



## Effect of a saponin-based surfactant on water absorption, processing characteristics and in vitro ruminal fermentation of barley grain

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### Abstract

The effects of a saponin-based surfactant on tempered barley grain were studied in three in vitro experiments. In Experiment 1, barley kernels were tempered at 220 or 246 g/kg moisture with surfactant included at 0, 60 or 120  $\mu\text{l}/\text{kg}$  dry matter (DM). Surfactant increased ( $P < 0.05$ ) the rate of water uptake during the initial 2 h of tempering, but not if tempering was extended beyond 4 h. Experiment 2 evaluated effects of surfactant on barley grain processing characteristics. Surfactant was included at 0, 60, 120, or 240  $\mu\text{l}/\text{kg}$  DM during tempering of barley (190 g/kg moisture; 2 or 4 h), then the barley was processed with rollers set 2.032, 2.223 or 2.413 mm apart. Surfactant did not affect ( $P > 0.05$ ) the processing index (PI) of the barley or the particle size distribution. Increasing the roller distance from 2.032 to 2.413 mm increased ( $P < 0.05$ ) the PI and the proportion of particles with 3.35–4.75 mm diameter, and reduced ( $P < 0.05$ ) the proportions of particles larger than 4.75 mm or smaller than 2.36 mm. No surfactant  $\times$  roller distance interactive effects on processing characteristics were observed. In Experiment 3, barley was tempered (190 g/kg moisture; 2 h) with surfactant at 0 or 120  $\mu\text{l}/\text{kg}$  DM and processed with rollers at 2.032 or 2.413 mm, then incubated in batch culture with surfactant supplemented at 0 or 360  $\mu\text{l}/\text{kg}$  DM. Apparent in vitro dry matter digestibility (IVDMD) and gas production were higher ( $P < 0.01$ ) with rollers at 2.032 mm than at 2.413 mm. Irrespective of substrate particle size, applying surfactant during tempering and/or prior to incubation reduced ( $P < 0.05$ ) the 4 h IVDMD, accumulation of volatile fatty acids (VFA) and reducing sugars, and molar

*Abbreviations:* DM, dry matter; IVDMD, in vitro dry matter disappearance; PI, processing index; RS, reducing sugars; VFA, volatile fatty acids.

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percentage of acetic acid in the VFA, but it increased ( $P < 0.05$ ) the molar percentage of butyric acid. The acetate:propionate ratio at 4 h was reduced ( $P < 0.05$ ) by surfactant applied during (120  $\mu\text{l/kg DM}$ ) and/or after (360  $\mu\text{l/kg DM}$ ) tempering if the barley had been rolled at 2.032 mm, but at 2.413 mm roller spacing this effect on acetate:propionate was observed only with the maximum application of surfactant (120 + 360  $\mu\text{l/kg DM}$ ). Despite enhancing the initial rate of water absorption by barley kernels, surfactant applied during tempering did not affect the processing characteristics of barley. Added during or after tempering, surfactant reduced the extent of IVDMD measured at 4 h, but not at 24 h.

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## 1. Introduction

The husk and pericarp layers of barley kernels have been identified as the most important factor limiting digestion of whole barley grain by ruminants (McAllister et al., 1994). It is necessary, therefore, that barley be physically processed to breach this barrier before it is fed to cattle, in order that the internal components of the grain are exposed to ruminal microorganisms (Wang and McAllister, 2000). Excessive processing, however, can lead to grain being digested too rapidly, giving rise to digestive disturbances such as acidosis and bloat (Owens et al., 1997).

Barley grain is most commonly processed by rolling, a method in which the grain is passed between platen rollers set at a fixed distance, resulting in whole kernels being crushed or cracked. The degree of physical damage imparted on the kernels at a given roller setting is affected by factors such as moisture, kernel uniformity and kernel hardness. These factors can vary substantially in barley purchased from commercial sources, and so feedlot operators often identify proper processing of barley grain as the greatest challenge in quality control during preparation of diets. Tempering is the process of adding moisture to the grain prior to processing, which is often used to reduce the variation in grain particle size that results from rolling.

Wang et al. (2003) found that including a surfactant in the water used for tempering did not affect barley grain processing characteristics, but it improved animal performance, especially when barley with a processing index of <72% was fed. The mechanism by which the surfactant enhanced the growth of the steers was not known. The present study was undertaken, therefore, to evaluate effects of a saponin-based surfactant on water uptake during tempering of barley grain and on the processing characteristics and ruminal fermentation of the grain.

## 2. Materials and methods

### 2.1. Experiment 1

The effect of surfactant on the rate of water absorption into barley grain kernels was assessed in a  $2 \times 3$  factorial experiment. The surfactant used was GrainPrep<sup>TM</sup> (AgriChem,

Inc., Ham Lake, MN, USA), which is based on saponins from *Yucca schidigera*. The product has a pH of 6.6, specific gravity of 1.25, viscosity (at 60 °C) of 184 cP, and is completely soluble in water. Barley grain (mixed varieties; 920 g/kg DM) was sieved to remove broken kernels and contaminants, and 100 g amounts were weighed into eighteen 750 ml flasks (triplicate flasks for each of six treatments). Aqueous solutions of surfactant were prepared to deliver 0, 5.5 or 11 µl of surfactant in 18 or 22 ml of solution, which yielded surfactant treatment rates of 0, 60 or 120 µl/kg barley DM and total moisture contents (endogenous barley moisture plus added water) of 220 or 246 g/kg. The surfactant solutions were applied to the grain using a single nozzle sprayer. The flasks were covered during tempering to prevent water evaporation and shaken manually every 30 min to ensure homogenous hydration. Subsamples (approximately 10 g) of barley were collected from each flask after 20, 40, 60, 120, 240 and 360 min of tempering and blotted dry using paper towelling. Additional surface drying was achieved using a hand-held blow dryer (no heat) for 30 s. The grain was weighed immediately, dried in a forced-air oven (105 °C) for 24 h and weighed again. The difference between these two weights, corrected for the original moisture content of the grain, was assumed to be water absorbed by the grain kernels during the tempering period. The experiment was completed at room temperature and repeated once each day for consecutive days.

## 2.2. Experiment 2

The processing characteristics of barley grain tempered with and without surfactant were investigated in a study with a 4 × 3 factorial arrangement of treatments. Barley grain (mixed varieties; 895 g/kg DM) was cleaned and sieved to isolate kernels with 2.80–4.75 mm diameter for use in the study. The cleaned grain was weighed into 12 plastic buckets (9 kg per bucket) and mixed with 950 ml of surfactant solution, producing an initial moisture level of approximately 190 g/kg. Four surfactant solutions were used, prepared to deliver 0, 60, 120 or 240 µl surfactant/kg barley DM. Triplicate buckets were prepared for each concentration of surfactant. Buckets were covered during tempering and mixed manually every 30 min to ensure homogenous hydration. Duplicate subsamples (approximately 750 g) from each bucket were collected after 2 and 4 h of tempering and rolled using a laboratory scale roller (Model R250.6, Kal Rob Machining, Picture Butte, AB, Canada) with rollers set at distances of 2.032, 2.223 or 2.413 mm. The rolled grain was dried in a forced-air oven at 65 °C for 48 h. Particle size distribution of each lot was determined by dry sieving described by Wang et al. (2003). The processing index (PI) of the rolled grain (Yang et al., 2000) was calculated as:

$$\text{PI}(\%) = \frac{\text{volume weight (g/l) after rolling}}{\text{volume weight (g/l) before rolling}} \times 100 \quad (1)$$

## 2.3. Experiment 3

The effects on barley grain fermentation of applying surfactant during tempering and/or immediately prior to incubation were assessed in an *in vitro* ruminal incubation with a

2 × 2 × 2 factorial arrangement of treatments. Four substrates were prepared by tempering barley grain with 0 or 120 µl surfactant/kg DM, then rolling at 2.032 or 2.413 mm and drying as described in Experiment 2. Each of the substrates was loaded into 24 replicate 150 ml serum vials (500 mg DM/vial).

Inoculum for the *in vitro* incubation was prepared immediately prior to the incubation using ruminal fluid from two steers fed a barley grain:barley silage-based finishing diet. The steers were cared for in accordance with guidelines established by the [Canadian Council on Animal Care \(1993\)](#). Ruminal fluid was collected 2 h after the morning feeding. Equal volumes from each steer were combined and strained through four layers of cheesecloth, and the filtrate was mixed with two volumes of mineral buffer ([Menke et al., 1979](#)).

To begin the incubation, 0.2 ml of buffer or 9 ml/l surfactant in buffer ([Menke et al., 1979](#)) was added to each grain-loaded vial, followed directly by 50 ml of inoculum. This yielded treatments of 0 or 360 µl surfactant/kg substrate DM. Vials containing no grain were also included in each incubation as controls. The vials were sealed immediately and affixed to a rotary shaking platform in a 39 °C incubator. The anaerobic inoculation and incubation procedures used were those described by [Wang et al. \(2000a\)](#).

Gas production during the fermentations was measured by a water displacement apparatus ([Fedorak and Hruday, 1983](#)). Triplicate treatment and control vials were removed from the incubation after 0, 4, 12 and 24 h, and the contents of the serum vials were transferred to centrifuge tubes. The supernatant from centrifugation at 500 × g (10 min, 4 °C) was prepared for analysis of volatile fatty acids (VFA) and reducing sugars (RS) as described by [Wang et al. \(2002\)](#). The pellets were washed by resuspension in 30 ml of 0 °C water and re-sedimented by centrifugation (500 × g, 10 min, 4 °C). After three wash cycles, the pellet was dried for DM determination.

#### 2.4. Calculations and statistical analyses

Average values from triplicates in each of the three consecutive repeats (Experiment 1) and average values from the duplicate subsamples of each of the three replicate buckets (Experiment 2) were used for statistical analysis. In Experiment 3, apparent *in vitro* DM digestibility (IVDMD) of barley grain at each of the incubation timepoints was calculated as the difference between the weights (DM basis) of the barley grain used as substrate and that remaining in the dried pellets.

Analysis of variance of data from all three experiments was performed using the MIXED model of [SAS \(1995\)](#). Treatment effects were determined by examining the significance of the difference among means using LSMEANS with PDIFF ([SAS, 1995](#)).

### 3. Results

#### 3.1. Experiment 1

Absorption of water by the barley kernels increased ( $P < 0.01$ ) as the tempering time increased from 20 to 360 min ([Fig. 1](#)). Moisture uptake by kernels (as g/kg DM) was higher ( $P < 0.01$ ) when water for tempering was added at 22 ml/100 g barley (moisture

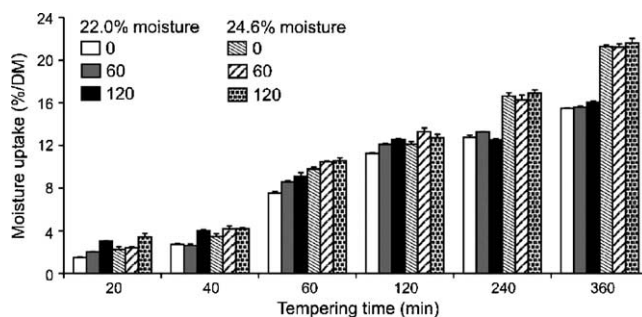


Fig. 1. Absorption of water by barley kernels during 360 min of tempering from two initial moisture contents (18 or 22 ml water added per 100 g barley DM, yielding 220 and 246 g/kg moisture, respectively) with saponin-based surfactant included at 0, 60 or 120  $\mu\text{l}/\text{kg}$  barley DM. Bars indicate standard error.

content 246 g/kg after tempering) than at 18 ml/100 g barley (moisture content 220 g/kg after tempering). At both initial moisture levels, more ( $P < 0.05$ ) water was absorbed into the kernel during the initial 2 h (i.e., at 20, 40, 60 and 120 min sampling times) when surfactant was included than when the barley was tempered with water only, but this effect did not occur at 240 or 360 min. The surfactant mediated increase in water absorption by barley grain was similar ( $P > 0.05$ ) between the two initial moisture levels.

### 3.2. Experiment 2

No surfactant  $\times$  roller distance interactive effects occurred on either the PI or the particle size distribution of the processed grain. Therefore, average values for each surfactant application rate and roller distance are presented independently in Table 1. Including surfactant at 60, 120 or 240  $\mu\text{l}/\text{kg}$  DM during tempering for 2 or 4 h did not affect PI or particle size distribution of the rolled grain, but these characteristics were affected ( $P < 0.05$ ) by roller distance. The percentages of smallest and largest particles ( $< 2.80$  and  $\geq 4.75$  mm in diameter) decreased ( $P < 0.05$ ) as roller spacing was increased from 2.032 to 2.413 mm, and the percentage of intermediate sized particles increased ( $P < 0.05$ ). Accordingly, the barley PI also increased ( $P < 0.05$ ) as roller spacing was increased. Tempering for 4 h resulted in decreased ( $P < 0.05$ ) percentages of largest ( $\geq 4.75$  mm) and smallest ( $< 2.80$  mm) particles and increased ( $P < 0.05$ ) proportions between 4.75 and 3.35 mm, as compared with tempering for 2 h. Tempering time did not affect the percentage of particles with diameters from 3.35 to 2.80 mm. Processing indices were higher ( $P < 0.05$ ) when barley was tempered for 4 than for 2 h, irrespective of roller setting.

### 3.3. Experiment 3

Initial solubilities (i.e., IVDMD at 0 h of incubation) were similar ( $P > 0.05$ ) across all substrate types, averaging  $59.3 \pm 9.8$  g/kg. In the absence of surfactant (during tempering or incubation), greater IVDMD ( $P < 0.01$ ) and gas production ( $P < 0.01$ ) were observed with

Table 1

Effects of saponin-based surfactant applied during tempering and spacing of rollers during processing on the processing index of the barley grain and on the distribution of sizes of processed particles

	Surfactant added ( $\mu\text{l}/\text{kg}$ barley DM)				S.E.M. <sup>a</sup>	Roller setting (mm)			
	0	60	120	240		2.032	2.223	2.413	SEM
Tempering for 2 h									
Processing index <sup>b</sup>	69.23	67.94	67.42	69.02	0.575	60.00 a	69.56 b	75.64 c	1.718
Screen size (mm) <sup>c</sup>									
4.75	5.34	5.85	5.87	5.41	0.206	9.98 b	4.36 a	2.51 a	0.706
3.35	64.67	65.02	64.46	64.97	0.257	60.06 a	67.52 b	66.75 b	0.933
2.80	21.71	21.36	21.22	21.49	0.151	16.79 a	21.45 b	26.05 c	0.921
2.36	5.28	4.85	5.50	5.10	0.162	7.34 b	4.67 a	3.54 a	0.336
1.70	1.66	1.63	1.66	1.74	0.085	3.55 b	0.97 a	0.49 a	0.181
< 1.70	1.33	1.28	1.28	1.29	0.064	2.26 b	0.97 a	0.65 a	0.090
Tempering for 4 h									
Processing index	70.43	73.48	72.86	73.59	0.893	62.20 a	75.80 b	79.76 c	1.322
Screen size (mm)									
4.75	4.62	4.82	4.52	4.79	0.208	9.27 c	3.39 b	1.41 a	0.626
3.35	67.27	68.65	67.56	67.82	0.394	65.10 a	69.98 b	68.40 b	0.575
2.80	21.87	20.56	21.75	21.60	0.319	15.98 a	21.53 b	26.82 c	1.228
2.36	4.20	4.00	4.15	3.89	0.196	5.66 c	3.80 b	2.66 a	0.316
1.70	1.07	1.05	1.09	1.04	0.098	2.33 b	0.60 a	0.27 a	0.223
< 1.70	0.96	0.93	0.93	0.92	0.096	1.66 b	0.70 a	0.44 a	0.094

Within a row and main effect (surfactant level or roller spacing), values followed by different letters alphabets (a–c) differ ( $P < 0.05$ ).

<sup>a</sup> S.E.M.: standard error of the mean (calculated across surfactant levels or across roller spacings).

<sup>b</sup> Processing index (PI) was calculated as: [volume weight after rolling (g/l)/volume weight before rolling (g/l)  $\times$  100%].

<sup>c</sup> Values shown are the proportions of total sample weight (%) retained on the sieve with mesh size shown or passing through the 1.70 mm sieve.

barley rolled at 2.032 mm versus 2.413 mm, at 4, 12 and 24 h (Table 2). Accumulations of VFA and RS, however, were only higher ( $P < 0.05$ ) at 12 h.

Including surfactant during tempering (120  $\mu\text{l}/\text{kg}$  DM) reduced ( $P < 0.01$ ) IVDMD and accumulations of VFA and RS at 4 h, irrespective of roller setting, compared with no surfactant. With barley rolled at 2.032 mm, VFA and RS concentrations were also lower ( $P < 0.05$ ) at 12 h. Surfactant applied during tempering did not affect gas production. Similarly, including surfactant in only the incubation (360  $\mu\text{l}/\text{kg}$  DM) reduced ( $P < 0.01$ ) IVDMD and accumulations of VFA and RS during the first 4 h, irrespective of the roller setting that had been used to process the barley.

The effects of adding surfactant (360  $\mu\text{l}/\text{kg}$  DM) to the incubations, in addition to having including it (at 120  $\mu\text{l}/\text{kg}$  DM) during tempering, were minimal. With both roller settings, dual application of surfactant (120 + 360  $\mu\text{l}/\text{kg}$  DM) did not affect IVDMD or gas production at 4, 12, or 24 h, and RS concentration only increased ( $P < 0.05$ ) at 24 h, compared with surfactant added during tempering only (120  $\mu\text{l}/\text{kg}$  DM). At roller setting 2.413 mm,

**Table 2**  
 Effect of including saponin-based surfactant during tempering and/or during in vitro incubation on fermentation parameters arising from in vitro ruminal incubation of barley grain processed with rollers set at 2.032 or 2.413 mm<sup>a</sup>

	Rollers set at 2.032 mm				Rollers set at 2.413 mm				S.E.M. <sup>b</sup>
	0 µl/kg DM <sup>c</sup>		120 µl/kg DM <sup>c</sup>		0 µl/kg DM <sup>c</sup>		120 µl/kg DM <sup>c</sup>		
	0 µl/kg barley DM <sup>d</sup>	360 µl/kg barley DM <sup>d</sup>	0 µl/kg barley DM <sup>d</sup>	360 µl/kg barley DM <sup>d</sup>	0 µl/kg barley DM <sup>d</sup>	360 µl/kg barley DM <sup>d</sup>	0 µl/kg barley DM <sup>d</sup>	360 µl/kg barley DM <sup>d</sup>	
<b>Apparent in vitro DM disappearance (g/kg)</b>									
4 h	198.2 c	120.3 a	108.9 a	132.4 ab	159.1 b	113.3 a	105.6 a	105.7 a	14.11
12 h	504.9 b	441.6 b	468.4 b	464.9 b	300.7 a	341.5 a	328.8 a	305.1 a	27.47
24 h	742.0 b	768.9 b	732.9 b	775.7 b	648.6 a	670.2 ab	649.3 a	691.3 a	25.61
<b>Gas production (ml/g DM)</b>									
4 h	46.88 cd	42.48 c	46.75 cd	51.53 d	22.17 a	22.64 a	28.21 ab	30.79 b	2.119
12 h	158.76 b	161.05 b	162.59 b	167.72 b	111.24 a	119.70 a	113.75 a	113.16 a	4.539
24 h	279.50 b	278.97 b	282.69 b	280.95 b	248.09 a	249.21 a	242.30 a	255.09 a	5.465
<b>Volatile fatty acids (µmol/ml)</b>									
4 h	57.82 c	39.80 a	45.12 b	43.43 ab	54.46 c	42.62 ab	45.38 b	37.83 a	2.612
12 h	79.06 ee	85.27 e	57.21 b	63.73 bc	68.61 cd	71.47 cd	75.26 de	44.06 a	3.166
24 h	104.26	103.48	95.38	99.86	102.20	107.25	91.81	98.33	5.168
<b>Reducing sugars (µmol/ml)</b>									
4 h	1.82 d	1.01 ab	1.09 bc	1.39 c	1.67 d	0.80 a	0.88 ab	1.16 bc	0.906
12 h	1.07 b	0.76 a	0.82 a	0.76 a	0.83 a	0.78 a	0.74 a	0.74 a	0.070
24 h	0.69 b	0.69 b	0.55 a	0.69 b	0.66 b	0.56 a	0.55 a	0.67 b	0.030

Within a row, values followed by different letters (a–f) differ ( $P < 0.05$ ).

- <sup>a</sup> All measurements were corrected for blanks containing no substrate.
- <sup>b</sup> S.E.M.: standard error of the mean.
- <sup>c</sup> Surfactant added during tempering.
- <sup>d</sup> Surfactant added to incubation.

treatment with 120 + 360  $\mu\text{l}$  surfactant/kg DM resulted in lower ( $P < 0.05$ ) VFA concentrations at 4 and 12 h compared with 120  $\mu\text{l}$ /kg DM only.

Inclusion of surfactant during tempering and incubation (120 + 360  $\mu\text{l}$ /kg DM) did not affect IVDMD at 4, 12 or 24 h, compared with adding surfactant to the incubation only (360  $\mu\text{l}$ /kg DM), but gas production and RS accumulation at 4 h increased ( $P < 0.05$ ), and VFA accumulation at 12 h was decreased ( $P < 0.05$ ). These trends were similar between roller settings.

In the absence of surfactant, the proportion of propionic acid in the VFA produced from barley rolled at 2.413 mm was lower ( $P < 0.05$ ) at 4 and 12 h than that from barley rolled at 2.032 mm (Table 3), and the proportion of acetic acid was higher ( $P < 0.05$ ) at 24 h. Molar proportions of butyric acid were not affected by roller setting.

Compared with no surfactant, applying surfactant during tempering (120  $\mu\text{l}$ /kg DM) reduced ( $P < 0.05$ ) the molar proportion of acetic acid at 4 h, and increased ( $P < 0.05$ ) the molar proportion of butyric acid after 24 h of incubation of barley, whether rolled at 2.032 or at 2.413 mm. When the 2.413 mm roller setting was used, these effects on acetic and butyric acids were also evident ( $P < 0.05$ ) at 24 and 4 h, respectively. Applying surfactant during tempering did not affect ( $P > 0.05$ ) the molar proportion of propionic acid, irrespective of roller setting.

Including surfactant in the incubation only (360  $\mu\text{l}$ /kg DM) reduced ( $P < 0.05$ ) the molar proportion of acetic acid at 4 h (and at 24 h, with roller setting 2.413 mm only) and increased ( $P < 0.05$ ) the molar proportion of butyric acid at 4 h, compared with no surfactant. If the barley had been tempered with surfactant (120  $\mu\text{l}$ /kg DM), further effects ( $P < 0.05$ ) of adding surfactant to the incubation (increased proportion of acetic acid; decrease in butyric acid) at 4 h were evident only when roller setting 2.032 mm was used.

#### 4. Discussion

This study demonstrates that including a steroidal saponin-based compound during tempering increases the rate of water absorption into barley grain. Saponins are surfactants that reduce hydrophobicity in the husk and pericarp layer of the kernel, thereby accelerating uptake of moisture (McEntyre et al., 1998). In commercial trials, a saponin-based surfactant markedly reduced the tempering time of corn (Johnson and Greer, 1996), suggesting a similar effect of the surfactant on the uptake of water by corn kernels. Other surfactants, such as Tween 80 and Span 80, have also been shown to increase water uptake into wheat kernels (Aksenova et al., 1993; Dunaeva et al., 1991).

Data from the present study indicate that the surfactant-mediated increase in moisture uptake rate occurred in the initial period (i.e., up to 2 h) of contact between the grain and the water, and that surfactant effects were diminished by natural absorption of moisture when the tempering period was extended beyond 4 h. In contrast, Experiments 1 and 2 showed that a tempering period of 4 h is needed for sufficient amount of water to be absorbed into the kernel and to produce uniform particles during rolling. It has been suggested that the optimum time for cold tempering (i.e., at room temperature) is 12 h (Hinman and Sorensen, 1994; Bradshaw et al., 1992). The present results suggest that the time required for tempering can be substantially reduced by including a tempering agent.

Table 3

Effect of including saponin-based surfactant during tempering and/or during in vitro incubation on molar percentages of principal VFA arising from in vitro ruminal incubation of barley grain processed with rollers set at 2.032 or 2.413 mm<sup>a</sup>

	Rollers set at 2.032 mm				Rollers set at 2.413 mm				S.E.M. <sup>b</sup>
	0 $\mu$ l/kg DM <sup>c</sup>		120 $\mu$ l/kg DM <sup>c</sup>		0 $\mu$ l/kg DM <sup>c</sup>		120 $\mu$ l/kg DM <sup>c</sup>		
	0 $\mu$ l/kg barley DM <sup>d</sup>	360 $\mu$ l/kg barley DM <sup>d</sup>	0 $\mu$ l/kg barley DM <sup>d</sup>	360 $\mu$ l/kg barley DM <sup>d</sup>	0 $\mu$ l/kg barley DM <sup>d</sup>	360 $\mu$ l/kg barley DM <sup>d</sup>	0 $\mu$ l/kg barley DM <sup>d</sup>	360 $\mu$ l/kg barley DM <sup>d</sup>	
Acetate (mol/100 mol)									
4 h	52.6 d	49.0 ab	50.7 bc	48.0 a	52.8 d	50.9 c	49.2 bc	51.0 c	0.68
12 h	46.3	47.0	45.8	47.2	46.1	45.7	47.0	47.2	0.95
24 h	43.6 a	44.5 a	42.4 a	44.8 ab	46.8 b	44.4 a	42.6 a	42.6 a	0.86
Propionate (mol/100 mol)									
4 h	21.5 d	21.2 cd	21.3 d	21.2 cd	20.6 bc	20.3 ab	20.1 ab	20.0 a	0.19
12 h	23.5 c	23.8 c	23.6 c	23.2 bc	22.2 a	22.7 ab	22.2 a	22.1 a	0.23
24 h	23.7	23.3	23.6	23.7	23.8	24.0	23.4	23.6	0.33
Butyrate (mol/100 mol)									
4 h	17.8 a	19.8 c	18.7 ab	2.05 cd	18.1 a	19.1 bc	19.1 bc	20.3 cd	0.36
12 h	20.6	20.1	20.4	19.7	20.9	21.2	20.5	2.0	0.87
24 h	19.9 ab	20.7 bc	22.1 c	20.7 bc	18.8 a	20.0 ab	21.4 bc	21.0 bc	0.63
Acetate:propionate ratio									
4 h	2.45 c	2.31 ab	2.35 ab	2.28 a	2.56 d	2.50 cd	2.56 d	2.41 bc	0.04
12 h	1.97	1.98	1.95	2.02	2.08	2.03	2.14	2.04	0.06
24 h	1.91	1.91	1.80	1.92	1.96	1.85	1.83	1.91	0.07

Within a row, values followed by different letters (a–d) differ ( $P < 0.05$ ).

<sup>a</sup> All measurements were corrected for blanks containing no substrate.

<sup>b</sup> S.E.M.: standard error of the mean.

<sup>c</sup> Surfactant added during tempering.

<sup>d</sup> Surfactant added to incubation.

Further study is necessary to define the optimal duration of tempering with the surfactant.

In commercial feedlots, barley is usually tempered at moisture levels ranging from 180 to 200 g/kg. The processing characteristics of barley grain at that moisture content (Experiment 2) were unaffected by including surfactant at up to 240  $\mu\text{l/kg}$  DM during 2 or 4 h of tempering. This suggests that the enhanced water uptake associated with surfactant application (Experiment 1) was not sufficient to substantively alter the processing characteristics of barley grain. Under commercial feeding conditions, therefore, including surfactant during tempering at the levels investigated here would not be expected to alter the processing characteristics of feed barley. This is supported by our earlier observation that this surfactant applied at the same rate (i.e., 60 ml/t DM) did not affect barley-processing characteristics (Wang *et al.*, 2003).

Despite no effects on barley processing characteristics, *in vitro* incubation (Experiment 3) demonstrated that IVDMD was reduced at 4 h by surfactant, whether applied before or after rolling the barley. Concurrent surfactant effects on accumulations of VFA and RS in the incubation liquid were observed. Effects of surfactant on initial rate of barley digestion were similar across roller settings, but this was not likely due to surfactant effects on particle size distribution, given the lack of effects of surfactant on processing characteristics described above. The pattern of fermentation responses to particle size (i.e., roller setting 2.032 mm versus 2.413 mm) differed from the fermentation response to surfactant. The lower initial IVDMD observed in association with increased particle size due to more widely spaced rollers was accompanied by decreased gas production and molar proportion of propionic acid in total VFA, whereas the reduced 4 h IVDMD in response to surfactant mainly reduced accumulation of VFA and RS and the molar percentage of acetic acid. This suggests that the mechanisms by which feed particle size and surfactant impact ruminal fermentation differ.

The effects of surfactant on ruminal fermentation were similar, irrespective of the timing of the application (i.e., during tempering or after processing), which is also indicative of the surfactant exerting a direct effect on the rumen microbial ecosystem. This is supported by earlier findings that the saponin upon which this surfactant is based enhanced the activity of ruminal microbes associated with starch digestion and utilization, while inhibiting ruminal fibre digestion (Wang *et al.*, 2000a, 2000b) and that it decreased rumen protozoal numbers (Wang *et al.*, 1998; Wallace *et al.*, 1994).

Although the effects of surfactant on 4 h IVDMD and VFA and RS concentrations were independent of substrate particle size, the surfactant-reduced acetate:propionate ratio was evident at all levels of surfactant application when barley was rolled at 2.032 mm, but only at the highest application rate (480  $\mu\text{l/kg}$  DM) when barley was rolled at 2.413 mm. This suggests that effectiveness of surfactant for regulating ruminal digestion of barley grain may be greater with (smaller) rather than larger particle sizes, which is also consistent with our earlier study (Wang *et al.*, 2003). The present study revealed that the positive effect of the surfactant on performance of feedlot steers reported by Wang *et al.* (2003) was probably the combined result of a lower initial rate of digestion that reduced the occurrence of acidosis, a reduced acetate:propionate ratio that increased energy efficiency, and increased microbial N efficiency as found by Zinn *et al.* (1998).

The effects of surfactant application were evident only during the first 4 h of incubation. This is likely due to the microbial inactivation of the steroidal saponin in the surfactant,

presumably by deglycosylation of saponin to sapogenin, as observed previously (Wang et al., 2000b; McAllister et al., 2001), rather than to adaptation of the ruminal microorganisms to the surfactant. Wang et al. (2000b) found no adaptation of the principal ruminal bacteria involved in starch and fibre digestion to this saponin.

## 5. Conclusions

Including a saponin-based surfactant in the water during tempering of barley increased the initial rate of water uptake by the kernels, but this effect was not apparent if tempering was prolonged beyond 4 h. The surfactant did not directly affect processing characteristics of the barley grain, yet it reduced in vitro digestion of barley grain measured at 4 h (but not at 24 h), probably due to direct effects of the surfactant on the rumen microbial population.

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