DIETARY SUPPLEMENTATION STRATEGIES FOR THE CAPTIVE WHITE-TAILED (*ODOCOILEUS VIRGINIANUS*) BREEDING DOE

By:

Ashlee Marie Adams

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ABSTRACT

Captive breeding and hunting of white-tailed deer in Texas is a popular business enterprise with significant economic impact. Stress in captive white-tailed breeding does may compromise their performance, alter immunity and increase death loss. Major stressors in captive deer include handling, weather extremes and poor nutrition. To alleviate stressors and the associated reduced performance cost to owners and breeders, it is necessary to research non-invasive methods to improve deer performance. Such methods may be dietary inclusion of feed additives comprising probiotic or plant-based essential oils, or dietary inclusion of a supplement known to have immunomodulatory effects in other species. Two experiments were performed to test these methods. Experiment 1 was conducted from 15DEC2015 to 8MAR2016 at a private deer breeding facility near Canadian, TX and does (n = 71) were weighed, palpated and blood sampled on d 0, 42 and 84 to determine weight maintenance, rib flesh score (RFS) and complete blood count via automated hemocytometer, respectively. Deer were randomly allocated to 1 of 6 treatment pens according to pre-assigned chute entry order. The two treatment diets consisted of a commercial deer breeder diet with no additive (CON) or the same breeder diet with a dietary immunomodulator (OmniGen-AF, Phibro Animal Health) included at 3.51 g/kg of feed (OG). Treatment diets were supplied via self-feeders located in each pen. The OG does lost less (P = 0.45) weight, numerically, throughout the study

and tended ($P = 0.10$) to consume less feed; indicating that the OG does had increased feed efficiency compared to does consuming the CON treatment. Further, OG-treated does tended to have a greater ($P = 0.09$) RFS on d 42. There were no treatment differences for CBC variables ($P \geq 0.32$); however, total leukocytes, neutrophils and neutrophil: lymphocyte decreased with time (day effect, $P \leq 0.02$). These CBC data may provide a foundation for automated CBC reference range in captive white-tailed does. Experiment 2 was conducted from 23Jun2016 to 15Sep2016 at the same study site using the same experimental methods as Exp. 1 except blood was not collected. The two treatment diets consisted of a complete feed without additive (CON) and the same breeder diet with a phytogenic nutritional supplement included (CG; Climate Guard, Delacon). From d 0 to 84 there was not a difference ($P \geq 0.19$) in ADG or BW gain, but numerically, CG does weighed more and CG-treated does gained BW; whereas, CON lost BW during the 84-d trial. There was a tendency ($P = 0.06$) for RFS to be greater in CG-treated does on d 42, and a difference existed ($P < 0.01$) by d 84 for CG to have greater RFS. Overall, breeding does receiving the phytogenic nutritional supplement in their diet consumed more feed, gained BW and exhibited increased RFS during the 84-day observation period. Results of these experiments indicate that nutritional supplements may improve performance in captive deer, but further research is needed to determine the most effective supplement type during different seasons and their impact on stress.

Key words: performance, phytogenic, probiotic, supplement, white-tailed deer

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SIGNATURE PAGE

Approved:

_____

[Chairman, Thesis Committee] [Date]
Dr. John Richeson

_____

[Chairman, Thesis Committee] [Date]
Dr. Ty Lawrence

_____

[Chairman, Thesis Committee] [Date]
Dr. Jenny Jennings

_____

[Head, Major Department] [Date]

_____

[Dean, Major Department] [Date]

_____

[Dean, Graduate School] [Date]

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CHAPTER I

INTRODUCTION

White-tailed deer breeding for hunting and recreational purposes is a growing industry for Texas and rural America. The deer industry has grown both in popularity and economic impact in recent years (Hewitt, 2011; Cain, 2016). Texas permitted deer breeding facilities has grown from 203 in 1996 to 1,395 in 2014, and the inventory has increased by 13,083 deer from 2012 to 2016 (Crossley, 2017). Hunting and breeding operations contribute an economic impact of $652 million annually for Texas from white-tailed deer facilities, and $3 billion nationwide from all cervid facilities. They also provide an estimated 29,199 jobs in rural America (Anderson et al., 2007a; Anderson et al. 2007b). Stressors such as poor nutrition, extreme weather, and predation may negatively affect deer performance, reproductive rates, immunity, and therefore survival (Verme, 1965; Verme, 1967; Verme 1969; Morrison, 1983; Griffin and Thompson, 1998; Haigh et al., 2005). To meet dietary needs of deer in a captive environment, supplemental feed must be provided to compensate for the loss of forage available to browse (Lewis et al., 1998; Hewitt, 2011). Limited research exists on the potential benefit of dietary supplements in captive deer. Blood variables such as complete blood count are often used for analysis of some of these effects; however, there is little consistency in reference ranges for deer. Therefore, it is

beneficial to analyze a complete blood count to establish ranges for deer as a tool for management decisions (Jones and Allison, 2007). Extreme heat or cold is known to have a negative impact on the performance and physiology of ruminants (Morrison, 1983; Young, 1983; Webster 1983; Griffin, 1989; St-Pierre et al., 2003; Baumgard and Rhoads, 2012). Predation, including extensive interaction with human caretakers, is the main stressor with captive deer, due to quick reactions to trauma that could lead to exhaustion and sometimes mortality (Griffin and Thompson, 1998; Haigh et al., 2005). Providing a nutritional supplement in pelleted form is an increasingly common approach to enhancing deer nutrition, and therefore survival (Lewis et al., 1998; Hewitt, 2011). The objective of these preliminary studies was to test the effects of a live yeast probiotic and a phytogenic formula on weight management, body condition, feed intake, and blood variables (probiotic experiment only) when supplemented in a pelleted feed to white-tailed breeding does. However, it is important to note that there was not a direct measure of stress evaluated, but some effects of stress could be linked to the variables measured. The selected live yeast probiotic, *saccharomyces cerevisiae*, has been demonstrated to provide beneficial health effects in other ruminant species (Wang et al., 2007; Grochowska et al., 2012; Ryman et al., 2013; Buntyn et al., 2016). Phytogenic supplements can comprise many facets of plant-based compounds, and the current product, Climate Guard, contains primarily essential oils, and saponin, which have shown beneficial characteristics in wild deer (Nagy et al., 1964; Oh et al., 1967; Rochfort et al., 2008), and other ruminant species (Newbold et al., 2004; Castillejos et al., 2006; Rochfort et al., 2008; Holtshausen et al., 2009). The following studies are preliminary for these products tested in captive deer.

CHAPTER II:

REVIEW OF LITERATURE

WHITE-TAILED DEER AND THE DEER INDUSTRY

Cervidae is the family composed of animals that shed antlers annually, including elk/red deer, moose, caribou/reindeer, white-tailed and mule deer, and other region-specific species. White-tailed deer are further classified as *Odocoileus virginianus*. The genus *Odocoileus* includes white-tailed deer and mule deer native to North America, and translates to 'teeth well hollowed'. Species *virginianus* reflects the location of the New World (Virginia in the 1500's), and commonly referred to as 'Virginia Deer' (Hewitt, 2011). White-tailed deer have avoided extinction through small genetic variations by selection, and survived three large faunal changes in North America. They consume a diverse diet as they adapt to environments, so it is difficult to classify their diet to one type (i.e. grazers, browsers, etc.). Deer are the most widely distributed of wild ruminants that are found on all continents except Antarctica (Van Soest, 1994). White-tailed and mule deer have a smaller rumen at ten percent of their body weight (BW) compared to 23 percent in red deer, and half the rumen volume of sheep (Geist, 1998). White-tailed deer are concentrate selectors with a simple, or low fiber, rumen (Hofmann, 1988). Concentrate selectors evolved early and adapted to their food plants before the grasses developed and

spread. These animals select plants or plant parts that are rich in highly nutritious and easily digestible plant cell contents; such as starch, plant protein, fat, and oil (Church, 1993). Grazing habits of various deer species have been studied recently. Grouping and movement patterns of mule deer were larger at dawn and dusk in open habitats (Fernanda Mejia Salazar et al., 2016). Fallow deer (*Dama dama L.*), observed under intensive pasture confinement, concentrated feeding activity to the morning and evening, and grouped together more in the evening. The analysis of these behaviors on individual farms will help improve management techniques (Mattiello et al., 1996). Managers ultimately need to create an environment adapted for the species of interest to ensure productivity. Ruminants need to experience thermal comfort, physical comfort, behavioral satisfaction and disease control. To evaluate these needs research is conducted to analyze the association between an environmental variable and performance criteria or health (Webster, 1983). Communication habits are also important when making management decisions. White-tailed deer communicate through audible, chemical and visual cues. They have few and quieter vocalizations than other cervid species, and visual cues through posture are engaged at close range. Olfaction is the most important method of communication in white-tailed deer as secretions and glands produce socially significant odors (Hewitt, 2011).

Deer management has reached its pinnacle using captive deer facilities which occur where allowed by state law (Hewitt, 2011). Full confinement in pens or small pastures where a high level of productivity is expected, such as deer farming, resembles conventional livestock management (NRC, 2007). The deer breeding industry, like other livestock industries, includes production and consumption of animal products. The primary product of the Texas deer industry is white-tailed deer for game harvest. Deer breeding

facilities are designed for reproduction and growth, while hunting preserves and game ranches are the consumption segment. Breeding stock are marketed to other breeders, and stocker deer are sold to game ranches for hunting purposes. Hunting is the final market for this industry, so producers selectively breed for improved genetics for trophy white-tailed deer (Anderson et al., 2007a; Anderson et al. 2007b). According to the Texas Parks and Wildlife Department (TPWD), White-tailed Deer Federal Aid Report 2016, more bucks were harvested annually than does from the 2004-2005 hunting season to 2015-2016 hunting season (Cain, 2016). This indicates the hunter's preference of gender for harvest due to trophy antler demand, but may not be beneficial as a culling method. Selecting for females sired by better quality males may positively effect a population (Lockwood et al., 2005). Does are bred, or cloned, for breeding stock versus bucks, due to the ability to collect and store semen from trophy bucks for artificial insemination.

Texas is only a portion of the national cervid farming industry, but it is the largest among the United States (Anderson et al., 2007a). The Agricultural and Food Policy Center's research report for 2007 evaluated the economic impact of the Texas deer breeding industry, and the United States cervid farming industry. These areas are some of the fastest growing livestock industries in rural America. Expenditures for operations averaged $306,000 for Texas deer breeding operations, and $101,000 for nationwide cervid breeding operations. Texas may be higher due to the variation in species used for the nationwide survey, and some small operations may not have a big economic impact. After incorporating feed, veterinary supplies, fuel and other purchases, operations have a total economic impact of $523 million annually for Texas, and $2.3 billion nationwide. The addition of hunting components increases the economic impact to $652 million and $3

billion, respectively. Another important factor to economic impact is the number of jobs created by the industry, and these operations contributed 29,199 jobs in rural America in 2007 (Anderson et al., 2007a; Anderson et al. 2007b).

According to Paul Crossley, of TPWD deer breeder program, the number of permitted deer breeding facilities has grown from 203 in 1996 to 1,395 in 2014. Deer inventory of these breeding facilities has increased by 13,083 deer from 2012 to 2016. Therefore, the average number of deer per captive facility has grown from 79 in 2012 to 86 in 2015 (Crossley, 2017). Wild deer in Texas have had an average herd size of 20 from 2005 to 2015 (Cain, 2016); however, herd size increases within breeding facilities (Crossley, 2017). A survey of United States wildlife agencies revealed that there are approximately 5,600 breeding facilities containing 163,000 white-tailed deer. Texas contains the most facilities and deer at 1,332 and 100,000, respectively (Adams et al., 2016). With extensive variation in captive deer management between and within states, Adams et al. (2016) suggested it may be time to set nationwide regulations as they relate to animal welfare, human health and safety, disease risks, and our hunting heritage. Much like the Beef Quality Assurance (BQA) program that is familiar to farmers and ranchers as a resource for improved management techniques in cattle, the Quality Deer Management (QDM) program educates managers on positive implications to their operations. Other state and area wildlife agencies will continue to help improve management practices, and act as a liaison to future government regulation in the captive deer industry (Hewitt, 2011).

Diseases for white-tailed deer are categorized 2-fold: (1) those with a potential to directly or indirectly reduce or eliminate populations, and (2) those with significant human health concerns that may reduce hunter participation or public support for hunting (Hewitt,

2011). The most publicly recognized and current disease of concern is chronic wasting disease (CWD), a transmissible spongiform encephalopathy (TSE), which also includes bovine spongiform encephalopathy and Creutzfeldt-Jakob disease in humans. The primary concern with this disease is the potential transmission to other wildlife and domestic livestock. Advanced cases of CWD consist of weight loss, emaciation, and loss of abdominal and subcutaneous adipose tissue. Diagnosis is identified by an abnormal prion protein in lymphoid and brain tissues by immunohistochemistry (Hewitt, 2011). Agencies such as TPWD and Texas Animal Health Commission (TAHC) are developing management strategies to address risks, and protecting big game resources in Texas from CWD in captive or free-ranging cervid populations. However, breeders may be reluctant to have their herd tested due to undesirable consequences if a positive CWD is found, such as reduced sales in breeding stock and drastic reduction in land value.

WHITE-TAILED DEER NUTRITION

To understand protein and energy requirements for white-tailed deer, changes in body composition was first studied by sacrificing wild deer of varying ages in different seasons (Robbins et al., 1974). A later experiment monitored monthly changes in feeding behaviors and BW and reported that weight and mean daily rate of feed intake increased with age and were affected by season; this study reported that the DMI of New Hampshire white-tailed deer was greatest from July to October (Holter et al., 1977). In 1981, Cowan et al. postulated that research to that point had failed to accurately quantitate energy requirements due to variation in experimental animals, techniques with

various error, and methodology and interpretation of results. However, like other livestock industries during early research, nutritional management is based upon the best estimates of nutrient requirements. Extensive research in sheep and other cervids can be used for nutritional guidelines in species that have not been extensively explored such as white-tailed deer, because all are closely related ruminants (Cowan and Clark, 1981). Although, deer may not be as efficient at utilizing cellulose as cattle, and caution should be used when comparing data for management techniques (Van Soest, 1994). DeLiberto et al. (1989) evaluated free-range white-tailed does in Oklahoma for changes in physiologic and metabolic parameters during different seasons. Kidney fat index was highest during winter, packed cell volume was least in summer, total volatile fatty acid and acetate was greatest during spring and summer, and rumen acetate:propionate (A:P) ratio was less in winter than in summer. Therefore, it was concluded that deer nutritional status declined from winter to summer, and management and nutritional supplementation should be focused during warmer seasons (DeLiberto et al., 1989). The differing results from these studies demonstrate how management strategies should change according to the herd's needs, seasonal changes, and if they are captive or free-range.

Due to the laws of thermodynamics and conservation of energy, animal physiologists study energy balance in various animal species. Feeding animals a diet with nutrients less than the amount required for body maintenance results in use of fat and other tissues to supply energy, and maintenance level is established by the reserves available to the animal (Van Soest, 1994). Structural and non-structural carbohydrates are the primary energy components of white-tailed deer diets. Structural carbohydrates make up a majority of the diet and include cellulose and hemicellulose found in plant cell walls.

Non-structural carbohydrates include cellular contents, starch and simple sugars. Cervids and other ruminant animals have developed a symbiotic relationship with microorganisms to digest otherwise indigestible structural carbohydrates (Hewitt, 2011). Saliva aids in mastication and swallowing, and contains buffers to aid in rumen fermentation which requires a basic and anaerobic environment. To further aid in digestion of structural carbohydrates, deer ruminate or "chew their cud" similar to other ruminant animals (Hofmann, 1989; Hewitt 2011). Rumen microbes ingest carbohydrates as an energy source and the by-products of fermentation in the form of volatile fatty acids are absorbed and utilized by the deer (Hewitt, 2011). Volatile fatty acid absorption rates and location differ based on rumen pH and microbial species preference on the type of VFA produced (Dijkstra, 1994). When rumen microflora pass out of the rumenoreticular compartment, reformulated proteins are digested in the acidic abomasum into amino acids then absorbed in the small intestine (Van Soest, 1994; Hewitt, 2011). To maximize digestive efficiency relative to nutrient requirements and availability, white-tailed deer are able to rapidly alter the microbial population, and possibly function, of their digestive system. Deer accumulate fat reserves before winter and use those resources during times of nutritional restriction. If the winter period and reduced nutritional status is not abnormally long, deer are able to recover and starvation is typically low (Hewitt, 2011). Assessing body condition is a method to subjectively quantify the status of fat and protein components of an animal's body (Russel et al., 1969). Fat is important to assess because it represents energy reserve deposited when dietary intake is above maintenance requirements (Hewitt, 2011). Providing a nutritional supplement is an increasingly common approach to enhance deer nutrition and performance. Whereas, baiting is

supplemental feeding with the intent to bring deer to a specific place so they can be censured, trapped, photographed, or harvested (Hewitt, 2011). Income from hunting leases with white-tailed deer has motivated ranchers to manage for deer and cattle simultaneously. White-tailed deer in South Texas experience a limited quality of diet from rangeland, which could limit population due to late summer forage availability (Meyer et al., 1984). A progressively common management practice in Texas and the southeastern United States, is to provide free-ranging white-tailed deer a pelleted supplemental feed for increasing reproductive rates, population density, antler size, body mass, and survival (Lewis et al., 1998). However, supplemental feed programs for free-ranging white-tailed deer benefit males more than females, due to competitive feeding habits around the feeder. Therefore, efficiency of the feed-delivery systems should be considered in management strategies specific to the operation (Bartoskewitz et al., 2003). Many benefits were observed in a 5-year study on a supplemented white-tailed deer herd. With feeder-supplied pellets, the herd grew from 23 to 159 animals, improved nutrition accelerated body growth and shortened the time to maturity, and *in utero* productivity doubled without damage to rangeland. Therefore, there was an increased carrying capacity from 0.09 to 0.63 deer/ha (Ozoga and Verme, 1982).

When white-tailed deer and angora goats were offered a high-quality supplement, they ate selectively to increase average diet quality (Murden and Risenhoover, 1992). When penned white-tailed deer were offered a protein-energy supplement during winter, they had reduced weight loss and death from starvation (Ullrey et al., 1975). Male white-tailed deer that were restricted protein had lower body mass than those with a high-quality diet containing greater protein. However, deer were able to compensate their diet

if a higher quality forage was available later in the year (Asleson et al., 1997). Insufficient protein reserves in Texas white-tailed does compromised neonatal fawns' immunocompetence, only when malnutrition was chronic and protein reserves were depleted (Sams et al., 1995). White-tailed does consuming a high nutrition diet produced more fawns per birth than those with low nutrition (Verme, 1965; Verme, 1967; Verme, 1969). As expected, there are numerous benefits to enhanced nutrition in captive or free range white-tailed deer.

Northern white-tailed deer were evaluated for energy requirements (Holter et al., 1977). Net energy required daily for maintenance was 106 ± 11, 97 ± 10 and 125 ± 30 kcal/kg $BW^{0.75}$ for all 15 months of the study, December through April, and May through October, respectively. Daily metabolizable energy requirements for maintenance were 148 ± 19, 153 ± 21, and 162 ± 53 kcal/kg $BW^{0.75}$ (Holter et al., 1977). More recently, a study measuring maintenance requirements for white-tailed deer in Southern Texas may contribute a relevant reference for research performed in this area. The study was conducted over a 30-day period from November 13 to December 13, 1997, at a deer research operation in Kingsville, TX. Daily digestible energy intake requirements for body mass maintenance ranged from 196 ± 40 at trial initiation to 85 ± 10 kcal/kg $BW^{0.75}$ at trial completion. This study suggests that digestible energy maintenance requirements decline during extended periods of energetic restriction, and results from Holter et al. (1977) may overestimate requirements for deer in southern areas. South Texas is a semiarid environment with variable rainfall patterns, so lower maintenance requirements may be an adaptive response to their environment (Strickland et al., 2005). Calpain and calpastatin enzymes, in ratio, have been used as an indicator for meat tenderness in beef

cattle. Calpastatin is an inhibitor of calpain, such that cattle with increased calpain have increased tenderness. Thus, genes coding for calpain (CAPN1) and calpastatin (CAST), located on the bovine chromosomes 29 and 7 respectively, are candidates for meat tenderness testing (Curi et al., 2008). Furthermore, testing for these enzymes may also link to maintenance energy requirements, which could aid in management techniques in deer facilities. Heat stress may increase maintenance requirements in deer as observed in sheep (Whittow and Findlay, 1968; Ames et al., 1971) and cattle (McDowell et al., 1969; Morrison, 1983; Beede and Collier, 1986; Huber, 1996).

BLOOD PARAMETERS IN DEER

Hematological parameters are an important method to compare animals' physiological state and for monitoring health status; this methodology was recently summarized by Jones and Allison (2007). A complete blood count (CBC) panel indicates concentrations of red blood cells, total white blood cells and differential (lymphocytes, neutrophils, monocytes, eosinophils and basophils) counts, platelet count, hematocrit, and hemoglobin levels. Red blood cells (RBC), or erythrocytes, primarily serve to carry oxygen and carbon dioxide between tissues and lungs. A complete evaluation of RBC includes a packed cell volume (PCV) or hematocrit percentage, RBC count, mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC). The PCV represents the percentage of RBC in whole blood. Increased PCV, or polycythemia, is a common indicator of dehydration, transport stress, or acute mental stress stimulation (Jones and Allison, 2007). Platelets, or thrombocytes, form the initial

hemostatic cap to damaged veins and maintain vascular integrity. Thrombocytopenia, or platelet deficiency, can indicate many mechanisms of infection, or can arise from long exposure to an anticoagulant, thus confounding results (Jern et al., 1989; Pearson, 1991; Jones and Allison, 2007; Friend et al., 1998; Mohammadi et al., 2007). Haptoglobin binds free hemoglobin from damaged RBCs to protect tissues from toxic properties of free iron, and increases under a wide variety of traumatic or inflammatory conditions in ruminants (Hirvonen et al., 1996; Peterson et al., 2004; Jones and Allison, 2007). Leukocytes, or white blood cells, consist of neutrophils, eosinophils, basophils, monocytes, and lymphocytes. The differential white blood cell count is more significant than total white blood cell count due to increases and decreases in individual cell types that could occur simultaneously, that can result in total count being unaffected (Polizopoulou, 2010). Neutrophils are the first-line of defense in innate immunity as they migrate into damaged tissue for phagocytosis. Therefore, elevated levels may indicate an active infection, and neutropenia occurs with severe inflammation as neutrophils translocate into tissue. However, stress and the resulting increase in cortisol can decrease L-selectin on the neutrophil to reduce translocation and increase neutrophil concentration in blood (Weiss and Perman, 1992; Mohammadi, 2007; Jones and Allison, 2007; Wang et al., 2007). Lymphocytes are primarily T cells or B cells of adaptive immunity, but also comprise innate natural killer cells and these lymphocyte types cannot be differentiated from a CBC analysis. Lymphopenia may occur with acute viral or bacterial infection, or stress. An increased neutrophil:lymphocyte ratio (NLR) is, therefore, an indication of severe stress (Radostits, 1994; Mohammadi, 2007; Jones and Allison, 2007). Eosinophils are granulocytes that increase in concentration during severe parasitic infection (Abbas et

al., 2007). Basophils are present in small numbers and not often used for diagnostics in CBC (Jones and Allison, 2007). Lastly, monocytes enter tissue and differentiate into macrophages that are capable of phagocytosis of infectious organisms. Their numbers are variable and difficult for diagnosis, but increased numbers may accompany chronic inflammation, or a stress response (Jones and Allison, 2007).

In deer, CBC reference ranges have not been clearly defined due to different experiment designs and restraint techniques used to collect data. Five types of artiodactyls, or even-toed ungulates, were anesthetized and blood samples were collected for comparison. Peinado et al. (1991) postulated that total white blood cell counts and the ratio between different cells can be affected by age, exposure to diseases, breed, and management practices (Peinado et al., 1999). In 1979, Wesson et al. investigated blood endocrine measures in deer at the time of restraint. They concluded that glucocorticoids would be valuable to measuring excitability of white-tailed deer and evaluating the usefulness of anesthetizing drugs and handling methods (Wesson et al., 1979). When clinical blood parameters were compared between white-tailed deer capture methods, a majority of parameters were influenced rapidly by capture method, but not by season (Sams et al., 1993). This suggests that CBC parameters established from different capture methods cannot be reliably used for comparative analysis (Sams et al., 1993, Marco and Lavin, 1999). Marco and Lavin (1999) concluded similar findings in red deer; RBC, PCV, hemoglobin concentration, and segmented neutrophil, lymphocyte, monocyte and total leukocyte counts were increased in animals captured by physical means. Eosinophil count was greater in animals captured using anesthesia (Marco and Lavin, 1999). Many methods have been used to collect blood samples from deer. Physical capture includes

box traps, pens, hand nets, drop nets, and long nets. Chemical capture methods primarily use firearms for darting anesthetizing drugs or harvest (Jones, 1984). The Merck Veterinary Manual is a frequently used source to reference CBC ranges of different animal species (Merck, 2016). However, due to the lack of consistent information, there are not CBC reference ranges published in this source for deer. However, some experiments are presented here to begin comparative research for establishment of reference ranges. Early studies compared deer to other small ruminants such as sheep, which are studied more intensely, for reference guides (Kitchen et al., 1967). It is important to understand the differences in hematological variables with capture methods, age, and analysis of variables. A large study of penned white-tailed deer were caught in a chute for blood sampling and blood cells were evaluated manually and reported by age group. Hematology and serum chemistry did not exhibit changes with age after one year; however, season and body condition are the primary influences on blood chemistry. This predictability allows blood data to be used to examine questions concerning physiological status of deer (Johnson et al., 1968). A small sample of white-tailed deer were rifle-harvested in order to determine herd health through analyzing blood variables, the results are shown below (Table 2.1; Creekmoore and Creekmoore, 1999). Results revealed elevated lymphocyte and monocyte levels that may be due to chronic infections or a physiological response to handling or fear. Without any sign of chronic infection it was determined stress was the cause of this increase in lymphocyte and monocyte concentration (Kirk and Bristner, 1985; Creekmoore and Creekmoore, 1999). The CBC values found in the study are similar to those found in a more recent study by Seal et al. (1981); blood was collected from 59 unsedated white-tailed deer at 15 zoos and

hematological parameters were established using manual counting techniques. These data are compared to deer that were rifle harvested below, and few variables differed with sampling techniques (Table 2.1; Seal et al., 1981; Creekmoore and Creekmoore, 1999).

Table 2.1 Normal Range of Hematologic Values for unsedated (Seal et al., 1981) and harvested (Creekmoore and Creekmoore, 1999) White-tailed deer.

Blood Parameter[1]	**Units**	**Seal et al. 1981[5]**	**Creekmoore and Creekmoore, 1999[6]**
Hematocrit	% Vol.	39-58	39.8-60.0
Hemoglobin	g/dL	14.5-22.5	15.7-22.0
MCH[2]	pg	7-15	-
MCHC[3]	g/dL	34-40	35.7-39.4
MCV[4]	fL	28-38	34.6-40.0
Red blood cells	10^6/µL	10.0-16.7	-
White blood cells	/µL	1,000-4,200	1,100-4400
Basophils	/µL	<10	-
Eosinophils	/µL	<100	140-820
Lymphocytes	/µL	600-1,800	30-1,580
Monocytes	/µL	<10	50-90
Neutrophils	/µL	600-2,800	640-2,550

[1]Only hematological blood chemistry parameters are included.
[2]Mean corpuscular hemoglobin, picograms (pg)
[3]Mean corpuscular hemoglobin concentration
[4]Mean corpuscular volume, femtoliters (fL)
[5]Data collected from sedated captive white-tailed deer
[6]Data collected from rifle-harvested free ranging white-tailed deer

Like white-tailed deer in the US, chital (axis axis) deer are farmed in Australia, and there is a similar need for hematological and serum biochemical reference data in this

species. Chapple et al. (1991) collected blood from 37 unsedated deer over a 12-month period. Using manual hematological techniques, they concluded that regular handling of animals changed hematological values over time. The most prominent changes were in RBC parameters and serum levels of glucose and muscle enzymes, which decreased over time suggesting that initial values were elevated above normal levels and the deer adapted to handling. Those hinds handled three times a week had decreases in Haptoglobin, RBC, and hematocrit overtime, with initial values being similar to stags (Chapple et al., 1991).

EFFECTS OF STRESS IN DEER

Pacek and Palkovits (2001) defined stress as the "sum of all biological reactions to physical, emotional, or mental stimuli that disturb an individual's homeostasis". Selye (1950) described stress in four stages: (1) an immediate activation of the sympathetic-adrenomedullary axis (SA), as the "alarm reaction", (2) activation of the hypothalamic pituitary-adrenal axis (HPA) as the "resistance phase", (3) adrenal hypertrophy, thymic and lymphoid atrophy, and gastrointestinal ulceration stage, and (4) exhaustion phase and death. Noradrenergic sympathetic nerve fibers are distributed into specific compartments of both primary and secondary lymphoid organs, where immediate secretion of norepinephrine can occur adjacent to immune cells. Glucocorticoids act on almost all tissues and organs to regulate almost every component of the inflammatory and immune response. Physiological concentrations of glucocorticoids regulate the immune response

by thymic T cell selection, immunospecificity and T helper cell development. Farmed deer expressed a physiological state synonymous to Selye's exhaustion phase within days or even hours of capture. Successful adaptation of this wild species to farming is demonstrated by the reduction (25%) of fatal infectious diseases (Griffin and Thompson, 1998). Nevertheless, many psychological and physical stressors are observed in the farm setting and include overcrowding, physical restraint, transport, infection, extremes in climate, establishment of hierarchies, and breeding (Griffin, 1989). The adjustment of animals to a new stressor reduces the intensity of HPA activation upon subsequent exposure. Recurring exposure to the same stressor reduced the adrenal response in several models of stress (Espmark and Langvatn 1985; Robert et al., 1989). Hershberger and Cushwa (1983) examined three levels of stress and their effects on deer restricted from feed then refed. Minimum stress treatment meant that deer were weighed, sexed, aged and tagged after capture then released to an isolated 0.2 ha pen with minimal disturbance for the remainder of the study. Moderately stressed deer had the same methods applied at capture but with additional human interaction for 30 minutes; three times per week an individual would walk in the pen and deer were forced to move and expend energy. Severely stressed deer were handled weekly to determine body temperature, BW, body condition, and blood was sampled by strapping deer to a table. Minimum stressed deer were without food for 23 days; they lost weight for an average of eight days and died after losing 26% of initial weight. Moderately stressed deer were without food for 18 days and died during the refeeding period. Severely stressed deer were without food 15 days and the majority died prior to refeeding while the remainder died within five days of refeeding (Hershberger and Cushwa, 1983). Most white-tailed deer can survive periods without food, but ruminants

depend on an active rumen microbial population for survival. After feed restriction deer were offered unlimited quantities of corn and/or hay, and the authors concluded that fasted deer should not be fed *ad libitum* fermentable carbohydrates such as flaked or cracked corn because of the accumulation of lactic acid in the rumen. Overall, supplemental feeding, with minimal disturbance, should begin prior to starvation damage, and should be cautious during winter interactions with deer (Hershberger and Cushwa, 1983). Stressors can be defined as acute, lasting minutes to days, or chronic, enduring weeks to months. Acute stressors such as predation or minor injuries may enhance most immune responses, while chronic stressors, such as habitat modification, reduce immune responses (Loerch and Fluharty, 1999; Martin, 2009; Dragos and Tanasescu, 2010). Enhancements in immunity, or immunostimulatory events, will help animals to recover or survive from stressors (Martin, 2009). A survey of white-tailed deer farmers outlined trauma as the leading cause for morbidity and mortality in the deer population of United States and Canada (Haigh et al., 2005).

Environmental Stress

Environmental stress is described when an animal's environment does not achieve thermal comfort, physical comfort, or behavioral satisfaction (Webster, 1983). Economic losses from decreased performance (feed intake, growth, etc.), increased mortality, and decreased reproduction due to weather stress in various livestock species were evaluated by St-Pierre et al. (2003). Annual losses in dairy, beef, swine, and poultry, exposed to temperatures outside their zone of thermal comfort (ZTC), were estimated between 1.7 and $2.4 billion annually (St-Pierre et al., 2003). Heat stress reduces growth rate and is intensified by high humidity, thermal radiation, and low air movement. Diet and breed

selection affects the degree of adverse response (Morrison, 1983). During heat stress, systemic, cellular, and molecular changes are conserved amongst different species. Heat stress alters postabsorptive carbohydrate, protein, and lipid metabolism independently of reduced feed intake. These changes effect energy utilization during heat stress, which could be the primary reason for reduced ruminant animal productivity during warm seasons (Baumgard and Rhoads, 2012). Cold stress may also have the same reprioritization effect through increased generation of body heat to compensate for cold stress. Over time, animals will increase thermal insulation, appetite and basal metabolic intensity, and alter digestive functions when exposed to cold stress (Young, 1983). A relatively new area of study is in heat shock proteins (HSPs), which may be important modifying factors in cellular responses to a variety of physiologically relevant conditions such as hyperthermia, oxidative stress, metabolic challenge, exercise, and aging. Findings of these HSPs suggest they are critical for both normal cellular function and survival at both normal and elevated temperatures, and after stress (Kregel, 2002).

Capture Myopathy

Capture myopathy is a term used to describe a syndrome caused by capture, restraint, and transport stress in deer. Clinical signs include depression, metabolic acidosis, muscular stiffness, paralysis, lack of coordination, and sudden death (Chalmers and Barret, 1982). Delayed acute capture myopathy was used to describe the syndrome observed in three roe deer captured by drive-nets and transported to an enclosure for scientific purposes (Montane et al., 2002). Simultaneous deaths occurring at 48 hours, 60 hours, and 8 days after capture, coincided with human disturbance. The negative prognosis indicators between capture and immediately prior to death are indicative of a

second stress episode that probably consisted of human pursuit inside the enclosure. Hematological and biochemical data did not show a difference at capture and after transport. However, kidneys were dark and swollen in all animals, the bladder contained dark brown, turbid urine suggesting myoglobinurea, and greyish noduli in the caudal lobes of the lungs. Muscles appeared grossly normal with microscopic evidence of mild to severe degradation of skeletal muscle fibers. Fibers with central nuclei, marked exudation of eosinophilic material into the interstitial space, and the presence of some macrophages and polymorphonuclear neutrophils between the degenerated muscle fibers were observed (Montane et al., 2002). Regardless of technique, capture of white-tailed deer can impose significant stress. Restraint and additive stressors can ultimately lead to this complex degenerative disease of the skeletal muscle known as capture myopathy (Hewitt, 2011). A five–year study of 415 white-tailed deer quantified handling on rates of capture myopathy (Beringer et al., 1996). Deer with capture myopathy died within 26 days of capture, and the majority of these were within 9 days of capture. It was concluded that deer that were blinded and exposed to less noise seemed to have less exertion associated with capture myopathy (Beringer et al., 1996).

IMMUNOMODULATORY SUPPLEMENTATION

An immunomodulator is any agent or compound that modifies the immune response or function. Therefore, there are many compounds that fall under this category, and only a few relevant compounds will be discussed in this review. A commercially available feed supplement containing yeast (*Saccharomyces cerevisiae*), fungal cell wall

components, and B-complex vitamins (OmniGen-AF, Phibro Animal Health), as an alternative to antibiotics, was found effective in stimulating the immune system when challenged with various stressors (Ryman et al., 2013). With the European ban on antibiotic feed use, live preparation of *Saccharomyces cerevisiae* was presumed safe as a zootechnical additive according to the European Food Safety Authority (EFSA, 2014). In the US, *Saccharomyces cerevisiae* is considered generally regarded as safe (GRAS) through the US Food and Drug Administration (FDA). Under the category of GRAS, there are no quantitative restrictions for their use under Section 201 of the Food Drug and Cosmetics Act (FDCA 2013). OmniGen-AF contains natural products of yeast and fungal organisms. Fungal cell wall components and yeast contain pathogen-associated molecular patterns (PAMPs), which interact with toll-like receptors and activate the innate immune system (Heine and Ulmer, 2005). The supplement also contains B-complex vitamins which are generally synthesized by rumen organisms and are related to microbial synthetic capability (Van Soest, 1994). Gut mucosa is among the most metabolically active tissue in the body. The volatile fatty acid (VFA) and glucose metabolism by this tissue could limit nutrient availability to peripheral tissues. Probiotics are also proposed to stabilize the intestinal microflora to benefit the host animal. This is valuable during periods of stress when reduction in animal performance is associated with alterations in microbial populations caused by stress (Parker, 1990). Newbold et al. (1996) suggested that presence of respiring yeast would be beneficial to rumen microflora through oxygen-consuming activity of rumen fluid via adhesion of cellulolytic rumen bacteria to cellulose for probiotic activity. Live yeast is a probiotic because it is capable of competing with other starch utilizing bacteria to prevent lactate accumulation in the rumen and provide growth factors

stimulating ruminal populations of cellulolytic bacteria (Chaucheyras et al., 1995; Lynch and Martin, 2002; Retta, 2016). Therefore, inclusion of *Saccharomyces cerevisiae* yeast fed in a high-starch diet may be beneficial in reducing the risk of acidosis and increase fiber degradation (Guedes et al., 2008). Consequently, they compete for nutrient utilization with pathogenic microbes having a positive influence on gut microflora to improve ruminal end products such as microbial protein and VFA that improve ruminal digestibility. Therefore, probiotics may decrease acidosis, improve forage digestion, and enhance growth of the host animal. By using probiotics as an alternative to antibiotics, there may be a similar reduction in morbidity and mortality via enhanced immune responses that protect animals against pathogens (Retta, 2016). Live yeast and other species of bacterial cells have been added to feed to assist rumen fermentation, but some may not survive in the rumen unless a proportion of the population survives through continuous feeding. Since these organisms cannot digest structural carbohydrates they are involved in some cross-feeding of essential factors that might be limiting to the main rumen population including amino acids, peptides, vitamins, and enzymes (Van Soest, 1994).

There have been various studies evaluating the live yeast strain contained in OmniGen-AF on many ruminant species. To examine the mechanism of action of OmniGen-AF, Wang et al. (2004) fed immunocompromised sheep to analyze innate immune function. There were five treatments used in this experiment: (1) control, (2) immunosuppressed with dexamethasone injection, (3) immunosuppressed plus OmniGen-AF inclusion, (4) immunosuppressed plus a pathogen challenge (*Aspergillus fumigatus*), and (5) immunosuppressed plus pathogen challenge and OmniGen-AF inclusion. OmniGen-AF augmented innate immune function by restoring normal expression of

neutrophil L-selectin. Therefore, it has potential to improve herd health in ruminant livestock, and possibly replace a portion of the antibiotics fed (Wang et al., 2004). A similar study was conducted in order to analyze additional blood variables. OmniGen-AF in rations of immunosuppressed sheep caused increased circulating concentrations of both neutrophils and lymphocytes. Therefore, the product can alter innate immune function due to the effects manifested in neutrophils (Wang, et al., 2007). Sheep supplemented with a live yeast culture of *Saccharomyces cerevisiae* tended to have increased total VFA concentration, but did not experience a change in ruminal pH or ammonia concentration (Grochowska, et al., 2012). A meta-analysis of studies over dried live *Saccharomyces cerevisiae* effects on sheep was conducted by Sales, 2011. This meta-analysis did not elucidate an improvement in feed conversion or growth of supplemented sheep (Sales, 2011). However, it was suggested that dried *Saccharomyces cerevisiae* fed to rearing slaughter lambs improved their immunity and alleviated effects of pre-slaughter stress (Sowinska et al., 2016). Paired with an ionophore, active yeast supplementation had no influence on the dry matter intake (DMI) and digestibility of nutrients in diets of finishing lambs (Pienaar et al., 2015). Yeast supplemented into cannulated dairy goats altered their eating behavior such that they preferentially consumed a less fibrous diet when offered a high fiber and low fiber diet, and increased the duration of eating and DMI (Desnoyers et al., 2009). Dairy heifers supplemented with OmniGen-AF exhibited greater binding and internalization of *Escherichia coli*, and increased L-selectin and IL-8R mRNA expression in leukocytes (Ryman et al., 2013). This illustrates that this dietary supplement can be used as an additional management tool to enhance udder health in dairy heifers by enhancing the innate immune response against pathogens that cause mastitis (Ryman et al., 2013).

Beef cattle supplemented with OmniGen-AF during the first 28 days of the feeding period, or the entire feeding period, did not have improved feedlot performance or carcass characteristics, but had an altered metabolic and immune profile. Serum concentration of cortisol was decreased, and pro-inflammatory cytokines were altered in treated steers. These results suggest that inclusion of this supplement may mitigate effects of stress-induced immunosuppression thereby improving host resistance to disease challenge, as was shown in a lipopolysaccharide immune challenge (Buntyn et al., 2016). A meta-analysis of the effects of *Saccharomyces cerevisiae* fermentation products on feedlot performance and carcass traits revealed that receiving cattle had improved ADG and G:F, and finishing cattle had improved BW, ADG, G:F, and carcass grades when supplemented (Wagner et al., 2016).

Riyanti et al. (2015) conducted a preliminary study to determine which *Saccharomyces cerevisiae* strain had the greatest population in the rumen. All strains evaluated in the study reduced protozoa population, and strain NRRL 12618 was selected because it had the highest population and increased rumen bacterial population; it was then used as a probiotic for experiment 2. Supplemented in vitro, this strain stabilized rumen pH and produced the greatest ammonia concentration and bacterial population in the rumen. In combination with rumen bacteria isolate probiotic, total VFA and isovalerate was detected. Therefore, the authors concluded that the NRRL 12618 strain of *Saccharomyces cerevisiae* has potential as a probiotic yeast because it increased fermentability, and decreased A:P (Riyanti et al., 2015).

Inclusion of *Saccharomyces cerevisiae* in the diet of ruminants such as sheep, beef cattle, and dairy heifers resulted in an improved immune response after exposure to various

stressors. The consistency of these results in ruminant species may indicate similar responses in other ruminants such as deer. However, there were inconsistent results between ruminant species for performance and carcass traits; thus, research within the species of interest is required for more conclusive findings.

PHYTOGENIC SUPPLEMENTATION

In recent years, the public has increased concern over antibiotic use in livestock production due to possible contribution to antibiotic resistant bacteria. Consequently, ruminant nutritionists have explored alternative methods for altering ruminal metabolism to improve animal productivity and feed efficiency. Plant-based medicine was commonly and almost exclusively used until the early 20th century when synthetic medicines were discovered and produced more efficiently; however, risks involved in resistant bacteria manifested from antibiotic administration has renewed interest in plant extracts. Phytogenic feed additives consist of a variety of spices, herbs, and products derived from them, and are primarily classified as essential oils (Benchaar, et al., 2008). Due to the ban on antibiotic feed additives in the European Union (and growing concern in the United States), phytogenic feed additives have gained increasing interest for use in the livestock sector. These additives vary due to botanical origin, composition, and processing. Current scientific evidence suggests significant potential to use phytogenic supplements to improve animal health of ruminants (Mirzaei and Venkatesh, 2012). Over 200,000 structures of plant secondary metabolites (PSM) have been defined. These PSM describe chemical compounds in plants that are not involved in the biochemical processes of plant reproduction and growth but may provide benefit in vivo. Plant extracts such as essential

oils, saponins, tannins, organosulfur compounds, and many others have potential to reduce methane production in the rumen (Patra and Saxena, 2010). Deer saliva has tannin-binding proteins to inhibit tannin response in these animals, which explains why no differences are observed when deer are supplemented with tannins (Van Soest, 1994; Rochfort et al., 2008). The best delivery method for phytogenic products will depend on compound stability, bioavailability, potency, safety and economics. Supplementation of plant materials offers a means to alter lipid composition in animal products due to altered fatty acid composition, and may be beneficial to human health (Rochfort et al., 2008).

The range of essential oils and their components is complex and makes consistent positive responses difficult to elucidate (Benchaar et al., 2008). The term ‘essential oil’ is derived from ‘Quinta essentia’ an effective component of a drug by Paracelsus Von Hobenheim, the Swiss medical pioneer (Guenther, 1948). Ironically, they are not ‘essential’ for nutrition, nor are they oils. Essential oils are aromatic volatile compounds with an oily appearance (Burt, 2004). Essential oils exhibit one of their antimicrobial effects by altering permeability of membranes disrupting ion transport and interacting with membrane proteins (Benchaar et al., 2009). Essential oils are mixtures of many secondary metabolites and there are likely a number of modes of action; such as permeating membranes of Gram-positive bacteria more easily than Gram-negative due to outer layer differences in cell wall (Burt, 2004), lipophilic properties (Dorman and Deans, 2000), or interacting with electron transport, ion gradients, protein translocation, and other enzyme-dependent reactions (Ultee et al., 1999; Dorman and Deans, 2000). Essential oils mediate methane by suppression of methanogens and hydrogen producing microorganisms in the rumen; this is depicted in many studies as outlined in a review by Patra and Sexena (2010).

Inclusion of essential oils in the diet may have no effect or decrease VFA concentration at high doses ensuing reduced nutrient digestion. There are varying reports on these PSM due to dosing, chemical structure, dietary composition, and molecular weight which is not usually described clearly in literature. The alteration of micro-organisms to PSM in the rumen is a limitation, which reduces the efficiency of compounds over time (Patra and Saxena, 2010). Essential oils supplemented to cannulated dairy cows decreased total VFA concentration, although these compounds also modified rumen microbial fermentation at higher doses (Castillejos et al., 2006). Sheep fed dietary essential oil compounds had altered protein degradation and amino acid deamination from inhibiting rumen microorganisms (Newbold et al., 2004). Sheep and deer were supplemented with essential oils isolated from Douglas fir needles and rumen microbial activity was analyzed (Oh et al., 1967). Rumen microbial activity, measured in terms of total gas production, was promoted slightly or had no effect, but all components promoted ruminal microbial activity in sheep. Therefore, the deer may have become adapted to such essential oils due to the area they lived in and their dietary selection, where Douglas fir trees were accessible as a food source throughout the year (Oh et al., 1967). Nagy et al. (1964) evaluated the effects of essential oils of sagebrush on deer rumen microbial function collected from rifle-euthanized deer. There was a rather general antibacterial range for both gram-positive and gram-negative bacteria tested and increased amounts of oils reduced the total production of VFA. When compared to those of a cannulated steer, the VFA concentrations were unlike those of deer; therefore, rumen reaction conclusions made about steers may not be the same in deer, and must be researched (Nagy et al., 1964).

Saponins form stable foams in aqueous solutions comparable to soaps, hence the name ‘saponin’. Like essential oils, saponins affect cell membrane permeability by formation of a micelle-like aggregation, and possibly more complex, unknown interactions (Hart et al., 2008). Membrane-permeabilising activity is a common action of many saponins whether steroid or triterpenoid (Francis et al., 2002). However, due to limited information presently available it is difficult to establish a clear functionality of saponins. Many factors, if altered slightly, can change the interactions saponins have with animal systems, and dietary constituents could be a large contributor to that variation. Individual compounds and their well-defining derivatives should be studied extensively in vivo (Francis et al., 2002). For this review, a few relevant studies on saponin interaction have been presented. Many responses arising from saponins may result from effects on enteric microorganisms. However, the microorganisms can also metabolize the saponins (Gutierrez et al., 1959; Makkar and Becker, 1997; Hart et al., 2008). The major effects of saponins are to alter composition of the rumen microbial population, which results in modification of rumen fermentation (Patra and Saxena, 2009). Through inhibition of rumen protozoa, saponins inhibit methanogenesis and may suppress activity of methanogens. Saponins might increase propionate production by rechanneling hydrogen from methane to propionate and decrease A:P, which is beneficial for efficient ruminal metabolism. However, some saponins could cause toxicity in animals (Patra and Saxena, 2010). Most studies reported an increase in feed intake as a response to saponin supplementation in dairy cows and sheep (Abreu et al., 2004; Holtshausen et al., 2009). Cannulated steers administered plant-derived saponin supplementation had increased rumen dry matter (DM) and neutral detergent fiber (NDF) digestibility of forage, reduced protozoa concentrations,

and increased daily outflow of microbial crude protein. This indicates increased rumen fermentation rate which impacts animal performance via increased energy and amino acid supply to the small intestine (McMurphy et al., 2014). In vitro studies concluded that saponins from a soapberry tree can decrease rumen protozoa, and decrease rumen ammonia and methane concentration (Hess et al., 2003). However, there are many types of saponins, and diet differentiation has an effect on interaction in ruminant species (Rochfort et al., 2008).

Many PSM have been defined, but this review is focused on those found in the product evaluated in research of the current thesis (Climate Guard). Essential oils altered rumen microbial fermentation in sheep and dairy cows, and certain essential oils had an antibacterial effect in supplemented deer. Saponin supplementation increased feed intake in dairy cows and sheep, and alters rumen microbial fermentation. However, research on compounds and species of interest are necessary to make comparative conclusions.

CONCLUSIONS FROM THE LITERATURE

The growing white-tailed deer industry has led to an interest in research in these species to improve their nutrition and management strategies, while mitigating the negative effects of stress. Blood parameters in deer have not been established due to variation in capture methods, and should be explored for further comparative research and ultimately allow assessment of health or stress status. Researchers have studied probiotic supplementation to improve ruminant health and performance. Live yeast supplementation

has been shown to improve immune responses in ruminants, but less consistent results exist for growth performance. Phytogenic compounds such as essential oils and saponins have altered rumen microbial fermentation, but there are many varieties of these compounds and interactions are likely; therefore, species-specific research should be considered when making supplementation decisions.

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CHAPTER III

DIETARY SUPPLEMENTATION STRATEGIES FOR THE CAPTIVE WHITE-TAILED (*ODOCOILEUS VIRGINIANUS*) BREEDING DOE

ABSTRACT

Stress from extreme weather, predation or poor nutrition in captive white-tailed breeding does may compromise their performance, alter immunity and increase death loss. A potential method to mitigate stress-induced losses in captive deer may be dietary inclusion of a supplement known to have immunomodulatory effects in other species (OmniGen-AF, Phibro Animal Health). Experiment I was conducted over an 84-d period (15DEC2015 to 8MAR2016) at a private deer breeding facility in Texas. Two treatment diets were offered *ad libitum* via self-feeders located in each treatment pen (experimental unit) and consisted of: 1) a commercial deer breeder diet (AntlerMax, Purina Animal Nutrition) with no additive (CON) or 2) the same breeder diet with OmniGen-AF included (OG). On d 0, white-tailed does (n = 72) were assigned randomly to treatment pens of similar size (n = 3 pens/ treatment) according to their chute entry order. A randomly selected subset population (6 does/pen) was used to assess rib flesh score (RFS) and complete blood count (CBC) on d 0, 42, and 84 and BW was determined for all deer on these days. The RFS was subjectively determined by palpating the ribs. Blood was collected via jugular venipuncture into evacuated tubes containing EDTA and analyzed

within 4-h using an automated hemocytometer to determine CBC variables. There was a tendency ($P = 0.10$) for the OG treatment to have greater BW on d 84; however, both OG and CON lost BW from d 0 to 42 and d 42 to 84. Nevertheless, OG treated does tended to have a greater ($P = 0.09$) RFS on d 42. There were no treatment differences ($P \geq 0.32$) for CBC variables; however, day effects were observed for several CBC variables. Total leukocytes, neutrophils and neutrophil:lymphocyte (NLR) decreased with time (day effect; $P \leq 0.02$). Total leukocytes were 7.58, 4.55, and 5.34 K/ μL on d 0, 42, and 84, respectively. The NLR was 1.76, 1.21, and 0.78 on d 0, 42, and 84, respectively. Supplementation with OmniGen-AF had limited effect on BW and did not alter CBC variables measured in white-tailed breeding does used in this study. Overall, there were decreases in total leukocytes and other CBC variables with time, and these data may provide a foundation for CBC reference range in captive white-tailed does.

Another method may be dietary inclusion of feed additives comprised primarily of plant-based essential oils and saponins. Experiment II was conducted over an 84-d period (23Jun2016 to 15Sep2016) at a private deer facility near Canadian, TX. Breeding age white-tailed does (n=67) were administered 1 of 2 treatment diets that consisted of: 1) a commercial breeder diet (AntlerMax, Purina Animal Nutrition) without additive (**CON**) and 2) the same breeder diet with nutritional supplement included (Climate Guard, Delacon; **CG**). Body weight (BW) and rib flesh score (RFS) were recorded on d 0, 42, and 84. Average daily gain and BW gain was determined for interim periods and overall. The RFS was determined by a trained evaluator using an ordinal scale. Daily temperatures were recorded and retrieved from the NOAA weather database for the Hemphill County Airport in Canadian, TX. Feed offered per doe over the 84-d study was 142.13 kg for CG and

109.80 kg for CON ($P = 0.31$). No difference ($P \geq 0.18$) was observed in BW, ADG, or BW change during the 84-d study period. A numerical increase in BW was observed for CG on d 42 and 84, and d 0 to 42 and overall ADG and BW change was numerically increased for CG treated does. On d 42, RFS tended ($P = 0.06$) to be greater in CG-treated does, and a difference ($P < 0.01$) was detected on d 84 for CG to have improved RFS. Results indicate that CG treated does had increased feed intake and improved performance and body condition.

INTRODUCTION

Captive breeding and hunting of white-tailed deer in Texas is increasing in popularity and economic impact. The primary product of this deer industry is harvesting trophy bucks; therefore, perhaps the greatest loss to this industry is mortality prior to harvest due to lost income opportunity (Anderson et al., 2007a; Anderson et al., 2007b; Hewitt, 2011; Adams et al., 2016; Cain, 2016; Crossley, 2017). To alleviate the negative effects of weather stress and poor nutrition that will ultimately improve survivability, it is necessary to research non-invasive methods to improve deer performance and reduce stress. One such method may be dietary inclusion of a feed additive comprising live yeast, which has been reported to have beneficial health effects in other species (Wang et al., 2004; Wang et al., 2007; Desnoyers et al., 2009; Grochowska et al., 2012; Ryman et al., 2013; Pienaar et al., 2015; Buntyn et al., 2016). Antibiotics have both health and performance benefit in ruminants, but have recently been banned in European countries and are under increasing scrutiny in the United States due to potential of antibiotic resistant bacteria. As an alternative to antibiotics, a commercial immunomodulatory feed

additive (OmniGen-AF, Phibro Animal Health) containing the live yeast *Saccharomyces cerevisiae* was reported to be effective in stimulating the immune system of other ruminant species; such as sheep (Wang, et al., 2007), slaughter lambs (Sowinska et al., 2016), dairy heifers (Ryman et al., 2013), and beef cattle (Buntyn et al., 2016). There are inconsistent results between ruminant species for performance traits, but increased performance was seen in sheep (Sales, 2011), and dairy goats (Desnoyers et al., 2009) supplemented with live yeast. By using probiotics rather than antibiotics there is a potential to realize beneficial health and performance effects while simultaneously addressing antibiotic resistance concerns (Retta, 2016). The present experiment sought to determine if a dietary immunomodulator (OmniGen-AF) in a pelleted complete feed altered captive deer body condition, performance and hematological variables. By improving these characteristics via dietary supplementation, it may mitigate negative responses to stressors, thus improving deer welfare and survivability during times of extreme weather or nutritional stress.

Another non-antibiotic alternative may be dietary inclusion of a feed additive comprising phytogenic compounds. Phytogenics are natural growth promoters that comprise plant based products such as essential oils and saponins that are effective in altering rumen microbial fermentation, and have demonstrated antimicrobial properties (Benchaar, et al., 2008; Hart et al., 2008; Patra and Sexena, 2010). By using phytogenic additives as an alternative to antibiotics, morbidity and mortality may be similarly improved via enhanced nutritional status that prepare ruminants such as sheep (Abreu et al., 2004), dairy cows (Holtshausen et al., 2009), and cannulated steers (McMurphy et al., 2014) for poor weather conditions or reactions to other stressors (Rochfort et al., 2008).

However, there are few, if any studies to support these results in deer. The current study objective was to determine if a dietary immunomodulatory (OmniGen-AF) or a phytogenic feed supplement (Climate Guard) in a pelleted complete feed alters captive deer body condition and performance. By improving these characteristics, deer may be able to mitigate negative responses to stressors, thus improving welfare and ability to survive through weather or nutritional stressors. These experiments are preliminary to understanding the interactions and effects of the respective products within deer.

MATERIALS AND METHODS

EXPERIMENT I

Experiment I was conducted over an 84-d period (15DEC2015 to 8MAR2016) at a private deer breeding facility near Canadian, Texas. Treatment diets were offered *ad libitum* via self-feeders (3-way pen feeder with fawn drop, All Season Feeders®, San Antonio, TX) located in each treatment pen (experimental unit) that were high-fenced (2.59 meters) and approximately 0.34 hectares in size. Treatment diets consisted of a commercial deer breeder diet (AntlerMax® without dried kelp inclusion, Purina Animal Nutrition, Gray Summit, MO) with no additive (**CON**) or the CON diet with an immunomodulatory product (OmniGen-AF, Phibro Animal Health, Teaneck, NJ) included at 3.51 g/kg (**OG**). OmniGen-AF is a patented proprietary product containing yeast (*Saccharomyces cerevisiae*), fungal cell wall components, and B-complex vitamins. Treatment diets were formulated and pelleted on the same day at a Purina Mills facility in Lubbock, TX. Feed grab samples were collected from random bags of feed within

treatment, and composited to determine proximate analysis of the treatment diets at a commercial laboratory (Servi-Tech Laboratories, Amarillo, TX). On d 0, mature white-tailed does (n = 72) were assigned randomly to treatment pens of similar dimension (n = 3 pens/ treatment, 12 deer/pen) according to chute entry order. Random numbers were generated for treatment allocation, treatment pen assignment and subset allocation. A randomly selected subset population (6 does/pen) was used to assess rib flesh score (RFS) and complete blood count (CBC) on d 0, 42, and 84; however, BW was determined for all deer on these days. The BW was recorded individually using a Delclayna Tunnel System (Deer Store, Little Falls, MN) with an electronic scale (EziWeigh5i, TruTest Inc., Mineral Wells, TX). The subset population was restrained in an enclosure (Papa Deerhandler, Deer Store, Little Falls, MN) for RFS evaluation and jugular blood collection. The RFS was determined by a trained evaluator by palpating the ribs and an ordinal subjective score was assigned using a 1 to 5 scale. The RFS was described according to Purina Animal Nutrition body condition scoring system for deer (Table 3.1). Briefly, a RFS of 1 was poor with majority of ribs prominent, a 2 was lean and thin with many ribs visible, a 3 was prime conditioned with ribs slightly visible, a 4 was heavy carrying extra weight and ribs were not visible, and a 5 was considered obese with ribs layered in fat. Blood was collected via jugular venipuncture into 4 mL tubes containing 7.2 mg K2-ethylenediaminetetraacetic acid (EDTA) (Becton, Dickinson and Company, Franklin Lakes, NJ). Blood was analyzed within 4-h using an automated hemocytometer (Idexx, Procyte Dx, Westbrook, ME) at the West Texas A&M University Animal Health Laboratory to determine CBC variables. Statistical analysis was performed using SAS version 9.4 with pen identified as the experimental unit. The BW,

BW change, and CBC data were analyzed with PROC MIXED. A repeated statement (day) was used for CBC variables to evaluate effects of treatment, day, and their interaction and compound symmetry was selected as the covariance structure. The CBC variables were tested for normal distribution using PROC UNIVARIATE. If normal distribution was not present for a variable, then that variable was $\log_2$ transformed and tested again to determine if normality was improved. The $\log_2$-transformed data was used for statistical analysis if the Shapiro-Wilk *P*-value was greater than alpha ($P \geq 0.05$), and non-transformed LSmeans were reported. To analyze the categorical RFS data, PROC NPAR1WAY was used and the Wilcoxon *P*-value was selected to determine if a treatment effect existed. For BW and CBC data, PDIFF was used to determine treatment differences, and the alpha level was set at 0.05 with trends considered between 0.06 and 0.10 for all analyses. Means of CBC variables for comparison to other species was analyzed with PROC UNIVARIATE.

EXPERIMENT II

Experiment II was performed at the same facility as experiment I, and was conducted over an 84-d period (23JUN016 to 15SEP2016). Breeding age white-tailed does (n=67) were stratified by pregnancy status, which was determined via ultrasound at chute entry, and allocated randomly to 1 of 6 high-fenced (2.54 meters) treatment pens of similar size (approximately 0.34 hectares; n=3 pens/treatment). One pen per treatment consisted of pregnant does, which fawned later in the study; this was balanced in order to mitigate effects of pregnancy status on treatment outcomes. The two treatment diets were offered *ad libitum* via self-feeders located in each treatment pen (experimental unit). Treatment diets consisted of a complete breeder diet (AntlerMax® without dried kelp

inclusion, Purina Animal Nutrition, LLC) without additive (**CON**) and the same breeder diet with phytogenic nutritional supplement included at 0.56g/kg of feed(Climate Guard, Delacon, Steyrigg, Austria; **CG**). Diets were manufactured at Purina Mills in Lubbock, TX on 21Jun2016. The BW was recorded individually, and does were restrained in an enclosure for rib flesh score (RFS) evaluation; BW and RFS were recorded on d 0, 42, and 84, and BW change was determined for interim periods and overall. The RFS was determined by a trained evaluator by palpating the ribs and a subjective score was assigned using a 1 to 5 scale derived from the Purina Animal Nutrition body condition scoring system for deer (Table 3.1). Daily temperatures were retrieved and recorded from the NOAA weather database for the Hemphill County Airport, Canadian TX (Figure 3.2) to report high, low, and average ambient temperatures during the observation period. Statistical analysis was performed using SAS version 9.4 with pen identified as the experimental unit. The BW, BW change, and feed offered data were analyzed with PROC MIXED. To analyze RFS as ordinal data PROC NPAR1WAY was used and the Wilcoxon *P*-value was selected to determine treatment effect. For each analysis, PDIFF was used to determine treatment differences, and an alpha level was set at 0.05 and a trend was considered between 0.06 and 0.10. Additionally, feeder visits throughout the day were recorded using game cameras (Browning Recon Force Full HD Platinum Series, Prometheus Group, Raleigh, NC) directed at each the feeder. Cameras were programmed to record a digital image with detected movement. Images were viewed individually and feeder visits were counted manually from recorded images. A visit was considered when deer were observed eating within the legs of the feeder. Using recorded weather data, five consecutive days of warm ambient temperatures (23AUG2016 to

28AUG2016) were chosen for evaluation of feeder visits. Visits were analyzed using PROC MIXED with repeated measures, day effects were evaluated, autoregressive was used as the covariance structure, and mean number of visits/head/pen was determined for 2-hour daylight intervals (Figure 3.4).

RESULTS AND DISCUSSION

EXPERIMENT I

Proximate analysis of treatment diets revealed a dry matter (DM) protein content of 20.0 and 18.9% for CON and OG, respectively (Table 3.2). Total digestible nutrients (TDN) and acid detergent fiber (ADF) varied slightly between treatments on a DM and as fed basis. However, manufactured feeds may differ, even under the same label, as determined by the similarities in the nutrients, and may be where this slight variation was derived (NRC, 2007). In general TDN and ADF are indicators of relative digestibility (Church, 1993; NRC, 2007). On d 0, BW was numerically, but not statistically different (P = 0.22) with an average of 48.7 kg for CON and 52.0kg for OG-treated does. There was a tendency (P = 0.10) for the OG supplemented does to have greater BW by d 84, with 47.4kg and 51.3kg BW for CON and OG, respectively. Differentiation of weights on d 0 occurred due to the inability to stratify by BW, and deer were randomly assigned to treatment. Therefore, it is important to analyze the change in BW through the experiment. Both OG and CON lost BW from d 0 to 42 and d 42 to 84. Overall, CON does lost 1.29 kg while OG lost 0.68 kg during the 84-d experiment, but was not different (P = 0.43). Although, it should be noted that the animal subjects were mature breeding does and large changes in

BW was not expected. Experiment I occurred during winter months and weight loss could be attributed to natural weight loss documented for white-tailed deer during this time. Free-ranging deer accumulate fat reserves before winter and use those resources during times of nutritional restriction when forage is dormant (Hewitt, 2011). Previous research on supplementation of deer during cold seasons reported reduced weight loss (Ullrey et al., 1975; Lewis et al., 1998). Improved digestion, enhanced growth, and increased duration of eating when supplemented with live yeast have been observed in sheep (Sales, 2011) and dairy goats (Desnoyers et al., 2009). Does given OG supplement were offered less (P = 0.10) feed for consumption throughout the feeding period (Figure 3.1). Feed disappearing from the feeder is shown as feed offered for consumption. Feed efficiency may have been increased for OG because OG does had less feed offered and lost less BW.

Feeding animals a diet with nutrients less than the amount required for body maintenance results in use of fat and other tissues to supply energy, and maintenance level is set by the reserves available to the animal (Van Soest, 1994). Therefore, the following calculations may predict performance outcomes based on predicted maintenance energy requirements and provided results. Few resources are available on maintenance energy requirements for white-tailed deer; therefore, some previous studies may be helpful in estimation. Northern white-tailed deer had a net energy for maintenance (NE_m) at 106 ±11, 97±10 and 125±30 kcal/kg $BW^{0.75}$ for all 15 months of the study, December through April, and May through October, respectively (Holter et al., 1977). More recently, a study measuring maintenance requirements for white-tailed deer in Southern Texas found that NE_m ranged from 196±40 kcal/kg $BW^{0.75}$ at trial initiation (13NOV) to 85±10 kcal/kg $BW^{0.75}$ at trial completion (13DEC). This suggests that digestible energy maintenance

requirements decline during extended periods of energetic restriction, and the previous study using northern white-tailed deer may overestimate requirements for deer in southern areas (Strickland et al., 2005). Furthermore, established maintenance energy requirements for small ruminants (sheep and goats) was determined at 129.7 kcal/kg $BW^{0.75}$ and 150 kcal/kg $BW^{0.75}$ for cattle (Salah et al., 2014). In a review of maintenance energy requirements for cervids (white-tailed deer, caribou, red deer, and elk) NE_m was established at 0.056 mcal/kg $BW^{0.75}$(NRC, 2007), but may not be a valid representation of white-tailed deer requirements alone, due to energy expenditure differences in large cervids versus smaller cervids (Parker et al., 1984). For the following calculations of daily intake versus maintenance requirements, the requirements are averaged from Strickland et al. (2005), and 140.5 kcal/kg $BW^{0.75}$ will be used. The proximate analysis for treatment diets was calculated with cattle as the assumed species, which may be an overestimate for deer in winter months (Ozoga and Gysel, 1972). Beginning BW of CON does was 48.7 kg and OG does was 52.0 kg. On a kg $BW^{0.75}$ basis, the resulting value is 18.44 and 19.36 for CON and OG, respectively. The previous value multiplied by 140.5 kcal/kg $BW^{0.75}$ results in an estimated daily maintenance energy requirement of 2,591 and 2,720 kcal for CON and OG, respectively. Feed offered per day was 1.41 and 1.21kg for CON and OG-treated does per day, respectively (Figure 3.1). According to the diet proximate analysis, as fed NE_m for feed is 1,480 and 1,390 kcal/kg for CON and OG, respectively (Table 3.2). Multiplied by feed offered per day shows CON does consumed 2,087 kcal/d which is 80% of the calculated maintenance energy requirement; OG does consumed 1,682 kcal/d which is 62% of the calculated maintenance energy. Therefore, both treatments should have lost weight, as is shown in the results, but OG does should have lost more weight; this difference from

the results of the experiment suggests there are confounding factors present. However, probiotics compete for nutrient utilization with pathogenic microbes having a positive influence on gut microflora to improve ruminal end products such as microbial protein and VFA that improve ruminal digestibility. Therefore, decreasing the energy needed to consume to meet dietary requirements (Van Soest, 1994; Retta, 2016). However, estimated maintenance energy requirements do not predict these results, and are calculated for each treatment below.

Using the information provided from this experiment, a NE_m for captive does during winter months can be calculated. The average of Beginning BW and end BW of does gives a midpoint BW of 48.06 kg for CON, and 51.69 kg for OG. On a kg $BW^{0.75}$ basis, that is 18.25 kg and 19.28 kg for CON and OG, respectively. These values divided by the kcal of feed consumed per kg, as given above, produced an NE_m of 114.33 kcal/kg $BW^{0.75}$ for CON, and 87.25 kcal/kg $BW^{0.75}$ for OG-treated does. Therefore, with consideration that the feed is on an offered basis and not consumed, it appears that OmniGen-AF supplementation may reduce NE_m for captive does, and may explain reduced feed offered with a reduced loss in BW. However, energy expenditures in animals can change with temperature (Buchanan et al., 2003), and inflammation response (Carroll and Forsberg, 2007). These data may be helpful when calculating energy needed for maintenance for captive deer.

Nevertheless, OG supplemented does tended to have a greater (P = 0.09) rib flesh score (RFS) on d 42 (Table 3.3). Increased RFS suggests that the deer consuming OG diets utilized feed more efficiently and were able to store more energy. Fat is important to assess because it represents stored energy reserves above maintenance requirements (Hewitt,

2011). Other ruminant species have had inconsistent feed efficiency results, but this is the first experiment that we are aware of that evaluated OG supplementation in deer, and replication of the experiment is necessary to substantiate our observation of improved feed efficiency with OG supplementation.

Day effects ($P \leq 0.03$; Table 3.4) were observed for several CBC variables; however, there were no treatment differences ($P \geq 0.32$; Table 3.5). Total leukocytes, neutrophil count, percent neutrophils, and neutrophil:lymphocyte (NLR) decreased with time (day effect; $P \leq 0.01$); however, percent eosinophils and percent lymphocytes increased with time (day effect; $P = 0.03$). Total leukocytes were 7.58, 4.55, and 5.34 K/μL on d 0, 42, and 84, respectively. However, differential white blood cell counts may be more specific for diagnosis than total white blood cell count due to increases and decreases in individual cell types that could occur simultaneously, leaving total count unaffected (Polizopoulou, 2010). Total neutrophils were 4.37, 2.23, and 1.82 K/ μL on d 0, 42, and 84, respectively. Percent neutrophils were 53.33, 45.38, and 34.43% on d 0, 42, and 84, respectively. Stress and the resulting increase in cortisol can decrease L-selectin expression on neutrophils in a manner that reduces translocation and increases neutrophil concentration in the blood (Weiss and Perman, 1992; Jones and Allison, 2007). Although we did not measure stress variables such as cortisol some CBC variables can indicate stress such as NLR (Mitlohner et al., 2002). The NLR was 1.76, 1.21, and 0.78 on d 0, 42, and 84, respectively. Percent eosinophils were 4.64, 5.90, and 7.57 on d 0, 42, and 84, respectively. Percent lymphocytes were 38.75, 44.86, and 52.11 on d 0, 42, and 84, respectively (Table 3.4). Elevated lymphocyte counts occur several days after an initial infection, because they are part of the adaptive immune response (Abbas et al., 2007).

Without any sign of chronic infection, stress may be the cause of this increase in lymphocyte concentration (Kirk and Bristner, 1985). Eosinophils are granulocytes that increase in concentration during severe parasitic infection (Abbas et al., 2007). Marco and Lavin (1999) concluded similar findings in red deer when eosinophil count was greater in animals captured using anesthesia versus physical means (Marco and Lavin, 1999). This agrees with the findings in the current experiment; does were entering breeding season, a time when they are handled the most, and were minimally handled since the previous breeding season. However, there is not a published reference range for deer CBC data due to the differences in results from various capture techniques (Seal et al., 1981; Sams et al., 1993; Creekmoore and Creekmoore, 1999; Marco and Lavin, 1999). Ranges from previous studies are provided in Table 2.1 for unsedated captive white-tailed deer in zoos (Seal et al., 1981), and free-ranging white-tailed deer that have been rifle harvested (Creekmoore and Creekmoore, 1999). The values between those studies were similar and have some similarity to the current study. Variables that differ include; increased white blood cells, increased eosinophils and decreased eosinophils observed in the current study compared to unsedated deer and within the range of harvested deer from the aforementioned studies. Neutrophils were greater than these ranges on d 0, but within the previously reported reference range for the remaining sample days (d 42 and 84). These data may provide a foundation for CBC reference range in captive white-tailed does sampled in a chute. A comparison of CBC results from this study and those of other ruminant species show some similarities to those of cattle, sheep, and lambs (Table 3.6). However, to determine if CBC values are outside of a “normal” range for deer, one must consider the capture method employed.

EXPERIMENT II

Proximate analysis of treatment diets revealed a DM CP content of 18.2 and 20.9% for CON and CG, respectively (Table 3.8). Other variables were similar within the analysis. Both treatment diets contained the same complete feed except the phytogenic supplement added to the CG diet. There was no difference ($P = 0.96$) in d 0 BW between treatments, which indicates an even distribution of doe BW stratification. A numerical difference ($P = 0.19$) was observed for d 42 BW; CON and CG averaged 49.43 and 52.92 kg BW, respectively. Likewise, BW on day 84 was numerically greater for CG (54.13) vs. CON (50.33 kg), but not statistically different ($P = 0.18$). From d 0 to 42 there was not a difference in BW change ($P = 0.19$) but there was a numerical difference; BW change was -1.76 kg for CON and 1.14 kg for CG treated does from d 0 to 42. From d 42 to 84, there was not a difference in BW change ($P = 0.93$). From d 0 to 84 there was not a statistical difference in BW change ($P = 0.46$) but there was a numerical difference; BW change was -0.66 kg for CON and 2.27 kg for CG-treated does. Overall, does receiving the phytogenic supplement gained BW through the observation period and CON-treated does lost BW. Climate Guard contains a combination of essential oils, saponins and other plant extracts. Essential oils and saponins alter cell membrane permeability resulting in an antimicrobial effect, by disrupting ion transport and interacting with membrane proteins (Hart et al., 2008). Both essential oils, and saponins are multifaceted in their interaction depending on the substance and the type of diet, so the exact mechanism to cause weight gain is difficult to elucidate (Patra and Saxena, 2010). Feed intake and BW may have increased for CG due to altered rumen microbial fermentation (Abreu et al., 2004; Holtshausen et al., 2009; Patra and Saxena, 2010). As shown in Figure 3.2, the

ambient temperature was above 32°C for 63 d and greater than 37° C for 14 d; therefore, it is likely that heat stress was occurring during the study period. The d 0 RFS was not different ($P = 0.22$) between treatments. There was a tendency ($P = 0.06$) for RFS to be greater in CG-treated does on d 42, and a difference ($P < 0.01$) was detected by d 84 for CG to have greater RFS (Table 3.7). Feed offered per doe over the 84-d study was 1.31 and 1.69 kg for CON and CG, respectively ($P = 0.31$; Figure 3.3). Results suggest that CG-treated does had increased feed intake and improved performance. Other studies have reported an increase in feed intake as a response to saponin supplementation in dairy cows and sheep (Abreu et al., 2004; Holtshausen et al., 2009). Saponins may increase propionate production by rechanneling of hydrogen from methane to propionate thereby decreasing acetate:propionate (A:P), which is beneficial for ruminant nutrition (Patra and Saxena, 2010). However, the mechanism is not fully understood without knowing the specific plant products used in the proprietary supplement, and without extensive rumen sampling to determine microbial alteration.

For the following calculations of daily intake versus maintenance requirements, the formula from Strickland et al. (2005) of 140.5 kcal/kg $BW^{0.75}$ will be used. The proximate analysis for treatment diets was calculated with cattle as the assumed species. Beginning BW of CON does was 51.60 kg and CG does was 51.78 kg. On a kg $BW^{0.75}$ basis is the resulting values are 19.25 and 19.30 for CON and CG, respectively. These values multiplied by 140.5 kcal/kg $BW^{0.75}$ results in a daily maintenance energy requirement of 2,705 kcal for CON, and 2,712 kcal for CG. Feed offered per day was 1.31 and 1.69 kg for CON and CG-treated does per day, respectively (Figure 3.3). According to the diet proximate analysis, as fed NE_m for feed is 1,520 and 1,500 kcal/kg

for CON and CG, respectively (Table 3.8). Multiplied by feed offered per day shows CON does consumed 1,991 kcal/d which is 74% of the calculated maintenance energy requirement; CG does consumed 2,535 kcal/d which is 94% of the estimated maintenance energy. Therefore, both treatments should have lost weight throughout the experiment, which is correct for CON, but CG-treated does gained weight in the results from this experiment. Using the information provided from this experiment, a NE_m for captive does during summer months can be calculated. The average of beginning BW and end BW of does gives a midpoint BW of 50.97 kg for CON, and 52.96 kg for OG. On a kg $BW^{0.75}$ basis, that is 19.08 and 19.63 kg for CON and OG, respectively. This divided by the kcal of feed consumed per kg, as given above, produced an NE_m of 104.39 kcal/kg $BW^{0.75}$ for CON, and 129.14 kcal/kg $BW^{0.75}$ for OG-treated does. Therefore, it appears that Climate Guard supplementation may increase NE_m for captive does in summer months. Inclusion of essential oils in the diet may have no effect or decrease VFA concentration at high doses ensuing reduced nutrient digestion. The alteration of micro-organisms to PSM in the rumen is a limitation, which reduces the efficiency of compounds over time (Patra and Saxena, 2010). The major effects of saponins are to alter composition of the rumen microbial population, which results in modification of rumen fermentation (Patra and Saxena, 2009). Most studies reported an increase in feed intake as a response to saponin supplementation in dairy cows and sheep (Abreu et al., 2004; Holtshausen et al., 2009). This may explain why feed offered was increased, as well as NE_m. This experiment contained one pen in each treatment with pregnant does to mitigate any affects this may have on treatment differences, but pregnant does may have increased the total NE_m because they may have an increased daily maintenance energy requirement (Ullrey et al.,

1969). It is important to breeders to meet nutritional needs of white tailed does because their nutrition is reflected on reproductive rates, and fawn health (Verme, 1965; Verme, 1967; Verme, 1969; Sams et al., 1995).

Feeder cameras provided information into feeding habits of captive white-tailed breeding does. Visits to feeders were greater ($P < 0.01$) in the 18:00 to 20:00 interval, or near dusk (Figure 3.4). This agrees with natural feeding habits studied in free-ranging white-tailed deer with a supplemental feeder (Ozoga and Verme, 1982), free-ranging mule deer (Fernanda Mejia Salazar et al., 2016), and captive fallow deer (Mattiello et al., 1996). Treatment differences were not reported, because cameras were not calibrated and camera placement issues were discovered; therefore, this information is provided as a means to show the eating behavior of captive deer at this facility.

CONCLUSIONS

White-tailed breeding does supplemented with a dietary immunomodulator (OG) had improved body condition on d 42 and tended to reduce amount of feed offered. Combined, these results suggest an improved feed efficiency with OG inclusion. Those that received a phytogenic nutritional supplement (CG) in their diet had increased BW and RFS during the 84-d observation period. This essential oil product may be beneficial to supplement during times of possible heat stress as a means to maintain or improve BW and increase feed intake in captive white-tailed deer. Few statistically significant differences were noted with these experiments; therefore, studies with additional replication and samples are needed to corroborate the numerical differences observed. However,

performance data on mature does is not expected to change significantly over time. Changes in NE_m was noticed in both supplemented diets when compared to CON diets. These data may be helpful when calculating energy needed for maintenance for captive deer consuming these diets. Both products may have potential to improve management of captive deer; overall, this study is preliminary to further research needed to support these conclusions.

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TABLES

Table 3.1. Rib flesh score derived from Purina Animal Nutrition body condition scoring system for deer.

Score	Description
1-Poor	Emaciated. Majority of ribs are prominent. Spine appears sharp. Belly is tucked high with hollow flanks.
2-Lean	Thin. Many ribs visible. Spine evident, but not sharp. A small amount of observable fat.
3-Prime	Strong, muscular, and healthy. Ribs slightly visible. Spine is visible, but not prominent. Belly has a slight depression with no fat rolls.
4-Heavy	Healthy, but carrying a few extra pounds. Ribs are not visible. Spine is not readily seen. Flank is full with slight fat rolls developing.
5-Obese	Ribs are layered in fat. Spine lies buried in fat. Fat rolls are clearly evident at the midline and brisket.

Table 3.2. Diet composition for experiment I.[1]

	Treatment[2]			
	CON		OG	
Item	Dry	As Fed	Dry	As Fed
Analysis Results[3]				
Moisture, %	-	11.1	-	11.1
Dry Matter, %	-	88.9	-	88.9
CP, %	20.0	17.8	18.9	16.8
ADF, %	22.2	19.8	25.3	22.5
TDN, %	70.4	62.6	67.8	60.3
NE_m, Mcal/kg	1.65	1.48	1.56	1.39
NE_g, Mcal/kg	1.03	0.93	0.97	0.86
NE_l, Mcal/kg	1.61	1.43	1.54	1.37
DE, Mcal/kg	3.10	2.76	2.99	2.67

[1]Analysis performed at Servi-Tech Laboratories, Amarillo, TX.

[2]CON= control (complete feed without additive); OG= OmniGen-AF (complete feed with OmniGen-AF at an inclusion rate of 3.51 g/kg of feed).

[3] CP= Crude Protein; ADF= Acid Detergent Fiber; TDN= Total Digestible Nutrients; NE_m= Net Energy for Maintenance; NE_g=Net Energy for Gain; NE_l= Net Energy for Lactation; DE= Digestible Energy.

Table 3.3. Effect of OmniGen-AF on body weight, body weight change, and rib flesh score of white-tailed breeding does for experiment I.

	Treatment[1]			
Item	CON	OG	SEM	*P*-Value
Body weight[2], kg				
D 0	48.7	52.0	3.60	0.22
D 42	47.9	51.7	1.83	0.22
D 84	47.4	51.3	1.31	0.10
Change in body weight[2], kg				
D 0 to 42	-0.82	-0.35	0.39	0.45
D 42 to 84	-0.47	-0.33	0.69	0.89
D 0 to 84	-1.29	-0.68	0.50	0.43
Rib Flesh Score[3]				
D 0	2.25	2.36	-	0.80
D 42	2.56	3.22	-	0.09
D 84	2.39	2.89	-	0.20

[1] CON= control (complete feed without additive); OG= OmniGen-AF (complete feed with OmniGen-AF at an inclusion rate of 3.51 g/kg of feed).

[2] Analyzed using PROC MIXED of SAS.

[3] Rib flesh score derived from Purina Animal Nutrition body condition scoring system for deer: 1=poor, 2=lean, 3=prime, 4=heavy, 5=obese; Analyzed using PROC NPAR1WAY of SAS, Wilcoxon for *P*–value, and LSmeans generated using ANOVA.

Table 3.4. Main effect of sampling day on complete blood count variables in white-tailed breeding does in experiment I.

	Day				Contrasts[1]	
Variable[2]	0	42	84	SEM	Linear	Quadratic
White blood cells, K/µL	7.58[a]	4.55[b]	5.34[b]	0.77	0.01	0.02
Neutrophils, K/µL	4.37[a]	2.23[b]	1.82[ab]	0.60	0.09	0.01
Neutrophils, %	53.33[a]	45.38[a]	34.46[b]	3.69	<0.01	0.76
Lymphocytes, K/µL	2.70	2.22	2.80	0.34	0.79	0.13
Lymphocytes, %	38.75[b]	44.86[b]	52.11[a]	3.70	0.01	0.90
Neutrophil/lymphocyte	1.76[a]	1.21[ab]	0.78[b]	0.24	<0.01	0.84
Monocytes, K/µL	0.21	0.16	0.29	0.05	0.29	0.20
Monocytes, %	2.93[a]	2.91[a]	5.78[b]	0.90	0.04	0.24
Eosinophils, K/µL	0.30	0.30	0.41	0.08	0.24	0.56
Eosinophils, %	4.64[b]	5.90[ab]	7.57[a]	1.40	0.01	0.62
Basophils, %	0.12	0.28	0.33	0.09	0.10	0.60
Red blood cells, M/µL	14.78	14.64	15.40	0.62	0.48	0.55
Hemoglobin, g/dL	18.66	18.56	19.23	0.80	0.56	0.65
Hematocrit, %	50.69	51.11	53.84	2.61	0.37	0.70
MCV, fL	34.10	34.16	34.80	0.73	0.39	0.68
MCH g/dL	12.68	12.66	12.47	0.20	0.46	0.74
MCHC g/dL	37.33	37.47	36.01	1.14	0.39	0.55

[a,b] LSmeans within a row with differing superscripts differ ($P \leq 0.05$).

[1]Contrasts analyzed using PROC MIXED of SAS, with a repeated statement of day.

[2]Basophil count and platelet count excluded due to lack of fit of statistical model; variables analyzed using an automated hemocytometer (Idexx, Procyte Dx; bovine species selected). Data that were not normally distributed was $\log_2$-transformed for statistical analysis. LSmeans and SEM reported from non-transformed data. MCV= Mean corpuscular volume; MCH= Mean corpuscular hemoglobin; MCHC= MCH concentration.

Table 3.5. Main effect of treatment on complete blood counts in white-tailed breeding does in experiment I.

	Treatment[1]			
Variable[2]	CON	OG	SEM	P-value
White blood cells, K/µL	5.86	5.79	0.83	0.95
Neutrophils, K/µL	2.82	2.80	0.66	0.52
Neutrophils, %	45.80	42.98	2.74	0.51
Lymphocytes, K/µL	2.45	2.69	0.36	0.65
Lymphocytes, %	44.04	46.44	3.22	0.63
Neutrophil/lymphocyte	1.39	1.10	0.20	0.52
Monocytes, K/µL	0.23	0.2	0.03	0.51
Monocytes, %	4.30	3.44	0.57	0.32
Eosinophils, K/µL	0.34	0.33	0.08	0.64
Eosinophils, %	5.62	6.45	1.49	0.65
Basophils, %	0.22	0.26	0.06	0.62
Red blood cells, M/µL	15.15	14.73	0.55	0.61
Hemoglobin, g/dL	19.2	18.43	0.78	0.52
Hematocrit, %	52.84	50.92	2.36	0.59
MCV, fL	34.66	34.05	0.79	0.61
MCH g/dL	12.71	12.5	0.15	0.39
MCHC g/dL	36.86	37.02	1.03	0.92

[1] CON= control (complete feed without additive); OG= OmniGen-AF (complete feed with OmniGen-AF at an inclusion rate of 3.51 g/kg of feed); analyzed using PROC MIXED of SAS with a repeated statement of day; compound symmetry used as the covariance structure.

[2]Basophil and platelet count excluded due to lack of fit of statistical model; variables analyzed using an automated hemocytometer (Idexx, Procyte Dx; bovine species selected). Data that were not normally distributed was $\log_2$-transformed for statistical analysis. LSmeans and SEM reported from non-transformed data. MCV= Mean corpuscular volume; MCH= Mean corpuscular hemoglobin; MCHC= MCH concentration.

Table 3.6. Hematological results from experiment I and reference ranges reported for other ruminants.

Variable[1]	Deer[2]	Sheep[3]	Goat[3]	Cow[3]
Neutrophils, K/µL	2.81	2.0-15	0.7-6	0.6-4
Neutrophils, %	44.93	28-47	10-50.0	15.0-33
Lymphocytes, K/µL	2.56	3.8-16.5	2.0-9	2.5-7.5
Lymphocytes, %	44.90	39-62	40-55	45-75
Neutrophil/lymphocyte	1.28*	0.53-0.909	0.35-0.67	0.24-0.533
Monocytes, K/µL	0.23	0-1.0	0-0.75	0-0.9
Monocytes, %	3.91	2-10.0	0-6.0	0-8.0
Eosinophils, K/µL	0.34	0-1.5	0-1	0-2.4
Eosinophils, %	6.03	0.5-11	0-10.0	0-20
Basophils, K/µL	0.01	0-0.3	0-0.12	0-0.2
Basophils, %	0.23	0-2	0-3	0-2
Red blood cells, M/µL	14.94	9.0-15.0	8.0-18.0	5.0-10.0
Hemoglobin, g/dL	18.78*	9.0-15.0	8.0-12.0	8.0-15.0
Hematocrit, %	51.87*	27-45	22-38	24-46
MCV[3], fL	34.40	28.0-40.0	16.0-25.0	40-60
MCH[4] g/dL	12.61	8.0-12	5.2-8	11.0-17
MCHC[5] g/dL	36.90	31-34	30-36	30-36
Platelets, K/µL	42.00*	300-600	800-1100	100-800

An asterisk (*) indicates variables outside of the range of other species.
Other variables from this experiment are within the ranges of at least one species listed.

[1]Variables from the current experiment; means generated using PROC UNIVARIATE of SAS.

[2]Retrieved from Merck Veterinary Manual hematologic reference ranges (Merck, 2016).

[1] MCV=Mean corpuscular volume; MCH=Mean corpuscular hemoglobin; MCHC= MCH concentration.

Table 3.7. Effect of phytogenic nutritional supplement on body weight, rib flesh score, and body weight change of white-tailed breeding does in experiment II.

	Treatment[1]			
Item	CON	CG	SEM	*P*-value
Body Weight[2], kg				
Day 0	51.60	51.78	3.43	0.96
Day 42	49.43	52.92	2.22	0.19
Day 84	50.33	54.13	2.40	0.18
Body Weight Change[2], kg				
Day 0 to 42	-1.76	1.15	1.86	0.19
Day 42 to 84	0.97	1.17	2.01	0.93
Day 0 to 84	-0.66	2.27	3.58	0.46
Rib Flesh Score[3]				
Day 0	3.64	3.36	-	0.22
Day 42	2.88	3.26	-	0.06
Day 84	2.88	3.88	-	0.01

[1] CON= control (complete feed without additive); CG= Climate Guard (complete feed with inclusion of Climate Guard at 0.56g/kg of feed).

[2] Analyzed using PROC MIXED of SAS.

[3] Rib flesh score: 1=poor, 2=lean, 3=prime, 4=heavy, 5=obese; Analyzed using PROC NPAR1WAY of SAS for *P*–value, and means generated using ANOVA.

Table 3.8. Diet composition for experiment II.[1]

	Treatment[2]			
	CON		CG	
Item	Dry	As Fed	Dry	As Fed
Analysis Results[3]				
Moisture, %	-	8.7	-	9.3
Dry Matter, %	-	91.3	-	90.7
CP, %	18.2	16.6	20.9	18.9
TDN, %	70.4	64.3	70.4	63.9
NE_m, Mcal/kg	1.65	1.52	1.65	1.50
NE_g, Mcal/kg	0.99	0.95	1.03	0.95
NE_l, Mcal/kg	1.61	1.47	1.61	1.45
DE, Mcal/kg	3.10	2.84	3.10	2.84

[1]Analysis performed at Servi-Tech Laboratories, Amarillo, TX.

[2]CON= control (complete feed without additive); CG= Climate Guard (complete feed with OmniGen-AF at an inclusion rate of 0.56 g/kg of feed).

[3] CP= Crude Protein; ADF= Acid Detergent Fiber; TDN= Total Digestible Nutrients; NE_m= Net Energy for Maintenance; NE_g=Net Energy for Gain; NE_l= Net Energy for Lactation; DE= Digestible Energy.

FIGURES

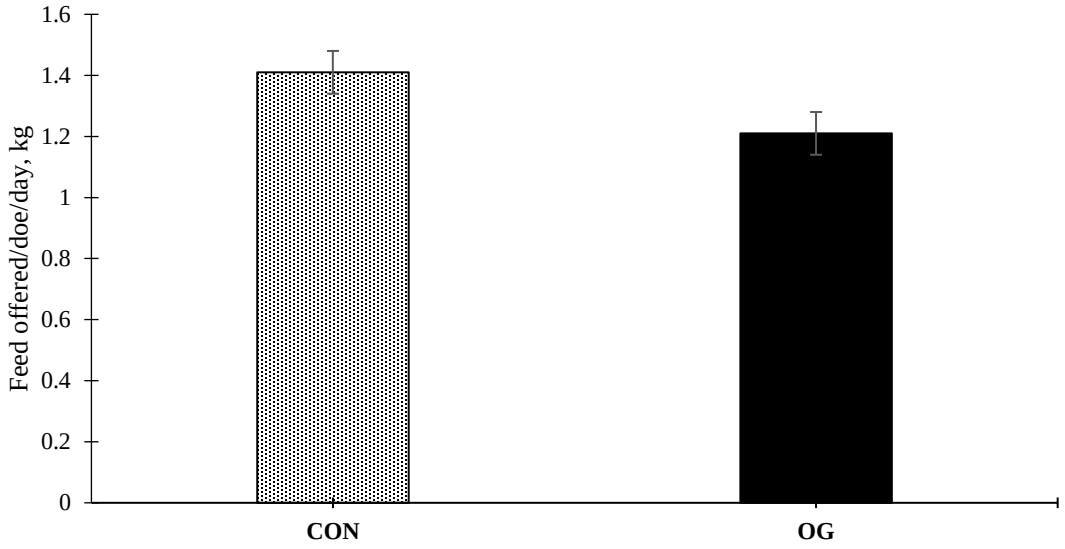

Figure 3.1. Effect of OmniGen-AF on kg feed offered to white-tailed breeding does through the observation period of experiment I. OG (complete feed with OmniGen-AF included at a rate of 3.51 g/kg of feed) does tended ($P = 0.10$) to utilize less feed than CON-treated does (complete feed without additive included).

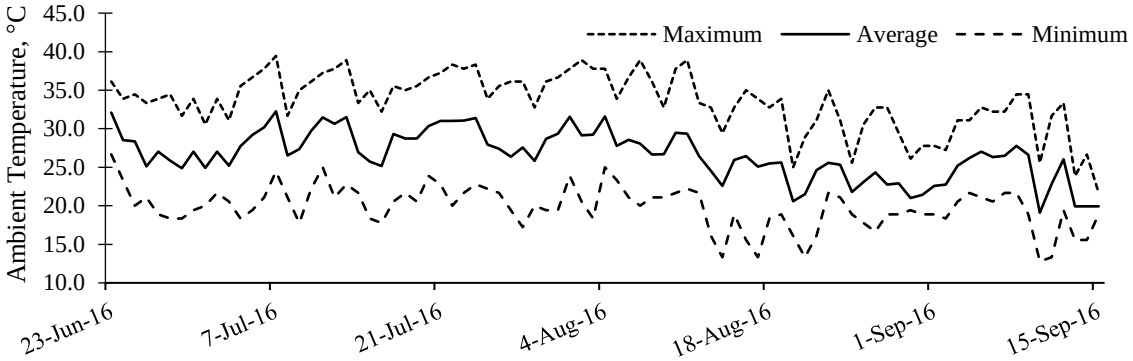

Figure 3.2. Minimum, maximum, and average ambient temperature for the observation period of experiment II. Data retrieved from the NOAA weather database for the Hemphill County Airport, Canadian, TX.

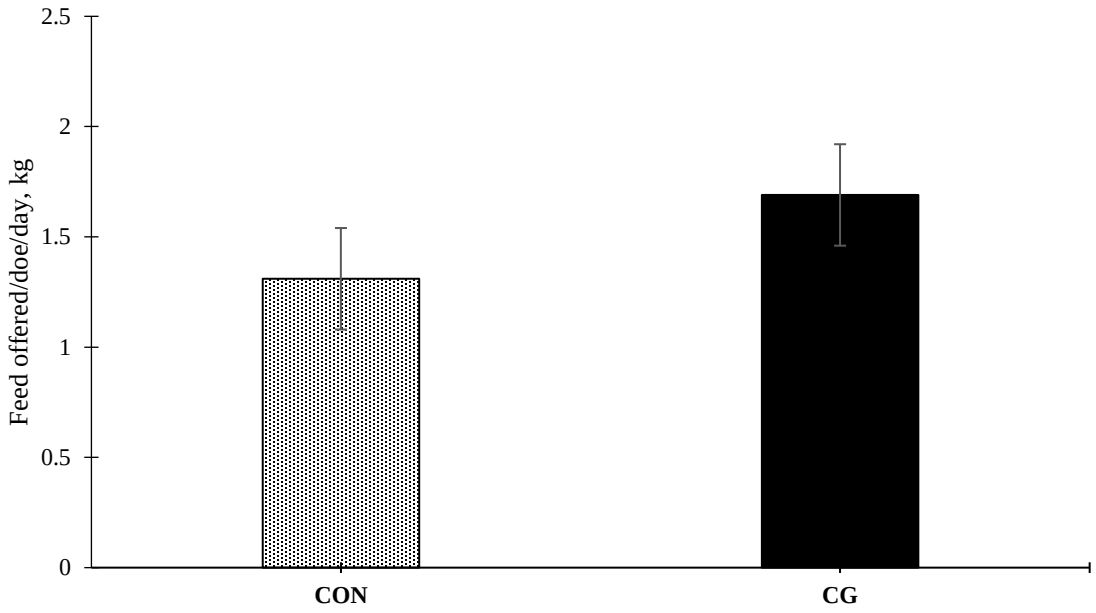

Figure 3.3. Effect of phytogenic nutritional supplement on kg feed offered to breeding does through the observation period of experiment II. CON (control; complete feed without additive) does utilized less feed numerically ($P = 0.31$) than CG (Climate Guard; complete feed with climate guard included at 0.56g/kg of feed) does.

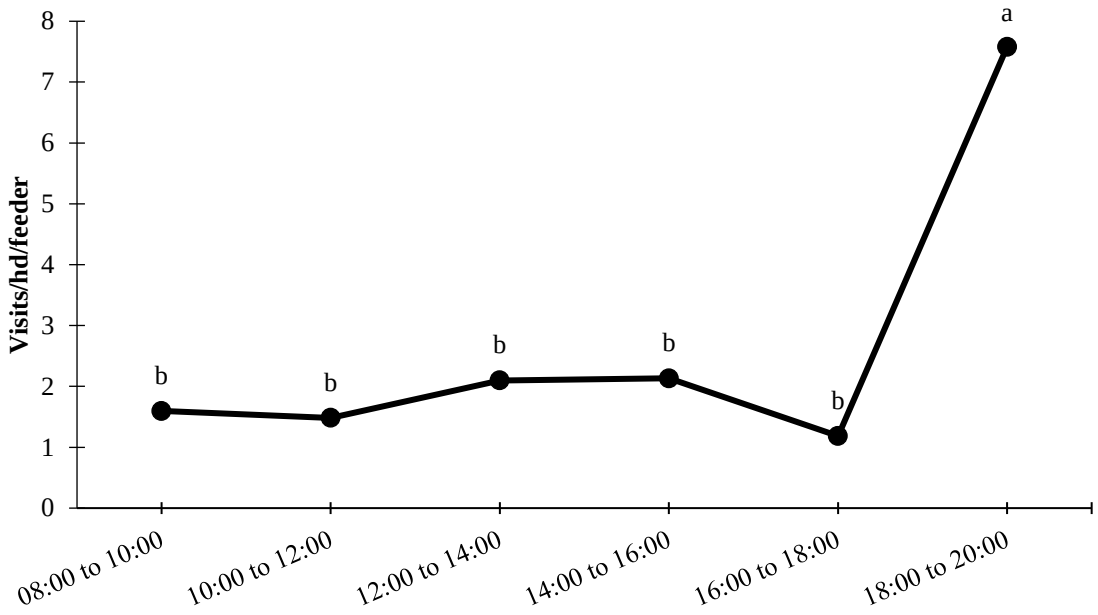

Figure 3.4. Effect of time of day on visits to feeder for captive white-tailed breeding does in experiment II. Means with uncommon superscript letters differ, $P < 0.01$. Feeder visits throughout the day were recorded using game cameras (Browning Recon Force Full HD Platinum Series, Prometheus Group, Raleigh, NC) viewing the feeder. Cameras were programmed to take a digital image with detected movement. Images were viewed individually and visits to feeder were counted. A visit was considered when deer were observed eating within the legs of the feeder. Using recorded weather data, five consecutive days of warm ambient temperatures were chosen for evaluation (23AUG2016 to 28AUG2016). Visits were analyzed using PROC MIXED of SAS with a repeated measure of day, using autoregressive as the covariance structure, and visits/head/pen recorded in 2-hour daylight intervals are displayed.