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Research Article

Effects of coating whole cottonseed with starch on *in vitro* ruminal fermentation

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Abstract

The purpose of this study was to evaluate the effects of coating whole cottonseed (WCS) with varying quantities of starch on *in vitro* ruminal fermentation. *In vitro* fermentation experiments were conducted over two days, with three replicates per day (n = 6). Each trial consisted of three treatments (control, 1% starch, and 5% starch). The control was an uncoated WCS, the 1% starch treatment consisted of WCS coated with 1% starch, and the 5% starch treatment was WCS coated with 5% starch. For the 5% starch treatment, the pH of the rumen fluid was lowest, total gas production was highest during the initial stage, NH_3-N content was 25% lower than that of the control ($P<0.05\%$), the acetate ratio at 48 h was reduced by 7.0%, and the propionate ratio increased 7.5% compared to that of the control ($P<0.05$). For the 1% starch treatment, the pH was not different from that of the other treatments except at 3 h after the start of incubation, and total gas production and NH_3-N content were not different from those of the control. Consequently, coating WCS with 5% starch altered the *in vitro* ruminal fermentation parameters; however, 1% starch treatment did not alter ruminal pH, total gas production, NH_3-N, or VFA composition, relative to the control.

Keywords: Whole cottonseed; coating; starch; ruminal fermentation; volatile fatty acid

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Introduction

Whole cottonseed (WCS) is commonly added to cattle feed as a source of energy, fibre, and protein. The chemical composition of WCS, as published by NRC (2000) is 23.5% crude protein (CP), 50.3% neutral detergent fibre (NDF), 40.1% acid detergent fibre (ADF), and 19.3% ether extract (EE). The high fibre concentrations provided by the lint and hull fractions are desirable for maintaining effective NDF levels in the diet (Clark and Armentano, 1993). The lint surrounding WCS is made up almost entirely of cellulose, and is completely digested in the rumen by rumen-dwelling microorganisms (Arieli, 1998). Its capacity for providing energy while increasing effective fibre levels makes WCS a unique livestock feed. Because of the adequate effective NDF and high energy density provided by WCS, it is suitable for animals requiring high levels of dietary energy, such as dairy cows and finishing beef cattle (Coppock et al., 1987).

Even though the nutritional value of WCS is excellent, the lint portion of WCS causes the seeds to stick together, or bridge, making it very difficult to handle using modern mechanized feeding systems

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(Laird et al., 1998). To improve the handling characteristics of WCS, pelleting (Bernard and Amos 1985), extruding (Bernard and Calhoun, 1997; Pena et al., 1986), and acidification (Coppock et al., 1985) methods were researched. Physical treatments like pelleting and extruding cause the dynamic release of free oil from seeds. Effused free oil reduces cellulolytic bacteria activity, and leads to decreased fiber digestion (Pires et al., 1997). Acidification of WCS decreases fat digestibility in the rumen (Coppock et al., 1985).

Recent studies (Laird et al., 1997a&b; Laird et al., 1998) have focused on methods of coating WCS with gelatinized corn starch in order to improve its handling characteristics. The application of a 3% to 5% coating of starch was determined to adequately coat the WCS for easier handling (Laird et al., 1997a, 1998). However, Bernard et al. (1999) reported that feeding WCS coated with 5% starch to dairy cattle increased the activity of amylolytic bacteria, and proportionately decreased the activity of cellulolytic bacteria and acetate, all of which cumulatively led to a decrease in milk fat percentage. Thus, the addition of readily fermentable carbohydrates to the diet alters ruminal fermentation and depresses milk fat production (Poore et al., 1993). When WCS coated with 2.5% starch was fed to dairy cattle (Bernard et al., 1999), dry matter intake, milk yield, and milk composition did not differ from that of the control animals. Thus, even though starch could be a good coating material for WCS, the negative effects of a 5% starch coating on rumen fermentation indicates a need for further research.

Imported coated WCS is expensive. Thus, we have attempted to develop an economic domestic method of coating WCS. The hypothesis of this study was that coating WCS with 1% starch would be effective in improving WCS handling, without having a negative effect on ruminal fermentation. Thus, this study was conducted in order to determine the effect of coating WCS with 1% starch on *in vitro* ruminal fermentation characteristics, compared to those associated with a 5% starch coating.

Materials and Methods

Experimental design

In vitro fermentation was performed over 2 days, with three replicates per day (n = 6). Fermentation trials consisted of three treatments (control, 1% starch, and 5% starch). The control consisted of uncoated WCS, the 1% starch treatment was WCS coated with 1% starch and 1% $CaCO_3$, and the 5% starch treatment was WCS coated with 5% starch and 1% $CaCO_3$. The $CaCO_3$ was added to increase the hardness of the coating.

Coating WCS

All WCS used for incubation trial were de-linted. The de-linting process was carried out using the Biradarpatil and Macha (2009) method. 90 ml of reagent-grade H_2SO_4 (Daejung, Cheongju, Korea) per kg of WCS was added to the WCS, then allowed to react for 8 min. H_2SO_4 –treated and de-linted WCS was washed with running water for 12 h, then dried for 48 h at room temperature. To coat the WCS, 20% (w/w) de-ionized water, 1% or 5% (w/w) gelatinized corn starch (Suprexcorn, FFA, Korea), and 1% (w/w) $CaCO_3$ (A91541, Ducsan, Korea) was added to the WCS, then completely mixed. Next, the mixture was dried in a dry oven (DF-135D1, Doriscience, Bucheon, Korea) at 120˚C for 30 min. The coated WCS were periodically stirred (at about 5-min intervals) during drying. After drying, the coated WCS were allowed to cool to room temperature, then stored in open plastic bags for 3 d to allow for further equilibration to ambient temperature and moisture. The coated WCS were ground using a Wiley Mill until capable of passing through a 6-mm screen (SE263, FOSS, Sweden), prior to *in vitro* fermentation. The uncoated WCS were treated in the same manner, and used as a control.

Chemical analysis

Representative samples were dried at 65˚C for 48 h, and ground using a sample mill until capable of passing through a 1-mm filter (Cemotec, Tecator, Sweden). The DM fraction was quantified by drying and weighing the samples. The CP, EE, NDF, ADF, crude ash, and starch contents were determined using the AOAC method (2000). The non-fibrous carbohydrate content was calculated as 100 – (NDF% + CP% + EE% + crude ash%). The chemical composition of the treated WCS is presented in Table 1. The true starch levels for the 1% starch and 5% starch treatments were an average of 1.32% and 5.38%, respectively. Because 1% $CaCO_3$ was added to both 1% starch and 5% starch coatings, the ash levels were higher than in the control.

Ruminal fermentation trial

Rumen fluid was collected from a cannulated Hanwoo heifer (400 kg), which was fed a formulated concentrate mix at 6.8 kg/d, and sudangrass silage at 4 kg/d, twice a day, at 09:00 and 18:00. Collected rumen fluid was immediately strained through four layers of surgical gauze and placed in vacuum bottles under oxygen-free conditions. The sealed rumen fluid was immediately transported to the laboratory. Upon arrival in the laboratory, McDougall's buffer solution and rumen fluid (39˚C, CO_2 bubbling) were mixed at a 4:1 ratio, placed in a 20-L pyrex bottle, and pre-incubated in a water bath at 39˚C for 24 h. Incubation was conducted according to the method described by Tilley and Terry (1963). Then, 0.4 g of each sample was placed into a separate 100 ml glass syringe (Y1010-100C, China) with rumen inoculum and rumen buffer

(1:3 ratio). Then, the air in the glass syringe was removed, and the syringe sealed, after which the mixture was incubated in a shaking incubator (HK-SIL 25C, Hwaseong, Korea) at 39˚C for 0, 3, 6, 9, 12, 24, or 48 h. Total gas, pH, ammonia nitrogen (NH_3-N), and volatile fatty acids (VFA) were analyzed after incubation.

In vitro ruminal fermentation parameter analysis

Total gas production in each glass syringe was measured at different stages using a gauge, while pH was measured with a pH meter (HI 9321, Hanna Instrument, Portugal) after the uncapping of each syringe. Each sample of incubated fluid was filtered through four layers of surgical gauze prior to analysis. The NH_3-N (Lopez et al., 1998) and VFA (Ding et al., 2006) concentrations were analyzed with a UV spectrometer (S-1100, Scinco, Korea) and gas chromatography (Trace GC ultra, Thermo, Italy) respectively.

Statistical analysis

Data were subjected to one-way analysis of variance using the general linear model procedure (Statistix7, 2000). A comparison of the means of the control, 1% starch treatment, and 5% starch treatment was made using Tukey's multiple range test (Statistix7, 2000). Significance was indicated when P<0.05.

Results and Discussion

In vitro ruminal fermentation parameters

pH

The effects of the starch concentration of WCS coatings on *in vitro* ruminal pH are presented in Figure 1. The pH of the 5% starch treatment at incubation times of 3 h to 48 h was the lowest among the three treatments (P<0.05). At almost all incubation times except 3 h, the pH of the 1% starch treatment was not different from that of the control. The addition of dietary starch has previously been reported to decrease ruminal pH (Krause et al., 2003). Bernard et al. (2001, 2003) reported that pH linearly decreased in *in vitro* trials, as the concentration of starch in WCS coatings was increased. The addition of starch, which fermented rapidly in the rumen, decreased pH via an accumulation of VFA and lactate (Russell et al., 1992). This suggests that the pH of the 5% starch treatment was lowest because the starch concentration in the coating was highest.

Total gas production

The effects of different starch concentrations in WCS coatings on *in vitro* ruminal total gas production are presented in Figure 2. The total gas production with 5% starch treatment at 6 h of incubation was highest among treatments (P<0.05). However, those at incubation times of more than 9 h were not different among treatments. Though total gas production was highest with 5% starch treatment at 6 h of incubation, the pH was the lowest among the treatments (P<0.05). It was assumed that the 5% starch treatment promoted the growth and decomposing activity of amylolytic bacteria during the early fermentation stage, and as a result, the pH decreased. As the phenomenon continued, cellulolytic bacterial activity was depressed, and thus, fibre digestibility may have decreased. A ruminal pH 6.2 is considered to be the normal range of rumen fermentation (Mentens, 1997), in the present study, though the addition of 5% starch decreased the ruminal pH, the values remained within the normal range.

Table 1: Chemical composition of whole cottonseed (WCS), with or without a coating of gelatinized corn starch

Item	WCS	WCS coated with	
		1% Starch	5% Starch
	…… % DM basis ……		
Dry matter	89.5	89.5	89.4
Crude protein	24.2	24.3	23.5
Crude ash	4.7	5.7	5.5
Ether extract	19.4	19.1	18.8
Neutral detergent fibre	42.8	42.5	40.8
Acid detergent fibre	38.4	38.1	35.7
Hemicellulose	4.4	4.4	5.1
Non-fibrous carbohydrate	8.9	8.4	11.4
Starch	0.37	1.32	5.38

Table 2: Effect of coating whole cottonseed (WCS) with gelatinized corn starch on volatile fatty acid (VFA) composition, after 48 h of rumen incubation.[1)]

Item	Control	Coated with		SE	P Value
		1%Starch	5%Starch		
Total VFA, mM	44.57	41.67	46.03	1.57	0.2243
mol/100 mol					
Acetate	53.23[a]	52.56[a]	46.70[b]	1.78	0.0035
Propionate	25.25[b]	27.62[b]	32.80[a]	0.95	0.0001
Iso-butyrate	4.24	4.07	4.68	1.00	0.8303
Butyrate	13.23	11.79	11.81	1.43	0.5390
Iso-valerate	3.37	3.95	4.01	0.77	0.6637
Acetate/propionate	2.05[a]	1.90[a]	1.43[b]	0.07	0.0001

[1)]Least square means of 6 observations; [a,b,c] Means within the same row with different superscripts are significantly different (P<0.05)

NH_3-N concentration

The effects of starch-coating WCS on *in vitro* ruminal NH_3-N are presented in Figure 3. NH_3-N was not different among treatments at incubation times of 0 to 24 h. NH_3-N of the 5% starch treatment at an incubation time of 48 h was 349 ppm, which was about

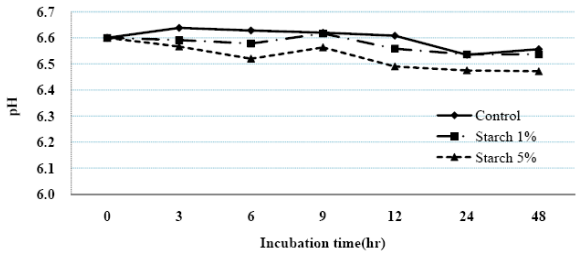

Fig. 1: Effect of coating whole cottonseed with gelatinized corn starch on *in vitro* rumen pH [means of 6 observations; [a,b,c] Means within the same incubation time with different superscripts are significantly different (P<0.05)

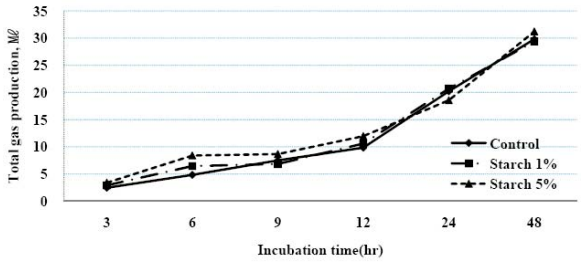

Fig. 2: Effect of coating whole cottonseed with gelatinized corn starch on *in vitro* rumen total gas production [means of 6 observations; [a,b,c]Means within the same incubation time with different superscripts are significantly different (P<0.05)

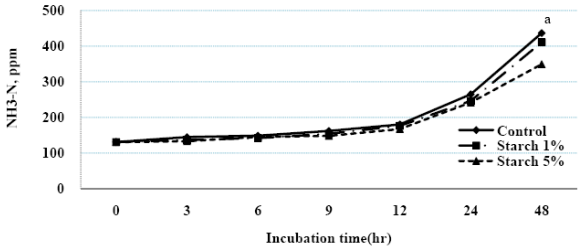

Fig. 3: Effect of coating whole cottonseed with gelatinized corn starch on *in vitro* rumen ammonia concentration [means of 6 observations; [a,b,c]Means within the same incubation time with different superscripts are significantly different (P<0.05)

25% lower than that (466 ppm) of the control (P<0.05). Generally, ammonia in rumen fluid is produced by microbes, ammonia consumption, and microbial growth activity during the amino acid decomposition process (Russell et al, 1992; Swartz et al, 1994). It has been suggested that, because cellulolytic microbes use ammonia as their primary nitrogen source, and amylolytic microorganisms use both ammonia and peptides as their primary nitrogen source (Russell et al., 1992), the starch coating of WCS promotes amylolytic bacterial activity, causing the pH and ammonia of the 5% starch treatment to be the lowest among the treatments. Based on the above results, the addition of up to 5% starch to the WCS coating increased the total gas production during the initial stage of incubation, decreased ruminal pH, and decreased NH_3-N at an incubation time of 48 h. However, the addition of 1% starch did not affect total gas production, pH, or NH_3-N compared to the control.

In vitro ruminal VFA composition

The effects of coating WCS with starch on *in vitro* ruminal VFA are presented in Table 2. The total VFA was not different among treatments. The acetate ratio of the 5% starch treatment decreased 7.0% and propionate increased 7.5%, compared to the control (P<0.05). Acetate content decreased linearly as starch levels increased (P<0.05). The acetate: propionate ratio of the 5% starch treatment was lower than that of the control (P<0.05). In a previous study, when cows were fed WCS coated with 5% starch (Bernard et al., 1999), the acetate ratio of the rumens decreased, and the propionate ratio increased compared with the control. This agrees with the results of our study. However, in the *in vitro* ruminal fermentation study (Bernard et al., 2001), total VFA increased linearly with starch levels, which differs from the total VFA results of this study. The total VFA and molar proportions of the VFA of the 1% starch treatment were not different from those of the control. The addition of starch rapidly decreased ruminal pH, through both the activation of amylolytic bacterial growth, and the increased molar proportions of propionate (Krause et al., 2003). Thus, cellulolytic bacterial growth was inhibited. In the presence of increased amounts of readily fermentable carbohydrates, cellulolytic bacterial activity was depressed (Hoover, 1986) and ruminal digestion of ADF and NDF was reduced (Cameron et al., 1991). In a feeding trial which provided 15% WCS coated with 5% starch to lactating dairy cows (Bernard et al., 1999), milk fat production was depressed, because of the decreased molar proportion of acetate and the 7.7% decrease in NDF digestibility, from 47.5% to 40.1%, compared to the control. In a follow-up study (Bernard et al., 1999) conducted to examine the effects of coating WCS with 2.5% starch on the performance of lactating dairy cows, milk fat production was not depressed, but dry matter intake tended to be lower than when the cows were fed uncoated WCS. As mentioned above, coating WCS with the lowest effective concentration of starch is vital, because the starch coating is rapidly degraded in the rumen, and may have detrimental effects on pH, VFA, and milk fat composition (Poore et al., 1993; Bernard et al., 1999).

Conclusions

Coating WCS with 5% starch altered the *in vitro* ruminal fermentation parameters. However, 1% starch treatment did not alter ruminal pH, total gas production,

NH_3-N, or VFA composition. Coating WCS with 1% starch produced desirable *in vitro* ruminal fermentation parameters, and overcame the undesirable effects associated with coating WCS with 5% starch. Furthermore, coating WCS with 1% starch has the advantage of reducing coating cost, relative to the cost of using 5% starch. In the future, additional *in situ* or *in vivo* trials are required to further confirm these *in vitro* results.

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