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Effects of Micronization on the *In situ* and *In vitro* Digestion of Cereal Grains

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ABSTRACT : The effects of micronization on *in situ* and *in vitro* nutrient disappearances of wheat, barley and corn were investigated in a series of experiments. In Experiment 1, chemical composition and *in situ* dry matter disappearance (DMD) of six varieties of wheat were determined. In addition, an *in vitro* study was completed using ground micronized and unmicronized wheat (var. Kansas). In Experiment 2, three varieties of wheat (Kansas, Sceptre and Laura) and in Experiment 3, three cereal grains (wheat, barley and corn) were either micronized for 1 min to attain internal kernel temperatures of 90-100°C or not (controls), and DM, protein and starch disappearances were estimated. In Experiment 2, an *in vitro* study was also completed using ground micronized and unmicronized wheat (var. Kansas). Wheat samples varied with respect to crude protein (10.0-21.2%), starch (61.6-73.9%), NDF (8.5-11.8%), volume weight (753-842 g/L) and kernel hardness (0.0-32.0). Rate ($p = 0.003$) and extent ($p = 0.001$) of i*n situ* DMD differed among wheat varieties. Correlations between *in situ* kinetics, and chemical and physical properties of wheat varieties showed that protein content was negatively correlated with the rate of disappearance ($r^2 = -0.77$). Micronization of all grains markedly reduced ($p = 0.001$) the rate and extent of DM, and protein disappearances as compared to control samples. Micronization increased ($p<0.05$) the digestion of starch in wheat. However, release of ammonia into the incubation medium was markedly reduced ($p<0.05$), suggesting that micronization increased the resistance of protein to microbial digestion. Disappearances of DM, protein and starch differed ($p = 0.001$) among cereal grains with wheat>barley>corn. Micronization reduced the rate of DM disappearance ($p = 0.011$) and slowly degradable protein fractions ($p = 0.03$), however, increased ($p = 0.004$) slowly degradable starch fractions of all three cereals. Examination of *in situ* samples by scanning electron microscopy confirmed that microbial colonization focused on starch granules in micronized grains, and that the protein matrix exhibited resistance to microbial colonization. These results suggest that micronization may be used to increase the ruminal escape value of protein in cereal grains, but may lead to increased starch digestion if grains are finely ground. (**Key Words :** Cattle, Rumen, Micronization, Corn, Barley, Wheat)

INTRODUCTION

Although the use of by-products arising from biofuel industry has been recently increasing (Dilorenzo and Galyean, 2010), cereal grains are still the foundation of the North American feedlot industry. Corn is the principal grain used in the diets of feedlot cattle in the United States and eastern Canada, whereas barley accounts for the majority of grain fed to cattle in western Canada. Wheat is occasionally used in feedlot diets, but is often restricted to 50% of the dietary dry matter or less due to fear of it leading to digestive disturbances such as acidosis (Owens et al., 1998) or bloat (Cheng et al., 1998). All cereal grains are routinely rolled to increase ruminal and total tract starch digestibility and improve the efficiency of their utilization by feedlot cattle (Owens et al., 1997). Wheat differs dramatically in its kernel properties ranging from soft to hard varieties making it necessary for processing procedures for wheat to be carefully monitored to avoid the generation of the fines can that augment digestive disturbances.

Micronization, is a dry-heat process that generates infrared electromagnetic short waves as a result of burning propane over ceramic tile or nichrome wire elements (Mercier, 1971). The procedure has been widely used to process grains for both livestock and human consumption (Shiau and Yang, 1982; McCurdy, 1992; Metussin et al., 1992; South and Ross, 1993). Early studies on micronized sorghum (Croka and Wagner, 1975) and hard red winter wheat (Aimone and Wagner, 1977) showed that

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micronization increased *in vitro* DM digestibility, gas production and degree of starch gelatinization as compared to dry-rolling. In broilers, micronization of wheat at temperatures between 90-100°C was found to improve feed efficiency whereas temperatures of 120°C decreased feed efficiency (Niu et al., 2003). Micronization has also been shown to improve the utilization of corn and sorghum by poultry, possibly by lowering fiber levels in the diet (Douglas et al., 1991).

In ruminants, micronization has been shown to reduce the *in situ* DM disappearance of both full-fat canola seed (Wang et al., 1997) and flaxseed (Mustafa et al., 2002). Such a response may be desirable for cereal grains as it could modulate the rate of acid production, possibly reducing the severity of sub-clinical acidosis. Therefore, a series of experiments was conducted to first examine differences in the fermentation properties of various varieties of wheat and secondly to examine the ability of micronization to regulate the ruminal fermentation of cereal grain.

MATERIALS AND METHODS

Experiment 1

Chemical and physical properties of wheat : Cultivated wheat (*Tritcum spp.*) varieties were obtained from the wheat breeding program of the Saskatchewan Wheat Pool. The first experiment involved six varieties of wheat, i.e., Canada Prairie Spring (CPS) red (Biggar), CPS white (Genesis), Canada Western (CW) red winter (Norstar), CW soft white spring (Owens), CW durum (Kyle) and CW red spring (Katepwa). Wheat samples were dried at 105°C for 24 h to determine DM. Crude protein (CP) was estimated from Kjeldahl N (Method 7.025, AOAC, 1984). Starch was determined by enzymatic hydrolysis of α-linked glucose polymers as described by Herrera-Saldana et al. (1990), except that amyloglucosidase (Boehringer-Mannheim, Dorval, PQ) was substituted for glucoamylase. Neutral detergent fiber (NDF) was determined using the procedure of Van Soest et al. (1991), with sodium sulfite omitted from the procedure. Alpha-amylase was added to wheat samples to solubilize starch and facilitate filtering during NDF analysis. Thousand kernel weights (TKW) were determined with a Count-A-Pak Seed Counter (Seedburo Equipment Co., Chicago, IL, USA). Kernel hardness (KH) was estimated using the particle size index procedure, in which lower numbers indicate harder kernels (American Association of Cereal Chemists, 1983).

In situ evaluation of wheat varieties : The wheat grains were halved transversely with scalpels prior to study as described previously (McAllister et al., 1990). Duplicate 5-g samples were incubated ruminally in monofilament nylon bags (8 cm×5 cm; 53 μm pore size) in each of two ruminally cannulated Holstein steers (405±4.0 kg) for 2, 4, 8, 12, 24, 48 and 96 h, such that four replicate bags were incubated for each time point. Additional samples were incubated for 30 min at 39°C in autoclaved ruminal fluid to estimate DM disappearance not attributable to microbial digestion (0 h). Bags were washed, dried (70°C for 48 h) and weighed as described by McAllister et al. (1990) to determine *in situ* DM disappearance. The steers were fed a diet of 80% concentrate (barley: corn: wheat, at equal portions) and 20% forage (cubed alfalfa hay) at 08:00 h and 16:00 h daily. Steers were adapted to the diet for 14 d prior to the experiment and were cared for in accordance with the standards set by the Canadian Council on Animal Care (CCAC, 1993).

In addition to determination of DM disappearance, protein and starch disappearance were estimated by analysis of the digestion residues. Digestion kinetics of DM, protein and starch were determined, without correction for microbial protein, using the equation of Ørskov and McDonald (1979).

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

where, a = rapidly soluble fraction (%), b = slowly digestible fraction (%), c = fractional rate of disappearance (%/h) and t = time of incubation (h), with the constraint that $a+b \leq 100\%$. The non-linear procedure PROC NLIN of SAS (2007) was used to provide estimates from a, b, and c.

Experiment 2

Micronization of wheat : Wheat was micronized through exposure to infrared radiation for a period of 60 sec in order to achieve internal kernel temperatures of 100°C. Whole wheat was micronized in a natural gas-fired Micro-Red 20 micronizer (Micronizing Company Limited, Suffolk, UK). For all comparisons of micronized vs. control, single lots of three varieties of wheat, CW durum (Sceptre), CW red spring (Laura) and soft red winter (Kansas) were divided into two lots, one of which was micronized and the other served as a control. Varieties were specifically selected to examine the effect of micronization on wheat with a range of kernel hardness. Prior to micronization, sufficient water was added to increase the moisture level of the grain to approximately 15%, followed by micronization for a period of 1 min. This achieved internal kernel temperatures of 100°C and resulted in a final moisture content of 7-9%.

In situ and in vitro evaluation of micronized vs. control wheat : The chemical and physical properties of all three varieties (Sceptre, Laura and Kansas) of micronized and control wheat were determined as described in Experiment 1. The percentage of gelatinized starch in micronized wheat was determined using the glucoamylase method of Chiang and Johnson (1977). The effect of micronization on *in situ*

disappearance of the three varieties of wheat was examined using the same technique as described in Experiment 1. An additional set of bags was incubated in the rumen of each steer, but was excluded from washing and prepared for examination by scanning electron microscopy (SEM). The determination of DM, protein and starch disappearances were performed for micronized vs. control wheat samples as described in Experiment 1.

Inoculum for the *in vitro* study was prepared using ruminal digesta collected from the steers used in the three *in situ* experiments. The anaerobic *in vitro* technique as described by Wang et al. (2000) was used. Briefly, solid ruminal contents and ruminal fluid, were separated by straining whole digesta through two layers of cheesecloth. Fractions were mixed 1:1 (w/v) and blended together for two 30-s periods in a Waring blender (Waring Products Division, New Hartford, CT, USA). The homogenate was strained through four layers of cheesecloth and diluted 1:1 (v/v) with McDougall's buffer (McDougall, 1948). Particles ranging from 2-3 mm in diameter were collected by grinding and sieving micronized and control soft red winter (Kansas) wheat. Inoculum (20 ml) was added to ground wheat (0.5 g) which had been placed in 45-ml serum vials. Triplicate vials were incubated at 39°C and removed from the study after 0, 4, 8, 12, 24 and 48 h. Microbial digestion was arrested by adding 1 ml of 5% mercuric chloride to each vial. Vials were stored at -40°C until being analyzed for starch using the procedure described above and for ammonia using the procedure described by Weatherburn (1967).

Scanning electron microscopy (SEM) : Wheat samples from the *in situ* incubations were prepared for SEM examination according to Bae et al. (1997). Briefly, samples were fixed for at least 3 h with 5% (v/v) glutaraldehyde (J. B. EM Services Inc., Dorval, QC) in 0.1 M sodium cacodylate buffer (pH 7.2). Specimens were washed five times in sodium cacodylate buffer, dehydrated in a graduated ethanol series and critical point dried (Polaron E3100, Polaron Equipment Limited, Watford, England) using liquid CO_2 as the transitional fluid. The specimens were affixed to aluminium specimen mounts with colloidal silver paste, air dried overnight and sputter-coated with gold (approx. 15 nm thicknesses; Denton Vacuum Desk-1, Denton Vacuum Inc., Cherry Hill, NJ, USA). Samples were examined with a Hitachi S-570 scanning electron microscope using an accelerating voltage of 7-10 kV and photographed using Kodak T-Max 100 panchromatic film.

Experiment 3

Micronization - comparison among grain types : A third nylon bag experiment was conducted using the steers as described in Experiment 1 and the effect of micronization on *in situ* disappearance of CW durum wheat, barley (*Hordeum vulgare*) and corn (*Zea mays*) were compared. Samples were analyzed and prepared for SEM as described above.

Statistical analyses

Means for grain *in situ* degradation were analyzed using the GLM procedure with steers treated as blocks (SAS, 2007). Models included variety (Experiment 1), variety, treatment and their interaction (Experiment 2), grain type, treatment and their interaction (Experiment 3). Proc Corr was use to examine potential correlation between chemical/physical and *in situ* parameters of the wheat varieties. When the p-value for the F-statistic was ≤0.05 the PDIFF procedure of SAS was used to separate means.

RESULTS

Experiment 1

Chemical and physical properties of wheat : The chemical and physical characteristics of the six varieties of wheat examined are presented in Table 1. Ranges were especially wide for CP (10.0-18.8%) and KH (0.0-35.5) with lower numbers of KH indicating harder kernels. CW durum (Kyle) showed the lowest KH (KH = 0.0) and the highest CP (18.8%) among varieties (Table 1). Less variation was observed in starch content (61.6-73.9%), NDF (8.5-10.9%), TKW (32.1-39.7 g) and volume weight (VW)(781-842 g/L).

In situ disappearance of wheat : *In situ* DM disappearance differed among varieties (Table 2). The greatest difference occurred between Owens (soft white spring) and Kyle (Durum). The DM disappearance was higher ($p<0.05$) from Owens than from Kyle. The DM disappearances observed for the other varieties were generally intermediate to these two varieties. At 96 h of incubation, the DM disappearance varied ($p = 0.003$) among varieties and it exceeded 95.0% for all varieties.

There was no difference in the rapidly soluble ($p = 0.72$) or slowly degradable ($p = 0.91$) fractions of DM disappearance among wheat varieties, but differences ($p = 0.001$) were observed in the rates of DM disappearance (Table 2). Again, the greatest difference was observed between Kyle (7.1%/h) and Owens (11.1%/h). In general, correlations between *in situ* DM kinetics, and the chemical and physical properties of wheat were poor. The highest correlations was between VW and the slowly degradable fraction ($r^2 = 0.81$) of DM disappearance. Protein concentration of the wheat varieties was negatively correlated ($r^2 = -0.77$) with the rate of DM disappearance.

Experiment 2

Chemical and physical characteristics of micronized vs. control wheat : Micronization numerically reduced % starch

Table 1. Chemical composition and physical characteristics of wheat varieties

Wheat variety	Type	DM (%)	CP (%)	Starch (%)	NDF (%)	TKW[1] (g)	VW[1] (g/L)	KH[1]	Gelatinization (%)
Biggar	CPS red	91.1	12.4	72.7	10.1	38.7	842	15.0	-
Genesis	CPS white	91.1	15.5	73.9	10.1	34.3	817	26.5	-
Norstar	CW red winter	91.0	10.0	73.2	8.5	35.7	835	21.0	-
Owens	Soft white spring	91.1	13.0	67.6	9.7	37.6	781	35.5	-
Kyle	CW durum	91.3	18.8	70.8	9.6	39.7	826	0.0	-
Katepwa	CW red spring	91.2	13.9	61.6	10.9	32.1	794	12.5	-
SD		10.1	2.9	4.6	0.8	2.9	23.9	12.2	
Untreated									
Sceptre	CW durum	88.0	19.5	67.4	10.6	36.8	762	0.0	-
Laura	CW red spring	90.5	21.2	64.8	11.2	28.7	753	17.0	-
Kansas	Soft red winter	90.5	13.6	69.7	11.8	34.5	770	32.0	-
SD		1.4	4.0	2.4	0.6	4.2	8.5	16.0	-
Micronized									
Sceptre	CW durum	92.1	19.9	65.6	12.7	36.1	631	3.0	72.1
Laura	CW red spring	92.3	22.7	62.6	13.8	27.5	581	12.5	64.7
Kansas	Soft red winter	90.8	14.7	68.1	12.9	34.8	635	19.0	49.6
SD		0.81	4.1	2.8	0.5	4.6	30.1	8.0	11.5

[1] TKW = Thousand kernel weight; VW = Volume weight (as-fed basis); KH = Kernel hardness (as estimated by particle size index; lower number indicates harder kernel.

Table 2. Effect of variety and micronization on the *in situ* dry matter disappearance (%) of wheat

Wheat variety	Type	Incubation time (h)	Kinetic parameter[1]		
		96	(a)	(b)	(c)
Experiment 1					
Biggar	CPS red	96.4	0.6	95.6	8.4
Genesis	CPS white	96.5	0.8	95.8	8.0
Norstar	CW red winter	96.2	1.0	94.8	8.9
Owens	Soft white spring	96.7	0.7	97.0	11.1
Kyle	CW durum	96.7	1.0	95.6	7.1
Katepwa	CW red spring	95.7	0.1	96.0	8.2
SE[2]		0.1	0.2	0.5	0.1
Effect of variety (p)		0.003	0.715	0.91	0.001
Experiment 2					
Untreated					
Sceptre	CW durum	90.5	12.3	75.5	4.1
Laura	CW red spring	87.6	12.1	73.6	4.4
Kansas	Soft red winter	83.7	9.7	72.0	6.2
Micronized					
Sceptre	CW durum	72.1	7.1	82.7	1.6
Laura	CW red spring	65.8	6.7	70.6	2.0
Kansas	Soft red winter	73.9	13.3	66.7	2.2
Effect of micronization (p)		0.001	0.009	0.93	0.001
Effect of variety (p)		0.47	0.07	0.25	0.21
Micronization×variety (p)			0.658	0.529	0.539
SE[2]		2.55	0.64	4.04	0.53

[1] (a) = rapidly soluble fraction; (b) = slowly degradable fraction; (c) = fractional rate constant at which (b) is degraded.
[2] SE = Standard error of the mean.

and VW, and increased % DM and % NDF of three varieties of wheat (Table 1). Micronization numerically increased KH of Laura (KH = 12.5) and Kansas (KH = 19.0) but decreased it (KH = 3.0) for Sceptre. The % gelatinization resulting from micronization tended to be increased for Sceptre (72.1%) and Laura (64.7%), which had higher protein content (19.5 and 21.2% CP for Sceptre and Laura, respectively) and harder kernels (0.0 and 17.0 KH for Sceptre and Laura, respectively) than Kansas (CP, 13.6% and KH, 32.0) (Table 1).

In situ evaluation of micronized vs. control wheat : In Experiment 2, the DM disappearance differed among varieties at 0 h (p = 0.001), 8 h (p = 0.05) and 24 h (p = 0.002) of incubation (data not shown). However, wheat varieties had no effect (p>0.05) on *in situ* kinetics of DM disappearance in Experiment 2 (Table 2). Varietal differences (p<0.05) were observed in the disappearance of wheat protein (from 0 h to 24 h, data not shown) and starch (from 0 h to 96 h) as well as in the rapidly soluble fractions of protein and starch (p = 0.001). The rate of starch disappearance also differed (p = 0.001) among varieties (Table 3).

Micronization reduced the DM disappearance of all three wheat varieties at all time points (p<0.05) except 0 h (p = 0.71) in Experiment 2 (data not shown). The rate of DM disappearance of wheat was reduced (p = 0.001) as a result of micronization (Table 2). Micronization reduced *in situ* disappearance of protein (p<0.01) and starch (p = 0.001) as well as the rate of disappearance (p = 0.001) of both nutrients (Table 3). Interestingly, the impact of micronization on the rapidly soluble fractions of DM (p = 0.009), protein (p = 0.10) and starch (p = 0.003) differed among varieties with the soluble fractions being reduced in durum wheat (Sceptre) and increased in soft wheat (Kansas; Tables 2 and 3). The slowly degradable fraction of protein decreased (p = 0.04) whereas, that of starch increased (p = 0.02) as a result of micronization in all three wheat varieties. Micronization also reduced the rates of DM, starch and protein disappearance (Table 3).

In vitro digestion of wheat : Micronization increased

Table 3. Effect of micronization on the *in situ* disappearance (%) of wheat protein and starch

Wheat variety	Type	Incubation time (h)	Kinetic parameter[1]		
		96	(a)	(b)	(c)
Protein					
Untreated					
Sceptre	CW durum	92.9	8.1^{a}	85.7	3.5
Laura	CW red spring	91.2	2.7^{bc}	83.7	5.1
Kansas	Soft red winter	88.9	0.8^{c}	86.8	5.3
Micronized					
Sceptre	CW durum	57.9	5.3^{d}	69.0	2.2
Laura	CW red spring	49.9	5.8^{ad}	58.6	2.1
Kansas	Soft red winter	53.9	3.9^{bd}	81.5	1.0
Effect of micronization (p)		0.001	0.10	0.04	0.001
Effect of variety (p)		0.38	0.001	0.33	0.51
Micronization×variety (p)			0.004	0.508	0.095
SE[2]		3.1	0.57	5.85	0.5
Starch					
Untreated					
Sceptre	CW durum	95.3^{a}	17.3^{c}	78.3	3.9
Laura	CW red spring	97.6^{a}	24.3^{a}	73.8	4.3
Kansas	Soft red winter	85.9^{b}	10.4^{b}	74.4	6.0
Micronized					
Sceptre	CW durum	80.8^{c}	14.4^{c}	85.6	1.5
Laura	CW red spring	84.1^{bc}	14.5^{c}	81.7	2.0
Kansas	Soft red winter	83.6^{bc}	14.0^{c}	79.8	2.0
Effect of micronization (p)		0.001	0.003	0.02	0.001
Effect of variety (p)		0.06	0.001	0.28	0.001
Micronization×variety (p)			0.915	0.003	0.001
SE[2]		1.62	0.7	2.2	0.16

[1] (a) = rapidly soluble fraction; (b) = slowly degradable fraction; (c) = fractional rate constant at which (b) is degraded.
[2] SE = Standard error of the mean.

(p<0.05) the *in vitro* digestion of wheat starch (Figure 1). However, *in vitro* ammonia concentrations were lower (p<0.05) in incubations with micronized wheat than with control wheat (Figure 1).

Experiment 3

In situ disappearance of micronized vs. control cereal grains : In Experiment 3, the DM disappearance differed (p = 0.001) with grain type throughout the period of *in situ* incubation (Table 4). As expected, DM disappearances from wheat and barley were more rapid and more extensive than DM disappearance from corn (Table 4). Disappearance of protein (p = 0.001; except in 8 h of incubation, p = 0.92) and starch (p = 0.001) also differed at all time points (data not shown). In the observation of *in situ* kinetics, the slowly degradable fraction of DM (p = 0.04) and starch (p = 0.001), and the rapidly soluble fraction of protein and starch (p = 0.001) varied among grains (Tables 4 and 5). Generally these parameters illustrated that the disappearance of these parameters among grains exhibited the pattern of wheat> barley>corn.

Micronization reduced DM disappearance of all grains between 2 and 48 h (p<0.05) and this effect was most pronounced for wheat (data not shown). Rate of DM disappearance (p = 0.011) declined for wheat and barley, but increased for corn as a result of micronization. As observed in Experiment 2, micronization reduced (p = 0.001) the rapidly soluble fraction, but did not alter the slowly degradable fraction (p = 0.18) of DM disappearance of all cereals. Consistent with observations in Experiment 2, micronization reduced (p<0.01) the disappearance of cereal protein and starch in most of the time points of incubations (data not shown). Similarly, the slowly degradable protein fractions of the three cereals were reduced (p = 0.03) and the slowly degradable starch fractions were increased (p = 0.004) by micronization. Micronization did not alter (p = 0.42) the soluble protein fraction in the cereal grains, but did reduce (p = 0.001) the soluble starch fraction (Table 5).

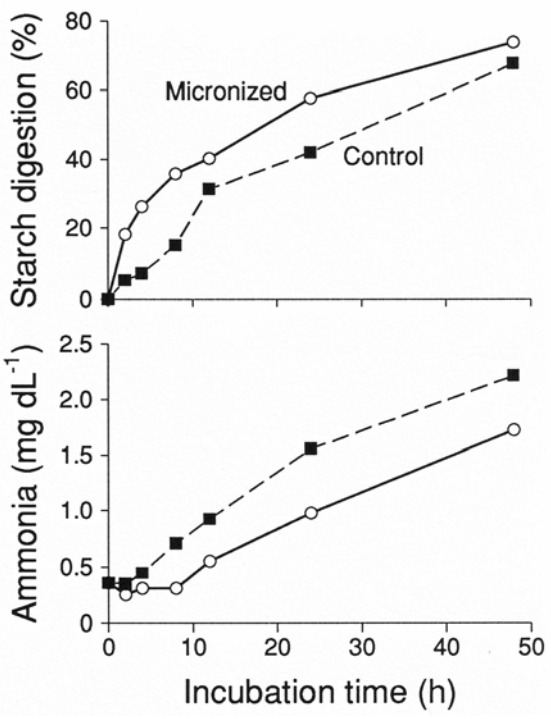

Figure 1. *In vitro* starch digestion and ammonia concentrations of soft red winter wheat var. Kansas over a 48 h incubation period. Each time point had three replicate vials for each treatment, error bars are sufficiently small that they lay within line symbols.

Electron microscopy of micronized vs. control wheat : Although micronization did gelatinize wheat starch, changes in the structural characteristics of the endosperm or starch granules as a result of micronization were not

Table 4. Effect of micronization on the *in situ* DM disappearance (%) of cereal grains

Grain	Incubation time (h)	Kinetic parameter[1]		
	96	(a)	(b)	(c)
Untreated				
Barley	59.8	9.6	49.3	5.7
Corn	38.9	10.2	62.0	0.7
Wheat	67.6	10.9	56.8	5.0
Micronized				
Barley	60.5	8.4	52.2	4.5
Corn	38.6	7.2	62.1	2.8
Wheat	59.1	7.0	70.7	1.4
Effect of micronization (p)	0.248	0.001	0.18	0.011
Effect of grain (p)	0.001	0.868	0.04	0.27
Micronization×grain (p)	0.046	0.009	0.227	0.003
SE[2]	1.93	0.40	3.45	0.65

[1] (a) = rapidly soluble fraction; (b) = slowly degradable fraction; (c) = fractional rate constant at which (b) is degraded.
[2] SE: Standard error of the mean.

Table 5. Effect of micronization on the *in situ* disappearance (%) of protein and starch from cereal grains

Grain	Incubation time (h)	Kinetic parameter[1]		
	96	(a)	(b)	(c)
Protein				
Untreated				
Barley	63.2	11.6	52.9	4.9
Corn	41.6	16.6	49.2	1.1
Wheat	71.8	16.2	53.8	5.5
Micronized				
Barley	48.7	11.5	38.3	4.1
Corn	39.9	15.4	43.3	3.3
Wheat	43.3	15.3	42.5	1.7
Effect of micronization (p)	0.001	0.419	0.03	0.435
Effect of variety (p)	0.001	0.001	0.88	0.207
Micronization×variety (p)	0.004	0.8524	0.717	0.078
SE[2]	2.21	0.72	3.8	0.85
Starch				
Untreated				
Barley	74.8	6.8	67.5	6.8
Corn	46.1	5.6	40.3	5.7
Wheat	80.3	16.4	64.1	4.8
Micronized				
Barley	76.9	2.3	73.4	5.5
Corn	42.4	3.8	58.0	1.9
Wheat	72.9	4.5	84.0	8.8
Effect of micronization (p)	0.274	0.001	0.004	0.88
Effect of variety (p)	0.001	0.001	0.001	0.59
Micronization×variety (p)	0.353	0.010	0.344	0.448
SE[2]	2.2	1.0	3.5	2.1

[1] (a) = rapidly soluble fraction; (b) = slowly degradable fraction; (c) = fractional rate constant at which (b) is degraded.
[2] SE = Standard error of the mean.

apparent (Figure 2A and B). After 2 h of ruminal incubation, the endosperm of control wheat was sparsely colonized (Figure 2C), whereas bacterial colonization of micronized wheat tended to be concentrated in starch granule cavities within the protein matrix (Figure 2D). After longer periods of ruminal incubation (from 12 to 48 h), protozoa were commonly associated with the endosperm of both control and micronized wheat (Figure 3A and B) and bacteria were consistently present on the protozoal surfaces (arrows). Occasionally, protozoa appeared to penetrate the surface of the endosperm (Figure 3A). Bacterial digestion of wheat starch granules from the inside out was observed in both control (picture not shown) and micronized wheat (Figure 3C). Most commonly, the endosperm was colonized by a variety of bacterial morphotypes, but occasional observations were made of areas colonized predominantly by a single morphotype (Figure 3D).

Electron microscopy of cereal grains : Micronizing barley greatly increased the resistance of protein in the endosperm to digestion. In untreated barley, the protein was often extensively colonized and largely degraded prior to digestion of the starch granules (Figure 4A), whereas in some areas, the protein matrix of micronized barley remained intact even after 48 h in the rumen with starch granules no longer present (Figure 4B).

DISCUSSION

Wheat varieties

The high genetic variability of wheat (*Triticum spp.*), arising in part from its tetraploid or hexaploid genome (Chantret et al., 2005), has enabled this cereal grain to be cultivated in many regions of the world. The kernels produced by these varieties differ widely in their physical (Barlow et al., 1973; Morris, 2002; Chantret et al., 2005) and chemical properties (Bowland, 1974). Although this variation is desirable with regard to its end uses (i.e., bread, pasta, cake flour) for humans, it poses challenges for its use in ruminant diets. This variation was most pronounced in protein content and KH which presumably reflects the

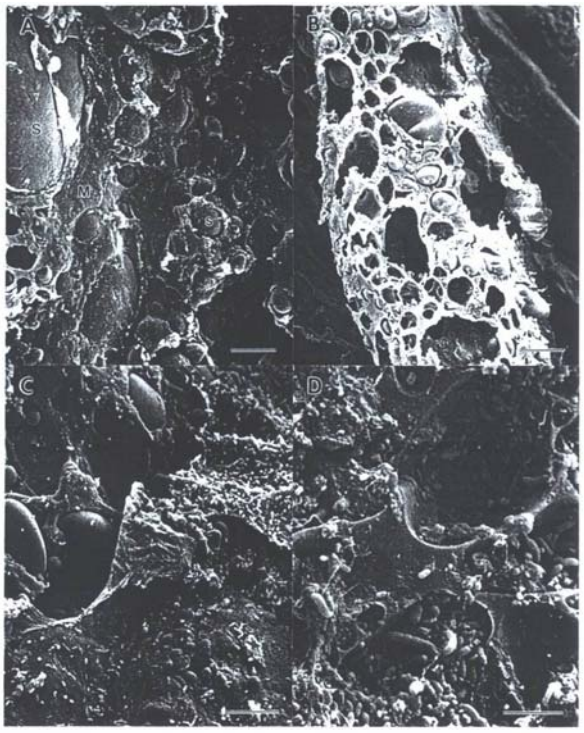

Figure 2. SEM of the endosperm of unmicronized (A) and micronized (B) wheat, prior to incubation in the rumen. The endosperm contains starch granules (S) embedded in a protein matrix (M). The integrity of the starch granules and protein matrix are maintained following micronization. SEM of the endosperm of unmicronized (C) and micronized (D) wheat after 2 h of incubation in the rumen. Bacteria were colonizing the protein matrix in control wheat, whereas in micronized wheat, colonization was concentrated in the starch granule cavities. Bars in A and D = 5 μm; in B and C = 10 μm.

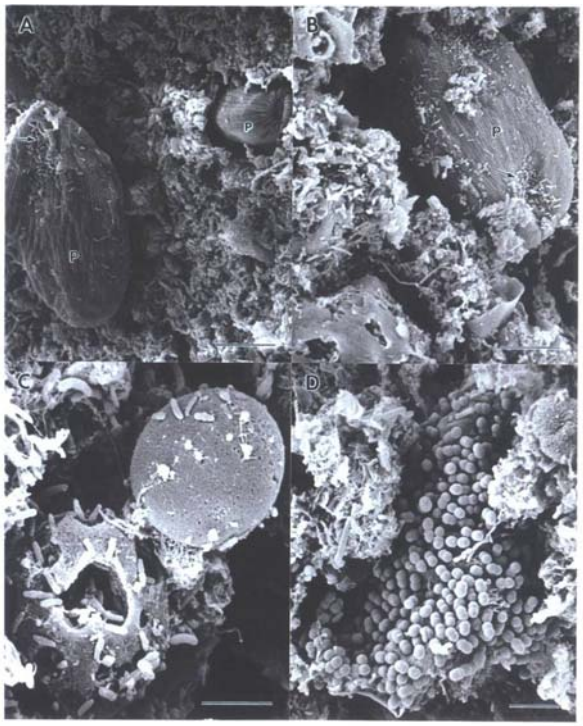

Figure 3. SEM of unmicronized (A) and micronized (B, C, D) wheat after 48 h of incubation in the rumen. Bacterial colonization of the surface of the endosperm is extensive in control (A) and in micronized (B) wheat. Protozoa (P) with adherent bacteria (arrows) were often associated with the endosperm surface in both control (A) and micronized (B) wheat. Bacterial digestion of starch granules was observed to progress from the inside out in micronized (C) sample and in control samples (picture not shown). In most instances, the endosperm was colonized by a variety of bacteria, but small areas were seen occasionally (D) which were colonized predominantly by a single bacterial morphotype. Bars in A and B = 10 μm; in C and D = 5 μm.

Figure 4. SEM of unmicronized (A) and micronized (B) barley after 48 h of incubation in the rumen. The protein matrix in the unmicronized endosperm is heavily colonized and digested. Bacteria have accessed starch granules, and have begun to colonize and penetrate them (A, arrows). In micronized barley, the protein matrix has resisted digestion and a "cast" remains, in which the original location of digested starch granules is evident. Bars = 20 μm.

interaction between proteins and starch granules in the endosperm of wheat (Barlow et al., 1973). Kernel hardness could be a particularly important property as hard kernels may be more susceptible to shattering and generating the fine particles that are often associated with subclinical acidosis (Owens et al., 1998) and bloat in feedlot cattle (Cheng et al., 1998). Feed wheat that consists of a mixture of varieties could present significant processing challenges.

Rates of DM disappearance were lowest for CW durum var. Kyle (7.1%) and highest for soft white spring var. Owens (11.1%), varieties that also exhibited the greatest difference in kernel hardness. Increased kernel hardness can be a reflection of the relative affinity between starch and protein within the endosperm (Barlow et al., 1973) and it appears that increased kernel hardness is associated with a decline in the rate of DM disappearance. This observation suggests that the nature of endosperm protein may influence the digestive properties of wheat in the rumen. However, when all varieties were considered, correlations between *in situ* rate of DM disapperance and KH were relatively low (r^2 = 0.53), indicating that KH may be only one of many factors that determine the rate of wheat digestion in the rumen. Others have reported substantial variation in the fermentation kinetics among wheat samples obtained from various sources, but were unable to attribute this variation to anyone chemical parameter (Lanzas et al., 2007). These authors emphasized the impact that processing may have on the fermentation dynamics of wheat, a variable that we carefully controlled in our study by cross-sectioning each individual wheat kernel with a scalpel.

Protein was the chemical parameter that was most highly correlated with rate of wheat digestion (r^2 = -0.77), further implicating protein characteristics as a factor that influences the digestive properties of wheat in the rumen. Two major proteins, puroindolines A and B associated with the fribilin protein complex on the surface of wheat starch granules have a central role in kernel hardness (Morris, 2002) and in determining the *in situ* disappearance of wheat starch (Swan et al., 2006). Differences in ruminal fermentation characteristics among varieties of sorghum (Streeter et al., 1990; Kotarski et al., 1992), corn (Hibberd et al., 1982; Philippeau et al., 1999) and barley (Boss and Bowman, 1996; Bowman et al., 2001) have been reported. To our knowledge, studies with wheat have been limited to comparisons of a few varieties (Bris and Dyer 1967; Varner and Woods, 1975), and no evaluations of varietal differences within a single experiment have been previously reported.

Micronization

Because both micronized and control wheat originated from a single batch of each variety, it was not possible to statistically test for differences in chemical composition. Visualization of starch granules using SEM identified no discernable changes in starch granule structure, but it was evident that this treatment altered some of the measured chemical and physical properties of the three varieties. Micronization tended to increase the NDF, reduce the amount of measurable starch and volume weight, and increase KH. Others have reported that micronization decreased the measurable amount of starch in wheat (Zarkadas and Wiseman, 2001) with only minor alterations to the structure of starch granules (White et al., 2008).

As observed in Experiment 1, soft red wheat (Kansas) exhibited the highest rate of digestion of the three varieties examined. In most instances, *in situ* disappearances of DM, protein and starch were reduced by micronization in all three wheat varieties. However, these reductions were more pronounced for soft wheat than for the other two varieties, suggesting that micronization altered the properties of the endosperm in soft wheat in manner that more closely resembles that of the harder varieties. This greater reduction may have been partially related to the fact that starch in micronized Kansas was less gelatinized than the starch in micronized Laura or Sceptre (Table 1). It has been reported that micronization-mediated digestion changes of starch in wheat may be related to the nature of the endosperm with alterations of proteins within the fribilin complex possibly playing a central role (White et al., 2008).

In contrast to *in situ* results, micronization increased starch digestion of finely ground wheat *in vitro*. Similarly, Aimone and Wagner (1977) concluded that greater gas production from micronized wheat in the initial stages of incubation was a reflection of increased starch digestion, but that *in vitro* DM disappearance decreased as a result of reduced protein digestibility. Micronizing sorghum denatures the protein in the endosperm and may increase its resistance to microbial digestion (McNeill et al., 1975). If a similar scenario is occurring when wheat is micronized, effective use of micronization to slow the digestion of wheat may require integrity of the protein matrix. Starch granules in ground micronized wheat are exposed to digestion and although the endosperm protein may be resistant to digestion, it fails as a physical barrier to protect starch granules from microbial attack. Under these conditions the greater gelatinization of starch in micronized wheat may have contributed to increased starch digestion. Furthermore, if starch was much more readily available for digestion than protein, it could have led to proliferation of amylolytic bacteria a factor that could further accentuate differences between the extent of starch and protein digestion. The fact that *in situ* and *in vitro* experiments yielded opposite results, illustrates that *in vitro* experiments may be of limited value for characterizing the digestive nature of grains if they are processed differently than the grains to be fed.

For *in situ* studies, the structural integrity of the endosperm was maintained because the wheat kernels were sectioned, rather than ground. Scanning electron microscopy revealed little visible change in the structural properties of the endosperm in micronized wheat. However, microbial colonization tended to focus on starch granules and there was visible evidence that the protein matrix in micronized wheat was more resistant to microbial colonization. Under these conditions, it appeared that the nature of the endosperm protein, as opposed to the degree of starch gelatinization, dictated the digestive characteristics of wheat in the rumen. Micronization did not alter the colonization patterns of all the ruminal microorganisms, however, as protozoa were observed to be associated with the endosperm of both micronized and control wheat.

Cereal species

Among cereals, *in situ* disappearance of DM, protein and starch were higher in wheat, and the observed ranking was wheat>barley>corn which is in agreement with previous studies (Nordin and Campling, 1976; McAllister et al., 1990). Wheat and barley are more rapidly fermented in the rumen than corn (Nordin and Campling, 1976). The protein matrix in barley and wheat is readily penetrated by a variety of proteolytic bacteria; however the protein matrix of corn is extremely resistant to microbial attack (McAllister et al., 1990). Micronization reduced *in situ* DM, protein and starch disappearances among cereals and its effect was most pronounced for wheat as compared to other grains.

CONCLUSIONS

In conclusion, wheat varieties differed in their rates of DM, protein and starch disappearance in the rumen, with hard wheat tending to be digested at a slower rate than soft wheat. Improving wheat as a feed grain by selection for a slower rate of digestion in the rumen may prove challenging as all varieties examined exhibited digestion rates that was more rapid than that of corn. Micronization appeared to alter the nature of the protein matrix and slow the *in situ* rate of starch digestion in all varieties of wheat examined. Consequently, micronization may prove to be an effective method to modulate the rate of acid production during the fermentation of wheat in the rumen. Differences in responses in the digestion of starch observed between *in situ* and *in vitro* experiments emphasize the importance of considering particle size when assessing the impact of heat-based processing techniques on cereal grains.

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