



## Plasma gossypol dynamics in white-tailed deer: Implications for whole cottonseed as a supplemental feed

S.L. Bullock<sup>a,\*</sup>, D.G. Hewitt<sup>a</sup>, R.L. Stanko<sup>a</sup>, M.K. Dowd<sup>b</sup>, J. Rutledge<sup>c</sup>, D.A. Draeger<sup>d</sup>

<sup>a</sup> Department of Animal and Wildlife Science, Texas A&M University – Kingsville, 700 University Boulevard, MSC 218, Kingsville, TX 78363, USA

<sup>b</sup> USDA-ARS, Southern Regional Research Center, New Orleans, LA 70124, USA

<sup>c</sup> Texas Parks and Wildlife Department, Carrizo Springs, TX 78834, USA

<sup>d</sup> Comanche Ranch, Carrizo Springs, TX 78834, USA

### ARTICLE INFO

#### Article history:

Received 27 December 2009

Received in revised form 27 May 2010

Accepted 31 May 2010

Available online 6 July 2010

#### Keywords:

Free-ranging deer  
Gossypol  
Gossypol stabilization  
Plasma gossypol  
Supplemental feed  
White-tailed deer  
Whole cottonseed

### ABSTRACT

Whole cottonseed (WCS) is a potential supplemental feed for white-tailed deer (*Odocoileus virginianus*) in rangeland conditions because of its high digestible energy and protein content, moderate fiber content, and resistance to degradation in moist conditions. WCS also contains the polyphenolic secondary metabolite gossypol, which reduces palatability to non-target monogastric species but may be of concern for deer nutrition. Plasma gossypol stabilization when fed a constant dry matter intake, plasma gossypol depletion after WCS was removed from the diet, and the relationship between WCS consumption and plasma gossypol concentration was studied in 10 mature male ( $N=5$ ) and female ( $N=5$ ) captive white-tailed deer. Consumption of WCS by 73 free-ranging white-tailed deer (59 males, 14 females) was estimated using results of the captive study. Plasma gossypol concentrations declined exponentially and averaged  $0.74 \mu\text{g}/\text{mL}$  35 days after WCS was removed from the diet. Plasma gossypol concentration was linearly related to WCS consumption ( $P < 0.001$ ), with females having  $0.35 \mu\text{g}/\text{mL}$  greater ( $P = 0.04$ ) plasma gossypol than males for a given rate of dry matter consumption. All female and 93% of male white-tailed deer captured in WCS supplemented pastures had detectable plasma gossypol. Female averaged  $1.88 \mu\text{g}/\text{mL}$  of plasma gossypol and males averaged  $4.84 \mu\text{g}/\text{mL}$  of plasma gossypol. Based on the captive deer data, these plasma values suggest an average WCS consumption of  $\sim 2.6 \text{ g}/\text{kg BW}/\text{day}$  for female free-ranging deer and  $\sim 5.6 \text{ g}/\text{kg BW}/\text{day}$  for male free-ranging deer. Inferentially, a large proportion of free-ranging white-tailed deer in rangeland conditions will consume WCS, with females consuming  $125 \text{ g WCS}/\text{day}$  and males consuming  $428 \text{ g WCS}/\text{day}$ . That plasma gossypol levels decrease rapidly after cottonseed is removed from the diet suggests that the long withdrawal periods often used prior to breeding season may not be needed. However, although 93% of gossypol was eliminated from the animals after a five-week withdrawal period, a small amount of gossypol can still be detected. While our preliminary data on these animals suggests that these levels are not detrimental to animal health or reproduction, ranch managers may want to take a conservative approach to the feeding of WCS until these questions are answered.

© 2010 Elsevier B.V. All rights reserved.

### 1. Introduction

White-tailed deer (*Odocoileus virginianus*) are important ecologically, economically, and recreationally in North America. Increasing numbers of landowners supplement or replace traditional agricultural income by leasing hunt-

\* Corresponding author. Tel.: +1 270 724 2185.

E-mail address: [sarah.lynn.bullock@hotmail.com](mailto:sarah.lynn.bullock@hotmail.com) (S.L. Bullock).

ing rights or selling hunts for white-tailed deer. Because landowner income is influenced by productivity of deer herds, many deer managers provide supplemental feed (Lewis and Rongstad, 1998; Bartoskewitz et al., 2003) to increase body mass, antler size, and reproductive performance.

Whole cottonseed (WCS), a by-product from the cotton (genus *Gossypium*) ginning process, is considered a good feedstuff for ruminants due to its high content of digestible energy and protein and moderate fiber (Risco and Chase, 1997). Because WCS does not degrade or mold as readily as pelleted feeds in moist/damp conditions, it may represent a viable supplement on rangelands where infrastructure may not be present to protect feed from the elements and where it is not possible to regularly monitor feed condition. However, WCS contains a polyphenolic pigment gossypol, which can cause toxicosis if consumed at high doses over several weeks (Risco and Chase, 1997).

Gossypol is more toxic to monogastric animals because they lack foregut microorganisms that help to detoxify plant compounds (Reiser and Fu, 1962; Smith, 1992; Risco and Chase, 1997). Therefore, monogastric animals such as feral pigs (*Sus scrofa*) and raccoons (*Procyon lotor*), which are often non-target consumers of supplemental feed, may be reluctant to eat large amounts of WCS (Taylor, 2003) unless climatic conditions cause them to pursue WCS as an alternative forage. Although adult ruminants have mechanisms to protect against toxicity of many plant secondary chemicals, high gossypol doses can overwhelm neutralizing mechanisms causing toxicosis (Lindsey et al., 1980; Smalley and Bicknell, 1982; Randel et al., 1992; Risco et al., 1993). In male ruminants, gossypol toxicity can manifest as reproductive problems, such as increased sperm abnormalities, reduced sperm motility, reduced scrotal circumference, and suppressed testosterone concentrations in cattle (Smalley and Bicknell, 1982; Risco et al., 1993; Chenoweth et al., 1994), goats (Solaiman et al., 2009), fallow deer (Brown, 2001), and red-deer (Gizejewski et al., 2008). Female ruminants appear less susceptible to gossypol induced reproductive problems, although bovine embryos cultured *in vitro* were sensitive to gossypol (Brocas et al., 1997). Physiological symptoms of gossypol toxicity in livestock include sudden death, dyspnea, erythrocyte fragility, and anemia (Lindsey et al., 1980; Smalley and Bicknell, 1982; Morgan et al., 1988; Gray et al., 1993; Solaiman et al., 2009).

Understanding plasma gossypol dynamics in white-tailed deer will aid in assessment of WCS as a supplement for deer and in designing feeding programs using WCS to meet goals of deer managers. The objectives of this study were to determine the time necessary for plasma gossypol concentration to stabilize in white-tailed deer fed a constant daily dry matter (DM) of WCS, determine the time necessary for plasma gossypol concentrations to decline after WCS is removed from the diet, and evaluate the relationship between WCS consumption and equilibrium plasma gossypol concentration. Plasma gossypol concentrations found in captive deer were used to estimate average daily DM intake of WCS by free-ranging deer.

## 2. Materials and methods

### 2.1. Animals and facilities

Experiments with captive deer were conducted at the Albert and Margaret Alkek Ungulate Research Facility at Texas A&M University – Kingsville, in Texas with five mature (>3 years of age) males and five mature females white-tailed deer. Female deer used were pregnant having been bred between December 2007 and February 2008. Procedures were approved by the Texas A&M University – Kingsville Institutional Animal Care and Use Committee (protocol approval #2006-01-17). All captive deer were treated for internal parasites (Ivermectin: Merial, Division of Merck and Co., Rahway, NJ) before experimentation.

Free-ranging white-tailed deer were sampled for plasma gossypol determination from a private ranch (28°38'25"N, 100°09'52"W) in the western Rio Grande Plains of Dimmit and Maverick Counties, Texas. Elevations average 250 m with level to rolling topography. Average annual rainfall in the nearest town, Carrizo Springs, Texas, is 56 cm (Leffler, 2008), but is highly variable with a coefficient of variation of annual precipitation approaching 40% (Norwine, 1978). Clay to loamy sand soils support diverse shrubs and savanna communities representative of Tamaulipan thornscrub (Fulbright, 2001). Common woody species are black brush (*Acacia rigida*), brasil (*Condalia hookeri*), cenizo (*Leucophyllum frutescens*), granjeno (*Celtis pallida*), guajillo (*Acacia berlandieri*), guayacan (*Guayacum angustifolium*), kidneywood (*Eysenhardtia texana*), mesquite (*Prosopis glandulosa*), twisted acacia (*Acacia schaffneri*), and white brush (*Aloysia gratissima*). Cacti common to the area are prickly pear (*Opuntia engelmanni*) and tasajillo (*Opuntia leptocaulis*). Common grass genera of the area consist of *Aristida*, *Bouteloua*, *Chloris*, *Eragrostis*, *Pappaphorum*, *Pennisetum*, and *Tridens* spp.

### 2.2. Dose–response methodology

Ten mature captive-raised white-tailed deer (5 males; 5 females) with prior experience consuming WCS were weighed, randomly assigned to one of the five test diets, and placed in individual pens (4 m × 5 m) for 10 weeks beginning on 5 May 2008. The five test diets had a target DM composition of 10–50% WCS in 10% increments with chopped alfalfa constituting the remainder of the diet. Total gossypol concentration of the WCS was 0.56% (58% (+)-isomer; 42% (–)-isomer) on a DM basis. One female deer assigned to 50% WCS diet refused to eat the target amount of WCS, and after 1 week was reassigned to a diet with a lower percent WCS. The female deer originally assigned to this lower WCS diet was reassigned to the 50% diet. The first day of the trial for the female deer assigned to the 50% diet started on day 12 when the new diet was assigned. The female deer that refused to consume the higher proportion of WCS had consumed the appropriate amount of WCS required in her new diet. Eight (4 males; 4 females) of the 10 deer had been consuming a non-WCS diet for over 6 months prior to the trials. The remaining 2 deer (1 male; 1 female) had been consuming a non-WCS diet for 35 days; therefore, plasma gossypol concentrations of 1.07 and 0.80 µg/mL were present in these animals at the beginning of the trial. Water was available ad libitum throughout the experiment. Orts were removed at 09:00 h daily, dried, and weighed. Body weight (BW) was recorded every 10 days. Plasma samples were collected via jugular venipuncture every 9–12 days at 18:00 h. Plasma gossypol level was considered to have reached a steady-state, if concentrations differed by <0.75 µg/mL over two sample periods (20 days).

### 2.3. Gossypol depletion methodology

On 11 June 2007, 10 captive mature white-tailed deer (5 males; 5 females) that were used in the dose–response trial were separated by sex, placed in two 0.1-ha communal pens, and fed diets of approximately 50:50 ratio of WCS:chopped alfalfa (*Medicago sativa*). On 12 September 2007, deer were moved to individual pens and fed the 50:50 ratio of WCS:chopped alfalfa on a DM basis through 30 October 2007. After 20 weeks of consuming 50% WCS, plasma samples were collected via jugular venipuncture, deer were then returned to their respective 0.1 ha communal pens, and diets were changed to ad libitum chopped alfalfa and a commercial pellet containing no cottonseed products. Commercial pellets were formulated to meet the nutritional requirements of white-tailed deer (441 Droptine 17%, Lindner's Feed and Milling, Comfort, TX). Water was provided ad libitum throughout the experiment. Plasma samples

were collected every 7 days at 18:00 h for 5 weeks to determine plasma gossypol depletion.

#### 2.4. Collection of blood samples and chemical analysis

Heparinized BD Vacutainer™ tubes (Becton Dickinson, Franklin Lakes, NJ) were used to collect whole blood. Blood samples were placed in a refrigeration unit for 2–4 h and centrifuged at  $1400 \times g$  and  $37^\circ\text{C}$  for 20 min. One milliliter aliquots of plasma were freeze dried and stored at  $-70^\circ\text{C}$  until analyzed for gossypol.

Samples of WCS and alfalfa were collected periodically throughout experiments. Samples of identical feedstuffs (i.e. WCS or alfalfa) were mixed and analyzed for aflatoxin and gossypol concentration. Samples of WCS were sent to Pope Testing Laboratories, Inc. (Irving, Texas) for aflatoxin analysis. Aflatoxin was determined using an immunoaffinity column (Aflatest®) method reported by AOAC International (1995). No detectable aflatoxin was found in any WCS used in experimentation.

Gossypol in WCS was determined by separating representative samples into hull and kernel fractions. A slightly modified version of the HPLC method reported by Hron et al. (1999) was used to determine gossypol in the kernel fraction. Kernel gossypol was then converted into WCS gossypol by accounting for the weight fractions, given that there is no significant amount of gossypol in cottonseed hulls (Yu et al., 1993).

Plasma gossypol was determined by a similar procedure, but scaled-down to accommodate the processing of samples in small volume tubes. Briefly, plasma (1 mL) was transferred into a 2-mL microcentrifuge tube. After freeze-drying the sample, 380  $\mu\text{L}$  of a complexing reagent (85:5:10 (v/v/v) of dimethylformamide/*R*-(–)-2-amino-1-propanol/glacial acetic acid) and one glass bead (3 mm diameter) were added to the tube. The sample was pulverized with a Beadbeater-8 mill (Bio-spec Products, Bartlesville, OK) for 2 min at 90% maximal speed. After brief centrifugation to ensure that all of the solids were fully wetted by the complexing reagent, the tube was heated in a dry-block bath at  $95^\circ\text{C}$  for 30 min to promote the formation of a gossypol Schiff's base complex with the complexing amine. Upon cooling, 1.52 mL of HPLC mobile phase (65/35 acetonitrile/phosphate buffer; 10 mM; pH = 3) was added; the tube was briefly vortex mixed and was centrifuged for 5 min at  $12,000 \times g$  to pelletize the particulate matter. A 1 mL sample of the clear supernatant was then transferred to an HPLC vial for analysis.

For seed and deer plasma samples (both captive and free-ranging), the gossypol complex was detected with an HP series 1100 HPLC system (Hewlett-Packard Company, Palo Alto, CA) consisting of an autoinjector, isocratic pump, and photodiode array detector. A SGE Inertsil ODS-2 column (4 mm i.d.  $\times$  100 mL) was used to separate the individual gossypol enantiomers. The HPLC mobile phase was pumped at 1 mL/min. The full spectrum of the detector (210–700 nm) was monitored to identify the elution peaks corresponding to the gossypol Schiff's bases, and 254 nm was used for analytical determinations. Standard response curves were developed for the (+)- and (–)-gossypol from a preparation of gossypol:acetic acid (1:1), a racemic compound that contains 89.64% gossypol.

#### 2.5. Free-ranging deer analysis

Free-ranging white-tailed deer were sampled from a private ranch near Carrizo Springs, TX. Treatment deer were captured via net gun from helicopter within a 6300 ha area with WCS supplemented ad libitum since February 2006. Whole cottonseed was fed in welded wire feeders, 53 cm in diameter and 122 cm high, at a density of 1 feed station/81 ha. Control deer were captured from a 7500 ha area with no supplemental feed. On 7 May 2008, blood samples were taken from 14 female deer captured from pastures supplemented with WCS and 15 female deer from unsupplemented pastures as controls. Plasma gossypol concentrations were assessed for females from both WCS supplemented and unsupplemented pastures. On 15 October 2008, 59 male white-tailed deer were collected by net gun fired from a helicopter in pastures supplemented with WCS, a blood sample taken, and plasma gossypol measured.

#### 2.6. Statistical analysis

The relationship between WCS consumption and stabilized plasma gossypol concentration was assessed with linear regression analysis with SAS 9.1.3 (SAS Institute Inc., Cary, NC). Differences between the sexes in gossypol depletion over time were assessed with the MIXED pro-

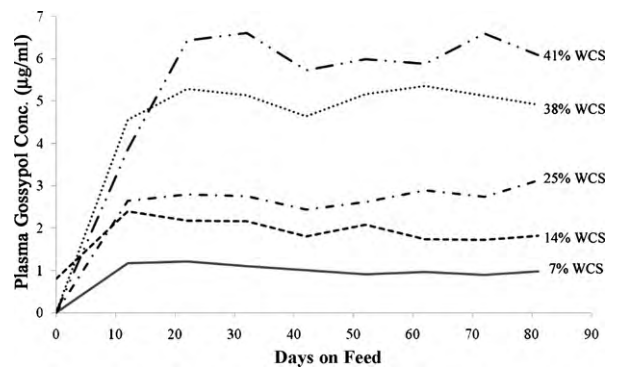


Fig. 1. Plasma gossypol concentration trends over 81 days for five mature captive-raised male white-tailed deer consuming whole cottonseed (7–41%) diet with chopped alfalfa (93–59%) constituting the remainder of the diet.

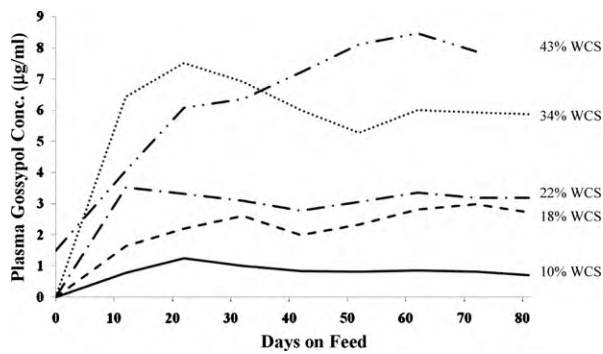
cedure of SAS for repeated measures with a first-order autoregressive model and a Kenward–Roger correction (Littell et al., 2006). Week, sex, and week  $\times$  sex were included in the model to assess if plasma gossypol concentration responded similarly in males and females. CONTRAST and ESTIMATE statements were used to determine when concentrations of plasma gossypol did not change between sampling periods, suggesting either depletion or a change in dynamics of plasma gossypol, depending on whether the concentration reached zero. Means are presented with standard errors (S.E.). Differences between group means were considered significant if  $P \leq 0.05$ .

### 3. Results

#### 3.1. Dose–response

Male white-tailed deer in captivity on average consumed 1047 g (SE  $\pm$  141 g) of WCS and 326 g (SE  $\pm$  77 g) of alfalfa daily on a DM basis. Female white-tailed deer in captivity on average consumed 589 g (SE  $\pm$  43 g) of WCS and 207 g (SE  $\pm$  51 g) of alfalfa daily on a DM basis. Consumption of WCS ranged from 1.52 to 7.25 g/kg BW/day, depending on the target amount of WCS in the diet. There was a significant decrease in total DM intake (g/kg BW/day) of male deer as the proportion of WCS increased ( $t_3 = 3.84$ ,  $P = 0.03$ ,  $y = 1.26 - 0.05x$ ). Female deer did not differ in total DM intake as the proportion of WCS in the diet increased ( $t_3 = 0.31$ ,  $P = 0.78$ ). For deer fed diets containing  $\leq 25\%$  WCS, plasma gossypol stabilized in both sexes after about 32 days (Figs. 1 and 2). Plasma gossypol concentration of the males fed 38% dietary WCS also stabilized within 32 days. However, one male and one female deer consuming diets containing 41 and 34% dietary WCS, respectively, required 62 days to reach steady plasma gossypol concentrations (Figs. 1 and 2). The remaining female fed 43% dietary WCS did not stabilize until the last sampling period at day 72 (Fig. 2).

There was a positive linear relationship between plasma gossypol concentration and DM WCS consumption for all deer ( $t_7 = 8.22$ ,  $R^2_{\text{adj}} = 0.88$ ,  $P < 0.001$ ; Fig. 3). Sexes differed slightly with females having 0.35  $\mu\text{g}/\text{mL}$  greater ( $t_1 = 2.60$ , SE  $\pm$  0.53,  $P = 0.04$ ) plasma gossypol than males for a given DM consumption of WCS. No interaction was detected between sex and WCS consumption ( $t_1 = 1.60$ , SE  $\pm$  0.25,  $P = 0.16$ ; Fig. 3). Plasma gossypol concentrations increased



**Fig. 2.** Plasma gossypol concentration trends over 81 days for 5 mature captive-raised female white-tailed deer consuming a whole cottonseed (10–43%) diet with chopped alfalfa (90–57%) constituting the remainder of the diet.

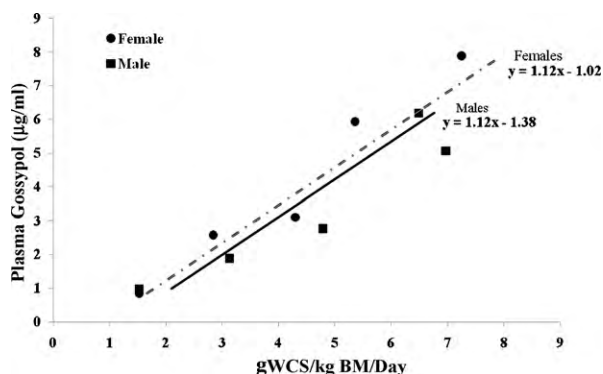
1.1  $\mu\text{g}/\text{mL}$  ( $\text{SE} \pm 0.14$ ) for each 1 g WCS/kg BW/day increase in intake, regardless of sex (Fig. 3).

### 3.2. Gossypol depletion

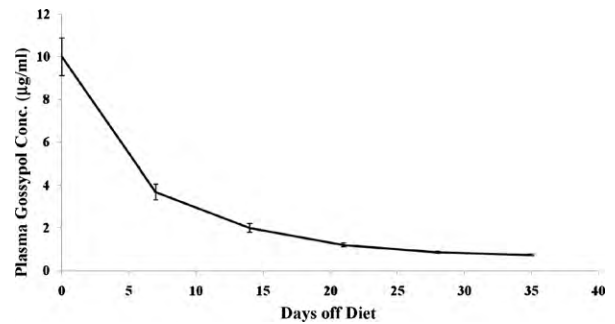
Depletion of plasma gossypol did not differ between sexes ( $F_{1,16.3} = 0.13$ ,  $P = 0.728$ ) and therefore data were combined. Initially, the animals had an average gossypol plasma concentration of 10.0  $\mu\text{g}/\text{mL}$ . Relative to this initial concentration, plasma gossypol decreased by 63% ( $-6.30 \mu\text{g}/\text{mL}$ ,  $t_{34.6} = 18.12$ ,  $\text{SE} \pm 0.35$ ,  $P < 0.001$ ), 17% ( $-1.73 \mu\text{g}/\text{mL}$ ,  $t_{36.8} = 4.82$ ,  $\text{SE} \pm 0.36$ ,  $P < 0.001$ ), 8% ( $-0.77 \mu\text{g}/\text{mL}$ ,  $t_{34.6} = 2.16$ ,  $\text{SE} \pm 0.35$ ,  $P = 0.04$ ), 3% ( $-0.33 \mu\text{g}/\text{mL}$ ,  $t_{34.6} = 0.92$ ,  $\text{SE} \pm 0.35$ ,  $P = 0.36$ ), and 2% ( $-0.13 \mu\text{g}/\text{mL}$ ,  $t_{34.6} = 0.37$ ,  $\text{SE} \pm 0.35$ ,  $P = 0.72$ ) over a 5-week period (Fig. 4). At day 35, plasma gossypol averaged 0.74  $\mu\text{g}/\text{mL}$  ( $\text{SE} \pm 0.40$ ).

### 3.3. Free-ranging deer

All female white-tailed deer captured in WCS supplemented pastures ( $N = 14$ ) had detectable plasma gossypol, which averaged 1.88  $\mu\text{g}/\text{mL}$  (range = 0.91–4.28  $\mu\text{g}/\text{mL}$ ). This concentration of plasma



**Fig. 3.** Relationship between plasma gossypol concentration and whole cottonseed (WCS) consumption of mature captive-raised male ( $N = 5$ ) and female ( $N = 5$ ) white-tailed deer consuming between 7 and 43% WCS diet ( $R^2_{\text{adj}} = 0.88$ ). Relationship for males is indicated by a solid line, while female relationship is indicated by a dashed line.



**Fig. 4.** Combined depletion of plasma gossypol, with associated standard error, in 10 mature captive-raised white-tailed deer (five females and five males) across 35 days following a 5-month diet comprised of 50% whole cottonseed.

gossypol equated to an estimated WCS consumption of 125 g/day (range = 93–221 g/day) or 2.60 g/kg BW/day (range = 1.72–4.74 g/kg BW/day) based on the correlations obtained from the captive deer. No females captured in unsupplemented pastures had detectable plasma gossypol.

Fifty-five of 59 (93%) male deer had measurable plasma gossypol. Average plasma gossypol concentration of the 55 male animals was 4.84  $\mu\text{g}/\text{mL}$  (range = 0.92–11.08), suggesting an estimated WCS consumption of 5.55 g/kg BW/day (range = 2.06–11.12), which equated to gossypol consumption of 31 mg/kg BW/day, based on the correlations obtained for captive deer. Daily WCS consumption for the 55 males was estimated at 428 g (range = 128–686 g). Eighteen males had plasma gossypol concentrations exceeding 6.19  $\mu\text{g}/\text{mL}$ , which is the upper bounds of the relationship established with deer in captivity. These 18 males had an average plasma gossypol concentration of 7.91  $\mu\text{g}/\text{mL}$  ( $\text{CI} = 7.27\text{--}8.55$ ); therefore, males exceeding the highest established consumption were said to consume at least 6.76 g WCS/kg BW/day.

## 4. Discussion

Daily DM consumption of WCS in the dose-response study stabilized during the first 2 weeks and remained constant thereafter. Plasma gossypol in captive deer stabilized after 4 weeks of feeding at low to moderate levels of WCS ( $\leq 25\%$ ), which is similar to the 4–6 weeks observed for plasma gossypol concentrations to plateau in beef heifers (Velasquez-Pereira et al., 1998), dairy cows (Mena et al., 2001), and lambs (Dowd and Calhoun, 1996) that were fed either cottonseed or cottonseed meal. These results indicate that gossypol absorption and elimination take several days, or even weeks, to equilibrate within an animal. Once equilibrated, measurements of plasma gossypol should not be sensitive to small variations in daily intake of WCS and estimates of WCS intake derived from plasma gossypol concentration will represent average WCS intake over several days. The long time needed for gossypol to reach a steady-state in the plasma could be explained by the complex interaction of rumen microbe adaptation, tissue storage, rates of gastrointestinal absorption, availability of substrates for conjugative biotransformation, detoxification enzyme concentration



and activity, and pathways of biotransformation (Smith, 1992).

Male white-tailed deer decreased total DM intake as WCS intake increased, which can be partially explained by high digestible energy (19.2 kJ/g) in whole cottonseed (Bullock, 2009). These results indicate that when males consume diets with a larger percent of WCS, energy requirements were met with less DM. In support, white-tailed deer consuming a 50% WCS diet with alfalfa consumed 7.7% more digestible energy than deer eating a pelleted diet designed to nutritional requirements of white-tailed deer (Bullock, 2009).

Feeding patterns suggested that too large of a proportion of WCS in the diet resulted in some reluctance to accept the feed (Provenza, 1995), possible related to the bitterness of the seed, gossypol toxicity, aversion to seed's high oil content, or a combination of these factors. When feed was presented each morning, white-tailed deer preferentially ate WCS if the proportion was  $\leq 25\%$  of total daily intake. If WCS was  $>25\%$  of the diet, however, the chopped alfalfa was eaten first before consuming the WCS portion of the diet. Thus, diets consisting of  $>25\%$  WCS may have been challenging the deer's ability to metabolize, excrete (Marsh et al., 2006), and store gossypol, causing the deer to preferentially consume alfalfa.

The difference in plasma gossypol concentration between males and females for a given amount of WCS consumed may indicate that small differences exist in gossypol metabolism by sex. This difference may be due to larger rumen capacity and slower rumen turnover rate in males (Barboza and Bowyer, 2000). Slower ruminal turnover may give the microorganisms more time to bind or metabolize gossypol. Faster passage rate in females may have been exacerbated because they were in their last trimester of pregnancy during experimentation. Passage rate increases in gravid females ruminants (Graham and Williams, 1962; Faichney and White, 1988; Gunter et al., 1990).

Plasma gossypol concentrations declined by 80% during the first 14 days after WCS was removed from the diet; however, some gossypol was still detectable in plasma of all deer after 35 days (0.74  $\mu\text{g}/\text{mL}$ ). Similarly, plasma gossypol concentrations of white-tailed deer consuming a 37% WCS diet declined from 8.53 to 0.42  $\mu\text{g}/\text{mL}$  in 5 weeks (K. A. Kelley, Texas A&M University-Kingsville, unpublished data). Lambs behaved similarly when removed from a cottonseed meal-based diet (Dowd and Calhoun, 1996) in that plasma gossypol levels declined by greater than 90% but some levels of gossypol were still detected 6 wk after having cottonseed meal removed from the diet. For comparison, plasma gossypol clearance rate is slower than that of commercial anthelmintics. Doramectin<sup>®</sup> declined to 0 ppb in plasma of white-tailed deer after 14 days (Pound et al., 2004b). Ivermectin<sup>®</sup> given to deer orally or subcutaneously took 21 and 14 days, respectively, to clear from the blood (Pound et al., 2004a). One explanation for the slow depletion of plasma gossypol is that gossypol accumulates in liver, kidney, heart, and other tissues (Dowd and Calhoun, 1996; Gamboa et al., 2001; Velasquez-Pereira et al., 1998). Gossypol stored in organ tissue may replenish plasma gossypol as it is excreted.

Quantifying supplemental feed consumption by free-ranging cervids has been exceedingly difficult. Because gossypol is unique to cotton plants and because plasma gossypol is a function of WCS consumption, reliable estimates of WCS consumption by free-ranging deer are possible. This technique will enable assessment of the effectiveness of supplemental feed programs and differences in WCS use by individual deer, gender, and age. For example, a higher proportion of white-tailed deer ate WCS (males = 93%; females = 100%) than ate a pelleted supplement (males  $<60\%$ ; females  $\leq 30\%$ ) on three ranches in southern Texas (Bartoskewitz et al., 2003). These three ranches had been providing pelleted feed for  $>5$  years and had feeder densities ranging from 1/165 to 1/405 ha. Two of the ranches had timed feeders that distributed a limited amount of feed/day, while the third ranch provided feed ad libitum. The greater proportion of deer eating WCS may have been affected by differences in available forage, feed site density, feeder type, hunting pressure, and season. Nonetheless, the density of feeders (1/81 ha) used to feed WCS as a supplement on our study site was remarkably effective, as measured by the percent of deer eating the supplement.

Thirty-three percent (18 of 55) of the free-ranging males consuming cottonseed had plasma gossypol concentrations greater than the upper limit of the regression developed using captive deer (6.19  $\mu\text{g}/\text{mL}$ ), which may bias the estimated consumption of WCS for individual free-ranging males. Plasma gossypol concentrations of females fell within the range of data from the captive deer study suggesting estimates of consumption are reliable.

## 5. Conclusions

A large proportion of the free-ranging white-tailed deer in rangeland conditions of our study consumed WCS. Because WCS has high concentrations of digestible energy, protein, and oils (Bullock, 2009), WCS may be particularly beneficial during summer, when nutrient requirements are high because female deer are raising fawns and males are in the early stages of building reserves for the breeding season. Compounding these nutritional challenges, quality of forage eaten by deer may be poor during summer in southern Texas rangelands (Meyer et al., 1984). Supplementing deer with WCS may also be valuable during winter, a period of elevated mortality in males (Webb et al., 2007), induced in part by the stress of breeding in late autumn and associated large declines in BW.

Understanding plasma gossypol dynamics in white-tailed deer can aid in assessing WCS as a supplement and designing feeding programs to meet deer managers' goals. Because of uncertainties about the effects of gossypol on reproduction in cervids, many deer managers cease feeding WCS several weeks or even months before the breeding season to reduce the likelihood of reproductive problems in males. Data from this research indicates that plasma gossypol levels decrease rapidly in deer after the removal of cottonseed from the diet, but that some small levels remains even after 5 weeks. Our preliminary data from these animals suggests that these gossypol levels are not detrimental to sperm function or animal health (report in

preparation). However, a formal study is needed to determine the levels of plasma gossypol that causes detrimental problems and the likely levels and duration of WCS feeding that would lead to these problems. As a conservative recommendation, managers may want to continue to remove WCS from deer diets for >5 weeks before deer breeding season.

## Acknowledgements

We thank Dan Friedkin and the Comanche Ranch, Houston Safari Club, Quail Unlimited, and Texas Parks and Wildlife Foundation for their financial support. For assistance in data collection, we are thankful to Robert Kaiser, Chuck Coats, Donny Kahl, Niki Perry, and a multitude of student volunteers. We are also grateful to Andy Rodomski and Susan M. Cooper for their helpful comments and revisions to the manuscript. This is publication number # 10-115 from the Caesar Kleberg Wildlife Research Institute.

## References

- AOAC: Assn. of Official Analytical Chemists, 1995. Official Methods of Analysis. 16th ed. AOAC, Washington, DC.
- Barboza, P.S., Bowyer, R.T., 2000. Sexual segregation in dimorphic deer: a new gastrocentric hypothesis. *J. Mammal.* 81, 473–489.
- Bartoskewitz, M.L., Hewitt, D.G., Pitts, J.S., Bryant, F.C., 2003. Supplemental feed use by free-ranging white-tailed deer in southern Texas. *Wildl. Soc. B* 31, 1218–1228.
- Brocas, C., Rivera, R.M., Paula-Lopes, F.F., McDowell, L.R., Calhoun, M.C., Staples, C.R., Wilkinson, N.S., Boning, A.J., Chenoweth, P.J., Hansen, P.J., 1997. Deleterious actions of gossypol on bovine spermatozoa, oocytes, and embryos. *Biol. Reprod.* 57, 901–907.
- Brown, C.G., 2001. Evaluation of whole cottonseed consumption on growth and reproductive function in male cervids. M.S. Thesis, Texas A&M University, College Station, TX.
- Bullock, S.L., 2009. Nutrition and physiology of white-tailed deer consuming whole cottonseed. M.S. Thesis, Texas A&M University – Kingsville, Kingsville, TX.
- Chenoweth, P.J., Risco, C.A., Larsen, R.E., Velez, J., Tran, T., Chase Jr., C.C., 1994. Effects of dietary gossypol on aspects of semen quality, sperm morphology and sperm production in young Brahman bulls. *Theriogenology* 42, 1–13.
- Dowd, M.K., Calhoun, M.C., 1996. Uptake and depletion of gossypol by tissues of Rambouillet lambs. In: Proceedings of the 45th Oilseed Conference, New Orleans, LA, March 10–12.
- Faichney, G.J., White, G.A., 1988. Rates of passage of solutes, microbes and particulate matter through the gastrointestinal tract of ewes fed at a constant rate throughout gestation. *Aust. J. Agric. Res.* 39, 481–492.
- Fulbright, T.E., 2001. Human-induced vegetation changes in the Tamulipan scrub of La Frontera. In: Webster, G.L., Bahre, C.J. (Eds.), *Changing Plant Life of La Frontera: Observations on Vegetation in the United States/Mexico Borderlands*. University of New Mexico Press, Albuquerque, NM, pp. 166–175.
- Gamboa, D.A., Calhoun, M.C., Kuhlman, S.W., Haq, A.U., Baily, C.A., 2001. Use of expander cottonseed meal in broiler diets formulated on digestible amino acid basis. *Poultry Sci.* 80, 789–794 (abstract).
- Gizejewski, Z., Szafranska, B., Stepiewski, Z., Panasiewicz, G., Cierieszko, A., Doprowski, H., 2008. Cottonseed feeding delivers sufficient quantities of gossypol as a male deer contraceptive. *Eur. J. Wildl. Res.* 54, 469–477.
- Graham, N.M., Williams, A.J., 1962. The effects of pregnancy on the passage of food through the digestive tract of sheep. *Aust. J. Agric. Res.* 13, 894–900.
- Gray, M.L., Greene, L.W., Williams, G.L., 1993. Effects of dietary gossypol consumption on metabolic homeostasis and reproductive endocrine function in beef heifers and cows. *J. Anim. Sci.* 71, 3052–3059.
- Gunter, S.A., Judkins, M.B., Krysl, L.J., Broesder, J.T., Barton, R.K., Rueda, B.R., Hallford, D.M., Holcombe, D.W., 1990. Digesta kinetics, ruminal fermentation characteristics and serum metabolites of pregnant and lactating ewes fed chopped alfalfa. *J. Anim. Sci.* 68, 3821–3831.
- Hron, R.J., Sr., Kim, H.L., Calhoun, M.C., Fisher, G.S., 1999. Determination of (+), (–), and total gossypol in cottonseed by high-performance liquid chromatography. *J. Am. Oil Chem. Soc.* 76, 1351–1355.
- Leffler, J., 2008. Handbook of Texas online, <http://www.tshaonline.org/handbook/online/articles/DD/hcd9.html> (accessed 2.10.08).
- Lewis, T.L., Rongstad, O.J., 1998. Effects of supplemental feeding on white-tailed deer, *Odocoileus virginianus*, migration and survival in northern Wisconsin. *Can. Field Nat.* 112, 75–81.
- Lindsey, T.T., Hawkins, G.E., Guthrie, L.D., 1980. Physiological responses of lactating cows to gossypol from cottonseed meal rations. *J. Anim. Sci.* 63, 562–573.
- Littell, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, R.D., Schabenberger, O., 2006. SAS for mixed models, 2nd ed. SAS Institute Inc., Cary, NC, p. 188.
- Marsh, K.J., Wallis, I.R., Andrew, R.L., Foley, W.J., 2006. The detoxification limitation hypothesis: where did it come from and where is it going? *J. Chem. Ecol.* 32, 1247–1266.
- Mena, H., Santos, J.E.P., Huber, J.T., Simas, J.M., Tarazon, M., Calhoun, M.C., 2001. The effects of feeding varying amounts of gossypol from whole cottonseed and cottonseed meal to lactating dairy cows. *J. Dairy Sci.* 84, 2231–2239.
- Meyer, M.W., Brown, R.D., Graham, M.W., 1984. Protein and energy content of white-tailed deer diets in the Texas coastal bend. *J. Wildl. Manage.* 48, 527–534.
- Morgan, S., Stair, E.L., Martin, T., Edwards, W.C., Morgan, G.L., 1988. Clinical, clinicopathologic, pathologic, and toxicologic alteration associated with gossypol toxicosis in feeder lambs. *Am. J. Vet. Res.* 49, 493–499.
- Norwine, J., 1978. Twentieth century semiarid climates and climatic fluctuations in Texas and Northeastern Mexico. *J. Arid Environ.* 1, 313–325.
- Pound, J.M., Miller, J.A., Oehler, D.D., 2004a. Depletion rate of injected and ingested ivermectin from blood serum of penned female white-tailed deer. *J. Med. Entomol.* 41, 65–68.
- Pound, J.M., Miller, J.A., Oehler, D.D., 2004b. Depletion rate of doramectin from blood serum of penned female white-tailed deer. *J. Med. Entomol.* 41, 942–945.
- Provenza, F., 1995. Post-ingestive feedback as an elementary determinant of food preference and intake in ruminants. *J. Range Manage.* 48, 2–17.
- Randel, R.D., Chase Jr., C.C., Wyse, S.J., 1992. Effects of gossypol and cottonseed products on reproduction of mammals. *J. Anim. Sci.* 70, 1628–1638.
- Reiser, R., Fu, H.C., 1962. The mechanisms of gossypol detoxification by ruminant animals. *J. Nutr.* 76, 215–218.
- Risco, C.A., Chenoweth, P.J., Larsen, R.E., Velez, J., Shaw, N., Tran, T., Chase Jr., C.C., 1993. The effect of gossypol in cottonseed meal on performance on hematological and semen traits in postpubertal brahman bulls. *Theriogenology* 40, 629–642.
- Risco, C.A., Chase, Jr., C.C., (1997) *Gossypol*, In: (Ed.), *Handbook of Plant and Fungal Toxicants*. CRC Press Inc., Boca Raton, FL, pp. 87–98.
- Smalley, S.A., Bicknell, E.J., 1982. Gossypol toxicity in dairy cattle. *Comp. Cont. Educ. Pract.* 4, 381–387.
- Smith, G.S., 1992. Toxicification and detoxification of plant compounds by ruminants: an overview. *J. Range Manage.* 45, 25–30.
- Solaiman, S.G., Gurung, N.K., McCarary, Q., Goyal, H., McElhenney, W.H., 2009. Feeding performance and blood parameters of male goat kids fed EasiFlo® cottonseed. *Small Rumin. Res.* 81, 137–145.
- Taylor, B.D., 2003. Whole cottonseed: voluntary use by deer and avoidance by non-target species. M.S. Thesis, Angelo State University, San Angelo, TX.
- Velasquez-Pereira, J., McDowell, L.R., Risco, C.A., Prichard, D., Martin, F.G., Calhoun, M.C., Williams, S.N., Wilkinson, N.S., Ogebe, P., 1998. Effects on performance, tissue integrity, and metabolism of vitamin E supplementation for beef heifers fed a diet that contains gossypol. *J. Anim. Sci.* 76, 2871–2884.
- Webb, S.L., Hewitt, D.G., Hellickson, M.W., 2007. Survival and cause-specific mortality of mature male white-tailed deer. *J. Wildl. Manage.* 71, 555–558.
- Yu, F., Barry, T.N., Moughan, P.J., Wilson, G.F., 1993. Condensed tannins and gossypol concentrations in cottonseed and in processed cottonseed meal. *J. Sci. Food Agric.* 63, 7–15.