† Maturity stages for grasses: 1, boot stage; 2, bloom stage; for lucerne: 1, prebloom; 2, early bloom; 3, late bloom.

In the previously mentioned study by Dehority & Scott (1967), they also combined six of the nine cultures in all possible combinations of two and measured cellulose digestibility in the twelve forages (Table 2). When *Bacteroides ruminicola* H8a, a non-cellulolytic, was combined with any of the major cellulolytics, i.e. *F. succinogenes*, *Ruminococcus albus* or *R. flavefaciens*, cellulose digestion was significantly increased. Combining the weakly cellulolytic *Butyrivibrio fibrisolvens* H10b with a second species did not increase digestibility and in two instances significantly reduced digestibility. This was surprising because of the marked ability of H10b to digest forage hemicellulose. Similar decreases were observed with the combination of A3c plus B34b and 7 plus B1a. When all six cultures were combined in the same fermentation tube, the mean cellulose digestion for the twelve forages was 54·6% which was significantly lower ($P<0·05$) than the mean value of 59·8% determined in digestion trials with sheep (Dehority, 1973).

Stewart *et al.* (1979) measured dry matter digestibility (DMD) of straw using five pure cultures of rumen bacteria either singly or all combined. Dry matter (DM) loss was greatest with all organisms combined (44%), followed in order by the three individual cellulolytic species, *F. succinogenes* (42·3%), *R. flavefaciens* (34·7%), *R. albus* (25·9%). The other species, *B. fibrisolvens* and *Lachnospira multiparus*, solubilized only minimal amounts. Incubation of the same straw with rumen contents resulted in a 56·8% loss of DM. In later studies from this same laboratory, using different strains of *F. succinogenes* and *R. albus*, *R. albus* solubilized more DM than the other two species (Graham *et al.* 1985; Kolankaya *et al.* 1985). Some evidence of synergism was observed. Akin & Rigsby (1985) found their strain of *R. flavefaciens* to be more active in solubilizing DM from orchardgrass (*Dactylis glomerata*) than specific strains of *R. albus*, *L. multiparus* or *B. fibrisolvens*. *Clostridium longisporum* (a minor rumen cellulolytic bacterium) was

Table 2. *Extent of forage cellulose digestion obtained with pure cultures of rumen cellulolytic bacteria singly or in all combinations of two*†

(Mean values for twelve forages (eight grass and four lucerne (*Medicago sativa*) samples))

	Cellulose digestion (%)					
Organism 2 . . .	A3c	7	B34b	B1a	H10b	H8a
Organism 1						
A3c	61·9	63·1	44·7**	62·2	63·5	62·2**
7		44·4	41·2	39·9*	40·3*	48·8**
B34b			44·1	43·5	46·1	47·0*
B1a				36·3	32·1*	42·2**
H10b					8·7	6·1
H8a						1·6

A3c, *Fibrobacter succinogenes*; 7, *Ruminococcus albus*; B34b and B1a, *R. flavefaciens*; H10b, *Butyrivibrio fibrisolvens*; H8a, *Bacteroides ruminicola*.

Within a horizontal row mean values were significantly different with respect to the mean cellulose digestibility obtained from that bacterial strain alone: $*P<0{\cdot}05$, $**P<0{\cdot}01$.

† Values from Dehority & Scott (1967) and Dehority (1973).

essentially unable to solubilize DM from barley straw, whereas *R. albus* degraded 20–28% (Varel *et al.* 1989). Combining *R. albus* with either *C. longisporum* or the methanogen, *Methanobacterium smithii*, did not increase DM solubility.

In a recently reported study by Osborne & Dehority (1989), three strains of rumen bacteria characterized as using only a single forage polysaccharide, i.e. *F. succinogenes* A3c cellulolytic, *B. ruminicola* H2b hemicellulolytic, and *L. multiparus* D15d pectinolytic, were used singly and in all combinations to study cellulose, hemicellulose and pectin digestion from two maturity stages of orchardgrass. In contrast to the previous results from this laboratory (Dehority & Scott, 1967), cellulose digestion by *F. succinogenes* A3c was not increased by adding either of the non-cellulolytic organisms. The reason for this discrepancy is not known; however, different strains of *B. ruminicola* were used in the two studies.

With regard to cellulose digestion in forages, it can be concluded from the reported studies that both positive and negative synergism can occur between bacterial species. Although rate of passage may decrease the extent of cellulose digestion in the rumen, total tract digestibility exceeds that obtained by combining up to six bacterial cultures and may reflect some possible hind-gut fermentation.

Protozoa. Because of our inability to culture rumen ciliates axenically, little information is available on cellulose digestion by the protozoa and possible synergism with other rumen microbes. Seven of nine in vivo studies have reported a decrease in cellulose digestion with defaunated animals; however, the decrease was generally quite small (Veira, 1986; Williams & Coleman, 1988). In contrast, Soetanto *et al.* (1985) and Romulo *et al.* (1986) observed increases in DM and cellulose or acid-detergent fibre digestion from Dacron bags in defaunated animals. A concomitant increase in the concentration of sporangia and fungal zoospores was also noted.

Studies by Coleman *et al.* (1976) and Coleman (1978, 1985, 1986), using cell-free protozoal extracts, strongly support the concept that rumen protozoa are cellulolytic. However, some question concerning the possible contribution of intracellular bacteria to protozoa cellulose digestion still remains.

Fungi. Cellulose digestion in the rumen by the recently discovered anaerobic fungi is extensively documented (Bauchop, 1981; Orpin & Joblin, 1988). Three genera have been described, *Neocallimastix*, *Sphaeromonas* and *Piromonas*; and almost all strains isolated to date appear to be cellulolytic (Hebraud & Fevre, 1988; Orpin & Joblin, 1988; Phillips & Gordon, 1988; Gordon & Phillips, 1989).

Bernalier *et al.* (1988) found that the fungus *Neocallimastix* could digest more cellulose alone than in combination with cellulolytic bacteria. Combining *R. flavefaciens* with *Neocallimastix* reduced cellulose digestion, while essentially no difference was found when *F. succinogenes* and the fungus were combined. Adding *S. ruminantum* to *Neocallimastix* appeared to increase the rate but not extent of purified cellulose digestion. Similar results were obtained by Richardson *et al.* (1986) in synergism studies on straw digestion. Digestibility was increased by co-culture with *F. succinogenes* and decreased with either *R. flavefaciens* or *R. albus*.

Digestion of cellulose and solubilization of straw by anaerobic rumen fungi were increased in co-culture with methanogens (Bauchop & Mountfort, 1981; Fonty *et al.* 1988; Joblin *et al.* 1989). However, this would appear to be an example of synergism through end-product utilization rather than 'unmasking'.

HEMICELLULOSE DIGESTION

Bacteria. Digestion of forage hemicellulose by ruminants was recognized as far back as the early 1900s. This activity was eventually traced to the rumen microbial population and studied in vitro with mixed cultures (Dehority *et al.* 1962). One of the more interesting findings was that, similar to cellulose digestion from forages, rate and extent of hemicellulose digestion decreased markedly with plant maturity. Studies were subsequently initiated on the degradation of isolated hemicelluloses by pure cultures of rumen bacteria (Dehority, 1965). All the cellulolytic species were able to degrade (change to a form soluble in acidified ethanol (800 ml/l)) the isolated plant hemicelluloses, regardless of whether they were able to utilize them as an energy source. Where applicable, rates of utilization were considerably slower than rates of degradation (Dehority, 1967).

Dehority & Scott (1967) measured hemicellulose digestion from two maturity stages each of bromegrass (*Bromus inermis*) and lucerne by pure cultures of both cellulolytic and non-cellulolytic rumen bacteria. Extent of digestion ranged from 0 to 53%, and varied with strain, forage type and forage maturity. In a later study (Coen & Dehority, 1970), both degradation (solubilization) and utilization of forage hemicellulose were measured. A portion of these findings, shown in Table 3, would support the following conclusions: (1) a major cellulolytic species (B34b), extensively degraded both grass and lucerne hemicellulose; (2) *B. ruminicola* (H8a) and *L. multiparus* (D15d) were unable to degrade or utilize grass hemicellulose, however, they were able to degrade and utilize lucerne hemicellulose to a limited extent; (3) both degradation and utilization decrease with forage maturity; (4) marked synergism in both degradation and utilization was observed by combining a degrading, non-utilizing cellulolytic strain (B34b) with a utilizing strain (*B. ruminicola* or *B. fibrisolvens*); (5) no synergism was observed when the cellulolytic strain was combined with *L. multiparus* and an actual decrease in utilization was noted with lucerne as a substrate and (6) if the hemicellulose was isolated from fescue (*Festuca pratensis*) grass, it could be almost completely degraded and utilized

Table 3. *Percentage degradation (deg) and utilization (utl) of hemicellulose from two stages of bromegrass (*Bromus inermis*), lucerne (*Medicago sativa*), fescue grass (*Festuca pratensis*) and isolated fescue grass hemicellulose**

	Forage†								Isolated fescue hemicellulose‡	
	Brome 1		Brome 2		Lucerne 1		Fescue			
Strain	deg	utl	deg	utl	deg	utl	deg	utl	deg	utl
B34b	77·8	0	61·1	0	56·3	2·1	66·6	3·0	88·5	0
H10b	51·9	41·3	32·5	27·1	35·4	34·1	44·8	38·0	87·5	83·8
H8a	4·7	6·1	5·0	6·1	33·6	33·9	2·7	2·0	82·0	80·4
D15d	2·2	4·8	3·1	2·6	49·5	23·2	4·0	1·3	1·7	1·7
B34b+H10b	81·3	69·6	65·8	58·5	61·9	43·2	67·3	64·8	91·3	87·8
B34b+H8a	84·1	80·3	70·3	67·2	59·6	54·8	69·0	67·7	93·9	87·0
B34b+D15d	78·3	3·5	62·9	2·1	61·8	14·9	67·9	3·9	87·0	3·8
All	83·5	78·7	70·6	67·3	61·8	58·4	67·6	65·9	87·4	85·7

B34b, *Ruminicoccus flavefaciens*; H10b, *Butyrivibrio fibrisolvens*; H8a, *Bacteroides ruminicola*; D15d, *Lachnospira multiparus*.

* Values from Coen & Dehority (1970).

† Agronomic description: Brome 1, boot stage; Brome 2, bloom stage; Lucerne 1, prebloom.

‡ Hemicellulose was isolated from the same stand of fescue grass as listed under forage.

by all species except *R. flavefaciens* and *L. multiparus*, and (7) the combination of all four organisms was no better than the best combination of two organisms. With these bacterial cultures, it seems obvious that the hemicellulose must either be solubilized out of the forage or isolated chemically, before it is available to the utilizers. The synergistic effect clearly seems to be the result of 'unmasking' or freeing the hemicellulose.

Very similar results were obtained by Morris & van Gylswyk (1980), who measured degradation and utilization of pentose from teff (*Eragrostis tef*)-hay cell walls with pure cultures of hemicellulose-utilizing rumen bacteria. Chesson *et al.* (1986) also observed considerable loss or solubilization of xylose and arabinose from cell walls of perennial (*Lolium perenne*) and Italian ryegrass (*Lolium multiflorum*) by the three major cellulolytic species. In both studies, *B. ruminicola* was very limited in its ability to solubilize DM from the cell walls, confirming the previous observations of Coen & Dehority (1970). Differences in the ability of *R. albus* and *C. longisporum* to solubilize hemicellulose from barley straw, lucerne and ryegrass have been reported by Varel *et al.* (1989).

In the recently published study by Osborne & Dehority (1989), details of which were described previously, hemicellulose degradation and utilization from the two maturity stages of orchardgrass were also determined. Although a different species of cellulolytic bacteria and different strain of hemicellulolytic bacteria were used, the synergistic response was almost identical to previous results (Coen & Dehority, 1970).

Protozoa and fungi. Information on hemicellulose digestion by the rumen protozoa and fungi is quite limited. Hemicellulase activity has been demonstrated in cell-free extracts from a number of protozoal species (Orpin, 1983–4; Williams & Coleman, 1985; Williams, 1986), and all three species of rumen fungi have been shown to digest both purified xylan and hemicellulose from intact forages (Orpin & Letcher, 1979; Orpin & Hart, 1980; Orpin, 1983–4; Hebraud & Fevre, 1988; Phillips & Gordon, 1988; Gordon &

Table 4. *Percentage degradation (deg) and utilization (utl) of purified pectin and pectin from two maturity stages each of lucerne (*Medicago sativa*) and bromegrass (*Bromus inermis*)**

			Forage†							
	Purified pectin		Lucerne 1		Lucerne 3		Brome 1		Brome 2	
Strain	deg	utl	deg	utl	deg	utl	deg	utl	deg	utl
B34b	30·1	4·0	70·5	30·4	54·3	26·6	71·3	29·8	35·5	8·1
D31d	74·9	47·2	31·3	29·1	29·3	24·1	43·3	49·7	1·0	2·6
D16f	91·8	82·0	67·5	57·3	54·4	53·1	55·3	49·7	46·7	45·3
B34b+D31d			83·2	82·3	74·0	74·3	72·6	70·1	52·5	53·0
B34b+D16f			78·4	74·2	67·4	64·5	68·8	54·3	43·7	34·8

B34b, *Ruminicoccus flavefaciens*; D31d, *Bacteroides ruminicola*; D16f, *Butyrivibrio fibrisolvens*.

* Values from Gradel & Dehority (1972).

† Agronomic description: Lucerne 1, prebloom; Lucerne 3, late bloom; Brome 1, boot stage; Brome 2, bloom stage.

Phillips, 1989; Theodorou *et al.* 1989). However, the contribution of their hemicellulolytic activity to possible synergism in the rumen remains to be elucidated.

PECTIN DIGESTION

Bacteria. Dehority *et al.* (1962), using mixed-culture fermentations, were able to show that both rate and extent of pectin digestion decreased markedly as the lucerne plant matured. Using pectin as a substrate, the major pectinolytic species which could be isolated from the rumen were *B. fibrisolvens*, *B. ruminicola* and *L. multiparus* (Dehority, 1969).

Quite unexpectedly, it was found that some non-pectin utilizing strains could degrade or solubilize pectin, very similar to the situation previously observed with the hemicelluloses (Dehority, 1965; Coen & Dehority, 1970; Gradel & Dehority, 1972). This activity was later confirmed in the studies of Morris & van Gylswyk (1980), using teff-hay cell walls as substrate. Representative values from the study by Gradel & Dehority (1972) on digestion of pectin from intact forages is shown in Table 4. *R. flavefaciens* B34b degraded a limited amount of purified pectin; but was essentially unable to utilize it as an energy source. In contrast, this organism extensively degraded pectin from the intact forage and could utilize up to 30%. The two other species, *B. ruminicola* D31d and *B. fibrisolvens* D16f, extensively degraded and utilized purified pectin. Combining either D31d or D16f with B34b generally increased degradation or utilization over the value obtained with either organism alone, and in some cases, true synergism was observed. For example, a synergistic increase was obtained in utilization by combining B34b and D31d for both stages of lucerne and brome 2. In contrast, the combination of B34b and D16f on brome 2 actually resulted in a decrease in both degradation and utilization compared with D16f alone.

Pectin digestion was also measured in the study by Osborne & Dehority (1989). Their findings, for two maturity stages of orchardgrass, are shown in Table 5. Quite unexpectedly, *B. ruminicola* H2b, the hemicellulose utilizer, utilized more pectin than the pectinolytic strain, *L. multiparus* D15d. The ability of these organisms to degrade

Table 5. *Percentage degradation (deg) and utilization (utl) of purified pectin and pectin from two maturity stages of orchardgrass (*Dactylis glomerata*)**

	Orchardgrass					
	Immature		Mature		Purified pectin	
Strain	deg	utl	deg	utl	deg	utl
A3c	68·5	0	61·2	4·3	17·9	9·5
H2b	54·9	46·1	40·9	29·5	12·1	5·1
D15d	18·9	6·8	28·3	13·1	87·1	73·2
A3c+H2b	83·9	75·3	76·2	61·9	17·9	8·1
A3c+D15d	78·3	0	66·7	4·8	87·8	73·2
H2b+D15d	56·6	49·4	47·2	33·6	87·9	73·4
A3c+H2b+D15d	85·4	76·8	73·1	58·6	88·7	73·5

A3c, *Fibrobacter succinogenes*; H2b, *Bacteroides ruminicola*; D15d, *Lachnospira multiparus*.
* Values from Osborne & Dehority (1989).

and utilize purified pectin was re-investigated, and the results, shown in the last two columns of Table 5, were quite similar to those previously reported (Dehority, 1969). These findings would raise a question as to whether those organisms isolated with a purified polysaccharide substrate may or may not be representative of the important or functionally active species which are present in the rumen.

Protozoa. Orpin (1983–4) has summarized the information available on the occurrence of the pectin-degrading enzymes found in cell-free extracts of different genera and species of rumen ciliates. Pectinesterase (*EC* 3.1.1.11) and polygalacturonase (*EC* 3.2.1.15) activity occurs in the *Isotrichidae* and *P. multivesiculatum*, while endopectate lyases occur only in the higher ophryoscolecids (Coleman *et al.* 1980; Orpin, 1983–4; Williams, 1986). Entodinia appear to be devoid of any enzymes active against pectin (Coleman *et al.* 1980).

Fungi. The rumen fungi apparently are not capable of utilizing pectin for growth (Orpin, 1988). Although cell-free enzyme preparations from fungi could release reducing saccharides from pectin, polygalacturonic acid was not degraded and would not support growth (Williams & Orpin, 1987).

CONCLUSIONS

Most of the studies conducted to date would directly or circumstantially support the 'masking' theory as an explanation for the microbial synergism observed in the digestion of forage polysaccharides. For the bacteria, the extent of synergism appears to be greatest with the hemicelluloses, followed by pectin and then cellulose. Presumably the synergistic increase in digestibility would be time-related, i.e. one organism first degrades part of the forage polysaccharides and the second organism either utilizes the solubilized material or can now physically reach the remaining insoluble structural carbohydrates. Osborne & Dehority (1989) attempted to study this by sequential addition of the second culture after 30 d; however, no differences were observed regardless of whether they were added simultaneously or sequentially in either order. However, the first organism was found to be still viable after 30 d, which probably would

offset the sequential addition. Measurement of growth by the individual strains over time might provide more reliable information on this point. Work is now in progress to develop the methodology which would allow counting each organism individually in a co-culture.

If digestibility is limited because access to the polysaccharides is restricted, then penetration of plant tissues by fungal rhizoids might provide another form of microbial synergism (Ho *et al.* 1988; Akin *et al.* 1989). Physical disruption of the resistant tissues would allow the rumen microbes greater access to digestible portions of the plant.

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