Journal of Animal and Veterinary Advances 8 (7): 1372-1376, 2009
ISSN: 1680-5593

The Effects of Cracking Whole Cottonseed on Apparent Nutrient Digestibilities, N and Energy Retention and *in vitro* Dry Matter Disappearance When Fed to Goats

[1]B.E. Hagens, [1]C.W. Dunaway, [2]N.C. Whitley and [1]B.J. Rude
[1]Department of Animal and Dairy Sciences, Mississippi State University, MS 39762, USA
[2]Department of Agriculture, University of Maryland Eastern Shore, MD 21853, USA

Abstract: The objective of this study was to determine apparent nutrient digestibilities and nitrogen retention of a cracked cotton seed diet compared to a whole cottonseed diet. To determine apparent nutrient digestibilities and N retention, 10 male goats (28±5.6 kg) were housed in metabolism crates and fed diets consisting of 22.5% alfalfa pellets, 56% milo, 1.5% mineral premix and either: 20% cracked cottonseed (CRACKED); or 20% whole cottonseed (WHOLE). Dry matter intake was greater for wethers consuming WHOLE compared to those consuming CRACKED ($p = 0.0057$, 0.92 and 0.58 kg day^{-1}, respectively; $p = 0.0560$, 3.20 and 2.36% BW day^{-1}, respectively). There were no differences ($p>0.10$) between WHOLE and CRACKED for DM digestibility (76.8 and 73.7%, respectively) and OM (77.8 and 75.4%, respectively). However, ash digestibility was greater ($p = 0.0083$) for WHOLE compared to CRACKED (58.8 and 44.0%, respectively). Neutral detergent fiber tended to be more ($p = 0.0683$) digestible by animals fed WHOLE (55.6%) than CRACKED (42.8%). Digestibility of ADF was not different ($p>0.10$) for wethers consuming WHOLE and CRACKED (38.8 and 35.7%, respectively). Wethers consuming WHOLE (84.9%) digested more ($p = 0.0051$) fat than those fed CRACKED (76.0%). Additionally, crude protein was more digestible ($p = 0.0002$) for WHOLE (75.5%) compared to CRACKED (65.2%) and N retention was greater ($p = 0.0005$) by goats fed WHOLE (15.8 g day^{-1}) compared to those fed CRACKED (1.2 g day^{-1}). To further investigate, the effect of cracking cottonseed a timed (0.5-48 h) IVDMD was performed. Degradation of DM was different for all incubation times ($p = 0.0001$; from 0.5-48 h) for cracked cottonseed compared to whole cottonseed. These results indicate that cracking cottonseed has a negative influence on apparent nutrient total tract digestibilities of whole cottonseed.

Key words: Goat, whole cottonseed, digestibility, cracking, energy retention, dry matter

INTRODUCTION

For >200 years, cotton has played a key role in the history and development of American agriculture. Raw cotton can produce lint to be used in various manufacturing processes such as textile production. Oil extracted from the seed is a valuable energy source. Cottonseed products such as Whole Cottonseed (WCS), Cottonseed Meal (CSM) and Cottonseed Hulls (CSH) and linters are often fed to livestock. The amount of energy, protein and fiber has made cottonseed a desirable component for cattle diets (Poore and Rogers, 1995), but bulkiness has limited inclusion in rations. To simplify handling of cottonseed, researchers have investigated methods of removing linters from cottonseed. Delinted cottonseed is the unprocessed and unmodified oilseed that has been separated from all cotton fibers. Delinted whole cottonseed is referred to as black, slick, pima, or acid treated. Pima is a type of cotton (*Gossypium barbadense*) known for extra long-staple with seeds that are naturally linter free.

Sullivan *et al.* (1993b) conducted a study to compare the feeding value of whole Pima cottonseed and cracked Pima cottonseed with that of whole cottonseed (*Gossypium hirsutum*) for lactating cows. Compared to *G. hirsutum* cottonseed, whole Pima cottonseed contained more protein, lipid and oleic acid and had less NDF, ADF and linoleic acid. The researchers theorized that the greater density of these nutrients in the whole Pima cottonseed may have allowed the seed to drop to the ventral portion of the rumen and pass further along the digestive tract without rumination, whereas fuzzy seed remain longer in the dorsal region of the rumen, resulting in more efficient rumination (Sullivan *et al.*, 1993b).

Objectives of this study were: to determine the effects of cracking cottonseed on DM intake, apparent nutrient digestibilities, N and energy retention and *in vitro* degradation rates.

Corresponding Author: Brian J. Rude, Animal and Dairy Sciences, P.O. Box 9815, Mississippi State, MS 39762, USA

MATERIALS AND METHODS

Animals and diets: Ten male goats were randomly assigned and adapted to one of two experimental diets. Animals in each group were housed by treatment in 3×3 m pens for 14 days for diet adaptation. Goats were then placed into individual metabolism crates for 3 days crate adaptation, followed by a 7 days period for collection of feces and urine. Daily feed samples were collected during morning and afternoon feedings. Feed offered was 120% of the previous day's intake, with half given at 0700 and the balance given at 1600. Animals had *ad libitum* access to both feed and water. Diets were a total mixed ration consisting of 22.5% alfalfa pellets, 56% milo and 1.5% mineral premix on an as fed basis (Table 1). The remaining 20% of the diet consisted of either cracked cottonseed (CRACKED) or whole cottonseed (WHOLE). The trial was conducted at the University of Maryland Eastern Shore Farm, Princess Anne. Acid delinted whole cottonseed was donated to the Department of Animal and Dairy Sciences at Mississippi State University by Delta and Pineland Company, Greenwood, MS. The portion of the cottonseed to be cracked was crushed by hammer milling. The whole and cracked cottonseed was shipped to Princess Anne, Maryland for diet preparation.

Sample collection and processing: Total orts were collected, weighed and composited daily. Fecal output was weighed daily, sampled at 10% and composited. Total urine output was measured and 10% was collected and composited daily. Urine collection containers had 2 N H_2SO_4 added to acidify urine to prevent ammonia volatization. All urine was standardized such that 5% of the samples were 2N H_2SO_4. Samples of all feedstuffs used for diets were collected (Table 1). All samples (feed, orts, feces, urine and feedstuffs) were stored at -20°C until laboratory analysis.

An *in vitro* study was conducted using either whole or cracked cottonseed. Samples were placed in ANKOM *in situ* filter bags, with an average pore size of 50±15 μm. The procedure performed was modified from Cherney *et al.* (1997). Briefly, 0.5 g of either cracked or whole cottonseed was placed into each filter bag. Duplicate samples were placed in an artificial rumen environment for 0, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 18, 24 and 48 h. Two vessels (2 L each) were flushed with CO_2 and 1500 mL McDougall's (1948) artificial saliva was placed in each vessel. Vessels were rotated in an ANKOM[II] Daisy incubator over night at 52°C. Approximately, 1 L of rumen fluid was collected from a fistulated steer into a pre-warmed insulated container and immediately transported to the laboratory. A Waring® blender was flushed with CO_2 and filled with rumen fluid. Rumen fluid was blended for 2 sec and then strained through 4 layers of cheesecloth into a warmed 1 L beaker flushed with CO_2 for approximately 5 sec. Strained rumen fluid (375 mL) was added to each vessel that contained 1500 mL of artificial saliva from the previous day. Vessels were placed and maintained inside a daisy incubator to ensure constant temperature of 52°C for 48 h. Duplicate filter bags containing samples of cracked or whole cottonseed were placed inside vessels for 0, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 18, 24 and 48 h. To ensure no associative affects on digestion of the cottonseed, each vessel (artificial rumen) contained either only whole or cracked cottonseed samples. These procedures were repeated 3 times (total of 4 runs). After laboratory analysis, samples incubated for 0-48 h were corrected by subtracting the values obtained for 0 h incubation.

Table 1: Nutrient composition of feedstuffs and diets containing cracked and whole cottonseed consumed (DM basis)

Diet	DM	Ash	OM	CP	NDF	ADF	HC[1]	EE[2]
Cracked	88.87	5.01	94.99	13.86	22.66	13.56	9.10	18.06
Whole	89.44	5.51	94.49	17.94	26.82	14.57	12.25	22.23

[1]HC: Hemicellulose; [2]EE: Ether Extract

Chemical analysis: Fecal samples were dried in a forced-air oven at 60°C for 48 h in preparation for analysis. Air dried fecal samples, feed, feedstuffs and orts were ground to pass a 2 mm screen in a Wiley Mill® (Arthor H. Thomas, Philadelphia, PA). Feed, feedstuffs, orts and fecal samples were analyzed for DM, ash, OM, CP and Ether Extract (EE) according to AOAC (2003) and for NDF and ADF as described by Komarek *et al.* (1994). Gross energy of feed, orts, feces and urine was determined using an isoperibol oxygen bomb calorimetry (Parr Instrument Co., Moline, IL). Urine samples were analyzed for gross energy by adding 1.0 mL of urine to saturate a 1 g pellet of cellulose powder. A blank cellulose pellet (no urine added) was used to determine gross energy of cellulose powder. Gross energy of the cellulose was subtracted from the gross energy of urine saturated pellets.

After 48 h inside the *in vitro* vessels, samples were removed and immediately rinsed with clear water for 5 sec 3 times and placed on a lab counter top to air dry over night. The dried sample bags were analyzed for DM (AOAC, 2003) and either NDF and ADF (Komarek *et al.*, 1994), or CP (AOAC, 2003).

Statistical analysis: Data from the digestibility trial was analyzed using GLM procedures of SAS (2002) as a completely randomized design replicated over time (3 runs). When treatment effect was significant ($p<0.05$) mean difference were separated using Fisher's protected least significant difference. Individual animal was the experimental unit (n = 5/treatment).

All data from the *in vitro* trial was analyzed using the MIXED procedures of SAS (2003) with repeated measures in a completely randomized design with means separated by Turkey's honestly significant difference.

RESULTS AND DISCUSSION

Diet composition and intake: Nutritional composition of WHOLE and CRACKED diets is presented in Table 1. The 2 diets were calculated to be isonitrogenous and isocaloric. However, upon analysis of the 2 diets, CP for the WHOLE (17.94%) was greater than CRACKED (13.86%). This could be due in part to small particulate matter being lost during the cracking process. The CRACKED diet (18.06%) contained less fat than the WHOLE (22.23%), this could be due to the cracking process of the cottonseed through the hammer mill where oil may have adhered to the equipment. These com-positions were similar to those reported by Sullivan *et al.* (1993b).

Dry matter intake was greater (p = 0.0057) for weathers consuming WHOLE compared to those consuming CRACKED (0.92 and 0.58 kg day^{-1} respectively; p = 0.0560, 3.2 and 2.36% BW day^{-1}, respectively, Table 2). Because of this difference of DM intake between groups, treatment effects were tested using analysis of covariance to determine if there were correlations between digestibilities and DM intake. There were no significant correlations between DM intakes. Therefore, the differences are assumed to be due to treatment effects and not significantly influenced by difference of DM intake. The DM intake expressed as a percentage of body weight was similar to that reported by Moreira *et al.* (2004) for delinted WCS when compared to fuzzy WCS and for whole Pima cottonseed to *G. hirsutum* cottonseed (Sullivan *et al.*, 1993a, b). During the current trial, some wethers consuming WHOLE sorted through their diet and refused the whole cottonseed, thus, consuming more alfalfa and milo. The cottonseed may have been less palatable to wethers in this trial. Therefore, wethers consuming CRACKED could not sort out the cottonseed, which may have reduced their overall intake.

There were no differences (p>0.10) for apparent digestibilities (Table 3) of DM or OM by goats. However, apparent ash digestibility was greater (p = 0.0083) for animals consuming WHOLE compared to CRACKED. Apparent digestion of NDF tended to be greater (p = 0.0683) for animals fed WHOLE compared to CRACKED but, apparent digestibility of ADF was not influenced by processing (p>0.10). Wethers consuming WHOLE digested more (p = 0.0051) fat than those fed CRACKED. Crude protein was more digestible (p = 0.0002) for WHOLE compared to CRACKED. Sullivan *et al.* (1993b) compared whole Pima cottonseed to short staple

Table 2: Average body weight and dry matter intake of goats consuming diets containing cracked or whole cottonseed

		DMI	
Diet	BW (kg)	kg day^{-1}	BW% day^{-1}
Crack	25.9	0.58^{a}	2.36^{a}
Whole	28.8	0.92^{b}	3.20^{b}
SEM	2.38	0.066	0.268
p<0.05	0.4172	0.0057	0.0560

^{a,b}:Means within column lacking common superscripts differ (p<0.06)

cottonseed and reported that fat digestibility was less for whole Pima diets than for *G. hirsutum* cottonseed. They postulated that digestibility of fat increases by processing cottonseed, without altering protein availability. Furthermore, cottonseed furnished a much larger portion of fat versus CP in the diets and the overall digestibility of the cottonseed effected fat digestion to a greater extent than CP digestion. However, in the current study, fat availability as well as CP decreased when the cottonseed was processed. Smith *et al.* (1981) found no difference for crude fiber digestibility when WCS was fed at increasing amounts up to 25% of diets for lactating cows.

Nitrogen retention was greater (p = 0.0005) for goats fed WHOLE compared to those fed CRACKED (Table 4). Energy retention was greater (p = 0.0032) for wethers consuming the WHOLE than for those consuming CRACKED. Gaseous losses were not included in calculation of retained energy. Wethers receiving the WHOLE digested and retained more N than wethers receiving CRACKED. Losses of N in feces and urine may have been the result of lower efficiency of ruminal protein utilization. Excess ammonia production in the rumen may have been excreted in the urine. Urea may also be recycled via saliva and reenter the rumen (Bach *et al.*, 2005). It is not uncommon for ruminants to synthesize urea N in excess of the N apparently digested (Lobley and Lapierre, 2001), implicating that animals are in a positive N balance. Nitrogen recycled into the gastrointestinal tract can be used for microbial protein synthesis in the rumen and provide amino acid to the host (Marini *et al.*, 2004). The loss of energy, thereby decreasing energy retention by wethers consuming CRACKED may have been influenced by the decreased N retention. According to Nocek and Russell (1988), when the rate of protein degradation exceeds the rate of carbohydrate fermentation, large quantities of N can be lost as ammonia and conversely, when the rate of carbohydrate fermentation exceeds protein degradation rate, microbial protein synthesis can decrease.

Figure 1 shows cracked and whole cottonseed (DM basis) *in vitro* digestion. After 0.5 h of *in vitro* incubation, approximately 20% of cracked cottonseed was degraded and only approximately 10% of the whole cottonseed was degraded. Cracked cottonseed continued to degrade at a constant rate until 12 h; after 12 h

Table 3: Apparent nutrient digestibilities of goats fed diets containing cracked or whole cottonseed

Diet	DM	Ash	OM	CP	NDF	ADF	HC[1]	EE[2]	Energy
Cracked	73.7300	43.9500[a]	75.3700	65.1600[a]	42.7700	35.130	55.1300[a]	76.0200[a]	73.8500
Whole	76.7900	58.7700[b]	77.8400	75.4600[b]	55.6200	38.790	73.9700[b]	84.9200[b]	77.8200
SEM	1.5180	3.0090	1.4740	1.1490	4.3170	5.496	3.4250	1.6470	1.5630
p-value	0.1913	0.0083	0.2697	0.0002	0.0683	0.7024	0.0046	0.0051	0.1107

[ab]Means within column lacking common superscripts differ ($p<0.01$); [1]HC: Hemicellulose; [2]EE: Ether Extract

Table 4: Nitrogen and energy retention of goats consuming diets containing cracked or whole cottonseed

Diet	N (g day^{-1})	Energy (kcal day^{-1})
Crack	1.1800[a]	1897.0000[a]
Whole	15.7700[b]	3417.0000[b]
SEM	1.8400	258.2000
p-value	0.0005	0.0032

[ab]Means within column lacking common superscripts differ ($p<0.01$)

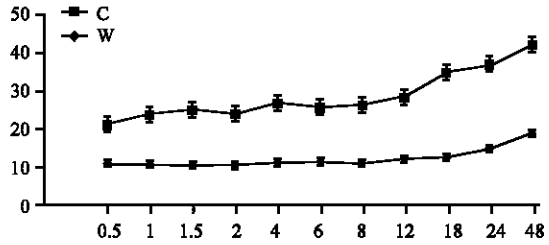

Fig. 1: *In vitro* DM disappearance (%) of Whole (W) and Cracked (C) cottonseed. Means within time differ ($p<0.05$). [a]Means within same time frame differ ($p<0.05$)

degradation of the cracked cottonseed began to degrade at a faster rate. Similarly, whole cottonseed was digested at a constant rate until about 18 h, after which it was more rapidly degraded. Apparent digestibility of DM ($p<0.05$) *in vivo* was different (76.8 and 73.7%) for WHOLE and CRACKED, respectively. *In vitro*, the greatest disappearance was only 43%. Furthermore, only cottonseed was incubated for digestion, not the entire diet.

Figure 2 and 3 show the degradation rates of NDF and ADF in whole and cracked cottonseed. There were no differences for NDF or ADF disappearance until after 12 h of *in vitro* ruminal degradation. After 12 h of *in vitro* incubation, both NDF and ADF in cracked cottonseed were digested to a greater extent than whole cottonseed. For the *in vivo* trial, wethers consuming the whole cottonseed diet sorted through their diet, refusing whole cottonseed. The wethers consuming the cracked cottonseed did not sort through their diet. However, *in vivo*, the rate of degradation was greater for wethers consuming WHOLE and this could be due to the act of mastication initiating breakdown for digestion did not occur *in vitro*. According to Allen (1997), effective fiber is the fraction of the feed that stimulates chewing; chewing stimulates saliva secretions, which buffers acidic

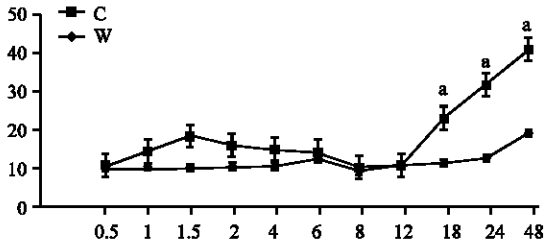

Fig. 2: *In vitro* neutral detergent fiber disappearance (%) of Whole (W) and Cracked (C) cottonseed. [a]Means within same time frame ($p<0.05$)

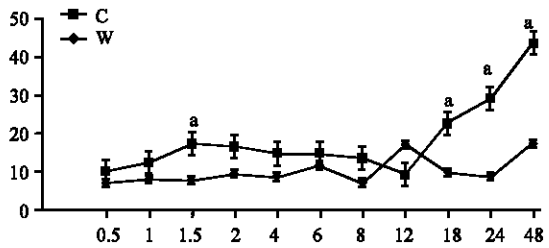

Fig. 3: *In vitro* acid detergent fiber (%) of Whole (W) and Cracked (C) cottonseed. [a]Means within same time frame differ ($p<0.05$)

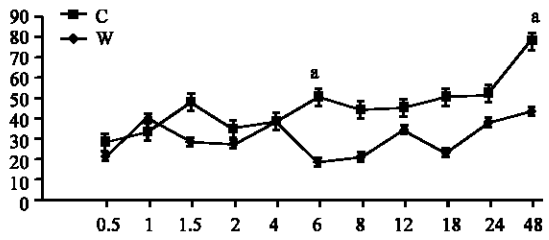

Fig. 4: *In vitro* CP disappearance (%) of Whole (W) and Cracked (C) cottonseed

end products of fermentation and helps prevent depressions of DM intake, ruminal motility, microbial yield and fiber digestibility.

In vitro degradation of protein for whole and cracked cottonseed was not different ($p<0.05$, Fig. 4.) from 0.5- 4 h. At 6 h, the cracked cottonseed began degrading rapidly whereas the whole cottonseed did not. *In vitro*, CP was degraded more rapidly for cracked cottonseed compared to whole cottonseed. However, *in vivo*, wethers

consuming WHOLE digested CP to a greater extent than those consuming CRACKED. The alfalfa pellets and milo may have caused the cracked cottonseed to escape rapidly and not have as much time to degrade in the rumen. *In vitro*, CP remained in the artificial rumen and was subjected to degradation, allowing the cracked cottonseed to digest at a faster rate than the whole cottonseed.

IMPLICATIONS

Diets containing cracked delinted cottonseed had reduced CP and fat compared to diets with whole delinted cottonseed, which may be partly attributed to the cracking process. Apparent nutrient digestibilities of CP and fat were reduced for the diets with cracked cottonseed compared to that with whole cottonseed. The amount of N retained by goats consuming diets with cracked cottonseed was less than for the wethers consuming diets with whole cottonseed. This may have been because the wethers consuming diets with whole cottonseed also retained more energy; therefore, energy and N may have been more effectively used by the microbial population in the rumen. Energy from the diet containing cracked cottonseed may have been available at a time the N was not available for the rumen microbes. This is supported by NDF and ADF being degraded more rapidly *in vitro* for cracked cottonseed. Thus, if any N recycling was taking place, energy was not available when saliva urea entered the rumen. When energy is limited, microorganisms degrade feed protein to ammonia and ammonia uptake by ruminal microorganisms is limited. The *in vitro* trial indicated that cracking cottonseed enhanced DM, NDF, ADF and CP degradation.

When making a recommendation for feeding delinted cottonseed, cracking may not be beneficial; however, more research to elucidate the effect of cracking should be conducted.

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