EFFECT OF SELECT ADDITIVES ON RUMINAL FERMENTATION OF WHOLE COTTONSEED COATED WITH GELATINIZED CORN STARCH

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Introduction

Whole cottonseed provide a unique blend of energy from oil, protein, and fiber which can be used as an ingredient for feeding cattle. However, whole cottonseed do not handle well in mechanized handling systems commonly used in feed mills and on many farms. Coating whole cottonseed with gelatinized corn starch sticks the lint to the hulls creating a product that flows well though mechanized handling systems. This product is commercially sold as Easiflo™ cottonseed and is currently being used by many feed mills and livestock producers who could not previously utilize cottonseed.

In our initial research, we observed a reduction in fiber digestibility when the coated cottonseed were fed to lactating cows (Bernard et al., 1999). This has been observed by other researchers as well. To increase fiber digestibility, we have been examining the select feed additives included in the coating applied to whole cottonseed. Inclusion of urea in the coating did improve ruminal digestibility and numerically increased the flow of amino acids to the small intestine (Bernard et al., 2003). This trial was conducted to determine the potential of other additives including yeast culture and malate to enhance the digestibility of whole cottonseed.

Materials and Methods

A replicated completely randomized design *in vitro* fermentation study was conducted to determine the effect of select additives on mixed ruminal microbial fermentation of whole cottonseed (WCS) with and without a gelatinized starch coating. Treatments in the replicated completely randomized design trial were arranged as a 2 x 2 x 2 x 4 factorial arrangement to provide two levels of gelatinized corn starch (0 or 2.5%), two levels of urea (0 or 0.25%), two levels of yeast culture (0 or 2%) and four levels of malate (0.0, 0.9, 1.8, and 2.7%). All treatments were prepared from sub-samples of one lot of WCS. Treatments were prepared in the Cotton Incorporated laboratory in Raleigh under the direction of Mr. Tom Wedegaertner and shipped to the University of Georgia where in vitro fermentations were conducted.

Ruminal fluid was collected from a steer as described by Martin and Streeter (1995) Particle-free ruminal fluid was anaerobically transferred (20% vol/vol) to a medium (pH 6.7) containing 292 mg of K_2HPO_4, 240 mg of KH_2PO_4, 480 mg of $(NH_4)_2SO_4$, 480 mg of NaCl, 100 mg of $MgSO_4{\cdot}7H_2O$, 64 mg of $CaCl_2{\bullet}2H_2O$, 4,000 mg of Na_2CO_3, and 600 mg of cysteine hydrochloride per liter (Russell and Martin, 1984; Russell and Strobel, 1988). Particle-free fluid and medium were mixed and 40 mL transferred anaerobically to 160-mL serum bottles containing 0, 0.4, 0.8 or 1.2 g of treated WCS that had been ground to pass through a 1 mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA). The bottles were sealed (CO_2 atmosphere) with butyl-rubber stoppers and aluminum caps to contain the gas pressure and placed in a 39°C water bath and periodically mixed.

After 24 h of incubation, 0.5 mL of gas was removed from each of the bottles, and hydrogen (H_2) and methane (CH_4) measured on a Gow Mac thermal conductivity series 580 gas chromatograph (Gow Mac Instrument, Bridgewater, NJ) equipped with a Porapak Q column (60°C, 20 mL/min of N_2 carrier gas). The bottles were uncapped and pH was measured immediately. Bottles were then emptied into centrifuge tubes, centrifuged (10,000 x *g* at 4°C for 15 min), and the cell-free supernatant removed and stored at -20°C. Volatile fatty acids in the supernatant were measured by HPLC. Ammonia was measured by a modified colorimetric method (Chaney and Marbach, 1962; Russell and Jeraci, 1984). L-lactate was analyzed by the enzymatic method of Hohorst (1965). All experiments were performed on two days with two experiments per day (n = 4).

Analysis of variance was conducted using PROC GLM procedure (SAS, 1992) for a replicated completely randomized design. Effects of replicate, starch level, urea level, yeast culture, malate and their interactions were included in the model. The effect of each additive within each starch level was accessed in second analysis. The model was similar to that described above except that starch and the corresponding interactions were not included in the second model. Significance was declared at $P < 0.05$.

Results and Discussion

Starch

Results of coating WCS with 2.5% gelatinized corn starch are presented in Table 1. Coating WCS with gelatinized corn starch decreased pH ($P < 0.0001$), concentrations of methane ($P < 0.0001$) and ammonia ($P < 0.001$), and molar proportions of acetate ($P < 0.0001$) and isovalerate ($P < 0.0001$). Concentrations of hydrogen ($P < 0.01$) and total VFA ($P < 0.0001$), and molar proportions of propionate ($P < 0.0001$) and valerate ($P < 0.0001$) were greater for WCS coated with 2.5% gelatinized corn starch. No differences were observed in molar proportions of butyrate due to starch. These results are consistent with previous research conducted in our laboratory (Bernard et al., 1999; Bernard et al., 2001).

Urea
Including urea decreased pH ($P < 0.0025$), methane concentrations ($P < 0.0022$) and molar proportions of isovalerate ($P < 0.0012$) and tended to decrease molar proportions of propionate ($P < 0.08$, Table 2). No differences were observed in concentrations of hydrogen, ammonia or total VFA or molar proportions of acetate, butyrate, or valerate. Ammonia concentrations increased linearly as urea increased from 0, to 0.25 and 0.5% in the gelatinized corn starch coating applied to WCS (Bernard et al., 2001).

Yeast Culture
Supplemental yeast culture deceased pH ($P < 0.0001$) and hydrogen concentrations ($P < 0.0003$), acetate to propionate ration ($P < 0.0001$) and molar proportions of acetate ($P < 0.0001$) and isovalerate ($P < 0.0001$) whereas concentration of hydrogen ($P < 0.0003$) and molar proportions of propionate ($P < 0.0001$), butyrate ($P < 0.012$) and valerate ($P < 0.0001$) increased. No differences were observed in concentrations of methane or total VFA.

Malate
Addition of malate to WCS decreased the ratio of acetate to propionate ($P < 0.0001$) by increasing molar proportions of propionate ($P < 0.0001$). Methane concentrations were highest ($P < 0.0195$) when 0 or 2.7% malate was included in the fermentations compared with 0.9 and 1.8%. No other changes in fermentation were noted for the addition of malate. Inclusion of malate into fermentations of soluble starch and cracked corn have been shown to increase pH, concentrations of total VFA, and molar proportions of propionate (Martin and Streeter, 1995). The WCS fermented in the current study would not be expected to decrease pH as greatly as the media used in the previous study by Martin and Streeter (1995) and may have minimized some of the effects noted in their trial. However, the increase in propionate is consistent with their results. Total concentrations of VFA were numerically higher with malate, but were not significantly higher ($P > 0.10$).

Interactions of additives
There were numerous interactions of individual additives with starch. To better access the effects of these additives the data were re-analyzed within each starch level. Fermentation results for WCS coated with 2.5% gelatinized corn starch are presented in Table 5.

The final pH was decreased ($P < 0.0001$) by the addition of urea and yeast culture to WCS without any starch coating (Table 5). There was an interaction of urea and yeast culture ($P < 0.0001$) due to lower pH for the combination of 0.25% urea and 2% yeast compared with the other treatments. An interaction ($P < 0.0058$) was also observed between urea and yeast culture for hydrogen concentrations due to higher hydrogen concentrations for the combination of 0.25% urea and 2% yeast culture and 0% urea and 0% yeast compared to the other treatments. Yeast culture decreased ammonia and total VFA concentrations ($P < 0.0001$). Malate decreased ($P < 0.0001$) acetate to propionate ratios and increased molar proportions of propionate ($P < 0.0001$) in a linear manner. Molar proportion of butyrate was decreased by urea ($P < 0.0022$), yeast culture ($P < 0.0001$), and malate

(P < 0.0032). Both urea and yeast culture decreased (P < 0.0001) molar proportions of butyrate, but the decrease was greatest for the combination of 0.25% urea and 2% yeast culture resulting in an interaction (P < 0.0116). Molar proportion of valerate was highest for the combination of 0.25% urea and 2% yeast culture (P < 0.0116) compared to the other treatments.

Collective these results indicate that the combination of urea and yeast culture alter fermentation of uncoated WCS resulting in slightly lower pH, decreased total VFA, increased molar proportions of propionate with a corresponding decrease in butyrate. Since propionate is utilized as an energy substrate more efficiently than butyrate, these changes would presumably be favorable.

Final pH of WCS coated with gelatinized corn starch was not affected by addition of urea, yeast culture or malate (Table 6). Hydrogen concentrations decreased by urea (P < 0.0077) and malate (P < 0.0308) and increased by yeast culture (P < 0.0002). Methane concentrations were lower with urea (P < 0.0016). An interaction of urea, yeast culture, and malate (P < 0.0012) was observed for methane because of higher concentrations for 0% urea and lowest concentrations for 0.25% urea, 2% yeast and intermediate levels of malate. Concentrations of ammonia were decreased with addition of urea (P < 0.0048) and malate (P < 0.0008) and increased with yeast culture (P < 0.0341). An interaction (P < 0.0001) of these additives was observed because of higher ammonia concentrations for the combinations of 0% urea, 0% yeast culture and 0% malate and 0.25% urea, 2% yeast culture, and 2.7% malate compared with the other treatments.

Total VFA was higher with yeast culture (P < 0.0022) and malate (P < 0.0039). Interactions of urea and yeast culture (P < 0.0015) and urea, yeast culture, and malate (P < 0.0064) were observed because of a greater increase with the addition of yeast culture without urea than when urea was included in the fermentation. Yeast culture decreased molar proportions of acetate (P < 0.0001) and increased propionate (P < 0.0001) and butyrate (P < 0.0001) resulting in lower acetate to propionate ratios (P < 0.0001). Malate increased molar proportions of propionate and the ratio of acetate to propionate. No additional interactions were observed for molar proportions of individual VFA.

Conclusions

Coating WCS with 2.5% gelatinized corn starch alters the concentration of fermentation end products and molar proportions of individual VFA compared to uncoated WCS. Inclusion of urea in the fermentations reduced concentrations of methane and molar proportions of isovalerate which reduces energy loss through the production of methane and increases the postruminal supply of leucine because of reduced degradation. Inclusion of yeast culture favored greater production of propionate and valerate and lower production of acetate which is more energetically favorable. The combination of urea and yeast was more effective in reducing methane concentrations, but total VFA concentrations were not as high for this combination. Inclusion of malate to WCS increased molar proportions of propionate and reduced the acetate to propionate ratio.

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Table 1. Effects of coating whole cottonseed with gelatinized corn starch on ruminal fermentation.

	Starch, %			
Item	0	2.5	SE	P
pH	6.50	6.34	0.008	<0.0001
H_2, m*M*	0.02	0.03	0.004	0.01
CH_4, m*M*	3.50	2.51	0.09	<0.0001
NH_4, mg/L	55.98	45.78	2.12	0.0009
Total VFA, m*M*	35.73	59.52	1.70	<0.0001
	-------------------- % of total VFA --------------------			
Acetate	60.99	57.22	0.45	<0.0001
Propionate	22.69	26.70	0.25	<0.0001
Butyrate	13.00	12.93	0.21	NS
Isovalerate	2.58	1.65	0.08	<0.0001
Valerate	1.27	1.63	0.05	<0.0001
Acetate:Propionate	2.70	2.18	0.03	<0.0001

Table 2. Effects of urea on ruminal fermentation of whole cottonseed.

Item	Urea, %		SE	P
	0	0.25		
pH	6.44	6.40	0.008	0.0025
H_2, m*M*	0.025	0.019	0.004	NS
CH_4, m*M*	3.20	2.82	0.09	0.0022
NH_4, mg/L	50.33	51.43	2.12	NS
Total VFA, m*M*	47.75	47.50	1.70	NS
	-------------------- % of total VFA --------------------			
Acetate	58.90	59.31	0.46	NS
Propionate	25.01	24.38	0.25	0.08
Butyrate	13.11	12.82	0.21	NS
Isovalerate	2.29	1.94	0.08	0.0012
Valerate	1.47	1.43	0.05	NS
Acetate:Propionate	2.41	2.48	0.03	NS

Table 3. Effects of yeast culture on ruminal fermentation of whole cottonseed.

	Yeast Culture, %			
Item	0	2.0	SE	P
pH	6.44	6.40	0.76	<0.0001
H_2, m*M*	0.012	0.032	0.004	0.0003
CH_4, m*M*	3.05	2.97	0.09	NS
NH_4, mg/L	53.39	48.37	2.12	0.096
Total VFA, m*M*	47.51	47.74	1.70	NS
	-------------------- % of total VFA --------------------			
Acetate	60.53	57.68	0.46	<0.0001
Propionate	23.91	25.48	0.25	<0.0001
Butyrate	12.60	13.34	0.21	0.012
Isovalerate	2.24	1.99	0.08	0.019
Valerate	1.27	1.63	0.05	<0.0001
Acetate:Propionate	2.56	2.32	0.03	<0.0001

Table 4. Effects of malate on ruminal fermentation of whole cottonseed.

	Malate, %					
Item	0	0.9	1.8	2.7	SE	P
pH	6.43	6.42	6.42	6.41	0.01	NS
H_2, m*M*	0.020	0.018	0.029	0.021	0.006	NS
CH_4, m*M*	2.98	2.83	2.89	3.33	0.12	0.0195
NH_4, mg/L	55.78	50.78	47.81	49.15	3.00	NS
Total VFA, m*M*	45.55	45.80	48.46	50.69	2.41	NS
	------------------ % of total VFA ------------------					
Acetate	60.00	58.97	59.23	58.21	0.64	NS
Propionate	22.79	24.26	25.36	26.37	0.36	<0.0001
Butyrate	13.41	13.07	12.95	12.44	0.29	NS
Isovalerate	2.16	2.17	2.10	2.04	0.11	NS
Valerate	1.43	1.48	1.50	1.40	0.08	NS
Acetate:Propionate	2.68	2.47	2.36	2.25	0.04	<0.0001

Table 5. Effect of supplemental urea, yeast culture, and malate on fermentation of whole cottonseed coated with 2.5% gelatinized corn starch.

Urea, %	0				0				0.25				0.25				
Yeast, %	0				2				0				2				
Malate, %	0	0.9	1.8	2.7	0	0.9	1.8	2.7	0	0.9	1.8	2.7	0	0.9	1.8	2.7	SE
pH	6.35	6.36	6.37	6.33	6.35	6.33	6.32	6.33	6.35	6.67	6.36	6.33	6.38	6.33	6.32	6.30	0.03
H_2, m*M*	0.013	0.000	0.050	0.050	0.038	0.063	0.075	0.025	0.000	0.000	0.003	0.000	0.025	0.025	0.063	0.038	0.014
CH_4, m*M*	2.15	2.95	2.90	2.20	2.18	2.90	2.80	3.50	2.70	2.05	2.25	2.50	2.40	2.05	2.15	2.55	0.22
NH_4, mg/L	50.10	47.28	40.60	42.03	91.83	47.00	38.40	38.56	40.03	40.05	39.90	44.48	34.63	37.15	37.68	62.75	4.98
Total VFA, m*M*	31.80	38.75	62.48	66.15	73.30	61.08	59.78	79.18	58.10	65.70	54.20	62.68	51.53	58.18	65.25	64.20	5.60
	------------------------- % of total VFA -------------------------																
Acetate	61.41	59.24	60.43	58.08	54.19	53.66	54.13	52.36	62.53	61.98	61.25	59.65	56.10	53.73	53.26	53.55	1.49
Propionate	23.61	25.85	26.05	28.31	27.38	27.36	27.37	33.10	22.80	24.61	24.65	26.14	25.18	28.16	28.41	28.17	1.02
Butyrate	11.82	11.69	10.97	10.77	14.59	14.57	14.53	13.92	11.74	11.04	11.41	11.50	15.06	14.54	14.54	14.27	0.60
Isovalerate	1.58	1.59	1.49	1.47	1.73	2.08	1.86	2.07	1.54	1.27	1.45	1.48	1.81	1.57	1.63	1.80	0.23
Valerate	1.35	1.57	1.23	1.34	1.94	2.08	2.01	1.95	1.34	1.00	1.22	1.32	1.80	1.97	1.94	1.95	0.26
Acetate: Propionate	2.60	2.30	2.33	2.05	2.00	1.98	1.98	1.60	2.75	2.55	2.48	2.30	2.25	1.90	1.88	1.90	0.10