EFFECT OF RUMINAL MICROBIAL COLONIZATION ON CEREAL GRAIN DIGESTION

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The objective of this study was to examine the microbial digestion and colonization of whole (W), halved (H) and quartered (Q) cereal grains within the rumen. Barley (*Hordeum vulgare*L.), maize (*Zea mays* L.), sorghum (*Sorghum bicolor* (L.) Moench), and wheat (*Triticum aestivum* L.) were placed in nylon bags in the rumen of a fistulated steer. In sacco DM disappearance (ISDMD) of W grain was lower ($P<0.001$) than that of H or Q grain. Once sectioned, wheat was most susceptible to microbial digestion followed by barley, sorghum and maize, respectively. Scanning electron microscopy showed that microbial colonization of W grain was restricted to fractured areas of the pericarp. Fracturing of the pericarp is necessary to allow rumen bacteria to gain access to the rapidly digestible nutrients of the endosperm. Initial colonization (2 h) of the endosperm of H and Q grains by rumen bacteria tended to be between large starch granules. After 24 h of exposure in the rumen, the endosperm of barley, wheat and sorghum was colonized by a variety of rumen bacteria. In contrast, regions of the germ and horny endosperm in maize were not colonized. The sequential colonization of the endosperm, culminating in the establishment of complex microbial consortia, is required for the digestion of cereal grains.

Key words: Cereal, rumen bacteria, digestion, processing, concentrate

[Effet de la colonisation microbienne du rumen sur la digestion de grains de céréales.] Titre abrégé: Digestion séquentielle de céréales par des bactéries ruminales.
Cette étude a pour objet d'examiner la digestion et la colonisation microbienne de grains de céréales entiers (W), coupés en deux (H) et en quatre (Q) parties dans le rumen. On a mis de l'orge (*Hordeum vulgare* L.), du maïs (*Zea mays* L.), du sorgho (*Sorghum bicolor* (L.) Moench) et du blé (*Triticum aestivum* L.) dans des sacs de nylon dans le rumen d'un bouvillon fistulé. La digestion in sacco de matière sèche du grain entier (W) est plus faible que celle du grain H ou Q. Une fois sectionné, le blé est plus sensible à la digestion microbienne, suivi par l'orge, le sorgho et le maïs respectivement. La microscopie à balayage électronique montre que la colonisation microbienne du grain W est restreinte aux zones fracturées du péricarpe. La fracture du péricarpe est donc nécessaire pour permettre aux bactéries ruminales d'avoir accès aux substances nutritives facilement digestibles de l'endosperme. La colonisation initiale (2 heures) de l'endosperme des grains H et Q par les bactéries ruminales a tendance à se faire entre les gros granules d'amidon. Au bout de 24 heures d'exposition dans le rumen, l'endosperme de l'orge, du blé et du sorgho est colonisé par diverses bactéries ruminales. À l'inverse, certaines zones du germe et de l'endosperme cornu du maïs ne sont pas colonisées. La colonisation séquentielle de l'endosperme qui culmine par l'établissement d'une flore microbienne complexe est donc nécessaire pour la digestion des grains de céréales.

Mots clés: Céréale, bactéries ruminales, digestion, transformation, concentré

Historically, ruminants have obtained the majority of their energy requirements from forages. Recently, greater demands for animal products have increased the need for improvements in the production and growth rates of ruminants. As a result, cereal grains, which have a higher metabolizable energy content than forages, have been used to meet the greater energy demands associated with increased ruminant production. Nordin and Campling (1976) and Moran (1986) have shown that cereal grains differ in their susceptibility to digestion by rumen microorganisms. Nordin and Campling (1976) demonstrated that whole cereal grains are virtually indigestible within the rumen. If these cereal grains are rolled or ground their susceptibility to microbial attack in the rumen is increased (Theurer 1986).

Starch is the principal component of cereal grains. Minato and Suto (1979) suggested that bacterial attachment may be an important requirement for microbial digestion of starch in the rumen. A recent study (McAllister et al. 1990) has shown that a variety of bacterial morphotypes have the capacity to attach and digest barley starch. In contrast, the starch granules of partially digested maize were colonized nearly exclusively by large and distinctive coccoid cells resembling *Sarcina*. The different bacterial morphotypes associated with the digestion of barley and maize starch may account for some of the differences observed between the digestion of maize and barley in the rumen.

Barley, maize, wheat and sorghum are the major cereal grains fed to ruminants in North America. It is likely that the rates of digestion of these feedstuffs in the rumen are largely dependent on the extent and efficiency of microbial colonization of these cereals. This study was conducted to further characterize the relative digestive rates of these important feeds, and to examine their in vivo microbial colonization and digestion. Scanning electron microscopy (SEM) was used to obtain a fuller understanding of the relationship between these two occurrences.

MATERIALS AND METHODS

A nylon bag digestion trial was carried out using a ruminally canulated Holstein steer fed twice daily (08:00 h and 16:00 h) on a 70% concentrate (50:50 barley:maize), 30% forage (cubed alfalfa hay) diet. Total intake was restricted to 10 kg DM d^{-1}. The steer was adapted to the diet for 7 d prior to the trial.

Maize, barley, sorghum and wheat kernels were halved and quartered with a scalpel. For measuring the exposed interior surface of sectioned kernels, a Tracor Northern 8502 Image Analyzer was used. Quartered or halved kernels were fixed in petri dishes using a silicone glue. Cut surfaces of the kernels were oriented perpendicular to the viewing plane. Image input of the kernels was via a Dage 81 high resolution camera. To enhance the discrimination of the cut surface, a 5% aqueous staining solution of toluidine blue and Azure A, in equal proportions, was applied. Digital grey scale images of the kernels were acquired and binary images were created by grey scale intensity threshold to obtain an exact representation of the sectioned surface area. The analysis performed provided the cut surface area of 10 halved or quartered kernels for each cereal grain. The total exposed interior surface area of the cereal grains included the germ, endosperm and thickness of the pericarp.

Six sets of quadruplicate nylon bags containing 3-g samples of whole (W), halved (H) or quartered (Q) barley, maize and wheat were placed in the rumen. Sorghum was incubated in the rumen only in W or H forms because of problems in sectioning the small seeds into quarters. Bags were made of monofilament nylon mesh (53-μm pore size; 41% open area) and measured 80 × 50 mm. A quadruplicate set of bags for each grain was removed after 2, 4, 8, 12, 24 and 48 h incubation in the rumen. Triplicate bags were also incubated in autoclaved rumen fluid for 0.5 h, to correct for washing losses. Immediately after incubation, bags were washed under cold tap water until the water appeared clear. Three bags from each time period were dried at 70°C for 48 h and weighed to determine in sacco dry matter disappearance (ISDMD).

The contents of the fourth bag were prepared for SEM as follows: grain specimens were fixed for 3 h in 5% glutaraldehyde in 0.1 M cacodylate (pH 7.2). After five washes in cacodylate buffer the specimens were dehydrated in a graduated ethanol series, critical-point dried (Cohen et al. 1968), mounted on aluminum stubs with silver paste and sputter-coated with gold. Specimens were viewed using a Hitachi S-570 scanning electron

microscope. Ilford FP4 panchromatic film was used to photograph the specimens.

The experiment was analyzed using the general linear model (GLM) procedure of the Statistical Analysis System Institute, Inc. (SAS) (1985). The model included species of cereal (df=3), process (df=2), species × process (df=6), time (df=6), species × time (df=18), process × time (df=12), species × process × time (df=36) and residual error (df=169). Differences among means were tested for significance using the least square means (LSMEAN) procedure of SAS (1985).

Estimates of rate of ISDMD were obtained for halved and quartered grains using the equation

$$p = a + b\,(1+e^{-ct})$$

(Orskov and McDonald 1979). The absence of an asymptote in the digestion of whole cereal grains, within the time period examined, prevented the calculation of rate estimates. The nonlinear regression (NLIN) procedure of SAS (1985) was used to obtain best fit curves to calculate ISDMD rates.

RESULTS

Whole kernels were virtually indigestible within the rumen (Fig. 1a). After 48 h of incubation in the rumen, the ISDMD for barley, maize, sorghum and wheat was 11, 14, 19 and 23%, respectively.

The ISDMD of halved kernels (Fig. 1b) was greater ($P<0.001$) than that of whole kernels. The four cereal species exhibited different ($P<0.001$) rates of ISDMD. Wheat exhibited the highest ISDMD followed by barley, sorghum and maize, respectively (Table 1). After 12 h of incubation in the rumen, the amount of ISDMD for halved wheat, barley, sorghum and maize was 50, 31, 25 and 13%, respectively (Fig. 1b). Sectioning of the cereal grains into quarters increased the interior surface area available for colonization (Table 2). The greater area available for microbial digestion resulted in an accelerated ($P<0.05$) rate of ISDMD for barley and wheat but the rate of ISDMD was not significantly different between halved and quartered maize kernels (Table 1). Quartering of the kernels tended to cause a decrease in the slowly degradable fraction of DM (Table 1). After 12 h of incubation in the rumen, the ISDMD for quartered wheat, barley, and maize was 60, 51, and 15%, respectively (Fig. 1c).

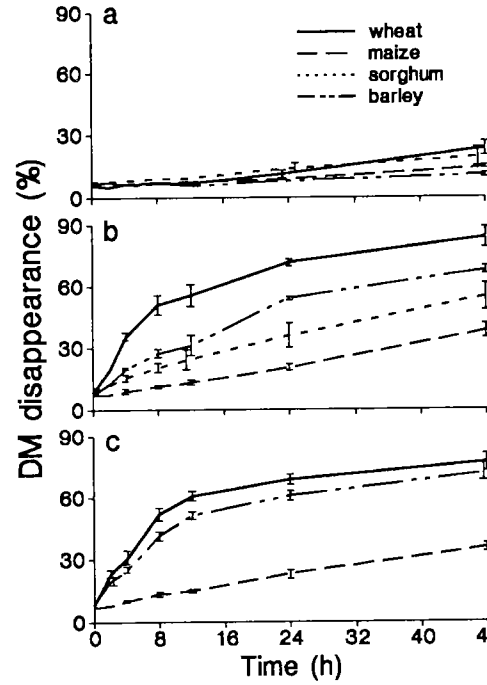

Fig. 1. Dry matter disappearance of cereal grains incubated in the rumen. (a) Whole cereal grains, standard error of mean (SEM) = ± 1.1. (b) Cereal grains sectioned into halves, SEM = ± 3.1. (c) Cereal grains sectioned into quarters, SEM = ± 2.2.

Examination by SEM showed that the outside surface of the kernels was almost devoid of bacteria throughout the incubation period, indicating extreme resistance to bacterial colonization. Even after 48 h of incubation in the rumen, the colonization of whole barley was restricted to damaged areas of the husk (Fig. 2).

Sectioning the kernels allowed bacteria access to the nutrient-rich components of the endosperm. After 2 h of incubation in the rumen, a limited number of bacteria had colonized the endosperm of the kernels, although few starch granules exhibited signs of amylolysis. The starch granules of most of the kernels, e.g., wheat (2 h), were colonized by a morphologically varied bacterial population

Table 1. Parameter estimates of the rate of dry matter disappearance for cereal grains in halved or quartered forms†

Cereal grain	Processing‡					
	H			Q		
Parameter§	a	b	c	a	b	c
B¶	6.18	71.97	0.042	8.01	63.59	0.089
SE	1.17	5.76	0.007	1.32	2.24	0.009
M¶	6.00	100.00	0.007	6.91	67.12	0.017
SE	0.46	0.00	0.001	0.52	24.63	0.006
W¶	7.59	75.17	0.089	6.83	68.64	0.121
SE	2.04	3.84	0.012	1.67	2.36	0.012
S¶	9.94	81.01	0.016	–	–	–
SE	1.58	35.56	0.011			

†Parameters calculated from the fitted equation: $p = a+b(1\text{-}e^{\text{-}ct})$ using ISDMD at 0, 2, 4, 8, 12, 24 and 48 h. Where p = amount of ISDMD at time (t), a = rapidly soluble fraction, b = slowly degradable fraction, c = fraction rate constant at which b is degraded.
‡H, halved; Q, quartered.
§Value ± asymptotic standard error.
¶B, barley; M, maize; W, wheat; S, sorghum.

Table 2. The effect of sectioning of cereal grains on the exposure of interior surface area†

Cereal grain‡	Exposure of interior surface (mm^2)	
	H	Q
B	6.51 ± 0.37	22.09 ± 1.13
M	24.11 ± 0.88	52.10 ± 1.32
W	6.59 ± 0.36	20.59 ± 0.80
S	9.91 ± 0.64	–

†Values are presented as mean of ten kernels ± standard error. Interior surface area consists of germ, endosperm and thickness of the pericarp.
‡See abbreviations in Table 1.

(Fig. 3). Bacteria were attached to starch granules by their extensive glycocalyces (arrows). In contrast, the areas between starch granules in maize (8 h) were colonized by rod-shaped bacteria, whereas the surfaces were colonized by large cocci (Fig. 4). In the later stages of digestion (24 h), the maize starch granules were colonized by a variety of adherent microcolonies (Fig. 5). By this time (24 h), the starch granules observed in other grains (e.g., sorghum, Fig. 6) also exhibited evidence of extensive bacterial amylolytic digestion. After 48 h of incubation in the rumen, the interior of most of the kernels, except for maize, was almost completely digested and was covered by a thick biofilm of primarily rod-shaped bacteria (Fig. 7). In maize, the floury endosperm was colonized by a variety of bacteria, but the germ was virtually uncolonized even after 48 h of exposure in the rumen (Figs. 8, 9). Additionally, the maize horny endosperm exhibited few signs of digestion (Fig. 10) and closer examination showed that this region was only sparsely colonized by rumen bacteria (Fig. 11).

DISCUSSION

The present work indicates, as did previous studies (Nicholson et al. 1971; Galyean et al. 1976; Morgan et al. 1988), that whole kernels of cereal grains are poorly digested by rumen bacteria. Examination by scanning electron microscopy showed that the kernel surface effectively resisted colonization and prevented rumen bacteria from gaining access to the endosperm. When kernels are fed to cattle, mastication damages the outside surface and improves the digestibility of whole kernels, a factor which is not considered by the nylon bag method. However, many whole kernels escape damage by mastication, maintain their resistance to microbial colonization, and are not digested (Nicholson et al. 1971; Wilson et al. 1973). Thus, the disruption of the pericarp by physical processing is required for efficient microbial digestion of cereal grains by cattle (Hale 1980).

Sectioning of the kernels greatly increased dry matter disappearance from nylon bags.

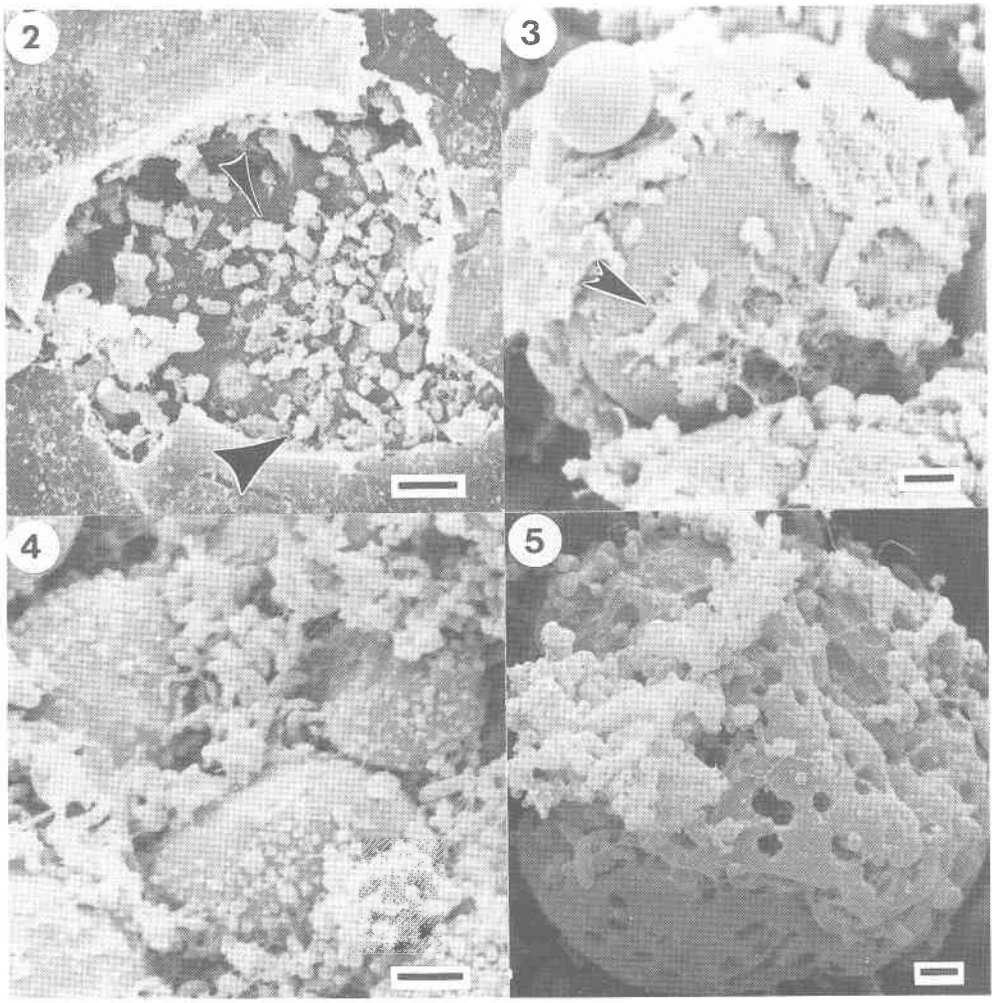

Fig. 2. SEM of the husk of barley after 48 h exposure in the rumen. Note the extensive bacterial colonization of the damaged area (arrows), while intact areas are virtually devoid of bacteria. Bar = 3 μm.
Fig. 3. SEM of the endosperm of sectioned wheat after 2 h exposure in the rumen. The starch granule is colonized by a variety of bacterial morphotypes. Bacteria attach to the granule by their glycocalyces (arrow). Bar = 3 μm.
Fig. 4. SEM of the floury endosperm of sectioned maize after 8 h exposure in the rumen. The areas between starch granules are colonized by rods while the surfaces are colonized by coccoid bacteria. Bar = 3 μm.
Fig. 5. SEM of starch granule from sectioned maize after 24 h of exposure in the rumen. At a later stage in digestion the starch granule is colonized by a variety of bacteria. Bar = 1 μm.

The observed ranking of ISDMD in sectioned grains, i.e., wheat > barley > sorghum > maize, is in agreement with the results of Nordin and Campling (1976). Microbial response to differences in the chemical and physical nature of the grain is likely responsible for differences in their digestibility in the rumen. Initially, bacteria tend to colonize

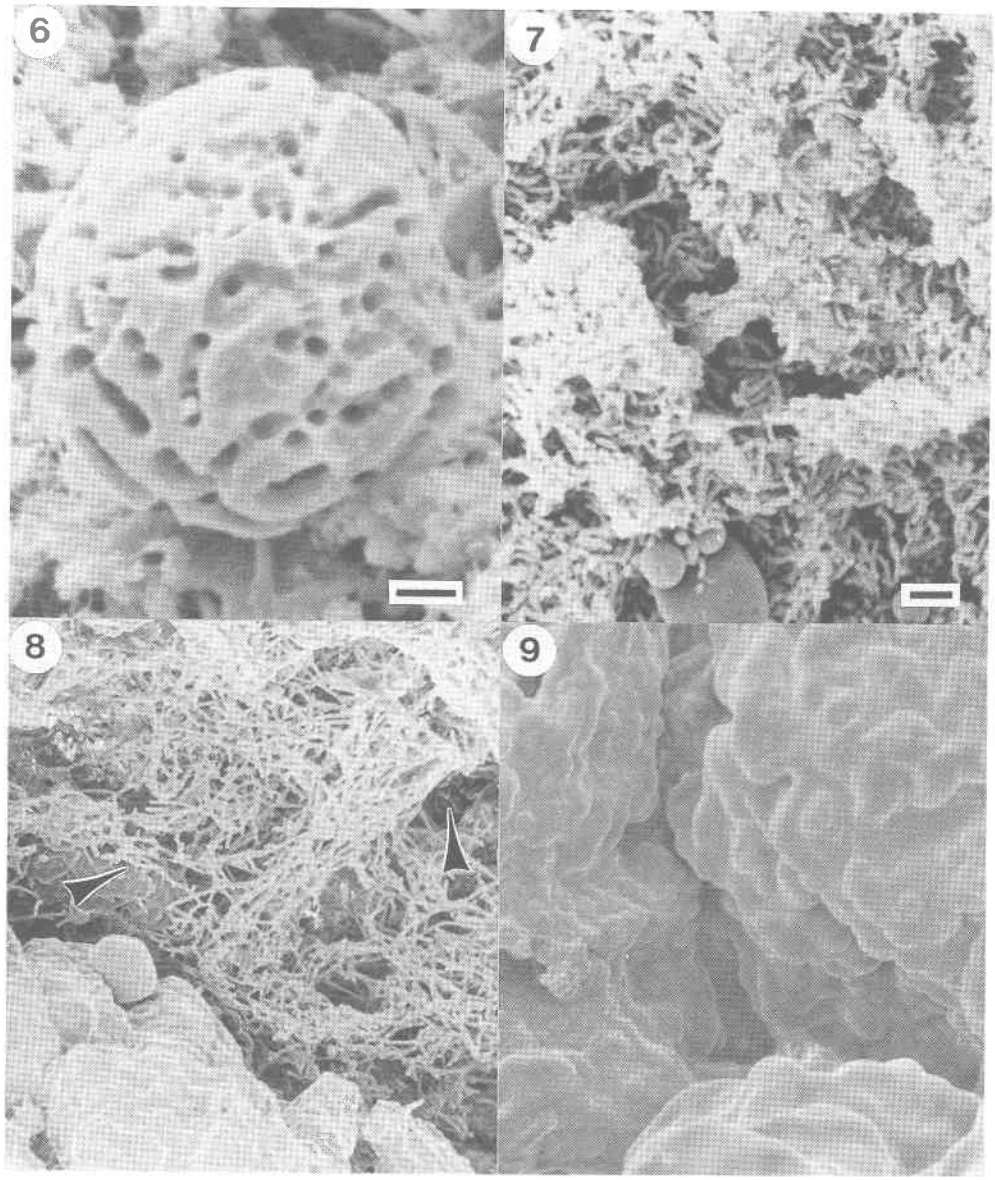

Fig. 6. SEM of a starch granule from sectioned sorghum after 24 h of incubation in the rumen, showing extensive pitting as a result of bacterial amylolysis. Bar = 2 μm.

Fig. 7. SEM of the endosperm of sectioned wheat after 48 h of incubation in the rumen. The endosperm is almost completely digested. The remainder is covered by a thick biofilm of principally rod shaped bacteria. Bar = 4 μm.

Fig. 8. SEM of the interior of sectioned maize after 48 h of exposure in the rumen. Bacteria colonize regions of the floury endosperm (arrows) but fail to colonize the germ. Bar = 10 μm.

Fig. 9. SEM of the germ of the same preparation as in Figure 8. Note the absence of bacterial colonization. Bar = 4 μm.

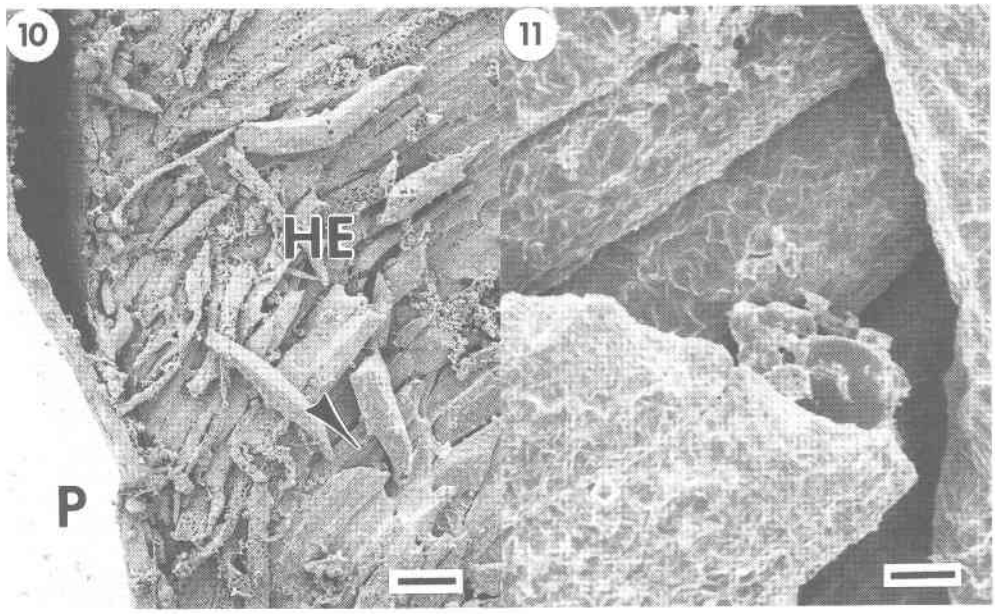

Fig. 10. SEM of the sectioned maize after 48 h of exposure in the rumen. Regions of the horny endosperm (HE) and pericarp (P) show little signs of microbial digestion. Bar = 760 μm.
Fig. 11. SEM of the horny endosperm of the same preparation as indicated by the arrow in Fig. 10. Note the virtual absence of bacteria. Bar = 76 μm.

the regions between starch granules. Only a few starch granules showed evidence of amylolytic digestion. As digestion proceeds (8 h), colonization increases and as was found previously (McAllister et al. 1990), maize starch granules are colonized almost exclusively by coccoid bacteria. In contrast, the starch granules of the other major cereals are colonized by a wide variety of bacteria.

Although the interior of most cereal grains was rapidly colonized, regions of the germ and horny endosperm of maize were sparsely colonized, even after 48 h of incubation in the rumen. The estimates of ruminal escape for protein of maize, sorghum and barley are 65, 52 and 21%, respectively (NRC 1985). The maize germ contains 17–20% protein, whereas the horny endosperm contains 8–10% protein (Earle et al. 1946). The resistance of these protein structures to microbial attack, as demonstrated by SEM, appears to contribute to the greater ruminal escape of maize protein. The presence of zein protein in the horny endosperm and the high oil content of the germ likely contributes to the resistance of these structures to microbial digestion. Previous workers (Ely et al. 1967) have shown that zein protein resists microbial digestion, while Peterson et al. (1975) demonstrated that oil could be used to increase the resistance of dietary protein to microbial attack. Similarly, the amount of starch that escapes rumen fermentation is also greater in maize than in the other cereals (Orskov 1986). The resistance of the horny endosperm to microbial digestion appears to result in more maize starch passing to the small intestine. The reduced ISDMD of maize in comparison with other cereal grains is in part due to the resistance of the germ and horny endosperm to bacterial colonization and digestion.

After 48 h of incubation in the rumen, there was no difference between the ISDMD of halved and quartered kernels. However, the rate of ISDMD of quartered barley and wheat was greater than that of halved barley and wheat. Quartering of the kernels increased the surface area available for initial bacterial

colonization and digestion. The increase in exposed surface of quartered maize did little to enhance the rate of ISDMD, likely because of the limited bacterial colonization and digestion of the germ and horny endosperm.

In the later periods of digestion (24, 48 h), most of the starch granules exhibited extensive digestive pits as a result of amylolytic digestion. Initial colonizers of the starch granules proliferated into large adherent microcolonies. The entire endosperm of most kernels, with the exception of maize, was covered by a bacterial biofilm in which several bacterial morphotypes were observed.

In conclusion, bacterial colonization and digestion are essential processes for the efficient utilization of cereals by ruminants. The sequential colonization of the cereal kernels begins with the substrate-specific colonization of selected regions of the endosperm by bacteria. As digestion proceeds, these adherent bacteria proliferate and develop into large microcolonies. In later stages of digestion, release of nutrients yields a more uniform microenvironment which supports a less site-selective colonization. This results in the formation of a biofilm that completely covers the endosperm. The chemical properties of the kernel, as was shown by the lack of microbial colonization and digestion of the germ and horny endosperm in maize, are important factors in determining susceptibility to bacterial colonization and digestion. If colonization is not possible, as in undamaged cereal grains, these feedstuffs are virtually indigestible in the rumen. The development of methods to control bacterial colonization could prove to be an effective means of regulating the rate of cereal digestion in ruminants.

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