production ecology of the marine picoplankton community. While other marine organisms may assimilate a limited amount of stored nitrogen by the expansion of metabolic pools (*14*), the existence of functionally distinct macromolecular reserves of this element in a member of the marine phytoplankton appears to be a novel observation. Not only is the potential productivity of marine cyanobacteria enhanced by their ability to accumulate substantial reservoirs of nitrogen, but the same strategy may be of further significance in that the availability of nitrogen to potential competitors is restricted.

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Alkaline Hydrogen Peroxide Treatment Unlocks Energy in Agricultural By-Products

Abstract. *Lignocellulosic residues (wheat straw, corncobs, and cornstalks) were treated with a dilute alkaline solution of hydrogen peroxide and suspended in cattle rumen in situ to measure microbial degradation. The rate and extent of dry matter disappearance were markedly increased as a result of the treatment. Results in vivo indicate that this treatment increases the fermentability of wheat straw structural carbohydrates such that this agricultural by-product may be considered an acceptable energy source for the ruminant animal. Treatment of wheat straw allowed more complete bacterial colonization and more rapid degradation of the cell wall.*

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Cellulose has a gross energy content equal to that of starch in cereal grains. However, the close physical and chemical association between structural carbohydrates and lignin and the crystalline arrangement of the cellulose polymer in plant cell walls effectively prevent extensive degradation by cellulolytic microorganisms in the digestive tract of ruminants (*1*). Recently it was demonstrated (*2*) that dilute alkaline solutions of hydrogen peroxide, required by fungi and bacteria to degrade lignin (*3*), partially delignify lignocellulosic materials while simultaneously reducing cellulose crystallinity. Alkaline hydrogen peroxide treatment increases the susceptibility of cellulose in agricultural residues to enzymatic and microbial degradation, suggesting that the treatment may be useful for improving the efficiency with which lignocellulosic materials are digested by ruminants. The poor digestibility of lignocellulose by the ruminant has been identified as a major obstacle to animal protein production in the face of an expanding world population (*4*).

Alkaline hydrogen peroxide treatment (*5*) increased the rate of digestion of corncobs (*Zea mays*), cornstalks, and wheat straw (*Triticum aestivum*), as measured by an in situ procedure (*6*), from 3.76, 4.34, and 2.98 percent per hour to 6.64, 7.18, and 5.96 percent per hour, respectively. The treatment also doubled the extent of digestion of these materials over 48 hours from 47.5, 59.6, and 38.3 percent to 95.4, 95.6, and 88.6 percent, respectively.

Three experiments were conducted in vivo to determine nutrient digestion coefficients and digestible and metabolizable energy contents of feed containing alkaline hydrogen peroxide–treated wheat straw. In experiment 1, 12 growing lambs (average initial weight, 22.5 kg) were assigned to one of four diets containing treated or untreated wheat straw at 36 or 72 percent of dry matter (*7*). After the first experimental period, lambs were rerandomized and the experiment was repeated. Fifteen days were allowed for adjustment to the diet and 5 days for total collection of feces and urine in each period. Within each treatment group, lambs were provided with the amount of feed equal to 90 percent of the intake of the animal with the lowest consumption ad libitum in that group. Experiment 2 was conducted with mature sheep (average initial weight, 63.5 kg) in a 4 × 4 Latin-square design. Diets similar to those in experiment 1 were fed. Each feeding period consisted of 10 days for diet adjustment and 6 days for feed and fecal collections. All sheep were fed similar amounts of dry matter daily across treatments such that dietary intake would not confound nutrient digestibility. Experiment 3 was conducted with the same animals and similar diets and experimental design as in experiment 2 to measure the digestibility of treated wheat straw by sheep fed at a voluntary intake rate (feed intake similar to that expected in a practical situation). All diets were balanced to meet National Research Council recommendations for growing lambs (*8*). The energy content of feed, feces, and urine (experiment 1) was determined by oxygen bomb calorimetry (*9*). The fiber content of feed and feces was determined by the method of Goering and Van Soest (*10*), with α-amylase added during the neutral detergent fiber analysis to aid in filtration (*11*).

In experiment 1 (Table 1), dry matter intake and digestibility were increased such that lambs fed the diets containing 36 and 72 percent treated wheat straw consumed 122 ± 35.8 and 335 ± 35.8 g/day more digestible dry matter, respectively, than did lambs fed untreated

wheat straw. Neutral detergent fiber (cellulose, hemicellulose, and lignin), acid detergent fiber (cellulose and lignin), and cellulose digestibilities were approximately twice as great for lambs fed the treated wheat straw diets as for those fed the untreated wheat straw diets. The digestible and metabolizable energy content of diets increased approximately 1.5 times as a result of treatment. Animals consuming the diet containing 72 percent untreated wheat straw lost an average of 106 g of body weight per day, whereas lambs consuming the diet containing 72 percent treated wheat straw gained weight at a rate (235 g/day) comparable to that of lambs consuming a diet composed predominantly of corn (241 g/day).

In experiment 2, the digestibilities of dry matter, neutral detergent fiber, and acid detergent fiber were greatly increased by treatment with alkaline hydrogen peroxide (Table 1). Cellulose digestibility increased from 56 to 85 percent and from 57 to 86 percent for diets containing low and high levels, respectively, of treated straw.

The results of experiment 3 indicate that treatment of wheat straw overcomes the intake restrictions imposed by this agricultural residue in its native form. The data on digestibility (Table 1) indicate that sheep can extensively digest the treated material when it is fed to them ad libitum, supporting the contention that treatment of lignocellulosic material with alkaline hydrogen peroxide results in a feedstuff that may be used for production purposes.

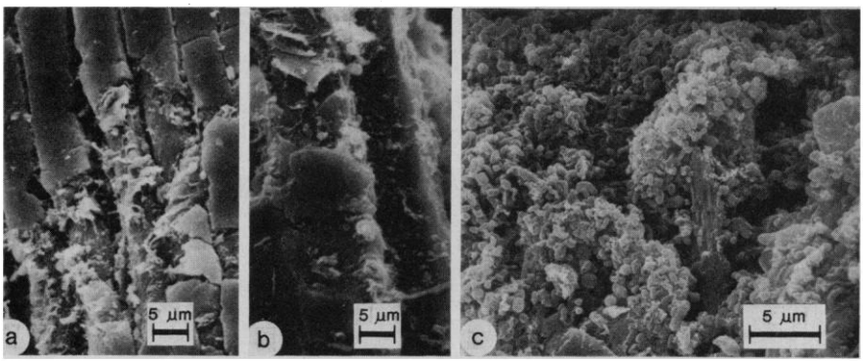

Fig. 1. Scanning electron micrographs of straw particles isolated from the rumen of fistulated mature sheep fed diets containing 72 percent untreated wheat straw (a and b) or 72 percent wheat straw treated with alkaline hydrogen peroxide (c).

The degree of microbial colonization of wheat straw in the rumen, as affected by treatment, was estimated by examining samples of ruminal contents from sheep being fed diets containing 72 percent untreated or treated wheat straw. Because cellulose degradation in the rumen requires attachment of cellulolytic bacteria to the substrate (*12*), straw particles were collected from ruminal fluid and examined by scanning electron microscopy (*13*).

The pattern of attachment of ruminal bacteria to untreated wheat straw particles was very similar to that observed previously with other lignocellulosic substrates (*14*). Bacteria were attached primarily to cut or broken tissue edges, along the inner surfaces of some cells, and in areas that had been physically damaged during feed preparation or by chewing (Fig. 1, a and b). Large areas of the straw particles were almost completely devoid of attached bacteria. In contrast, straw particles obtained from sheep fed treated wheat straw were uniformly covered by a dense population of bacteria (Fig. 1c). Although the factors regulating the attachment of ruminal

Table 1. Feed intakes, apparent digestibilities, digestible and metabolizable energy values, and weight gains of lambs and mature sheep consuming diets containing alkaline hydrogen peroxide–treated and untreated wheat straw at two different concentrations. Values are means.

Treatment and percent of wheat straw in diet	Feed intake (g/day)	Apparent digestibility (%)				Digestible energy (percent of gross energy)	Metabolizable energy (percent of gross energy)	Weight gain (g/day)
		Dry matter	Neutral detergent fiber	Acid detergent fiber	Cellulose			
				Experiment 1				
Alkaline peroxide								
36	985*	72.2*	63.4†	60.2†	64.8†	72.1†	69.6†	241
72	769*	67.4*	73.6†	72.6†	76.6†	66.5†	63.8†	235*
None								
36	863	53.4	28.0	30.0	26.8	52.8	50.1	202
72	434	48.5	43.0	44.3	43.4	48.8	43.2	−106
Standard error	35.8	2.55	3.87	4.04	4.46	2.62	3.33	23.0
				Experiment 2				
Alkaline peroxide								
37	1048	86.4†	85.6†	78.4†	84.5†			
72	1070	79.3†	84.3†	79.3†	85.7†			
None								
37	1026	68.3	54.7	49.1	56.0			
72	1034	56.1	48.7	48.2	56.5			
Standard error	7.2	2.77	4.52	3.96	4.17			
				Experiment 3				
Alkaline peroxide								
33	2234	82.7†	78.6†	73.9†	77.8†			
70	2526*	70.7†	72.9†	68.0†	74.6†			
None								
33	2271	68.4	49.4	42.2	43.8			
70	1297	58.0	51.6	49.5	54.3			
Standard error	253.2	3.70	4.57	3.08	2.79			

*Significantly different from corresponding value for untreated wheat straw ($P < 0.05$, least significant difference test). †$P < 0.05$, factorial arrangement of main effect.

microorganisms to lignocelluosic substrates are unknown, it is clear that alkaline hydrogen peroxide treatment removes a significant barrier to attachment, allowing more rapid cell wall colonization and digestion.

The finding that alkaline hydrogen peroxide renders plant fibers more digestible by ruminants suggests that many alternative feed sources, including crop residues and other cellulosic plant biomass, may be used in animal production. Utilization of cellulose in this way makes available a nearly inexhaustible feed supply. Furthermore, the ability to feed highly digestible cellulosic materials to ruminants would eliminate competition between present animal production practices and the demands of an expanding world population for cereal grains.

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7. In experiment 1, low-straw diets were composed of wheat straw (36 percent, treated or untreated), ground corn (46.2 percent), soybean meal (11.7 percent), liquid cane molasses (5.5 percent), calcium carbonate (0.7 percent), and vitamin supplement (0.2 percent); high-straw diets were composed of wheat straw (72 percent, treated or untreated), ground corn (3.5 percent), soybean meal (18.2 percent), liquid cane molasses (5.5 percent), calcium carbonate (0.2 percent), dicalcium phosphate (0.4 percent), and vitamin supplement (0.2 percent). Diets fed in experiments 2 and 3 were essentially the same as those in experiment 1, except that urea was added (0.5 percent at the expense of soybean meal) to ensure optimal ruminal ammonia levels.
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13. Samples (~25 ml) were withdrawn directly from the rumen through a fistula, and the suspended straw particles were collected by centrifugation, washed in 0.1*M* phosphate buffer (*p*H 7.0), and fixed in 4 percent glutaraldehyde for 24 hours. The samples were postfixed in 2 percent OsO_4 for 3 hours, dehydrated by sequential ethanol washes (25 percent concentration steps), and critical-point-dried (CO_2). After being coated with gold and palladium, the straw particles were examined with an ISI model SS130 scanning electron microscope.
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Synthesis of Fibrils in Vitro by a Solubilized Cellulose Synthase from *Acetobacter xylinum*

Abstract. *A digitonin-solubilized cellulose synthase was prepared from* Acetobacter xylinum. *When this enzyme was incubated under conditions known to lead to active synthesis of 1,4-β-D-glucan polymer (cellulose), electron microscopy revealed that clusters of fibrils were assembled within minutes. Individual fibrils are 17 ± 2 angstroms in diameter. Evidence that the fibrils were freshly synthesized and cellulosic in nature was their incorporation of the tritium from UDP-[^{3}H]glucose (UDP, uridine 5′-diphosphate), their binding of gold-labeled cellobiohydrolase, and an electron diffraction pattern with 004, 200, and 012 reflections (characteristic of cellulose synthesized in vivo) but missing 110 and $\bar{1}10$ reflections. The small size of the fibrils is atypical of native* A. xylinum *cellulose microfibrils. The fibrils synthesized in vitro resemble, in morphology and size, the fibrillar cellulose produced when* A. xylinum *is cultured in the presence of agents that interfere with the normal process of crystallization of the microfibrils. The solubilized enzyme unit may therefore be producing a basic fibrillar structure that, in vivo, interacts laterally with other fibrils to produce native cellulose microfibrils.*

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Cellulose, a polymer of 1,4-β-D-glucan, is the most abundant biopolymer on Earth. It is the primary component of such products as wood, cotton, and paper. Cellulose exists in nature as microfibrils, which form the structural framework of the cell walls of plants, some algae, and certain fungi (*1*). Among the few bacteria that synthesize cellulose, *Acetobacter xylinum* has been studied intensely because it produces abundant amounts in growth medium. The cellulose is extruded from a row of pores aligned along the longitudinal axis of the cell (*2*) and forms an extracellular pellicle, which consists of ribbons of cellulose microfibrils. Freeze etching of *A. xylinum* cells has revealed in the outer membrane a row of particles, complementary to the pores, which may take part in the synthesis of cellulose (*3*).

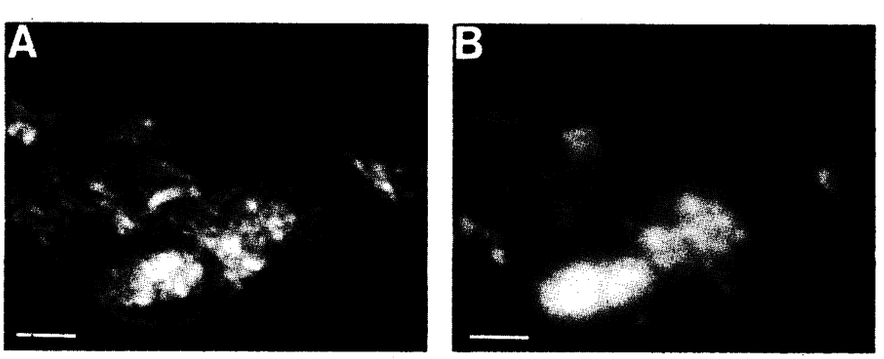

Fig. 1. (A) Dark-field microscopy of in vitro reaction product. Scale bar, 1.0 μm. (B) Fluorescence microscopy image of the product in the presence of 0.5 percent Tinopal LPW (Ciba-Geigy). Scale bar, 1.0 μm.