

1                   **Cottonseed Meal and Whole Cottonseed:**  
2                   **Optimizing Their Use in Dairy Cattle Rations**

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5                   **Dr. Millard C. Calhoun**

6                   **Professor Emeritus**

7                   **Texas Agricultural Experiment Station**

8                   **Texas A&M University System**

9                   **San Angelo, TX**

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## Introduction

Cottonseed and cottonseed meal are excellent feeds. Cottonseed is high in protein, energy and fiber, and is a good source of phosphorus and vitamin E. Cottonseed meal is high in protein, and is a good source of fiber and phosphorus. However, both contain gossypol, a polyphenolic binaphthyl dialdehyde that can produce toxic effects in animals. The unique physical characteristics of cottonseed, along with its nutritional value, have resulted in its being extensively used in diets for lactating dairy cattle, but concerns about gossypol have made producers reluctant to use cottonseed meal when cottonseed is being fed. The purpose of this report is to address the issue of feeding cottonseed and cottonseed meal to lactating dairy cattle, and contains information on the effects of gossypol and recommendations for safely using these cotton by-products in cattle diets. A companion paper, prepared for this conference, contains information on the nutrient and gossypol composition and variation in these components of cottonseed and cottonseed meal.

## Literature Review

Several reports cover the use of cotton by-products in diets of lactating dairy cattle (Smith et al., 1981; Coppock et al., 1987; Coppock and Wilks, 1991; Arieli, 1998). Smith et al. (1981) collected data from 55 commercial dairies that were feeding whole cottonseed. There was no apparent affect of feeding up to 2.9 kg of cottonseed dry matter per cow per day on calving interval or on the incidence of displaced abomasum, ketosis, milk fever or retained placenta. A summary of 18 feeding trials (Coppock et al., 1987) with whole cottonseed showed no significant reduction ( $P > 0.05$ ) in dry matter intake when whole cottonseed was included up to 25% of the ration. Milk yield response was variable, but usually there was a small positive effect.

1 In eight of thirteen trials, milk fat percentage was increased and in about half the trials milk  
2 protein percentages were significantly lower than the controls. In a subsequent review article,  
3 Coppock and Wilks (1991) stated that inclusion of about 15% of whole cottonseed in the diet of  
4 lactating cows often results in small increases in milk yield, milk fat percentages and decreases  
5 in milk protein percentages. This is also the level recommended by Arieli (1998).

6 The literature on feeding cotton by-products to ruminants and gossypol toxicity was  
7 extensively reviewed by Calhoun and Holmberg in 1991. The review covered cotton and its by-  
8 products, oil extraction processes, gossypol in cottonseed and meal, gossypol analysis and  
9 gossypol poisoning. At that time, it was believed that free gossypol was the toxic form of  
10 gossypol and bound gossypol was not available to ruminants and was not toxic. Consequently,  
11 recommendations for using cottonseed and cottonseed meal in animal diets were based on free  
12 gossypol (Berardi and Goldblatt, 1980; Calhoun and Holmberg, 1991). Total gossypol, which  
13 includes both free and bound gossypol was seldom determined. The recommended safe levels of  
14 free gossypol for ruminants proposed by Calhoun and Holmberg in 1991 are given in Table 1.  
15 These authors acknowledged their recommendations were conservative because of uncertainty  
16 about many of the factors known to influence gossypol toxicity.

17 More recently Rogers and Moore (1995) reported that it was safe to feed higher levels of free  
18 gossypol in the diet when the source of free gossypol was whole cottonseed instead of cottonseed  
19 meal. For example, for mature cows and bulls the recommended safe levels of free gossypol in  
20 the diet were 900 and 1,200 ppm when whole cottonseed was fed compared with 200 and 600  
21 ppm, respectively, when the source of free gossypol was cottonseed meal. The higher levels of  
22 free gossypol recommended for diets containing cottonseed reflects the fact that free gossypol in

1 unprocessed whole cottonseed is extensively bound during digestion in the rumen and is less  
2 available than free gossypol in cottonseed meal (Calhoun, 1995).

3 However, in studies with cattle and sheep, in which different sources of free gossypol  
4 (gossypol acetic acid, cottonseed and cottonseed meal processed by different methods) were fed,  
5 it was observed that plasma and tissue levels of gossypol and toxicity often were not correlated  
6 with either the free gossypol content of the feed or free gossypol intakes (Calhoun et al.,  
7 1990a,b; Calk, 1992; Calhoun and Wan, 1995; Wan et al., 1995). These studies demonstrated  
8 that free gossypol is of limited value for making recommendations about levels of cottonseed and  
9 cottonseed meal that can be fed safely to ruminants.

10 Prior to 1980, gossypol was seldom mentioned in reports about feeding cottonseed or  
11 cottonseed meal to mature cattle. It was known that young cattle, prior to the development of a  
12 fully functional rumen, were susceptible to gossypol poisoning (Calhoun and Holmberg, 1991),  
13 and that mature cattle were very tolerant; because, during digestion of cottonseed and cottonseed  
14 meal, gossypol was bound in the rumen so that it was mostly unavailable. However, Lindsey et  
15 al. (1980) demonstrated that the ability of the rumen to detoxify gossypol can be exceeded when  
16 very high intakes of free gossypol are consumed by mature cows.

### 17 **Gossypol Toxicity in Cattle.**

18 Gossypol poisoning has been described for calves (Holmberg et al., 1988; Risco et al., 1992)  
19 and mature cattle (Lindsey et al., 1980; Smalley and Bicknell, 1982). Sudden deaths in what  
20 were believed to be healthy groups of animals has been a frequent observation at the beginning  
21 of natural disease occurrences. However, serum chemistry changes that allow recognition of  
22 impending toxicological effects of gossypol ingestion prior to the development of clinical signs

1 have not been reported (Holmberg et al., 1988; Morgan et al., 1988; Risco et al., 1992). Gross  
2 lesions after death are primarily related to the effects of cardiovascular failure. The most  
3 common and prominent change is increased fluid in the abdominal and thoracic cavities  
4 (Holmberg et al., 1988; Calhoun and Holmberg, 1991).

5 Increased fragility of erythrocytes in hypotonic saline solutions has been observed  
6 consistently in cattle (Lindsey et al., 1980; Mena, 1996; Mena, 1997) fed cottonseed or  
7 cottonseed meal. Changes in erythrocyte fragility are sensitive to gossypol intake and occur  
8 prior to signs of gossypol toxicity. The effect of gossypol on erythrocyte fragility is reversible, as  
9 values gradually return to normal (2 to 3 mo) when gossypol is eliminated from the diet (Mena et  
10 al., 2004). Although the change in osmotic fragility of erythrocytes is very useful for assessing  
11 gossypol intake in ruminants fed gossypol experimentally, its value in assisting in a differential  
12 diagnoses of gossypol toxicity under field conditions is questionable because many other  
13 hemolytic agents/conditions are known which would be difficult to eliminate as possible causes.

#### 14 **Gossypol Analysis.**

15 The official methods for free and total gossypol are spectrophotometric procedures based on  
16 the reaction of aniline with gossypol to form dianilinogossypol (AOCS 1985 a,b). Although  
17 widely used, these procedures are only applicable to the determination of gossypol in cottonseed,  
18 cottonseed cake and untreated cottonseed meals. They are unsuitable for the determination of  
19 gossypol in mixed feeds because of interferences with other components of feeds (Pons, 1977),  
20 and are not selective. They measure not only gossypol, but also gossypol analogues and  
21 gossypol derivatives, with an available aldehyde group, that are soluble under the conditions of  
22 the methods. The most serious limitation of spectrophotometric procedures is they do not

1 determine the (+) and (-) isomers of gossypol. The gossypol molecule exhibits optical activity  
2 because of steric hindrance to rotation about the internaphthyl bond (Matlin et al., 1988).

3 High performance liquid chromatography (HPLC) procedures also have been used to  
4 measure free and total gossypol in cottonseed and cottonseed meal (Hron et al., 1990). They are  
5 selective for gossypol and are 50 to 100 times more sensitive than the official methods (Pons,  
6 1977; Hron et al., 1990). The HPLC procedures of Hron et al. (1990) were adapted to measure  
7 the (+) and (-) isomers of gossypol by substituting a chiral amine, (R)-2-amino-1-propanol, for  
8 3-amino-1-propanol in the complexing reagent used for gossypol extraction (Hron et al., 1999).  
9 This procedure was used to survey the isomer content of gossypol in seed of cotton varieties  
10 being grown in the United States and in cottonseed meals and cottonseed hulls by Forster and  
11 Calhoun (1995), and since 2001, has been used to determine total and (+)- and (-)-gossypol in  
12 seed of cotton varieties included in the National Cotton Variety Tests (NCVT, 2001). The  
13 proportion of isomers varies with the type of cotton, cotton variety and growing conditions.  
14 Seeds of commercial Pima cottons (*Gossypium barbadense*) currently being grown in the United  
15 States contain a slight excess of the (-) isomer; whereas, seed of Upland cottons (*Gossypium*  
16 *hirsutum*) contain an excess of the (+) isomer. Because they are selective and very sensitive,  
17 HPLC procedures can be used to determine gossypol in feeds.

### 18 **Gossypol Availability and Toxicity.**

19 An HPLC procedure for determining (+)- and (-)-gossypol in plasma and tissues of animals,  
20 developed by Kim and Calhoun (1995), has been used to measure plasma gossypol levels in  
21 numerous studies in which ruminants were fed various sources of gossypol. Plasma gossypol  
22 levels depended on the source and level of each gossypol isomer in the diet, processing

1 conditions, diet composition, dry matter intake and rumen development. Gossypol acetic acid,  
2 when placed directly into the rumen in a gelatin capsule, was much more toxic to lambs than free  
3 gossypol from either cottonseed meats or cottonseed meals in the diet (Calhoun et al, 1990a).  
4 Generally the free gossypol in cottonseed is extensively bound in the rumen and is much less  
5 available to cattle and sheep than free gossypol in cottonseed meal, regardless, of the process  
6 used for oil extraction (Calhoun and Wan, 1995). However, the free gossypol in cottonseed was  
7 much more available than free gossypol in cottonseed meals when these were mixed with a milk  
8 replacer and given to milk-fed lambs (Calhoun and Wan, 1995).

9       Increasing the level of protein in the diet of lambs decreases gossypol availability, but the  
10 effect is rather modest (Calhoun, unpublished data). Formation of a complex between the free  
11 epsilon amino groups of lysine and the aldehyde groups of gossypol may be responsible for the  
12 decrease in availability (Reiser and Fu, 1962), but the addition of lysine to the diets of sheep and  
13 cattle does not decrease the availability of gossypol (Calk et al., 1992; Blauwiekel et al., 1997).  
14 The addition of iron as ferrous sulfate effectively binds gossypol and decreases its availability to  
15 sheep and cattle (Calk et al., 1992; Mena, 1997). Digestible dry matter intake in relation to  
16 gossypol intake from cottonseed or cottonseed meal also is important. As dry matter intake  
17 increases in relation to the amount of cotton by-products consumed, gossypol levels in the  
18 plasma of cattle and sheep decrease. Conversely, as dry matter intake decreases in relation to the  
19 amount of cotton by-products consumed, gossypol availability increases and the potential for  
20 gossypol poisoning also increases (Calhoun, unpublished data). Results of a study with Holstein  
21 cows in early lactation (30 to 120 days) at North Carolina State University support the possibility  
22 that feed intake may be an important factor in determining plasma gossypol levels.(Blackwelder

1 et al., 1998). In this study, cows were fed cottonseed and cottonseed meal at constant  
2 percentages in a total mixed ration and there was a much as a twofold difference in intakes of  
3 cottonseed and cottonseed meal. However, there was no correlation between gossypol intakes  
4 and plasma gossypol values.

5 Certain types of heat processing appears to alter gossypol binding in seed, so that gossypol  
6 bypasses the rumen detoxification process. Roasted cottonseed, for example, is higher in  
7 available gossypol than unprocessed cottonseed (Bernard and Calhoun, 1997). This suggests  
8 cottonseed that has gone through a heat process is likely to be more toxic. In cottonseed,  
9 gossypol is contained in discrete structures called pigment glands. Heating seed does not rupture  
10 pigment glands, suggesting the enhanced availability of gossypol in heated seed is due to a  
11 chemical reaction between gossypol and other constituents in the pigment gland which produces  
12 a gossypol complex that bypasses the normal detoxification process occurring in the rumen. The  
13 mild coating and drying conditions used to produce EasiFlo cottonseed, cottonseed coated with 3  
14 to 5% cornstarch, does not appear to elevate levels of available gossypol (Bernard et al., 1999).

15 Several reports have advocated processing cottonseed by cracking or grinding as a means of  
16 increasing its feeding value (Sullivan et. al., 1993a,b; Pires et. al., 1997; Ariela, 1998). This is  
17 now the accepted practice when Pima seed is fed to dairy cows. However, breaking the seed  
18 coat also increases gossypol availability (Mena et al., 1997; Santos et al., 2002; Prieto et al.,  
19 2003).

20 At present, the only way to assess gossypol availability is to feed the product and measure  
21 gossypol in blood and tissues. This is time consuming and expensive. The need for a simple,  
22 quick procedure for available gossypol is particularly critical with cottonseed because of the

1 variable availability of gossypol in processed cottonseed. In contrast, the availability of gossypol  
2 in cottonseed meals can be predicted by considering both total and free gossypol content (Wan et  
3 al., 1995). However, because of the reactivity of gossypol and the diversity of the types of  
4 binding that can occur between gossypol and other constituents of the seed, it may be difficult to  
5 characterize these complexes sufficiently to develop a simple chemical procedure for available  
6 gossypol.

### 7 **Plasma Gossypol in Lactating Dairy Cattle Fed Cottonseed and Cottonseed Meal.**

8 Using plasma gossypol to assess the gossypol status of lactating dairy cattle fed cotton feed  
9 products was first proposed by Calhoun (1995). Since then, a large number of samples have  
10 been collected at dairies in the United States and analyzed for gossypol at the Texas Agricultural  
11 Experiment Station's Nutrition and Toxicology Laboratory in San Angelo, TX. Gossypol was  
12 measured in plasma and the cottonseed products being fed, and participating dairies were asked  
13 to provide information on days in milk, daily milk production, ingredient composition of the diet,  
14 and the amount of each cottonseed product fed per cow per day. This was done to establish safe  
15 levels of cottonseed, cottonseed meal and cottonseed hulls for use in dairy rations.

16 Plasma gossypol levels plateau in about 35 to 42 days when either cottonseed, cottonseed  
17 meal or combinations of cottonseed and cottonseed meal are introduced into the rations of dairy  
18 cows in mid-lactation (Mena, 1996; Mena, 1997). In contrast, plasma gossypol levels plateau  
19 after 100 days in milk in cows sampled at commercial dairies. The difference is probably due to  
20 the fact that dry matter and gossypol intakes are increasing in early lactation; whereas, this is not  
21 the case with cows in mid-lactation. The importance of this is that, in order to obtain meaningful  
22 plasma gossypol data for comparison purposes, blood samples should be collected after 100 days

1 in milk, and when the cows have been on the same diet for at least 35 days.

2 The positive isomer appears first in plasma when cattle are started on diets containing  
3 cottonseed or cottonseed meal (Mena, 1997). The minus isomer then appears, and both isomers  
4 accumulate until a plateau is reached that reflects the level and availability of gossypol in the diet  
5 and the proportion of isomers in the sources being fed. In cows sampled at commercial dairies  
6 the proportion of the minus isomer increases as total gossypol increases in plasma. This has  
7 been a consistent finding regardless of whether the source of gossypol is Upland or Pima seed.  
8 When gossypol is removed from the diet, plasma gossypol levels gradually decrease,  
9 approaching zero in 28 days. The positive isomer disappears at a faster rate than the minus  
10 isomer. This is reflected in a decrease in the proportion of the positive isomer and a  
11 corresponding increase in the proportion of the minus isomer in plasma after gossypol is  
12 removed from the diet (Mena, 1997).

13 The variability in plasma gossypol levels of cows consuming the same diet can be quite  
14 large. However, cows maintain their relative ranking over time even when changes in the source  
15 and or level of gossypol in the ration results in a change in the average plasma gossypol levels of  
16 the group being sampled.

17 Serum was obtained from 20 Holstein cows at a commercial dairy in Midland, Texas that  
18 were being fed a total mixed ration (TMR) which contained whole, Upland seed (15.0%) and  
19 cottonseed hulls (3.4%). Ten cows were in early lactation ( $69 \pm 7.3$  d post-partum) and 10 were  
20 in late lactation ( $410 \pm 24.0$  d post-partum). The whole cottonseed contained 0.68% total  
21 gossypol with 57.2% (+)- and 42.8% (-)-gossypol. The hulls contained 0.05% total gossypol  
22 with 57.7% (+)- and 42.3% (-)-gossypol. Total gossypol intakes ranged from 32.5 g/d for the

1 cows in early lactation to 27.2 g/d for the cows in late lactation. Serum gossypol levels were not  
2 different for the cows in early ( $2.7 \pm .38 \mu\text{g/ml}$ ) and late ( $3.0 \pm .25 \mu\text{g/ml}$ ) lactation. Serum total  
3 gossypol, for the 20 cows, ranged from 1.2 to 5.7  $\mu\text{g/ml}$ . Although not sampled, it is likely that  
4 serum gossypol levels would have been slightly higher for cows in mid-lactation. This  
5 cottonseed was fed to these cows for 8 to 10 months without signs of gossypol poisoning  
6 (Calhoun et al., 1995).

7 Three lactating Holstein cows in the University of Arizona dairy herd died suddenly during  
8 the spring of 1994. These cows were receiving a TMR containing 15% of ammoniated, whole,  
9 Upland cottonseed. Gossypol poisoning was considered a possibility and samples of the  
10 cottonseed and plasma were analyzed for gossypol. Plasma gossypol was  $10.5 \pm .72 \mu\text{g/ml}$  (N =  
11 29) with a range of 4.2 to 19.4  $\mu\text{g/ml}$ . Decreasing the ammoniated seed to 10% of the diet  
12 decreased plasma gossypol to  $7.8 \pm 0.42 \mu\text{g/ml}$  (N = 30) with a range of 3.8 to 11.8  $\mu\text{g/ml}$ . After  
13 these cows were switched to ammoniated seed (10% of the diet), which was delivered to the  
14 University Dairy in September, 1994, plasma gossypol was 0.27  $\mu\text{g/ml}$  (N = 30) with a range of  
15 0.08 to 0.58  $\mu\text{g/ml}$  (Table 3; 2-3). The ammoniated seed analyzed in the spring of 1994  
16 contained 0.52% free and 0.68% total gossypol (whole seed basis). The load of ammoniated  
17 seed delivered to the University Dairy in September contained 0.22% free and 0.34% total  
18 gossypol. Seven samples of whole Upland seed, that had not been ammoniated, were obtained  
19 from the same area in Arizona during 1993-94. These averaged 0.66% free and 0.69% total  
20 gossypol.

21 There is a previous report of suspected gossypol poisoning in dairy cows fed ammoniated

1 seed at six Arizona dairies in the late spring and mid-summer of 1980 (Smalley and Bicknell,  
2 1982). Cottonseed was being fed at 2.7 to 4.5 kg per cow daily. Mortality was as high as 10%  
3 and clinical signs and histopathologic changes were consistent with gossypol poisoning. The  
4 same amount of non-ammoniated seed was fed to these herds in previous years, and there were  
5 no deaths diagnosed as gossypol poisoning. Smalley and Bicknell (1982) believed measuring  
6 gossypol in cottonseed was of limited value because levels in ammoniated seed were lower,  
7 ranging from 0.17 to 0.68% in cottonseed meals, compared with 0.91% gossypol in meals from  
8 non-ammoniated seed. On a whole, seed basis the gossypol values would have been  
9 approximately 0.09 to 0.37% for the ammoniated and 0.50% for untreated seed. In some areas  
10 where aflatoxin contamination of cottonseed is a problem, ammoniation has been used routinely to  
11 reduce aflatoxin levels to less than 20 ppb. With the exception of the few cases already  
12 described, the extensive use of ammoniated cottonseed in the diets of dairy cattle has not resulted  
13 in problems with gossypol. There is no way to know what happened to increase gossypol  
14 availability in these cases, but it would be wise to monitor plasma gossypol levels in cows fed  
15 ammoniated cottonseed to insure that problems do not reoccur.

16 In a study conducted at the University of Arizona (Mena, 1996; Mena et al., 2001), plasma  
17 gossypol was determined in 30 Holstein cows fed diets containing Upland cottonseed and  
18 cottonseed meal for 42 days. Six cows were fed each of the following treatments: (1) a soybean  
19 meal control diet with no gossypol; (2) 900 ppm total gossypol from good quality whole, linted  
20 cottonseed; (3) 900 ppm total gossypol from an expander solvent cottonseed meal; (4) 900 ppm  
21 total gossypol, with equal amounts of gossypol from cottonseed and cottonseed meal and (5)  
22 1800 ppm total gossypol, with equal amounts of gossypol from cottonseed and cottonseed meal.

1 The cottonseed contained 0.69% total and 0.66% free gossypol (whole seed, as fed basis) and the  
2 cottonseed meal contained 1.26% total and 0.09% free gossypol (as fed basis). The plasma  
3 gossypol response to free gossypol intake was 3.8 times greater when the source of free gossypol  
4 was cottonseed meal indicating the availability of free gossypol in cottonseed meal was much  
5 greater than free gossypol in cottonseed. The opposite was true for total gossypol. Plasma  
6 gossypol when the combinations of cottonseed and cottonseed meal were fed indicate available  
7 gossypol may be additive, regardless of source. Compared with the control group, cows fed the  
8 TMR with 1800 ppm total gossypol had higher ( $P < .05$ ) milk yield (32.6 vs 29.5 kg/d). There  
9 was no evidence of gossypol toxicity in the cows in this experiment.

10 In a second study conducted at the University of Arizona (Mena, 1997), plasma gossypol was  
11 determined in 40 Holstein cows fed the same cottonseed and cottonseed meal treatments as in the  
12 first study. However, eight cows received each treatment and the duration of the study was  
13 increased to 84 days. Average plasma gossypol levels were lower across all treatments than for  
14 the corresponding treatments in the first study. The reason for this is not known, but the other  
15 results were similar. The cows fed the TMR with 1800 ppm total gossypol had higher ( $P < 0.05$ )  
16 milk yield than the control group (32.6 vs 30.6 kg/d). Available gossypol appeared to be  
17 additive, regardless of whether the source was cottonseed or cottonseed meal, and there was no  
18 evidence of gossypol toxicity in the cows in this experiment.

19 The practical consequences of greater gossypol availability in cracked Pima seed was evident  
20 in the occurrence of gossypol poisoning in a herd of Holstein cows at a commercial dairy in the  
21 El Paso, Texas area.. The herd involved was milking around 400 cows and was among the top  
22 producing herds in the state. Cracked Pima was the only type of cottonseed being fed and had

1 been in the ration at levels of 3.6 to 4.1 kg per cow per day for several months before there were  
2 problems. However, within a few days after starting to feed from a new load of seed, average  
3 milk production dropped from 36.2 to 27.2 kg/day. Eventually the owner of the herd was forced  
4 to dry some cows earlier than planned because of low production and 19 cows died. The gross  
5 pathology and histopathology were consistent with gossypol poisoning (Dr. M.J. Behr,  
6 Veterinary Pathologist, New Mexico Department of Agriculture, Veterinary Diagnostic Services,  
7 Albuquerque, NM, personal communication).

8 Samples of the cotton by-products being fed and plasma from eight cows were analyzed for  
9 gossypol. On the day blood samples were collected the cows were switched from cracked Pima  
10 to whole Upland seed. Twenty-eight days later blood was collected from 20 cows, including  
11 seven of the original cows, and analyzed for gossypol. Plasma gossypol averaged  $12.2 \pm 1.3$   
12  $\mu\text{g/ml}$  for the cows sampled initially and ranged from 6.5 to 16.0  $\mu\text{g/ml}$ . Seventy one percent of  
13 the gossypol in the plasma was the (-) isomer. When the cows were sampled 28 days after  
14 switching to whole Upland seed, plasma gossypol averaged  $4.6 \pm 1.3 \mu\text{g/ml}$  and ranged from 2.4  
15 to 7.6  $\mu\text{g/ml}$ , and the minus isomer had decreased from 71 to 66% of plasma gossypol. Feed  
16 intake and milk production gradually returned to normal after the herd was switched to whole  
17 Upland seed, and there did not appear to be any long-term adverse effects on production and  
18 reproduction.

19 Although measuring gossypol in plasma has proven to be an effective way to assess the  
20 availability of gossypol in the diet of lactating dairy cattle, controlled studies that relate plasma  
21 gossypol levels to known effects of gossypol are lacking. With the exception of the work of Dr.  
22 Santos and coworkers at the University of California (Santos et al., 2002; 2003), in the numerous

1 studies with lactating dairy cattle in which plasma gossypol was measured, the sources and levels  
2 of cotton feed products being fed and the lengths of the feeding periods were not adequate to  
3 provide this information (Mena, 1996; Mena, 1997; Bernard and Calhoun, 1997; Blauwiekel et  
4 al., 1997; Blackwelder et al., 1998; Bernard et al., 1999; Prieto et al., 2003). In the California  
5 study, 813 cows on three commercial dairy farms were assigned to one of two diets starting at 13  
6  $\pm$  11 days in milk for a 170 day experiment. One diet contained 10% whole Upland cottonseed  
7 (WCS) and the other a 1:2 blend of WCS and cracked Pima seed (CPS). Actual average  
8 cottonseed intakes, on an as fed basis, were 2.7 kg/d for the WCS diet, and 0.8 kg/d of WCS and  
9 1.9 kg/d of CPS for WCS/CPS blend. Average free gossypol intakes were 17.5 and 22.8 g/d for  
10 the WCS and WCS/CPS diets respectively. Although the free and total gossypol content of the  
11 diet increased only 32% when CPS replaced a portion of the WCS, the average plasma total  
12 gossypol tripled in cows fed the WCS/CPS blend (8.33 vs 2.65  $\mu$ g/ml).

13 In this study, there were no effects of type of diet or dietary gossypol intake on lactation  
14 performance, culling rate or incidence of health problems; however, overall reproduction was  
15 adversely affected by feeding the WCS/CPS blend. Although the interval from calving to first  
16 postpartum AI was similar for the two treatments, and diet had no effect on the first postpartum  
17 conception rate, fertility decreased after the first AI and there was an increased incidence of  
18 abortion in cows fed the WCS/CPS blend (Santos et al., 2002; 2003). The authors concluded  
19 that when diets increase plasma gossypol to concentrations above 5 to 10  $\mu$ g/ml, reproductive  
20 functions might be compromised and fertility depressed.

### 21 **Summary and Recommendations**

22 Guidelines for using plasma gossypol values to establish safe levels of cotton by-products in

1 diets of lactating dairy cattle are given in Table 2. Currently, 5  $\mu\text{g}/\text{ml}$  is considered the safe  
2 upper limit for plasma gossypol in dairy cattle fed cotton feed products for extended periods.  
3 The safe upper limit for the minus isomer is 3.5  $\mu\text{g}/\text{ml}$  of plasma. These values are  
4 approximately one-half the values observed in cattle where gossypol poisoning was suspected,  
5 and were arbitrarily selected to provide a margin of safety. Deaths attributable to gossypol were  
6 suspected in two herds; in one, total plasma gossypol averaged  $10.5 \pm 0.7 \mu\text{g}/\text{ml}$  ( $N = 29$ ) and in  
7 the other total plasma gossypol averaged  $12.2 \pm 1.3$ . Levels of the minus isomer were  $6.6 \pm 0.4$   
8  $\mu\text{g}/\text{ml}$  of plasma in the first herd and  $8.5 \pm 1.0 \mu\text{g}/\text{ml}$  of plasma in the second herd. Based on  
9 this information, whenever the average for a group of cows exceeds 11.0  $\mu\text{g}/\text{ml}$  for total  
10 gossypol or 8.0  $\mu\text{g}/\text{ml}$  for (-)-gossypol, deaths due to gossypol poisoning are a distinct  
11 possibility. Because of the difficulty in making a positive diagnosis of gossypol poisoning, the  
12 deaths of a few animals in a large herd might not be linked to the cotton by-products being fed.  
13 Likewise, since it is a problem to get high producing dairy cows to conceive it might not be  
14 readily apparent that gossypol is contributing to the problem.

15 Current recommendations for using cotton feed products in diets of dairy cows are 2.3 to 3.6  
16 kg of whole seed per day, or about 15% of the diet dry matter, and no more than 5.4 kg per day  
17 of all cotton feed products, i.e., cottonseed, cottonseed meal and cottonseed hulls (about 22.5%  
18 of the diet dry matter). In dairy herds fed a TMR with 15% good quality, whole, linted  
19 cottonseed, total plasma gossypol averages 1.5 to 3.5  $\mu\text{g}/\text{ml}$ , and generally does not exceed 5.0  
20  $\mu\text{g}/\text{ml}$  when all cotton feed products are no more than 22.5% of the diet. Practical feeding  
21 experience over many years has established that these levels are safe when cows are fed good

1 quality, unheated, whole Upland seed and meal and hulls derived from Upland seed, but recent  
2 events indicate the same levels may not be safe when seed is processed.

3 Because of the greater availability of gossypol when Pima seed is cracked or ground, it is  
4 obvious different recommendations are needed when Pima seed is fed. No more than 1.8 kg/day,  
5 or about 7.5% of the diet dry matter, of cracked or ground Pima should be fed for extended  
6 periods to dairy cattle. No other cotton feed products should be fed along with the Pima.  
7 However, 1.4 kg/day of cracked or ground Pima can be fed in combination with 1.4 to 1.8 kg/day  
8 of good quality, whole, unprocessed Upland cottonseed. Including the Pima seed as part of total  
9 mixed ration (TMR) is preferable to using either a partial TMR, where a portion of the roughage  
10 is fed separately, or including the Pima seed in a grain mix that is fed separately from the  
11 roughage component of the diet. Because of concerns about particle size and gossypol  
12 availability, plasma gossypol probably should be determined when feeding finely ground Pima  
13 seed. Coarsely cracking Pima seed so that the seed coat is just broken should be adequate to  
14 prevent undigested seed in the feces.

15

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Table 1. Recommended safe levels of free gossypol in ruminant diets. <sup>1</sup>				
		Age	Free gossypol	
	Rumen development	week	ppm in diet	mg/kg LW/d
	Preruminant	0 to 3	100	1.1
	Transition <sup>2</sup>	3 to 8	200	2.3
	Functional			
	Post- weaning	8 to 24	200	3.6
	Mature <sup>3</sup>	> 24	600	6.8

<sup>1</sup> Information in this table is from Calhoun and Holmberg (1991).

<sup>2</sup> Transition from preruminant to functional ruminant begins when animals start to consume dry feed.

<sup>3</sup> This level is considered safe for females used for breeding. The recommended safe level for males used for breeding is 200 ppm.

Table 2. Guidelines for using plasma gossypol to establish safe levels of cotton by-products in diets of lactating dairy cattle. <sup>a</sup>			
Plasma gossypol, µg/ml			
Total gossypol	(-)-gossypol		Comments
5 or less	3.5 or less		Safe
6 to 8	4 to 5.5		Caution
9 to 11	6 to 8		Danger
More than 11	More than 8		Gossypol poisoning

<sup>a</sup> Plasma gossypol values in this table are based on averages for groups of cows in peak lactation that were fed the same ration for a sufficient period to allow plasma gossypol values to plateau. Values appear to plateau at about 100 days in milk. Whenever source(s) and level(s) of cotton by-products in the diet change, a minimum of 4 wk is required for plasma levels to reach a new plateau.

Appendix Table 3. Nutrient values for whole, linted seed of Upland cottons (*Gossypium hirsutum*).<sup>1</sup>

Item	NCPA 1995 <sup>2</sup>			Dairy NRC 2001 <sup>3</sup>			Dairy One 2004 <sup>4</sup>		
	N	Mean	SD <sup>5</sup>	N	Mean	SD	N	Mean	SD
Dry matter, %	83	91.6	0.89	1059	90.1	4.6	1034	91.4	3.0
Crude protein, %	83	23.0	0.98	1124	23.5	2.6	859	25.0	6.1
Sol. protein, % of CP							413	27.5	12.8
Degrad. protein, % of CP							57	47.3	6.2
ADICP, %				4	1.9	0.1	136	1.96	0.58
NDICP, %				71	2.4	1.2	137	2.55	0.69
ADF, %	83	38.9	3.59	1024	40.1	4.4	782	38.1	9.93
NDF, %	83	47.3	3.54	953	50.3	5.8	790	49.6	10.94
Crude fiber, %	83	29.5	2.07				89	23.7	7.08
Crude fat, %	83	20.1	0.95	27	19.3	1.4	456	22.5	4.65
Ash, %	83	3.8	0.23	193	4.2	2.1	227	4.4	0.56
Calcium, %	83	0.14	0.016	928	0.17	0.08	603	0.20	0.057
Phosphorus, %	83	0.56	0.055	928	0.60	0.08	603	0.76	0.187
Magnesium, %	83	0.35	0.020	928	0.37	0.04	602	0.40	0.073
Potassium, %	83	1.14	0.067	928	1.13	0.07	602	1.21	0.103
Sodium, %	83	0.008	0.007	928	0.02	0.02	602	0.024	0.009
Chloride, %				148	0.06	0.03	136	0.09	0.018
Sulfur, %	83	0.20	0.023	424	0.23	0.04	417	0.25	0.069
Copper, ppm	83	7	1.3	928	7	3	600	7	2.4
Iron, ppm	83	50	11.5	928	94	185	600	85	73.3
Manganese, ppm	83	15	2.2	928	18	13	600	17	9.0
Zinc, ppm	83	33	3.5	928	37	18	600	37	12.7
Molybdenum, ppm	83	1.6	0.52	919	1.3	0.6	600	0.58	0.47

<sup>1</sup> Values are on a 100% dry matter basis.

<sup>2</sup> Research sponsored by the National Cottonseed Products Association (Calhoun et al., 1995).

<sup>3</sup> National Research Council, Nutrient Requirements of Dairy Cattle 6th Revised Edition, 2001.

<sup>4</sup> Information accessed at <http://www.dairyone.com> on February 20, 2004.

<sup>5</sup> Standard deviation.

Appendix Table 1. Nutrient composition of cottonseed meal.<sup>1</sup>

Item	NCPA 1995 <sup>2</sup>			Dairy NRC 2001 <sup>3</sup>			NCPA 2002 <sup>4</sup>			Dairy One 2004 <sup>5</sup>		
	N	Mean	SD <sup>6</sup>	N	Mean	SD	N	Mean	SD	N	Mean	SD
Dry matter, %	66	89.1	0.93	180	90.5	1.90	14	91.6	0.71	114	91.6	2.69
Crude protein, %	66	47.6	1.99	158	44.9	4.1	14	43.8	0.84	114	42.5	6.38
Sol. protein, % CP	---	---	---	---	---	---	---	---	---	48	13.5	5.29
Degrad. protein,	---	---	---	---	---	---	---	---	---	10	50.8	---
ADICP	---	---	---	8	1.8	0.5	---	---	---	19	2.4	0.65
NDICP	---	---	---	7	3.3	0.9	---	---	---	19	5.1	2.90
Crude fiber, %	66	11.2	1.47	---	---	---	14	11.7	1.98	28	12.0	2.00
ADF, %	66	17.3	2.70	58	19.9	5.4	---	---	---	80	21.3	7.59
NDF, %	66	24.5	3.61	47	30.8	9.0	---	---	---	81	31.3	9.82
Crude fat, %	66	2.2	0.89	113	1.9	2.2	14	2.9	1.61	66	6.5	4.66
Ash, %	66	7.5	1.00	44	6.7	0.7	---	---	---	26	7.7	1.02
Calcium %	66	0.22	0.03	185	0.20	0.1	14	0.21	0.02	68	0.35	0.11
Phosphorus, %	66	1.20	0.14	185	1.15	0.1	14	1.10	0.07	68	1.20	0.26
Magnesium, %	66	0.66	0.06	65	0.61	0.11	14	0.64	0.03	67	0.62	0.11
Potassium, %	66	1.72	0.11	185	1.64	0.38	14	1.56	0.07	67	1.60	0.25
Sodium, %	66	0.14	0.09	97	0.07	0.06	14	0.16	0.10	67	0.19	0.20
Chloride, %	---	---	---	3	0.07	---	---	---	---	16	0.33	0.02
Sulfur, %	66	0.44	0.03	30	0.40	0.11	14	0.41	0.02	51	0.44	0.04
Copper, ppm	66	12	2	59	14	3	14	13	2	67	15	8

Iron, ppm	66	126	39	60	149	47	14	51	7	67	182	97
Manganese, ppm	66	20	27	61	24	11	---	---	---	67	26	12
Zinc, ppm	66	64	68	55	67	15	14	60	8	67	70	29
Molybdenum,	66	2.5	0.8	18	3	0.8	---	---	---	67	1.1	0.5

<sup>1</sup> Values are on a 100% dry matter basis.

<sup>2</sup> Research sponsored by the National Cottonseed Products Association (Calhoun et al., 1995).

<sup>3</sup> National Research Council, Nutrient Requirements of Dairy Cattle 6th Revised Edition, 2001.

<sup>4</sup> Information accessed at <http://www.dairyone.com> on February 20, 2004.

<sup>5</sup> Standard deviation.