Variation in the Nutrient and Gossypol Content of Whole Cottonseed and Cottonseed Meal

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Introduction

Cottonseed and cottonseed meal are excellent feeds that are widely fed to ruminant animals. The safe and effective use of these in diets for dairy cattle requires good nutritional information on which to base feeding decisions and an understanding of factors which contribute to variation in their nutrient and gossypol content. Type of cotton, variety and growing conditions certainly are important sources of variation but harvesting and storage conditions and procedures used for oil extraction also have an impact. Equally important, but possibly not fully appreciated, is variation due to the analytical laboratories and procedures used to determine the composition of cottonseed and cottonseed meal. Because new cotton varieties are continually being introduced by cotton breeders and oil extraction procedures change with time, it is important to know the effects of these changes on the composition of cottonseed and cottonseed meal. This report summarizes available information on the nutritional and gossypol content of whole cottonseed and cottonseed meal, and factors that contribute to variation in composition; including the role of laboratories and procedures used for analysis.

Types of Cottonseed

Seed of two types of cotton are fed to dairy cattle in the United States, Upland (*Gossypium hirsutum*) and Pima (*Gosssypium barbadense*). Approximately 98% of cotton acreage in the United States is Upland, but the two percent planted to Pima is concentrated in the San Joaquin Valley of California, in close proximity to the California dairy industry. Seed of Upland cottons, commonly called white or fuzzy cottonseed, have short cotton fibers still attached to the seed coat; whereas, seed of Pima cottons are basically bare, black seed without attached short fibers.

Pima seed is sometimes called black seed; however, some delinted Upland seed is available for feeding and the bare seed coat is also black. Upland seed is fed primarily as whole seed, just as it comes from the gin. In contrast, Pima seed generally is cracked or ground prior to feeding.

Nutrient Composition of Upland Cottonseed

Several sources contain information on the composition of Upland seed (Calhoun et al., 1995; NRC, 2001; Dairy One, 2004). This information is presented in Table 1 and includes the number of samples analyzed and the standard deviation for each mean. In the study reported by Calhoun et al. (1995), eighty three samples of whole cottonseed were collected from 31 cotton oil mills and analyzed for nutrient and gossypol content. Samples were collected at the beginning, middle and end of the 1993-94 crushing season. Samples were composited over five working days and sampled regularly across shifts for each date. All samples were sent to the Texas Agricultural Experiment Station's (TAES) Nutrition and Toxicology Laboratory at San Angelo, TX. The first 28 samples received were sub-sampled and submitted to the Northeast DHIA Forage Testing Laboratory (Dairy One) in Ithaca, NY for nutritional analyses.

The results were highly variable. The variability was believed to be related to the lint and high oil content of the seed, which made it difficult to obtain a representative sub-sample for analysis. To address this problem, seed were separated into lint and hulls and meats fractions using a kitchen blender and a series of screens. The fractions were ground through a 1 mm screen and then recombined in the correct proportions for each nutrient to be measured, i.e., dry matter, crude protein, fat, etc., and sent to Dairy One for analysis. A sub-sample of the original seed was also sent to Mid-Continent Laboratories (Jackson, MS) for crude protein and fat analysis. Mid-

Continent was selected as a reference laboratory because it used the official methods of the American Oil Chemists Society (AOCS) for protein and fat, and because of its consistent outstanding performance with cottonseed analysis, over a period of several years, in the Laboratory Proficiency Program administered yearly by AOCS.

Initially, the crude protein content of the 28 cottonseed samples analyzed by Dairy One averaged 27.6 ± 0.73% (DM basis) and ranged from 20.8 to 34.1%. After preparation, to obtain a representative sub-sample, and being re-analyzed by Dairy One, the samples averaged 22.4 ± 0.20% crude protein, and ranged from 20.7 to 25.8%. These were in excellent agreement with the values reported by Mid-Continent (22.8 ± 0.18% crude protein, with a range of 21.5 to 26.0%). The correlation between Mid-Continent and Dairy One (prepared cottonseed samples) for crude protein was $r = 0.89$.

A decrease in variability was also evident for fat when the samples were prepared for analysis prior to sending them to Dairy One. Initially, the fat content was 16.7 ± 0.45% (DM basis) and ranged from 14.3 to 21.8%. After preparation, to obtain a representative sub-sample, the same samples averaged 17.9 ± 0.23% fat, and ranged from 16.0 to 21.2%. The agreement with the analyses done by Mid-Continent was not as good ($r = 0.64$) as for crude protein. Mid-Continent values for fat were consistently higher ($P < 0.05$) than Dairy One and averaged 20.1 ± 0.18%, and ranged from 18.8 to 21.8%.

The crude protein and crude fat values reported in Table 1 for Calhoun et al. (1995) were determined by Mid-Continent Laboratories, and differ from the original report. In the original report the analyses were done by Dairy One; crude protein was 22.4% with a SD of 1.06, fat was 17.9% with a SD of 1.24. All other nutrients were determined by Dairy One. Acid detergent fiber (ADF), neutral detergent fiber (NDF) and crude fiber were determined after cold acetone extraction of seed, as described by Van Soest and Robertson (1980). This was done because of the effect of the high fat content of the seed on the results of the detergent fiber analyses. Cold acetone extraction decreased ADF from 44.3 to 38.9%, NDF from 54.0 to 47.3% and crude fiber from 31.4 to 29.5%.

The website for Dairy One includes a feed composition library for samples analyzed in their forage laboratory (Dairy One, 2004). Their data for whole cottonseed in Table 1are for samples analyzed during the period 5/01/2000 thru 4/30/2004. The standard deviations for all nutrients are much larger than those reported by Calhoun et al. (1995) for the same constituents, which is consistent with the greater variability for cottonseed samples analyzed by Dairy One previously mentioned in this report. With the exception of a few nutrients there is not a lot of difference between values reported by the three sources. Crude protein and fat are higher for Dairy One than for Calhoun and NRC. Neutral detergent fiber is lower for Calhoun than for Dairy One and NRC, probably reflecting the use of cold acetone extraction by Calhoun.

Each year, in September, the USDA Agricultural Marketing Service-Cotton Program publishes a list of cotton varieties planted, along with an estimate of the percentage of each variety planted in each state. In addition, seed data (oil, nitrogen, gossypol) have been included in National Cotton Variety Test (NCVT) publications since 1977. Consequently, there are considerable data for these seed constituents for many cotton varieties. Unfortunately, many of the commercially important cotton varieties are not included in these tests. For those that are, about one year is required to analyze and report the data, which means the seed most likely were used before the information was available. The result is that cottonseed with markedly different physical characteristics and/or chemical composition could be used without livestock feeders or processors being aware of these changes.

Percentages of acres planted to commercially important Upland cotton varieties in 2001, that were included in the 2001 National Cotton Variety Tests (NCVT, 2001), number of test locations for each variety, and variation in oil and crude protein content of seed are presented in Table 2. The total number of varieties listed in Cotton Varieties Planted in the United States in 2001 was 166 (USDA AMS, 2001). Fifty-one varieties were listed in the NCVT publication for 2001, and of these 30 were raised commercially. Only varieties with greater than 0.05 percent of total acres planted are included in Table 2 (24 varieties). These represent 54.1% of cotton acreage in 2001, and provides a cross section of the major brands planted (Paymaster, 37.1%; Deltapine, 30.7%; Stoneville, 12.1%; Sure-Grow, 7.8% and FiberMax, 4.5% of U.S. cotton acreage). Transgenic varieties, genetically engineered varieties resistant to worms, herbicides or both, accounted for about 78% of the Upland cotton planted in the United States in 2001. Eleven transgenic varieties are included in Table 2. These are designated by the suffixes B , for Boll Guard, and R , for Roundup Ready.

Samples of Upland seed were submitted from every cotton-growing region in the United States except Arizona and California. Four varieties, designated as National Standards, were grown at all locations where Upland varieties were tested. In 2001, seed data were available from

22 locations for the National Standards (Acala Maxxa, All Tex Atlas, Deltapine NU 33B and Sure-Grow 747. In the High Quality Region, which includes eight locations and covers eight states across the cottonbelt from the east coast to Texas, the same eighteen varieties were grown at all locations. However, only the four National Standards were planted commercially.

It is obvious from examination of Table 2 that there is as much variation within a variety as there is between varieties for oil and crude protein. Much of this appears to be associated with the location where the cotton is grown. Regardless, there are significant differences between varieties and between locations. Averaged across all locations the percentages of oil in the National Standards were: 19.9^{c}, $21.0^{b,}$ 21.1^{b} and 22.9^{a} for Sure-Grow 747, Deltapine Nu 33B, Acala Maxxa, and All Tex Atlas, respectively (averages without a common superscript are significantly different at $P < 0.05$). Crude protein percentages for the National Standards were: 21.6^{c}, 22.4^{b}, 23.0^{b} and 25.8^{a} for Deltapine Nu 33B, Sure-Grow 747, All Tex Atlas and Acala Maxxa, respectively. Averaged across the four National Standard varieties there were significant location differences for oil and crude protein, but there was not a clear geographical pattern to these differences. However, oil content was lowest at Bossier City, LA (18.8%) and highest for Artesia, NM (23.7%), and crude protein content was lowest at Tunica, MS (19.8%) and highest at Lubbock, TX (26.9%). There were significant differences between varieties and between locations for the High Quality Region, but the only differences of commercial importance were the ones already discussed for the four National Standards.

Oil and crude protein values for seed from the transgenic cottons were similar to values for conventional cottons. There are no obvious differences; however, the varieties necessary to make direct comparisons, i.e., Deltapine 451 vs Deltapine 451 BR planted at the same location(s), were not included in the tests. There were several locations in the southwestern states where cotton was irrigated and comparison with the same varieties grown without irrigation was possible. Irrigation increased the average oil content of the four National Standards from 20.2 to 22.6%, but crude protein content was unchanged.

Gossypol Content of Upland Cottonseed

Total and free gossypol determined by the Official Methods of AOCS (AOCS, 1985a,b) are essentially the same in recently harvested and properly stored whole cottonseed (Robinson et al., 2001). Although total gossypol in cottonseed, determined spectrophotometrically by the official method of AOCS, is highly correlated with total gossypol determined by high performance liquid chromatography (HPLC) (Hron et al., 1999), the HPLC procedure gives values that are slightly lower than the official method (Table 3). However, poor agreement between laboratories is a serious problem, even when laboratories use the official methods of AOCS for determination of gossypol. The problem can be much worse when a laboratory uses a high performance liquid chromatography (HPLC) procedure that does not include a complexing reagent, such as an amino propanol, in the solvent used for extracting gossypol. For example, the procedure used by Woodson-Tenent Laboratories from 1988 to 2000 to analyze gossypol in cottonseed for the NCVT involved direct injection into the HPLC of an aqueous acetone (30% water and 70% acetone) extract of ground cottonseed meats. In 2001, Woodson-Tenent Laboratories switched to the HPLC procedure of Hron et al. (1999), which uses 2-amino propanol in the complexing reagent for determination of gossypol, and gossypol levels in seed essentially doubled compared with values reported by NCVT in recent years (NCVT, 1998; 1999). An additional advantage is that complexing gossypol with 2-amino propanol allows separation of (+)- and (–)-gossypol by HPLC. Thus, starting with 2001, levels of both (+)- and (–)-gossypol are reported for seed of cotton varieties in the NCVT (NCVT, 2001).

Variation in total gossypol content, and minus gossypol, expressed as a percentage of total gossypol, in meats of seed of commercially important Upland cotton varieties, that were included in the 2001 NCVT, are presented in Table 3. There is much more variation among and within varieties for total gossypol then there was for either oil or protein in seed. As a group the FiberMax varieties are consistently low and the Stoneville varieties consistently high compared to the other major brands of cotton. Deltapine brand varieties and Paymaster brand varieties tend to be intermediate between FiberMax and Stoneville, but individual varieties within these brands were quite variable The four Sure-Grow varieties cover the spectrum from low to high. There is much less variation in the proportion of the minus isomer of gossypol. An excess of the minus isomer is a characteristic of Pima cottons that are grown commercially in the United States (Percy et al., 1996) and suggests *Gossypium barbadense* genetics may have been used in the development of FiberMax 958.

There were significant differences among varieties and among locations for total gossypol and the proportion of the minus isomer of gossypol. Averaged across all locations, the percentages of total gossypol in meats, for the National Standards were: 1.05^{d}, 1.25^{c}, 1.32^{b} and 1.69^{a} for Acala Maxxa, Sure-Grow 747, All Tex Atlas and Deltapine Nu 33B, respectively. Values for minus gossypol, expressed as a percentage of total gossypol, were: 37.0^{d}, 41.2^{c}, 42.7^{b}

and 44.5[a] for Acala Maxxa, Deltapine Nu 33B, All Tex Atlas and Sure-Grow 747, respectively. Averaged across the four National Standard varieties there were significant location differences for total gossypol. Tipton, OK had the lowest average total gossypol (0.98%) and University City, NM had the highest (1.62%). Test locations in the southeast, central and high plains areas of Texas had consistently low total gossypol values. Far west Texas and New Mexico locations had consistently high total gossypol values. The National Standards grown in the Mississippi Delta and along the east coast tended to have high seed gossypol. The proportion of the minus isomer was not correlated with total gossypol, and there were no obvious geographical patterns.

In order to express gossypol values on a whole seed basis it is necessary to know the percentage of meats in the seed. This was not determined by Woodson-Tenent Laboratories; however, we routinely determine the percentage of meats in samples of whole Upland seed received in the TAES Nutrition and Toxicology Laboratory in San Angelo. Over a period of about 10 years, these have averaged 51.7 ± 0.48% and ranged from 48.1 to 57.3%. Using the average, total gossypol levels in whole seed can be estimated by multiplying the values for total gossypol in meats in Table 3 by 0.517. With this calculation, total gossypol in whole seed ranged from 0.52 to 1.01% for the 24 varieties in Table 3. For comparison purposes the 83 samples of whole Upland seed in Table 1 averaged 0.66 ± 0.01% total gossypol, the (–) isomer of gossypol was 38.8 ± 0.26% of total gossypol (Calhoun et al., 1995).

Nutrient Composition of Pima Cottonseed

With the exception of the values for oil, nitrogen and gossypol reported yearly since 1977 in the NCVT publications, little information is available for Pima seed. However, two recent studies were conducted to provide this information (DePeters et al., 2000; Robinson et al., 2001). The percentages of crude protein and fat reported by DePeters et al. (2000), for 10 samples of Pima seed collected in 1998 from one location in California, and by Robinson et al. (2001), for 26 samples of Pima seed collected during the fall of 1999 at 10 cotton gins located in the southwest USA, were much higher than for values reported for Pima seed in the 1998 and 1999 NCVT publications (NCVT, 1998, 1999). In contrast, free gossypol levels reported by both DePeters et al. (2000) and Robinson et al. (2001) were much higher than those reported by NCVT.

In order to re-evaluate the nutrient composition of Pima seed, 50 samples that were originally submitted to my laboratory for gossypol analysis by consulting nutritionists, and veterinarians working with commercial dairies in California, New Mexico and Texas; animal scientists at universities and cotton breeders were analyzed. Crude protein and crude fat were determined for these samples by Mid-Continent. All other nutrients were determined by Dairy One. Acid detergent insoluble crude protein (ADICP), neutral detergent insoluble crude protein (NDICP), ADF and NDF were determined after cold acetone extraction of ground Pima seed, as described by Van Soest and Robertson (1980). All minerals were determined in duplicate. The results of these analyses are presented in Table 4. Information from the study of Robinson et al. (2001) are included in Table 4 for comparison purposes.

Crude protein and crude fat were higher and more variable in the Pima seed analyzed by Robinson et al. (2001). Acid detergent fiber and NDF values, after cold acetone extraction (TAES), were similar for both sets of samples; however, the ranges were much greater for ADF and NDF in Robinson's samples. Acid detergent fiber and NDF in the TAES samples were decreased 13.1% and 19.6%, respectively by cold acetone extraction. It is not know if Robinson's samples were extracted with acetone prior to fiber analysis. Values for the various minerals in both sets agree fairly well. Probably, the only important difference is for phosphorus. The average value reported by Robinson et al. (2001) is 43.0% higher than the TAES value (1.03 vs 0.72%).

Table 5 gives the percentages of acres planted to six commercially important Pima cotton varieties in 2001, that were included in the 2001 NCVT, at three locations (El Paso, TX, Las Cruces, NM and Maricopa, AZ). The oil and crude protein content of seed summarized by variety and location are also presented. Values actually reported in the NCVT are percentages of oil and nitrogen in whole seed, on an as received moisture basis. These were converted to a 100% DM basis by dividing by 0.93 and nitrogen was converted to crude protein by multiplying by 6.25. Oil and nitrogen were determined for the NCVT program by Woodson-Tenent Laboratories, Inc. (Little Rock, AR) using the official methods of AOCS. The six varieties tested accounted for 89.9% of Pima acreage in 2001. Variation among varieties was fairly modest for both oil and protein. This was not unexpected because many of the varieties currently being grown are selections from Pima S-6 and S-7. Oil content of the seed was similar across growing locations, but there was a significant location effect for crude protein. Seed from cotton grown in Maricopa contained 35.8% more protein than seed from the same varieties grown in El Paso, and 11.0% more than seed from cottons grown in Las Cruces.

The oil and protein analysis done by Woodson-Tenent Laboratories, Inc for the NCVT was evaluated by submitting seed of three varieties grown at three locations to Mid-Continent Laboratories. Using a paired t-test the percentage of oil reported by NCVT was higher ($P < 0.01$) than Mid-Continent Laboratories (24.3 vs 23.5%), but the absolute difference was fairly small (0.8%). Protein values were not significantly different. Considering that separate sub-samples were analyzed by the two laboratories, this certainly is acceptable agreement.

Gossypol Content of Pima Cottonseed

Total gossypol, and (–)-gossypol, expressed as a percentage of total gossypol, in meats of seed of commercially important Pima cotton varieties grown at three locations in the 2001 NCVT, are summarized in Table 6. Since the procedure involves drying the cottonseed meats at 180° F for four hours prior to determining gossypol, the gossypol values reported by Woodson-Tenent Laboratories are assumed to be on a 100% DM basis. The range of values for total gossypol for the six varieties was 0.95 to 1.58% of meats DM, and (–)-gossypol, as a percentage of total gossypol, ranged from 47.9 to 55.6%. Varietal differences were fairly modest, and most of the variation appeared to be associated with the location where the cotton was grown. Total gossypol was lower ($P < 0.05$) at Maricopa (1.02%) compared with El Paso (1.39%) and Las Cruces (1.35%). In order to express gossypol values on a whole seed basis it is necessary to know the percentage of meats in the seed. This was not determined by Woodson-Tenent Laboratories; however, the percentage of meats was determined by TAES for the 50 seed samples reported in Table 5 and averaged 63.3 ± 0.22%. Using this figure, total gossypol levels in whole seed can be estimated by multiplying the values for total gossypol in Table 6 by 0.633.

Based on this calculation, total gossypol on a whole seed basis ranged from 0.60 to 1.00%. For comparison purposes the 50 samples of Pima seed in Table 5 averaged 0.93 $\pm$ 0.016% total gossypol and ranged from 0.70 to 1.24%. The (–) isomer of gossypol was 52.2 $\pm$ 0.19% and ranged from 49.2 to 55.3%.

Nutrient Composition of Cottonseed Meal

Table 7 presents information on the nutrient composition of cottonseed meals from three sources (Calhoun et al., 1995; NRC, 2001; Waldroup and Kersey, 2002). The first two sources, which are labeled NCPA 1995 and NCPA 2002, respectively, present results from two studies sponsored by NCPA. The data listed under NCPA 1995 are for 66 samples of expander solvent cottonseed meals collected from 25 cottonseed oil mills in the United States during the 1993-1994 processing year (Calhoun et al., 1995). The data listed under NCPA 2002 are for samples of expander solvent cottonseed meals, produced during the 2000 crop year, by 14 cottonseed oil mills in the United States. Representative samples were obtained from outgoing shipments of cottonseed meal over a two-month period during peak processing times (Waldroup and Kersey, 2002). The data listed under NRC 2001 is for cottonseed meal, solvent, 41% crude protein (International Feed No. 5-01-630) from the 7th Revised Edition of Nutrient Requirements of Dairy Cattle (NRC, 2001).

The details on the procedures used for sample collection and analysis are described in the respective sources. The majority of the cottonseed oil mills in the United States participated in the collection of samples for the two NCPA studies. These are considered the best sources of information for the nutrient composition of cottonseed meals being produced in the United

States. According to NRC (2001), their information on the nutrient composition of feeds was compiled from commercial laboratories, literature data, and unpublished data provided by university researchers. The NRC data is widely used by nutritionists, and was included in Table 7 to facilitate comparison with the results of the NCPA studies.

During the last 15 years there have been significant changes in the processing and utilization of cottonseed. The number of conventional oil mills extracting oil from cottonseed decreased from 50 in 1990 to less than 14 today (NCPA, 2004), and the percentage of cottonseed crushed for oil decreased from 63 to less than 45. Almost all of the cottonseed meals currently being produced in the United States use expanders in the direct solvent process; therefore, only data for expander solvent cottonseed meals are presented in Table 7. However, information from a small number of mechanically (expeller) processed cottonseeds meals is contained in the original sources (Calhoun et al., 1995; Waldroup and Kersey, 2002). Expanders were incorporated into the direct solvent process to facilitate oil extraction, and to bind free gossypol.

There are not a lot of differences in the data for individual nutrients reported for the three sources. Crude protein was higher for NCPA (1995) than for NCPA (2002) and NRC (2001). ADF and NDF were lower in the NCPA (1995) samples of cottonseed meal compared with the NRC data. There is some variation in values reported for sodium and iron but these most likely reflect variable amounts contributed from the manufacturing process.

Gossypol Content of Expander Solvent Cottonseed Meal

Gossypol values for 41 expander solvent cottonseed meals produced by 13 oil mills during the period 2001-2003 are summarized in Table 8. A separate summary is given for each oil mill.

Overall, AOCS free gossypol averaged 0.135% of DM for the complete set, with a standard deviation of 0.058%. The range was 0.060 to 0.324%. In 1995, Calhoun et al., reported very similar free gossypol values for 66 expander solvent cottonseed meals (0.14% with a SD of 0.04%). AOCS Total gossypol for the 41 samples averaged 1.383% with a SD of 0.209%. These values are slightly higher than those reported previously by Calhoun et al.(1995) for expander solvent cottonseed meals (1.16% with a SD of 0.14%). The average for minus gossypol, expressed as a percentage of total gossypol, was slightly higher for the most recent set (42.8 vs 41.7%), but this small difference was not significant.

Although not statistically analyzed, because of the small number of samples from most plants, it is obvious there are important differences between oil mills in the free gossypol content of the expander solvent cottonseed meals they produce. There is much less variation within plants than between plants. Undoubtedly these differences reflect the overall process used for oil extraction in the different oil mills, including the amount of soapstock added back to the meal prior to the desolventizer-toaster. It is also likely the gossypol content of the cottonseed being processed contributes to variation in free gossypol in the meal.

Discussion and Summary

Prior to 1980 almost all cottonseed was processed by the oil mills, and little was fed directly to livestock. Since then, the amount fed as whole seed, primarily to cattle, has increased from 15% in 1980 to about 55% in 2003. This has been accompanied by a corresponding decrease in the amount of cottonseed crushed by the oil mills from about 80% in 1980 to about 40% in 2003, and a decrease in the number of oil mills from 74 in 1980 to 14 in 2002 (NCPA, 2004). The cottonseed processing industry established trading rules that enabled pricing cottonseed based on defined quality and quantity factors (NCPA, 2000). Official chemists licensed by the U.S. Department of Agriculture issued grade certificates based on the analyses of these quality (% foreign matter and % moisture, on an as received whole seed basis, and free fatty acids, expressed as a percentage of the oil) and quantity (% oil and % ammonia) factors, that were used by the oil mills in trading cottonseed. Cottonseed grade certificates were sent to the USDA Agricultural Marketing Service - Cotton Division in Memphis, TN and an annual report was issued summarizing the quality of cottonseed by quality factors for each state and the United States (USDA AMS, 1998). The 1980 report contained information for 38,224 seed samples. The number of grade certificates issued each year decreased as the number of oil mills decreased, reaching 4,996 in 1998. At that time, the reports ceased because of lack of interest. The explanation appears to be competition for seed from the dairy industry, which does not appear overly concerned about cottonseed quality.

There was very little change in the yearly averages for oil and ammonia (protein) in cottonseed from 1980 to 1998. Although this is the case, it is important to keep in mind that considerable variation exists among varieties in cotton seed composition, that commercial varieties are continually changing, and that location where the cotton is grown also affects composition. Because of the difficulty in preparing a representative sample for analysis and the need for reliable oil (energy) and protein values for cottonseed, it is recommended that cottonseed be submitted to an AOCS certified laboratory for “Feed Grade Cottonseed Analysis”. The cost is around $20.00/sample and includes determination of % foreign matter, % moisture,

% oil and % crude protein, on an as received whole seed basis, and free fatty acids, expressed as a percentage of the oil.

Gossypol, a toxic polyphenolic binaphthyl dialdehyde, occurs throughout the cotton plant, but is concentrated in pigment glands present in cottonseed (Berardi and Goldblatt, 1980). Because of restricted rotation about the bond that joins the two naphthalene groups of the molecule, gossypol exists naturally as a mixture of two sterioisomers, (+)- and (–)-gossypol. The minus isomer appears to have the greatest biological activity and is the isomer responsible for infertility in males (Matlin et al., 1985). High levels of terpenoid aldehydes, like gossypol, as well as a number of other secondary plant metabolites in the vegetative parts of the cotton plant are desirable because of the protection provided against a number of plant pests.; whereas, gossypol in seed is undesirable, because of its toxicity to animals (Bell, 1986). Considerable progress has been made toward eliminating gossypol from seed, while at the same time maintaining or increasing gossypol levels in the rest of the plant (Benedict, 2002); however, we are several years away from commercial varieties without gossypol in seed. In the meantime we are confronted with a number of varieties that have very high levels of gossypol in the seed, that are being grown commercially. It is important to know where these are being grown, and to check gossypol levels in seed. The recommended analysis is total gossypol by the AOCS official method. The major problem with deciding on a laboratory to determine gossypol is that it is not included in the laboratory proficiency program for cottonseed analysis. Since 1993, my laboratory has been a reference laboratory for gossypol analysis, and we have analyzed several thousand cottonseed samples.

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Table 1. Nutrient values for whole, linted seed of Upland cottons (*Gossypium hirsutum*).[1]

	Calhoun et al.(1995)[1]			Dairy One[2]			2001 Dairy NRC[3]		
Item	N	Mean	SD[4]	N	Mean	SD	N	Mean	SD
Dry matter, %	83	91.6	0.89	1244	91.3	2.31	1059	90.1	4.6
Crude protein, %	83	23.0	0.98	1053	24.8	4.79	1124	23.5	2.6
Sol. protein, % CP				480	26.8	11.38			
Degrad. prot. % CP				59	47.4	8.09			
ADICP, %				170	2.0	0.72	4	1.9	0.1
NDICP, %				151	2.6	0.99	71	2.4	1.2
ADF, %	83	38.9	3.59	962	39.4	8.60	1024	40.1	4.4
NDF, %	83	47.3	3.54	968	51.2	9.46	953	50.3	5.8
Crude fiber, %	83	29.5	2.07	109	25.1	7.70			
Crude fat, %	83	20.1	0.95	545	21.2	4.66	27	19.3	1.4
Ash, %	83	3.8	0.23	287	4.3	0.67	193	4.2	2.1
Calcium, %	83	0.14	0.016	723	0.20	0.14	928	0.17	0.08
Phosphorus, %	83	0.56	0.055	723	0.74	0.19	928	0.60	0.08
Magnesium, %	83	0.35	0.020	722	0.39	0.08	928	0.37	0.04
Potassium, %	83	1.14	0.067	722	1.20	0.13	928	1.13	0.07
Sodium, %	83	0.008	0.007	722	0.02	0.01	928	0.02	0.02
Chloride, %				149	0.09	0.11	148	0.06	0.03
Sulfur, %	83	0.20	0.023	481	0.25	0.07	424	0.23	0.04
Copper, ppm	83	7	1.3	720	6.8	3.66	928	7	3
Iron, ppm	83	50	11.5	720	82	155.5	928	94	185
Manganese, ppm	83	15	2.2	720	16.1	11.28	928	18	13
Zinc, ppm	83	33	3.5	720	35.8	15.48	928	37	18
Molybdenum,									

ppm	83	1.6	0.52	720	0.57	0.50	919	1.3	0.6

[1] Values are on a 100% dry matter basis.
[2] Information accessed at http://www.dairyone.com on August 10, 2004.
[3] National Research Council, Nutrient Requirements of Dairy Cattle 6th Revised Edition, 2001.
[4] Standard deviation.

Table 2. Percentages of acres planted to commercially important Upland cotton varieties in 2001, that were included in the 2001 National Cotton Variety Tests (NCVT, 2001), number of test locations for each variety, and variation in oil and crude protein content of seed.[1]

	Acres[2]		Oil, % DM			Crude protein, % DM		
Variety	%	N	Mean	SD[3]	Range	Mean	SD[3]	Range
Acala Maxxa	0.75	22	21.1	0.84	16.8 - 22.5	25.8	2.20	21.8 - 28.8
All Tex Atlas	0.98	22	22.3	1.72	18.9 - 25.7	23.0	1.76	20.5 - 27.4
Deltapine 50	0.37	6	21.6	0.51	20.8 - 22.0	22.6	1.59	20.0 - 23.6
Deltapine 451 BR	6.39	8	21.6	0.82	20.4 - 22.7	19.7	1.31	17.7 - 21.2
Deltapine 2156	0.21	8	22.8	2.20	21.3 - 26.1	24.4	0.21	24.2 - 24.6
Deltapine 5415 R	2.47	3	18.3	3.65	15.0 - 22.2	19.4	1.37	18.2 - 20.9
Deltapine Nu 33 B	1.66	24	21.0	1.42	19.0 - 24.3	21.6	2.11	19.0 - 22.8
Fibermax 832	2.73	4	21.7	0.38	21.2 - 22.0	23.7	1.43	22.0 - 25.1
Fibermax 958	0.45	4	22.2	1.78	20.6 - 23.8	21.1	1.17	20.8 - 22.7
Fibermax 966	0.24	8	23.3	1.07	21.7 - 24.8	21.7	1.05	20.6 - 23.3
Fibermax 989	0.88	3	23.2	1.24	22.3 - 24.6	20.9	2.82	18.1 - 23.7
Paymaster 1218 BR	10.72	4	22.2	0.97	21.0 - 23.2	21.8	0.78	20.7 - 22.6
Paymaster 1560 B	0.16	8	20.3	1.06	19.0 - 21.7	22.6	2.39	19.7 - 25.6
Paymaster 2145 R	0.81	4	22.6	0.96	21.6 - 23.9	25.8	1.53	23.9 - 27.6
Paymaster 2326 R	11.44	4	21.2	2.49	18.9 - 24.4	24.5	1.34	23.4 - 26.0
Phytogen PSC 355	0.77	8	22.8	0.95	21.4 - 23.9	22.2	1.21	19.7 - 23.8
Sure-Grow 105	0.18	4	21.3	0.34	20.9 - 21.7	20.3	0.99	19.1 - 21.5
Sure-Grow 125	0.43	4	18.9	1.61	17.8 - 21.3	22.8	1.40	21.6 - 24.9
Sure-Grow 501 BR	0.06	4	20.4	0.94	19.3 - 21.6	21.9	1.32	19.9 - 22.9
Sure-Grow 747	0.60	21	19.9	1.62	16.8 - 23.6	22.4	2.05	19.2 - 27.8

Stoneville BXN 47	3.27	4	21.3	0.34	20.9 - 21.7	20.3	0.99	19.1 - 21.5
Stoneville 474	0.78	8	20.4	0.58	19.5 - 21.5	23.9	2.16	20.9 - 27.2
Stoneville 4793 R	1.20	4	20.5	0.87	19.2 - 21.1	22.0	1.38	21.2 - 24.1
Stoneville 4892 BR	5.75	8	21.1	0.86	20.0 - 22.6	21.2	1.57	19.2 - 24.5

[1] Cotton Varieties Planted 2001 Crop, USDA AMS, Cotton Program, Memphis, TN.
[2] Percentage of U.S. cotton acreage planted to these varieties in 2001.
[3] Standard deviation.

Table 3. Variation in total gossypol (TG), and minus gossypol (as a percentage of TG) in meats of seed of commercial Upland cotton varieties in the 2001 National Cotton Variety Tests (NCVT, 2001).[1, 2]

	TG, % of meats DM			Minus gossypol, % of TG		
Variety	Mean	SD	Range	Mean	SD	Range
Acala Maxxa	1.05	0.15	0.78 - 1.27	37.0	1.84	33.9 - 40.7
All Tex Atlas	1.32	0.20	0.95 - 1.70	42.7	1.75	39.6 - 45.8
Deltapine 50	1.27	0.18	1.05 - 1.55	35.2	2.43	33.3 - 40.0
Deltapine 451 BR	1.57	0.13	1.33 - 1.69	39.0	1.19	38.0 - 41.6
Deltapine 2156	1.04	0.15	0.91 - 1.25	42.6	1.72	40.6 - 44.1
Deltapine 5415 R	1.30	0.15	1.14 - 1.42	45.5	2.24	43.0 - 47.4
Deltapine Nu 33 B	1.39	0.21	1.13 - 1.96	41.2	2.02	37.1 - 45.0
Fibermax 832	1.01	0.14	0.81 - 1.15	42.1	3.31	37.4 - 45.1
Fibermax 958	1.13	0.11	1.00 - 1.26	51.0	1.93	49.1 - 53.5
Fibermax 966	1.13	0.11	0.90 - 1.22	47.4	1.23	46.7 - 48.6
Fibermax 989	1.16	0.12	1.03 - 1.25	44.2	2.15	42.7 - 46.7
Paymaster 1218 BR	1.27	0.08	1.16 - 1.33	39.3	1.14	38.3 - 40.8
Paymaster 1560 B	1.28	0.29	0.90 - 1.68	42.4	1.65	39.8 - 44.8
Paymaster 2145 R	1.09	0.21	0.86 - 1.37	41.0	2.35	38.4 - 43.1
Paymaster 2326 R	1.11	0.27	0.88 - 1.49	42.6	2.93	38.6 - 45.0
Phytogen PSC 355	1.59	0.16	1.31 - 1.84	39.3	1.29	37.4 - 41.7
Sure-Grow 105	1.90	0.17	1.74 - 2.12	41.9	1.70	39.4 - 43.1
Sure-Grow 125	1.09	0.14	0.92 - 1.25	42.3	1.36	41.3 - 44.2
Sure-Grow 501 BR	1.53	0.07	1.45 - 1.59	42.3	2.04	40.3 - 45.0
Sure-Grow 747	1.25	0.22	0.82 - 1.70	44.5	1.61	41.8 - 46.9
Stoneville BXN 47	1.95	0.26	1.62 - 2.25	39.9	1.02	38.4 - 40.7
Stoneville 474	1.55	0.19	1.30 - 1.88	39.7	2.32	37.2 - 42.3
Stoneville 4793 R	1.71	0.25	1.33 - 1.86	41.2	1.27	39.8 - 42.9

Stoneville 4892 BR	1.76	0.16	1.59 - 2.12	41.2	1.21	41.0 – 42.8

[1] Values in the NCVT were for decorticated cottonseed (meats), on a DM basis.

Table 4. Nutrient composition of Pima seed submitted to the Texas Agricultural Experiment Station's Nutrition and Toxicology Laboratory at San Angelo, TX, compared with values reported by Robinson et al. (2001) for Pima seed.[1]

	TAES[2]				Robinson et al., 2001			
Item	N	Mean	SD	Range	N	Mean	SD	Range
Dry matter, %	50	92.7	0.39	91.8 – 93.5	29	93.3	0.65	92.1- 94.3
Crude protein, %	50	24.6	1.41	20.5 - 26.7	29	29.1	3.38	19.8 - 34.5
Sol. protein, % of CP	40	22.9	4.58	11.0 - 32.0	29	26.2	3.83	14.0 - 32.0
Crude fat, %	50	24.1	1.42	20.6 - 27.6	29	27.1	3.96	20.0 - 34.0
ADICP, % of CP	21	2.1	0.33	1.8 - 2.9				
NDICP, % of CP	21	2.1	0.25	1.8 - 2.9				
ADF, %	21	31.8	1.63	28.9 - 34.4	29	31.3	6.38	20.0 - 44.5
NDF, %	21	41.1	1.56	37.8 - 43.6	29	44.4	7.55	36.6 - 58.1
Lignin, %	50	17.2	2.24	12.4 - 25.0				
Ash, %[2]	40	4.4	0.23	3.9 - 4.9				
Calcium, %[5]	50	0.24	0.03	0.19 - 0.30	29	0.19	0.02	0.16 - 0.23
Phosphorus, %	50	0.72	0.08	0.53 - 0.91	29	1.03	0.13	0.88 -1.26
Magnesium, %	50	0.37	0.02	0.32 - 0.42	29	0.44	0.04	0.40 - 0.50
Potassium, %	50	1.35	0.08	1.11 - 1.50	29	1.29	0.08	1.19 - 1.38
Sodium, %	50	0.02	0.01	0.00 - 0.04	29	0.01	0.01	0.01 - 0.01
Chloride, %	50	0.09	0.04	0.02 - 0.17	29	0.09	0.03	0.02 - 0.19
Sulfur, %	50	0.25	0.04	0.17 - 0.39	29	0.31	0.04	0.21 - 0.46
Copper, ppm	50	9	1.5	7 - 15	29	9	1.37	7 - 12
Iron, ppm	50	45	9.0	29 - 68	29	55	19.36	35 - 130
Manganese, ppm	50	12	1.2	10 - 15	29	14	1.34	12 - 17
Zinc, ppm	50	34	5.4	25 - 56	29	41	6.23	31 - 52
Molybdenum, ppm	13	1.1	0.15	1.00 -1.40	29	1.2	0.55	1.0 - 2.3

[1] Nutrient values are on a 100% dry matter basis.
[2] Crude protein and crude fat in TAES samples were determined by Mid-Continent Laboratories, Inc., Jackson, MS. All other nutrients in TAES samples were determined by Dairy One, Ithaca, NY. Acid detergent insoluble crude protein (ADICP), neutral detergent insoluble crude protein (NDICP), acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined after cold acetone extraction of ground Pima seed.

Table 5. Percentages of acres planted to commercially important Pima cotton varieties in 2001, that were included in the 2001 National Cotton Variety Tests (NCVT), and variation in oil and crude protein content of seed grown at three locations[1, 2]

	Acreage[3]	Oil, % DM			Crude protein, % DM		
Item	%	Mean	SD[4]	Range	Mean	SD	Range
Variety							
Deltapine 744	4.49	25.6	0.47	25.3 - 26.3	23.9	3.33	20.6 - 27.2
Phytogen 57	24.12	25.9	0.45	25.4 - 26.2	24.5	4.58	19.4 - 28.3
Phytogen 76	23.62	23.8	1.04	22.6 - 24.6	23.7	3.33	20.1 - 26.7
OA 325 (DP-HTO)	12.56	25.7	0.28	25.5 - 26.0	24.7	2.36	22.2 - 26.9
OA 340	8.15	25.7	0.65	25.1 - 26.4	24.1	3.83	20.2 - 27.8
Pima S-7	16.98	25.2	0.57	24.6 - 25.6	23.7	3.28	20.2 - 26.6
Location							
El Paso		25.5	0.91	24.2 - 26.4	20.4[a]	0.93	19.4 - 22.2
Las Cruces		25.4	0.46	25.3 - 26.0	24.6[b]	0.68	24.0 - 25.8
Maricopa		25.1	0.90	22.6 - 26.2	27.3[c]	0.68	26.6 - 28.3

[1] 2001 National Cotton Variety Test. USDA ARS, Stoneville, MS.
[2] Values in the NCVT were oil and nitrogen in whole seed, as received DM basis. These were converted to a 100% DM basis by dividing by 0.93. Nitrogen was converted to crude protein by multiplying by 6.25.
[3] Cotton Varieties Planted 2001 Crop, USDA AMS, Cotton Program, Memphis, TN, August 2001.
[4] Standard deviation.

Table 6. Variation in total gossypol (TG), and minus gossypol, expressed as a percentage of total gossypol, in meats of seed of commercially important Pima cotton varieties grown at three locations in 2001, and included in the 2001 National Cotton Variety Tests (NCVT).[1]

	TG, % of meats DM[2]			Minus gossypol, % of TG		
Item	Mean	SD[3]	Range	Mean	SD	Range
Variety						
Deltapine 744	1.35	0.27	1.06 - 1.58	51.7	0.62	50.9 - 52.1
Phytogen 57	1.20	0.22	0.95 - 1.35	52.8	0.52	52.3 - 53.3
Phytogen 76	1.28	0.23	1.02 - 1.42	49.9	1.95	47.9 - 51.8
OA 325 (DP-HTO)	1.18	0.14	1.02 - 1.28	52.1	1.83	50.0 - 53.2
OA 340	1.22	0.15	1.06 - 1.35	54.8	1.38	53.2 - 55.6
Pima S-7	1.28	0.23	1.02 - 1.47	52.4	1.35	51.5 - 53.9
Location						
El Paso	1.39[b]	0.10	1.28 - 1.58	52.2	2.55	47.9 - 55.6
Las Cruces	1.35[b]	0.10	1.24 - 1.47	52.4	0.68	51.7 - 53.2
Maracopa	1.02[a]	0.04	0.95 - 1.06	52.2	2.30	50.0 - 55.7

[1] 2001 National Cotton Variety Test. USDA ARS, Stoneville, MS.
[2] Values in the NCVT were for decorticated cottonseed (meats), on a DM basis. The percentage of meats in whole seed was not determined by NCVT.
[3] Standard deviation.
[a,b] Means without a common superscript are different ($P < 0.05$).

Table 7. Nutrient composition of cottonseed meal.[1]

	NCPA 1995[2]			NCPA 2002[3]			NRC 2001[4]		
Item	N	Mean	SD[5]	N	Mean	SD	N	Mean	SD
Dry matter, %	66	89.1	0.93	14	91.6	0.71	180	90.5	1.90
Crude protein,	66	47.6	1.99	14	43.8	0.84	158	44.9	4.1
ADICP							8	1.8	0.5
NDICP							7	3.3	0.9
Crude fiber, %	66	11.2	1.47	14	11.7	1.98			
ADF, %	66	17.3	2.70				58	19.9	5.4
NDF, %	66	24.5	3.61				47	30.8	9.0
Crude fat, %	66	2.2	0.89	14	2.9	1.61	113	1.9	2.2
Ash, %	66	7.5	1.00				44	6.7	0.7
Calcium %	66	0.22	0.03	14	0.21	0.02	185	0.20	0.1
Phosphorus, %	66	1.20	0.14	14	1.10	0.07	185	1.15	0.1
Magnesium, %	66	0.66	0.06	14	0.64	0.03	65	0.61	0.11
Potassium, %	66	1.72	0.11	14	1.56	0.07	185	1.64	0.38
Sodium, %	66	0.14	0.09	14	0.16	0.10	97	0.07	0.06
Chloride, %							3	0.07	
Sulfur, %	66	0.44	0.03	14	0.41	0.02	30	0.40	0.11
Copper, ppm	66	12	2	14	13	2	59	14	3
Iron, ppm	66	126	39	14	51	7	60	149	47
Manganese,	66	20	27				61	24	11
Zinc, ppm	66	64	68	14	60	8	55	67	15
Molybdenum,	66	2.5	0.8				18	3	0.8

[1] Values are on a 100% dry matter basis.
[2] Source of information (Calhoun et al., 1995).
[3] Source of information (Waldroup and Kersey, 2002).
[4] National Research Council, Nutrient Requirements of Dairy Cattle 7th Revised Edition, 2001.
[5] Standard deviation

the period 2001-2003.

pol[3]	Minus gossypol % of total gossypol	
ge	Mean	Range
1.469	51.6	50.9 -52.1
1.096	41.7	41.2 - 42.7
	44.8	
1.133	42.1	41.6 - 43.0
1.295	41.2	41.0 - 41.5
1.228	43.3	43.0 - 43.6
1.443	41.8	41.6 - 41.9
1.833	40.9	40.2 -41.8
1.302	41.4	40.8 - 41.9
1.152	41.5	38.9 - 44.6
1.240	43.4	43.4 - 43.5

1.453	42.4	42.2 - 42.9
1.080	42.7	41.8 - 43.6

ypol Ba 7-58 (AOCS, 1985a).
sypol Ba 8-78 (AOCS, 1985b).
graphy (Hron etal., 1999).