EFFECTS OF GOSSYPOL CONSUMPTION ON GROWTH TRAITS OF RED DEER STAGS AND SUPPLEMENTAL MELATONIN FOR ADVANCEMENT OF ESTROUS CYCLES IN RED DEER HINDS

A Thesis

by

SHANE LEE MORGAN

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 2011

Major Subject: Physiology of Reproduction

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ABSTRACT

Effects of Gossypol Consumption on Growth Traits of Red Deer Stags and Supplemental Melatonin for Advancement of Estrous Cycles in Red Deer Hinds.

(May 2011)

Shane Lee Morgan, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Ronald D. Randel

Experiment I studied the effect of dietary gossypol (G) on antler and body growth traits of red deer stags, whereas Experiment II studied the effect of exogenous melatonin on female red deer reproductive traits. Specifically in Experiment I, thirty mature red deer stags were randomly allotted by weight, body condition score, and age to one of three treatment groups (n=10 each): control (C; 5:6 soybean meal:corn), extruded cottonseed pellet (P; 0.04% Free G, 0.36% Total G) and whole cottonseed-soybean meal (WCS; 5:3 cottonseed:soybean meal, 0.96% Free G & Total G). The supplements were mixed to be isocaloric (1661g/d TDN) and isonitrogenous (620-637g CP/d). Stags were fed daily for 155 d from antler casting (2/26/09) until hard antler had been reached (7/31/09). Antlers were measured using the Safari Club International (SCI) scoring method once hard antler was achieved. Hard antlers where removed just above the burr and allowed to dry (60 d) before weighing. Average daily gain did not differ ($P > 0.10$) among dietary treatment groups. However, average antler weights from C (1.130 ± 0.068 kg) and P (1.297 ± 0.068 kg) were greater ($P < 0.04$) than WCS (1.041 ± 0.068

kg) weights upon completion of the trial. Although SCI measurements were numerically lowest for WCS, differences were not significant.

In Experiment II, 60 mature and 24 yearling red deer hinds were assigned to two treatments on August 3rd; one received melatonin implants (MEL: n=42), while the other served as a control group (CNTRL: n=42). Hinds were evenly distributed to treatment by lactation status, age and body condition score. Antlerless stags were placed with the hinds (1:14) to provide natural service breeding during the trial. Implants were verified to be functional by a serum melatonin assay. Pregnancy status was determined by ultrasonography on d 105 and verified again on d 150. MEL treatment hinds displayed lower ADG (0.003 ± 0.007 kg/d) than CNTRL (0.020 ± 0.007 kg/d) hinds during the trial ($P < 0.01$). No advancement of estrous cycles was observed in red deer hinds implanted in early August ($P > 0.10$); however, pregnancy rates for yearling hinds were increased 36.4% ($P < 0.04$).

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In closing, as an animal scientist I have come to realize that in our exploration of living systems, we often discover much about ourselves.

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CHAPTER I

INTRODUCTION

Crude drawings of large-antlered animals that line the walls of historic dwellings illustrate that man's infatuation with deer began long before recorded history (Whitley, 2009). The lure of these majestic animals and possibility of profitable small acreage farming has facilitated the development of an entire animal industry. The growth and popularity of the native and exotic deer industry has increased dramatically over the last two decades in the United States. In 2007, the Department of Agricultural Economics at Texas A&M University estimated the deer breeding industry to contribute over $3 billion to the U.S. economy (Anderson et al., 2007). The production of seed stock, hard antlers, velvet antler, venison and recreational hunting are the current focal points driving this relatively new agricultural industry. Although historically the members of the *Cervidae* or "deer" family have been managed as wildlife, principally through management of ecosystems, increasing numbers of production situations are more similar to domestic animal production and management systems. Similar to domestic livestock production systems, the deer industry can also be divided into extensive and intensive practices. However, in the deer industry these systems are more commonly referred to as deer ranching and farming.

This thesis follows the style of the Journal of Animal Science.

The term game ranching is often used interchangeably with deer farming although they are fundamentally different production-based systems. Ranching often refers to "larger" acreage plots in which animals are typically not handled and are maintained at or below carrying capacity. These systems are typically more habitat and population management-based systems, often designed around hunting or recreational operations. Some supplementation of range mineral, feed or parasite control may be provided to assist with herd health and maintenance in these systems. Deer farming is a relatively new enterprise in the U.S. that is quickly gaining popularity. The lure of majestic antlers and the possibility of profitable small acreage farming have facilitated the rapid growth of this industry. However, in such countries as New Zealand and Scotland, deer farming has been a significant division of livestock production for many years (Blaxter et al., 1974; Fisher and Fennessy, 1985). This transition from wild to captive animals has allowed populations of these species to be comparatively viewed as livestock. Therefore, deer farming is typically considered a more intensive production system due to increased human involvement in herd health, nutrition and reproduction.

The increase in knowledge and management practices needed by this growing animal industry has opened a new chapter in small ruminant research. Traditionally, modified domestic animal management practices have typically been used by many deer managers. However, cervids possess antlers, a unique characteristic that influences the majority of the industry's income. Unlike traditional livestock species, antler development traits are highly correlated with monetary value of the animal (in all but

venison production systems). This unique phenomenon increases the necessity for deer-specific management protocols.

CHAPTER II

OBJECTIVES

The objective of this research was to examine the effects of gossypol ingestion upon body and antler growth of red deer (*Cervus elephus*) males, while also exploring the use of melatonin implants in advancing the breeding season of female red deer. Since pressure and heat during the pelleting process are thought to reduce the biological activity of gossypol (Randel et al. 1991), a pelletized cottonseed product was evaluated as an alternative to whole cottonseed (WCS). Successful accounts of exogenous melatonin initiating early estrous in sheep and goats, suggest that deer species may also be subject to seasonal manipulation by melatonin treatments (Gordon, 2005). The goal of this research was to explore and aid in the advancement of managerial aspects for cervid populations. Hypothesis I**:** Dietary gossypol will negatively affect antler and body growth. Hypothesis II**:** Pelleting processes will minimize the negative consequences of feeding cottonseed products by decreasing free gossypol concentrations. Hypothesis III**:** Melatonin implants will simulate short photoperiods, thus advancing the initiation of the seasonal breeding period and resulting in earlier calving.

CHAPTER III

LITERATURE REVEIW

Red Deer

Red deer *(Cervus elephus)* are one of the most majestic and well-researched deer species in the world. Although native to Eurasia, populations have also been introduced into Africa, South America, North America, Argentina and most notably, New Zealand. There are currently 12 subspecies of red deer, with body weights ranging from 90 to 300 kg depending on subspecies and sex (Whitehead, 1993). Red deer are members of the *Cervidae* family, classified as ungulates by the possession of even numbered toes and a four-chambered stomach (ruminant). Both males (stags) and females (hinds) display seasonal alterations in diet, growth and reproduction (Geist, 1998). The most evident of these circannual rhythms is the stag's growth of antlers, which become calcified prior to the breeding season. Like most deer species, red deer are short-day breeders with anestrous ending usually around September. Hinds will breed as yearlings if they have achieved 65-70% of their mature body weight (Fisher and Fennessy, 1985). The length of the estrous cycle is 18-21 days, with estrus lasting 12-24 hours. Gestation length averages 230-235 days, with placentation occurring during the first trimester around five to six weeks (Adam et al., 1985). Behavioral changes associated with increased sex steroids return stags to the hinds' home ranges to compete for harems. Displays of territorial competition and dominance include: "roaring" vocalizations, glandular/urinary marking and aggressive fighting.

In many countries, red deer have been domesticated and extensively utilized for venison production and for velvet antler production, which is medicinally popular in many Asian cultures (Berry, 1995). The ability to hybridize with close relatives elk (*Cervus canadensis)* and sika (*Cervus nippon*) also enhances red deer popularity. Elk x red deer hybrids display greater average daily gains (ADG) and increased carcass size in contrast to red deer calves, making them economically beneficial to venison producers (Pearse, 1992). The adaptability of red deer to various grazing systems is another attractive species attribute. Similar to native deer, red deer favor a variety of browse, grasses and forbs (Mungall and Sheffield, 1994). Although preferring browse and forbs, grasses/hay can compose approximately 90% of their diet in grassland environments (Hunt and Hay, 1992).

Physiology of Seasonal Reproduction

Healthy deer display seasonal changes in appetite, growth and reproduction throughout the year. These annual variations are highly correlated with circulating concentrations of melatonin. Melatonin is synthesized and released by the pineal gland only during dark hours; therefore, the amount of melatonin present is inversely related to photoperiod. Deer, along with sheep and goats, are termed “short-day breeders” and experience a reproductive season during the short photoperiod days of the fall. During this time, shortening photoperiods and increasing dark periods reduce the inhibition time of the suprachiasmatic nucleus (SCN) and thus the pineal gland, allowing progressively more melatonin to be released (Hadley and Levine, 2007). Melatonin binds to the

premammillary (PMR) region of the hypothalamus allowing the release of gonadotropin releasing hormone (GnRH). Although GnRH-releasing neurons are spatially close to the PMR they are not in direct contact; therefore, the actual circuitry is unknown. However, it is hypothesized that the involvement of kisspeptin neurons are a likely key in the missing linkage (Revel et al., 2007).

Prior to seasonal reproduction, adequate quantities of the neuropeptide GnRH must be released from the hypothalamus in order to elicit a response from the anterior pituitary (Senger, 1997). The release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the anterior lobe of the pituitary must also be at concentrations capable of supporting folliculogenesis/spermatogenesis. This neuroendocrine network is often referred to as the hypothalamic-pituitary-gonadal, or HPG, axis (Senger, 1997). This physiological system regulates the initiation of elevated gonadal activity and the increased production of steroid hormones responsible for many secondary sexual characteristics observed during breeding seasons. These events are normally referred to as the "rut" in wild populations and are often associated with behavioral aggressiveness, receptiveness and mating. In many females of seasonal breeding species the first ovulation occurring post-anestrous is referred to as a silent ovulation, as it is not accompanied by behavioral estrus. Similar to the onset of puberty, progesterone from the silent ovulation corpus luteum (CL) "primes" the brain by increasing its sensitivity to estrogen (Asher, 1985). When estrogen from follicular growth of the following estrous cycle increases, it is accompanied by behavioral estrus (Senger, 1997). It is suggested that seasonal anestrous evolved as a natural form of

synchronization preventing the birth of calves/fawns during conditions of possible high mortality. Native deer calve/fawn during the spring when forage and temperature favor lactation and offspring growth (Blottner et al., 1996; Malpaux et al., 2001).

Melatonin

Melatonin is a structurally simplistic indoleamine found in animals, plants and even some microbes. The dominant source of mammalian melatonin is derived from the pineal gland. The mammalian pineal gland is regulated by neuronal transmitted signals from the SCN "clock" and photoperiodic stimuli perceived by the photoreceptors in the retina (Morgan, 2000). Rhythmic secretions of melatonin play a significant role in synchronization of seasonal and circadian functions. The neurohormone melatonin (N-acetyl-5 methoxytryptamine) is secreted from pinealocytes located in the pineal gland. During dark hours, cAMP-mediated transcription of the N-acetyltransferase (NAT) enzyme converts cellular serotonin into the hormone melatonin (Bernard et al., 1997). The melatonin rate-limiting enzyme's degradation is light-induced, thus timing daily or circadian rhythms (Stehle et al., 2001). The soluble indoleamine is secreted into peripheral blood and cerebral spinal fluid, where it is circulated throughout the organism (Malpaux et al., 2001). Recent evidence of a molecular mechanism for timing, involving temporal melatonin-controlled expression of clock genes in "calendar cells" has been explored. Various strategically placed calendar cells in the brain, pituitary gland and elsewhere are proposed to control components of seasonal physiology and timing (Lincoln et al., 2003).

Although considered primarily a neurohormone, melatonin is also an excellent antioxidant and in many lower life forms, this is its sole responsibility. The indoleamine is a scavenger of reactive oxygen species (ROS), with a particular role in the protection of nuclear and mitochondrial DNA (Tan, 2000; Reiter et al., 2001). Although the antioxidant has the ability to cross both cell membranes and the blood-brain barrier, unlike other antioxidants (Vitamin C) it does not undergo redox cycling. Antioxidant redox cycling allows the reacquisition of antioxidant properties post-ROS reaction. Melatonin forms stable end-products and is unable to reduce once reacted with free radicals; therefore, it is termed a terminal antioxidant (Tan, 2000).

Antlerogenesis

The development of antlers is a secondary sex characteristic in male deer. Antlerogenesis is the relocation of substantial amounts of mineral compounds from the skeletal system and diet into the growing antler. Antlers are not grown directly from the skull; instead, they form atop pedicles located on the frontal bones. Pedicles are comprised of antlerogenic cells located in the periosteum at the lateral crest of the frontal bone. This region of regenerative cells is known as the antlerogenic periosteum (AP) and was first observed during transplantation studies (Hartwig and Schrudde, 1974). These bony protrusions are not present at birth but begin to develop as males approach puberty (Fennessy and Suttie, 1985). Li et al. (2001) proposed that this remarkable regenerative and transplantable phenomenon was stem cell-based. Although pedicle initiation is triggered by elevated circulating concentrations of androgens, antler development is

associated with low androgen concentrations (Suttie et al., 1998). Deer antlers are grown by a combination of intramembranous and endochondral ossification from atop the pedicle to its genetically determined height. Antler cartilage is comprised primarily of collagen types I, II and X, with rapidly growing regions being comprised of different cells depending on maturation stage in the chondrocyte lineage (Price and Allen, 2004).

When circulating testosterone concentrations rise during the decreasing photoperiods (fall), antler mineralization and behavioral transformations occur. Testosterone is one of the most influential androgens having stimulatory effects on the pedicle and inhibitory effects on antler growth. Although circulating blood androgen concentrations are low during antler growth, its presence is crucial for normal antler development. Deficient testosterone during antlerogenesis will impede the processes of mineralization, velvet shedding and antler casting (Goss, 1983; Brown, 2001). It is known that steroid hormones can encourage cell proliferation by modulating growth factors such as IGF-1 and IGF-2. Both IGF-1 and IGF-2 receptors have been discovered in the developing antler with speculation that IGF-1 is responsible for linear growth, while IGF-2 is responsible for limiting lateral growth due to its ring-like binding (Sadighi et al., 1994). Parathyroid hormone-related peptide (PTHrP) has also been shown to regulate differentiation in several antler cell types. In combination with locally synthesized retinoic acids, these factors seem to play important roles in the local regulation of cell proliferation and differentiation in regenerative antlers (Price and Allen, 2004).

Gossypol

Gossypol (G) is a polyphenolic yellow pigment produced in pigment glands of the cotton plant. Although it is found throughout the plant, the toxic chemical is infamous for limiting cottonseed's nutritional applications. Plant gossypol is found in a mixture of two stereoisomers, (+) and (-), with the latter isomer believed to have the greatest biological activity (Calhoun et al., 1995). Within cottonseed products, "free" and "bound" gossypol can be also be found in a variable ratio depending on the manufacturing processes. Cottonseed is naturally relatively high in free gossypol (FG). However, mechanical processes catalyzed by pressure and heat bind FG aldehyde groups to available amino groups (Reiser and Fu, 1962). The bound gossypol is therefore rendered biologically inactive and is able to pass harmlessly through the gastrointestinal tract (Randel et al., 1991). Free gossypol aldehyde groups were shown by Reiser and Fu (1962) to display an affinity for ε amino groups, especially lysine. Two moles of lysine were shown to bind with every mole of FG, whereas bacterial protein failed to bind or eliminate significant amounts of FG (Reiser and Fu, 1962). Lysine is an important amino acid in the formation of tropocollagen, the basic structural unit of bone and cartilage. Lysine deficiencies have been shown to retard development, bone growth, bone mineralization and growth hormone concentrations in rodents (Cree and Schalch, 1985; Odutuga and Amballi, 2007).

In contrast to monogastric animals, ruminants are able to inactivate low levels of dietary gossypol in their rumen, therefore decreasing their vulnerability to gossypol poisoning (Kerr, 1989). However, a variety of tissues have reportedly been affected by

gossypol toxicity in ruminants. Lowered hemoglobin and increased erythrocyte fragility, respiration rates and plasma protein were documented in mature dairy cattle fed high gossypol diets (Lindsey et al., 1980). Randel et al. (1996) observed that most ovarian, follicular and embryo characteristics of Brahman heifers were not significantly affected by dietary gossypol (15 g FG/d). However, results did suggest that increased FG intake may negatively influence ADG, progesterone concentrations and embryo viability. Willard et al. (1995) reported 4 g FG/d fed to cows pre- and postpartum impaired fetal calf skeletal development, disrupted vitamin metabolism and lengthened postpartum intervals. However, when gossypol was removed, long-term performance of the calves and cows remained unaffected.

Although the effects of gossypol are often not easily observed in ruminants, its consequences can be detrimental to fertility. Gossypol-induced lesions along the mid-piece of bovine sperm cells can subsequently lead to secondary spermatozoa transformations during extra-gonadal movement (Chenoweth et al., 2000). Damage to the germinal epithelium, spermatozoa mitochondria, and sperm plasma membrane were also accredited to the consumption of gossypol in rams and bulls (Chase et al., 1994; Chenoweth et al., 2000). Earlier investigations of Mg/Ca-Mg ATPase activity and plasma membrane calcium uptake revealed that the three parameters were almost completely inhibited by 10 microM gossypol in both ram and bull sperm (Breitbart et al., 1984). Supporting evidences in mouse spermatogenic cells show that inhibition of calcium channels and acrosome reactions are perhaps partial mechanisms of the anti-fertility effects of gossypol (Shi et al., 2003). It is important to note that the anti-fertility

effects of gossypol are often overshadowed by its toxic effects, especially in nonruminants.

Most gossypol studies have primarily focused on domesticated livestock species. However, cottonseed's popular use as a deer supplement in the southern United States led to its nutritional evaluation in cervid populations. Research in male fallow deer (*Dama dama)* discovered that when offered cottonseed *ad libitum*, bucks would consume enough gossypol to reduce body and antler growth (Brown, 2001). Mapel (2004) reported that a daily consumption of 0.41g FG/d reduced BW and serum progesterone in fallow does but did not appear to affect reproductive performance.

CHAPTER IV

EFFECTS OF GOSSYPOL CONSUMPTION ON THE GROWTH TRAITS OF RED DEER STAGS

Introduction

The increased numbers of native and exotic deer produced throughout the country has facilitated a demand for supplemental feeding programs supporting stocking rates that are beyond carrying capacity. As a cost-effective way of increasing protein, energy and fiber, whole cottonseed (WCS) and cottonseed products are routinely used in supplemental or complete rations. The practice of providing seasonal or continuous cottonseed *ad libitum* to deer populations is exploited by many managers in the southern United States "cotton-belt." The qualities of inexpensive protein, minimal non-target species use and availability contribute to the appeal and use of cotton products (Buser and Abbas, 2001). Although cottonseed appears to be an ideal resource, its use may have adverse effects on performance traits such as fertility, body weight and antler growth (Brown, 2001; Mapel, 2004). The cytotoxin gossypol, which is naturally produced in the cotton plant, is responsible for restricting the nutritional applications of WCS and CSM in nonruminant diets (Calhoun et al., 1995). Although ruminants are less susceptible to gossypol toxicity than monogastrics (Kerr, 1989), impacts on antler and body growth traits in deer may be economically significant. Venison production systems are very similar to domestic food animal production systems, wherein greater weight gains often correlate with increased income. In contrast, antler-based production systems focus

primarily on an animal's ability to grow large antlers, a characteristic unique to cervids. Genetics from exceptionally antlered animals are often prized, thus increasing the monetary value of these animals and their offspring. Regardless of a production system's focus, negative effects on desired traits would also negatively impact a business's income potential. Therefore, further research is needed in the use of gossypol-containing products and their effects on cervids.

Materials and Methods

Animals and Experimental Design

Thirty mature red deer stags from the Texas AgriLife Research and Extension Center at Overton, Texas were assigned to one of three dietary treatments using a randomized design. Red deer stags were randomly allotted within treatment groups such that age, body weight and body condition score were evenly distributed. Dietary treatments were formulated as follows: control (C) was comprised of 5:6 soybean:corn mixture, extruded cottonseed pellet (P) containing 0.04% FG and 0.36% total G, and whole cottonseed-soybean meal (WCS) comprised of 5:3 cottonseed:soybean meal, with 0.96% FG and total G. Treatments were constructed to be isocaloric (1661g/d TDN) and isonitrogenous (620-637g CP/d). All diet rations were analyzed for gossypol content and measured by Pope Testing Laboratories, Inc. using official methods of the American Oil Chemist Society. The chemical composition of each ration is detailed in Table 1.

Stags were fed daily rations (C: 2.09kg, P: 2.36kg, WCS: 1.95kg/stag) for the duration of antler growth. Antler growth lasted ≈155 days, from antler

Table 1. Comparison of dietary treatment rations.

		Treatment		
Components	(% DM)	CONTROL	WCS	PELLET
Crude Protein		29.1	32.6	25.0
Acid Detergent Fiber		8.4	34.7	36.9
Neutral Detergent Fiber		14.4	41.8	45.1
Lignin		1.8	10.7	8.8
Crude Fat		2.5	14.6	6.6
Ash		4.1	4.8	4.2
TDN		81	81	68
Calcium		0.22	0.39	0.24
Phosphorus		0.48	0.56	0.68
Magnesium		0.2	0.36	0.47
Potassium		1.25	1.47	1.34
Sodium		0.012	0.080	0.011
Sulfur		0.24	0.27	0.28
Dry Matter		89.5	92.7	92.0
	(ppm)			
Iron		123	112	146
Zinc		37	46	35
Copper		9	14	7
Manganese		31	38	17
Molybdenum		2.0	1.9	1.4

casting in February until hard antler was reached in August. Mature stags (n = 10/treatment) were maintained on 0.809 ha Coastal bermudagrass pastures with free access to mineral, salt and water. Using a drop floor chute equipped with scale, body weights were recorded on days 0, 28, 56, and then postponed until hard antler (≈day 155) to avoid damaging of the velvet antler. Once antlers were mineralized and stripped of velvet, stags were brought into the drop floor chute for antler measurements. Antlers were measured using the Safari Club International (SCI) scoring method seen in

Appendix A. After scoring, mineralized antlers were removed just above the burr with obstetric cable. Removed antlers were allowed to dry for a minimum of 60 d before weights were recorded.

Stratified across three dates (August 10, 18, 26), mature stags received a human chorionic gonadotropin (hCG) challenge (1000 IU/stag) via intravenous injection to assess testosterone production. Since mineralization is triggered by an increase in testosterone (Goss, 1983), challenge dates were assigned to animals according to their time of antler mineralization. Although animals were stratified by treatment within challenge dates, due to maturity differences in the timing of antler mineralization they could not be stratified by age.

The following year all stags were managed together on mixed bermudagrass pasture for the duration of the antler growing season, with periodic supplementation and ad libitum access to minerals and water. Stag antlers were again measured using the SCI scoring method and the same procedure/facilities as reported previously. However, antlers were not removed at this time for purposes of value retention at sale; therefore, no antler weights were available for analysis.

Blood Collection and Assay

All blood samples were refrigerated at 4°C and allowed to clot overnight. The following morning, samples were centrifuged at 1400 x g for 30 min to yield serum. Serum was collected and stored at -20°C until analysis. In correspondence with body weights recorded on days 0, 28, 56, and at hard antler (≈day 155), blood samples (8 mL)

were also collected at the time of weight measurements via jugular vein puncture. Samples were processed and stored for later analysis of serum amino acid composition and/or gossypol concentrations.

Intravenous hCG challenge (1000 IU/stag) was used to assess stag testosterone production across all treatments. Blood samples were collected before and at hourly intervals post-challenge for six consecutive hours. Testosterone concentrations were determined using radioimmunoassay procedures (Chase et al., 1994).

Statistical Analysis

Initial BW and Final BW were used to calculate ADG during for duration of the trial. Body weight and ADG were the dependant variables of interest and were analyzed by class variables treatment and age using the MIXED model procedure of SAS (2002). Individual antlers were used to calculate an average single antler weight for each animal. Antler data (score and average weight) were analyzed by treatment and age using the MIXED procedure (SAS). Differences in SCI scores recorded for year one and two were analyzed using the MIXED procedure specific for repeated measures. Also utilizing SAS, repeated measures ANOVA was conducted for serum testosterone concentrations analyzed by challenge date, hour, and treatment using PROC MIXED with age as a covariate.

Results and Discussion

Body Weight and Average Daily Gain

To determine gossypol's effects on body growth characteristics, stag body weights were recorded at the initiation and upon completion of the trial. Initial and final body weights were influenced by age ($P < 0.001$) but did not differ between dietary treatments ($P = 0.42$; Tables 2 and 3). Calculated average daily gains were also not affected by treatment ($P > 0.10$) but did show a tendency to be influenced by age ($P = 0.10$). As expected, 2-year-old stags had lower ADG than 3- and 4-year-old stags (Table 4). As cervids mature, less nutrient partitioning is required for growth and development and therefore more can be utilized for body maintenance and reserves. Thus, older stags are able to have greater increases in energy reserves and BW in preparation for the breeding season. Although adverse physiological effects have been documented from feeding cottonseed, stags consuming up to 0.16 g/kg (BW) of FG displayed no ill effects on body growth characteristics. In fact, WCS stags had numerically greater ADG than either C or P treatment stags. Treatment rations were designed to be isocaloric and isonitrogenous; however, the high fat content of WCS diets (Table 1) may be partially responsible for increased ADG. In contrast, results from Brown (2001) reported decreased weight gains in male fallow deer when fed whole cottonseed *ad libitum* (FG: 0.35-0.51 g/kg (BW)). Two obvious reasons for the contrasting results are the differences in dose of FG/kg (BW) and species. Understandably, a higher intake of FG should result in decreased performance. In addition, differences in vulnerability may result from digestive or ruminal differences between the two species. Forage studies

Table 2. Distribution of initial body by age and treatment.

	Initial Body Weight (kg)		
Treatment[a]	Age 2	Age 3	Age 4
Control	98.52 ± 4.2	127.73 ± 4.8	129.24 ± 4.8
WCS	96.93 ± 4.2	118 ± 4.8	125.91 ± 4.8
Pellet	96.48 ± 4.2	123.94 ± 4.8	132.72 ± 4.8
Average	97.31 ± 2.3[b]	123.64 ± 2.6[c]	129.29 ± 2.6[c]

[a] No differences ($P > 0.10$) were detected among treatments.
[b,c] Least square means within a row differ ($P < 0.01$).

Table 3. Influence of treatment and age on final body weight.

	Final Body Weight (kg)		
Treatment[a]	Age 2	Age 3	Age 4
Control	127.58 ± 7.8	169.34 ± 9.1	180.53 ± 9.1
WCS	150.71 ± 7.8	165.56 ± 9.1	170.25 ± 9.1
Pellet	128.37 ± 7.8	170.25 ± 9.1	188.39 ± 9.1
Average	135.55 ± 4.8 [b]	168.38 ± 5.6 [c]	179.72 ± 5.6 [c]

[a] No differences ($P > 0.10$) were detected among treatments.
[b,c,] Least square means within a row differ ($P < 0.01$).

Table 4. Influence of treatment and age on average daily gain.

	Average Daily Gain (kg/d)		
Treatment[a]	Age 2	Age 3	Age 4
Control	0.188 ± 0.04	0.269 ± 0.04	0.331 ± 0.04
WCS	0.347 ± 0.04	0.304 ± 0.04	0.286 ± 0.04
Pellet	0.206 ± 0.04	0.299 ± 0.04	0.359 ± 0.4
Average	0.247 ± 0.02 [b]	0.291 ± 0.02 [b,c]	0.325 ± .02 [c]

[a] No differences ($P > 0.10$) were detected among treatments.
[b,c] Least square means within a row differ ($P < 0.05$).

conducted at Kerr Wildlife Management Area found fallow deer diets to consist of 54% browse, 30% grass and 12% forbs (Mungall and Sheffield, 1994); while contrasting red deer diets may contain up to 90% grasses and hay (Hunt and Hay, 1992). Therefore, it is possible that the red deer's ability to utilize lower quality forages increases their ability to tolerate moderate levels of gossypol. However, Reiser and Fu (1962) reported the ruminal mechanisms of gossypol detoxification to be independent of rumen anaerobic/aerobic liquor incubation, temperature, centrifugation or proteolytic enzymes. They concluded that without impacting total gossypol, incubational decreases in lysine and FG concentrations were the result of an amino acid-gossypol bond that formed and remained permanent during digestion. Therefore, it is important to reiterate the differences between dietary treatments, wherein the addition of (lysine-rich) soybean meal to the WCS diet was absent in rations utilized by Brown (2001) for fallow deer. Considering free gossypol's high affinity for ε-amino acids (i.e. lysine), it can be hypothesized that the greater availability of lysine in the WCS ration potentially resulted in the binding of more gossypol, thus decreasing its toxicity.

Testosterone Production

To assess testosterone-producing capabilities, blood samples were drawn at 6 hourly intervals post hCG administration. Stags were administered an hCG challenge once antlers were mineralized and removed. To compensate for hourly bleeding intervals, challenges were organized across three dates (Aug. 10, 18, 26) with 10 animals per challenge date. As expected, an hourly effect ($P < 0.001$) was observed for

testosterone production post-challenge, with peak testosterone production occurring at 2 hr post hCG injection for all treatments (Figure 1). No dietary treatment effect was detected on testosterone production ($P = 0.25$). These findings are in agreement with previous studies in fallow deer (Brown, 2001), in which testosterone production during hCG challenges was not suppressed by gossypol consumption. These results suggest dietary gossypol is not responsible for decreasing Leydig cell function or concentration in cervid species. Although testosterone concentrations were not affected by treatment, they were influenced by age ($P < 0.001$), with the age 3 stags having the highest average testosterone production (Figure 2). A date effect ($P = 0.002$) was also observed for testosterone production, with coinciding date* age ($P < 0.001$) and date*treatment ($P < 0.001$) interactions. As seen in Figure 3, increases in average testosterone were observed with later challenge dates. This trend is not unexpected due to seasonal increases in hypothalamic-pituitary-gonadal axis activity. Increased HPG activity is responsible for increased spermatogenesis, testicular growth and sex hormones (i.e. testosterone) (Senger, 1997). Thus, an increase in testosterone production could be expected with subsequent challenge dates leading up to rutting behavior. Although animals were stratified by treatment within challenge dates, due to maturity differences in the timing of antler mineralization, they could not be stratified by age. Mineralization is triggered by an increase in testosterone (Goss, 1983); therefore, challenge dates were assigned according to time of antler mineralization. Mature stags developed hard antlers earlier than their younger counterparts, allowing many of the younger stags to fall into later

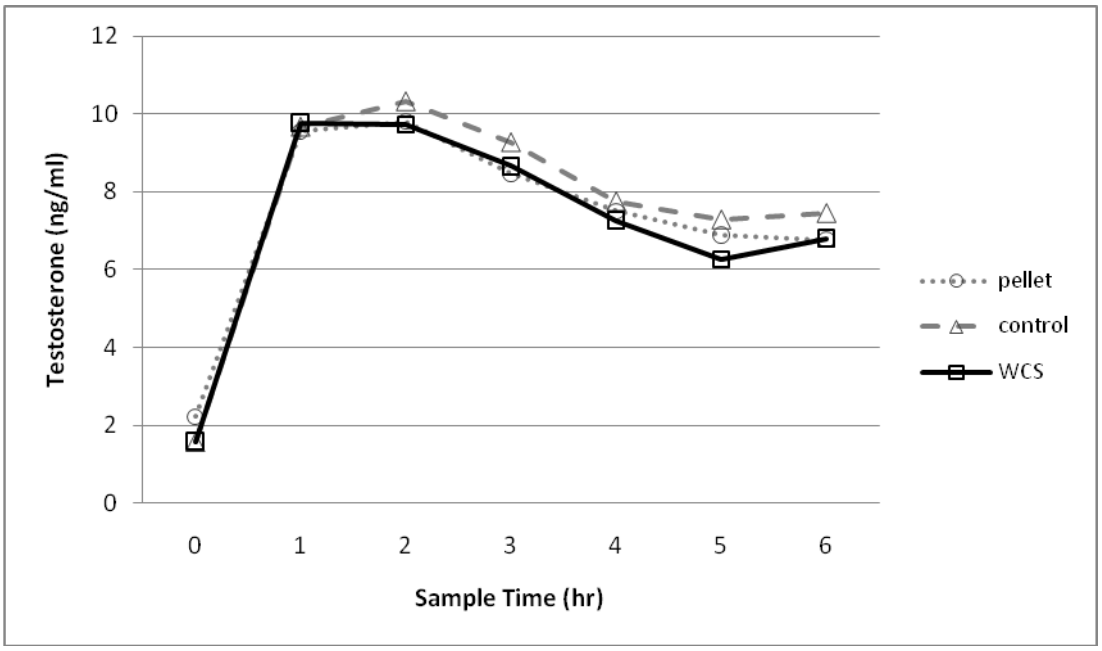

Figure 1. Influence of treatment on hCG-induced testosterone production ($P = 0.24$). Administration of 1000 IU hCG occurred immediately after 0 hr blood collection.

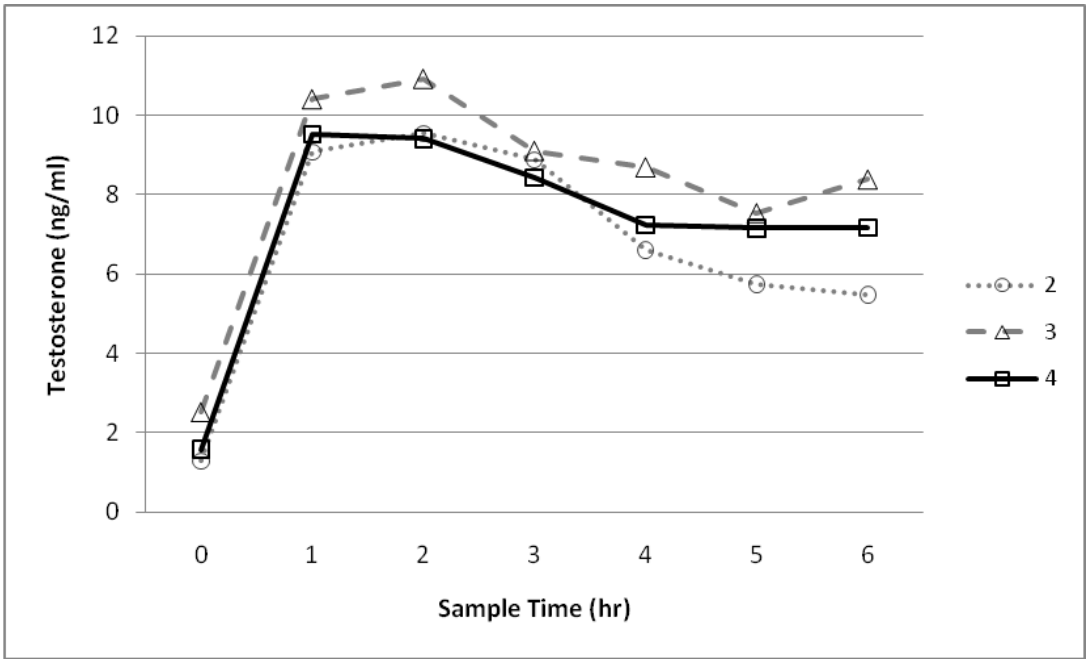

Figure 2. Influence of age on hCG induced testosterone production ($P < 0.01$). Administration of 1000 IU hCG occurred immediately after 0 hr blood collection.

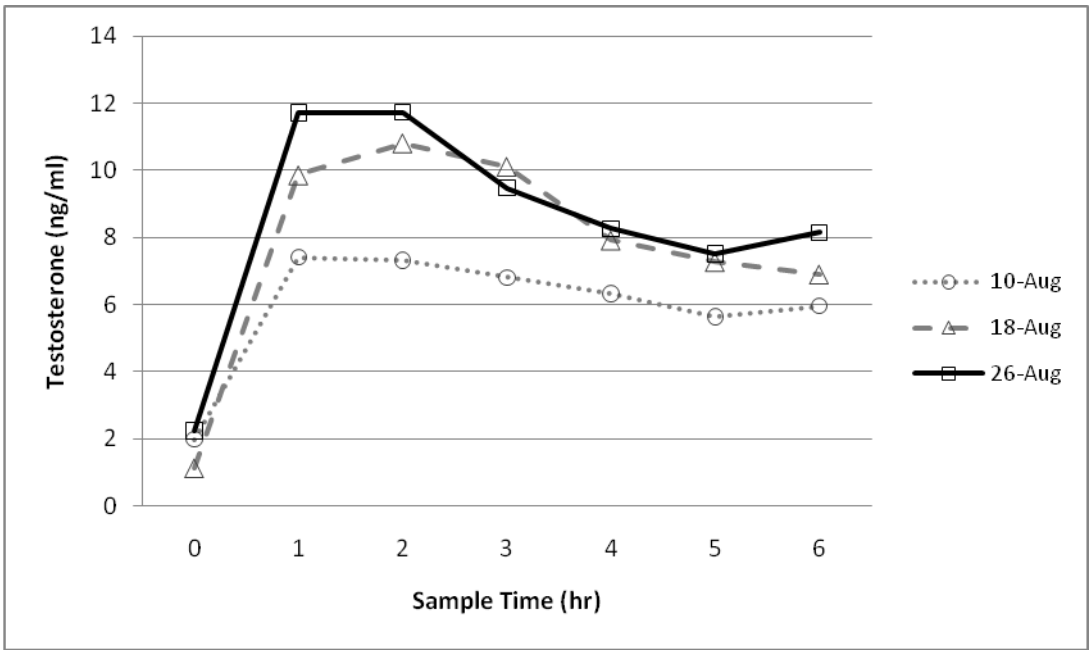

Figure 3. Influence of date on hCG induced testosterone production ($P < 0.01$). Administration of 1000 IU hCG occurred immediately after 0 hr blood collection.

challenge dates. Therefore statistical age*date interactions are linked to the ages found during each challenge.

Antler Development

Stags were placed on trial at the time of antler casting, with daily feeding and monitoring until the following antler mineralization (≈155d). Once velvet was shed, antlers were measured and scored using the Safari Club International method specific for red deer (Appendix A). After scoring, antlers were removed and weighed after a 60-d drying period. Antler weight was shown to be affected by dietary treatment ($P = 0.04$) and age ($P < 0.001$), with an age*treatment interaction ($P = 0.04$). As would be

expected, antlers from 2-year-old stags were lighter than their more mature 3- and 4-year-old counterparts (Table 5). Age and antler development often display a parabolic relationship in cervids with antler production typically peaking after mature body size is attained (Bender et al., 2003; Monteith et al., 2009). Dietary treatment results revealed P stags developed heavier antler weights than WCS ($P = 0.02$) and C stags ($P = 0.09$; Table 5). Although the numerically lowest average antler weights were recorded for WCS, they did not differ from C stags ($P = 0.37$). As seen in Table 5, 4-year-old antler weights for WCS stags were lower than C weights ($P = 0.02$), while also showing a tendency to be lower than P weights. Also shown in Table 5, 3-year-old stags in P (1.657 ± 0.124 kg) treatment had heavier antlers than either C or WCS stags. No treatment effect was observed for 2-year-old stag antler weights.

Safari Club International scores recorded before antler removal reveal that although SCI 1 measurements were numerically greater for C (186.22 ± 7.35 inches) and P (198.66 ± .7.6 inches) than WCS (183.73 ± 7.35 inches), differences were not significant ($P = 0.39$; Figure 4). As would be expected, age was shown to influence SCI 1 scores ($P < 0.001$). SCI 1 and the following year SCI 2 scores did not differ ($P = 0.42$), resulting in no influence of treatment on SCI 2 (Figure 4). In contrast to SCI 1, SCI 2 scores showed only a tendency to be influenced by age ($P = 0.07$; Figure 5). The smaller influence of age seen in SCI 2 is likely due to younger stags having increased in maturity and lessened their overall body growth requirements. Recalling the parabolic elationship of age and antler growth, as male cervids begin to mature, antler development begins to plateau and peak, exhibiting a less significant effect of age upon antler size.

Table 5. Influence of treatment and age on average antler weight.

Treatment	Antler Weight (kg) Age 2	Age 3	Age 4	Average
Control	$0.66 \pm 0.11^{a,x}$	$1.10 \pm 0.12^{a,y}$	$1.65 \pm 0.12^{a,z}$	1.13 ± 0.07^{a}
WCS	$0.67 \pm 0.11^{a,x}$	$1.23 \pm 0.12^{a,y}$	$1.22 \pm 0.12^{b,y}$	$1.04 \pm 0.07^{a,c}$
Pellet	$0.71 \pm 0.11^{a,x}$	$1.66 \pm 0.12^{b,y}$	$1.53 \pm 0.12^{a,y}$	$1.30 \pm 0.07^{b,d}$
Average	0.68 ± 0.06^{x}	1.32 ± 0.07^{y}	1.47 ± 0.07^{y}	

[a,b] Least square means within a column differ ($P < 0.05$).
[c,d] Least square means within a column differ ($P < 0.10$).
[x,y,z] Least square means within a row differ ($P < 0.05$).

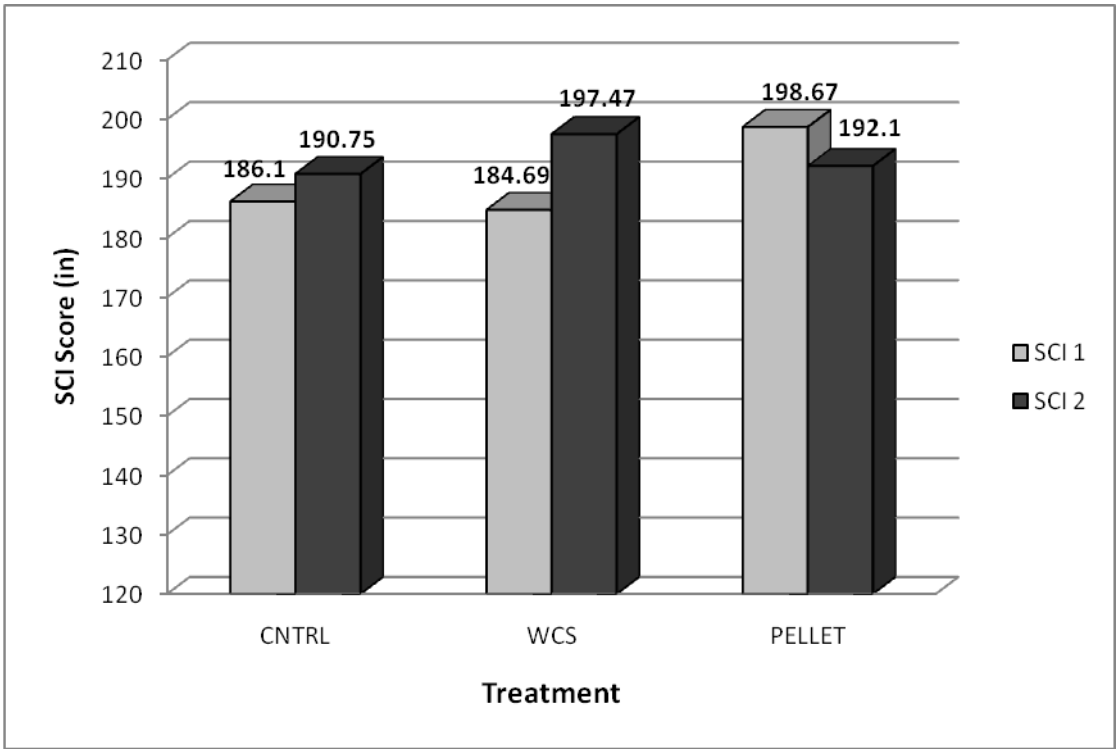

Figure 4. Influence of treatment on SCI scores 1 and 2 ($P > 0.10$). SCI 1 scores were recorded upon completion of dietary trial. SCI 2 scores were recorded for antlers grown the following year with no dietary treatments.

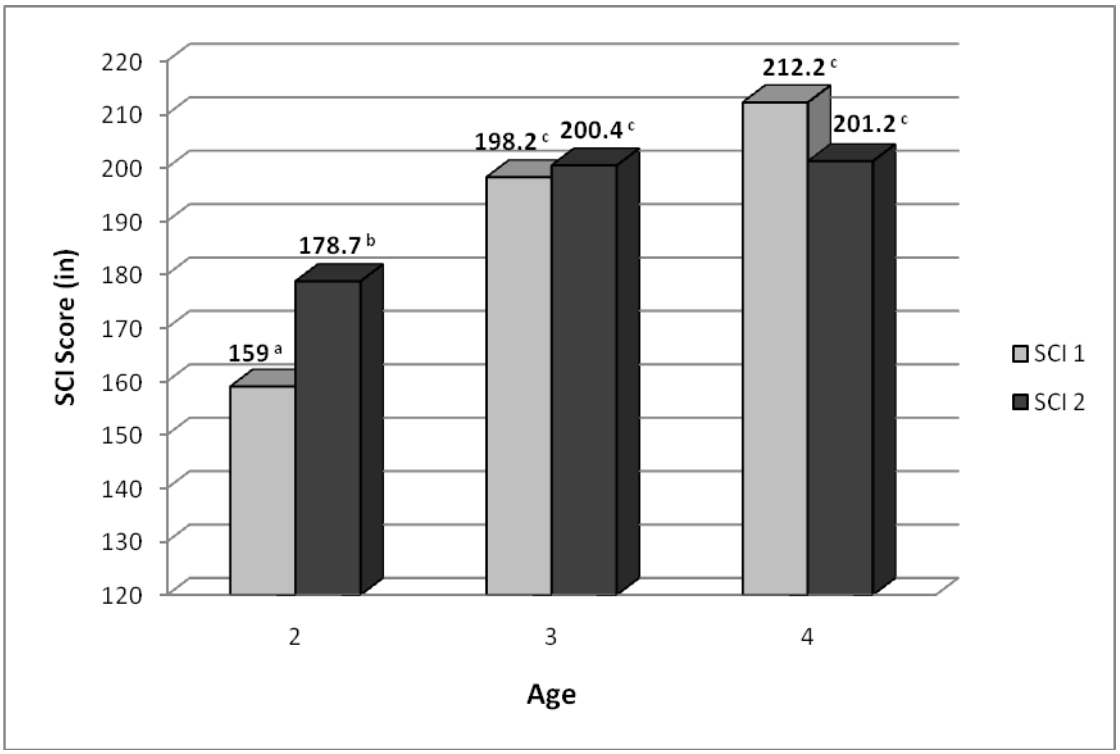

Figure 5. Influence of age on SCI scores 1 and 2. SCI 1 scores were recorded upon completion of dietary trial. SCI 2 scores were recorded for antlers grown the following year with no dietary treatments. [a,b,c] least square means differ with subscript ($P < 0.05$).

Although SCI scores were unaffected by dietary treatment, antler weight data suggest an increase in gossypol susceptibility in mature stags. These results are similar to Brown (2001) wherein 1-year-old fallow buck antlers were unaffected by whole cottonseed ingestion, while 2-year-old stag antlers were twofold lighter than control bucks. Unlike mature animals, young cervids partition nutrients primarily for body growth and development, leaving fewer nutrients available for antler production. Therefore, nutritional restraints would be difficult to document in currently under-emphasized traits such as antler production. In an attempt to explain the mechanics of

gossypol's effect on mature cervid antlers, we look to documented influences on bone and cartilage. Bone and antler mineralization has been linked to chondrocyte mitochondria and their reserves of calcium and phosphorus (Sayegh et al., 1974). Gossypol's documented detrimental effects on Ca^{2+} channels and mitochondria are perhaps reasons for decreased antler weight and density (Breitbart et al., 1984; Chenoweth et al., 2000). In addition, we are led to revisit the idea of gossypol's affinity for lysine. Lysine is an important amino acid in the formation of tropocollagen, the basic structural unit of bone, cartilage and antlers. Lysine is also considered one of the most limiting amino acids in ruminant diets (Richardson and Hatfield, 1978). Diets deficient in lysine have been shown to retard development, bone growth, bone mineralization and growth hormone concentrations in rodents (Cree and Schalch, 1985; Odutuga and Amballi, 2007). Therefore, the ruminal binding of gossypol to amino acids is perhaps one rationale for limiting nutrients and antler production. This may also attribute to the magnitude of differences seen in Brown (2001) and this study, wherein the high lysine content in the added soybean meal helped to minimize free gossypol concentrations. Supplemental iron is also considered to have detoxifying qualities in the presence of gossypol (Tone and Jensen, 1974). Deer were allowed free access to mineral during all trials conducted at the Texas AgriLife Research and Extension Center at Overton (Brown, 2001; Mapel, 2004; and the present study). Damage to body and growth traits suggest that *ad libitum* access to supplemental mineral is not sufficient for gossypol detoxification in cervids. Additionally and perhaps most notably, the pelletized

cottonseed product containing lower free gossypol concentrations showed no detrimental consequences on antler development.

Conclusion

While the daily consumption of whole cottonseed showed no negative effect on weight gain or SCI score, it did tend to reduce antler weight in 4-year-old red deer stags. The mechanics of gossypol's influence on antler development are not well understood, partially due to the fact that the nature of antlerogenesis regulation is still very much a mystery. However, considering gossypol's affinity for lysine, an important amino acid for body, bone and antler growth, it can be deduced that gossypol permanently binds dietary lysine, restricting available amino acids for growth and development. These findings, in conjunction with Brown (2001), may also indicate that the addition of soybean meal to whole cottonseed provides a degree of protection against gossypol toxicity, possibly due to the combined effect of detoxification and amino acid supplementation. These results imply that red deer in a non-antler affiliated production system can be supplemented with a daily (5:3) cottonseed-soybean ration, containing up to 0.16 g/kg (BW) of FG. Additionally, it was confirmed that the pelleting process decreases the amount of free gossypol, allowing these cottonseed products to be safely used in male red deer production systems.

CHAPTER V

EVALUATION OF MELATONIN IMPLANTS FOR ADVANCING ESTROUS CYCLES IN RED DEER HINDS AND CONCLUSION

Introduction

The seasonal events of antlerogenesis and reproduction are governed by rhythmic hormone concentrations. These cyclic hormone concentrations are entrained by annual changes in daylight length. Day lengths are measured by the amount of light hours present during the ≈ 24 hr period. This measurement of light hours is referred to as the photoperiod, with long days having more light hours and short days more dark hours. In the northern hemisphere, long days occur during the spring-early summer, while short days occur during the fall-early winter. Deer are "short-day breeders," displaying seasonal polyestrous followed by a period of anestrous during long days (Adam et al., 1986). Although the actual mechanisms involved in the control of seasonality are unclear, the neurohormone melatonin is believed to provide the biological signaling in response to photoperiod (Malpaux et al., 2001). Melatonin is only secreted during dark hours and is thus seen in higher concentrations during short days. Therefore, advancing the elevation of circulating melatonin concentrations *in vivo* could possibly advance the onset of seasonal estrous cycles in female deer. Breeding seasons are routinely used by traditional livestock managers to provide parturition during desired times. Altering seasonal periods of estrous would allow deer producers to select for optimum offspring timing or allow increased time intervals for assisted reproductive techniques. Many

assisted reproductive techniques involve surgical procedures (i.e. laparoscopic embryo transfer/artificial insemination) which require lengthy healing periods. Healing intervals often limit seasonal attempts at assisted reproductive techniques. Therefore, altering the perception of photoperiod could potentially increase productivity in these species which have been previously limited by their seasonal biology.

Materials and Methods

Animals and Experimental Design

Sixty mature and 24 yearling red deer hinds from the Texas AgriLife Research and Extension Center at Overton, Texas were assigned to two treatments using a randomized design. One treatment group received melatonin implants (MEL; n = 42), while the other served as a control group (CNTRL: n = 42). Designated MEL treatment hinds received a 24 mg melatonin implant (Regulin®) on August 3rd. Implants were inserted subcutaneously behind the base of the ear using a Regulin® applicator gun. Hinds were evenly distributed by treatment, lactation status (wet/dry), age (yearling, 2-years-old, mature) and body condition score, into two 1.6 ha bermudagrass pastures (n = 42 per pasture). Body condition score (BCS) was used as a reference for an animal's energy reserve or body fat deposition. Hind BCS was obtained by estimating fat deposition above the pelvis bones and vertebrae. A BCS of one represents a severely emaciated animal, while a ten would represent an obese animal. Three antlerless stags were also placed within each pasture of hinds to provide natural service breeding during the fall (n = 3:42). Using a drop floor chute, these stags were equipped with an *Ovine* ram crayon

marking harness so that estrus detection could be visually observed. However, due to breeding behavior and constant wallowing, breeding marks from crayons were rarely observed. Hinds were brought back in seven days post-implantation for weighing and blood collection. Stags were removed on November 15th, allowing for a 105 d breeding period. Gestation was determined by ultrasonography at the time of buck removal on November 15th and then again 45 days later to confirm all pregnancies.

Blood Collection and Assay

Post-implantation blood samples (8 ml) were collected via jugular vein puncture. Blood samples were refrigerated at 4°C and allowed to clot overnight. The following morning, samples were centrifuged at 1400 x g for 30 min to yield serum. Serum was collected and stored at -20°C until analysis. Melatonin concentrations were determined from serum using a melatonin research radioimmunoassay kit from Rocky Mountain Diagnostics, Inc. (REF: R-3900).

Statistical Analysis

Serum melatonin concentrations were analyzed by class variables treatment, lactation status and age using the MIXED model procedure of SAS (2002). Dependent variables body weight, body condition score and ADG were analyzed by treatment and age using the PROC MIXED procedure of SAS. Gestation determination by ultrasound was performed twice on all hinds, with the average estimated gestation length used for statistical analysis. Gestation was analyzed by treatment, lactation and age using the

MIXED model procedure (SAS). To determine differences in pregnancy rates, conception was analyzed by treatment using Chi square analysis procedures of SAS.

Results and Discussion

Serum Melatonin Concentration

Initially, to confirm the presence and functioning of melatonin implants, all blood samples were collected at noon for assaying. Melatonin assays verified the presence of implants, showing MEL hinds to have higher serum melatonin concentrations ($P < 0.001$) than CNTRL hinds (Figure 6). Melatonin concentrations also tended ($P = 0.06$) to be influenced by age, with 2-year-olds having the highest concentrations of melatonin followed by the yearlings and then the mature hinds (Figure 7). Although these results suggest an influence of age on implant serum melatonin concentrations, this is likely due to the small sample size of 2-year-old hinds.

Body Weight, Condition and Average Daily Gain

Initial body weights for hinds were not influenced by treatment ($P = 0.36$) but were affected by lactation status ($P = 0.002$) and age ($P < 0.001$). Initial BCS was also influenced by lactation ($P = 0.003$), with a tendency to be influenced by age ($P = 0.10$). As would be expected, yearling hinds had lower initial BW than 2-year-old and mature hinds (Figure 8). Unexpectedly, lactating hinds had higher initial BW than dry hinds (Figure 9), although initial BCS were ¾ of a condition score lower for lactating hinds (Figure 10). However, no influence of treatment was observed for initial

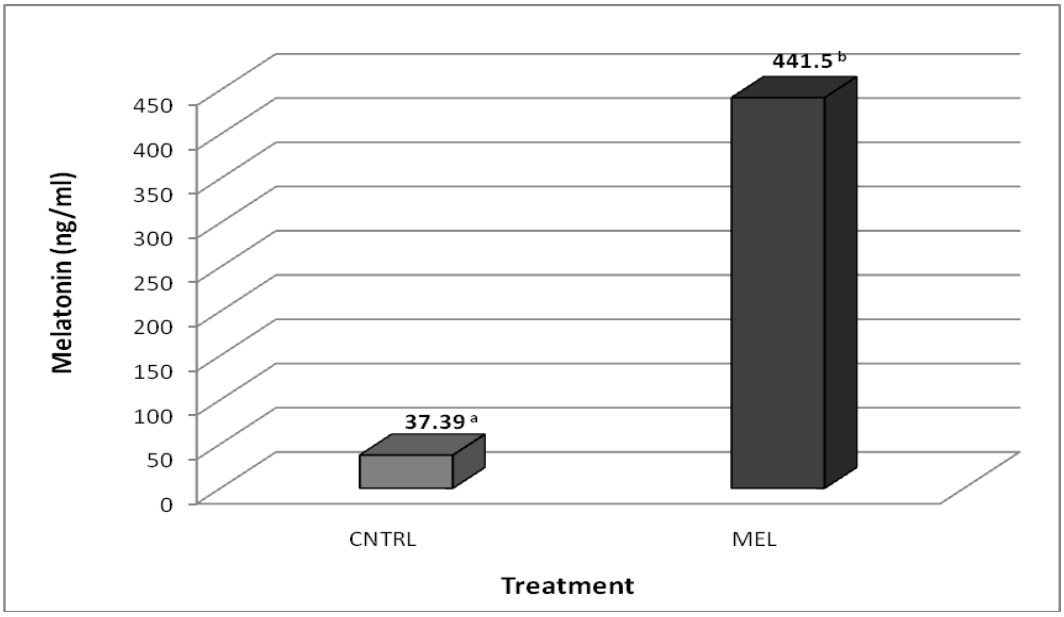

Figure 6. Influence of treatment on serum melatonin concentrations.
[a,b] least square means differ with subscript ($P < 0.05$).

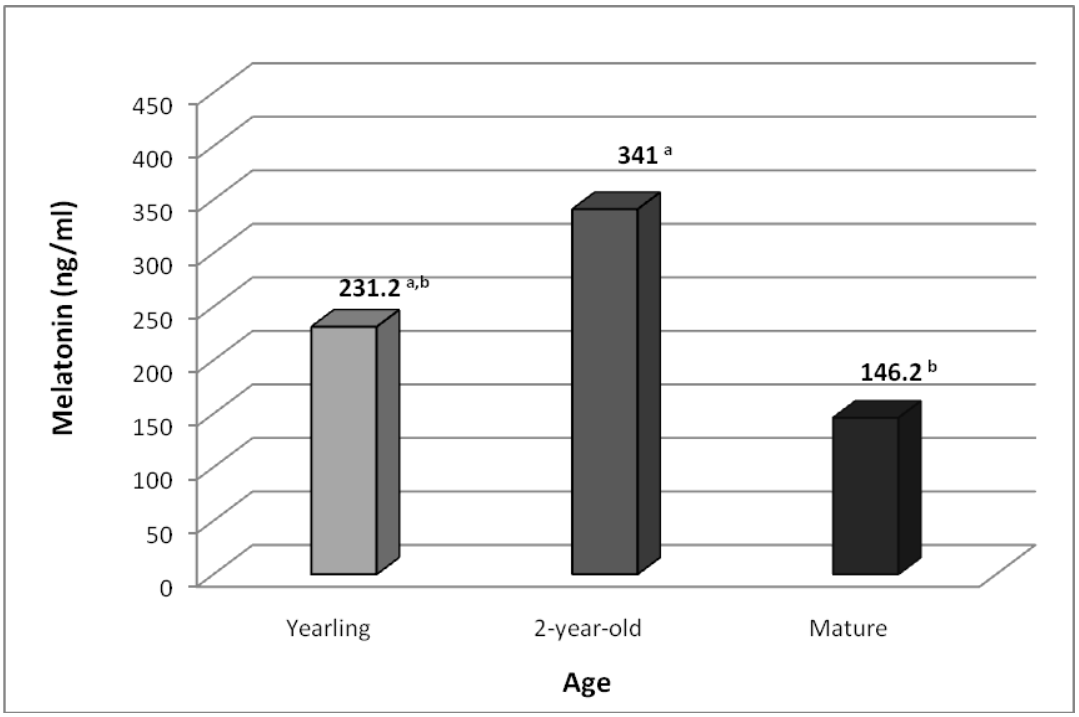

Figure 7. Influence of age on serum melatonin concentrations.
[a,b] least square means differ with subscript ($P < 0.05$).

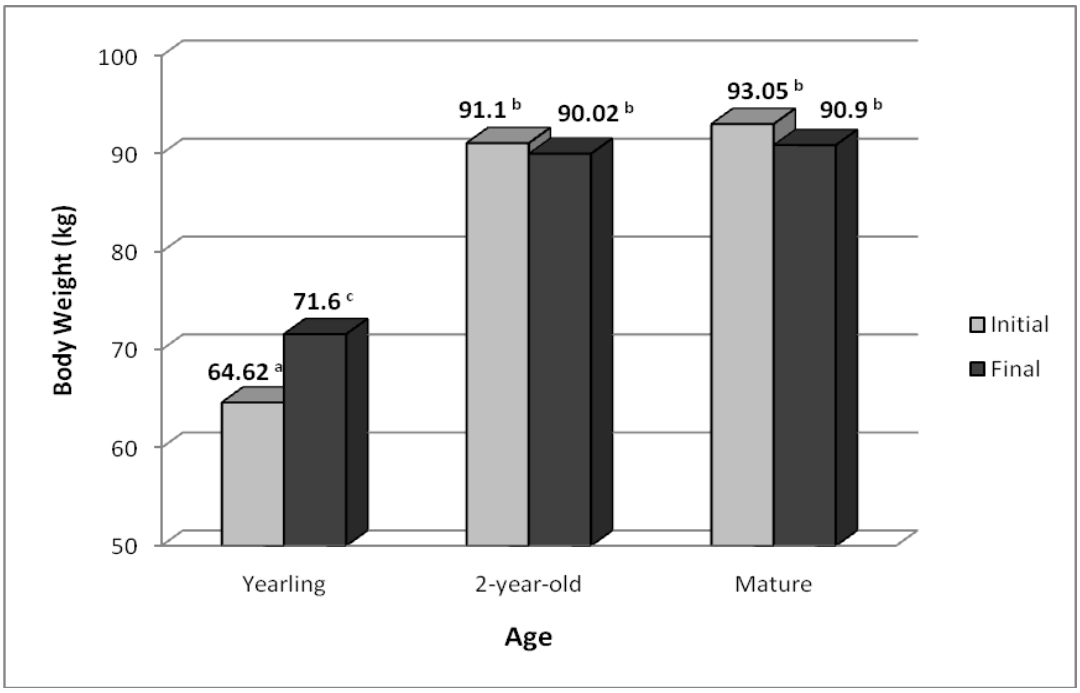

Figure 8. Influence of age on initial and final body weight.
[a,b] least square means differ with subscript ($P < 0.05$).

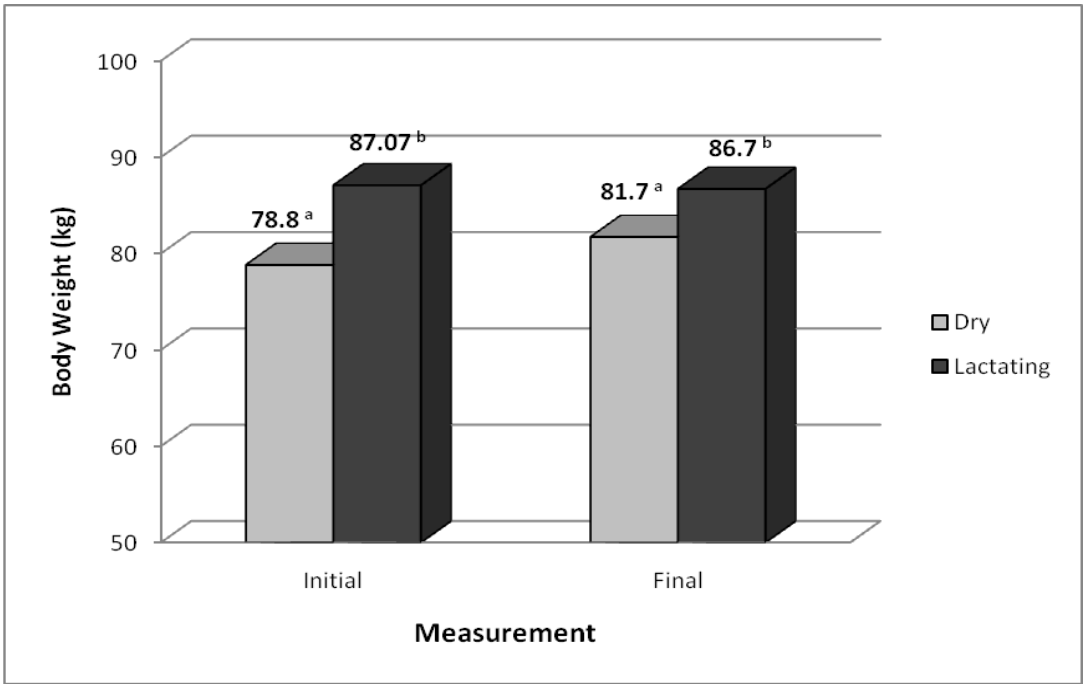

Figure 9. Influence of lactation status on initial and final body weight.
[a,b] least square means differ with subscript ($P < 0.05$).

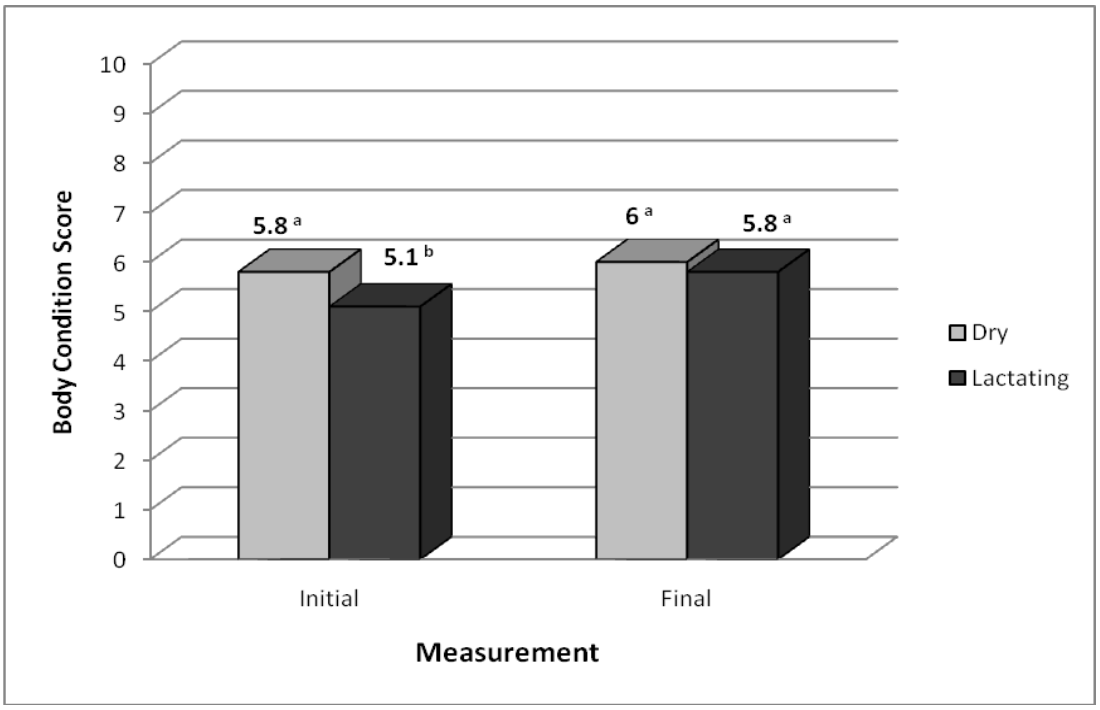

Figure 10. Influence of lactation status on initial and final body condition score. [a,b] least square means differ with subscript ($P < 0.05$).

BW (P=0.36) or initial BCS (P=0.64), demonstrating successful distribution of hinds within treatments. Final body condition scores were not influenced by treatment, age or lactation; however, an increase in BCS was observed for lactating hinds ($P = 0.01$; Figure 10). Final body weights showed a tendency ($P < 0.10$) to be influenced by treatment and lactation while also being affected ($P < 0.001$) by age. Yearling hind BW remained lower than 2-year-old and mature hinds during the trial, whereas 2-year-old and mature hinds showed no difference (Figure 8). These data suggest that hinds reach their mature BW during the mid to late part of their second year of life. Final BW recordings help to explain the lactation effect seen in initial BW. Lactating hinds again displayed higher BW than dry hinds (Figure 9) concluding that lactating hinds were

heavier than dry hinds throughout the trial. Interestingly, MEL hinds had lower final BW than CNTRL hinds although initial BW, initial BCS or final BCS did not differ (Figures 11 and 12). Calculated average daily gains confirmed CNTRL hinds gained more than MEL hinds ($P = 0.05$; Table 6). Declines in cervid weight gains have been correlated with seasonal appetite depression and lower feed conversion rates (Blaxter et al., 1974; Silver et al., 1969). Therefore, the resulting reduction in ADG may be a consequence of advancing the date for elevated melatonin concentrations and mimicking short photoperiod behavior. Average daily gain was also influenced ($P < 0.05$) by age and lactation status. Although lactating hinds were generally heavier, dry hinds exhibited greater ADG than their lactating counterparts (Table 7). These lower average daily gains are frequently seen in lactating animals due to the higher metabolic energy requirements for milk production. Mature and 2-year-old hinds expectedly had lower ADG than yearling hinds (Table 8). In order to breed as yearlings, hinds must achieve 65-70% of their mature body weight (Fisher and Fennessy, 1985). Whereas mature cervids often display reduced ADG during corresponding short photoperiods, high rates of gain are expected for developing yearlings leading into sexual maturity.

Reproductive Performance

Pregnancy was determined by ultrasonography 100 days post ear implantation and confirmed with a following ultrasound on day 145. Ultrasound dates were determined according to first observed stag breeding and crayon harness markings. As noted earlier, breeding harnesses were an unreliable indication of mating due to breeding

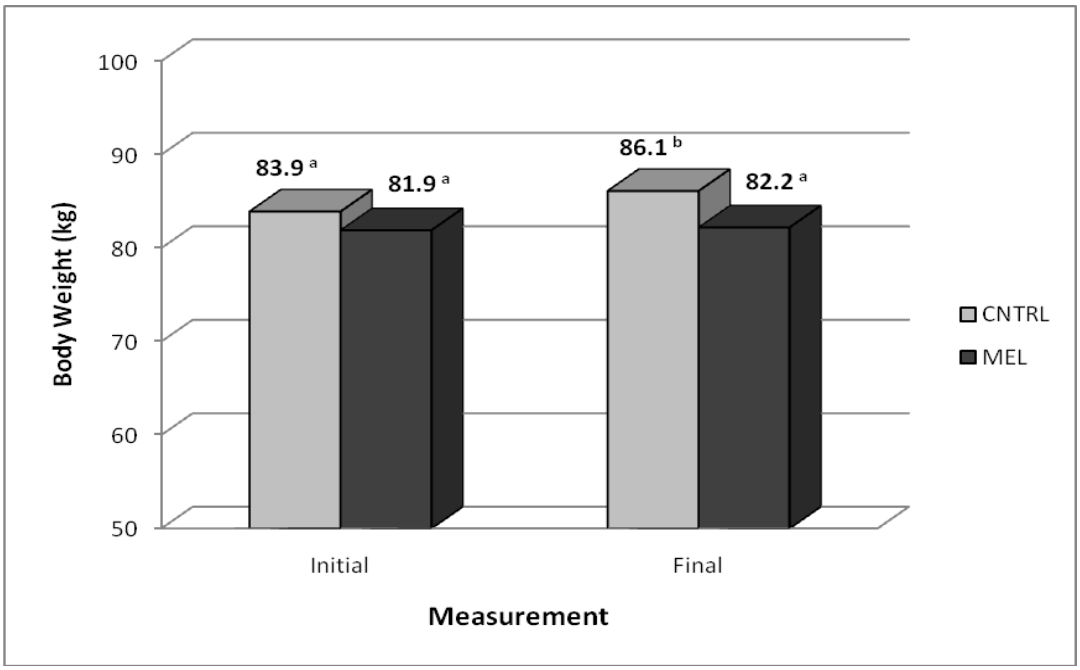

Figure 11. Influence of treatment on initial and final body weight.
[a,b] least square means differ with subscript ($P < 0.05$).

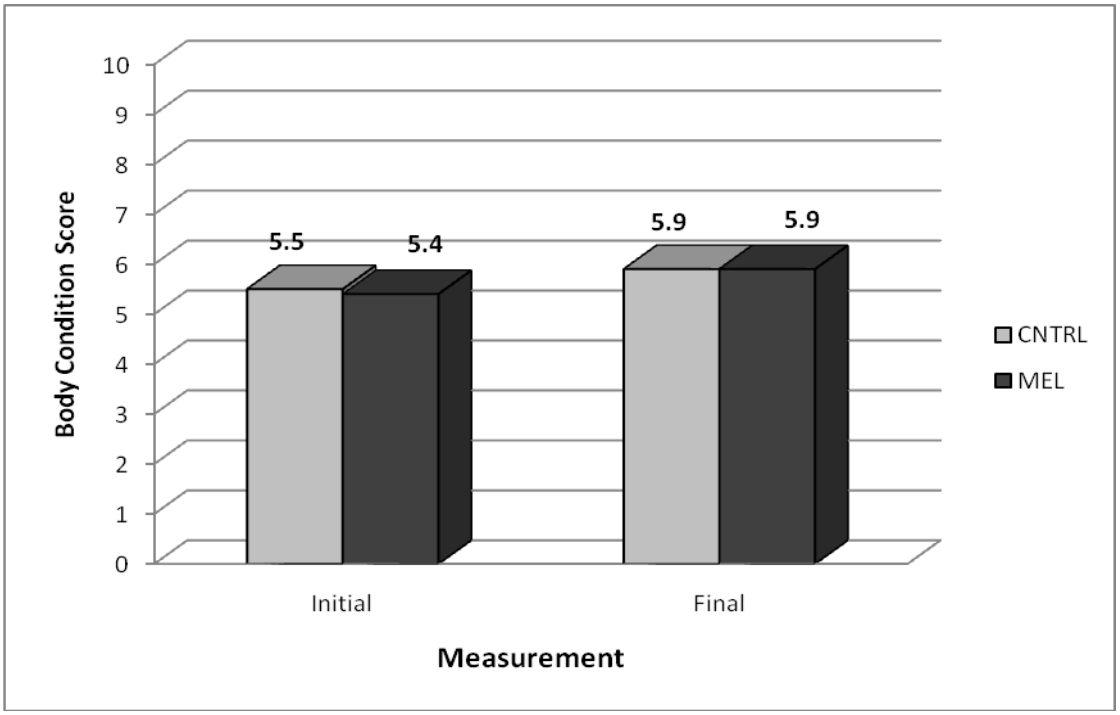

Figure 12. Influence of treatment on initial and final body condition score ($P > 0.10$).

Table 6. Influence of treatment on average daily gain.

	Average Daily Gain (kg/d)
CNTRL (n=42)	0.020 ± 0.007[a]
MEL (n=42)	0.003 ± 0.007[b]

[a,b] least square means within column differ ($P < 0.01$).

Table 7. Influence of lactation status on average daily gain.

		Average Daily Gain (kg/d)
Dry	(n=48)	0.027 ± 0.007[a]
Lactating	(n=36)	-0.003 ± 0.007[b]

[a,b] least square means within column differ ($P < 0.01$).

Table 8. Influence of age on average daily gain.

		Average Daily Gain (kg/d)
Yearling	(n=24)	0.066 ± 0.010[a]
2-year-old	(n=8)	-0.011 ± 0.014[b]
Mature	(n=52)	-0.012 ± 0.005[b]

[a,b] least square means within column differ ($P < 0.01$).

behavior and constant wallowing of both male and females. Pregnancy rates and gestation length were used for treatment analysis. Pregnancy was shown only to be affected by age ($P = 0.02$), with conception dates for yearlings being delayed 20-25 days in contrast to mature and 2-year-old hinds (Table 9). Interestingly, this delayed period corresponds with roughly the same length of an average estrous cycle in red deer (≈21d). We are unable to determine whether these findings are coincidental or an indication of an additional non-fertile estrous cycle in yearlings. Although a slight advancement in conception was observed in MEL hinds initially (Figure 13), differences were not significant throughout the breeding season ($P = 0.16$). Interestingly, peaks around day 10 and 60 suggest a possible synchronizing effect on MEL hinds (Figure 13). Nonetheless, pregnancy rates were affected by treatment ($P = 0.04$). Although mature and 2-year-old hinds boasted a 100% pregnancy rate, yearlings obtained only a 90% pregnancy rate (Figure 14). Yearlings from the MEL treatment attained a 100% pregnancy rate, whereas yearling CNTRL hinds achieved only a 63.6% pregnancy rate. No influence of yearling initial or final BW was discovered in relation to these pregnancy rates ($P > 0.10$). Although 24 mg melatonin implants failed to advance the conception dates in treated hinds over controls, there were greater pregnancy rates in yearlings. Mixed results have been reported on the use of melatonin in advancing seasonal female breeding. Successful studies indicate that the combination of timing and method of administration plays a crucial role in success. Implants and daily oral administrations of melatonin are two common methods reported to advance breeding and breeding behavior in many seasonal species (Gordon, 2005). The use of melatonin implants has also been reported to

Table 9. Influence of age on stage of pregnancy (time from conception).

	Pregnancy (days)
Yearling (n=24)	42.2 ± 6.5[a]
2-year-old (n=8)	67.6 ± 9.3[b]
Mature (n=52)	61.7 ± 3.6[b]

[a,b] least square means within column differ with subscript ($P < 0.01$).

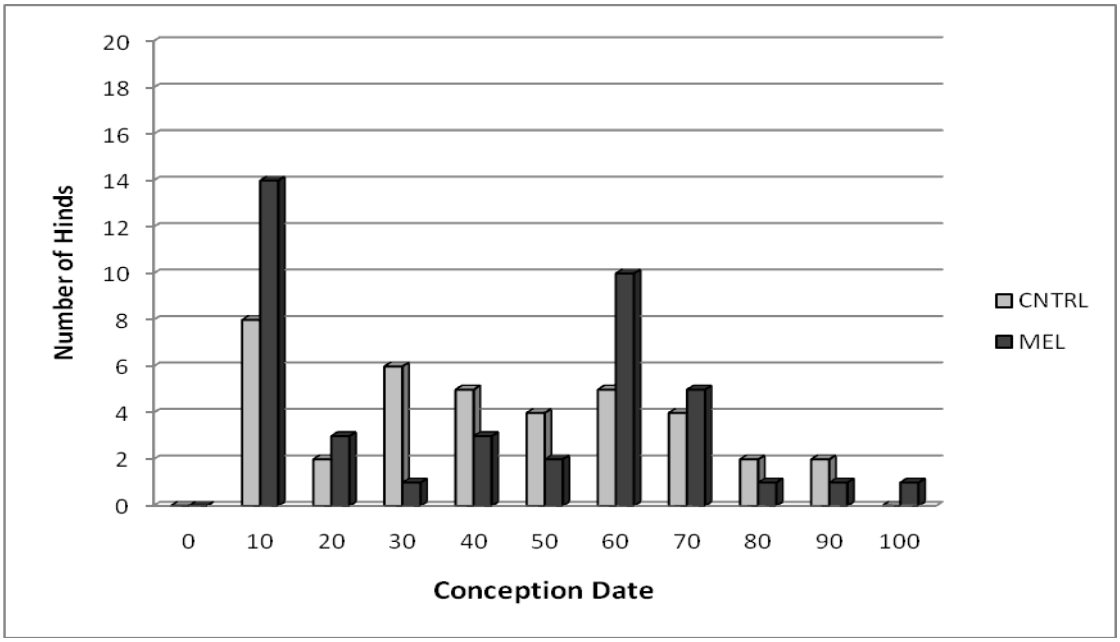

Figure 13. Influence of treatment on day of conception ($P > 0.10$). Melatonin implants were inserted on day 0 and pregnancy was determined on day 100, with verification on day 145.

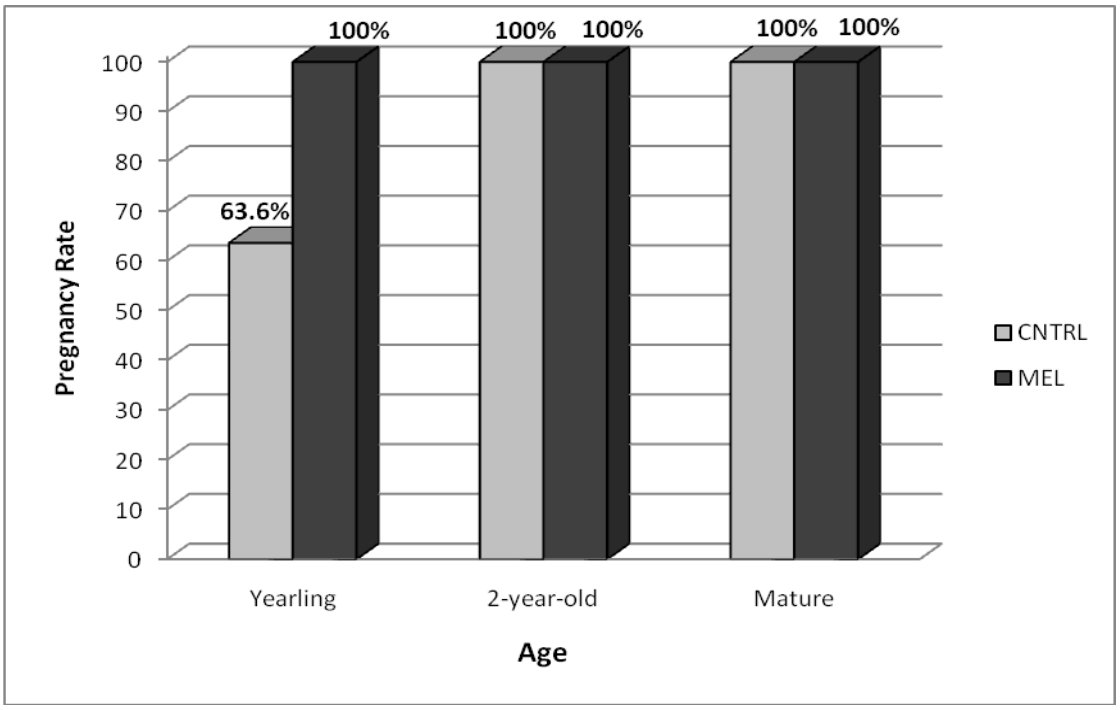

Figure 14. Influence of age on pregnancy rates ($P = 0.04$).

increase early conception rates in pre- and post-pubertal sheep and goats (Williams et al., 1992; Papachristoforou et al., 2007). Although melatonin implants increased pregnancy rates in yearling hinds, no advancement of conception was detected in this study. Implant dates were determined using calving dates from the previous year and a 230 day gestation period. The prior year calving records indicate that the date of conception for the first calf was during late September. However, as seen in Figure 13, many CNTRL hinds initiated earlier than normal estrous cycles in late August, nullifying any treatment affect. Earlier conception dates are hypothesized to be a result of increased nutrition and conditioning in contrast to prior year breeding animals.

Conclusion

It is often considered that the productivity of ovine, caprine and cervids is often limited by their inherent seasonal biology. Although melatonin treatments have previously been used to promote early breeding in many of these seasonal species, no advancement was observed in red deer hinds implanted in early August. However, pregnancy rates for yearling hinds were significantly increased by the insertion of melatonin implants prior to the breeding season. Additionally, low serum melatonin concentrations were present in early conceiving CNTRL hinds suggesting that melatonin is not the only biological signal for initiating estrous cyclicity. The use of melatonin implants may also have physiological effects on appetite and metabolism, causing reduced average daily gains similar to those seen during short photoperiods. Although breeding stags were deemed fertile at the time of introduction, earlier implantation dates may require stags to also receive melatonin. Nonetheless, further research is needed for the use of melatonin implants to advance seasonal estrous cyclicity in female cervids.

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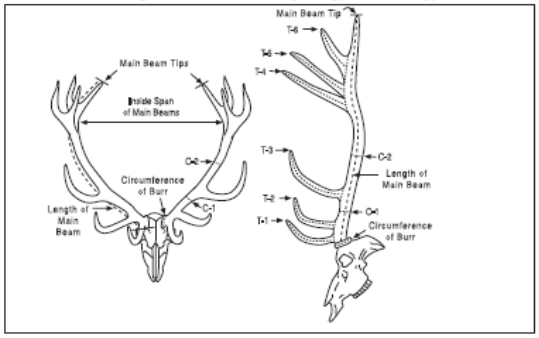
APPENDIX A

SAFARI CLUB INTERNATIONAL SCORING METHOD

SCI
FIRST FOR HUNTERS

SAFARI CLUB INTERNATIONAL
Method 20 Entry Form

For red deer and related deer *Includes Bukharan deer, Yarkand deer, hangul, Tibetan deer, shou, McNeill deer and Gansu deer. In these deer all tines will count in the score regardless of whether they are typical or non-typical.*



Hunter _____
How you want your name to appear in the Record Book

Membership No. _____ e-mail _____

Address _____

_____
City State Zip Country

Ph. (____) _____ (____) _____ (____) _____
Home Business Fax

I certify that, to the best of my knowledge, I took this animal without violating the wildlife laws or ethical hunting practices of the country or province in which I hunted. I also certify that, to the best of my knowledge, the laws of my country have not been violated by my taking or importing this animal.

Free-ranging ❑ Yes ❑ No

Signature _____

The acceptance or denial of all entries are at the discretion of Safari Club International, its Board and committees. Entries are subject to review by the Trophy Records Committee of SCI at any time. All photos and entries submitted to SCI become SCI's property.

Submit to: Safari Club International
4800 W. Gates Pass Rd., Tucson, AZ 85745 USA.

- ❑ **$35** Record Book processing fee
- ❑ **$55** Medallion Award processing fee *(Walnut plaque)* includes shipping & handling
- ❑ **$80** Record Book & Medallion Award processing fee includes shipping & handling

To enter Record Book and/or Medallion:

1) Add the appropriate entry processing fees together as necessary. *(Medallion fee includes shipping & handling.)*
2) All entries must be complete, signed by hunter and accompanied by fees and a photograph of the trophy.
3) Please clearly label back of photo with name of hunter, name and score of animal, and date taken.
   - ❑ For simple horns and unbranched antlers: include 1 photo
   - ❑ For animals with branched antlers: include enough photos so that all tines can be clearly seen.

Checks on U.S. banks only. Credit cards preferred. Entry fees are valid for 12 months from date of form located in lower right hand corner.

We Accept: ❑ MC ❑ Visa ❑ AMX ❑ Discover ❑ Diners Club

_____ _____
Card Number Expiration Date

Animal _____

Remeasurement? ❑ Yes ❑ No Former Score _____ Record No. _____

Date Taken _____
Month Day Year

❑ Rifle ❑ Handgun ❑ Muzzleloader ❑ Bow ❑ Crossbow ❑ Picked Up

Place Taken _____ _____
Country State or Province

Locality _____

Guide _____ Hunting Co. _____

I. Length of Main Beam		L	____/8	R	____/8
II. Length of Typical Tines on Lower Main Beam	**T-1**	L	____/8	R	____/8
	T-2	L	____/8	R	____/8
	T-3	L	____/8	R	____/8
III. Length of Non-Typical Tines on Lower Main Beam, if any *(Use back of form for additional tines)*	**NT-1**	L	____/8	R	____/8
	NT-2	L	____/8	R	____/8
IV. Length of All Other Tines *(Use back of form for additional tines)*	**T-4**	L	____/8	R	____/8
	T-5	L	____/8	R	____/8
	T-6	L	____/8	R	____/8
	T-7	L	____/8	R	____/8
	T-8	L	____/8	R	____/8
	T-9	L	____/8	R	____/8
	T-10	L	____/8	R	____/8
	T-11	L	____/8	R	____/8
	T-12	L	____/8	R	____/8
	T-13	L	____/8	R	____/8
	T-14	L	____/8	R	____/8
	T-15	L	____/8	R	____/8
	T-16	L	____/8	R	____/8
	T-17	L	____/8	R	____/8
	T-18	L	____/8	R	____/8
	Subtotal	L	____/8	R	____/8
V. Circumference of Burr		L	____/8	R	____/8
VI. Circumference of Main Beam	**C-1**	L	____/8	R	____/8
	C-2	L	____/8	R	____/8
	Subtotal	L	____/8	R	____/8
VII. Inside Span of Main Beams			____/8		
VIII. Total Score			____/8		

Supplemental Information

Total Number of Points (All tines plus beam tip) L _____ R _____

Official Measurer _____

Measurer No. _____ Email _____

Day Measured _____
Month Day Year

Signature of Measurer _____

 6/07

VITA

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