



## **GOSSYPOL EFFECTS IN ANIMAL FEEDING CAN BE CONTROLLED**

### **Abstract**

Gossypol, a substance found naturally in cottonseed and cottonseed products has been the subject of numerous research projects and articles in the past. However, controlling gossypol intake is still a concern in many livestock classes. This review article includes a summary of the classical information on limits and effects as well as results from recent research projects with immature and adult ruminant and non-ruminant species. The intent of this article is to provide practical information and guidelines for the safe and efficient use of cottonseed and cottonseed products.

Cottonseed meal has long been a popular and economic protein concentrate for animal feeding. The realities of least cost ration formulation and simple economic incentive have been the prime reason that this co-product of the cottonseed oil extraction industry has found its way into feeds for many classes of animals. Cottonseed meal has some natural limiting factors that must be considered for safe use. Chief among these factors are protein level and quality, fiber level and the gossypol content. Here, we want to review what is known about this last factor, the proper control of gossypol in animal feeds and limits of free gossypol when consumed by production animals.

Included in this review will be the effects of free gossypol in cottonseed meal, whole cottonseed or cottonseed hulls on non-ruminants, mature and immature ruminants and on the fertility of bulls and cows. The latest recommendations for safe levels of gossypol are also reviewed.

By Steve D. Martin

### **What is gossypol?**

A comment on cottonseed processing and the occurrence of gossypol may be helpful. Along with each 100 lb. of cotton fiber produced in the field, about 140 lbs. of seed are also produced. Thus, more seed than fiber is produced from an acre of cotton. About 1.5 percent of the approximately five million tons of seed produced annually in the U.S. are needed for planting, leaving the rest for processing or feeding. In the past five years the amount of cottonseed that has been crushed has amounted to between 40-50% of that available. The remainder is fed as whole seed. In a typical year, about 1.1 million tons of cottonseed meal is fed to livestock in the U.S. with excellent results.

Cottonseed oil, meal, hulls and linters are the four major products of cottonseed. Cottonseed meal (CSM) may be sold in the form of meal, cake, flakes or pellets. Most meal is sold at a 41% protein level and has been popular with stockmen for more than a century. Cottonseed hulls (CSH)

are used as a roughage source for ruminants. Hulls are comparable in quality to grass hay and require no further processing.

Gossypol is a polyphenolic yellow pigment found naturally throughout the cotton plant. In the seed, gossypol is found in small pigment glands. When the seed kernels are flaked and heated as part of the oil extraction process, these small glands are ruptured and the gossypol is released. Some of the gossypol will bind with components in the seed (Adams and Geissman, 1960; Jones, 1985). This is the reason for the terms “bound” and “free” gossypol. All of the gossypol in whole seed is in the free form (Ponset al., 1953), but as a result of processing, both free and bound gossypol will be present in meal (Jones, 1985). During processing, free gossypol is bound to cottonseed protein resulting in bound gossypol and unavailable amino acids. This binding reduces the protein quality, especially with regard to lysine availability. Lysine is believed to be the primary amino acid that is bound to free gossypol (Baliga and Lyman, 1957; Conkerton et al., 1957; Kuiken and Lyman, 1948).

The binding of free gossypol is important because, based on the traditional concepts, the free form is the toxic form. The degree of binding is also critical due to the importance of available lysine especially when meal is fed to non-ruminants. This creates the trade off in cottonseed meal where more bound gossypol results in a lower level of the already marginal lysine. It is unknown at this time if some of the bound gossypol is released in the gut of the animal and this is a fertile area for more research.

### **Gossypol levels in cottonseed products**

Whole cottonseed (WCS) has a significantly higher level of free gossypol than meal or hulls. In a survey completed by Texas A&M researchers, whole seed in Texas ranged from 0.47 to 0.63% free gossypol and meal ranged from 0.079 to 0.298% on an as-fed basis (Calhoun, 1989). Hulls were recently sampled for free gossypol and found to contain 0.06% on an as-fed basis (NCPA, unpublished data). Table I contains information concerning gossypol levels in various cottonseed products. There is a lack of understanding regarding free gossypol values in whole seed, and the basis for gossypol reporting. Commercial testing labs report free gossypol as a percent of the kernel only. The weight of hulls and linters has to be considered to calculate gossypol levels in whole seed. These weights can vary but both together generally make up 45% of the dry weight of gin-run whole seed (Tharp, 1948). Another possible area of confusion is the fact that even though all of the gossypol in whole seed is considered to be in the free state, analyses for free and total gossypol will not necessarily result in the same numbers. This is because there are two separate official analytical procedures for free and total gossypol even though in theory, they are measuring the same thing.

As indicated by the Texas A&M survey, there is quite a variation of free gossypol levels in cottonseed meal. This variability is due to different processing methods used to extract oil as well as some natural variation in cotton varieties and an influence of growing factors (Cherry et al., 1978). Recently, cottonseed meals from several oil mills in Texas were analyzed for free gossypol. The four processes and their respective free gossypol levels were, on an as-fed basis: mechanical (screw press), 0.02-0.05%; pre-press solvent, 0.02-0.07%; direct solvent, 0.1-0.5%; and, when expanders were used in the solvent process, 0.06-0.1% (Calhoun, 1989). The values found in this survey are in line with what the industry has historically seen in each of the common processing techniques (Jones, 1981). The mechanical (or screw press) method involves the most heat and pressure, thus the low free gossypol levels. The direct solvent method uses the least amount of heat and pressure, and results in higher free gossypol levels. The free gossypol levels for prepress are intermediate due to an intermediate amount of heat and pressure. The use of expanders in the solvent process is a new development in the industry that results in more efficient oil extraction. These adaptations of the familiar extruder are now common in solvent mills and subject the kernel to moist heat and moderate pressure before extraction. Mills that employ the direct solvent process alone without the use of an expander are becoming increasingly

uncommon in the U.S. Statements regarding the heat and pressure resulting from each process is generalities and the process at individual mills may vary. Table 1 contains a list of cottonseed products and their respective free and/or bound gossypol levels.

It has been known for many years and is well documented that the gossypol in cottonseed feed products could be toxic to some animals in certain situations if known precautions were not followed. Several good reviews exist of these observations and they can be consulted for specific information (Berardi and Goldblatt, 1980; Holmberg et al., 1988, and Feedstuffs, Dec. 21 and 28, 1981). Some of the classical signs of chronic gossypol toxicity seen in a variety of species as compiled by Berardi and Goldblatt (1980) are depression, loss of appetite, anorexia, “thumps” or labored breathing, dyspnea, weakness, emaciation, weight loss, diarrhea, vomiting, decreased egg size and hatchability in poultry, injury to the heart, liver and kidneys and widespread edema. These symptoms have been observed consistently in non-ruminants and occasionally in young ruminants or in mature ruminants with very high free gossypol intakes (Hudson et al., 1988).

### **Recommendations for non-ruminants**

The early knowledge of the dose level in swine, which was determined in the 1950s, allowed researchers to recommend the safe level of free gossypol in this specie. The limit in swine rations has been identified in several studies as 100 ppm (Tanksley and Knabe, 1981). The ration levels determined as safe for poultry are 50 ppm for layers and 100-150 ppm for broilers (Waldroup, 1981; NCPA, 1970). A study comparing CSM to soybean meal as a protein source in the concentrate for yearling horses showed no clinical signs of toxicity when the free gossypol concentration in the total ration was 115 ppm (Potter, 1981). In another study, weanling horses were not affected by a ration containing 343 ppm free gossypol (McCall, 1982). Gossypol tolerance in fish appears to be higher than in other animals and is also specie specific. For instance, Roehm et al. (1967) reported no effect when rainbow trout received 250 ppm of free gossypol. Growth of catfish, however, was not found to be inhibited at a level of 900 ppm of free gossypol per day (Dorsa et al., 1982). Tilapia seem so be more resistant to gossypol than other species of fish. A level of 1,800 ppm of free gossypol did not adversely affect this specie (Robinson et al., 1984). Some of the values quoted in this paragraph were reported as-fed, some on a dry matter basis and others were not specified. Tables 2 and 3 contain these and other data, and the as-fed/dry matter basis, when known, is included.

### **Using iron salts**

The addition of iron salts to cottonseed meal has long been known to allow the level of gossypol to be increased without toxicity to some non-ruminant species. It is thought that the iron binds to the free gossypol (NCPA, 1966). The recommendation is a 1:1 ratio of the iron itself to free gossypol for swine diets containing more than 100 ppm of free gossypol on an as-fed basis (Tanksley and Knabe, 1981). However, the safe limit even with the inclusion of iron is 400 ppm of free gossypol (NCPA, 1970). The recommendation is 1-2 ppm additional iron for each 1 ppm of free gossypol for broilers and a 4: 1 ratio of additional iron to gossypol is recommended for layers. Even with the inclusion of iron salts, it is suggested that the maximum gossypol levels be 400 ppm for broilers and 150 ppm for layers on an as-fed basis (Waldroup, 1981). Table 3 contains a summary of accepted free gossypol levels for swine and poultry, with and without iron salts. If iron salts are used, care must be taken to mix the salts well with either CSM or the final ration. As well, care must be taken in calculating the available iron if dried (exsiccated) ferrous sulfate ( $\text{FeSO}_4 \cdot \text{H}_2\text{O}$  with 32% iron) is used or if copperas ferrous sulfate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  with 20% iron) is used.

### **Gossypol and ruminants**

For many years, it was assumed that only non-ruminant animals were susceptible to gossypol toxicity. Reports earlier this century of injury to cattle being fed cottonseed products were thought to be gossypol related, but were eventually discovered to be a vitamin A deficiency (Adams and Geissman, 1960). Work by Reiser and Fu (1962) identified the mechanism by which functional ruminants avoid gossypol toxicity. These researchers found that the detoxification was a result of free gossypol becoming bound to soluble proteins in the rumen. The gossypol most likely binds to the epsilon-amino group of lysine. This bond is not broken by the proteolytic enzymes secreted in the lower gut.

It is known, however, that ruminants are susceptible to gossypol toxicity if the rumen detoxification process is somehow bypassed. Intravenous administration of gossypol in sheep resulted in similar symptoms to those seen in non-ruminants (Danke et al., 1965). Commercial beef cattle that are consuming cottonseed meal as a protein source in mixed feed or as a supplement are very unlikely to be in danger of a toxicity situation. There are at least two reasons for this: the rumen's detoxification abilities and the fact that a mature beef animal in a production situation usually won't receive enough CSM to tax the rumen's gossypol detoxification process.

Over the decades, the largest proportion of cottonseed meal has been fed to beef cattle and this is still the case. This extensive practical use did much over the years to build confidence in cottonseed meal and tempted dairy and sheep producers to use large amounts of meal almost all of it without incident. Cottonseed meal was included in rations or many classes of livestock like baby dairy calves, high-producing dairy COWS and young sheep. However, these additional uses for meal were not as well supported by scientific data, and the unsupported use of CSM in several types of rations has resulted in some apparent toxicity conditions reported in the farm press. We'll consider some of these instances and how to avoid them.

## **Dairy cows**

Today's high milk production levels have caused some new concern with regard to gossypol toxicity in ruminants. In order for a modern dairy cow to reach her genetic potential, her ration has to have a much higher protein (and energy) content than in the past. In many parts of the U.S., cottonseed and CSM can supply a major part of this requirement. The concern that this high level of meal feeding could tax the rumen's gossypol detoxification ability prompted research to answer this question. In their classical work, Lindsey et al., (1980) found gossypol in the plasma and livers of dairy cows fed a diet containing 45% direct solvent and screwpress process cottonseed meal. This level of meal was much higher than what is usually used commercially. However, the purpose of the study was to determine if the rumen could be overloaded with free gossypol. The fact that free gossypol was found in the plasma and livers, indicates that there is a limit to how much free gossypol can be detoxified in the rumen of lactating dairy cows. One cow receiving the highest level of free gossypol died, and symptoms and post-mortem inspection were both consistent with gossypol toxicosis. This study demonstrated that the consumption of 24.2 g of free gossypol per head per day for these high-producing Holsteins was too much. This translates into an intake of 19.4 mg/lb. body weight per day (42.7 mg/kg bodyweight per day). The study, however, did not reveal how much gossypol could be safely handled by the high producing milk cow. Observations in the study did lead the researchers to believe that a high free gossypol intake caused the animals to be more susceptible to ill effects caused by heat stress and other stresses incurred in today's production system.

When discussing gossypol in dairy cows, the popular practice of feeding whole cottonseed must be considered. Many dairymen feed whole seed to benefit from the convenient package of protein, energy and fiber. Very few feedstuffs available to U.S. dairymen are as nutrient-dense as whole cottonseed, and this accounts for its attractiveness. Rumination of whole seed causes a somewhat slower release of these nutrients which makes seed feeding even more beneficial. Cows being fed whole seed

frequently show increases in milk production as well as milk fat percentages along with a slight reduction in milk protein. As mentioned, 100% of the gossypol in whole cottonseed is in the free form. If too much seed is fed, it could cause the gossypol level in the rumen to reach or exceed the detoxification limit. The problem could be exaggerated when some amount of whole seed is fed in addition to a concentrate that contains CSM. The question is, how much gossypol is too much, or in practical terms, how much whole cottonseed or cottonseed meal is too much? In response to these kinds of questions concerning gossypol and dairy cattle, many articles have appeared in the farm press containing recommendations on how much whole seed can be fed. The usual safe recommendations vary from 5-8 lb. of whole seed per head per day (Bath, 1976; Coppock, 1984; Jimenez, 1979, 1980, 1981a, 1981b). There is still much to be learned about the mature ruminant's ability to detoxify gossypol.

High availability at certain times of the year, economic factors, and a lack of specific literature in the past may have led to the overuse of some cottonseed products. With the above cited recommendations in mind, cottonseed and cottonseed meal can be safely used as high-quality dairy feedstuffs.

### **Rumen function and gossypol**

The subject of gossypol with regard to young ruminants is an area of more uncertainty. In order for gossypol to be detoxified, the animal must have a functioning rumen. It is suggested by Wardrop and Coombe (1960) that the development of ruminants can be generally divided into three phases: (1) birth to three weeks old, a non-ruminant phase; (2) three to eight weeks old, a transitional phase; and (3) eight weeks and onward, a functioning ruminant. The National Research Council (NRC) recommendations for beef cattle agree with Wardrop and Coombe and state that the rumen becomes functional at six to eight weeks of age (NRC, 1984). These are certainly not hard and fast guides and will vary widely as milk and feed composition and consumption vary. Based on the knowledge of the development of ruminants, it would be reasonable to treat young lambs as well as calves as non-ruminants at least until six weeks of age and probably up to eight weeks or more so be safe. It is important to keep in mind that the wide ranges in the time it takes newborn sheep and calves to have enough rumen function to detoxify a significant free gossypol load have not been determined by controlled trials at this time. Currently, there are research projects in progress to better define safe gossypol levels in young calves and lambs and this information should be available in the near future.

### **Pre-ruminants**

There have been occasional press reports and scientific reports (Holmberg et al., 1988; Rogers et al., 1975) of what was assumed to be the practical impact of free gossypol on pre-ruminant calves. One report was from ranches in the U.S. which suffered actual calf losses that were diagnosed as having been caused by gossypol toxicity. Holmberg et al. (1988) reported on a typical situation. Calves were individually housed in hutches at one day of age and were fed a commercial milk replacer. The usual pattern was that on day seven, the calves were fed a 17% crude protein starter ration that contained 27% CSM. Free gossypol in the total ration ranged from 250 to 380 ppm. The calves were placed into group pens at 60 days of age and near that same time they were switched to a lower protein ration and the milk replacer was discontinued. The second ration was fed until the calves were 110 days old at which time they were sold. The basis for this high level of cottonseed meal feeding is not certain. One possibility is a ration formulation containing up to 27.9% CSM that was reported in at least two publications (Anonymous, 1968; Ensminger and Olentine, 1978).

In another published report, gossypol toxicosis occurred in young calves fed a liquid diet that contained 57% milk replacer and 43% CSM plus a little hay. The calves were fed this ration starting at three weeks of age and the free gossypol concentration in the diet dry matter was about 840 ppm. Some

of the calves receiving this ration died and others showed evidence of gossypol toxicity. The authors of the report have recommended that CSM should not be fed to young calves as part of a liquid feed (Rogers et al., 1975). This recommendation seems logical based on what is known about milk passage in young ruminants. When a young ruminant drinks milk, the esophageal groove closes and causes most of the liquid to bypass the rumen and go directly into the omasum. This of course would prevent any rumen detoxification of free gossypol regardless of rumen development.

It is certain that these practices did not have research support. In fact, there was literature available that would have indicated the possibility of problems from feeding rations containing 27% or 43% CSM (Hollon et al., 1958). Several research reports, one dating as far back as 1894 (Emery, 1894), indicated that young calves are sensitive to cottonseed meal poisoning (Hollon et al., 1958). Results of extensive research conducted in North Carolina to try to determine the safe level of dietary free gossypol for young calves indicated that the maximum concentration was 100 ppm. as was suggested for swine (Hollon et al., 1958). There was certainly adequate literature available on gossypol toxicity in young calves to prevent economics from being the sole factor determining levels of cottonseed meal use in baby calf rations.

In recent years, gossypol toxicity in young ruminants has received much attention in the popular farm press, even to the point of making producers and feed manufacturers wary of including the safe levels of CSM supported by available research data.

The symptoms of gossypol toxicosis reported in young ruminants are similar to those listed earlier for non-ruminants. These symptoms appear to be most prevalent after long-term feeding of unsafe levels (greater than 70 days, more than 100 ppm free gossypol). Death may result and may be preceded by the typical symptoms or it may be sudden. Fatalities have been reported to occur up to 30 days after the removal of CSM from the diet (Holmberg et al., 1988).

Based on the data currently available, the use of cottonseed meal in the diets of pre-ruminant calves or lambs requires careful attention to details and a precise understanding of what time period encompasses the pre-ruminant period. It appears that if any guess is to be made at a safe level, then the best guess at a recommendation for pre-ruminants is a maximum of 100 ppm in the total diet based primarily on the observation by Hollon et al. (1958) as well as on the observation that 100 ppm appears to be safe for growing swine. With baby calves consuming milk replacer, starter rations and sometimes hay, the gossypol concentration in the total diet is often difficult to calculate. It is important to remember that the recommendations are on a total intake basis. Also, considering recommendations on an amount per day basis instead of a percentage basis is best. With this in mind, the recommendation of less than 140mg of gossypol/cwt per day for young calves is useful (3.1 mg/kg bodyweight per day) (Hollon et al., 1958). Another important piece of information is the knowledge of the free gossypol concentration in the meal to be mixed. As noted earlier, the process by which oil is extracted from the seed determines to a large degree how much free gossypol will be in the meal. So, by knowing the normal ranges of gossypol in meals from the various processes, calculations can be performed to help prevent problems. Even better than using the normal ranges, testing of large individual lots of meal will help insure safe feeding. As with any feedstuff, the only sure way to know what is in the lot you are dealing with is to test that lot. Many analytical laboratories in the U.S. routinely test for free gossypol in meal or can direct feed manufacturers to labs that will perform the analysis, which is routine and not exorbitant in cost.

## **Lambs**

Young lambs are also fed meal in many of the cotton-producing areas. Lambs are not as likely to receive CSM as early as calves in the dairy industry. A common situation in sheep production is the need to wean lambs early at 6-8 weeks of age due to drought conditions often seen in sheep-producing

regions. Normal weaning age for range lambs is 16-20 weeks. Concern about feeding cottonseed meal to these early-weaned lambs was the reason for a research project recently completed in West Texas. Early-weaned lambs were fed rations containing the following types of meal: (1) mechanical; (2) direct solvent; (3) solvent with expanders, or (4) glandless, each at two protein levels (15-19%) for 98 days. The direct solvent meal contained 0.364% free gossypol, a higher than average value, and produced obvious signs of toxicosis and death in four out of 20 lambs when included at 23% of the high-protein ration. These four lambs were the only ones in the entire study showing visible symptoms of toxicosis.

A consistent finding in all lambs except those fed glandless cottonseed meal was an increase in red blood cell fragility, but this did not appear detrimental to the animals and feedlot performance was not affected in any of the remaining lambs. This highest level of free gossypol fed which caused the four deaths was 17.6 mg/lb. of body weight per day (38.7 mg/kg bodyweight per day) or approximately 820 ppm in the total diet dry matter (Calhoun et al, 1990; Calhoun, 1990). This work indicates that there is a limit to the amount of gossypol detoxified in the rumen of an early weaned lamb. This level was achieved with an unusually high free gossypol-containing direct solvent meal that comprised a higher percentage of the ration than was necessary to meet protein requirements (NRC, 1985).

Based on research completed in Oklahoma, workers had previously reported that 9.1-11.4 mg of gossypol/lb of body weight (20.0 mg/kg bodyweight per day) resulted in the death of feeder lambs before the end of a 30-day study (Morgan et al., 1988). The researchers in Texas fed graded levels up to twice this amount for twice the length of time before effects were seen. The difference seems to be attributable to the method of administration and the form of the gossypol. In the Oklahoma study, the gossypol was administered as gossypol acetic acid powder, which was dosed once per day. This would result in the rumen receiving the entire day's dose of free gossypol at one time. It is logical that this single, large dose of free gossypol could exceed the rumen's capacity for binding gossypol to soluble proteins. In the Texas study where a level of 17.6 mg of gossypol/lb of bodyweight was found to be toxic in four out of 20 lambs, the gossypol was contained in meal in mixed rations that were consumed throughout the day as in a production setting. Therefore, data from this study would more readily apply to the typical on-farm situation.

The Texas research group designed an experiment to determine if there were in fact differences in effect due to the route of gossypol administration. Eight-week-old lambs were fed one of four levels of free gossypol for 28 days. The gossypol was derived from either gossypol acetic acid administered by capsule, from direct solvent CSM or whole cottonseed. All lambs receiving gossypol at the two highest levels, 9.1 and 13.6 mg free gossypol/lb of body weight per day (20 and 30 mg/kg BW) died by day 15 of the study. This is consistent with the findings of Morgan et al.(1988). However, lambs receiving the same amount of gossypol from CSM or whole seed showed no clinical signs of gossypol toxicity and none died. The results of this study indicate that the single dose, oral administration of gossypol acetic acid is much more toxic than the same dose of gossypol fed in CSM or whole seed (Huston et al., 1990). Thus, results from studies using the single dose administration of gossypol acetic acid may not apply to the usual production situation where animals are receiving the gossypol dose through the consumption of CSM or whole cottonseed.

### **Lambs, cows and calves**

When considering early-weaned lambs, dairy cows and dairy calves, safe levels of free gossypol seem to be similar between the weaned lambs and mature cattle, while these levels differ from those that appear to be safe for young dairy calves. The reason for this appears to be the functionality of the rumen in each case. The 6- to 8-week-old weaned range lambs should have a functioning rumen. The new born dairy calf, however, is basically a non-ruminant, at least for the first several weeks of life. When put on

a bodyweight basis, the level of free gossypol resulting in toxicity in dairy cows as determined by Lindsey et al. (1980) is similar to the unsafe levels found for lambs by Calhoun et al. (1990). Both researchers found that levels around 18 mg/lb. of bodyweight (40 mg/kg bodyweight per day) resulted in deaths of some animals. In contrast, Hollon et al. (1958), based on their research suggested the limit for dairy calves at 1.4 mg/lb. of body weight (3.1 mg/kg bodyweight per day). It seems most obvious that the safe level of free gossypol for ruminants is mainly determined by the functionality of the rumen. Table 4 contains a summary of research results for ruminants.

### **Effects on fertility**

CSM has been a common source of supplemental protein for mature ruminants for many years. Much of this meal was fed to animals in the breeding herd. For years, brood cows and bulls have been helped through the winter with a couple of pounds of cottonseed cake or meal per day. As knowledge developed in recent decades regarding gossypol's effect on fertility in humans, questions have been raised regarding feeding large amounts of cottonseed or CSM to production animals. There is a limited amount of data in this area and projects are currently in progress to determine the effects of supplemental CSM or whole cottonseed on reproduction in mature ruminants. It is almost certain that the answer to this problem is not simply refraining from this type of supplementation. The intent of the research trials in this area is to determine how to use cottonseed products as a supplement for breeding animals and at the same time insure high reproductive efficiency. One thing hampering information on this subject is the preliminary nature of the research findings and subsequent recommendations. This is a relatively new area of research and as such, all the questions are not answered. It is also a very important area of study because of the important relationship between reproductive efficiency and profit.

### **Males**

The recent interest in gossypol's effect on male reproduction is a result of reports in the 1970s of the contraceptive effect of gossypol in men in China. These observations were the basis of research to determine the value of gossypol as a male anti-fertility agent in lab animals and humans. In the Chinese studies, a dose level of 20mg of free gossypol per day for two months resulted in infertility in 99.98% of the 4,000 men in the study (National Coordinating Group on Male Anti-Fertility Agents. 1978). Infertility resulting from gossypol has also been found to be reversible as early as six weeks after the removal of gossypol (Sotelo et al., 1982). The research area has been relatively active (Segal, 1985; Xue, 1985) and several researchers have attempted to determine if the results seen with men in China are applicable to other species.

Considering the large amount of cottonseed products fed to male breeding animals, projects were initiated to determine if whole seed or CSM feeding had any negative effects on reproductive ability in bulls and rams. Based on research completed up to this point, effects of gossypol (fed at levels discussed below) on male reproduction include immature spermatozoa, detached sperm heads and a decrease in number and mobility of spermatozoa. (Ruttle.1989). Some of the causes for these problems have been attributed to a degeneration of testicular tissue (Chase et al.. 1990a), a decrease in the number of sperm reaching maturation and an increase in the percent abnormal sperm. It was observed that it takes approximately six weeks after gossypol is removed from the diet for the semen to return to normal (Ruttle, 1989). In one study, yearling bulls fed whole seed that delivered 15 g of free gossypol per day for 60 days showed signs of a higher percent abnormal sperm (Stahnke. 1986).

In another research trial, young weaned bulls were fed whole seed, which delivered 27 g per day, or CSM which delivered 1.9 g free gossypol per day for more than 400 days. The bulls consuming whole seed were later in reaching puberty and bulls on both treatments had lower quality semen than bulls on a gossypol-free diet. Both sets of bulls recovered when gossypol was removed from the diet

(Chase et al., 1989). A critical factor in this study is the extremely long duration of intake of gossypol. The practice of feeding this type of ration to bulls every day for more than 400 days is not likely to occur in a commercial situation. Most commercial bulls would receive a few pounds of CSM per day before and during breeding season or through the winter which should not result in the accumulative effect seen in the bulls fed for over 400 days. In another research trial, 2.7 g of free gossypol was fed in a mixed ration containing whole cottonseed for 120 days with no adverse effects on any semen parameters that were measured (Smith et al., 1989).

Another study has recently been completed in Texas to determine if gossypol intake had any effects on testosterone secretion. Young bulls were used in the study and were fed diets containing whole cottonseed, CSM or soybean meal. Testosterone secretion measured before, at and after puberty was not affected by gossypol intake (Chase et al., 1990b).

In routine feeding practices, the use of 3-5 lb. of CSM fed either as a supplement or contained in a mixed feed is most unlikely to expose the breeding animals to the levels of gossypol needed to cause reproductive problems. The effects on semen quality seen in the research trials mentioned did not determine if it left the bulls infertile. It is important to point out at this time that it has not been proven that feeding CSM would negatively affect actual breeding percentages in a range or pasture breeding situation. It is, of course, more likely that the feeding of whole cottonseed might result in fertility problems compared with CSM simply because of the higher concentration of free gossypol. Simply put, more work needs to be done in this critical area.

## **Females**

It is reasoned that if gossypol has an effect on testicular function in males, it may also have an effect on the ovaries in females. In a review by Nomeir and Abou-Donia (1985), nine studies were cited where gossypol did not cause females of several species to become infertile. In one of these studies, Wu et al. (1981) observed altered follicle stimulating hormone secretion in female hamsters consuming gossypol. Many questions remain in the area of gossypol and female reproduction. There have been very few producer reports of reproductive problems that were thought to be due to cottonseed feeding. It is not at all certain that these problems could be due to gossypol or anything in the cottonseed, since there is a lack of scientifically controlled investigation. Reports of female dairy cow infertility have been uncommon and have not pointed to a specific problem.

## **Embryos**

There is limited data on the effect of gossypol on embryos. Zirkle et al (1988) discovered a dose-dependent effect of gossypol on bovine embryos, *in vitro*. This action, the result of the *in vitro* culturing of embryos in fluid containing from 1-30 micrograms of free gossypol appeared to be a physiological effect upon the embryo itself and not a mutational effect. It is unlikely that an embryo *in utero* could be exposed to that amount of gossypol when considering the dam's rumen detoxification ability. More work, preferably, *in vivo*, is needed to determine if this is a problem that could effect reproductive efficiency in a production situation.

## **Conclusion**

The one final word that can be said now is the classic acknowledgement that more information is needed in each of these specific production situations. The research trials planned and those in progress may help to answer the large number of questions that have been raised by the current emphasis on even greater animal productivity and the need for economic efficiency. Once again, it is certain that the answer is not the total removal of all cottonseed products from the rations of all producing classes.

Cottonseed, CSM and CSH have been shown to be fine sources of protein, energy and fiber and are palatable to many classes of livestock. The presence of gossypol has always required that cottonseed products be fed with attention and care if the maximum benefit is to be derived. The development of CSM as a protein concentrate over the last century has been dependent on applying carefully conducted research studies to actual feeding practices. This has been the firm basis of cottonseed product use in the past. In this regard, nothing has changed. Knowing the limitations of cottonseed product applications in animal feeding practices will allow for their safe and efficient use.

Editor's Note – Steve D. Martin is assistant to the director of research and education for the National Cottonseed Products Association. Reprinted with permission from Feedstuffs, August 6, 1990.

**TABLE 1. Gossypol Levels Seen in Common Cottonseed Products.**

Product	% total gossypol	% free gossypol	Reference
Cottonseed Kernel <sup>a</sup>	— 0.88-1.0 <sup>c</sup> 0.39-1.7 <sup>c</sup>	0.4-1.4 <sup>b</sup> 0.75-0.86 <sup>c</sup> 0.39-1.7 <sup>c</sup>	Altschul et al., 1958 Cherry et al., 1978 Pons et al., 1953
Whole Cottonseed	—	0.47-0.63 <sup>d</sup>	Calhoun, 1989
Delinted Whole Cottonseed	—	0.47-0.53 <sup>b</sup>	Calculated <sup>e</sup>
Cottonseed Meal	—	0.02-0.05 <sup>b</sup>	Berardi & Goldblatt, 1980
Screw Press	1.02 <sup>d</sup>	0.04 <sup>d</sup>	NCPA, 1970
Prepress Solvent	—	0.02-0.07 <sup>b</sup>	Berardi & Goldblatt, 1980
Direct Solvent	1.13 <sup>d</sup>	0.05 <sup>d</sup>	NCPA, 1970
Solvent (expander process)	—	0.1-0.5 <sup>b</sup>	Berardi & Goldblatt, 1980
Cottonseed Hulls	1.04 <sup>d</sup>	0.3 <sup>d</sup>	NCPA, 1970
Glandless Whole Cottonseed	—	0.06-0.1 <sup>d</sup>	Calhoun, 1989
	0.01 <sup>c</sup>	0.06 <sup>d</sup>	NCPA, Unpublished data
		—	Calculated <sup>f</sup>

<sup>a</sup> The kernel is reported to comprise approximately 55% of ginned cottonseed (Tharp, 1948).

<sup>b</sup> Dry matter/as fed basis not reported.

<sup>c</sup> Dry matter basis.

<sup>d</sup> As fed basis.

<sup>e</sup> Calculated from Cherry et al., 1978 and Norris, 1982.

<sup>f</sup> Calculated from Lusas and Jividen, 1987 and Tharp, 1948.

**TABLE 2. Reported "Effect" and "No Effect" levels of free gossypol in nonruminants from research trials.**

Class of Livestock	Free Gossypol Intake (ppm)		Reference
	Effect	No Effect	
Yearling horses	—	115 <sup>a</sup>	Potter, 1981
Weanling horses	—	348 <sup>a</sup>	McCall, 1982
Catfish	—	900 <sup>b</sup>	Dorsa et al., 1982
Tilapia	—	1,800 <sup>bc</sup>	Robinson et al., 1984
Rainbow trout	1,000 <sup>ac</sup>	250 <sup>ac</sup>	Roehm et al., 1967
Shrimp ( <i>Penaeus vannamei</i> )	—	170 <sup>b</sup>	Fernandez, 1987

<sup>a</sup> Dry matter/as fed basis not reported.

<sup>b</sup> Dry matter basis.

<sup>c</sup> Fed as gossypol acetic acid.

**TABLE 3. 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:1 ppm Free Gossypol)	Waldroup, 1981 NCPA, 1970
Layers	50	150 ppm (4 ppm Fe:1 ppm Free Gossypol)	Waldroup, 1981 NCPA, 1970
Swine	100	400 ppm (1 ppm Fe:1 ppm Free Gossypol)	Tanksley & Knabe, 1981 NCPA, 1970

**TABLE 4. Reported "effect" and "no effect" levels of free gossypol in ruminants from research trials.**

Class of Livestock	Free Gossypol Intake (ppm)		Reference
	Effect	No Effect	
Pre-ruminant calves	—	100 <sup>a</sup>	Hollon et al., 1958
Young lambs	824 <sup>b</sup>	—	Calhoun, 1989
Mature dairy cows	1,076 <sup>b</sup>	—	Lindsey et al., 1980

<sup>a</sup> Dry matter/as fed basis not reported.

<sup>b</sup> Dry matter basis.

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