UPDATE ON THE IMPACT OF DRY EXTRUDING COTTONSEED TO REDUCE AFLATOXIN AND GOSSYPOL LEVELS

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Abstract

Cottonseed is an economical source of protein and is commonly used in balancing livestock rations; however, its use is typically limited by protein level, fat content, gossypol, and the potential for aflatoxin. Cottonseed and mixtures of cottonseed and cotton gin by-products (CGBP) were extruded at relatively high temperatures to determine if the process would reduce the free-gossypol and aflatoxin levels in the material, without degrading the nutritional value of the original material. Various mixtures of cottonseed and CGBP were used in the gossypol test to confirm previous tests conducted at the U. S. Cotton Ginning Laboratory in Stoneville, Mississippi. The results from this test indicated a 71 to 78% reduction in free gossypol levels for the various mixing ratios, based of the official AOCS standard methods. This reduction is most likely under estimated, since the official methods for determining free-gossypol levels are not specific to gossypol and constituents from other feed ingredients can interfere with the spectrophotometric determination. Cottonseed with aflatoxin levels in excess of 650 ppb was used to determine the effect of extrusion temperature and increased processing on the aflatoxin and nutritional values of the contaminated cottonseed. The variability of aflatoxin in contaminated lots is extremely high and although these tests used relatively small lot sizes, numerous samples, and rigorous sample collection techniques, the variability associated with the analyses was a major issue. The largest amount of variation was encountered in the non-extruded material and after the material was extruded, the variability was greatly reduced. Aflatoxin levels decreased on the order of 32 to 45% (based on mean values) as the extrusion temperature was increased from 104 °C to 160 °C (220 to 320 °F). Most nutritional values were not significantly altered by the extrusion temperatures used in this test. Soluble protein was the exception; it was significantly decreased at the higher temperatures. Results from the test on multiple pass extrusion, indicated a decrease in aflatoxin levels and variability as the amount of processing increased on the order of 46 to 66% (based on mean values for four stages of processing compared to one stage of processing). Nutritional values associated with this test were not significantly altered by increased processing. Results from this study indicate that dry-extruding cottonseed to reduce gossypol and aflatoxin levels is an area of research, which should be further explored.

Introduction

Cotton gins nationwide produce approximately 8.5 million tons of cottonseed annually, of which about 1.86 million tons of whole cottonseed is fed to livestock. Whole cottonseed can provide a good supply of protein, fat, and fiber. For almost all feed ingredients, there is a negative correlation between fiber and energy; however, whole cottonseed is the exception to this rule. Cottonseed meal is a co-product of the cottonseed oil extraction industry and an estimated 1.5 million tons are utilized in livestock rations nationwide. Since cottonseed meal is primarily used as a protein source, the protein levels are carefully controlled during processing, yielding about a 41% protein level. Whole cottonseed and cottonseed meal have long been popular and economic sources of protein for ration formulations. However, there are limiting factors which must be considered when determining quantities of whole cottonseed and cottonseed meal in the formulation of rations. Significant considerations should be given to protein level and quality, fat content, gossypol, and aflatoxin.

Gossypols are pigments found naturally in many gossypium species, including cotton. At least 15 gossypol pigments or derivatives have been identified in cotton plant products (Berardi and Goldblatt, 1980). The predominately occurring pigment is polyphenolic binaphthyl aldehyde, which is yellow in color and referred to as gossypol (Calhoun, et al., 1991). Gossypol is located throughout the cotton plant, with highest concentrations in the roots and significant quantities in the seed. In the seed, gossypol is contained in small pigment glands. These glands appear as small pepper specks when a cottonseed meat is sliced open. When these glands are intact (not ruptured), virtually all the gossypol is biologically active and is said to be in its "free" form. The quantity of gossypol can vary depending upon variety and environmental conditions; however, the gossypol content, of commercial varieties grown throughout the Cotton Belt, have not substantially changed in the last 40 years, even with the development of "glandless" varieties (Calhoun and Holmberg, 1991). Gossypol levels in gin-run whole seed are about 0.6% by weight or 6000 ppm (Lusby et al., 1991a). Relatively large amounts of gossypol may be toxic when fed to livestock, especially in its free form, these amounts vary by: duration of feeding; species; breed; age; state of rumen development; feeding level; and method of feeding (Calhoun and Holmberg, 1991).

Gossypol was first discovered by J. J. Longmore in 1886 and purified in crystalline form in 1889 by L. Marchlewski (Jones, 1991). In 1915, Withers and Carruth identified gossypol as the cause of death in pigs and calves (Jones, 1991). With this discovery, gossypol became the principle suspect whenever problems arose in feeding cottonseed or cottonseed meal to livestock. By the 1930's, it was known that swine, poultry and young ruminants were very susceptible to gossypol poisoning, and mature ruminants were very tolerant. Beliefs of most ruminant nutritionists from the mid 1930's until 1980 stemmed from Morrison (1954), which stated: "cottonseed meal is one of the best protein supplements for dairy cows, beef cattle, and sheep". Morrison further stated "for calves under 3 to 4 months of age it is best not to use more than about 20% of cottonseed meal in the concentrate mixture". In 1975, gossypol toxicity developed (in a 700 cow dairy herd in Alabama) when large amounts of cottonseed meal were fed as the single source of protein to achieve high levels of milk production, resulting in the death of 25 mature cows. Lindsey et al. (1980) reported gossypol intoxication in mature dairy cattle consuming direct solvent extracted cottonseed meal containing high free gossypol. This research accompanied by periodic rediscoveries of gossypol poisoning in cattle (Woodward et al., 1933; Kuhlman et al., 1934; Leighton et al., 1953; Hollon et al., 1958; Rogers et al. 1975; Schuh et al., 1986; Holmberg et al., 1988), in sheep (Morgan et al., 1988 and Calhoun et al. 1990 a, b), and reports from China that gossypol was a potent antifertility agent for human males (Xue, 1985) renewed concerns about the safety of feedstuffs containing gossypol. Collectively, these studies substantiated the remarkable ability of ruminants (post-weaning) to tolerate large amounts of gossypol for extended periods. However, it is unfortunate that major emphasis has been placed on feeding gossypol at very high levels; to demonstrate an effect, without including sufficient lower levels to define safe levels. (Calhoun et al., 1991)

Animal sensitivity to gossypol is considerably different between species and classes of animals. In general, monogastric animal and ruminants, prior to development of normal rumen function, are more susceptible to gossypol poisoning than mature ruminants (Abou-Donia, 1976; Berardi and Goldblatt, 1980). Research has defined safe levels of free gossypol in diets for monogastric animals; however, the information available on safe feeding levels for ruminant animals is limited. Therefore, the recommended

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safe feeding levels for ruminant animals is very conservative. Typical recommended safe levels of free gossypol are presented in Tables 1 and 2, for non-ruminant, and Table 3, for ruminants. In general, limited amounts of cottonseed meal can be used in swine and poultry rations when properly managed; however, no whole cottonseed should be used. For cattle, sheep, and goats with normal rumen function cottonseed and cottonseed meal can generally be safely used when utilizing the products to meet protein requirements (i.e. "to balance rations"), fat content may be the limiting factor when considering these products (Calhoun et al., 1991).

Virtually all the gossypol in whole seed is in the free form (unbound). A Texas A&M survey reported that free gossypol levels in whole cottonseed ranged from 0.47 to 0.63% (4700 to 6300 ppm) (Calhoun et al., 1989). Even though all the gossypol in whole cottonseed is considered to be in the free state, analyses for free and total gossypol will not necessarily result in the same numbers. This is due to the use of two separate official analytical procedures for free and total gossypol. The goal in processing is to rupture the pigment glands containing gossypol, so that the gossypol binds with proteins, thus decreasing the free gossypol content. The degree of binding is also critical, since the process reduces protein quality and amino acid availability, especially with regard to lysine availability. Lysine is reported to be the primary amino acid bound to gossypol (Baliga and Lyman, 1957; Conkerton et al., 1957). Bound gossypol is generally considered as unavailable to the animal; however, researchers have always been concerned about bound gossypol toxicity but have not found enough evidence to include it along with free gossypol (Jones, 1991). The total gossypol content of processed cottonseed is not affected by processing; it is equal to the sum of the free plus the bound gossypol (Berardi and Goldblatt, 1980). However, during oil extraction part of the gossypol is removed with the oil while the outstanding gossypol remains with the meal. The extent of binding varies with processing method; Table 4 lists the typical free gossypol levels associated with various processes.

Cotton gin by-products (burrs, stems, leaves, soil, etc.) present a major problem for the ginning industry. With an approximate 2.8 million tons produced annually nationwide, researchers are exploring alternative economic uses for this material. Thomasson, et al. (1998) conducted a preliminary study to determine the feasibility of expansion processed cottonseed and CGBP mixtures as a potential valuable livestock feed. The study used an Anderson 11.4 cm (4.5-inch) Expander Cooker and focused on mixing ratios of 50:50, 75:25, and 90:10 (% cottonseed: % CGBP). This work has shown that a simple, relatively low-cost expansion process can be used to produce a livestock feed from cottonseed and CGBP with reduced free gossypol levels (about 90%) in the final product. Pesticide residues from certain persistent compounds would prohibit feeding this material to animals. Check the labels of all products used on the cotton crop to verify that the CGBP product can be fed without trace accumulations. Lactating dairy cows should not be fed the mixture.

Aflatoxin in cottonseed may limit its use. Aflatoxins, secondary metabolites of the fungus *Aspergillus flavus* and *A. parasiticus* are acute toxins to most animals and are the most hepatoxic and hepatocarcinogenic natural agents known (Smith, 1969). *Aspergillus flavus* is universal and propagates in any substratum capable of supporting fungal growth, especially in warm humid environments (Forgacs and Carll, 1965). These fungi can infect crops before and after harvest and produce aflatoxins, thereby contaminating foods and feeds (Goldblatt, 1970; Jelinek et al. 1989). Because the production of aflatoxin is so dependent upon environmental conditions, the amount actually produced will vary widely from sample to sample and year to year.

The first report of aflatoxin toxicity appeared in 1961, when contaminated peanut meal was linked to the deaths of over 120,000 turkeys and other poultry (Blount, 1961). Keyl and Booth (1971) conducted the first comprehensive study on the effects of feeding aflatoxin to livestock and poultry. This work established the levels of aflatoxin required to produce recognizable growth effects in swine, beef and dairy cattle, and broilers and laying hens. However, the methodology available then could not detect low part per billion levels; therefore, the data on transmission of aflatoxin residues in the meat were not as definite. More recent studies have established that residues of aflatoxin can be found in animal tissue (Furtado et al., 1982; Trucksess et al., 1982; Richard et al., 1983; Trucksess et al., 1983; Chen et al., 1984; Richard et al., 1986). These studies suggested that low levels of aflatoxin contaminated feed (400 ppb for cattle and swine and 150 ppb from turkeys) will result in detectable aflatoxin residues in the liver, kidney, and muscle; aflatoxin is eliminated from the animal tissue in a relatively short time (4 days for swine, 14 days for turkeys, and 21 days for cattle); and chickens can handle large doses (2000 ppb) with little effects, and after 2 days, no aflatoxin is detected in the tissue.

Mycotoxins are regulated by the Food and Drug Administration (FDA). The Federal Grain Inspection Service (FGIS) has a Memorandum of Understanding to report any over-tolerance results it finds to FDA. In 1969, the FDA set an action level for aflatoxins at 20 ppb for all foods, including animal feeds, based on the agency's aim of limiting aflatoxin exposure to the lowest possible level. Due to animal feeding studies in the 1970's and 1980's, the agency revised its action level in 1982 to 300 ppb for aflatoxins in cottonseed meal intended for used as a feed ingredient for beef cattle, swine, and poultry (regardless of age or breeding status). The action level for cottonseed meal, and other animal feeds and feed ingredients intended for dairy animals, for animals species or uses not previously specified, or when the intended use is not known remained at a level of 20 ppb.

The health impacts of aflatoxin are much less precise than regulatory limits or guidelines suggest. There are no clear-cut safe levels, since the levels vary with each individual animal. The generally recommended level of aflatoxins in feed is 0 ppb. However, aflatoxin-contaminated feed can be tolerated by some animals, particularly mature ones. In general, ruminants are able to tolerate higher levels of aflatoxins and longer periods of low-level intake than simple-stomached animals. In order of susceptibility, ducklings are first followed in order by turkeys, pigs, calves, mature cattle, and sheep (Smith, 1969). The response of ruminants to aflatoxin-contaminated feed depends upon the level of toxin present, age, and species. Young, rapidly growing ruminants are more susceptible than are mature ruminants. Ingestion of aflatoxins at levels lower than FDA action levels may cause some undesirable side effects, and is dependent on such factors as age, sex and general health of the animals; obviously, the higher the level of contamination, the greater the risk in feeding to animals.

Aflatoxin detection tests are inherently variable. This can be attributed to sampling, subsampling, and analytical variability. Whitaker et al. (1974; 1976; 1979) indicated that sampling variability, especially for small sample sizes, is the largest source of error in determining aflatoxin variability. Sampling error is large because aflatoxin is found only in a small percentage (less than 0.1%) of the kernels in the lot (Whitaker and Wiser, 1969). In addition, of the 0.1% a single seed concentration may be extremely high. Because of the extreme range in aflatoxin concentrations among individual seeds in a contaminated lot, the variation between replicated lots is large. About 90% of the error associated with detection tests is due to sampling. Once the sample has been taken from the lot, the sample must be prepared for aflatoxin extraction. The entire sample must be thoroughly mixed before the subsample is collected (Dickens and Whitaker, 1982). The subsampling variance is not as large as the sampling variance due to the large number of mixed particles in the subsample. Next, the aflatoxin is extracted by official methods (AOAC, 1980; Nesheim, 1979). These methods involve several steps such as solvent extraction, centrifugations, drying, dilutions, and quantification, which can result in considerable variation among replicated analyses on the same subsample extract. Analytical variability generally accounts for only a small portion of the total error.

The only way to achieve a more precise estimate of the true lot concentration is to reduce the total variation associated with the tests. Sampling error can be greatly reduced by collecting a more representative sample from the lot. This can be accomplished by taking 10 or more random samples from the lot, the larger the number of samples the better. Subsampling error can be reduced by thoroughly mixing the sample prior to collecting the subsample and increasing the sample size. Replicating the tests will reduce the analytical variability.

There are several proposed methods of processing cottonseed to reduce aflatoxin levels. Ammoniation is a relatively common process used in Arizona and California; however, the process is not an FDA-approved practice so the ammoniated material must be used on-farm or sold for use within the state. With proper treatment, the process has been shown to reduce aflatoxin concentrations by 95% or more. Blending is another alternative that has been used to reduce moderate concentrations of aflatoxin. The FDA does not permit the blending of contaminated and uncontaminated commodities, but does allow the mixing of different levels of contaminated commodities. Irrespective of the processing method, the final product must be retested and fall within the regulatory limits and be properly labeled. The literature on ammoniation and blending is quite clear on the effectiveness of the processes, but there are several conflicting reports on the reduction of aflatoxin levels due cooking, extruding, or in general terms, processes that utilized relatively high temperatures and pressures. For example, Fischback and Campbell (1965) reported that it was necessary to raise the temperature to 300 ºC (575 ºF) or higher to decompose aflatoxins and even then reductions are limited. Goldblatt (1966) stated that a temperature of 100 ºC (212 ºF) decreased aflatoxin content. A recent report by Kenkel and Anderson (1999) suggests that roasting temperatures of 143 to 149 ºC (290 to 300 ºF) can reduce aflatoxin levels by 40 to 50%, in corn. Very limited recent information is available in the literature on the effects of temperature and pressure on aflatoxin. Since the mid 1980's much of the research on aflatoxins has focused on the development of novel biocontrol strategies and/or the development of elite crop lines "immune" to aflatoxin producing fungi.

Purpose

The purpose of this study was to determine if the dry-extrusion process would decrease both aflatoxin levels in cottonseed and gossypol levels in mixtures of CGBP and cottonseed.

Materials and Methods

The study was comprised of four sections: gossypol; preliminary aflatoxin; effects of extruder temperature on aflatoxin levels; and the effects of multiple pass extrusion on aflatoxin levels. The gossypol section was a continuation of work by Thomasson, et al. (1998) and is the basis for utilizing mixtures of CGBP and cottonseed in lieu of strictly cottonseed. The gossypol trials focused on determining if the dry-extrusion process would reduce gossypol levels in various mixtures of CGBP and cottonseed and determining the resulting nutritional values of the extruded mixtures. A small preliminary test was conducted to determine if aflatoxin levels in contaminated cottonseed were affected by the extrusion process, this test was the justification for completing the remaining sections of this study. The third section of this study focused on effects of extruder temperature on changes in aflatoxin levels. Variations in nutritional values were also considered in this section of the study. The final section of this study evaluated the effects of multiple pass extrusion on the changes in aflatoxin levels and nutritional values. The process of extruding the material multiple times was a simplified means of testing the effects of increased dwell time. The alternative to this method was reconfiguring the extruder for each of the respective dwell times of interest.

Extrusion Equipment

The commercial-size dry-extruding machinery at the Insta-Pro International Research and Development Facility in Des Moines, Iowa. All sections of this study utilized the Insta-Pro Model 1500 dry-extruder followed by an Insta-Pro air type belt drier to cool the material. This dry-extruder is a single screw adiabatic extruder that generates heat through friction. It is commonly referred to as a high temperature, short-time extruder, which can achieve temperatures up to 180 ºC (356 ºF) in less than 20 seconds. The inside diameter of the barrel is 16.5 cm (6.5-inches) and the overall length is 107 cm (42-inches), with a constant diameter screw. The barrel was configured with two compression chambers, as shown in Figure 1. Compression is accomplished by changing the pitch of the worm flights and shear is achieved by selecting the size of the steamlocks and screw flight, and adjusting the nose bullet and cone in the last chamber of the barrel. The barrel wall and steamlocks are grooved to allow more mixing and shearing. (N. W. Said, 1998, Personal Communication)

The material is fed into the extruder through a top electronic controlled volumetric feeder equipped with an agitator, which provides a uniform and free-flowing material. Once the material enters the inlet chamber, it is forced into the first steamlock by the screw. Grooves in the steamlock walls allow for a gradual build-up in pressure as the material passes through the compression chambers. When the material reaches the last chamber containing the nose bullet and cone, an estimated maximum pressure of 2,756 kPa (400 psia) is achieved.

Dry-extrusion is a process, which applies pressure and shear to the material being extruded; in addition, the material is being internally mixed in the extruder, to create a more-uniform final material. The mixing process, along with pressure and shear, produce frictional forces between the material particles and between the particles and the internal barrel components of the extruder, heating the product being extruded. These four characteristics of dry-extrusion are dependent on one another; therefore, these characteristics will be lumped together and defined as the dry extrusion process. The dry extrusion process will be quantified in terms of extruder temperature (this parameter is based on the four characteristics previously discussed).

Sampling

Sampling is crucial in any experiment and even more so when the test media is extremely variable. The sampling procedures for the gossypol and aflatoxin tests were generally based on the FDA Office of Regulatory Affairs Inspectional References: Investigations Operations Manual's guide for mycotoxin sample size and the discussion of sampling aflatoxin contaminated material, presented in the introduction section of this paper. The guidelines for collecting samples from a suspect bulk lot of cottonseed are: collect 15 random 2 kilogram (4.4 lb) samples from the lot for a total sample weight of 30 kilograms (66 lb); the samples may be mixed prior to shipping the sample to the analytical laboratory. The guidelines for collecting samples as a follow-up to a positive analysis are: collect 50 random 0.5 kilogram (1.1 lb) samples from the lot for total sample weight of 25 kilograms (55 lb); the samples may not be mixed prior to shipping the samples to the analytical laboratory. These guidelines are recommended when testing relatively large amounts of material (semi-truck loads etc.), as compared to the lot sizes used in this study.

Due to the amount of manual material handling, time, and resources required for these replicated extrusion tests, relatively small lot sizes were essential. A key for determining lot sizes for these tests were the required amount sample needed for the various analyses. Several analytical laboratories were contacted to discuss required samples sizes needed for aflatoxin, gossypol, and nutritional analyses. Gossypol and nutritional analyses required about 1 to 1.5 kilograms (2.2 to 3.3 lb) samples. Sample sizes required for gossypol and nutritional analyses are small compared to the amount required for aflatoxin analyses, due to the large variability and

other aspects associated with aflatoxin analyses. Most laboratories generally require a minimum of 2 kilograms (4.4 lb) of material per sample and at least ten samples, which are mixed together at the laboratory, for a representative aflatoxin analyses for truck loads or other large quantities of material. A sample size of 1 to 1.5 kilograms (2.2 to 3.3 lb) (below that required in a typical elevator, oil mill, or feedlot setting) was selected because the material used for these tests have already tested positive for high levels of aflatoxin contamination; the entire amount of contaminated material used in the test was mixed before and during lot preparation; 15 to 20 random subsamples were combined for each sample; the tests were replicated; and analyses was performed by two laboratories. Lot sizes for the gossypol, preliminary aflatoxin, secondary extruder temperature, and multiple pass extrusion tests were 90, 90, 70, and 140 kilograms (200, 200, 150, and 300 lb), respectively.

Samples for the gossypol, aflatoxin, and nutritional analyses were collected in essentially the same manner; however, the number of samples varies by test and analysis and are discussed later in the respective sections. During lot preparation, initial samples were collected to determine pre-processing levels. These samples were composed of a minimum of 15 random subsamples, which were collected as the bulk material was being mixed and distributed between individual lots. After processing the material in the gossypol, preliminary aflatoxin, and extruder temperature tests, the entire extruded lot was placed on a large piece of cardboard and the material was spread out uniformly before the subsamples were collected. During the multiple pass extrusion tests, the extruded material was collected in several plastic tubs where subsamples were collected throughout the extruded material, before the material was reprocessed. Throughout the remaining sections of this paper, the word "sample" refers to a collection of subsamples, which were collected as previously discussed.

Gossypol

The gossypol section of this study required about 850 kilograms (1870 lb) of CGBP and 600 kilograms (1320 lb) of cottonseed. The CGBP (not including motes from the upper moting system of a gin stand or lint cleaner waste) and cottonseed were collected during the ginning of typical spindle picked Mid-South seed cotton. Burdette Gin Company in Burdette, Mississippi supplied the CGBP and the cottonseed was collected at the U. S. Cotton Ginning Laboratory (USCGL), USDA/ARS, in Stoneville, Mississippi.

Various mixing ratios of CGBP and cottonseed were used in this study. Thomasson et al. (1998) suggested that mixing ratios with less than 25% cottonseed produced a loose and fluffy product, unacceptable for its intended use as a livestock feed. Therefore, a mixing ratio of 25% cottonseed and 75% CGBP was used as a base level. The mixing ratios, in terms of percent cottonseed to percent CGBP, were 25:75, 30:70, 40:60, 50:50, and 60:40. Based on these ratios, lots consisting of 90 kilograms (200 lb) of total material were mixed thoroughly.

The test consisted of three replications, requiring a total of 15 test lots. After all the lots were prepared, random numbers were assigned to each lot to indicate the processing order. A target extrusion temperature of 132 ^{o}C (270 ^{o}F) was selected. Since the lots differ in composition, water must be added during processing to maintain a relatively constant temperature. This is due to the cottonseed having relatively high oil content in comparison to the CGBP; the oil acts a lubricant allowing the material to more easily pass thru the barrel of the extruder. After the extrusion process, the material was collected and uniformly spread out on a piece of cardboard.

Several samples were collected before and after the extrusion process. During the mixing process, ten cottonseed and five CGBP samples were randomly collected. Nutritional analysis was performed on five of the cottonseed and five of the CGBP samples, while gossypol analyses were performed on the remaining samples. When the test procedures were originally developed, samples for gossypol analyses were to be collected from the CGBP bulk material and from each of the prepared lots. However, these samples were omitted in the final set of procedures, based on Dr. Calhoun's recommendation that using the official AOCS methods for gossypol analyses on these samples would produce highly variable and erroneous results, due to the non-homogeneity of the material. Prior to processing each lot, one mixed sample was collected for nutritional analyses. After processing, one sample was collected for gossypol analysis and one sample was collected for nutritional analysis. Gossypol analyses were performed at the Agricultural Research and Extension Center in San Anglo, Texas by Dr. Calhoun and the nutritional analyses were preformed by Dairy One in Ithaca, New York.

Two methods were utilized in the gossypol analyses. The official AOCS standard methods were utilized in determining the total and free gossypol level, while a high performance liquid chromatographic (HPLC) procedure was used to determine the isomer percentages. The official method for free gossypol is based on extraction with an acetone-water mixture (70:30), reaction with aniline and measurement of the gossypol-aniline reaction product in a spectrophotometer at 440 nm (AOCS 1985a). The official method for total gossypol is based on reacting free and bound gossypol in a sample with 3-amino-1-propanol in dimethyl formamide solution to form a gossypol-diamino propanol complex. This reacted with aniline to form the gossypol-aniline reaction product which is measured the same as for free gossypol (AOCS 1985b). These procedures measure gossypol, gossypol analogs, and gossypol derivatives having an available aldehyde function, and although not specific for gossypol appear to be satisfactory for use with cottonseed and cottonseed meal. However, the procedure for free gossypol has been found unsatisfactory when applied to mixed feeds, over-predicting the actual levels found in the material. Extraction with aqueous acetone results in incomplete recovery of free gossypol from feed mixtures and removes other feed constituents, which interfere in the subsequent spectrophotometric determination (Pons and Hoffpauir, 1957). High performance liquid chromatographic (HPLC) procedures have been developed which are more specific for gossypol (Hron et al., 1990); however, this is not an official method for determining free and total gossypol. (Calhoun and Holmberg, 1991)

Preliminary Aflatoxin

The preliminary aflatoxin section of this study required about 900 kilograms (1980 lb) of aflatoxin-contaminated cottonseed. The Anderson Clayton Corporation in Stanfield, Arizona, supplied the cottonseed, the cotton variety and production location were not identified. The seed was drawn from a certified pile that tested positive for aflatoxin contamination at a level of 1,005 ppb. While preparing the material for transport, grab samples were collected and analyzed for aflatoxin content. The reported (uncertified) level was 1,650 ppb.

This section focused on the dry-extrusion process at six processing temperatures. The temperatures were 104, 116, 127, 138, 149, and 160 ^{o}C (220, 240, 260, 280, 300, and 320 ^{o}F). Only a single replication of the temperatures was performed, since the primary purpose was to determine the feasibility of conducting further tests (i.e. is extrusion a possible means of reducing aflatoxin levels) and to determine the temperature levels that should be associated with further testing. The test lots consisted of 90 kilograms (200 lb) of cottonseed per lot and prior to creating the lots, the shipping bags were cut open so the seed would fall on the floor. Shovels were used to crudely mix the seed. The lots were created by scooping seed randomly from the pile. Once the lots were generated, random numbers were assigned to each lot, indicating the processing order. During the extrusion process, the remaining contaminated seed was used initially and between lots to adjust the extruder temperature to the proper level. After each lot was extruded, the material was uniformly spread out on a piece of cardboard for sample collection.

During lot preparation for this section, three random samples were collected for initial aflatoxin analyses. After each lot was processed, three random samples were collected for aflatoxin analysis. All the samples in this section were shipped to Dairy One in Ithaca, New York, for analyses.

The aflatoxin analyses in this section and throughout the rest of the study utilize the following procedure prior to using a Veratox testing kit, which is marketed by the Neogen Corporation: the entire sample is ground to a level where the material could pass through a No. 20 sieve and the ground sample is thoroughly mixed. The Veratox for aflatoxin is a competitive direct enzyme-linked immunosorbent assay that allows the user to obtain exact concentrations in parts per billion. Free toxin, in the sample is allowed to compete with enzyme-labeled toxin (conjugate) for the antibody binding sites. After a wash step, substrate is added which reacts with the bound enzyme conjugate to produce a blue color. The test is read in a microwell reader to yield optical densities. The detection range for the kit is 5 to 50 ppb; therefore, if the contamination level is above 50 ppb sample dilution is required.

Extrusion Temperature

The extrusion temperature section of this study required 850 kilograms (1870 lb) of contaminated cottonseed. The Chickasha Cotton Oil Company in Casa Grande, Arizona supplied roughly 1,850 kilograms (4070 lb) of aflatoxin-contaminated cottonseed for use in this section and the multiple pass extrusion section. The cottonseed was produced in Maricopa County, Arizona and the cottonseed initially tested positive for aflatoxin with levels in excess of 650 ppb.

Based on the information obtained from the preliminary aflatoxin test, this section focused on extrusion temperatures of 104, 132, and 160 °C (220, 270, and 320 °F). Four replications were performed for a total of twelve lots. The test lots consisted of 70 kilograms (154 lb) of cottonseed per lot and prior to creating the lots, the shipping bags were cut open so the seed would fall on the floor. Shovels were used to crudely mix the seed. The lots were created by scooping seed randomly from the pile. Once the lots were generated, random number were assigned to each lot, indicating the processing order. During the extrusion process, the remaining contaminated seed (remaining after the multiple pass extrusion lots were generated) was used initially and between lots to adjust the extruder temperature to the proper level. After each lot was extruded, the material was uniformly spread out on a piece of cardboard for sample collection.

During lot preparation for this section and the multiple pass extrusion section, 23 random samples were collected. Seventeen of these samples were used to determine the initial aflatoxin content of the cottonseed and the remaining 6 samples were used to determine the initial nutrient values of the cottonseed. After processing, 5 random samples were collected. Four of these samples were used for aflatoxin analyses and the remaining sample was used for nutritional analyses. All the nutritional, 10 of the initial aflatoxin samples, and three of the aflatoxin samples collected after extrusion were shipped to Dairy One in Ithaca, New York for analyses. The remaining samples were sent to the Neogen Corporation in Lansing, Michigan. The laboratory procedures were discussed previously in the preliminary aflatoxin section.

Multiple Pass Extrusion

The multiple pass extrusion section of this study required about 400 kilograms (880 lb) of aflatoxin-contaminated cottonseed. The cottonseed was from the same bulk supply received from the Chickasha Cotton Oil Company, discussed in the extrusion temperature section. During these tests, each lot was extruded four times with samples for nutritional and aflatoxin analyses collected before each pass and after the final pass. This test required 140 kilograms (308 lb) of material per lot. Three replications were completed, requiring a total of three lots. Lot preparation was identical to the method used in the extrusion temperature section.

The target extrusion temperature for this section was 132 °C (270 °F). The method of processing was conducted in the following manner: a lot was extruded; the material was collected in plastic tubs; samples were randomly collected; the material was reprocessed by the extruder; and the process was repeated until the lot was processed four times. This method was repeated for each lot.

Initial nutritional and aflatoxin samples were collected as described in the extrusion temperature section (the extrusion temperature and multiple pass sections utilized cottonseed from the same bulk source). After each stage of processing (pass), 5 random samples were collected. Four of these samples were used for aflatoxin analyses and the remaining sample was used for nutritional analyses. All the nutritional and three of the aflatoxin samples collected after each stage of processing were shipped to Dairy One in Ithaca, New York for analyses. The remaining samples were sent to the Neogen Corporation in Lansing, Michigan. The laboratory procedures were discussed previously in the preliminary aflatoxin section.

Results

Gossypol

During the mixing ratio study, an internal extruder temperature range of 130 to 135 °C (266 to 275 °F) was maintained and approximately 95 to 100 amperes were used to operate the extruder. The water injection rates were 38, 38, 30, 11, and 8 liters per hour (10, 10, 8, 3, and 2 gallons per hour) for the 75, 70, 60, 50, and 40 percent CGBP mixtures, respectively. The production rates were 500, 568, 646, 750, and 791 kg per hour (1100, 1250, 1420, 1650, and 1740 lb per hour) for the 75, 70, 60, 50, and 40 percent CGBP mixtures, respectively. As expected, the water injection rates decreased and the production rates increased as the percent of cottonseed in the mixture increased.

Gossypol results are reported in an as fed basis, as shown in Table 5. Total and free gossypol levels for the non-extruded cottonseed (0.682 and 0.693, respectively) are consistent with values previously reported. The differences between the free and total values are attributed to analytical methods, since similar instances have been well documented and base the differences on determining values, which are theoretically the same, by two different official methods. Further, there were significant differences in the free and total gossypol levels for the various mixing ratios and the levels generally decrease with a decrease in the percentage of cottonseed in the mixture, which was expected since cottonseed contains more gossypol than the CGBP. The mean square error for the total gossypol test was 0.0007, resulting in an F-value of 153. The mean square error for the free gossypol test was 0.0001, resulting in an F-value of 2177.

Theoretically, the reported values minus the percent of CGBP in the mixture times the gossypol levels associated with extruded CGBP and this quantity divided by the percent of cottonseed in the mixture should equal the gossypol levels associated with the extruded cottonseed. When performing these calculations for total gossypol, values from 0.82 to 0.59 are obtained for mixtures containing 25 to 60% cottonseed. Theoretically, these values should be statistically equivalent to those obtained for the 100% non-extruded cottonseed; however, several significant differences were detected. Based on this information and the fact that total gossypol levels for the 25 and 30 extruded cottonseed mixtures were significantly higher than the 100% non-extruded cottonseed, it was determined that the gossypol analyses, using the official AOCS methods, overestimated the actual gossypol levels present in the material. These overestimates were expected. A more in depth discussion of the gossypol over estimations can be found in Hron et al., 1990).

Reductions in the free gossypol levels were from 71 to 78% for mixing ratios of 25 to 60% cottonseed. These reductions are most likely underestimated, since the overestimations associated with the free and total

gossypol levels could not be determined. Significant differences in the plus and minus isomer percentages were detected between the non-extruded and extruded mixtures. The differences were relatively small, less than a 1.4 difference between all the values. The mean square error and degrees of freedom associated with the isomer tests were 0.26 and 13, respectively.

Nutritional values for the extruded mixtures of CGBP and cottonseed are shown in Table 6. There are significant differences in several of the nutritional components, which were expected due to the varying amount of CGBP. Crude protein, net energy of maintenance, net energy of gain and total digestible nutrients significantly increased, and ash content decreased as the percent of cottonseed increased. Acid and neutral detergent fiber significantly increased with the addition of CGBP, but did not significantly with changing mixing ratios. Calcium, potassium, sodium, and iron significantly increased with increased CGBP content, while phosphorus and zinc significantly increased with increased cottonseed content. In general terms, the nutritional value of the product increased as the percent of cottonseed increased in the mixture.

Preliminary Aflatoxin

During the preliminary aflatoxin test, extrusion temperatures were maintained within +/- 1 °C (+/- 1.8 °F) of the target values. The water injection rates increased from 15 to 30 liters per hour (4 to 8 gallons per hour) and the amperage increased from about 65 to 90 amps as the temperature increased from 116 to 160 °C (240 to 320 °F). As the pressure and shear were increase by adjusting the extruder nose cone, required to increase temperature, water injection rates had to be increased to keep the material flowing through the barrel.

Significant difference in the aflatoxin levels were found between the non-extruded cottonseed and cottonseed extruded at 149 and 160 °C (300 and 320 °F); however, there were no significant differences between the 6 temperature treatments. Means and 95% confidence intervals are shown in Figure 2. A trend line is also included in Figure 2, which demonstrates the apparent decrease in aflatoxin levels with increased temperature. The sample variability was relatively high for the non-extruded material and extrusion temperatures of 104, 127, and 138 °C 220, 260, and 280 °F), indicating that additional replications are needed. Standard deviations ranged from 44 ppb for the non-extruded to 3 ppb for the material extruded at a temperature of 160 °C (320 °F). The F-value and p-value for the test were 2.26 and 0.1. Although initial aflatoxin levels, as determined by the supplier, were 1,005 ppb, the maximum level detected for non-processed cottonseed was 162 ppb and the maximum level detected in the extruded cottonseed was 123 ppb.

Based on the aflatoxin information, it was determined that additional tests would be required to determine if the extrusion process reduced aflatoxin levels in cottonseed. Additional test should focus on fewer extruder temperatures, but maintain the range of 104 to 160 °C (220 to 320 °F). Further, additional tests should include more observations per treatment and more test replications.

Extrusion Temperature

During the extrusion temperature test, temperatures were maintained within +/- 1 °C (+/- 1.8 °F) of the target values. The water injection rates were 15, 19, and 30 liters per hour (4, 5, and 8 gallons per hour) for treatment temperatures of 104, 132, and 160 °C (220, 270, and 320 °F), respectively. The extruder pulled an average current of 70, 80, and 86 amps for temperatures of 104, 132, and 160 °C (220, 270, and 320 °F), respectively. As the pressure and shear were increased, by adjusting the extruder nose cone, required to increase temperature, water injection rates had to be increased to keep the material flowing through the barrel. As expected, current and water requirements increased with increasing temperature.

Three times as many aflatoxin samples (3 replications) were analyzed by Neogen in the extrusion temperature study as compared to the preliminary aflatoxin test. Even with this increase there was substantial variability within the treatments, the mean square error for the test was 254,905 with 15 degrees of freedom. There were no significant differences in aflatoxin levels between the treatments, even at the 0.2 significance level; however, Figure 3 shows an apparent decrease in aflatoxin levels as well as a reduction in the 95% confidence intervals as the extrusion temperatures are increased. The mean, 95% confidence interval, maximum detected level, and the minimum detected level for aflatoxin associated with the non-extruded material was 475, +/- 542, 1630, and 12 ppb, respectively. The mean, 95% confidence interval, maximum detected level, and the minimum detected level for aflatoxin associated with an extrusion temperature of 160 °C (320 °F) was 109, +/- 16, 131, and 93 ppb, respectively. When comparing the values of the non-extruded material and the material extruded at 160 °C (320 °F), it appears that the aflatoxin levels as well as the variability are decreased by the extrusion process.

Nine times as many aflatoxin samples (3 replications and 3 observations per replication) were analyzed by Dairy One in the extrusion temperature study as compared to the preliminary aflatoxin test. Even with this increase there was substantial variability within the treatments, the mean square error for the test was 12,490 with 42 degrees of freedom. The error associated with the samples analyzed by Diary One was substantially lower than the error associated with the samples analyzed by Neogen, due to the greater number of samples analyzed. There were no significant differences in aflatoxin levels between the treatments, even at the 0.2 significance level; however, Figure 4 shows an apparent decrease in aflatoxin levels as well as a reduction in the 95% confidence intervals as the extrusion temperatures are increased. The mean, 95% confidence interval, maximum detected level, and the minimum detected level for aflatoxin associated with the non-extruded material was 143, +/- 137, 665, and 1 ppb, respectively. The mean, 95% confidence interval, maximum detected level, and the minimum detected level for aflatoxin associated with an extrusion temperature of 160 °C (320 °F) was 119, +/- 27, 218, and 57 ppb, respectively. When comparing the values of the non-extruded material and the material extruded at 160 °C (320 °F), it appears that the aflatoxin levels as well as the variability are decreased by the extrusion process.

The sample data received from Dairy One, gathered from the non-extruded material was dropped from the statistical analyses. This method is not statistically sound, since the test was not originally setup in this manner; however, it illustrates how the variability in the non-extruded material affected the test. When analyzing the data without the non-extruded data, significant differences were detected. The mean square error and degrees of freedom were reduced to 2,575 and 33, respectively, which yields a p-value of 0.018. The aflatoxin levels associated with the material extruded at temperatures of 132 and 160 °C (270 and 320 °F) were significantly lower those associated with an extrusion temperature of 104 °C (220 °F).

There were no significant differences in the majority of the nutritional values; however, there were a few notable differences. Crude protein, fiber, total digestible nutrient, net energies, and most of the mineral values exhibited relatively low variation between temperature treatments, as indicated by the corresponding p-values shown in Table 7. Soluble protein and copper were the exceptions. Soluble protein levels associated with an extrusion temperature of 104 °C (220 °F) were significantly higher than those at 132 and 160 °C (270 and 320 °F); however, no significant differences were detected between the 132 and 160 °C (270 and 320 °F) treatments. Copper values were significantly decreased as the extrusion temperature increased from 104 to 160 °C (220 to 320 °F). There were no significant differences in iron content of the extruded material; however, the iron value for the 104 °C (220 °F) treatment was relatively high (702 ppm) in comparison to the other treatments (148 and 179 ppm). Two of the iron values for the 104 °C (220 °F) treatment were 1,600 and 989 ppm, which

was substantially higher than any other samples. These high values may be explained by the fact that small rocks were found in the cottonseed. Rocks tend to "hang" in the barrel of the extruder, which can cause damage to the extruder by marring the inside of the barrel or the screw thereby potentially depositing iron into the extruded material. In general, the nutritional value of the cottonseed was increased by extruding the material at 132 °C (270 °F), due to the decreased soluble protein levels.

Multiple Pass Extrusion

During the multiple pass extrusion tests, extrusion temperatures were maintained within the range of 132 to 138 °C (270 and 280 °F). The water injection rates were constant at 19 liters per hour (5 gallons per hour) and the extruder current draw ranged from 72 to 78 amperes, for all stages of processing. Numerous nose cone adjustments were required to maintain a relatively constant and uniform flow rate for all the stages of processing. This is due to the changes in the physical makeup of the material, which is altered by each stage of processing.

Sample variability greatly affected the statistical analyses of the aflatoxin data obtained from Neogen, the mean square error and degrees of freedom for the analyses were 296,792 and 13, respectively. There were no detected significant differences in aflatoxin levels associated with increased processing; however, Figure 5 shows an apparent decrease in aflatoxin levels as well as a reduction in the 95% confidence intervals as the number of stages of processing increased. The mean, 95% confidence interval, maximum detected level, and the minimum detected level for aflatoxin associated with the non-extruded material was 785, +/- 542, 1630, and 12 ppb, respectively. The mean, 95% confidence interval, maximum detected level, and the minimum detected level for aflatoxin associated with four stages of processing was 182, +/- 61, 236, and 129 ppb, respectively. Not only were there reductions between the non-extruded and fourth stage of processing; but there were incremental decreases as the number of processing stages increased. When comparing the values of the non-extruded material and extruded material, it appears that the aflatoxin levels as well as the variability are decreased by increased processing and the largest difference was between no processing and two stages of processing.

Three times as many aflatoxin samples (3 observations per replication) were analyzed by Dairy One in the multiple pass extrusion temperature study as compared to those analyzed by Neogen. Increasing the number of samples substantially decreased the sample variability; the mean square error for the test was 13,305 with 41 degrees of freedom. There were significant differences in aflatoxin levels between one stage of processing and three and four stages of processing, at a 0.1 significance level (p-values were 0.061 and 0.056, respectively). Although there were no additional significant differences, there was a trend of decreasing aflatoxin levels with increased processing treatments as shown in Figure 6. The 95% confidence interval for the non-extruded material was substantially larger than the intervals associated the various stages of processing, shown in Figure 6. The mean, 95% confidence interval, maximum detected level, and the minimum detected level for aflatoxin associated with the non-extruded material was 143, +/- 137, 665, and 1 ppb, respectively. The mean, 95% confidence interval, maximum detected level, and the minimum detected level for aflatoxin associated with an material processed four times was 124, +/- 23, 180, and 60 ppb, respectively. When comparing the values of the non-extruded material and the material extruded multiple times, it appears that the aflatoxin levels as well as the variability are decreased by the extrusion process.

The sample data received from Dairy One, gathered from the non-extruded material was dropped from the statistical analyses. This method is not statistically sound, since the test was not originally setup in this manner; however, it illustrates how the variability in the non-extruded material affected the test. When analyzing the data without the non-extruded data, several significant differences were detected. The mean square error and degrees of freedom were reduced to 3,310 and 32, respectively, which yields a p-value of 0.0007. Significant differences in aflatoxin levels were detected for 1, 2, and 3 stages of processing, while no difference were detected between 3 and 4 stages of processing.

There were no significant differences in most of the nutrient values with respect to increased processing. Phosphorus, zinc, and sulfur were the exception. These values were significantly decreased when comparing one stage of processing to four stages of processing. Although not significant, the remaining mineral values and crude protein values appeared to decrease, while the fiber values appeared to increase with increased processing, as shown in Table 8. In general, increasing the number of stages of processing, while maintaining an extrusion temperature of 132 °C (270 °F), did not significantly impact the nutritional value of the extruded cottonseed.

Conclusions

Water injection rates decreased and production rates increased as the percent of cottonseed in the mixed products increased. For the tests using 100% cottonseed an increase in the water injection rates and power were required when increasing the extrusion temperature. As the texture of the material changed (the ratio of cottonseed to CGBP in the gossypol study and the material associated with various stages of processing in the multiple extrusion study), the nose cone had to be adjusted to maintain the targeted temperatures.

Reductions in the free gossypol levels ranged from 71 to 78% for mixing ratios of 25 to 60 percent cottonseed. These reductions are most likely underestimated, since the official methods of determining free and total gossypol overestimate the levels in mixed feeds. The nutritional value associated with the extruded mixtures of cottonseed and CGBP, increased as the percent of cottonseed increased in the mixture.

The preliminary aflatoxin test indicated that the extrusion process reduced aflatoxin levels and warranted further study. These additional tests showed that when collecting three observations per treatment (70 kg [154 lb] lots for a single stage of processing and 140 kg [308 lb] lots for four stages of processing) and performing multiple replications (4 replications in the temperature tests and 3 replications in the multiple pass study) the aflatoxin variability was still an issue. The variability associated with the non-extruded material significantly impacted the results of the tests; however, the aflatoxin variability was substantially lower for the extruded products. This decrease was attributed to the internal mixing that occurs during the extrusion process.

When comparing the effects of treatments and not accounting for the initial aflatoxin levels present in the products prior to the extrusion process, significant differences were detected between test treatments. In the extrusion temperature tests, reductions of 33 to 45% were found when comparing extrusion temperatures of 104 to 160 °C (220 to 320 °F). When extruding the material multiple times at an extrusion temperature of 132 °C (270 °F) reductions of 46 to 67% were found, these values are based on the mean values determined for the material processed by one stage of extrusion and material processed by four stages of extrusion.

The nutritional values associated with the extrusion temperature tests were not significantly changed by the increased temperatures, with the exception of soluble protein. Soluble protein was reduced as the extrusion temperature was increased. Nutritional values associated with the multiple pass extrusion study were not significantly changed by increasing the number of stages of process, with the exception of phosphorus, zinc, and sulfur. These values generally decreased with increased processing.

Based on the results of this study, further research should be conducted to determine the optimum extruder parameters required to achieve the largest reductions in gossypol and aflatoxin levels, with regards to economic feasibility.

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Disclaimer

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Table 1. Reported "Effect" and "No Effect" levels of free gossypol in non-ruminants, based on research trials.

	Free Gossypol Intake (ppm)		
Class of livestock	**Effect**	**No Effect**	**Reference**
Yearling horses	-	115 †	Potter, 1981
Weanling horses	-	348 †	McCall, 1982
Young lambs	824 §	-	Calhoun, 1989
Catfish	-	900 §	Dorsa et al., 1982
Tilapia	-	1800 §	Robinson et al., 1984
Rainbow trout	1,000 †	250 †c	Roehm et al., 1967
Shrimp (Penaeus vannamei)	-	170 §	Fernandez, 1987

† Dry matter/as fed basis not reported
§ Dry matter basis
Fed as gossypol acetic acid

Table 2. Currently accepted tolerance levels for free gossypol in poultry and swine, as fed basis.

Class of livestock	**Free Gossypol Intake (ppm)**	**Maximum Free Gossypol Intake with Iron Salts**	**Reference**
Broilers	100-150	400 ppm (1-2 ppm Fe: 1ppm Free Gossypol)	Waldroup, 1981
Layers	50	150 ppm (4 ppm Fe: 1 ppm Free Gossypol	Waldroup, 1981
Swine	100	400 ppm (1 ppm Fe: 1ppm Free Gossypol)	Tanksley & Knabe, 1981

Table 3. Recommended safe levels of free gossypol for ruminants.

Stage of rumen development	**Age**	**Free gossypol levels**	
		Ppm in diet	**Mg/lb/LW/day**
Preruminant	0-3 wk	100	1.1
Transition †	3-8 wk	200	2.3
Functional			
Post-weaning	8-24 wk	200	3.6
Mature §	>24 wk	600	6.8

† Transition from pre-ruminant to functional ruminant begins when animals start to consume dry feed (i.e., pasture, hay, concentrate)
§ This level is considered safe for females used for breeding. The recommended safe level for males used for breeding is 200 ppm free gossypol.

Table 4. Processing methods and their respective free gossypol levels, on an as-fed basis.

Processing Method	**% Free Gossypol**
Hydraulic †	0.04 - 0.10
Screw press †	0.02 – 0.05
Prepress solvent †	0.02 – 0.07
Direct solvent †	0.10 – 0.50
Expander solvent §	0.06 – 0.21

† Berardi and Goldblatt (1980)
§ Calhoun (1989)

Table 5. Percent free and total gossypol and isomer ratios for cottonseed and cotton gin by-products, based on mixing ratios. Results are reported in an as fed basis.

Product	**AOCS gossypol, %**		**Isomer % of total**	
Composition	**Free†**	**Total §**	**(+)**	**(-)**
100% Cottonseed				
Non-extruded	0.693a	0.682a	59.3a	40.7a
25% Cottonseed				
Extruded	0.060b	0.205e	57.9b	42.1b
30% Cottonseed				
Extruded	0.065bc	0.244de	58.4b	41.6b
40% Cottonseed				
Extruded	0.066bc	0.280cd	58.1b	41.9b
50% Cottonseed				
Extruded	0.056c	0.297c	58.2b	41.8b
60% Cottonseed				
Extruded	0.078b	0.356b	58.2b	41.8b

† Free gossypol determined by AOCS official method Ba 7-58.
§ Total gossypol determined by AOCS official method Ba 8-78.
(+) and (-) gossypol isomers were determined by HPLC using 2-amino-propanol as a complexing reagent.
AOCS – American Oil Chemist's Society.
Means in a row not having a letter in common are significantly different at =0.05 according to the Waller-Duncan's multiple range test.

Table 6. Nutritional values of non-extruded cottonseed and cotton gin by-products and various mixing ratios of extruded cotton gin by-products and cottonseed. †

	Non-extruded		Extruded				
Nutrient Value	**100% CS**	**100% CGBP**	**25% CS**	**30% CS**	**40% CS**	**50% CS**	**60% CS**
Crude Protein (%)	30.0a	15.5d	16.6cd	17.4cd	17.7cbd	19.1bc	20.1b
Adjustable Crude Protein (%)	30.0a	15.5d	16.6cd	17.4cd	17.7cbd	19.1bc	20.1b
Soluble Protein (%)	21.7a	19.0a	14.8a	16.5a	9.8a	23.0a	14.8a
Acid Detergent Fiber (%)	30.3b	46.5a	47.3a	47.1a	44.5a	46.7a	43.8a
Neutral Detergent Fiber (%)	41.5c	51.8b	56.0ab	58.1a	54.5ab	55.1ab	53.0ab
Total Digestible Nutrients (%)	80.0a	37.3f	41.5de	40.3e	42.8cd	44.5bc	45.7b
Net Energy of Maintenance (Mcal/kg)	2.09a	0.81f	0.92de	0.88e	0.95cd	0.99bc	1.01b
Net Energy of Gain (Mcal/kg)	1.54a	0.04e	0.18d	0.13de	0.22cd	0.26bc	0.31b
Calcium (%)	0.19e	2.11a	1.92ab	1.90ab	1.69bc	1.39cd	1.10d
Phosphorus (%)	0.84a	0.26e	0.41d	0.42d	0.49c	0.56b	0.61b
Magnesium (%)	0.40a	0.34b	0.35b	0.36ab	0.38ab	0.38ab	0.37ab
Potassium (%)	1.22c	1.61a	1.64a	1.63a	1.53ab	1.57ab	1.48b
Sodium (%)	0.004e	0.049ab	0.051a	0.047ab	0.042bc	0.035c	0.026d
Iron (ppm)	86e	1043a	554bc	631b	581b	400d	428dc
Zinc (ppm)	41a	30cd	29d	33bc	35b	33bc	35b
Copper (ppm)	5.67a	4.00b	4.33b	4.50b	5.00ab	4.50b	4.83ab
Manganese (ppm)	14e	83a	59bc	63b	59bc	48cd	44d
Molybdenum (ppm)	1.20b	1.73a	1.25b	1.47ab	1.50ab	1.42b	1.35b
Sulfur (%)	0.29c	0.39a	0.37ab	0.37ab	0.35ab	0.32bc	0.32bc
Ash (%)	-	-	12.1ab	12.5a	11.3b	9.1c	8.6c

† All values based on dry matter

§ Means in a row not having a letter in common are significantly different at =0.05 according to the Waller-Duncan's multiple range test.

-Not reported

Table 7. Nutritional values for cottonseed extruded at various temperatures.†

	Temperature (°C)			
Nutrient Value	**104**	**132**	**160**	**p-value**
Crude Protein (%)	22.5a	22.3a	22.3a	0.9937
Adjustable Crude Protein (%)	22.5a	22.3a	22.3a	0.9937
Soluble Protein (%)	18.0a	10.8b	11.0b	0.0005
Acid Detergent Fiber (%)	43.5a	41.0a	47.0a	0.2203
Neutral Detergent Fiber (%)	58.0a	56.3a	59.6a	0.6061
Total Digestible Nutrients (%)	78.8a	77.5a	79.5a	0.4981
Net Energy of Lactation (MCAL/LB)	0.93a	0.92a	0.94a	0.5224
Net Energy of Maintenance (MCAL/LB)	0.95a	0.93a	0.96a	0.5354
Net Energy of Gain (MCAL/LB)	0.65a	0.63a	0.65a	0.5176
Calcium (%)	0.15a	0.15a	0.15a	0.8563
Phosphorus (%)	0.57a	0.58a	0.56a	0.9257
Magnesium (%)	0.32a	0.32a	0.31a	0.9079
Potassium (%)	1.26a	1.28a	1.30a	0.7266
Sodium (%)	0.009a	0.010a	0.011a	0.6964
Iron (ppm)	702a	148a	179a	0.1689
Zinc (ppm)	33a	33a	34a	0.9703
Copper (ppm)	6.0a	5.3ab	4.5b	0.0288
Manganese (ppm)	23a	19a	19a	0.3402
Molybdenum (ppm)	1.68a	1.95a	1.88a	0.8894
Sulfur (%)	0.22a	0.22a	0.21a	0.3473

† All values based on dry matter

§ Means in a row not having a letter in common are significantly different at =0.05 according to the Waller-Duncan's multiple range tests.

p-values are base on F-test with 11 degrees of freedom.

Table 8. Nutritional values for cottonseed extruded multiple times. †

	Number of Time Extruded				
Nutrient Value	**1**	**2**	**3**	**4**	**p-value**
Crude Protein (%)	20.7a	17.4a	16.9a	16.5a	0.3867
Adjusted Crude Protein (%)	20.7a	17.4a	16.9a	16.5a	0.3867
Soluble Protein (%)	11.3a	10.3a	13.3a	9.3a	0.2557
Acid Detergent Fiber (%)	49.3a	48.8a	52.2a	50.8a	0.8124
Neutral Detergent Fiber (%)	60.8a	65.8a	65.2a	67.1a	0.5924
Total Digestible Nutrients (%)	78.3a	78.0a	77.3a	78.0a	0.8417
Net Energy of Lactation (MCAL/LB)	0.93a	0.93a	0.93a	0.92a	0.8272
Net Energy of Maintenance (MCAL/LB)	0.94a	0.93a	0.92a	0.93a	0.5909
Net Energy of Gain (MCAL/LB)	0.64a	0.63a	0.62a	0.62a	0.3691
Calcium (%)	0.15a	0.14a	0.13a	0.11a	0.1899
Phosphorus (%)	0.54a	0.46ab	0.41ab	0.35b	0.1476
Magnesium (%)	0.31a	0.28a	0.26a	0.22a	0.1746
Potassium (%)	1.29a	1.28a	1.20a	1.00a	0.2269
Sodium (%)	0.014a	0.011a	0.010a	0.007a	0.2247
Iron (ppm)	190a	206a	187a	389a	0.4265
Zinc (ppm)	33.3a	28.7ab	26.0ab	21.0b	0.0925
Copper (ppm)	4.3a	5.0a	4.0a	2.3a	0.3327
Manganese (ppm)	20.7a	20.3a	19.0a	17.3a	0.5745
Molybdenum (ppm)	2.03a	2.00a	1.63a	1.63a	0.5672
Sulfur (%)	0.20a	0.17ab	0.15b	0.16b	0.1013

† All values based on dry matter

§ Means in a row not having a letter in common are significantly different at =0.05 according to the Waller-Duncan's multiple range tests.

p-values are base on F-test with 11 degrees of freedom.

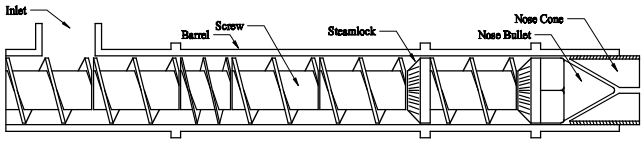

Figure 1. Extruder Barrel Cross-Section.

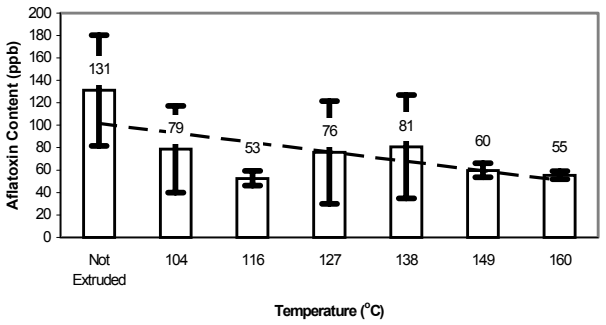

Figure 2. Aflatoxin means (columns) and 95% confidence intervals (bars) for the preliminary aflatoxin test, conducted at various extruder temperatures.

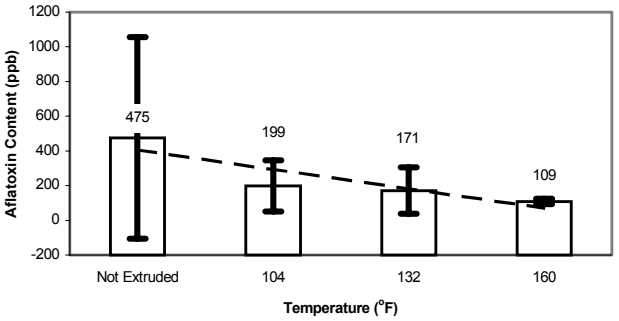

Figure 3. Aflatoxin means (columns) and 95% confidence intervals (bars) for the aflatoxin test, conducted at various extruder temperatures. Samples analyses performed by Neogen.

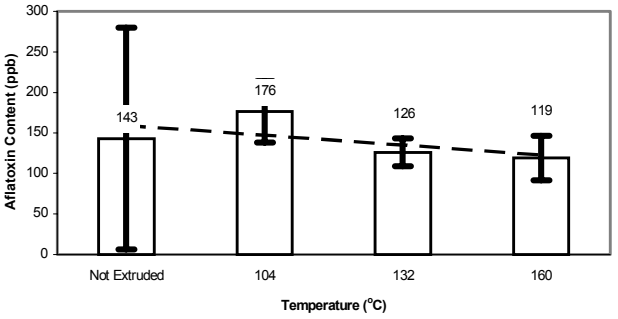

Figure 4. Aflatoxin means (columns) and 95% confidence intervals (bars) for the aflatoxin test, conducted at various extruder temperatures. Samples analyses performed by Dairy One.

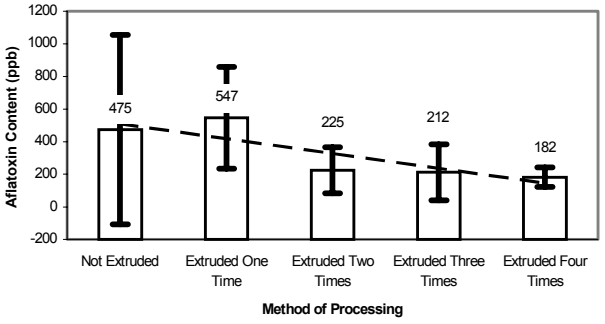

Figure 5. Aflatoxin means (columns) and 95% confidence intervals (bars) for the multiple pass aflatoxin test. Samples analyses performed by Neogen.

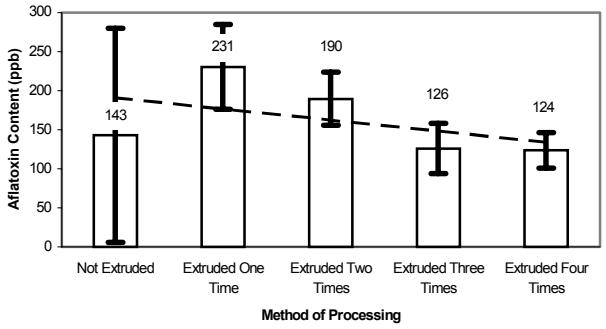

Figure 6. Aflatoxin means (columns) and 95% confidence intervals (bars) for the multiple pass aflatoxin test. Samples analyses performed by Dairy One.