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Disintegration of starch crystal structure by steam flaking may be responsible for the improvement of *in vitro* ruminal fermentation of steam flaked sorghum grains*

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ABSTRACT

The intact and steam-flaked sorghum grains were analysed for starch crystal structure, gas production kinetics and ruminal fermentation characteristics to investigate the disintegration of starch crystal structure by steam flaking responsible for the improved *in vitro* ruminal fermentation of steam flaked sorghum. All typical diffraction peaks at 2θ values of 15°, 17°, 18°, and 23° disappeared in the steam-flaked sorghum. The degree of starch gelatinization and the rate of gas production were significantly increased ($P<0.0001$) by steam-flaking processing. The steam-flaked sorghum showed increased ruminal molar proportion of acetic acid ($P<0.01$) and propionic acid ($P<0.01$), and decreased acetic: propionic acid ratio ($P<0.05$). The present results indicate that the disintegration of starch crystal structure, associated with increased starch gelatinization in steam-flaked sorghum, would be responsible for the improvement of *in vitro* ruminal fermentation of steam flaked sorghum grains.

KEY WORDS: steam-flaked sorghum, starch, crystal structure, ruminal fermentation, *in vitro*

INTRODUCTION

Sorghum is a widely used grain for feeding livestock. However, the natural starch granules of cereal grains are partially embedded in the protein matrix, and this may impair both the protein and starch digestibility (Streeter et al., 1993).

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Steam flaking, a physical processing of grains, can effectively improve the feeding value of sorghum grains by 12-15% *via* increasing starch digestibility in the rumen and in the whole digestive tract (Huntington, 1997). The possible mechanisms enabling the starch to be more accessible to digestive enzymes and microbes include: 1. the starch granules become swollen by water and heat during the steam processing, and 2. the protein matrix surrounding the endosperm of cereals is disrupted by flaking (Rooney and Pflugfelder, 1986). Moreover, earlier studies suggested that heat-moisture treatment might alter the thermodynamics of starch gelatinization as well as the crystal structure of grain starch (Wang et al., 1998). Although it has been well documented that steam flaking can increase starch gelatinization (Xiong et al., 1990), very little is known about its effects on starch crystal structure. Therefore, the objective of the present study was to determine the effects of steam-flaking processing on the starch crystal structure, *in vitro* gas production kinetics by rumen microorganisms and ruminal fermentation characteristics of sorghum grains.

MATERIAL AND METHODS

The sorghum grains were steam-flaked under the following conditions: retention time in steam chest was 60 min at 100°C. The average bulk density was 443 g/l. The intact and steam-flaked sorghum samples were ground to pass through a 1-mm screen for subsequent analyses. The nutrient composition of the intact and steam-flaked sorghum grain is presented in Table 1. The CP (crude protein) and ash contents of the samples were determined according to AOAC (1997). The NDF (neutral detergent fibre) and ADF (acid detergent fibre) contents were determined using methods of Van Soest et al. (1991). The starch content and the degree of starch gelatinization were measured according to the procedure described by Xiong et al. (1990).

Table 1. Nutrient compositions of the intact and steam-flaked sorghum

Type of sorghum	Nutrient compositions, % DM				
	CP	starch	NDF	ADF	ash
Intact	8.89	69.22	9.72	3.67	1.21
Steam flaked	9.01	68.94	9.89	3.85	1.09

Monochromatic Cu-Kα radiation (wave length = 0.1542 nm) was produced by a BDX3300 X-ray Powder Diffractometer. The intact and steam-flaked sorghum powder samples were exposed to the X-ray beam. The scanning regions of the diffraction angle 2θ were 5°-30°. Duplicated measurements were made at ambient temperature.

In vitro incubation was according to the procedure developed by Menke et al. (1979). Approximately 200 mg of intact and steam-flaked sorghum samples and 30 ml buffered rumen fluid were put into 100 ml calibrated glass syringes (HFT000025, Häberle Maschinenfabrik GmbH, Germany). Ruminal fluid was obtained from three fistulated native steers fed with diets consisting of 55% roughage (Chinese wild rye and lucerne pellets) and 45% mixed concentrate. The gas production was measured as the volume of gas in the calibrated syringes (3 syringes/treatment) and was recorded at 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 20, 24, 28, 32, 36, 40, 48, 56, 64 and 72 h incubation. Other incubations of the syringes (3 syringes/treatment) were terminated at 24 h for the analysis of fermentation parameters. The pH of the culture fluid was measured immediately. Eight ml of the fluid was collected for the analysis of ammonia nitrogen (NH_3-N) concentration by the method of Broderick and Kang (1980) and volatile fatty acid (VFA) concentration according to Li and Meng (2006). The same experiment was repeated twice on different days with ruminal fluid taken from three different native steers.

Data were subjected to statistical analysis using the GLM procedure of SAS (1999) for a randomized complete block design with treatment as the main effect. Runs served as blocks. Because the block effect was not significant (P>0.05), the data were pooled with six replicates for each group and analysed as a one-way experimental design model. Treatment effects were considered statistically significant at P<0.05.

RESULTS

As shown in Figure 1 and Table 2, the X-ray patterns of the intact sorghum starch gave the stronger diffraction peaks at around 2θ values of 15°, 17°, 18°, and 23°. It is in agreement with the previous finding that the sorghum starch is typically characteristic A-type. In contrast, no diffraction peak and thus crystal

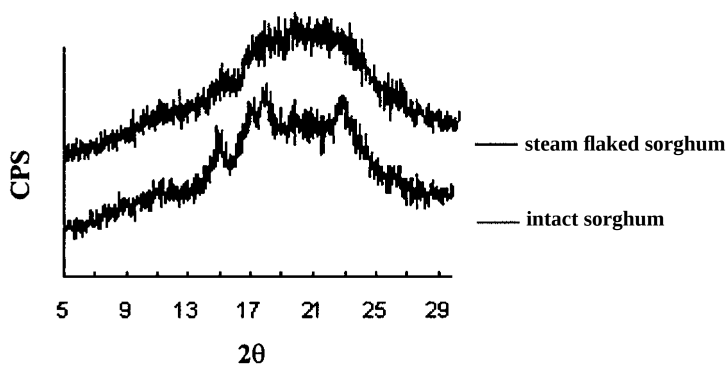

Figure 1. X-ray diffraction spectra of sorghum with/without steam flaking

structure was detected in steam-flaked sorghum starch. The starch gelatinization degree of steam-flaked sorghum increased from 23.13 to 91.18% as compared with that of the intact sorghum grains.

Table 2. X-ray diffraction spectra parameters of sorghum grain starch and the degree of starch gelatinization of sorghum with/without steam-flaking processing

Type of sorghum	2θ	INT [1]	d(A) [2]	I% [3]	Degree of starch gelatinization , %
Intact	15.056	178	5.88	422	23.13
	16.884	421	5.25	999	
	17.896	330	4.95	783	
	22.732	399	3.91	946	
Steam flaked	ND	ND	ND	ND	91.18

[1] INT is the intensity of diffraction peak; [2] d(A) is the lamellar distance; [3] I% is the relative intensity of diffraction peak; ND - not detected

As shown in Table 3, steam-flaking processing significantly increased ($P<0.0001$) the gas production rate of sorghum grains from 0.1104 to 0.1466 h^{-1}; whereas no evident differences ($P>0.05$) were observed in the 72 h total and potential gas production between processed and intact sorghum grain substrates.

Table 3. The effects of steam-flaking on *in vitro* gas production over 72 h and ruminal fermentation parameters after 24 h incubation of sorghum grains (observations, n=6)

Item	Type of sorghum		SEM	P-value
	intact	steam flaked		
72 h total gas production, ml	76.64	75.41	1.15	0.495
Potential gas production (A[1]), ml	74.13	72.36	1.10	0.278
K [1] (h^{-1})	0.1104	0.1466	0.002	<0.0001
Ruminal pH	6.62	6.63	0.18	0.716
Ammonia-N, mg/100 ml	22.92	23.31	1.05	0.8075
Total VFA, mMol/l	101.84	99.65	2.10	0.500
Molar proportion, mol/100 mol				
acetic acid	59.64	60.12	0.062	0.005
propionic acid	23.03	24.25	0.17	0.007
butyric acid	11.11	10.12	0.076	0.001
isobutyric acid	1.18	1.21	0.027	0.396
valeric acid	1.35	1.34	0.003	0.101
isovaleric acid	3.69	2.96	0.090	0.005
Acetic:propionic acid ratio	2.59	2.48	0.019	0.013

[1] the kinetic parameters for gas production were determined using the following one-component model: $y = A (1-e^{-kt})$, where y is the gas production (ml); A is the potential gas production (ml), or theoretically maximal gas production; k (h^{-1}) is the rate constant, an indication of the rate of gas production; t represents the time (h) that gas production was observed

The steam-flaking processing did not affect (P>0.05) the *in vitro* ruminal pH, ammonia-N and total VFA concentrations. However, steam-flaked sorghum grains showed higher molar proportions of acetic acid (P<0.01) and propionic acid (P<0.01), and a lower molar proportion of butyric acid (P=0.001) and acetic: propionic acid ratio as compared with those of intact sorghum grains.

DISCUSSION

The disappearance of the typical diffraction peaks and the increase of the starch gelatinization of steam-flaked sorghum suggested that the starch crystalline granular structure was disintegrated, and both inter- and intra-molecular hydrogen bonds were broken. Therefore, the steam-flaking processing causes looser structures of sorghum starch, which facilitates the starch to absorb moisture and swell (Holrm et al., 1988), and offered higher accessibilities of the starch granules to bacterial and digestive enzymes (Huntington, 1997), resulting in more rapid ruminal fermentation. In agreement with the present study, Theurer (1986) found that the digestion rate of starch in steam-flaked sorghum grains was three times faster than that of intact sorghum starch.

The steam-flaked sorghum grains showed increased molar proportions of acetic acid and propionic acid. Similar results were observed by Vik-Mo et al. (1973), who reported that the acetic acid and propionic acid absorbed by steers increased when steers were fed 77% steam-flaked sorghum-based diets. Such changes of ruminal fermentation are beneficial for enhancing the milk protein content in dairy cows (Chen et al., 1995) as well as improving the energy utilization of feedlot cattle (Theurer et al., 1999).

CONCLUSIONS

In this study, steam-flaking processing disintegrated the starch crystal structure and increased the degree of starch gelatinization as well as gas production rate of sorghum. Steam-flaking also increased ruminal molar proportions of acetic acid and propionic acid, but decreased acetic : propionic acid ratio. These results indicate that the disintegration of starch crystal structure in steam-flaked grains may be responsible for the improvement of *in vitro* rumen fermentation of steam flaked grains.

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