

# Effect of a saponin-based surfactant and aging time on ruminal degradability of flaked corn grain dry matter and starch<sup>1</sup>

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**ABSTRACT:** The objectives of this study were to investigate the effect of a saponin-based surfactant, Grain Prep surfactant (GP), and hot flake aging time on starch characteristics and ruminal DM and starch degradability of steam-flaked corn grain. In 2 experiments, the moisture content of incoming corn was automatically adjusted using the Grain Prep Auto Delivery System to 19.8% (Exp. 1) and 18.5% (Exp. 2). The application rate of GP was 22 mg/kg (as-is basis). Control corn was treated with water alone. Processed corn in Exp. 2 was stored in insulated containers for 0, 4, 8, or 16 h. Flaked corn samples were incubated in the rumen of lactating dairy cows for 0, 2, 4, 6, 16, or 24 h. In Exp. 1, GP increased, compared with the control, the soluble fraction and effective degradability (ED) of DM by 17.2 and 8.6%, respectively. The ED of cornstarch was increased by 6.7%. In Exp. 2, the concentration of soluble DM and starch were increased by GP by 15 and 24% compared with the control. The ED of DM and starch were also increased by 3 and 4%, respectively. No differences in

gelatinization temperatures were observed due to treatment, except that GP-treated grain had a slightly greater mean gelatinization enthalpy in Exp. 2. In a pilot study, DM degradability parameters were not affected by germination of the corn kernels. Aging of the hot flakes for up to 16 h resulted in a quadratic decrease in DM and starch ruminal degradability. The aging process affected starch gelatinization enthalpy values of flaked grain in a manner opposite to that observed for ruminal DM and starch degradation. This phenomenon was most likely explained by increased starch intramolecular associations or crystallinity associated with starch annealing, or both. This study confirmed our previous observations that Grain Prep surfactant increases flaked corn DM and starch degradability in the rumen. Because the rate of degradation was not affected by the surfactant, the increase in degradability was attributed mainly to increases in DM and starch solubility.

**Key words:** corn grain, grain processing, in situ, saponin

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## INTRODUCTION

The goal in processing cereal grains for cattle diets is to increase their digestibility and energy concentration. Steam-flaking of corn grain combines 3 important physical factors (water, heat, and shear force) to produce swelling, consequent gelatinization, and increased digestibility of cornstarch (Zinn et al., 2002). These factors facilitate enhanced water penetration, heat input, and

a thinner flake to increase starch availability in the rumen and total tract digestibility. A recent study by Sindt et al. (2006) reported a linear increase in starch availability with an increasing tempering moisture concentration from 0 to 12%. Similarly, the ruminally degradable fraction of barley DM was linearly increased with increasing grain moisture content from 9 to 22% [A. N. Hristov, T. A. McAllister (Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada), and D. Greer, unpublished data]. Thus, increased water penetration and moisture content of the grain before flaking would most likely result in enhanced starch availability.

Saponins, with their surface active, foam-forming properties (Cheeke and Shull, 1985) can enhance water penetration and consequently digestibility of processed grain and may trigger biochemical processes conducive to germination (Sobia and Ahmad, 2004). For example, corn (Johnson and Greer, 1996) or barley (Wang et al., 2005) grains treated with a saponin-based surfactant

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had markedly faster rates of hydration than untreated grains. In situ, solubility and degradability of flaked corn grain DM and starch were increased by a saponin-based surfactant (Hristov et al., 2004).

The objectives of this study were to investigate the effect of a saponin-based surfactant, Grain Prep surfactant (GP), and hot-flake aging time on starch characteristics and ruminal DM and starch degradability of steam-flaked corn grain.

## MATERIALS AND METHODS

### Corn Grain

The effect of GP on ruminal degradation of steam-flaked corn DM and starch was examined in 2 commercial feed processing plants. In both experiments, the moisture content of the incoming corn was automatically adjusted using the Grain Prep Auto Delivery System (AgriChem Inc., Ham Lake, MN) to a moisture content of 19.8 and 18.5% (Exp. 1 and 2, respectively). The average starch content (DM basis) of the 2 grains was  $71.0 \pm 0.50$  and  $74.0 \pm 0.61\%$ , respectively. In both experiments, the corn was discharged from the conditioning and mixing auger, where the moisture content adjustment was made, into a surge bin.

In Exp. 1, the surge bin was above the steam chest and the corn was held for an average of 45 min before flowing by gravity into the steam chest. Residence time in the steam chest where live steam was injected was approximately 20 min. The corn reached a temperature of 95°C approximately 10 min before discharge from the steam chest and rolling. In Exp. 2, the surge bins were at ground level and the corn was held for 12 to 16 h before it was conveyed to the steam chest with a bucket elevator. In both experiments, the steam-flaked grain below the rolls had gained approximately 4% moisture from the steam (corn treated with water only,  $3.9 \pm 0.7\%$ ; corn treated with the GP surfactant solution,  $4.2 \pm 0.5\%$ ).

There was no discernible moisture loss from rolling in Exp. 1, but approximately 20 g/kg was lost in the air lift that transported the grain flakes from under the rolls to the feed batching area. In Exp. 2, the control corn lost more moisture in rolling than did the GP-treated corn, 1.7 vs. 1.0% ( $P = 0.020$ ). The density of the finished flakes was 33.5 kg/100 L (Exp. 1) and 41.3 kg/100 L (Exp. 2). In both experiments, GP was injected into the conditioning water at a rate to produce a 750 mg/kg solution. The final GP concentration of the finished flake was estimated to be 22 mg/kg (as-is basis). Control corn was treated with water alone.

In Exp. 1, 3 samples were prepared for each treatment (water and GP) and preserved in ice. In Exp. 2, hot flakes were collected under the rollers, sealed in bags, and immediately immersed in ice (0-h flakes) or allowed to age in sealed insulated containers for 4, 8, or 16 h, after which time the flakes were placed in ice. Three replications were completed for each treatment (control and GP) and aging time (0, 4, 8, or 16 h). Thus, in both

experiments, 3 replicates per treatment were analyzed for rumen degradability.

### Germination Study

The following experiment was conducted to investigate the effect of kernel germination and GP on in situ degradability of corn grain. Five hundred grams of intact, untreated corn kernels with 10% moisture content were placed in jars and tumbled (rock tumblers, Covington Engineering, Redlands, CA) for 6 h with water to reach a grain moisture content of  $34.2 \pm 0.51\%$ . Treatments were 3 levels of GP: 0 mg/kg (water alone), 60 mg/kg, and 600 mg/kg (final concentration on an as-is basis; estimated at 10% grain moisture). These application levels were based on our previous in vitro data (Hristov et al., 2004). Kernels were placed on moist towels and allowed to germinate at room temperature (24°C). When >50% of the kernels germinated, the grain was placed on crushed ice and immediately processed for in situ DM degradability. Shoots emerged from 60 to 70% of the kernels in approximately 48 h. Two more treatments were prepared and incubated in situ without being allowed to germinate: 0 mg/kg GP (water) and 60 mg/kg GP. All treatments were prepared in duplicate and incubated in the rumen, as described in the next section, simultaneously. Grain was coarsely rolled using a laboratory-scale roller (Model M9-12, Kalrob Machining Ltd., Picture Butte, Alberta, Canada) before the in situ incubation. Grain from this study was incubated in the rumen for 0, 2, 4, 6, 16, 24, or 48 h, and only degradability of corn DM was estimated.

### In Situ Degradability

Animals used for the in situ experiments were cared for according to the guidelines of the University of Idaho Animal Care and Use Committee. The experiments utilized 3 ruminally cannulated (Bar Diamond, Parma, ID) lactating dairy cows. In Exp. 1 and the germination study, the cows were on average  $295 \pm 100$  d in milk and had average BW and daily milk yield of  $623 \pm 22.6$  and  $26.8 \pm 4.9$  kg. Cows used for Exp. 2 were on average  $213 \pm 89.5$  d in milk with average BW and daily milk yield of  $760 \pm 77.7$  and  $29 \pm 2.9$  kg. In Exp. 1, cows were fed (% of DM): alfalfa hay, 28.5; corn silage, 17.0; steam-rolled corn grain, 19.3; rolled barley grain, 12.3; dried distiller grains, 8.5; whole cottonseed, 4.4; shredded beet pulp, 3.5; extruded soybean meal, 4.4; and a mineral and vitamin supplement (Land O'Lakes, Saint Paul, MN), 2.1. In Exp. 2, the diet fed to the cows consisted of (% of DM): alfalfa hay, 34.9; corn silage, 21.8; cull peas, 14.0; steam-rolled corn grain, 8.7; rolled barley grain, 6.0; dried distiller grains, 5.4; whole cottonseed, 5.3; and a commercial supplement containing fat, ruminally protected methionine, minerals, and vitamins (Land O'Lakes), 3.9.

In both experiments, the diets were fed twice daily (0700 and 1600) for 14 d before and throughout the

**Table 1.** Ruminal in situ degradability of flaked corn grain DM and starch as affected by Grain Prep (GP) surfactant (Exp. 1)<sup>1</sup>

Item	Treatment		P-value <sup>2</sup>
	Water	GP	
DM			
Soluble, %	20.9 ± 1.30	24.5 ± 1.28	0.050
Potentially degradable (fraction <i>b</i> ), %	54.2 ± 1.62	56.4 ± 1.63	0.337
Rate of degradation of <i>b</i> , %/h	15.9 ± 1.41	15.8 ± 1.27	0.948
Effective degradability, %	60.3 ± 0.62	65.5 ± 0.62	<0.001
Starch			
Soluble, %	18.1 ± 1.48	20.4 ± 1.46	0.290
Potentially degradable (fraction <i>b</i> ), %	61.9 ± 1.82	65.7 ± 1.84	0.143
Rate of degradation of <i>b</i> , %/h	17.2 ± 1.44	16.4 ± 1.25	0.658
Effective degradability, %	64.1 ± 0.71	68.5 ± 0.71	<0.001

<sup>1</sup>Values are model estimates and associated SE; n = 186.

<sup>2</sup>Main effect of treatment.

duration of the study. Processed corn samples collected on the experimental sites were immediately sealed, refrigerated, and transported to the University of Idaho within 48 h. Flaked corn samples were sieved through a 4.75-mm sieve to remove fines before being incubated in the rumen. Grain samples, 5 g (as-is basis), were incubated in duplicate in 50- $\mu$ m, 10 × 20-cm polyester bags (Ankom Technology, Fairport, NY) for 0 (water-washed but not incubated in the rumen), 2, 4, 6, 16, or 24 h. Original samples and bag residues were analyzed for DM (oven drying at 60°C) and for starch using a starch assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland; McCleary et al., 1994). After ruminal incubation, the bags were washed in a household washing machine with cold tap water (3 cycles of 5 min each without a spin cycle).

### Differential Scanning Calorimetry

Gelatinization properties of ground, flaked, corn grain samples from Exp. 1 and 2 were analyzed using a TA Instruments 2920 Modulated Differential Scanning Calorimeter (New Castle, DE). Grain samples (10 ± 0.01 mg, DM basis) were weighed directly into stainless-steel sample pans, followed by the addition of deionized water (20  $\mu$ L). Pans were sealed, allowed to equilibrate overnight, and heated from 20 to 180°C at a rate of 10°C/min. An empty pan was used as a reference. The enthalpy change ( $\Delta H$ , a measure of the amount of energy required to bring about melting of a crystalline material) and gelatinization onset ( $T_o$ ), peak ( $T_p$ ), and completion ( $T_c$ ) temperatures were computed from the gelatinization endotherms.

### Statistical Analysis

Statistical analyses were performed using SAS (SAS Inst. Inc., Cary, NC). In Exp. 1 and 2, degradability data for duplicate bags incubated in each cow at each time point were averaged (thus, n = 3). In situ degradability parameters (soluble DM or starch, **fraction a**; poten-

tially degradable fraction, **fraction b**; rate of degradation of the degradable fraction, *c*; and effective degradability in the rumen, **ED**) were estimated according to the model of Ørskov and McDonald (1979; NLMIXED procedure of SAS) using dummy variable technique for treatment comparisons and contrasts.

Degradation curves from the germination study did not fit the exponential model of Ørskov and McDonald (1979) well. For this experiment, a linear model was used to fit the data:  $y = a + (c \times t)$  (average coefficient of determination,  $r^2 = 0.97 \pm 0.007$ ). The ED of corn DM was estimated assuming all DM was degradable in the rumen; i.e., potentially degradable DM (*b*) = 1 - *a*; ED of DM =  $a + \{(1 - a) \times [c/(c + k_p)]\}$ , where *c* and  $k_p$  are the rates of degradation and passage, respectively (Mathers and Miller, 1981).

In all experiments, a passage rate of 0.06/h was used to estimate ED. Replicated bags were averaged per cow and incubation time. Data on starch characteristics were analyzed using ANOVA. Statistical difference was declared at  $P \leq 0.05$ . When the overall treatment effect was  $P \leq 0.05$ , treatment means were separated by pairwise *t*-test (flake aging time data) or an LSD test (differential scanning calorimetry data).

## RESULTS AND DISCUSSION

In Exp. 1 and 2, the soluble fraction (fraction *a*) of flaked corn DM was greater ( $P = 0.050$  and  $0.003$ , Exp. 1 and 2, respectively) for GP-treated grain compared with the untreated control (Tables 1 and 2 and Figures 1 and 2). In Exp. 2, there was a significant ( $P = 0.009$ ) interaction between surfactant treatment and aging time for fraction *a*. Thus, GP-treated grain had greater proportion of fraction *a* DM than the control grain at aging times 0 ( $P < 0.001$ ) and 4 h ( $P = 0.033$ ) and tended to have ( $P = 0.091$ ) a greater proportion at aging time of 8 h (Table 3). Control and GP-treated corn aged for 16 h had similar ( $P = 0.192$ ) concentration of fraction *a* DM. No other interactions between the main effects were found for any of the DM or starch degradability param-

**Table 2.** Ruminal in situ degradability of flaked corn grain DM and starch as affected by Grain Prep (GP) surfactant (Exp. 2)<sup>1</sup>

Item	Treatment		<i>P</i> -value <sup>2</sup>
	Water	GP	
DM			
Soluble, <sup>3</sup> %	19.8 ± 0.70	22.7 ± 0.68	0.003
Potentially degradable (fraction <i>b</i> ), %	60.2 ± 1.35	60.6 ± 1.53	0.843
Rate of degradation of <i>b</i> , %/h	10.1 ± 0.64	9.3 ± 0.61	0.356
Effective degradability, %	57.5 ± 0.38	59.4 ± 0.38	<0.001
Starch			
Soluble, %	24.2 ± 1.02	29.7 ± 0.99	<0.001
Potentially degradable (fraction <i>b</i> ), %	60.4 ± 1.47	59.7 ± 1.85	0.782
Rate of degradation of <i>b</i> , %/h	12.3 ± 0.92	10.3 ± 0.90	0.122
Effective degradability, %	64.7 ± 0.50	67.2 ± 0.51	<0.001

<sup>1</sup>Values are model estimates and associated SE; n = 431.

<sup>2</sup>Main effect of treatment.

<sup>3</sup>Treatment × flake aging time interaction, *P* = 0.009; other interactions for DM or starch degradability parameters were not significant (*P* = 0.117 to 0.952).

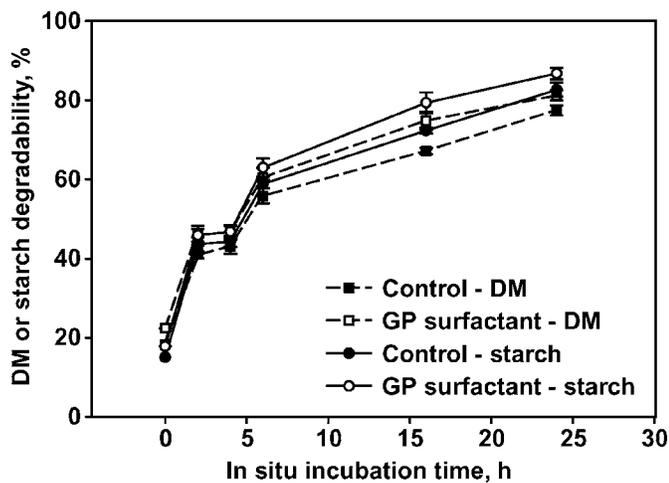
ters. The potentially degradable DM of the processed corn grain and its rate of degradation were not affected by treatment (*P* = 0.337 to 0.948). The ED of DM was increased (*P* < 0.001) by GP in both experiments: by 8.6% in Exp. 1 and by 3.3% in Exp. 2.

The concentration of soluble fraction of cornstarch was similar (*P* = 0.290) between the 2 treatments in Exp. 1, but was greater (*P* < 0.001) for GP compared with the control in Exp. 2 (Tables 1 and 2 and Figures 1 and 2). Similar to DM, the potentially degradable fraction of starch and its rate of degradation were not affected (*P* = 0.122 to 0.782) by treatment in both experiments. The ED of starch was greater (*P* < 0.001) for GP-treated corn compared with the control: by 6.9% in Exp. 1 and by 3.9% in Exp. 2.

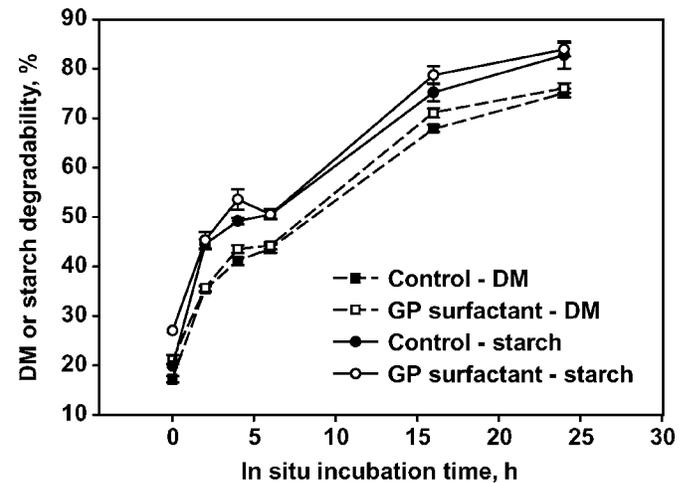
Results from these 2 experiments confirm our previous data with steam-flaked corn grain from a commercial

feed mill in OR (Hristov et al., 2004). In this earlier study, ruminal degradability of GP-treated grain DM and starch was increased over the control by 4.8 and 4.0%, respectively. Thus, in 3 separate studies we found unidirectional response in ruminal degradability characteristics to GP surfactant: increased solubility and ED of corn DM and starch.

In the literature, increased solubility and degradability of flaked corn have often been demonstrated to be a function of the extent of starch gelatinization. Enhanced penetration of moisture into the kernels by GP (Wang et al., 2005) may intensify the swelling and loss of birefringence and granule crystallinity associated with starch gelatinization (MacGregor and Fincher, 1993). Increased tempering moisture or grain moisture before processing resulted in increased cornstarch availability in vitro (Sindt et al., 2006) and the proportion of degrad-



**Figure 1.** Ruminal in situ degradability of flaked corn grain DM and starch as affected by Grain Prep (GP) surfactant in Exp. 1 (means ± SEM). Control and GP surfactant DM and starch degradation lines differed (*P* < 0.001).



**Figure 2.** Ruminal in situ degradability of flaked corn grain DM and starch as affected by Grain Prep (GP) surfactant in Exp. 2 (means ± SEM). Control and GP surfactant DM and starch degradation lines differed (*P* < 0.001).

**Table 3.** Proportion of the soluble in the rumen DM in control and Grain Prep (GP) surfactant-treated flaked corn grain as affected by flake aging time (Exp. 2)<sup>1</sup>

Item	Treatment		P-value <sup>2</sup>
	Water	GP	
Aging time, h			
0	21.9 ± 1.43	28.5 ± 1.37	<0.001
4	19.1 ± 1.40	23.2 ± 1.32	0.033
8	16.1 ± 1.42	19.4 ± 1.35	0.091
16	22.3 ± 1.35	19.7 ± 1.42	0.192

<sup>1</sup>Values are model estimates and associated SE; n = 431.<sup>2</sup>Main effect of treatment.

able barley grain DM in situ [A. N. Hristov, T. A. McAllister (Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada), and D. Greer, unpublished data]. Although solubility of starch in cereal grains is not equivalent to degradability, solubility of cornstarch does increase due to gelatinization (Zinn et al., 2002; Levine et al., 2004), and this process has been accompanied by increased rate of and total gas production (Solanas et al., 2005) and enzymatic degradability of the starch (Levine et al., 2004). In pigs, Medel et al. (2004) reported increased energy digestibility with increasing gelatinization of barley starch due to steam cooking and subsequent flaking. Mild processing methods (pelleting and expanding), however, may not produce starch gelatinization to the extent that will result in significant increase in digestibility (Svihus et al., 2005). More severe processes, such as extrusion and steam-flaking, will most likely result in more complete gelatinization and consequent increase in starch availability (Solanas et al., 2005; Svihus et al., 2005). However, in our study, no differences in gelatinization behavior ( $T_o$ ,  $T_p$ ,  $T_c$ , or  $\Delta H$ ) were observed between the water- and GP-treated corn samples in Exp. 1 (Table 4), though starch within grain samples was not fully gelatinized in the case of either treatment by the steam-flaking process (indicated by

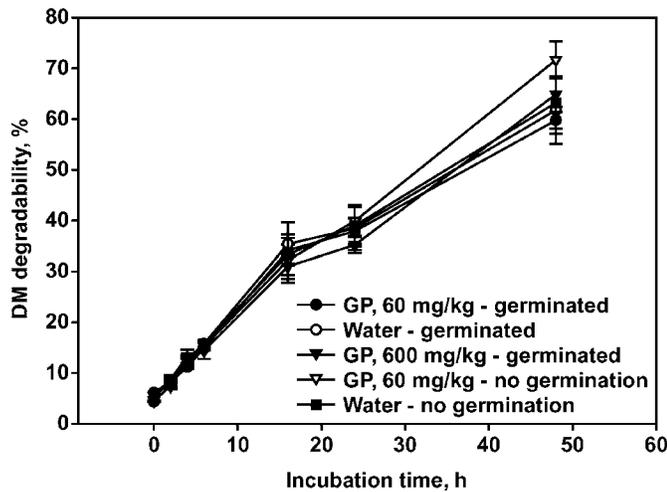
**Table 4.** Gelatinization properties of Grain Prep (GP) surfactant- and water-treated flaked corn grain (Exp. 1, n = 6; Exp. 2, n = 24)

Item	Treatment			P-value <sup>1</sup>
	Water	GP	SEM	
Exp. 1				
Onset temperature, °C	62.6	62.9	0.64	0.748
Peak temperature, °C	74.2	73.1	1.44	0.627
Completion temperature, °C	84.7	84.8	0.45	0.911
Enthalpy, J/g	1.2	1.6	0.38	0.564
Exp. 2				
Onset temperature, °C	65.0	65.1	0.17	0.758
Peak temperature, °C	74.5	74.5	0.12	0.805
Completion temperature, °C	83.5	83.9	0.23	0.197
Enthalpy, <sup>2</sup> J/g	2.5	2.8	0.07	0.005

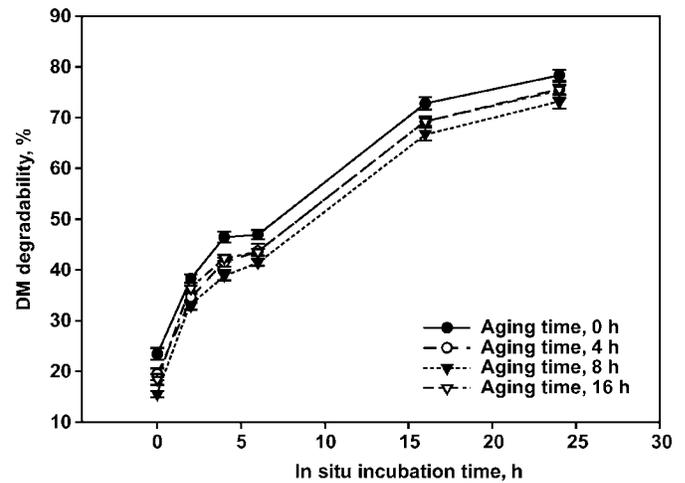
<sup>1</sup>Main effect of treatment.<sup>2</sup>Treatment × flake aging time interaction,  $P = 0.042$ .

the positive  $\Delta H$  values). For Exp. 2, no differences in gelatinization temperatures ( $T_o$ ,  $T_p$ ,  $T_c$ ) were observed between the 2 treatments. However, GP-treated grain possessed a slightly greater mean gelatinization enthalpy ( $P = 0.005$ ) compared with the control grain (Table 4), a result that would appear to be in conflict with the greater ruminal DM and starch degradability observed for the GP-treated (relative to water-treated) grain. Though there was a significant interaction between treatment and aging time for gelatinization enthalpy ( $P = 0.042$ ), the interaction was deemed to be nonsevere and of no practical significance, indicating no real differential response of treatment to the aging process. The fact that the extent of starch gelatinization did not consistently explain ruminal degradation patterns in Exp. 1 and 2 suggests that other factors may contribute to the increase in ruminal DM and starch degradability observed for the GP-treated grain.

The physical and perhaps biochemical factors involved during steam-flaking of grain are complex. Absorption of water, increased temperature, and mechanical disruption of the kernel outer layers may trigger processes conducive to natural germination of the seed. Thus, mechanical processing may activate the enzymatic metabolism of the embryonic tissues, which will result in conversion of starch into soluble sugars. Saponins from GP may enhance these processes through a dual mode of action: 1) as wetting agents, reducing surface tension and increasing water penetration; and 2) as bioactive agents, stimulating enzymatic processes in the seed. Saponins with their surface active, foam-forming properties (Cheeke and Shull, 1985) have been shown to enhance water penetration in the seed (Wang et al., 2005). In this latter study, GP increased the rate of water uptake by barley kernels during the initial 2 h of tempering (Wang et al., 2005). In contrast, Sindt et al. (2006) did not observe changes in tempered, steamed, or flaked corn moisture due to the addition of a saponin-based surfactant. Steroidal saponins (occurring in *Yucca schidigera*) are likely to affect biochemical reactions in living organisms. Although studies with cereal grains are scarce, biomedical research with other plants, mice, and fish has shown increased hormonal (El Izzi et al., 1992; Francis et al., 2002; Kim et al., 2003) and enzymatic (Koduri and Rillema, 1992) activities in the presence of saponins. Saponins (saponin mannitol, or from alfalfa) did not affect or reduced germination percentage and radicle growth of oats, barley, and rye (Luntz and Salguez, 1988, and Fernandez et al., 1986, respectively). However, a more recent study reported increased germination percentage of sorghum and pearl millet with the inclusion of 0.25% crude saponins from *Sapindus mukorossi* (Sobia and Ahmad, 2004). Our data showed no effect of GP on germination percentage of corn grain. In 40 h, 38% of the kernels (452 out of 1,190) on all treatments sprouted and, in 48 h, 67% (water) and 70% (GP treatments) of the kernels sprouted. In situ DM degradability parameters were also not affected by germination or GP treatment (effects of germination and GP treatment,



**Figure 3.** Effect of germination and Grain Prep (GP) surfactant on ruminal in situ degradability of corn grain DM (means  $\pm$  SEM). Degradation lines were not affected ( $P = 0.114$  to  $0.805$ ) by treatment.



**Figure 4.** Ruminal in situ degradability of flaked corn grain DM as affected by aging time in Exp. 2 (means  $\pm$  SEM). Degradation lines differed ( $P = 0.002$  to  $<0.001$ ), except for 4 vs. 16 h of aging ( $P = 0.954$ ).

respectively; Figure 3): soluble DM = 8.4 to 9.5% ( $P = 0.567$  and  $0.982$ ); rate of degradation = 4.5 to 4.9% ( $P = 0.958$  and  $0.987$ ); and ED = 48.5 to 49.8% ( $P = 0.923$  and  $0.982$ ). Interactions between the main effects were not significant for any of the in situ degradability parameters ( $P = 0.645$  to  $0.731$ ). Thus, the effect of GP on the soluble fraction of corn DM and starch found in these experiments cannot be explained by intensified germination processes in the seed.

Saponins may also act on the microbial activities in the rumen. The effects of these compounds on ruminal ammonia and propionate concentrations and certain microbial groups are well documented (Wallace et al., 1994; Hussain and Cheeke, 1995; Makkar et al., 1998; Hristov et al., 1999). At the concentrations employed in Exp. 1 and 2, however, ruminal effects of the saponins in GP are unlikely (Hristov et al., 1999; 2004; Lovett et al., 2006).

Aging of flaked corn significantly impacted fraction  $a$  of DM and starch (Table 5 and Figures 4 and 5). Fraction  $a$  of DM and starch decreased ( $P < 0.001$ ) quadratically with increasing aging time from 0 to 16 h; flakes aged for 4 and 8 h had decreased fraction  $a$  compared with the 0 h flakes ( $P = 0.004$  and  $< 0.001$ , respectively). The 16-h flakes had lower ( $P = 0.003$ ) fraction  $a$  DM compared with the 0-h flakes, but the proportion of this fraction increased ( $P = 0.018$ ) compared with the 8-h flakes. The effect on the soluble fraction resulted in similar trends for ED of corn DM; a decrease from 0 to 8 h ( $P < 0.001$ ) and then an increase ( $P < 0.001$ ) from 8 to 16 h aging time. The soluble starch also decreased quadratically ( $P < 0.001$ ) with aging time. Aging time had no effect on the potentially degradable fractions of starch and its rate of degradation ( $P = 0.214$  and  $0.873$ , respectively), but similar to DM, decreased quadratically the ED of

**Table 5.** Ruminal in situ degradability of flaked corn grain DM and starch as affected by aging time (Exp. 2)<sup>1</sup>

Item	Aging time, h				<i>P</i> -value <sup>2</sup>
	0	4	8	16	
<b>DM</b>					
Soluble, <sup>3</sup> %	25.2 $\pm$ 0.99 <sup>a</sup>	21.2 $\pm$ 0.96 <sup>b</sup>	17.7 $\pm$ 0.98 <sup>c</sup>	21.0 $\pm$ 0.98 <sup>b</sup>	<0.001
Potentially degradable (fraction $b$ ), %	60.2 $\pm$ 2.21	60.7 $\pm$ 2.06	60.5 $\pm$ 1.86	60.2 $\pm$ 2.02	0.999
Rate of degradation of $b$ , %/h	9.4 $\pm$ 0.93	9.5 $\pm$ 0.86	10.1 $\pm$ 0.87	9.9 $\pm$ 0.89	0.912
Effective degradability, <sup>3</sup> %	61.7 $\pm$ 0.54 <sup>a</sup>	58.2 $\pm$ 0.54 <sup>1</sup>	55.6 $\pm$ 0.53 <sup>c</sup>	58.3 $\pm$ 0.54 <sup>b</sup>	<0.001
<b>Starch</b>					
Soluble, <sup>3</sup> %	33.6 $\pm$ 1.36 <sup>a</sup>	25.9 $\pm$ 1.33 <sup>b</sup>	23.4 $\pm$ 1.59 <sup>b</sup>	25.1 $\pm$ 1.37 <sup>b</sup>	<0.001
Potentially degradable (fraction $b$ ), %	56.0 $\pm$ 2.21	61.9 $\pm$ 2.62	62.0 $\pm$ 2.44	60.2 $\pm$ 2.13	0.214
Rate of degradation of $b$ , %/h	11.6 $\pm$ 1.35	10.7 $\pm$ 1.20	11.0 $\pm$ 1.32	12.0 $\pm$ 1.29	0.873
Effective degradability, <sup>3,4</sup> %	70.4 $\pm$ 0.70 <sup>a</sup>	65.2 $\pm$ 0.71 <sup>b</sup>	63.2 $\pm$ 0.72 <sup>b</sup>	65.1 $\pm$ 0.71 <sup>b</sup>	<0.001

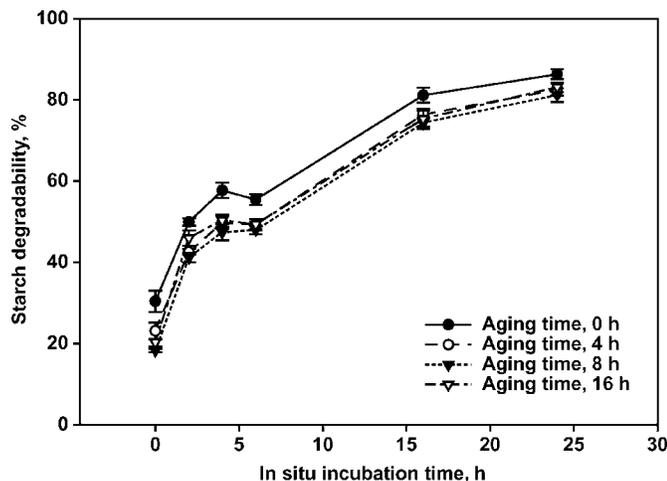
<sup>a-c</sup>Within a row, means without a common superscript letter differ ( $P < 0.05$ ).

<sup>1</sup>Values are model estimates and associated SE;  $n = 431$ .

<sup>2</sup>Main effect of aging time.

<sup>3</sup>Linear and quadratic effects of aging time ( $P \leq 0.001$ ).

<sup>4</sup>4 vs. 8 h of aging,  $P = 0.056$ .



**Figure 5.** Ruminal in situ degradability of flaked corn grain starch as affected by aging time in Exp. 2 (means  $\pm$  SEM). The 0-h degradation line differed ( $P < 0.001$ ) from the 4-, 8-, and 16-h degradation lines.

cornstarch ( $P < 0.001$ ). Flakes aged for 16 h tended to have greater ( $P = 0.061$ ) starch ED than flakes aged for 8 h.

There are several physical phenomena that might offer some explanation and insight into these observations. First, the aging process appeared to impact starch gelatinization enthalpy values of flaked grain (Table 6) in a manner inverse to that observed for ruminal DM and starch degradation. Gelatinization enthalpy was greater for flaked grain subjected to 4 h of aging compared with that processed without any aging. The noted increase in starch gelatinization enthalpy for the 4 h (relative to 0 h) flaked grain was likely caused by an annealing phenomenon, which involves ordering and perfection of starch crystallites held just below submelting temperatures in the presence of water or plasticizer (Tester et al., 2000). This annealing or molecular reordering has been demonstrated to influence gelatinization temperatures and increase gelatinization enthalpy values (Krueger et al., 1987). For aging, hot-flaked grain (approximately 84°C coming off the rollers) was stored in closed containers and allowed to gradually cool over the course of the aging period. It is estimated that flaked grain subjected to 4 h of aging had reached a tempera-

ture of approximately 59°C by the end of the aging period. Annealing could have conceivably occurred under such conditions and would provide a logical explanation for the observed increase in enthalpy values for flaked grain aged for 4 h. A greater presence of starch crystallites would be consistent with decreased ruminal degradation. Beyond 4 h, grain temperatures encountered during aging ( $<59^{\circ}\text{C}$ ) would likely not be sufficient for further significant annealing to occur. As results from Exp. 2 indicated (Table 5), solubility of corn DM and starch tended to increase between 8 and 16 h of aging. The increase in DM (and starch,  $P = 0.061$ ) ED between 8 and 16 h of storage observed in Exp. 2 is difficult to explain but could result from microbial or enzymatic degradation of starch. These observed trends were consistent with starch gelatinization enthalpy values, which generally tended to decrease at 8 and 16 h of aging.

Another phenomenon likely contributing to the observed decrease in cornstarch solubility and degradability with increasing aging time is the process of starch retrogradation. Upon storage, dispersed starch molecules (primarily amylose) from flaked grain may reassociate, which has an effect on starch availability opposite to gelatinization (Zinn et al., 2002). Storage of processed grain at low temperatures may enhance the process of starch retrogradation (Hoover, 1995). Retrograded starch is more resistant to enzymatic digestion (Hongtrakul et al., 1998; Gajda et al., 2005; Sindt et al., 2006) and may bypass to the large intestine undigested (Reid and Hillman, 1999; studies with pigs). Ward and Galyean (1999) reported a dramatic decrease in starch availability for steam-flaked corn stored in a holding bin compared with grain sampled immediately after being rolled. Unlike results from Exp. 2, however, these authors did not observe differences in in vitro digestibility of fresh and stored grain. Similarly, Zinn and Barrajás (1997) also did not detect differences in starch reactivity (solubility) and ruminal and total tract digestion of steam-flaked corn fed to cattle fresh or after being dried for 5 d. These discrepancies in the effect of storage on starch availability between experiments are difficult to explain. Our results indicate that solubility and degradability of cornstarch (due to annealing phenomenon, or starch retrogradation, or both) are decreased rapidly with storage of the hot flake. These ruminal effects, however, are subtle and may not correspond to increased total tract starch digestibility in vivo.

**Table 6.** Gelatinization properties of flaked corn grain as affected by aging time (Exp. 2;  $n = 24$ )

Item	Aging time, h				SEM	P-value <sup>1</sup>
	0	4	8	16		
Onset temperature, °C	65.0	65.0	65.3	64.8	0.24	0.408
Peak temperature, °C	74.3	74.6	74.8	74.4	0.17	0.247
Completion temperature, °C	83.4	83.7	84.3	83.4	0.32	0.152
Enthalpy, J/g	2.5 <sup>a</sup>	3.0 <sup>b</sup>	2.7 <sup>ab</sup>	2.5 <sup>a</sup>	0.10	0.009

<sup>a,b</sup>Within a row, means without a common superscript letter differ ( $P < 0.05$ ).

<sup>1</sup>Main effect of aging time.

In conclusion, treatment of steam-flaked corn grain with a saponin-based surfactant, GP, produced increased ruminal DM and starch effective degradability in 2 experiments at commercial feed preparation facilities. Because rate of degradation was not affected by treatment, we attribute the increase in degradability mainly to increases in DM and starch solubility. These effects could not be explained by enhanced gelatinization of cornstarch or stimulated germination of the kernels. Aging of the hot flakes for up to 16 h resulted in quadratic decrease in DM and starch ruminal degradability. This phenomenon was most likely explained by increased starch intramolecular associations or crystallinity associated with starch annealing, or both.

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