EFFECT OF COTTONSEED MEAL CONSUMPTION ON PERFORMANCE OF FEMALE FALLOW DEER

A Thesis

by

STEVEN LEE MAPEL

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2004

Major Subject: Physiology of Reproduction

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Approved as to style and content by:

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ABSTRACT

Effect of Cottonseed Meal Consumption on Performance of Female Fallow Deer.

(May 2004)

Steven Lee Mapel, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Ronald D. Randel

The objectives of this study were to determine the effects of gossypol ingestion on reproductive function and productivity of female fallow deer (*Dama dama*) by measuring endocrine function, pregnancy rates, and body weights of does and fawns. Sixty multiparous fallow does were randomly allotted into three groups corresponding to treatment diet that varied in respect to gossypol content. The does were then separated by treatment into pastures containing two fallow buck sires per pasture. The control group (SBMG), (containing no gossypol in diet) received 362 g soybean meal (SBM) $\cdot animal^{-1}\cdot day^{-1}$. The low gossypol group (CSML) was fed 227 g cottonseed meal (CSM; 0.09% free gossypol; determined by HPLC) and 181 g $SBM\cdot animal^{-1}\cdot day^{-1}$. The high gossypol group (CSMH) received 454 g $CSM\cdot animal^{-1}\cdot day^{-1}$. Diets were fed daily from 6/16/2003 to 11/20/2003. Blood samples were collected weekly from 8/14/2003 to 11/20/2003 for progesterone and gossypol analysis. Fawns born in June and July of 2003 were weaned 9/18/2003. Bucks were fitted with marking harnesses for the duration of the breeding season and heat marks were recorded daily for estrus detection. Ultrasonography, for pregnancy detection, was performed on 11/20/2003 and 12/15/2003. All groups lost weight from 8/14/03 to 11/20/03. SBMG lost less ($P<.05$)

weight than either CSML or CSMH. Final body weights were 2% greater (P<.02) in SBMG than in CSML or CSMH. Body condition from 8/14/03 to 11/20/03 did not differ (P>.1) between treatments. The pregnancy rate for all groups was 100%. There was no difference (P>.01) in time from weaning to conception (23 d) between treatments. Does in CSMH exhibited decreased (P<.02) progesterone concentrations. Consumption of CSM (free gossypol in amounts up to 0.81 mg·kg^{-1}BW; 0.41 g·animal^{-1}·day^{-1}).did not appear to affect reproductive performance of fallow deer.

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CHAPTER I

INTRODUCTION

The United States produces approximately 5.1 billion kg of cottonseed annually, (The Cotton Board, 2003). Whole cottonseed (WCS) and cottonseed meal (CSM) are valuable feedstuffs, providing important sources of protein and energy for ruminant animals. Cottonseed has been fed to livestock for over a hundred years and approximately 4 billion kg of cottonseed products are used as supplemental feedstuffs each year.

Recent years have shown an explosion in the management and supplemental feeding of cervids, primarily white-tailed deer (*Odocoileus virginianus*), to maximize antler growth and reproductive performance. Accordingly, an increasing portion of WCS is being fed as supplemental feed to deer populations. The positive qualities of cottonseed products include high protein (41% for cottonseed meal), low cost (Buser and Abbas, 2001), and low non-target species use of WCS (Rollins, pers. comm.). Consequently, many land owners practice seasonal and yearly ad-libitum feeding of cottonseed to deer. Cottonseed, however, may have negative implications for deer due to the toxic nature of gossypol found in WCS and CSM.

Gossypol is a chemical found in the yellow pigmentation of cotton plants genus, *Gossipium*. Monogastric animals are much more susceptible to gossypol poisoning than ruminants. Subsequently, monogastric animals usually display symptoms of toxicity at much lower dosages than ruminants. Although ruminants are more resistant to gossypol

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poisoning, excessive amounts of gossypol are toxic to ruminants (Kerr, 1989). Threshold and quantitative toxicity varies between species. Threshold and quantitative toxicity varies between species. Deleterious effects including; death, erythrocyte fragility, and lower hemoglobin, have been associated with gossypol have been reported in cattle (Lindsey et al., 1980). Erythrocyte fragility was observed in sheep fed whole cottonseed (Calhoun et al., 1990). Decreased body weights and antler growth were documented in fallow deer fed ad-libitum quantities of whole cottonseed (*Dama dama*; Brown 2001).

Reproductive efficiency is essential to maintain viable wild and farmed populations of deer. Replacement rates (i.e. fawn crop) are commonly used by wildlife managers and deer farmers as indicators of herd nutritional health. The results of these indicators in turn influence management decisions. Subsequently, the proper reproductive function and performance of deer populations is fundamental to the management of wild and farmed populations. The use of cottonseed meal in rations for cervids may negatively impact female reproductive function and performance. Therefore, evaluating dose dependent toxicity of gossypol in cervids is an important step in assessing the value of cottonseed meal as a supplemental feedstuff.

CHAPTER II

OBJECTIVES

The objectives of this study were to determine the effects of gossypol ingestion on reproductive function and productivity of female fallow deer (*Dama dama*) by measuring endocrine function, pregnancy rates, and body weights of does and fawns.

CHAPTER III

LITERATURE REVIEW

The Fallow Deer

Fallow deer are native to the Mediterranean countries of North Africa and Europe. Today fallow deer have a widespread range encompassing several continents. Since their introduction into Texas, populations have reached over 20,000 animals, with the greatest concentration in the Edwards Plateau region (Mungall, 1994).

Fallow deer are commonly farmed for venison production. An average adult female weighs between 56 and 63 kilograms (Whitehead, 1993). Fallow deer are short day breeders; mating occurs from September through February (Mungall and Sheffield, 1994). Mean estrous cycle length is 22.4 days and ranges between 20 to 27 days (Asher, 1985). Gestation period length was reported to be 234.2 days (Asher, 1985). Does usually reach puberty at 16 months and fawn at two years of age (Harrison and Hyett, 1954).

Physiology of Reproduction

Fallow does have a bicornuate uterus. Placetomes are larger, but fewer in number than those of cattle (Senger, 1997). Placental attachment is syndesmochorial and fertilization occurs at the ampullary-isthmic junction in the oviduct. Embryos recovered prior to d-8 are found in the oviduct or utero-tubal junction region (Asher et al., 1994). Embryo attachment in the uterus occurs between d-34 and 41 in red deer (Asher et al., 1994).

Endocrine function as it relates to reproductive cycles and pregnancy is still not fully understood. Fallow deer, much like sheep and most other deer species are seasonal breeding animals. Seasonal anestrous is known to be controlled in part by photoperiod through the hormone melatonin. During periods of darkness, melatonin is secreted by the pineal gland. As day length shortens, melatonin concentrations are elevated (Williams et al., 1996). As a result pulsatile secretions of gonadotropin releasing hormone (GnRH) from the hypothalamus are increased (Maulpaux et al., 1996). GnRH in turn acts on the anterior pituitary to stimulate the release of the glycoprotein gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH) (Karsch et al., 1987). The amount and frequency of pulsatile LH and FSH secretions are important for the recruitment and selection of oocytes and ovulation, thereby contributing to the regulation of the estrous cycle (Mapletoft, 1999). Luteinizing hormone and FSH stimulate the secretion of estrogen from developing follicles. Estrogen exhibits both positive and negative feedback effects on the hypothalamus and pituitary hormones (Clarke, 1995a,b). Estrogen also produces behavioral effects and is responsible for estrus behavior in females. Following ovulation, the follicle undergoes changes to become the corpus luteum. The corpus luteum secretes progesterone (P_4) in response to gonadotropins and contributes to the early maintenance of pregnancy (Bazer, 1992). Progesterone is also produced in increasing quantities by the placenta. After the first trimester, placental P_4 is sufficient to support pregnancy in the absence of the corpus luteum (Asher et al., 1994).

The physiology of maternal recognition is responsible for the maintenance of pregnancy. In the absence of fertilization, prostaglandin F2α (PGF2α) is secreted by the uterine cells (Bazer, 1992). Prostaglandin F2α is carried to the ovaries where it causes the degeneration of the corpus luteum (Bazer, 1992). In the absence of the corpus luteum, the estrous cycle begins anew. During a successful pregnancy, the embryo causes interferon tau to be released from the uterus. Interferon tau blocks the action of PGF2α, the corpus luteum continues to function, and the pregnancy is maintained (Bazer, 1992).

Ultrasonography has been used to detect pregnancy in fallow deer as early as d-33 of pregnancy. Willard et al. (1999) achieved greater than 97% accuracy detecting pregnancy at d-30 post breeding. Willard et al. (1998) also found that P_4 concentrations in pregnant fallow does increased from d-9 through d-203 of pregnancy. This study also showed that P_4 concentrations of pregnant does were greater than non-pregnant does. Consequently, progesterone concentrations can also be used to determine pregnancy as well (Sasser and Rudder, 1987). However, Asher et al. (1989) asserted that under stress conditions, the adrenal glands of fallow deer are capable of secreting significant amounts of P_4 (up to 8 ng/ml). This non-luteal source of P_4 can be responsible for the sometimes erratic P_4 profiles observed in fallow deer. This adrenal P_4 likely has an additive effect when combined with P_4 from luteal tissue and can result in false positive pregnancy diagnosis when P_4 concentrations are used to determine pregnancy.

Nutrition and Fertility

Morphophysiologically, fallow deer fall into the category of intermediate feeders, as classified by Hoffman (1985). According to this system, fallow deer fall between concentrate selectors such as white-tailed deer, and grazers represented by sheep and cattle. Hoffman (1989) reported that concentrate and intermediate feeders do not have the grazer's efficiency in digesting foods with high cellulose content (i.e. grass). Hoffman also theorized that they may; however, more efficiently metabolize materials with higher proportions of cell contents (i.e. concentrates). Shorter rumen retention times and ‘rumen bypass’ were reported as causation for this phenomenon. Other researchers have given evidence to support ‘rumen bypass’ (Rowell-Schafer et al., 1997, 2001; Wood et al., 2000). Concurrently, Huston et al. (1986) showed that white-tailed deer tend to have shorter retention times in the rumen and faster food particle turnover rates than either sheep or cattle. Nutrition is fundamental in achieving both reproductive competence and successful fertilization. Nutritional intake is highly correlated with overall health and body condition of animals (Kaur and Arora, 1995).

Dietary carbohydrates are broken down through the metabolic action of microorganisms in the rumen. The major energy source of ruminants is the subsequent production of the volatile fatty acids: acetate, butyrate, and propionate, by the microflora (Van Soest, 1982). Blood glucose is found at much lower concentrations in ruminants as compared to monogastric animals and is primarily synthesized from propionate (Boland et al., 2001). In review, Boland and Callaghan (1999) reported that both low energy intake as well as excessive concentrate intake can reduce fertility and early embryonic development. Glucose is transported to the fetus by facilitated diffusion (Hay, 1991). Bazer (1989) found that the sheep and swine allantois was primarily fructogenic, converting maternal glucose to fructose. This conversion results in fetal and allantoic fructose concentrations being higher than glucose.

Dietary protein is catabolized for its amino acids and utilized by the microflora for its nitrogen. However, excess nitrogen is associated with increased urea concentrations that can be detrimental to fertility (Boland et al., 2001). Concentrations of specific amino acids located in the allantoic and amniotic membranes have been shown to be paramount for fetal development (Bazer, 1989; Wu et al., 1995; Kwon et al., 2003).

Additionally, the placenta plays an important role in the transportation of amino acids. Amino acids are carried through the placenta by fascilitated transport (Hay, 1991). However, the transport of specific amino acids varies. Some amino acids are completely catabolized by the placenta and must be re-synthesized endogenously by the fetus (Goodwin et al., 1987).

Adequate nutrient intake is required for the energetically expensive physiological processes of gestation and lactation. Low food intake and/or availability can contribute to lowered fertilization rates and a high frequency of pregnancy termination (Kaur and Arora, 1995). Prepubertal anestrous in lambs and induced anestrous in cattle have been seen as a result of low nutrition diets (Boland et al., 2001). Prolonged under-nutrition has produced lower ovulation rates in sheep and is associated with decreased LH production (Foster et al., 1986). In addition, diets high in protein and energy have been shown to increase ovulation rate and litter size in ruminants (Boland et al., 2001).

In wild cervid populations, annual reproductive rates are highly correlated to food availability. Forage intake of fallow deer on the Kerr Wildlife Management Area were found to be composed of 54% browse, 30% grass, 12% forbs, and 4% other (Mungall and Sheffield, 1994). Additionally, quality and relative abundance of food items were found to be important selection criteria for populations of farmed fallow deer (Ulrika et al., 2001). Forage compositions in natural populations of white-tailed deer have been shown to change corresponding to geographic location and season (Kie et al., 1980). Populations of white-tailed deer have also demonstrated feed intake variability throughout the year (Wheaton and Brown, 1983). Free ranging populations of fallow deer are likely to demonstrate similar variation in forage selection.

Reproductive performance studies conducted in fallow deer by Willard et al. (1999) indicated no differences in pregnancy rates between yearling, 2-yr-old, and mature does. However, mature does have higher weaning rates than 2-yr-olds and yearlings, and yearlings have lower weaning rates than either 2-yr-olds or mature does. These researchers also demonstrated that does which failed to wean a fawn have heavier body weights and lower pregnancy rates than does that did wean a fawn at the onset of the breeding season. Willard et al. (1999) reported that heavier BW in does at breeding was associated with decreased fertility.

Gossypol

Gossypol, a phenolic binaphthyl aldehyde, was first isolated by Longmore in 1886 from cottonseed oil. The compound has a molecular weight and formula of 518.54 and $C_{30}H_{30}O_8$, respectively (Clark, 1928; Carruth, 1918). Abou-Donia (1976), in review, discussed three tautomer forms of gossypol: the aldehyde form, hemiacetal, and phenolic quinoid tautomer. Gossypol is also found in two sterioisomer forms, (+) and (-). The (-) isomer is considered to be the most biologically active (Calhoun et al., 1995). Within cottonseed meal, "free gossypol" and "bound gossypol" can be found in varying amounts depending on the processing method. During the processing of cottonseed, heat and pressure cause bond formation between gossypol and other molecules. This "bound gossypol" is considered to be physiologically inactive (Randel et al., 1992).

Gossypol is produced in glands throughout the cotton plant, but most gossypol is concentrated in the roots and seeds. These pigment producing glands can be identified as black specks located in the cottonseed meat (Buser and Abbas, 2001). Average gossypol content of cottonseed is 0.4 to 1.7% (Abou-Donia, 1976). On average, cottonseed meal contains 0.07% to 0.22% gossypol. The percent gossypol available in seeds varies with differing environmental conditions and processing methods (Calhoun et al., 1995). According to Pons (1953) gossypol concentration in seeds were directly related to rainfall and indirectly related to temperature. Additionally, Buser and Abbas (2001) reported up to 78% reduction in free gossypol in mechanically extruded cottonseed without significant reduction in nutrient values. Their methods used a single screw adiabatic extruder to reduce free gossypol concentrations.

Gossypol Toxicity

Gossypol affects a wide range of tissues and its toxicity varies between species. Abou-Donia (1976), in review, reported the oral LD_{50} in rats for a single dose to be 2,400 to 3,340 $mg \cdot kg^{-1}$. In dogs, 10 to 200 $mg \cdot kg^{-1} \cdot day^{-1}$ was enough to cause death in less than a month. Swine are extremely sensitive to gossypol. The oral LD_{50} was cited at 550 mg/kg. In studies conducted in dogs, treated animals showed signs of anorexia, diarrhea, and weight loss. Postmortem findings indicated enteritis, hydrothorax, edema, hydropericardium, and organ congestion. Postmortem examinations in rats found fluid accumulation in the body, hemorrhage of glands, gastritis, and congestion in the intestine and kidney.

Ruminants demonstrate a certain level of resistance to gossypol. Reiser and Fu (1962) suggested that the highly reducing environment of the rumen might be responsible for detoxification. It is postulated that gossypol binds to the free amino groups of miscellaneous proteins (Clark, 1928). Free gossypol seems to have an affinity for ε amino groups (Reiser and Fu, 1962). Two moles of lysine were shown to bind to every mole of free gossypol. This bound gossypol is not absorbed in the gastrointestinal tract and therefore is not harmful. In an additional experiment Reiser and Fu (1962) found that gossypol was not eliminated by bacteria and failed to bind significantly with bacterial protein. This led them to believe that the detoxification of gossypol by ruminants results from the subsequent binding of free gossypol to soluble proteins.

Despite detoxifying mechanisms, Kerr (1989) demonstrated that high doses of gossypol are toxic to ruminants. Mature dairy cows demonstrated symptoms of toxicity at 6.6 and 42.7 mg of free gossypol · kg of $BW^{-1} \cdot day^{-1}$ (Lindsey et al., 1980). Symptoms included death, reduced hemoglobin, erythrocyte fragility, increased respiration rates, and increased plasma protein. Gray et al. (1993) also reported increased erythrocyte membrane fragility in cattle consuming gossypol. Randel et al. (1996) and Willard et al. (1995) also documented erythrocyte fragility and reduced weight gains in heifers fed cottonseed meal. Thirumalesh et al. (1999); however, saw no effects on certain enzymes marking liver necrosis in calves fed less than 0.001% free gossypol. In an experiment performed with male fallow deer, treated animals were allowed to consume ad-libitum WCS (free gossypol in amounts up to 150 $mg \cdot kg^{-1}$ BW or 10.0 $g \cdot animal^{-1} \cdot day^{-1}$). These bucks showed reduced BW, body condition, antler mass, and circulating testosterone concentrations (Brown, 2001).

The biochemical mechanisms of gossypol toxicity have yet to be completely elucidated. However, several studies have demonstrated that gossypol produces uncoupling effects on the mitochondria (Abou-Donia and Dieckert, 1974; Reyes and Benos, 1988). Gossypol has also been shown to inhibit the electron transport complexes as well as other enzymes including, lactate dehydrogenase, adenosine triphosphate, protein kinase C, and adenylate cyclase (Breitbart et al., 1989; Teng, 1995; Olgiati et al., 1984). Cuellar and Ramirez (1993) hypothesized that gossypol may alter the lipid matrix in the mitochondrial membrane. In an in vitro study, gossypol substantially reduced interfacial membrane potential (Reyes et al., 1984). Lyman et al. (1959) reported that gossypol forms protein complexes and limits nutrient digestion and availability through its amino acid binding properties. Braham et al. (1967) concluded that gossypol caused anemia in pigs by binding with iron. In a 2001 study, Kolena et al. associated inhibitory effects of gossypol on cumulus expansion and progesterone synthesis with nitric oxide production.

In the last two decades a multitude of work has been published demonstrating the effects of gossypol on female reproduction (Randel et al., 1992). Lin et al. (1989) documented reduced progesterone concentrations and pregnancy inhibition in rats receiving intramuscular injection of gossypol. This study also found that exogenous progesterone administration prevented the inhibition of pregnancy associated with gossypol administration. In a previous study with rats, researchers found that administration of gossypol into one uterine horn terminated pregnancy in the ipsilateral horn only (Lin et al., 1985). In other research on pregnant rats, gossypol acetic acid (GAA) reduced serum progesterone concentrations and inhibited pregnancy (Yang and Wu, 1987). Effect of gossypol on follicular development and hormone secretion has been studied in several species. Bansode (1994) observed pycnosis, chromatolysis, and dissolution of granulosa cells indicating follicular degeneration in ovaries of bats (*Rhinopoma kinneari)* following 10 mg administration of GAA. In work conducted on Syrian female hamsters, Wu et al. (1981) reported altered quantities of FSH, estradiol (E_2), and P_4 in blood serum. In the same study; however, LH concentrations did not differ between groups. Ovulation frequency and pregnancy rates were also not effected by treatment.

In cattle, effects on endocrine function and embryo viability have varied. Zirkle et al. (1988) cultured bovine embryos that degenerated in a dose dependent manner when subjected to gossypol acetic acid (GAA). Stahringer et al. (1992) reported altered triiodothyronine (T_3) and thyroxine (T_4) concentrations in Brahman bulls fed WCS diets containing 15g of free gossypol. Randel et al. (1996) found that feeding CSM (5 g free gossypol$\cdot$animal$^{-1}\cdot$day^{-1}) and WCS (15 g free gossypol$\cdot$animal$^{-1}\cdot$day^{-1}) to heifers reduced weight gain and increased corpus luteum progesterone content. That study also found higher percentages of degenerative embryos in heifers receiving CSM. The administration of GAA to cultured bovine luteal cells inhibited hCG-induced cAMP and progesterone production in a dose dependent manner (Gu et al., 1989, 1990). In contrast, feeding cows up to 10 g free gossypol$\cdot$animal$^{-1}\cdot$day^{-1} did not significantly alter LH, E_2, P_4, or pregnancy rates (Gray et al., 1993). Gossypol has been reported to have an effect on the muscularis layer of the female reproductive tract. In that study, Lei and Xu (1993) hypothesized that smooth muscle contraction resulting from GAA exposure might be related to elevated prostaglandin production or increased Ca^{2+} influx Feeding cows 2 and 4 g of free gossypol day^{-1} did not significantly alter prepartum or postcalving body condition, body weight, or milk production. Thyroid hormone concentrations, (T_3) and (T_4) were not different between treatment groups (Willard et al., 1995).

In summary the effects of gossypol toxicity on tissues, endocrine profiles, and reproductive function varies between species. Most studies indicate that there is a dose dependent response affecting reproductive performance. Previous findings have demonstrated negative affects of ad-libitum consumption of WCS (free gossypol in amounts up to 150 $mg \cdot kg^{-1}$ BW or 10.0 $g \cdot animal^{-1} \cdot day^{-1}$) in male fallow deer (Brown 2001). More research is necessary to determine the effects and acceptable levels of gossypol intake from CSM for female cervids.

CHAPTER IV

MATERIALS AND METHODS

Sixty multiparous fallow does (average initial BW= 50.62 kg) from the Texas Agricultural Research and Extension Center at Overton, Texas, were randomly assigned into three treatment groups adjusted for BW. Two mature fallow bucks (average BW for trial= 80.0 kg) were randomly assigned into each of the three groups at the onset of the feeding trial. Antlers were removed prior to the onset of breeding. Each group was maintained on Coastal bermuda grass pasture and supplemented with salt, minerals, and water. All animals received Coastal bermuda grass hay when standing forage was limiting. The control group (SBMG; n= 20) was fed 362 g soybean meal (SBM) $\cdot$animal$^{-1}\cdot$d^{-1} (Table 1). The low-range gossypol group (CSML; n= 20), was fed 227 g of cottonseed meal (CSM) and 181 g of SBM$\cdot$animal$^{-1}\cdot$d^{-1} (Table 1). The high-range gossypol group (CSMH; n= 20) was fed 454 g CSM$\cdot$animal$^{-1}\cdot$d^{-1} (Table 1). Digestible protein and total digestible nutrients supplemented to each group can be seen in Table 1. The height of feeding troughs prevented access of feed supplementation to fawns. Thereby, fawns were not allowed to consume treatment diets. Gossypol content of CSM was measured by Pope Testing Laboratories Inc., Irving, Texas using the official methods of the American Oil Chemist Society protocols. Analysis of supplemented cottonseed meal revealed a total and free gossypol concentration of 1.9% and 0.09%, respectively. The cottonseed meal low group received 2.27 g and 0.2 g of total and free gossypol$\cdot$animal$^{-1}\cdot$d^{-1}, respectively (Table 2). The cottonseed meal high group received 4.54 g and 0.41 g of total and free gossypol$\cdot$animal$^{-1}\cdot$d^{-1}, respectively (Table 2). In late April, the fallow does were separated into 3 separate pastures corresponding to their treatment groups. Each pasture was surveyed daily from May 23, 2003 to July 1, 2003 in order to tag and weigh fawns as they were born. Bucks were placed into their allotted pastures corresponding to their treatment group on June 14, 2003. The treatment groups were supplemented, with the appropriate ration, beginning June 16, 2003 through November 20, 2003. Marking harnesses were maintained on bucks throughout the breeding season and heat marks were recorded daily. Body condition score (scale 1-10), BW measurement, and jugular venous blood samples were collected from SBMG (n= 17), CSML (n= 17), and CSMH (n= 16) at weekly intervals beginning August 14, 2003 through November 20, 2003 (Godfrey et al., 1988). Fawns born in June and July of 2003 were weighed weekly from August 14, 2003 until weaning on September 18, 2003. Does that died during the trial were removed from analysis (SBMG= 2; CSML= 0; CSMH= 1). Does that did not wean a fawn were also removed from analysis (SBMG= 1; CSML= 3; CSMH= 3). Sample sizes for SBMG, CSML, and CSMH were 17, 17, and 16, respectively. Fawns that died during the trial were also removed from analysis. Sample sizes for fawns reared by SBMG, CSML, and CSMH were 19, 17, and 17, respectively. Body condition score was used as an indicator of body fat deposition. A body condition score of one represents an emaciated animal. A score of ten would be representative of an obese animal. Generally when the vertebrae of the spine can be felt or seen the animal was assigned a score of four or less. When vertebrae and pelvis bones can not be readily distinguished by touch (due to the deposition of fat over these areas) a score of six or greater was assigned. Body condition was scored by two experienced technicians. Blood samples (n= 885) were centrifuged at 1800 r.c.f. for 30 min at 4° C to yield serum and stored at -20° C. Progesterone concentrations were determined using radioimmunoassay

Table 1. Dry matter feed supplementation by group.

Treatment	Supplement	DM Amt ($g \cdot an^{-1} \cdot d^{-1}$)	Protein (g)	TDN (g)
SBMG	SBM	326.9	144.9	255.0
CSML	SBM	163.4	68.6	127.5
	CSM	207.7	67.5	131.5
	Total	371.1	136.1	259.0
CSMH	CSM	415.4	135.0	262.9

Table 2. Total and free gossypol intake.

Treatment	Total Gossypol		Free Gossypol	
	$g \cdot an^{-1} \cdot d^{-1}$	$mg \cdot kg^{-1}$(BW)	$g \cdot an^{-1} \cdot d^{-1}$	$mg \cdot kg^{-1}$(BW)
SBMG	0.00	0.00	0.00	0.00
CSML	2.27	44.86	0.20	3.95
CSMH	4.54	89.72	0.41	8.10

(Williams, 1989). The average detection limit for the assay was 0.054 ng/ml. Intra- and inter-assay coefficients of variance were 7.26% and 2.44%, respectively. Additional blood plasma samples were taken at 28 d intervals, samples were sent to Dr. Millard Calhoun's lab, Texas A&M University Research Center, Uvalde, Texas for blood gossypol analysis. Plasma gossypol content was measured using high performance liquid chromatography (Kim and Calhoun, 1995). Ultrasonography, for pregnancy determination, was performed at buck removal (November 20, 2003) and again 25 days later (December 15, 2003; Sonovet 600®).Data were evaluated using SPSS (SPSS Inc., Chicago, Illinois). Body measurements, serum P_4 concentrations and plasma gossypol concentrations were analyzed for lactating does using General Linear Model's procedures specific for repeated measurements (SPSS Inc., Chicago, Illinois). Pregnancy rates were compared using Chi square frequency analysis procedures of SPSS (SPSS Inc., Chicago, Illinois).

CHAPTER V

RESULTS

Body Weight and Condition

Initial BW for SBM, CSML, and CSMH were 49.38 ± 1.11 kg, 51.08 ± 1.09 kg, and 51.42 ± 1.19 kg, respectively (Table 3). Final BW for SBM, CSML, and CSMH were 44.47 ± 0.93 kg, 43.86 ± 0.96 kg, and 43.71 ± 0.91 kg, respectively (Table 3). Average BW from August 14, 2003 to weaning (September 18, 2003) did not differ ($P>.10$) between groups (Figure 1). Average BW from weaning (September 18, 2003) to November 20, 2003 was greater ($P<.01$) for SBMG than for CSML or CSMH (Figure 2). Average BW from August 14, 2003 to November 20, 2003 was 2% greater ($P<.02$) for SBMG than either CSML or CSMH (Figure 3). From August 14, 2003 to November 20, 2003, SBMG lost an average of 4.90 ± 0.33 kg of BW. The CSML lost an average of 7.22 ± 0.27 kg, while CSMH lost an average of 7.84 ± 0.60 kg. SBMG lost less BW ($P<.05$) than either CSML or CSMH. No difference ($P>.10$) in BW loss was observed between CSML and CSMH (August 14, 2003 to November 20, 2003) (Figure 3). Average weekly weight loss was also less ($P<.01$) for SBMG (-0.35 ± 0.03 kg) than for CSML (-0.52 ± 0.03 kg) or CSMH (-0.55 ± 0.03 kg) (August 14 to November 20).

Body condition scores decreased from August 14, 2003 until weaning (September 18, 2003) and then increased thereafter (September 18, 2003 to November 20, 2003) (Figures 4, 5). No differences (P>.10) occurred in weekly body condition scores among the three groups (Figures 4, 5, 6). The average body condition scores over the extent of the trial for SBMG was 5.3 ± 0.1, CSML and CSMH were 5.2 ± 0.1 and 5.3 ± 0.1, respectively (Table 3).

Fawns were tagged and weighed within 24 hrs of birth. The average fawning dates for SBMG, CSML, and CSMH were Julian days 278.6 ± 2.0, 280.0 ± 1.4, and 281.6 ± 1.8; respectively. Average parturition date did not differ (P>.10) among groups (Table 4). Average fawn birth weights for SBMG, CSML, and CSMH were 4.67 ± 0.12 kg, 4.62 ± 0.13 kg, and 4.73 ± 0.13 kg, respectively (Table 4). Average birth weights among groups by sex can be seen in Table 5. No difference (P>.10) among groups in average daily gain (ADG) was detected from August 14, 2003 to September 18, 2003 (Table 4). Male fawns had heavier birth weights (P<.05) than female fawns across all groups (4.81 ± 0.61 kg; 4.50 ± 0.34 kg) (Table 5). However, BW gains between sexes did not differ (P>.1; Figure 7).

Table 3. Initial and final BW and average body condition score (BCS) of fallow does.

Treatment[a]	n	Initial BW	Final BW	Average BCS
SBMG	17	49.38 ±1.11	44.47±0.93	5.3±0.1
CSML	17	51.08±1.09	43.86±0.96	5.2±0.1
CSMH	16	51.42±1.19	43.71±0.91	5.3±0.1

[a]No differences (P>.1) were detected among treatments.

Table 4. Average parturition date of does, fawn birth weight, and fawn BW/d of age.

Treatment[a]	n	Julian date of parturition	birth weight (kg)	ADG (kg)
SBMG	17	278.6±2.0	4.67±0.12	0.23±0.02
CSML	17	280.0±1.4	4.62±0.13	0.24±0.02
CSMH	16	281.6±1.8	4.73±0.13	0.28±0.02

[a]No differences (P>.1) were detected among treatments.

Table 5. Average birth weight of male and female fawns.

		Birth weight (kg)		
Treatment	n (males)	Mean (kg)	n (females)	Mean (kg)
SBMG	10	4.85±0.17[a]	9	4.48±0.17[b]
CSML	11	4.77±0.16[a]	6	4.34±0.21[b]
CSMH	9	4.80±0.17[a]	8	4.64±0.19[b]

[a b]Means in rows with different subscripts differ (P<.05).

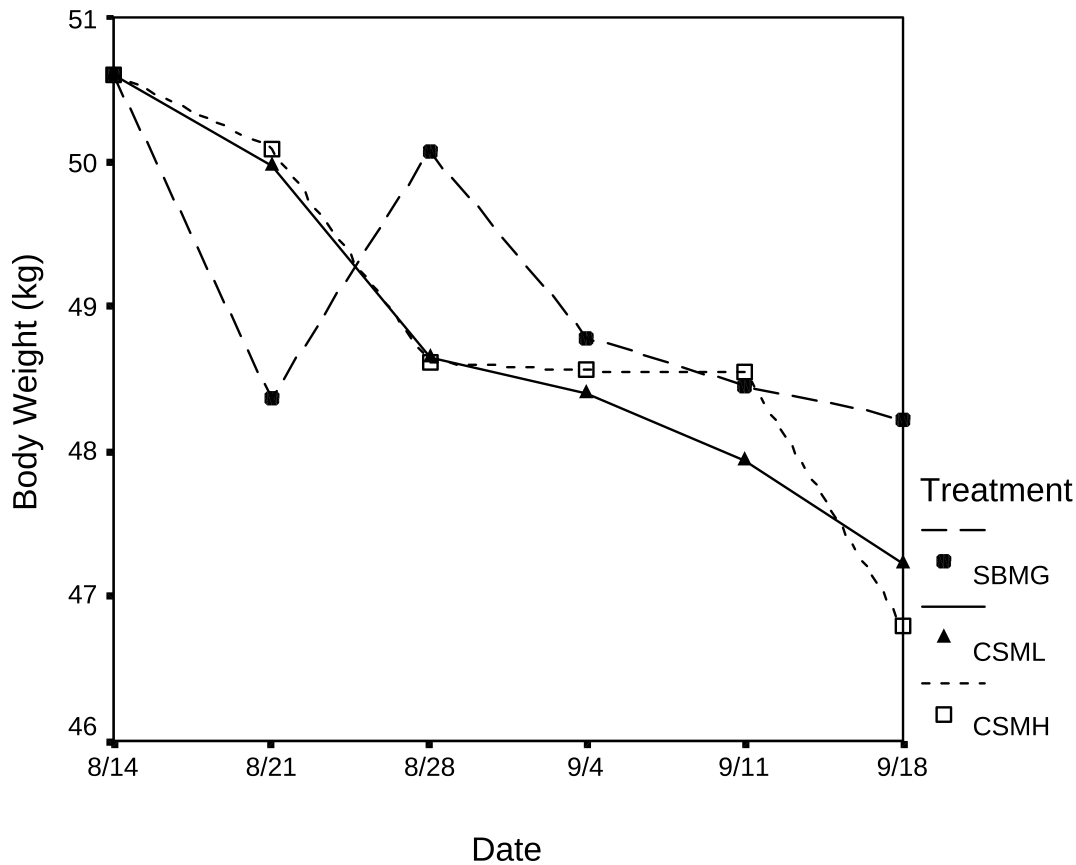

Figure 1. Estimated marginal means of doe BW from 8/21/03 to weaning (9/18/03) (pooled SEM= 0.13).

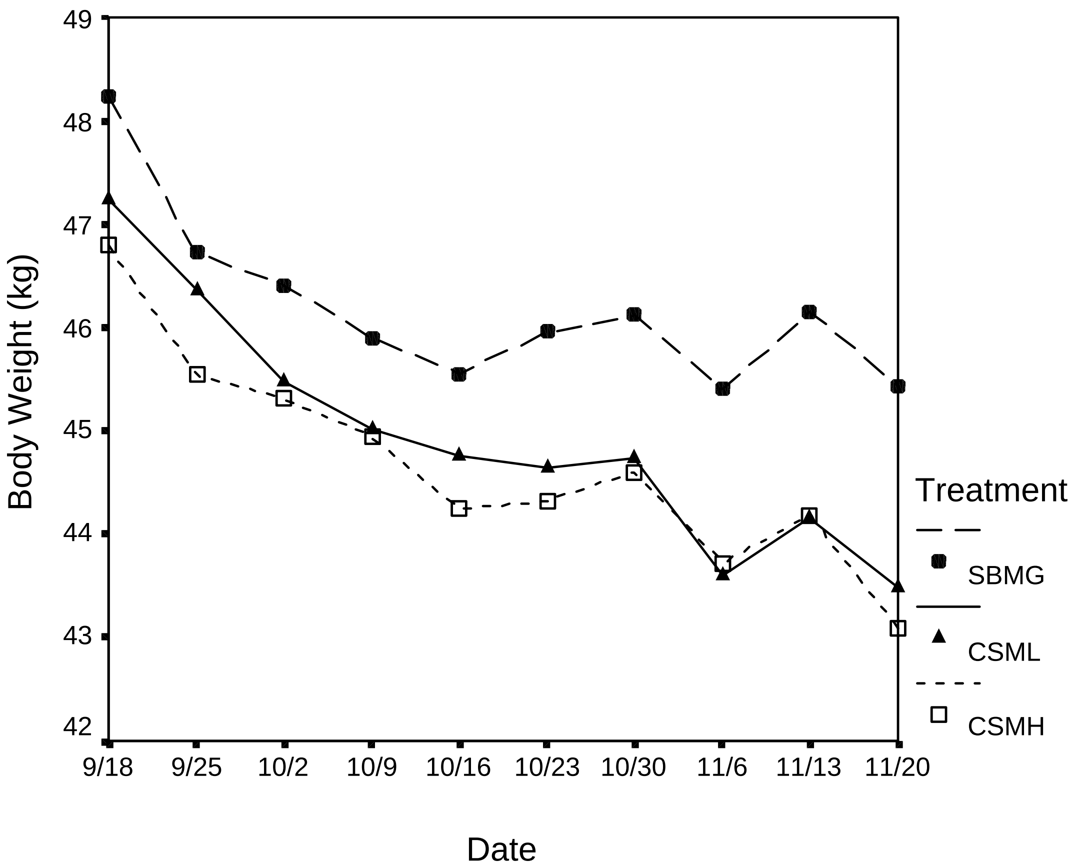

Figure 2. Estimated marginal means of doe BW from weaning (9/18/03) to 11/20/03 (pooled SEM= 0.16).

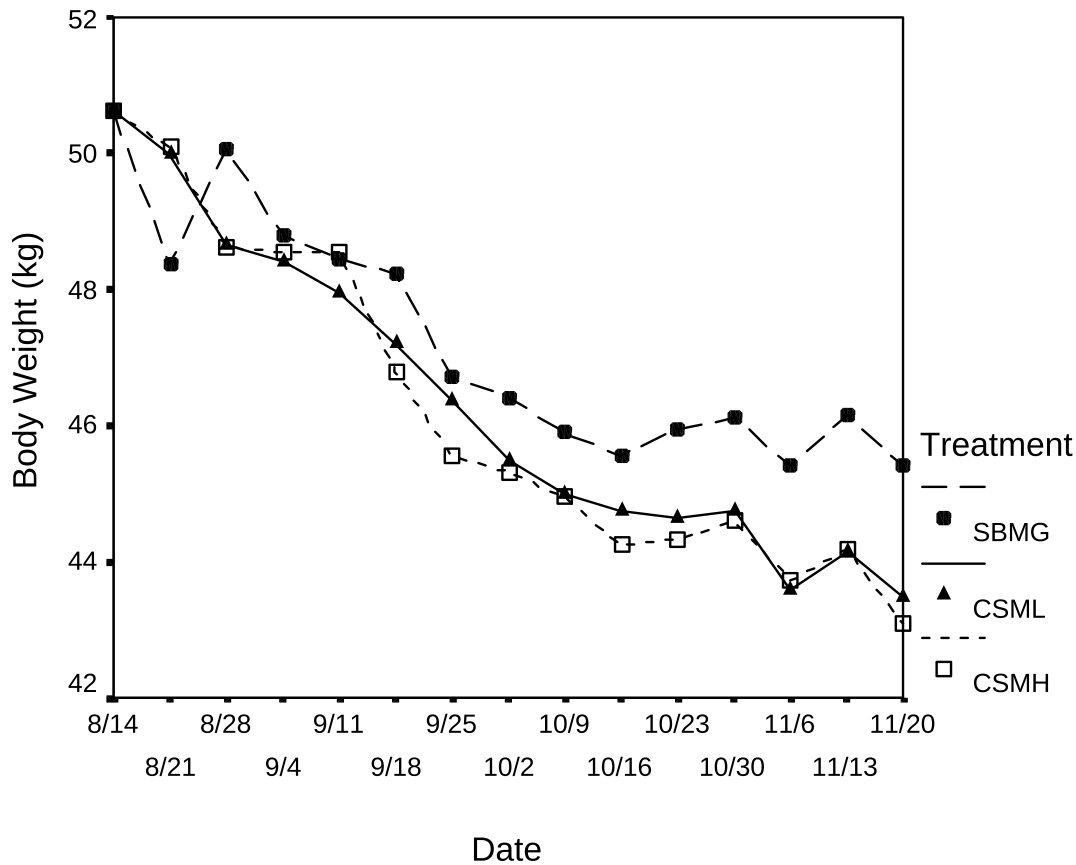

Figure 3. Estimated marginal means of doe BW from 8/14/03 to 11/20/03 (pooled SEM= 0.14).

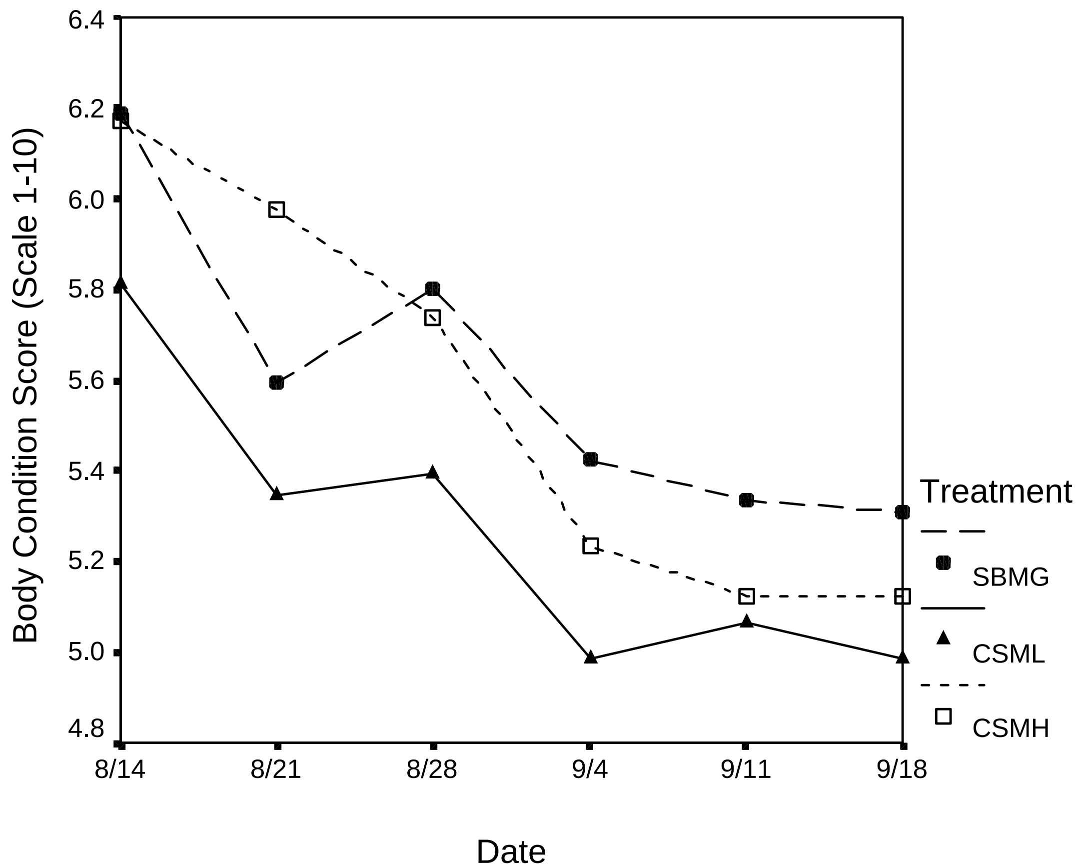

Figure 4. Estimated marginal means of body condition score among fallow does from 8/14/03 to weaning (9/18/03) (pooled SEM= 0.08).

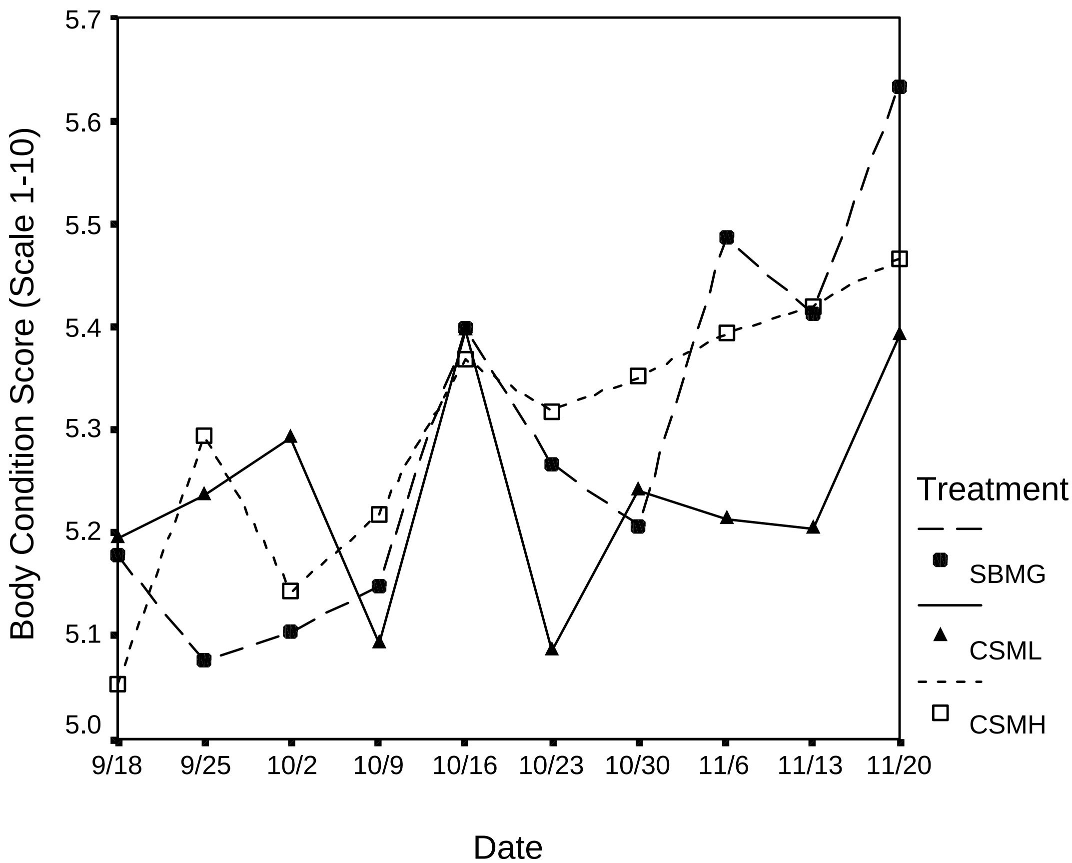

Figure 5. Estimated marginal means of body condition score among fallow does from weaning (9/18/03) to 11/20/03 (pooled SEM= 0.06).

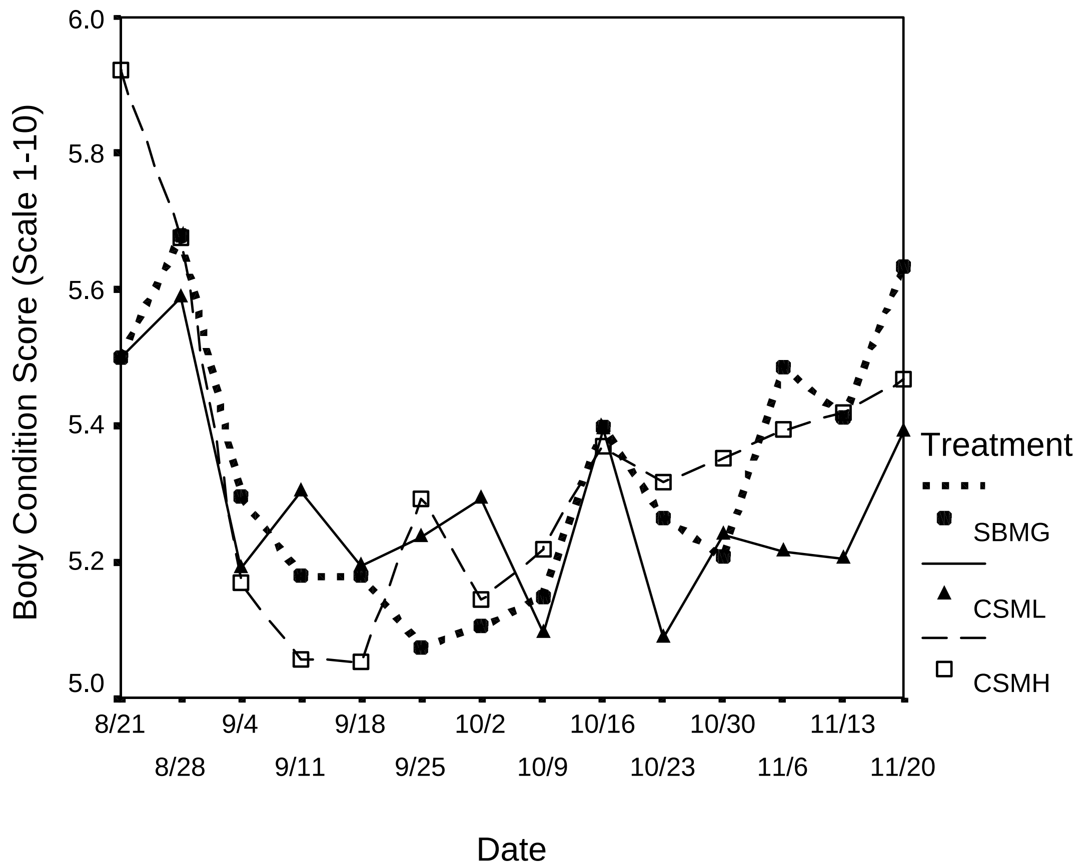

Figure 6. Estimated marginal means of body condition score among fallow does from 9/18/03 to 11/20/03 (pooled SEM= 0.10).

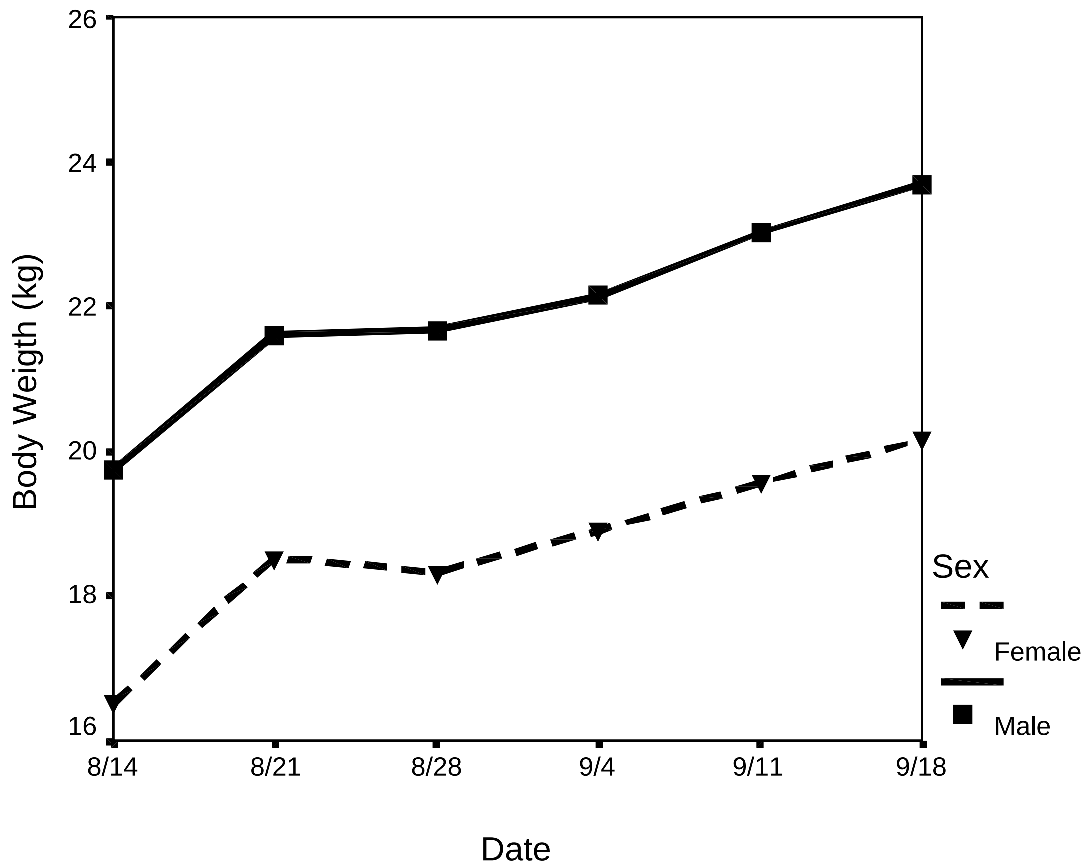

Figure 7. BW of male and female fawns from 8/14/03 to weaning (9/14/03) (pooled SEM= 0.90).

Reproductive Performance

Pregnancy rates, as determined by both ultrasonography and serum progesterone concentration, were 100% for all groups. Number of days from weaning to first estrus did not differ (P>.10) among groups. No difference was found (P>.10) among groups for the number of days from weaning to conception. The median conception date was October 9, 2003 for SBMG and October 12, 2003 for CSML and CSMH. The grand mean across all groups for time between weaning and conception was 23 d. Within lactating does among all groups, BW was negatively correlated (P<.01; R= -0.50) with time from weaning to conception (Figure 8). Does which were heavier at weaning (>47.0 kg BW) had approximately six fewer days (P<.01) to first estrus and conception than did lighter does (<47.0 kg BW). Within lactating does among all groups, body condition score was negatively correlated (P<.05; R= -0.41) with time from weaning to conception. Does with body condition scores less than or equal to five (27.24±1.25 d) had a longer interval to conception (P<.01) than does scoring greater than five (19.66±1.01 d) (Figure 9).

Progesterone concentrations were less (P<.01) for CSMH than for SBMG from August 14, 2003 to weaning (September 18, 2003) (Figure 10). Progesterone concentrations from August 14, 2003 to weaning (September 18, 2003) did not differ (P>.05) between CSML and CSMH. From weaning (September 18, 2003) to November 20, 2003, lower (P<.05) progesterone concentrations were observed for CSMH relative to SBMG and CSML (Figure 11). Serum progesterone concentrations for all groups averaged less than 2.5 ng/ml from August 14, 2003 to October 9, 2003. Progesterone concentrations increased thereafter, reaching a peak at November 13, 2003 (12.55 ± 3.73

ng/ml). Average progesterone concentrations in relation to estrus among all groups can be seen in Figures 12, 13, and 14.

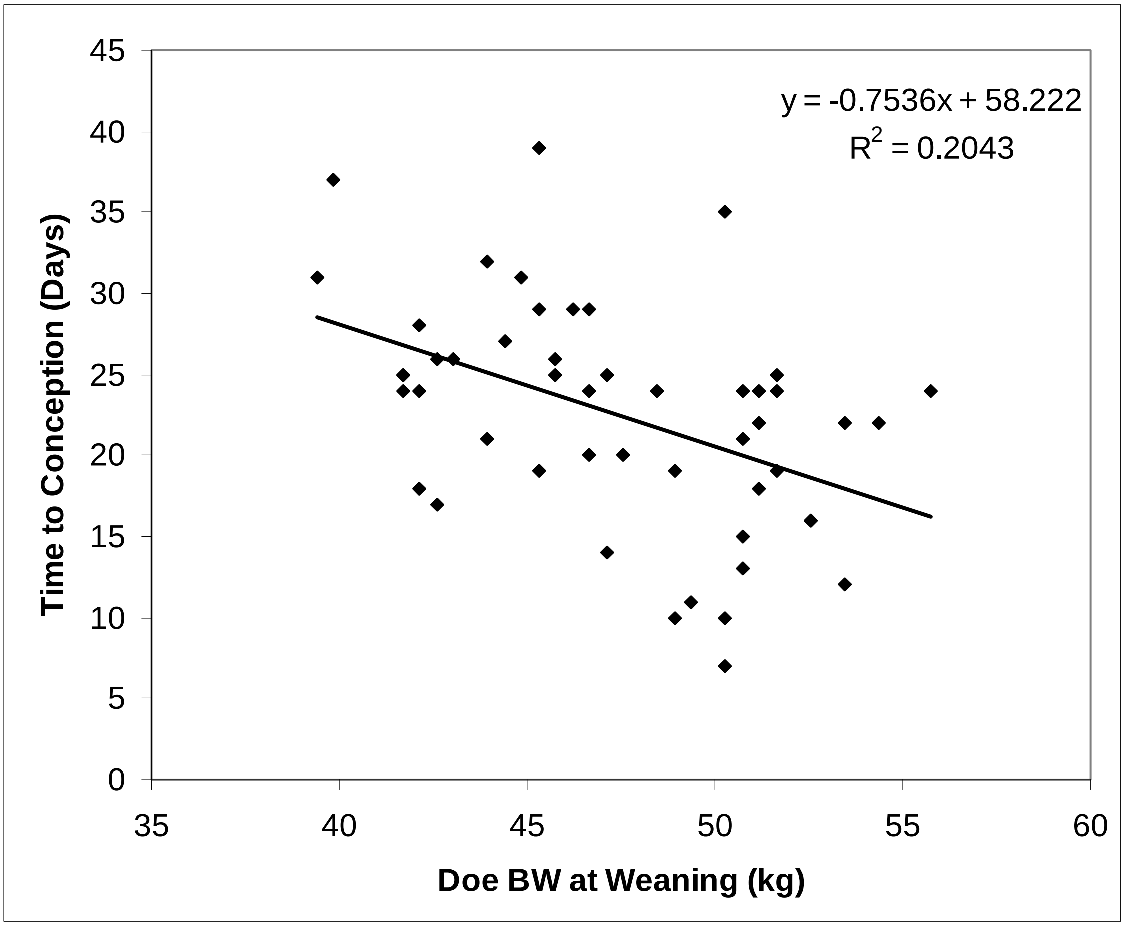

Figure 8. Time between weaning and conception in relation to BW of doe at weaning.

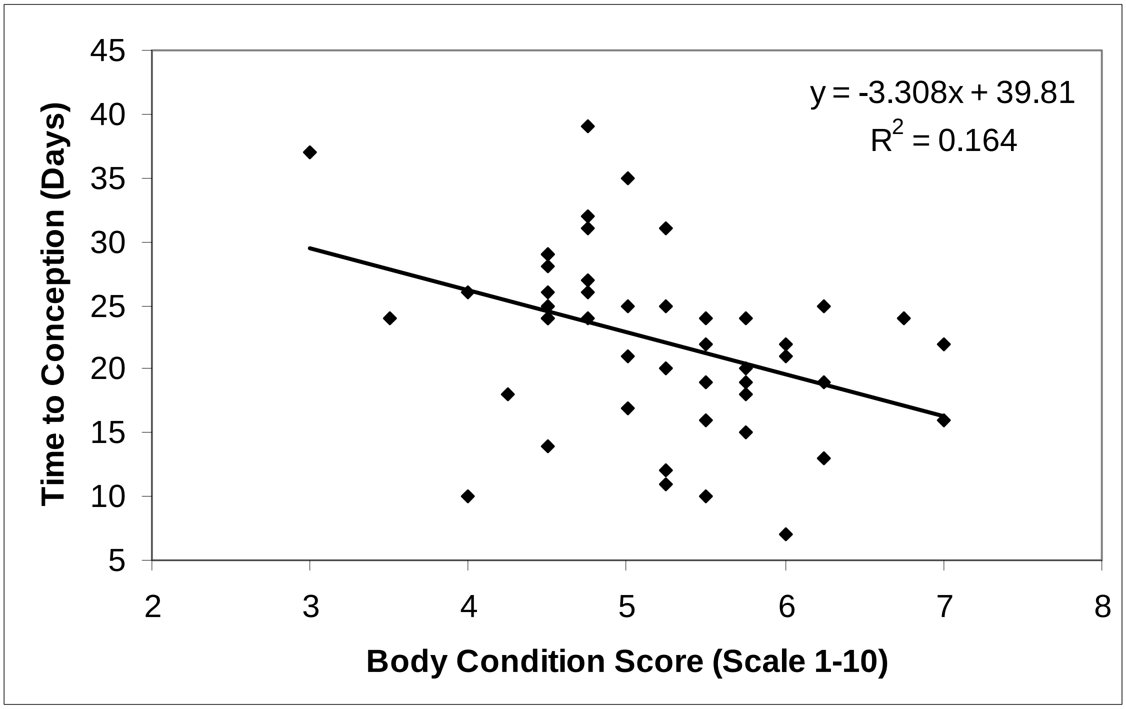

Figure 9. Time between weaning and conception in relation to body condition score at weaning.

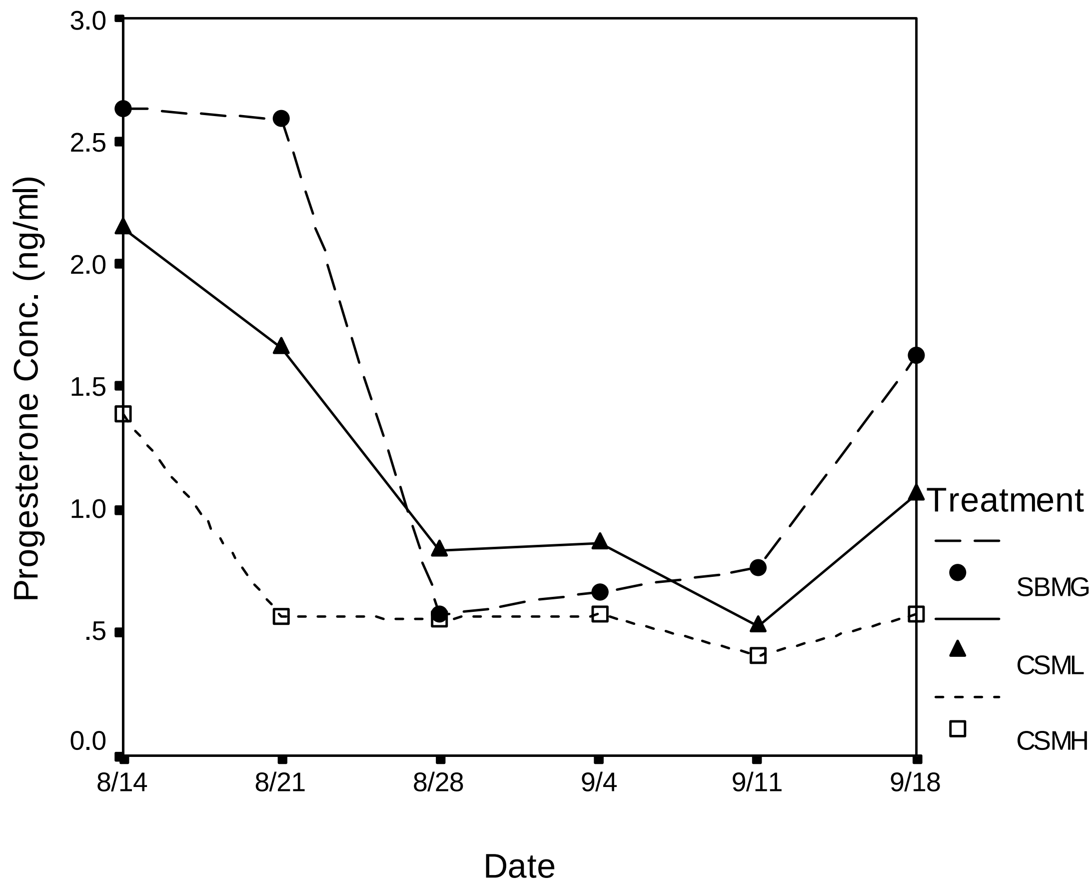

Figure 10. Estimated marginal means for serum progesterone concentration for lactating does from 8/14/03 to weaning (9/18/03) (SEM= 0.11).

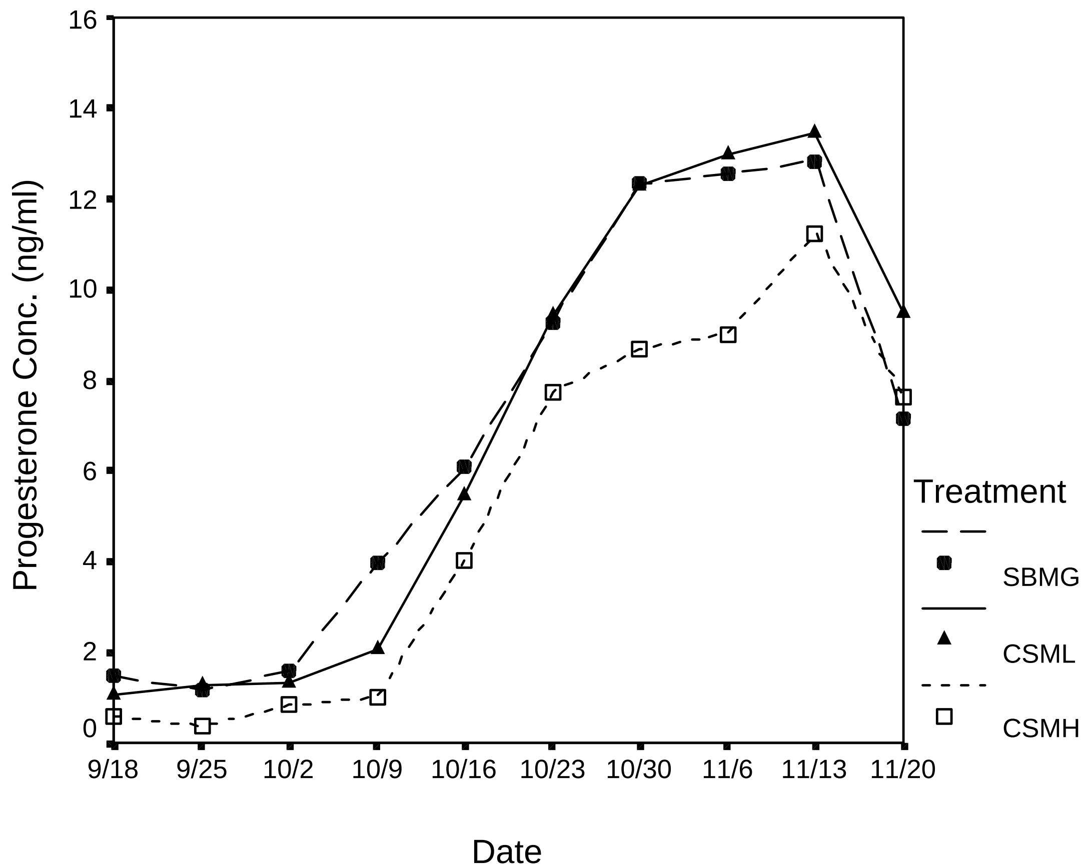

Figure 11. Estimated marginal means of serum progesterone concentrations among lactating does from weaning (9/18/03) to 11/20/03 (pooled SEM= 0.31).

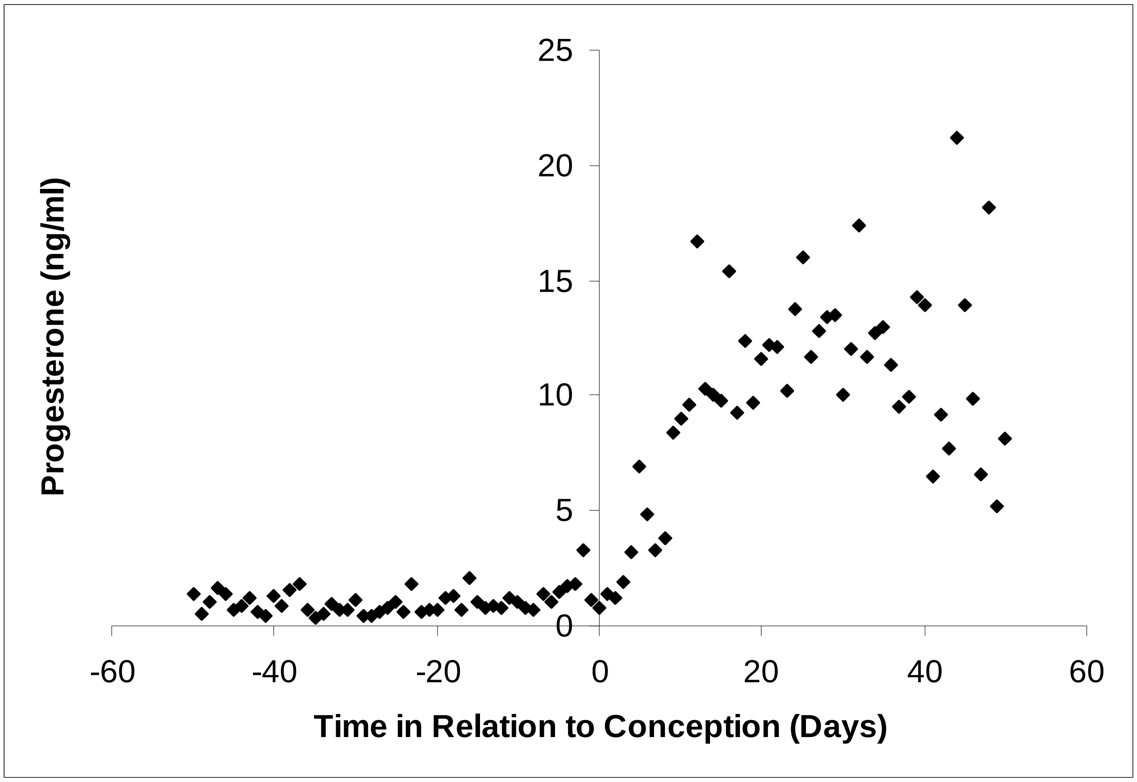

Figure 12. Profile of mean serum progesterone concentration relative to estimated date of conception (pooled SEM = 0.23).

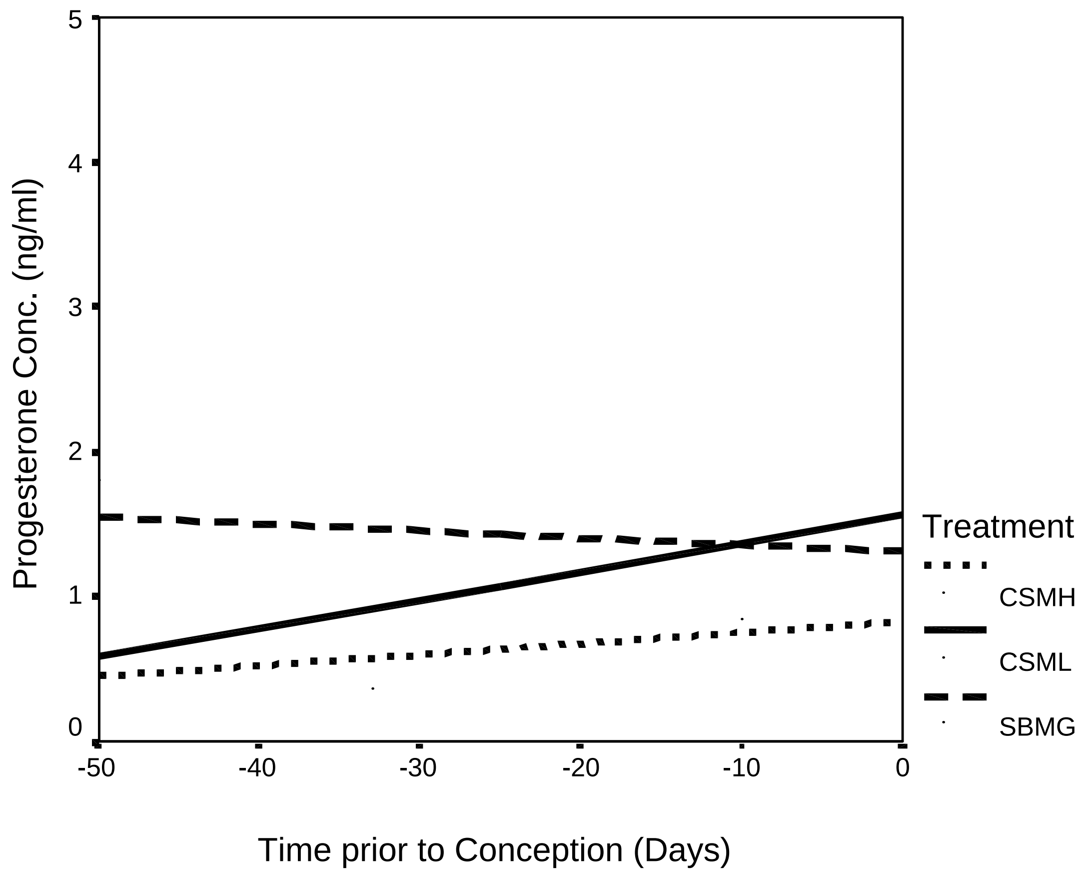

Figure 13. Serum progesterone concentrations from 50 days prior to conception up to conception by treatment. SBMG Rsq= 0.003, CSML Rsq= 0.085, CSMH Rsq= 0.026 (y = 0.12x + 1.21).

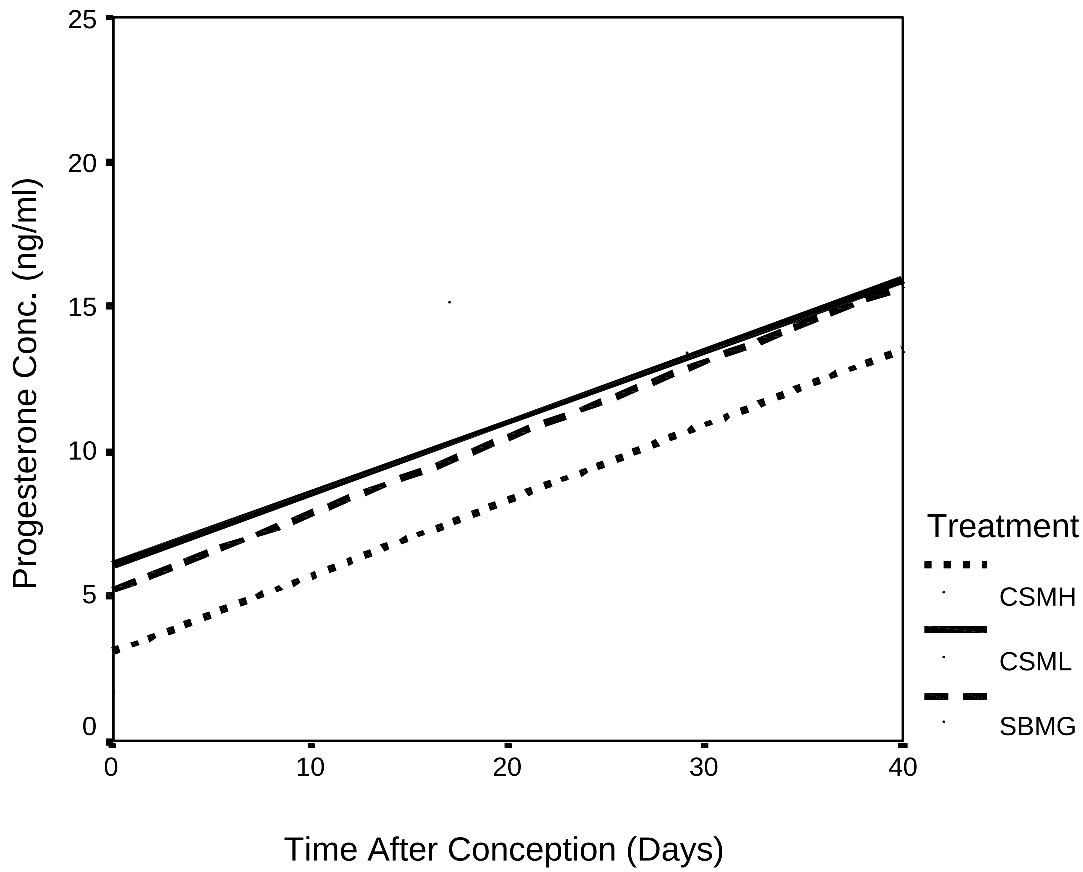

Figure 14. Serum progesterone profile from conception to 40 d pregnancy by treatment. Rsq SMBG = 0.33, Rsq CSML = 0.30, Rsq CSMH= 0.38 (y = .56x + 4.68).

CHAPTER VI

DISCUSSION

All does experienced reductions in BW throughout the experiment. Additionally, all does lost body condition before weaning and then gained body condition thereafter. This weight loss can be attributed to the normal physiological events that occur seasonally in fallow deer (i.e. lactation and seasonal food consumption patterns). Seasonally breeding deer will generally lose weight throughout the fall and winter and gain weight in the spring (Willard, 1996). When fed in excess, cottonseed meal products have been proven to cause deleterious physiological effects among animals (Abou-Donia 1976; Randel et al., 1992). Weight loss, anemia, and erythrocyte fragility are some of many symptoms associated with gossypol intake (Abou-Donia, 1976). Fallow does consuming CSM did have greater weight loss through the breeding season; however, these differences were apparently not substantial enough to affect animal health in regard to body condition, conception rate, or time to rebreeding. The difference in weight loss could be attributed to variation in metabolizing efficiency of fallow does in relation to the supplements. Prior research has demonstrated that ingested gossypol becomes concentrated in the liver mitochondria (Abou-Donia and Deichert 1974). Consequently, the metabolic cost of detoxification of CSM could also contribute to differences in weight losses between groups. The uncoupling effect of gossypol could also be responsible for loss of mitochondrial membrane potential that manifested itself as metabolic energy loss that might otherwise be used for protein or fat accretion. Gossypol has also been verified as an inhibitory agent of a growing number of enzymes. The potential physiological consequences involved are too many to be speculated on here, but could possibly result in reduced BW gains.

Previous trials conducted with fallow deer have indicated that heavier BW in does is associated with decreased fertility in relation to pregnancy rates (Willard et al. 1999). Willard et al., (1999) reported that non-lactating mature does were heavier than lactating mature does. Those non-lactating females were associated with decreased fertility as well. Mitchell and Brown (1974) found that wild red deer (*Cervus elaphus*) hinds that did not wean a fawn had higher fertility than hinds that did wean a fawn. Albon et al., (1986) showed that higher BW and body condition was correlated with higher pregnancy rates in farmed red deer. This experiment showed no correlation ($P>.1$) between BW and pregnancy rate or between lactational status and pregnancy rate. However, among lactating does, time to conception and first estrus was negatively correlated ($R= -.50$; $P<.01$) with weaning BW. Time to conception and first estrus was also negatively correlated ($R= -.41$; $P<.05$) with body condition scores at weaning. This would suggest that among lactating female fallow does, heavier BW and greater body condition are correlated with less time between weaning and conception and first estrus.

Studies done in rats confirm that gossypol administration can inhibit pregnancy and can also cause abortive effects if administered prior to implantation (Lin et al., 1985; Yang and Wu 1987). Research has also shown negative effects associated with reproduction in cattle. In vitro cattle research has verified that direct administration of gossypol can cause embryo degeneration (Zirkle et al., 1988). Randel et al. (1996) reported an increase in percentages of degenerative embryos recovered from Brangus heifers fed CSM (5 g free gossypol$\cdot$animal$^{-1}\cdot$day^{-1}). In vitro concentrations of gossypol

(5.0 μg/ml) disrupted bovine embryo development (Brocas et al., 1997). However, Brahman cows fed CSM (2 or 4 g free gossypol$\cdot$animal$^{-1}\cdot$day^{-1}) did not exhibit any determinable negative consequences concerning most cow or calf production variables (Willard et al., 1995). Brown (2001) demonstrated negative effects of free-choice supplementation of whole cottonseed to male fallow deer. A free gossypol intake in amounts up to 150 mg$\cdot$kg^{-1} BW (10.0$\cdot$g$\cdot$animal$^{-1}\cdot$day^{-1}) resulted in decreased body weight, body condition score, antler growth, and plasma testosterone concentration. In this study fallow does consuming relatively low concentrations of gossypol (8.1 mg$\cdot$kg^{-1}BW; 0.41 g$\cdot$animal^{-}1$\cdot$day^{-1}) via ingestion of CSM exhibited no negative effects in relation to pregnancy rates. This trial ceased prior to 45 d of pregnancy and therefore long term evaluations were not assessed in this experiment. Although the focus of this experiment was female reproductive performance, the high first estrus conception rates indicate that the fallow bucks were adequately fertile despite their consumption of CSM (free gossypol in amounts up to 5.1 mg$\cdot$kg^{-1}BW; 0.41 g$\cdot$animal^{-}1$\cdot$day^{-1}).

Pregnant rats fed 60 to 120 mg/kg BW of GAA exhibited reduced progesterone concentrations by day six (Yang and Wu, 1987). Gu et al. (1990) documented the inhibition of cAMP induced progesterone secretion in cultured bovine luteal cells by decreasing steroidogenic enzyme function. Gossypol appears to be a competitive inhibitor of adenylate cyclase as well. Adenylate cyclase is responsible in part for the regulation of cyclic AMP, an intracellular messenger, involved in a range of physiological processes including steroidogenesis (Olgiati et al., 1984). In contrast to other experiments, Randel et al. (1996) found increased luteal progesterone in heifers fed CSM (5 g free gossypol$\cdot$animal^{-}1$\cdot$day^{-1}). Although not consistent in its findings, previous research demonstrates that gossypol can alter progesterone synthesis and secretion. Cottonseed meal consumption (free gossypol in the amount of 8.1 mg·kg^{-1}BW; 0.41 g·animal^{-}1·day^{-1}) did appear to decrease serum progesterone concentrations in fallow does. However, Asher et al. (1989) demonstrated that the adrenal glands of fallow deer produce progesterone in response to stress. The inability to determine luteal derived progesterone from that of the adrenal glands diminishes the ability to evaluate luteal function. Asher et al. (1989) determined that adrenal progesterone secretion reaches a peak concentration (4-8 ng/ml) within 40 min following an ACTH challenge and then progressively decreases in concentration between 40 and 180 min. The timing and order in which animals were worked in this experiment could have contributed to the differences observed in serum progesterone concentrations among groups. Due to the subsequent complication of evaluating luteal progesterone concentrations from blood samples, considerations should be made regarding the timing and order in which animals are handled in order to control adrenal progesterone secretion. Randomization of the order in which animals are worked should minimize the effect of adrenal progesterone on circulating concentrations of progesterone across treatment groups.

Gossypol has been implicated in pre-implantation abortion among rats administered GAA (Lin et al., 1985; Yang et al., 1987). No abortions were detected by ultrasound during this trial. It has also been reported that gossypol can be transferred to neonates through the milk of lactating rats (Lin et al., 1992). Previously, Lindsey et al. (1980) found no such transfer of gossypol into the milk of lactating dairy cows fed CSM (free gossypol in amounts up to 6.6 mg·kg^{-1}BW; 42.7 mg·animal^{-}1·day^{-1}mg). Due to the relatively low concentrations of gossypol supplemented in this experiment, it is unlikely that any significant amount of gossypol was transferred to fawns through milk. There was no difference (P>.10) in birth weight, average date of parturition, or BW gain among fawns according to dam group. Fawn ADG did not differ amongst group, which would indicate that lactational performance among does was not affected by treatment. The higher birth weights of male fawns relative to female fawns found in this experiment is common for deer species (McEwen 1957).

The present study implies that multiparous fallow does can be supplemented with as much as 454 $g \cdot an^{-1} \cdot d^{-1}$ CSM, containing less than or equal to 0.09% free gossypol, without affecting reproductive performance or body condition.

CHAPTER VII

CONCLUSION

It appears that CSM can be of value for the supplementation of fallow deer and likely other deer species as well. However, careful consideration must be given to the amount of gossypol consumed to avoid harmful effects. At this time, the recommendation must be to limit daily free gossypol intake of fallow deer to less than 8.1 $mg \cdot kg^{-1}$BW (0.41 $g \cdot animal^{-}1$).

LITURATURE CITED

Abou-Donia, M.B. 1976. Physiological effects and metabolism of gossypol. Residue Rev. 61:126-160.

Abou-Donia, M.B., and J.W. Dieckert. 1974. Gossypol: Uncoupling of respiratory chain and oxidative phosphorylation. Life Sci. 14:1955-1963.

Albon, S.D., B. Mitchell, B.J. Huby, and D. Brown. 1986. Fertility in female red deer (*Cervus elaphus*): The effect of body composition, age, and reproductive status. J. Zool. 209:447-460.

Asher, G.W. 1985. Reproduction of farmed fallow deer (*Dama dama*). Pages 103-109 in Proc. Deer Course for Veterinarians, Ashburton, New Zealand.

Asher, G.W., M.W. Fisher, B.J. McLeod, and D.K. Berg. 1994. Reproductive physiology of cervids: A review. Page 257 in Proc. of Deer Course for Veterinarians, Queenstown, Australia.

Asher, G.W., A.J. Peterson, and D. Duganzich. 1989. Adrenal and ovarian sources of progesterone secretion in young female fallow deer, (*Dama dama*). J. Reprod. Fertil. 85:667-675.

Bansode, F.W. 1994. Genotoxic effects of gossypol acetate on the ovary of *Rhinopoma kinneari* wroughton (Microchiroptera: Mammalia). Contraception. 49:601-607.

Bazer, F.W. 1989. Allantoic fluid: Regulation of volume and composition. Pages 135-155. in Reproductive and Perinatal Medicine (Vol 11). Brace R.A., Ross M.G., and J.E. Robillard, eds. Perionatology Press, Ithaca, NY.

Bazer, F.W. 1992. Mediators of maternal recognition of pregnancy in mammals. Society for Experimental Biology and Medicine. 199:373-384.

Boland, M.P., and D.O. Callaghan. 1999. Nutrition and early embryonic development. Reprod. Dom. Anim. 34:127-132.

Boland, M.P., P. Lonergan, and D.O. Callaghan. 2001. Effect of nutrition on endocrine parameters, ovarian physiology, and oocyte and embryo development. Theriogenology. 55:1323-1340.

Braham, J.E., R. Jarquin, R. Bressant, J.M. Gonzalez, and L.G. Elias. 1967. Effect of gossypol on the iron-binding capacity of serum in swine. J. Nutrition. 93:241-248.

Breitbart, H., A. Mayevsky, and L. Nass-Ardon. 1989. Molecular mechanism of gossypol action on sperm mortality. Int. J. Biochem. 21:1097-1102.

Brocas, C., R.M. Rivera, F.F. Paula-Lopes, L.R. McDowell, M.C. Calhoun, C.R. Staples, N.S. Wilkinson, A.J. Boning, P.J. Chenoweth, and P.J. Hansen. 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.

Buser, M.D., and H.K. Abbas. 2001. Mechanically processing cottonseed to reduce gossypol and aflatoxin levels. Journal of Toxicology. 20:179-208.

Calhoun, M.C., J.E. Huston, S.W. Kuhlmann, B.C. Baldwin, B.S. Engdahl, and K.W. Bales. 1990. Free gossypol intake and erythrocyte fragility of lambs fed cottonseed meal processed by different methods. J. Anim. Sci. 68(Suppl. 1):53(Abstr.).

Calhoun, M.C., S.W. Kuhlmann, and B.C. Baldwin. 1995. Assessing the gossypol status of cattle fed cotton feed products Pages 95-101 in Proc. Pac. Northwest Animal Nutr. Conference. Washington State Univ., Pullman, WA.

Carruth, F.E. 1918. Contribution to the chemistry of gossypol, the toxic principle of cottonseed. J. Amer. Chem. Soc. 40:647-663.

Clark, E.P. 1928. Studies on gossypol II: Concerning the nature of Carruth's D gossypol. J. Biol. Chem. 76:229-235.

Clarke, I.J. 1995a. Evidence that the switch from negative to positive feedback at the level of the pituitary gland is an important timing event for the onset of the preovulatory surge in luteinising hormone. J. Endocrinol. 145:271-282.

Clarke, I.J. 1995b. The preovulatory LH surge: A case of a neuroendocrine switch. Trends in Endocrinol. and Metab. 6:241-247.

Cotton Board, The. 2003. Cotton economic data. National Cotton Council Economic Service Dept. Online.Available: http://www.cottonboard.org/index.cfm/4,355,62,54,html. Accessed Jan. 14, 2004.

Cuellar, A., and J. Ramirez. 1993. Further studies on the mechanism of action of gossypol on mitochondrial membrane. J. Biochem. 25:1149-1155.

Foster, D.L., F.J. Farsch, D.H. Holster, K.D. Ryan and S.M. Yellon. 1986. Determinants of puberty in a seasonal breeder. Rec. Prog. Horm. Res. 42:351-352.

Godfrey, R.W., F.M. Roquette, Jr., and R.D. Randel. 1988. Influence of cow weight change on cow reproductive performance and calf performance. J. Reprod. Agric. 1:221-227.

Goodwin, G.W., W. Gibboney, R. Paxton, R.A. Harris, and J.A. Lemons. 1987. Activities of branched chain amino acid amino-transferase and branched chain 2-oxo acid dehydrogenase complex in tissues of maternal and fetal sheep. Biochem. J. 242:305-308.

Gray, M.L., L.W. Greene, and G.L. Williams. 1993. Effects of dietary gossypol consumption on metabolic homeiostasis and reproductive endocrine function in beef heifers and cows. J. Anim. Sci. 70:3052-3059.

Gu, Y., C.J. Chang, Y. Rikihisa, Y.C. Lin. 1989. Inhibitory effect of gossypol acetic acid (GAA) on progesterone secretion in cultured bovine luteal cells. J. FASEB. 3:A866 (Abstr.).

Gu, Y., Y.C. Lin, Y. Rikihisa, 1990. Inhibitory effect of gossypol on steroidogenic pathways in cultured bovine luteal cells. Biochem. Biophys. Res. Comm. 169(2):455-461.

Harrison, R.J., and A.R. Hyett. 1954. The development of growth of the placetomes in the fallow deer (*Dama dama L.*). J. Anat. Lond. 88:338-355.

Hay, W.W. Jr. 1991. Energy and substrate requirements of the placenta and fetus. Proc. Nutr. Soc. 50:321-326.

Hofmann, R.R. 1985. Digestive physiology of the deer- their morphophysiological adaptation of their digestive tract. The Royal Soc. of New Zealand. 22:393-407.

Hofmann, R.R. 1989. Evolutionary steps of ecophysiological adaptation and diversification of ruminants: A comparative view of their digestive system. Oecologia. 78:443-457.

Huston, J.E., B.S. Rector, W.C. Ellis, and M.L. Allen. 1986. Dynamics of digestion in cattle, sheep, goats, and deer. J. Anim. Sci. 62:208-215.

Karsch, F.J., J.T. Cummins, G.B. Thomas, I.J. Clarke. 1987. Steroid feedback inhibition of pulsatile secretion of gonadotropin-releasing hormone in the ewe. Biol. Reprod. 36:1207-1218.

Kaur, H., and S.P. Arora. 1995. Dietary effect on ruminant livestock reproduction with particular reference to protein. Nutr. Res. Rev. 8:121-136.

Kerr, L.A. 1989. Gossypol toxicosis in cattle. Compend. Cont. Ed. Prac. Vet. 11:1139-1146.

Kie, J.G., L.D. Drawe, and S. Gretchen. 1980. Changes in diet and nutrition with increased herd size in Texas white-tailed deer. J. Range Mang. 33:28-34.

Kim, H.L. and M.C. Calhoun.1995. Determination of gossypol in plasma and tissues of animals. Inform 6:486 (Abstr.).

Kolena, J., S. Vrsanska, E. Nagyova, and M. Jezova. 2001. Gossypol inhibits follicle-stimulating hormone-and epidermal growth factor-stimulated expansion of oocyte-cumulus complexes from porcine preovulatory follicles. Physiol. Res. 50:627-630.

Kwon, H., G. Wu, F.W. Bazer, T.E. Spencer. 2003. Developmental changes of amino acids in ovine uterine and fetal fluids during pregnancy. Biol. Reprod. 68:1813-1820.

Lei, H.Q., Y.X. Xu. 1993. Effects of gossypol acetic acid on the motility of rat oviduct smooth muscle. Acta Pharmaceutica Sinica. 28:6-10 (Abstr.).

Lin, Y.C., Y.F. Hu, G.C. Chang, and P.P. Moh. 1992. Transfer of gossypol to neonatal rats via milk of nursing dams. Life Sci. 51:1657-1665.

Lin, Y.C., R. Pathmanayaki, Y. Rikihisa. 1985. Inhibition of embryo development in the unilaterally treated uterine horn of pregnant rats. Biol. Reprod. 32(Suppl. 1):143 (Abstr.).

Lin, Y.C., R. Rajamahendran, Y. Rikihisa, and F.C. Gwazdauskas. 1989. Effect of gossypol and its metabolite on in vitro early development of mouse embryos. Theriogenology. 31:727-740.

Lindsey, T.O., G.E. Hawkins, and L.D. Guthrie. 1980. Physiological responses of lactating cows to gossypol from cottonseed meal rations. J. Dairy Sci. 63:562-573.

Lyman, C.M., B.P. Baliga, and M. Slay. 1959. Reactions of proteins with gossypol. Arch. Biochem. Biophys. 84: 486-497.

Malpaux, B., C. Viguie, D.C. Skinner, J.C. Thiery, J. Pelletier, and P. Chemineau. 1996. Seasonal breeding in sheep: Mechanism of action of melatonin. Anim. Reprod. Sci. 42:109-117.

Mapletoft, R.J., and G.P. Adams. 1999. A new understanding of the bovine estrous cycle. Pages 459-466 in Proc. Southwest International Veterinary Symposium and Exposition. San Antonio, TX.

McEwen, L.C., E. French, N.D. Magruder, R.W. Swift, and R.H. Ingram. 1957. Nutrient requirements of the white-tailed deer. N. Amer. Wildl. & Nat. Res. Conf. 22:119-132.

Mitchell, B., and D. Brown. (1974). The effects of age and body size on fertility in female Red deer (*Cervus elaphus*). Proc. Int. Congr. Game Biol. 11(13E):89-98.

Mungall, E.C. 1994. Texotics Tables- Census Numbers. Exotic Wildlife Assoc. Publication- The Fence Line IV: 14.

Mungall, E.C., and W.J. Sheffield. 1994. Exotics on the range: The Texas example. Texas A&M University Press, College Station, Texas.

Olgiati, K.L., D.G. Toscano, W.M. Atkins, and W.A. Toscano. 1984. Gossypol inhibition of adenylate cyclase. Archs. Biochem. Biophys. 231:411-415.

Pons, W.A., C.L. Haffpauir, and T.H. Hopper. 1953. Influence of variety of cottonseed and environment. Ag. Food Chem. 1:1115-1118.

Randel, R.D., C.C. Chase, and S.J. Wyse.1992. Effects of gossypol and cottonseed products on reproduction of mammals. J. Anim. Sci. 70:1628-1638.

Randel, R.D., S.T. Willard, S.J. Wyse, and L.N. French. 1996. Effects of diets containing free gossypol on follicular development, embryo recovery, and corpus luteum function in brangus heifers treated with bFSH. Theriogenology. 45:911-922.

Reiser, R., and H.C. Fu. 1962. The mechanism of gossypol detoxification by ruminant animals. J. Nutr. 76:215-218.

Reyes, J., J. Allen, N. Tanphaichitr, R.A. Bellve, and J.D. Benos. 1984. Molecular mechanism of gossypol action on lipid membranes. J. Biol. Chem. 259:9607-9615.

Reyes, J., and D.J. Benos. 1988. Specificity of gossypol uncoupling: A comparative study of liver and spermatogenic cells. Am J. Physiol. 254:C571-C576.

Rollins, D. Personal Communication. Texas Agricultural Research and Extension Center, San Angelo, TX. Feb 2000.

Rowell-Schafer, A., R.R. Hofmann, M. Lechner-Doll, H.H.D. Meyer, S.P. Shirazi-Beechey, and I.S. Wood. 1997. The expression of intestinal sodium-glucose cotransporter in cervids. Z Saugetierkunde. 62(Suppl 2):170-175.

Rowell-Schafer, A., M. Lechner-Doll, R.R. Hofmann, W.J. Streich, B. Guven, and H.D. Meyer. 2001. Metabolic evidence of a "rumen bypass" or a "ruminal escape" of nutrients in roe deer (*Capreolus capreolus*). Comp. Bioch. Phys. A. 128:289-298.

Sasser, R.G., and C.A. Rudder. 1987. Detection of early pregnancy in domestic ruminants. J. Reprod. Fertil. 34:261-271.

Senger. P.L. 1997. Pathways to Pregnancy and Parturition. 1st ed. Current Conceptions Inc., Pullman, WA.

SPSS. 2001. SPSS for Windows (standard version 11.0.1). Chicago, IL.

Stahringer, R.C., D.A. Neuendorff, A.W. Lewis, J.P. Lewis, J.F. Edwards, and R.D. Randel. 1992. Effect of whole cottonseed feeding on thyroid function in beef heifers. J. Anim. Sci. 70(Suppl. 1):281(Abstr.).

Teng, C.S.1995. Gossypol-induced apoptotic DNA fragmentation correlates with inhibited protein kinase C activity in spermatocytes. Contraception. 52:389-395.

Thirumalesh, T., M.M. Kailas, and S. Yathiraj. 1999. Effect of feeding cotton seed extraction on haematological and biochemical values in crossbred calves. Indian J. Anim. Nutr. 16:366-368.

Ulrika, A., B. Biergersson, O. Leimar. 2001. The effect of food quality and relative abundance on food choice in fallow deer. Anim. Behav. 64:439-445.

Van Soest, P.J. 1982. The Nutritional Ecology of the Ruminant. O & B Books, Inc. Corvallis, OR.

Wheaton, C., and R.D. Brown. 1983. Feed intake and digestive efficiency of south Texas white-tailed deer. J. Wild. Mang. 47(2):442-450

Whitehead, G.K. 1993. The Whitehead Encyclopedia of Deer. Swan Hill Press. Shrewsbury, England.

Willard, S.T. 1996. Factors influencing pregnancy status, seasonal reproduction, and growth in farmed deer. Ph.D. Dissertation. Texas A&M Univ.

Willard, S.T., D.A. Neuendorff, A.W. Lewis, and R.D. Randel. 1995. Effects of free gossypol in the diet of pregnant and postpartum brahman cows on calf development and cow performance. J. Anim. Sci. 73:496-507.

Willard, S.T., S.J. Petty, R.G. Sasser, D.L. White, and R.D. Randel. 1999. Pregnancy detection and the effects of age, body weight, and previous reproductive performance on pregnancy status and weaning rates of farmed fallow deer (*Dama dama*). J. Anim. Sci. 1999. 77:32-38.

Willard, S.T., R.G. Sasser, J.T. Jaques, D.R. White, D.A. Neuendorff, and R.D. Randel. 1998. Early pregnancy detection and the hormonal characterization of embryonic-fetal mortality in fallow deer (*Dama dama*). Theriogenology. 49:861-869.

Williams, G.L. 1989. Modulation of luteal activity in postpartum beef cows through changes in dietary lipid. J. Anim. Sci. 67:785.

Williams, L.M., L.T. Hannah, C.E. Kyle, and C.L. Adam. 1996. Central melatonin receptors in red deer. General and Comparative Endocrinology. 104:1-6.

Wood, I.S., J. Dyer, R.R. Hofmann, and S.P. Shirazi-Beechey. 2000. Expression of the Na+/glucose co-transporter (SGLT1) in the intestine of domestic and wild ruminants. Eur. J. Physiol. 441:155:162.

Wu, G., F.W. Bazer, and W. Tou. 1995. Developmental changes of free amino acid concentrations in fetal fluids of pigs. J. Nutr. 125:2859-2868.

Wu, Y.M., S.C. Chappel, and G.L. Flickinger. 1981. Effects of gossypol on pituitary-ovarian endocrine function, ovulation and fertility in female hamsters. Contraception. 24:259-268.

Yang, Y.Q., and X.Y. Wu. 1987. Antifertility mechanisms of gossypol acetic acid in female rats. J. Reprod. Fert. 80:425-429.

Zirkle, S.M., Y.C. Lin, F.C. Gwazdauakas, and R.S. Canseco. 1988. Effect of gossypol on bovine embryo development during the preimplantation period. Theriogenology. 30:575-582.

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