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Effects of particle size of processed barley grain, enzyme addition and microwave treatment on *in vitro* disappearance and gas production for feedlot cattle

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Objective: The effects of particle size of processed barley grain, enzyme addition and microwave treatment on *in vitro* dry matter (DM) disappearance (DMD), gas production and fermentation pH were investigated for feedlot cattle.

Methods: Rumen fluid from four fistulated feedlot cattle fed a diet of 860 dry-rolled barley grain, 90 maize silage and 50 supplement g/kg DM was used as inoculum in 3 batch culture *in vitro* studies. In Experiment 1, dry-rolled barley and barley ground through a 1-, 2-, or 4-mm screen were used to obtain four substrates differing in particle size. In Experiment 2, cellulase enzyme (ENZ) from *Acremonium cellulolyticus* Y-94 was added to dry-rolled and ground barley (2-mm) at 0, 0.1, 0.5, 1, and 2 mg/g, while Experiment 3 examined the interactions between microwaving (0, 30, and 60 s microwaving) and ENZ addition (0, 1, and 2 mg/g) using dry-rolled barley and 2-mm ground barley.

Results: In Experiment 1, decreasing particle size increased DMD and gas production, and decreased fermentation pH (p<0.01). The DMD (g/kg DM) of the dry-rolled barley after 24 h incubation was considerably lower (p<0.05) than that of the ground barley (119.1 dry-rolled barley versus 284.8 for 4-mm, 341.7 for 2-mm; and 358.6 for 1-mm). In Experiment 2, addition of ENZ to dry-rolled barley increased DMD (p<0.01) and tended to increase (p = 0.09) gas production and decreased (p<0.01) fermentation pH, but these variables were not affected by ENZ addition to ground barley. In Experiment 3, there were no interactions between microwaving and ENZ addition after microwaving for any of the variables. Microwaving had minimal effects (except decreased fermentation pH), but consistent with Experiment 2, ENZ addition increased (p<0.01) DMD and gas production, and decreased (p<0.05) fermentation pH of dry-rolled barley, but not ground barley.

Conclusion: We conclude that cellulase enzymes can be used to increase the rumen disappearance of barley grain when it is coarsely processed as in the case of dry-rolled barley. However, microwaving of barley grain offered no further improvements in ruminal fermentation of barley grain.

Keywords: Barley Grain; Enzyme; Gas Production; *In vitro* Dry Matter Disappearance; Particle Size; Rumen Fluid

INTRODUCTION

In 2014, global production of cereal grains included 990 million metric tonnes (mt) of corn, 717 million mt of wheat, 478 million mt of rice, and 145 million mt of barley [1]. Barley is an important crop for animal production [2] and its utilization has been widely investigated for pigs [3], poultry [4,5], dairy cows [6] and beef cattle [7]. Koenig et al [7] reported microbial protein synthesis and nutrient digestibility of beef cattle fed barley-based diets containing from 743 to 893 g/kg dry matter (DM) barley.

Digestibility of whole barley grain by cattle is limited by the presence of its fibrous hull and intact pericarp, which can prevent bacterial attachment and digestion in the rumen [8]. Fracturing of the pericarp through grain processing is necessary to allow rumen bacteria to gain access to the rapidly digestible nutrients of the endosperm [9]. Consequently, rumen fermentation of barley grain by ruminants is affected by particle size resulting from processing, with extensive processing maximizing rumen availability. However, extensive processing can be costly and can increase the incidence of rumen acidosis in cattle [10]. Therefore, alternative technologies such as enzymes and heat-treatment may help optimize the efficiency of barley grain utilization by ruminants.

Use of fibrolytic enzyme additives may provide a unique opportunity to increase the ruminal digestibility of barley grain [11], particularly when grain is minimally processed. The barrier to microbial digestion created by the hull and beta-glucan within the endosperm may be eliminated in part by fiber degrading enzymes, thereby increasing access of ruminal microbes to the inner, more digestible starch with the endosperm [9]. Krause et al [11] showed that exogenous fibrolytic enzymes improved nutrient digestion by feedlot cattle fed high-concentrate diets that contained barley grain.

Processing using methods such as steam-rolling and steam-flaking, in which heat and moisture are applied to grain, can help gelatinize starch and may increase degradation by micro-organisms in the rumen [2]. A less expensive alternative to these heat treatments may be microwaving. Microwaving of barley grain has been shown to increase ruminal starch degradability [12]. However, feed grains are not routinely microwaved commercially in part because beneficial effects have not been widely identified.

The aim of the study was to investigate interactions between particle size, microwaving and the application of cellulases to barley grain. We hypothesized that microwave treatment would improve *in vitro* disappearance of barley grain, especially when subjected to fibrolytic enzyme additives, and that this combination of treatments would reduce the degree of particle size reduction needed to optimize rumen availability of barley.

MATERIALS AND METHODS

Animals and diets

Three *in vitro* batch culture experiments were conducted. In Experiments 1 and 2, rumen fluid (1 L per animal) was collected from four ruminally cannulated steers (average body weight, 645 kg; average rumen fluid pH, 5.9). In Experiment 3, rumen fluid was collected from two ruminally cannulated steers (average body weight, 700 kg; average rumen fluid pH, 6.4). The pH of rumen fluid was measured immediately after sampling using a pH meter (B20PI, SympHony Benchtop Meters; VWR, Edmonton, AB, Canada).

All steers were fed a total mixed diet for *ad libitum* consumption containing 860 dry-rolled barley grain, 90 maize silage, and 50 supplement g/kg DM basis. The steers had free access to water and a mineral block throughout the experiment. Rumen fluid was obtained before feeding, strained through 4 layers of cheesecloth into an insulated thermos and immediately transferred to the laboratory. Steers were cared for according to the guidelines of the Canadian Council on Animal Care (Ottawa, ON, Canada).

Experimental design, substrates and treatments

Experiment 1: *In vitro* batch culture incubations were performed to investigate effects of particle size reduction due to processing of barley grain on *in vitro* DM disappearance (DMD), gas production and fermentation pH. The study was conducted as a completely randomized design with four treatments (dry-rolled barley, 1-, 2-, and 4-mm barley) in two runs (each using pooled rumen fluid from 2 steers), with 3 replications per run. A sample of dry-rolled feed grade barley was obtained from the feed mill at the Lethbridge Research and Development Center (Lethbridge, AB, Canada). The sample contained 870 g/kg of DM comprised of 979 organic matter, 112 crude protein, 679 starch, and 281 neutral detergent fiber (aNDF) g/kg. The dry-rolled barley was then ground through a 1-, 2-, or 4-mm screen (SM100, Retsch GmbH, Haan, Germany).

Experiment 2: Dry-rolled barley and barley ground through a 2-mm screen from Experiment 1 were used to investigate effects of enzyme addition on *in vitro* DMD, gas production and fermentation pH. The study was performed as a completely randomized design with treatments arranged as a 2 (dry-rolled and 2-mm barley)×5 (0, 0.1, 0.5, 1, and 2 mg/g fresh matter [FM] of enzyme) factorial in two runs (each using pooled rumen fluid from 2 steers), with 3 replications per run. The enzyme (ENZ) powder was from *Acremonium cellulolyticus* Y-94 (Meiji Seika Pharma Co., Ltd., Tokyo, Japan). Activity was assayed by the manufacturer as 1,120 units/g microcrystalline cellulose and 7,460 units/g carboxymethyl cellulose at pH 5.6. In addition, this organism is known to express other fibrolytic activities, such as xylanase, β-glucosidase, β-xylosidase, and mannanase [13]. To add ENZ at the desired rates 0.4, 2, 4, and 8 g/L solutions were prepared with distilled water and the appropriate solution was applied at 0.25 mL/g barley FM, so as to add ENZ at a rate of 0.1, 0.5, 1, and 2 mg/g FM of both ground or dry-rolled barley.

Experiment 3: *In vitro* batch culture incubations were performed with dry-rolled barley and 2-mm ground barley using a completely randomized design with treatments arranged as a 3 (0, 30, and 60 s microwaving)×3 (0, 1, and 2 mg ENZ/g FM) factorial in a single run, with 3 replications per run. Only a single run was performed because the steers were no longer available.

Dry-rolled and ground barley samples from Experiment 2 were subjected to heating using a household microwave oven (Model DMW 799W, normal maximum output power 700 w;

Dandy Products Ltd, Guelph, ON, Canada) for the desired times. Following microwaving, all samples were cooled to room temperature. The ENZ used in Experiment 2 was added at the desired rates using solutions (0, 4, and 8 g/L, respectively) prepared with distilled water applied at 0.25 mL/g barley fresh weight after microwaving.

In vitro disappearance, gas production and fermentation pH procedures

In vitro DMD, gas production and fermentation pH were measured for each replicate of each treatment within each experiment. Approximately 1.0 g samples (fresh weight) were weighed into Ankom F57 fiber bags and three empty bags were used as blanks within each run. The appropriate amounts of ENZ were added to the individual barley samples within the filter bags and left over night. The bags with sample were sealed (Model H-306, Impulse, Uline, Pleasant Prairie, WI, USA) and placed into 100 mL serum bottles.

The incubations were conducted as described by Anele et al [14]. Strained rumen fluid (20 mL) and pre-warmed buffer solution (60 mL, [15]) were added into 100 mL serum bottles under CO_2. Bottles and bags without barley (and with enzyme) were used as blanks. Bottles were incubated at 39°C in an incubator (Model 1915, VWR Reach-In Incubator, Sheldon Manufacturing Inc., Comelius, OR, USA) for 1, 3, 6, and 24 h for Experiment 1, 24 h for Experiment 2, and 6 and 24 h for Experiment 3. After completing the incubation, bottles were placed in an ice water bath to impede microbial activity. Bags were removed from the vials, washed and dried in an oven at 55°C to determine DMD. The pH of the incubation medium was measured with a pH meter.

Gas production was investigated after 1, 3, 6, and 24 h in Experiment 1, after 24 h in Experiment 2, and after 6 and 24 h in Experiment 3. Headspace gas production was measured after 1, 3, 6, 14, and 24 h by inserting a needle (25 gage×22.2 mm, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) attached to a pressure transducer (Model3462 Fisher scientific, Pittsburgh, PA, USA). The gas was released from the bottles after measurement. Pressure values (p), corrected for gas released from the blanks were used to generate volume estimates using the equation of Mauricio et al [16].

$$Gas\ production\ (mL) = 0.18 + 3.697 \times p + 0.0824 \times p^2$$

Chemical analysis

The original dry-rolled barley sample was ground through a 2-mm screen to estimate analytical DM and ash as described by AOAC [17] and aNDF as described by Van Soest et al [18] using heat-stable ɑ-amylase (Termamyl 120 L, Novo Nordisk Biochem, Franklinton, NC, USA). The sample was ground finer (MM400, Retsch GmbH, Germany) prior to crude protein and starch analysis. The crude protein content (N×6.25) of samples was determined by flash combustion, chromatographic separation, and thermal conductivity (Carlo Erba Instruments, Milan, Italy). Starch was determined by enzymatic hydrolysis of alpha linked glucose polymer as described by Rode et al [6].

Statistical analysis

Data in Experiment 1 for *in vitro* DMD, gas production and fermentation pH at each incubation time were analyzed using the MIXED procedure of SAS (SAS Institute, Inc., Cary, NC, USA) with grain processing treatment (barleys ground through 1-, 2-, and 4-mm screens and dry-rolled barley grain) as a fixed effect in the model. When the effect of grain processing was significant (p<0.05), treatments were compared using least significant differences. Data in Experiment 2 for DMD, gas production and fermentation pH were analyzed separately for dry-rolled and ground barley using the MIXED procedure of SAS with a model that included the fixed effect of enzyme treatment (0, 0.1, 0.5, 1, and 2, mg/g FM). In Experiment 3, data for the same variables as above were analyzed for dry-rolled and ground barley separately using a mixed model that included the effects of microwaving (0, 30, and 60 s), ENZ addition (0, 1, and 2 mg/g FM) and their interaction. Orthogonal polynomial contrasts (linear and quadratic) were used to examine effects of enzyme dose (Experiments 2 and 3) and duration of microwaving. Significant effects of treatment were declared at p<0.05 and tendencies at 0.05<p<0.10.

RESULTS

Particle size of barley (Experiment 1)

Processing affected *in vitro* DMD after all incubation times (p<0.01, Table 1). After 1 and 3 h, DMD of barley ground through 1- and 2-mm screens was greater than for other barleys. There was no disappearance of the dry-rolled barley until after 6 h. After 6 h, DMD of 1-mm barley was still greater than dry-rolled and 4-mm barleys, but the DMD of the 2-mm barley was only greater than that of dry-rolled barley. After 24 h, DMD of all three ground barleys were similar, all of which were greater than for dry-rolled barley.

Gas production from the four barleys tended to differ after 1 h (p = 0.053), and differed after 3 and 24 h of incubation with the main difference being lower gas production from dry-rolled barley. The fermentation pH only differed among barleys after 24 h of incubation (p<0.01), with pH being greater for dry-rolled barley than for the other three barleys.

Enzyme addition and particle size of barley (Experiment 2)

The effect of ENZ differed for dry-rolled and ground barley. With dry-rolled barley, addition of ENZ increased (linear, p<0.01) the 24-h DMD in a quadratic manner (p = 0.04; Table 2). There was only a linear trend (p = 0.09) for differences in gas produc-

Table 1. *In vitro* DM disappearance (DMD), gas production and fermentation pH of dry-rolled and ground barley (Experiment 1)

Incubation time	Screen size of ground barley			Dry-rolled barley	SEM	p
	1-mm	2-mm	4-mm			
DMD (g/kg DM)						
1	52.7[a]	50.0[a]	7.7[b]	0.0[b]	0.6	<0.01
3	161.3[a]	118.8[a]	54.5[b]	0.0[c]	1.4	<0.01
6	118.7[a]	113.8[ab]	80.7[b]	0.0[c]	1.1	<0.01
24	358.6[a]	341.7[a]	284.8[a]	119.1[b]	2.5	<0.01
Gas production (mL/g DM)						
1	5.1[ab]	5.0[ab]	6.9[a]	3.3[b]	0.5	0.053
3	10.8[a]	9.5[ab]	4.7[ab]	1.4[c]	1.0	<0.01
6	23.0	29.9	18.8	12.5	2.5	0.26
24	61.7[a]	55.7[a]	42.2[a]	20.0[b]	4.2	<0.01
Fermentation pH						
1	5.77	5.76	5.77	5.80	0.04	0.999
3	5.66	5.68	5.70	5.73	0.03	0.91
6	5.58	5.59	5.62	5.69	0.04	0.82
24	5.43[b]	5.44[b]	5.50[b]	5.69[a]	0.04	<0.01

DM, dry matter; SEM, standard error of means; p, probability.
Means within a row with different letters differ (p<0.05).

tion, but fermentation pH decreased linearly (p<0.01) with enzyme addition, although the magnitude of change was small. With ground barley, ENZ addition had no effects on the variables measured.

Particle size, microwave treatment, and enzyme addition (Experiment 3)

There were no interactions between microwaving and enzymes, so only the main treatment effects are presented (Table 3). Addition of ENZ to dry-rolled barley linearly increased (p<0.01) DMD and gas production and linearly decreased (p<0.05) fermentation pH. The effects of ENZ occurred after both 6 and 24 h of incubation for these variables. In contrast, the effects of ENZ added to ground barley were limited to increased gas production after 6 h (linear effect, p = 0.03), and decreased fermentation pH after 6 h (linear effect, p = 0.051; quadratic effect, p<0.01) and 24 h (linear effect, p = 0.047) of incubation.

For both ground and dry-rolled barley, microwave treatment

had no effects on DMD. There was a linear trend (p = 0.055) for increased gas production for dry-rolled barley after 24 h incubation, but not for ground barley. For dry-rolled barley, fermentation pH after 6 h decreased quadratically and linearly (p = 0.02, respectively) with microwaving, and after 24 h of incubation only quadratic (p = 0.01) effects on pH were noted. However, with ground barley, there was only a trend (p = 0.09) for decreased fermentation pH at 6 h of incubation with microwaving.

DISCUSSION

It is well known that the source of rumen inoculum can affect the results of *in vitro* incubations, and thus it is recommended that donor animals be fed a diet that includes the substrate used in the *in vitro* assay [19]. Thus, in the present study rumen fluid from feedlot cattle fed a diet comprised of 860 g/kg DM dry-rolled barley grain was used. The pH of rumen fluid collected before

Table 2. The effect of enzyme on *in vitro* dry matter disappearance (DMD), gas production and fermentation pH of dry-rolled barley and barley ground through a 2-mm screen incubated for 24 h (Experiment 2)

	Enzyme addition (mg/g FM)					SEM	p	
	0	0.1	0.5	1	2		L	Q
Dry-rolled barley								
DMD (g/kg DM)	161.0	200.7	252.9	249.9	263.8	1.11	<0.01	0.04
Gas production (mL/g DM)	26.4	27.2	26.0	28.9	25.1	1.28	0.09	0.42
Fermentation pH	5.66	5.67	5.66	5.62	5.59	0.02	<0.01	0.67
Barley ground through a 2-mm screen								
DMD (g/kg DM)	426.1	455.6	428.2	426.1	426.1	1.52	0.85	0.53
Gas production (mL/g DM)	58.6	60.4	57.4	54.7	62.3	1.40	0.96	0.21
Fermentation pH	5.39	5.37	5.34	5.33	5.37	0.01	0.69	0.31

Enzyme, cellulase prepared from *Acremonium cellulolyticus* Y-95; FM, fresh matter; SEM, standard error of means; p, probability; L, linear; Q, quadratic; DM, dry matter.

Table 3. The effect of microwave and enzyme addition on *in vitro* dry matter disappearance (DMD), gas production and fermentation pH of dry-rolled barley and barley ground through a 2-mm screen incubated for 6 and 24 h (Experiment 3)

	Enzyme (mg/g FM)			p		Microwave (s)			SEM	p	
	0	1	2	L	Q	0	30	60		L	Q
Dry-rolled barley											
DMD											
6 h	69.0	89.4	117.4	<0.01	0.74	80.8	104.3	90.8	0.63	0.45	0.11
24 h	148.9	195.9	210.3	<0.01	0.28	191.6	187.5	176.0	0.84	0.36	0.80
Gas production											
6 h	7.1	11.5	14.3	<0.01	0.65	9.4	11.8	11.7	0.93	0.27	0.48
24 h	28.9	33.7	37.4	<0.01	0.81	30.1	34.1	35.8	1.31	0.055	0.63
Fermentation pH											
6 h	6.19	6.16	6.15	<0.01	0.37	6.19	6.15	6.16	0.01	0.02	0.02
24 h	6.08	6.02	6.03	0.048	0.11	6.08	6.01	6.05	0.01	0.21	0.01
Barley ground through a 2-mm											
DMD											
6 h	168.5	180.5	190.9	0.39	0.97	193.8	168.2	177.9	0.96	0.54	0.43
24 h	285.3	295.2	317.8	0.17	0.75	323.2	287.1	288.1	0.97	0.14	0.36
Gas production											
6 h	19.4	24.3	24.2	0.03	0.17	25.0	20.9	22.0	0.91	0.17	0.15
24 h	56.7	61.3	57.6	0.82	0.25	57.8	59.9	57.8	1.47	0.999	0.55
Fermentation pH											
6 h	6.12	6.05	6.09	0.051	<0.01	6.10	6.08	6.07	0.01	0.09	0.69
24 h	5.83	5.82	5.78	0.047	0.53	5.81	5.84	5.79	0.01	0.53	0.13

Enzyme, cellulase from *Acremonium cellulolyticus* Y-94; FM, fresh matter; p, probability; L, linear; Q, quadratic; SEM, standard error of means.

feeding was 5.9 in Experiments 1 and 2 and 6.4 in Experiment 3. Thus, the results are applicable to feedlot finishing cattle fed barley grain based diets.

Anele et al [14,20] reported effective rumen degradability (20 h mean retention time) of dry-rolled barley grain ranged from 228 to 250 g/kg DM depending upon degree of processing, while Anele et al [20] reported a mean of 390 g/kg DM for dry-rolled barley. Both those studies used rumen fluid from cattle fed 700 g/kg DM barley silage rather than a diet of primarily barley grain as in the present study. Using a longer incubation time of 48 h, Yang et al [21] reported *in vitro* DMD of a ground (1-mm) diet containing 451 g/kg DM steam-rolled barley after 48 h of incubation ranged from 699 to 742 g/kg DM. In Experiment 1, *in vitro* DMD of ground barley grain after 24 h of incubation ranged from 285 to 359 g/kg DM depending on extent of processing. These results are similar to the previous studies by Anele et al [14,20] where a relatively short rumen incubation time was assumed, as was used in the present study. It should be noted that conditions of the *in vitro* technique differed (i.e., source of rumen fluid, substrate to ruminal fluid plus buffer ratio, processing of grain) from those used in other studies, thus it is somewhat difficult to directly compare our results to those in the literature. However, the emphasis of our study was on relative differences between treatments to determine optimum processing conditions.

Particle size (Experiment 1)

The major difference in *in vitro* DMD due to processing was

between ground and dry-rolled barley which occurred at each incubation time, whereby DMD was substantially less for dry-rolled than ground barley. Differences in DMD also occurred between 1- and 4-mm barley in the early incubation times (1 to 6 h), but after 24 h of incubation these differences no longer existed. Thus, extent of particle size reduction during grinding had greatest effects in early stages of digestion, but were less important later in digestion. Anele et al [20] reported that processing (barley ground through 2-mm screen versus dry-rolled) increased the extent and rate of disappearance of DM and starch, which supports the results of the present study. Finely grinding barley is necessary when producing compound feed for beef cattle that is pelleted; however, grinding increases availability of starch. It can be concluded from the effects of grinding on increasing DMD, especially at early time periods, that feedlot cattle fed ground barley based diets may be at a greater risk of rumen acidosis because of greater starch availability. Koenig et al [7] reported that optimum processing index (defined as the weight of the grain after processing divided by the weight of the grain before processing) of dry-rolled barley was 0.61 when beef cattle were fed processed grain with barley silage 200 g/kg DM. Based on the results of our study, it is recommended to feed dry-rolled barley to feedlot cattle rather than barley ground through 1-, 2-, and 4-mm screens. However, research to determine the desirable proportion of ground barley in compound feeds to support cattle health is needed.

Enzyme addition (Experiments 2 and 3)

The enzyme product used in this study was selected because it had been shown previously to increase NDF degradation of rhodesgrass (*Chloris gayana* Kunth. cv. Callide) and guineagrass (*Panicum maximum* Jacq. cv. Natsukaze) [22] when used as a silage additive. In addition to endoglucanase activity, ENZ has greater β-glucosidase activity than some other products such as those derived from *Trichoderma viride* [23]. The linear increase in *in vitro* DMD of dry-rolled barley with increasing ENZ addition observed in Experiment 2 was confirmed in Experiment 3, and was consistent with the observed increase in total gas production (trend only in Experiment 2) and decrease in fermentation pH in both experiments.

The observed responses for ENZ addition to barley grain are consistent with other *in vitro* studies. Yang et al [21] reported for dairy cows, addition of xylanase and cellulase increased total tract DM digestion of total mixed diet comprised of 451 g/kg DM steam-rolled barley grain. Krause et al [11] reported acid detergent fiber digestion of rolled barley improved when using an enzyme mixture containing mainly cellulase and xylanase activities, while Colombatto et al [24] reported the contents of aNDF and hemicellulose of rolled barley were decreased with addition of xylanases and endoglucanases.

The minimal effects of ENZ when added to ground barley observed in Experiments 2 and 3 suggest that disruption of the fibrous hull via grounding negated the effect of cellulolytic enzymes. Grinding would be expected to remove the structural barrier to endosperm digestion by rumen microbes, although the chemical fiber matrix would remain unchanged. Applying ENZ to ground barley may have decreased the direct physical contact between enzyme and fibrous substrate, thereby reducing its effectiveness.

Microwaving

This study hypothesized that the effects of supplemented enzymes would be enhanced when used in combination with microwave treatment. Heat and moisture applied to grain can help gelatinize starch and may increase degradation by microorganisms in the rumen [2]. Contrary to our hypothesis there were no interactions between microwave treatment and enzyme addition. Yin and Walker [25] suggested that a hard gel is formed when amylose is released from starch granules during microwave heating, which could have rendered the microwave treatment ineffective in terms of increasing digestibility. Overall, the microwaving treatment as applied in the present study had relatively minor effects on barley grain fermentability, and cannot be recommended at this time.

IMPLICATIONS

Particle size reduction of barley by grinding increased *in vitro* DMD and gas production. Use of cellulolytic enzymes increased rumen disappearance of dry-rolled barley grain, but effects of enzymes on ground grain were minimal. Microwaving barley grain did not further improve *in vitro* fermentability of barley grain, even when used in combination with enzymes. The results indicate that cellulolytic enzymes can be used to increase the rumen availability of dry-rolled barley grain, but more extensive processing of grain eliminates the benefits of enzyme use.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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