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A Review: The Use of Livestock Protection Dogs in Association with Large Carnivores in the Rocky Mountains[1]

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Summary

Livestock protection dogs (LPDs) in the United States have helped to protect livestock herds from certain predators, but expanding large-carnivore populations pose new challenges, and the number of LPDs killed by large predators is increasing. We conducted a literature review to identify LPD breeds that may be more suited for use around large carnivores, such as gray wolves. The use of spiked collars to increase the survivability for LPDs in areas of coexistence with large carnivore populations is also discussed. This paper advances the adoption of techniques and LPD breeds used outside of the United States in areas where large carnivores exist with livestock production.

Key Words: Bears, Carnivores, Livestock, LPD, Protection Dogs, Wolves

Introduction

The traditional use of livestock protection dogs (LPDs) has been pivotal to the historical coexistence of gray wolves *(Canis lupus)* and domestic sheep (Rigg 2001) in Europe and Asia, but the use of LPDs in the Rocky Mountains of the United States is a relatively new venture. Most modern LPD use in the Rocky Mountains originated after the 1970s passage of the Endangered Species Act and the concurrent ban on the use of most poisons on public lands (Feldman 2007). Livestock producers searched for alternate methods of predator control at the same time efforts were initiated to protect large carnivores, such as grizzly bears *(Ursus arctos)* and gray wolves. LPDs of several breeds were imported into the United States under an organized program in the 1970s, and use of these dogs as a non-lethal tool has since expanded (Dohner 2007). LPDs have proven to be effective in reducing predation to livestock herds, including cattle, sheep, and goats, from various species of predators (Andelt 2004), but expanding populations of large carnivores provide new challenges. Livestock producers in areas with large carnivores are experiencing similar problems, whether in the United States or in other countries, including Finland, where there are no LPD traditions (Otstavel et al. 2009). Agricultural producers in the western United States have the highest reported economic losses due to wildlife damage, and those losses occur on the patchwork land ownership of public and private lands (Messmer 2009).

Effective management of predator damage at the edges – defined as the intersections of carnivores, people and livestock – are where efforts now need to be focused, using methods that allow the coexistence of livestock and large predators (Shivik 2006). While some advocate the adoption of new strategies and approaches to address wildlife damage concerns (Messmer 2009), we advocate the adoption of ancient approaches for use in this new world of large-carnivore recovery.

Western Wyoming provides a natural laboratory for the study of conflict with large carnivores, since it contains reintro-

duced gray wolf and recovering grizzly bear populations amid livestock production. As these species have reached biological recovery goals and expanded their ranges, conflicts with livestock have escalated (Sommers, et. al. 2010). Various methods to reduce conflict, both lethal and non-lethal, have been used. While smaller predator species, including the most common and most serious predator of livestock in the western United States, the coyote *(Canis latrans)*, are subjected to numerous control methods, recovering populations of large carnivores are granted special protections that limit the methods of control.

In general, as these rare, threatened or endangered species populations recover, managers seek more sustainable management options rather than automatic lethal control when conflicts arise. Effective conflict management seeks to prevent or reduce the frequency or severity of conflicts; deal with the individuals that cause the conflict (most often through removal); and increase tolerance for carnivores (through education, compensation, harvest, etc.) (Sillero-Zubiri et al. 2007).

All control methods, lethal or non-lethal, will fail at some time, in some situations. Control methods are simply tools in a toolbox, that when used help to reduce the amount or severity of conflict. One of the most important tools for resolving conflict is lethal control. Shivek (2006) noted that to ensure the successful reintroduction of some predators, it may be necessary to lethally control them.

Shivik (2006) called the use of LPDs "old technology of special note due to its recent popularity," while adding that guardian animals may be useful in a theoretic way as "continued understanding of their training and use may result in what amounts to the ultimate disruptive stimulus device."

Western Wyoming's pattern of land ownership includes the majority of acreage administered by federal agencies, with most of the remainder held in private ownership as ranches. The lowest elevation of this arid region is about 5,500 feet, and large acreages are needed to provide enough forage for cattle and sheep operations. Thus, most of the ranches graze their herds at least a portion of the year on federally administered land. Nearly 70 percent of the nation's sheep inventory is found in the western states, and an estimated 25 to 30 percent of all sheep in the United States graze on public land allotments (National Research Council 2008). Some range sheep herds spend nearly all year on public land, grazing in lower-elevation deserts free of deep snows in the winter, moving to higher-elevation mountain pastures as the snow melts in the summer. Sheepherders live in wagons alongside the herds, with camptenders checking on them every few days and bringing supplies to the herders. Similar to nomadic cultures in other areas of the world, range sheepherders in the western United States also practice transhumance, moving herds with the seasons and using LPDs to protect them (Gehring et al. 2010). Herds graze over hundreds of miles of range and often go unnoticed by the public although their grazing practices are regulated by federal agencies (Urbigkit 2008, National Research Council 2008). Most range- sheep operations utilize land that is primarily unfenced and unimproved, that involves long-distance movements from season to season and requires on-site herders (National Research Council 2008).

The LPDs live with the herds full time. Most herd's sheep are highly gregarious western, white-faced sheep, primarily of the Rambouillet breed. They have a strong flocking instinct, which helps in guarding against predation. Each herd, usually consisting of about 1,000 ewes and their lambs, will have two to five protection dogs (Andelt 2004). The sheep herd spreads out up to about one-square mile to graze during the day, but bed together in a tighter group at night (P. Arambel personal communication 2-21-2010).

Range-sheep producers in western Wyoming have individual ranch operations, with changing LPD populations. For example, one sheepman might place five dogs with each band of sheep, and three bands are trailed to separate mountain pastures. Some bands may retain all their dogs, while other bands may gain or lose dogs, so that one band may have two dogs and another may have seven. In other cases, the dogs associated with each band may not see other LPDs until late in the fall, when the sheep come off the mountain and the dogs converge at sorting pens. The dogs belonging to one ranch will travel with their sheep herds to a shared winter range, where herds and dogs from other ranches are encountered (some from Utah, Colorado, Idaho, and Wyoming). The LPD population constantly changes as the dogs mature, are hurt or killed. Some dogs may leave their herd to go with an adjacent herd, and owners will switch ownership of dogs,

Two Akbash females (yearling female on left, her five-year old mother on right) at play. Akbash have proven to be effective at guarding herds in large carnivore country, but the use of spiked collars on these dogs may improve their ability to survive aggressive encounters with wolves and are used in their country of origin for this purpose. Photo by Cat Urbigkit.

"borrowing" studs for breeding. No specific breed is maintained in this natural-breeding program, but the most fit dogs breed (males fight for breeding rights) and only the strong pups survive.

It has been reported that the most common LPDs used in the United States are Great Pyrenees, Akbash and Komondor, with Anatolian Shepherd, Maremma, Shar Planinetz used to a lesser extent (Andelt 2004). These dogs usually weigh from 75 to 100 pounds, and have been very successful at reducing predation from coyotes (Andelt 2004).

The need to achieve a balance of human contact with LPDs, so the dogs are bonded to their sheep while capable of being handled by their owners, was discussed in early bulletins for agriculturalists in the United States (Green and Woodruff 1983, Green and Woodruff 1990). Other early LPD researchers in the United States advised that LPDs should be left to bond with their animals, with little human contact (Lorenz and Coppinger 1988). Our observations in the Rocky Mountains indicate that the method limiting human contact has resulted in dogs well bonded to their sheep, but with little or no bond to their human owners. As a result, owners have LPDs that are shy of humans, and cannot even be caught by their owners for veterinary treatment. Similar results have been reported from other countries that used similar techniques, including Switzerland (Landry 2005).

In the United States, LPDs are used to protect against coyotes, but the original LPD breeds were developed in Asia and Europe to combat predation by large carnivores—brown bears and wolves (Smith et al. 2000). Smith summarized recorded accounts of LPDs and bears in the United States and several other countries, where LPDs successfully repelled bears, and losses were reduced when flocks were protected from bear depredation by LPDs.

While LPDs are successful at repelling black bears (*Ursus americanus*) and grizzly bears during most encounters (Andelt 2004), their effectiveness against wolves has met with mixed results. The number of conflicts between LPDs and wolves is increasing in the Rocky Mountains of the United States, with 83 LPDs killed by wolves in this region from 1985 to December 2005 (Bangs et al. 2006). Confirmed fatal wolf attacks on LPDs are only a fraction of all wolf-caused deaths, since many LPDs will simply disappear, with their fate unknown (Bangs et al. 2005).

This adult male Great Pyrenees livestock protection dog shows the battle scars of previous predator encounters. Great Pyrenees dogs often do not survive their encounters with wolves. Photo by Cat Urbigkit.

Most (11 of 18 = 61 percent) of the documented fatal wolf attacks on LPDs from 1995 through 2004 in the Yellowstone region of the Northern Rockies involved the killing of Great Pyrenees LPDs (Bangs et al. 2005). Researchers suggest that these conflicts involved LPDs that were outnumbered and outweighed by their wild counterparts (Bangs et al. 2005).

There are similar reports of wolves killing LPDs in France, and both hunting dogs and LPDs in Italy (Rigg 2001). Wolves in the United States have been taking their toll on hunting dogs, as well. There were 49 hunting dogs confirmed as killed by wolves in Wisconsin 2004 through 2006, with an additional 10 injured (Ruid et al. 2009). The 35 dogs confirmed to have been killed by wolves in Montana, Idaho and Wyoming during 2006 to 2008 included LPDs, hunting dogs, and pets (U.S. Fish and Wildlife Service 2009).

In one stunning case in Romania, wolves killed 157 adult LPDs from January 2001 to October 2002, with wolves consuming the majority of the carcasses in most cases, and leaving the nearby livestock unscathed. This situation appeared to be the result of one wolf pack that had specialized in preying on dogs (Mertens and Schneider 2005).

Materials and Methods

We conducted a literature review of references from around the world to gain insight to increasing the effectiveness of LPDs in wolf range in two ways: a) By identifying survival tools that may provide existing LPDs more protection from wolves to better their chances of staying alive to guard their herds; and b.) By identifying different LPD breeds more suited to facing wolves, that could be utilized in the Northern Rockies of the United States. In selecting LPD breeds, the following criteria were used:

- Must be canine-aggressive, so that the dogs are inclined to actively challenge wolves;
- Must not be human aggressive, since many herds graze on public lands for part of the year;
- Should originate in areas with large carnivores to take advantage of working characteristics similar to that to be faced in the Northern Rockies; and
- Must not be of small body size, so that the animals stand a better chance against large carnivores.

Some breeds that may work well in the United States were left off the list of potential breeds because of their rarity and/or prohibition of export out of countries of origin.

Results and Discussion

Just as wolves have been persecuted throughout the world, with wolf popula-

tions falling to near extinction in some countries, the LPDs that protected their flocks from wolf predation have fallen to the same fate (Rigg 2001). Without pressure from large carnivores and with livestock herds decreasing worldwide, use of LPDs decreased to the extent that there are now recovery programs in place (Rigg 2001) to get these dogs back onto their historic landscapes to address expanding large-carnivore populations.

The spread of communism in Europe and Asia brought with it an active campaign of collectivized agricultural policy, which worked to rid entire regions of its free people—the nomadic livestock cultures. Livestock and their guard dogs were killed or collectivized, and their nomadic herders and families were taken from the land (Gehring et al. 2010). When the herders became villagers, the cultures lost their old traditions (Ivanova 2009).

In the 1930s, demand for dog skins resulted in the destruction of the largest dogs available, rabies campaigns called for wholesale extermination of all dogs in certain regions, including LPDs (Ivanova 2009), as well as later intermixing of dog breeds and dog diseases taking tolls on native dog populations. The loss of interest in livestock husbandry, as well a decline in predator populations as a result of persecution by humans, also played a role in the reduction of working LPDs (Cruz 2009). Recent interest in dogs declared as members of "national" dog breeds has resulted in a greater demand for dogs in the pet trade than as working animals. Additionally, some LPDs are now being used and bred for dog fighting rather than guarding herds (Cruz 2009, Plakhova and Plakhova 2008).

Ovcharkas (both Central Asian and Caucasian) are used in the dog-fighting ring in current times, including in Central Asia and Russia. While there is much criticism of dog fighting, traditional dog fights in Asia are not like pit bull dog fights in America, which involve severe injury or the death of the dog. Dog fights involving LPDs in their historic context of nomadic people play an entirely different role—that of testing the best dogs as wolf fighters, and promoting the best dogs for breeding. Dogs with the proper drive, tenacity and strength needed to confront and kill a wolf are selected to pass their genes on to the next generation. Serious injuries are reported to occur rarely since these matches are conducted to observe traits, such as dominance display, agility and physical strength *(http://www.centralasianshepherd.us/cao_or_alabai.html, accessed Nov. 1, 2009)*.

Dogfights, which are called "wrestling" in Central Asia, serve to test a dog's level of canine aggression and have a set of stringent rules developed over centuries (Gasymzade and Azizov 2007). Any dog that is inactive or cries is determined to have lost, and human handlers end the match. Signs of submission end the fight as well. Traditional LPD dogfights or wrestling matches involve the dogs' controlled aggression, not blind fury as seen in pitbull-type fighting. In LPD matches, the fights begin and end quickly, and the result is a determination of the best dogs to fight wolves. One champion fighting dog, a Caucasian Ovcharka dog that lived in Russia in the 1930s, was believed to have killed 100 wolves in his lifetime, an enviable record (Gasymzade and Azizov 2007).

The willingness to aggressively challenge a wolf can be an important factor in a LPD's effectiveness. Sedefchev (2005) told the story of seeing a Great Pyrenees LPD chase a wolf a short distance before the dog left the wolf, but continued to bark. The researcher noted "the dogs show him with their behavior that they are not a real obstacle, and the wolf's success is just a question of time" (Sedefchev 2005). The story continued, "When the dogs chase the wolf with the intention to kill it, this means much more for the wolf" (Sedefchev 2005).

In contrast to the Great Pyrenees, the Karakachan dog of Bulgaria has been known to chase a wolf away from the flock for nearly a mile and a half. It has been reported that after such encounters, wolves leave that flock alone and turn their attention to other flocks (Dohner 2007). Dohner (2007) wrote, "The shepherds believe that their dogs must show this level of dedication to harassing and even attacking wolves in order to combat the strong predator pressure in the area; therefore they value physically strong, confident dogs."

a. Survival tools/spiked collars

Some breeds of LPDs already in use in the Northern Rockies, such as the Akbash, demonstrate proper canine aggression and have proven to be an effective LPD breed, but individuals of this breed have been killed by wolves. Our review found that in other regions of the world, where LPDs and wolves coexist, the use of spiked, anti-wolf LPD collars is common, and herders use several LPDs with each herd, the number varying from two to five.

The use of spiked, anti-wolf collars on LPDs has been reported from various regions of the world, including Italy, Poland, Romania, Spain, Turkey, and Portugal (Rigg 2001, Cruz 2009). Unfortunately, there is little detailed information in written literature about this LPD survival tool. Use of spiked collars has been very limited in the Rocky Mountains, based on a variety of factors including questions of how to properly use them; concern about the safety of the collars where there are barbed or woven wire fences and heavy brush, and frigid temperatures and snow; as well as the lack of access to the collars.

Iron, leather and webbing collars on sale in the Ankara market in Turkey. Photo courtesy Sir Terence Clark.

There are two general types of spiked collars—leather or fabric, and iron. Leather or fabric collars may be a safe alternative during the winter months when herders are concerned about harm to the dogs from having iron collars on their dog's necks with snow and cold temperatures. The heavy iron collars, if manufactured of a large enough size to drape over the lower portion of a LPD's neck, may provide enough room for the dog to slide the collar over its head should it become hung up in a fence or brush. Although herders live with the dogs in range-sheep outfits, the dogs are not always in sight and only may be seen once or twice a day. In this situation, ensuring the safety of the collars is an important consideration.

The use of spiked collars is worth exploring for existing LPDs in wolf country of the Northern Rockies. Spiked collars may reduce the amount of interpack aggression and dog fights in more aggressive breeds of LPDs guarding a herd. Spiked collars may also find a use in protecting hunting dogs in the United States as well.

b.) Potential wolf-fighting breeds

Using the criteria described earlier, our review determined the list of breeds with high potential to protect livestock herds in the Northern Rockies from large carnivore predation include Central Asian Ovcharka, Transmontano Mastiff, Karakachan, Kangal, and Shar Planinetz.

Central Asian Ovcharkas are believed to be one of the oldest breeds of dogs on Earth (Plakhova et al. 2008) and are raised in their countries of origin from birth with sheep. Central Asian Ovcharkas (also known as Central Asian Shepherds) have various names in their countries of origin, including Aziat in Russia, Alabai or Kopek or Volkodav ("wolf killer") in Turkmenistan, Tobet in Kazakhstan, Sage Koochee in Afghanistan, and Dakhtarma in Tajikistan, but they all fall into the Ovcharka group which was developed by peoples with a nomadic pastoral lifestyle over large territories (Plakhova and Plakhova 2008, Ivanova 2009). Herdsmen, called chobans, rejected dogs that were aggressive to humans (Ivanova 2009). Ivanova wrote: "The Central Asian Ovcharka was developed by the native peoples of Central Asia to fit their nomadic way of life. The specific nature of their way of life has determined the need for a reliable protection dog. This is an ancient livestock protection breed with innate instincts for guarding animals and property. In different countries with nomadic life and sheep breeding, there was a need for a reliable guard dog that did not make demands on the conditions of life" such as the limited resources involved in a subsistence lifestyle (Ivanova 2009). This breed is known for loyalty to people, and it has been reported that for centuries in their countries of origin, individual dogs of this breed that were aggressive to humans were killed (Bagiev 2006).

The Transmontano Mastiff of Portugal is a breed that originated in a pastoral livestock system where stock are grazed in uncultivated areas away from villages, with the continuous presence of wolves leading to its functional body structure of massiveness with long head and limbs, which enable it to travel with the herds (Cruz 2009). Ninety-five percent of the northern Transmontano LPD population is still used to protect extensive sheep flocks from wolf predation (Dohner 2007). An aggressive program to reduce wolf predation on sheep and cattle herds in Portugal's Montesinho Natural Park was begun in 1994, placing Transmontano Mastiff LPD pups with herdsmen. The result has been a reduction in the amount of damage caused by wolves (*www.caodegadotransmontano.org.pt, accessed 10/30/09*). The breed is described as quite reserved and docile, while not being highly aggressive.

The Karakachan Dog is an aboriginal LPD breed of Bulgaria, developed by nomadic people who practiced transhumance with their herds. After World War II, nationalization of land in Bulgaria began, with nomads forced into villages and livestock placed on state cooperative farms. Many dogs were killed, and when the state farms were discontinued in 1991, much of the livestock and LPDs were killed as well (Sedefchev and Sedefchev 2009). Official recognition of the Karakachan as a breed under Bulgarian law did not occur until 2005. A program was begun in Bulgaria in 1996 to conserve the Karakachan LPD and its original type and working abilities, and this program has focused on "conservation of predators, livestock, pastures and pastoral traditions: conservation of the unique symbiosis between all these elements" (Sedefchev and Sedefchev 2009). According to Sedefchev and Sedefchev: "We unite the conservation of a guardian and a predator, because evolutionarily they devel-

This is a yearling female Central Asian Ovcharka at work guarding sheep. The sheer size and canine aggression of this breed makes it an appropriate breed for consideration in areas of large carnivore populations. Photo by Cat Urbigkit.

Turkish Kangal LPD with iron collar in Turkey. Photo courtesy Sir Terence Clark.

oped together. Survival of the guardian depends on the survival of the predator and vice versa." The program, which places LPDs with shepherds, has resulted in a reduction of harm from predation of about 80 percent in areas inhabited by large carnivores (Sedefchev and Sedefchev 2009). Karakachan dogs of Bulgaria live amid one of the highest densities of bears and wolves in Europe (Dohner 2007), and react accordingly. The dogs work in teams and actively chase and harass wolves (Dohner 2007).

The Turkish Kangal is a LPD that is able to fight wolves, and rather than just try to deter the predators, Kangals reportedly prefer to kill wolves (Tepeli and Tepeli 2008). Kangals are famous for their fierce battles with predators, and many adult dogs in their country of origin carry battle scars (Dohner 2007). Kangal pairs are known to work as a team to attack a wolf, with the smaller female chasing and blocking, while the larger male rushes in and hits the wolf with his chest, knocking it to the ground. The animals are raised in villages with children and small animals, and are known for their steady temperament and gentle manner (Dohner 2007).

Macedonia's **Shar Planinetz or Sarplaninac (Shar)** is a slightly smaller LPD, but its heavy-boned structure and its canine aggression make this a viable breed for protecting flocks from wolves (Dohner 2007).

Conclusions

It is recommended that range sheep producers in the Northern Rockies try using groups of two to five of these LPDs to protect each of their herds, with the objective of outnumbering and outweighing wolves or other large carnivores encountered. Individual dogs will have different behavioral and guarding tendencies, so developing the proper mix of traits into a group of LPDs may take time and readjustment of the animals involved.

The proper use of spiked collars should be explored as well. Producers considering using spike collars in the United States need to have an understanding about proper collar use. Unanswered questions at this time include: are collars used on younger dogs to stop littermate fighting and does this have an impact on the dog's willingness to fight later on; are the collars worn by dogs year-round, or only when entering wolf areas/times; are they placed on dogs only when wolf presence is detected or predation begins; what's the difference between iron and leather; how/why is cloth held to iron collar. If the collars can be designed in such a manner as to not pose a safety risk to LPDs in the United States, then a program for their design, manufacture and distribution is needed.

Those seeking to acquire LPDs from their countries of origin should note that although "herding" dogs in the United States are defined as breeds such as the border collie, some LPDs are called "herding" dogs also, but in a different context. Some strains or lineages of certain breeds are called property protection dogs (Bagiev 2006). These dogs stay within defined property boundaries, and some are to protect the property from thieves. Other strains of the same breed are called herding dogs because they stay with livestock herds to protect them from wolves and no aggression towards humans is acceptable. The Causcasian Ovcharka is an example of a breed with these two different types of protection dogs, which is also reflected in the physical confirmation of the two (Bagiev 2006). The wolf-fighting or herding type is found in the North Caucasus where there are migratory sheep herds. Bagiev 2006 reports that "a pack of dogs resembling a pride of lions is present with each animal herd" and the dogs not only protect the herd, but control its movements as well.

Kazakhstan has the highest density of wolves in the world, and LPDs are the only reliable form of protection against predation there (Plakhova et al. 2008). The importance of LPDs is reflected in the Kazakh proverb, "The dog is more important than sheep" (Plakhova et al. 2008).

It should be noted that even if livestock producers use the most effective LPDs, there will inevitably be conflict between wolves and livestock, and their human and canine guardians. Instead of eliminating this conflict, management programs should aim to reduce the amount and severity of that conflict.

LPDs can reduce sheep depredation by 11% to 100%, and most livestock producers surveyed viewed LPDs as an economic asset, with high economic efficiency for a relatively low cost (Gehring et al. 2010). Livestock producers also note that the use of LPDs does not require assistance from government agencies or rely on advanced technology (Gehring et al. 2010).

We concur with the Gehring et al. (2010) recommendation that additional research on LPD behavior in terms of effectiveness for protecting livestock from large carnivores is needed, as is an information exchange between producers who use LPDs in the presence of large carnivores in their countries of origin and those who are new to the interaction of LPDs and large carnivores.

In writing about the use of livestock guard dogs in Asia, Ivanova 2009 explained the relationship of the nomadic culture, their herds, herd protectors, and the landscape upon which they all depend: "Experience accumulated during hundreds of years is passed on for generations and it has achieved a high degree of perfection that cannot be improved any further but only preserved". While livestock producers in the Northern Rockies have been able to use some of the same tools used by these nomadic cultures, what has been lacking is the full range of techniques, and knowledge of how to use them. Tapping into the knowledge and tradition of ancient LPD cultures will help to fill that void.

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Effects of Human Chorionic Gonadotropin on Serum Progesterone Concentration During the First Weeks After Mating, Components of Pre-implantation Complete Blood Counts, and Number of Offspring at Parturition in Ewes

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Summary

Human chorionic gonadotropin (hCG) may boost progesterone production and attenuate maternal-immune response against concepti in ewes, increasing prenatal survival. The present study examined effects of repeated hCG administration after mating on serum progesterone concentration and complete blood counts (CBC) during early gestation, as well as offspring numbers at parturition. Fifty-six ewes were synchronized and mated, then administered saline (CON) or 100 IU hCG on d 4, d 7, d 10, and d 13 after estrus (d 0). Ewes not conceiving were mated again at subsequent estrus. Progesterone was measured on d 4 to d 15 and CBC on d 7 and d 11. In ewes pregnant at treatment, serum progesterone was greater ($P < 0.050$) in hCG-treated ewes than CON from d 7 through d 15 (final sampling day), while in ewes conceiving at subsequent estrus (after treatment), progesterone was greater ($P < 0.050$) in hCG-treated ewes on d 11 through 15 only. On d 7, total white blood cells (WBC) and lymphocytes (LYM) were greater ($P < 0.050$), mean corpuscular volume ($P = 0.067$) tended to be greater, and eosinophil fraction of WBC tended to be less ($P = 0.068$) in hCG-treated ewes. On d 11, red blood cells and hemoglobin were reduced ($P < 0.050$) and hematocrit tended to be reduced ($P = 0.055$) in hCG-treated ewes. Additionally, neutrophil fraction of WBC was greater ($P < 0.050$) in pregnant ewes on d 7, total LYM were less ($P < 0.050$) in pregnant ewes on d 11, and LYM fraction of WBC was less ($P < 0.050$) in pregnant ewes on d 7 and d 11 than in non-pregnant ewes, independent of treatment. No difference ($P > 0.050$) was found between treatments for number of ewes pregnant from mating at estrus just before treatment, the first estrus after treatment, the second estrus after treatment, or the number of ewes not pregnant. Frequency of single or multiple lambs at parturition did not differ ($P > 0.05$) due to treatment in ewes pregnant from mating at any estrus. Repeated administration of hCG during the first two weeks after estrus increased serum progesterone concentration in pregnant and non-pregnant ewes, influenced components of CBC, but did not appear to influence lambing rate or number of offspring at parturition when administered at these doses.

Key words: Ewes, Complete Blood Counts, Human Chorionic Gonadotropin, Progesterone

Introduction

Progesterone insufficiency during embryonic implantation (McLaren, 1973), maternal-immune response toward the conceptus (Howell et al., 1994), or a combination of these and other factors may contribute to prenatal loss in many domestic-livestock species. Progesterone production is influenced by luteal size and maturation (Hunter and Southee, 1989). Thus, exogenous luteotropins, such as human chorionic gonadotropin (hCG), may increase progesterone concentration and reduce early-stage fetal loss in livestock, when administered early in pregnancy (Kelly et al., 1988; Willard et al., 2003; Szmidt et al., 2008). In addition to increasing progesterone, hCG may reduce maternal immune response against developing offspring, as demonstrated in mice (Fabris et al., 1977; Khil et al., 2007). The immunosuppressive ability of hCG appears to supersede that of increased progesterone alone (Fabris et al., 1977), however, the later may act as a partial intermediate of immunosuppressive function (Ramadan et al., 1997; Wulster-Radcliffe et al., 2003). As substantial prenatal loss can begin as early as d 8 of pregnancy in many livestock species (Nancarrow, 1994), hCG may be most effective when administered within the first two weeks after mating. Single injections of hCG have been reported to increase lambing rates and fetal size (Cam and Kuran, 2004), possibly by promoting increased-uterine secretions during early gestation (Nephew et al., 1994). However, effects of repeated hCG administration are less understood. The objective of this study was to examine effects of repeated injections of hCG on serum-progesterone concentrations in sheep during the first week after mating, on components of complete blood counts (CBC) during the period before implantation, and on number of offspring at parturition in ewes.

Materials and Methods

Animal preparation

All procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee. Before use, ewes were weighed (64.0 kg ± 1.2 kg) and examined for health, and a breeding soundness exam was performed on all rams. Animals had free access to water and shelter and were fed chopped alfalfa hay (approximately 2 kg/ewe) once daily at 0600 for the duration of the study. Experimental procedures were conducted at New Mexico State University, Las Cruces, N.M. (32° 19' 11" N, 106° 45' 55" W; elevation 1,219 m), and breeding began on September 5, 2008. All ewes were of mixed Suffolk x Hampshire breeding and were produced at New Mexico State University. Ewes ranged from 2 yr to 6 yr of age at breeding and were of average body condition.

Experimental procedure

Thirty-six mature, multiparous ewes and 18 nulliparous long-yearlings were used. On d -15 (d 0 = estrus), all ewes received a progesterone-impregnated intravaginal insert (EAZI-BREED CIDR, 0.3 g progesterone; Pharmacia and Upjohn, Co., Hamilton, New Zealand) to synchronize estrus. Inserts were removed on d -1 and ewes were joined with fertile rams during a 60-d breeding period. Ewes were stratified by age and treatment designation before being divided randomly into four breeding groups. Beginning on d 0, each group was held in an isolated pen for 48 h with one of four mature, fertile rams fitted with marking harnesses. After the 48-h mating period, ewes were pooled in a common pen and two rams were reintroduced (with marking harnesses) for 60 d. Treatments were randomly assigned after stratification by age. Thirty ewes received repeated injections of hCG (hCG; 100 IU in 1 mL physiological saline, i.m.; ProSpec-Tanny TechnoGene, Ltd, Rehovot, Israel, CAS: HOR-250) and the remaining ewes received repeated saline placebo injections (CON; 1 mL physiological saline, i.m.). Injections were administered just after blood collection on d 4, d 7, d 10, and d 13. Serum samples were collected (jugular venipuncture) into sterile vacuum tubes once daily at 0700 on d 3 to d 15. Whole-blood samples were also collected at 0700 on d 7 and d 11 (EDTA-containing vacuum tubes). Immediately after sampling, whole-blood samples were packaged on ice and shipped overnight to the Veterinary Diagnostics Services, Albuquerque, N.M., for CBC analysis, which included: white blood cell (WBC) number, red blood cell (RBC) number, hemoglobin (Hgb) concentration, hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelet number, and absolute number (and fraction of total WBC) of neutrophils (NEU), lymphocytes (LYM), monocytes (MON), eosinophils (EOS), and basophils (BAS). On d 15, all ewes received a cautionary dose of liquamycin (5 mL, s.c.; LA-200, Pfizer, Inc., New York, N.Y.), and were returned to the general flock for the remainder of gestation. Numbers of offspring were recorded at parturition. Each ewe was observed as having a single lamb, multiple lambs, or no lambs. Date of parturition was used to determine approximate date of breeding. At parturition, ewes were determined to have given birth to lambs conceived on the estrus just before treatment, the first estrus after treatment, or the second estrus after treatment.

Serum progesterone radioimmunoassay

After collection, blood samples for progesterone analysis were kept at room temperature for 30 to 60 min and then centrifuged (1,500 x g at 4° C for 15 min) to separate serum. After centrifugation, serum was stored in plastic vials at -80° C until assayed. Radioimmunoassay (Coat-A-Count, Siemens Medical Solutions Diagnostics, Los Angeles, Calif.; Schneider and Hallford, 1996) was used to quantify progesterone concentration in all serum samples (mean intra-assay CV = 6.5 percent over five assays; inter-assay CV = 5.9 percent).

Statistical analysis

Progesterone data were analyzed as a split-plot design using the mixed procedure of SAS (SAS Inst. Inc., Cary, N.C.) with repeated measure function. Treatment, pregnancy status, and treatment x pregnancy status were included in the main plot and day and associated interactions were in the sub plot. Blood CBC components were subjected to analysis of variance appropriate for a completely randomized design (glm procedure of SAS), and effects of treatment, pregnancy status, and treatment by pregnancy status were examined within sampling day. Pregnancy rates were sub-

jected to Chi square analysis using the frequency procedure of SAS. Fisher's exact test was used for frequency analysis in which more than 25 percent of cells contained expected frequencies of less than five. Number-of-offspring-per-ewe lambing was analyzed for treatment effects by cycle with the glm procedure of SAS. Length of estrous cycle in ewes conceiving on the first or second cycle after treatment was also analyzed by the glm procedure.

Results and Discussion

Serum progesterone concentration

No treatment x pregnancy status (at the time of treatment) x day interaction was observed ($P > 0.050$) for serum progesterone concentration. However, interactions were observed between treatment and pregnancy status ($P = 0.059$) and between day and pregnancy status ($P < 0.001$). Therefore, ewes pregnant at the time of treatment and those not pregnant were analyzed separately for serum-progesterone concentration. A subsequent treatment x day interaction was observed ($P < 0.050$) in both pregnant and non-pregnant groups, thus, serum-progesterone concentrations were examined for treatment effects within each day. In ewes pregnant at the time of treatment, serum-progesterone concentrations (Figure 1) were greater ($P < 0.050$) in hCG-treated ewes than in CON ewes beginning on d 7 and remained greater through the duration of the sampling period, but did not differ ($P > 0.050$) between treatment groups before d 7. In ewes not pregnant at the time of treatment (Figure 2), serum-progesterone concentration was greater ($P < 0.050$) in hCG-treated ewes beginning only on d 11, but still remained greater through the end of the sampling period. Additionally, serum-progesterone concentrations tended to be greater ($P = 0.108$) on d 9, but did not differ ($P > 0.050$) between treatments on d 4 through d 8 or d 10. Effect due to day was observed ($P < 0.001$) within both treatments in both pregnant and non-pregnant ewes, as expected, with curves following natural, temporal patterns.

Complete blood count components

No interactions were observed (P > 0.050) for treatment x pregnancy status

Figure 1. Serum progesterone concentration in pregnant ewes administered hCG or saline on days 4, 7, 10, and 13 after introduction of ram (treatment by day, $P < 0.001$; * denotes treatment differences, $P < 0.05$).

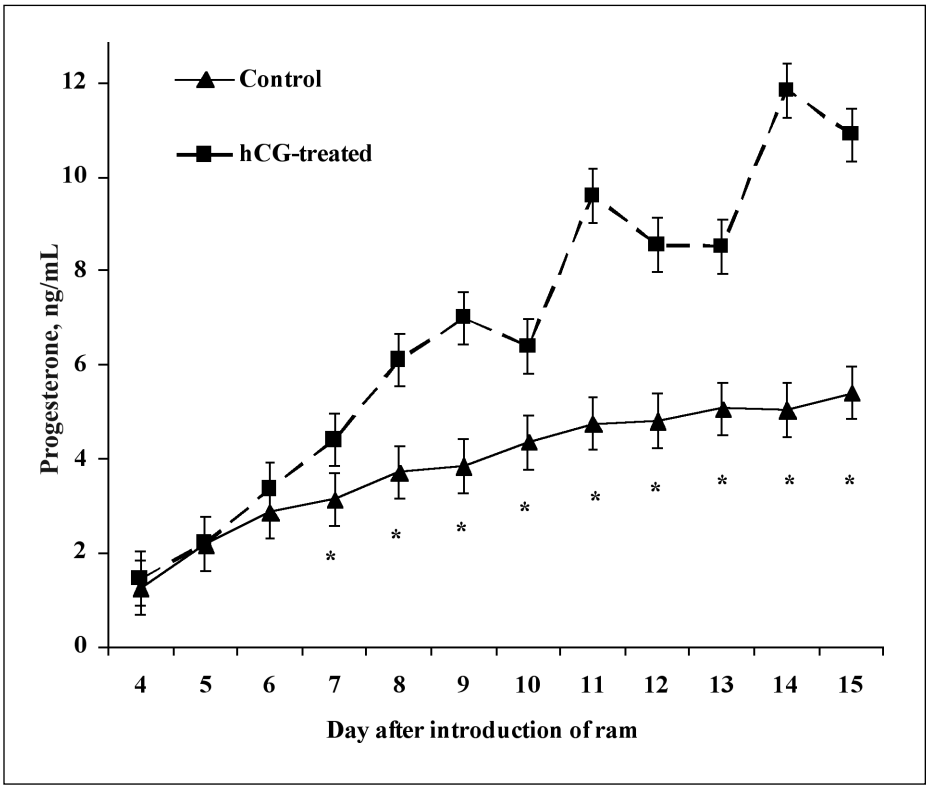

Figure 2. Serum progesterone concentration in non-pregnant ewes administered hCG or saline on days 4, 7, 10, and 13 after introduction of ram (treatment by day, $P = 0.002$; * denotes treatment differences, $P < 0.05$).

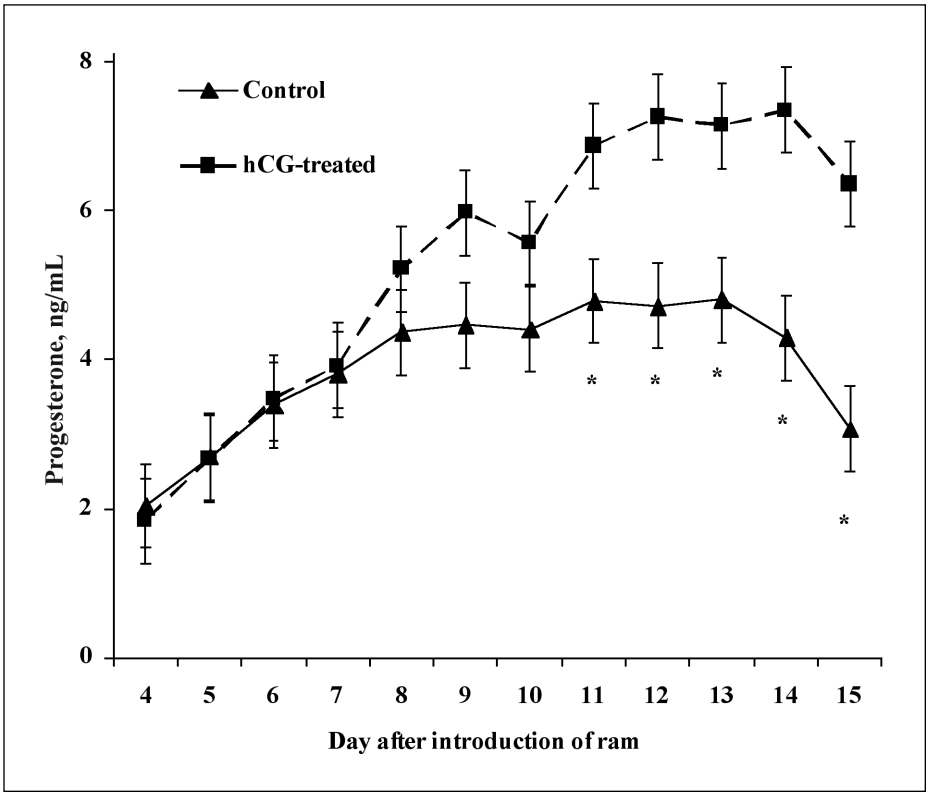

Table 1. Components of whole blood on day 7 after mating in ewes injected with hCG or saline on days 4, 7, 10, and 13 after estrus detection and mating.

Item	Control	hCG	SE[1]	*P*-value
White blood cells[2]	12.20	23.34	2.85	0.007
Red blood cells[3]	10.96	10.26	0.36	0.113
Hemoglobin[4]	11.96	11.58	0.43	0.468
Hematocrit[5]	33.00	32.11	1.25	0.555
MCV[6]	30.22	31.44	0.53	0.067
MCHC[7]	36.13	35.98	0.19	0.520
Platelets[2]	240.71	187.86	41.51	0.335

[1] Standard error (n = 15).
[2] Count * 10^3.
[3] Count * 10^6.
[4] g/dL.
[5] Percentage of total white blood cells.
[6] Mean corpuscular volume, fL.
[7] Mean corpuscular hemoglobin concentration, g/dL.

Table 2. Immune-cell measurements in whole blood on day 7 after mating in ewes injected with hCG or saline on days 4, 7, 10, and 13 after estrus detection and mating.

Item	Control	hCG	SE[1]	*P*-value
Neutrophils[2]	33.33	37.33	4.75	0.515
Lymphocytes[2]	55.44	54.89	4.92	0.927
Monocytes[2]	5.78	4.78	1.29	0.574
Eosinophils[2]	5.22	2.33	1.32	0.068
Basophils[2]	0.22	0.22	0.15	0.990
Absolute Neutrophils[3]	4.12	8.24	2.13	0.096
Absolute Lymphocytes[3]	6.69	13.63	1.49	0.002
Absolute Monocytes[3]	0.69	1.00	0.39	0.492
Absolute Eosinophils[3]	0.67	0.40	0.18	0.196
Absolute Basophils[3]	0.03	0.07	0.05	0.483

[1] Standard error (n = 15).
[2] Percentage of total white blood cells.
[3] Count * 10^3.

on d 7 or d 11 for any component of CBC, thus all CBC data are presented as main effects. On d 7 after estrus, numbers of WBC (Table 1) and LYM (Table 2) were greater ($P < 0.050$) in hCG-treated ewes than in CON. Additionally, MCV (P = 0.067) tended to be greater in hCG-treated ewes, and EOS fraction of WBC tended to be reduced ($P = 0.067$) in hCG-treated ewes compared to CON ewes. No differences ($P > 0.050$) were observed between treatments on d 7 for RBC, Hgb, or Hct; however, on d 11 (Table 3) RBC, Hgb, and Hct were reduced ($P < 0.050$) in ewes receiving hCG compared to controls. Additionally, WBC, LYM, MCV, and EOS fraction of total WBC (Table 4), which differed between treatments on d 7 ($P < 0.050$), did not differ at d 11 ($P > 0.050$). Despite treatment differences in total WBC at d 7, total counts of NEU, MON, EOS, and BAS did not differ ($P > 0.050$) between treatments on d 7 or d 11. Likewise, fraction of total WBC comprised by NEU, MON, LYM, and BAS did not differ ($P > 0.050$) between treatments on either day. The NEU fraction of WBC was greater ($P < 0.050$) on d 7 in ewes pregnant at the time of treatment, total LYM were less ($P < 0.050$) at d 11 in ewes pregnant at the time of treatment, and LYM fraction of WBC was less ($P < 0.050$) at d 7 and d 11 in ewes pregnant at the time of treatment compared to those not pregnant at this time. No effect ($P > 0.136$) due to age was observed on any immune component of CBC.

Lambing Results

No difference ($P = 0.546$) was detected between treatments in the number of ewes conceiving at the estrus immediately before treatment, at the first estrus after treatment, at the second estrus after treatment, or the number of ewes failing to deliver lambs (Table 5). Thirteen ewes in each treatment group conceived at the estrus before treatment, while four CON and seven hCG-treated ewes conceived at the first estrus after treatment, and four CON and three hCG-treated ewes conceived at the second estrus after treatment. Only two CON ewes failed to produce a lamb, while six hCG-treated ewes failed to produce offspring. At parturition, frequencies of single-lamb or multiple-lamb births (Table 6) were not different ($P = 0.420$) between treatments for ewes bred at any estrus. Number-of-live-lambs-per-ewe lambing was not different ($P > 0.174$) between treatments in ewes conceiving on any cycle. Additionally, length of estrous cycle did not differ ($P > 0.152$) between treatments in ewes conceiving on the first or second cycle after treatment.

Discussion

Data from this study indicate that repeated hCG administration during early pregnancy can increase circulating levels of progesterone in ewes. Findings support previous demonstrations in both pregnant (Nephew et al., 1994) and non-pregnant ewes (Gómez-Brunet et al., 2007). When hCG was administered to ewes on d 11.5 of pregnancy, Nephew et al. (1994) observed an immediate increase in progesterone concentration that lasted approximately 72 h. Although progesterone response in the current study was delayed 3 d from the initial injection in pregnant ewes, injections spaced 72 h apart succeeded in maintaining increased serum progesterone throughout the remainder of the sampling period. In non-pregnant ewes, increased serum progesterone concentration was delayed for almost one wk after initial injection. This observation was

Table 3. Components of whole blood on day 11 after mating in ewes injected with hCG or saline on days 4, 7, 10, and 13 after estrus detection and mating.

Item	Control	hCG	SE[1]	*P*-value
White blood cells[2]	15.41	19.32	2.37	0.225
Red blood cells[3]	11.01	9.77	0.30	0.005
Hemoglobin[4]	11.99	10.80	0.36	0.016
Hematocrit[5]	34.22	31.22	1.24	0.055
MCV[6]	31.00	34.86	0.58	0.176
MCHC[7]	35.16	34.86	0.40	0.502
Platelets[2]	205.80	243.80	31.40	0.374

[1] Standard error (n = 15).
[2] Count * 10^3.
[3] Count * 10^6.
[4] g/dL.
[5] Percentage of total white blood cells.
[6] Mean corpuscular volume, fL.
[7] Mean corpuscular hemoglobin concentration, g/dL.

Table 4. Immune-cell measurements in whole blood on day 11 after mating in ewes injected with hCG or saline on days 4, 7, 10, and 13 after estrus detection and mating.

Item	Control	hCG	SE[1]	*P*-value
Neutrophils[2]	32.89	28.22	3.23	0.279
Lymphocytes[2]	59.78	66.33	3.27	0.150
Monocytes[2]	3.56	2.11	0.87	0.197
Eosinophils[2]	3.78	3.22	1.12	0.710
Basophils[2]	0.00	0.11	0.06	0.330
Absolute Neutrophils[3]	5.22	5.04	1.05	0.885
Absolute Lymphocytes[3]	9.10	13.23	2.06	0.091
Absolute Monocytes[3]	0.58	0.43	0.15	0.493
Absolute Eosinophils3	0.51	0.3	0.13	0.899
Absolute Basophils[3]	0.00	0.03	0.02	0.330

[1] Standard error (n = 15).
[2] Percentage of total white blood cells.
[3] Count * 10^3.

Table 5. Number of ewes conceiving at the estrus just before treatment, the first estrus after treatment, the second estrus after treatment, or not pregnant after introduction of rams and administration (i.v.) of hCG or saline on days 4, 7, 10, and 13 after estrus detection of 1st cycle.[1,2]

Cycle	Control	hCG
Estrus before treatment[3]	13	13
1st estrus after treatment	4	7
2nd estrus after treatment	4	3
Not pregnant[4]	2	6

[1] Frequencies did not differ ($P = 0.546$).
2 Cycle was determined by lambing date.
3 Treatment was applied on day 4, 7, 10, and 13 of the 1st cycle only.
4 Ewes were not pregnant at day 75 ultrasound and were removed from the flock. In addition two mature ewes lost eartags between unltrasounding and lambing and were not included in lambing data.

similar to earlier work in which hCG given to non-pregnant ewes on d 0 did not increase progesterone concentration until d 8 (Gómez-Brunet et al., 2007). Although hCG half-life in circulation is approximately 22 h (Schmitt et al., 1996), hCG injections administered 72 h apart in the current study maintained increased progesterone from the day of initial observation through the end of the collection period in both pregnant and non-pregnant ewes, suggesting no desensitization to hCG during this period. This supports similar data involving repeated administration of hCG in cattle (Helmer and Britt, 1987).

Data from d 7 CBC contrast previous reports of an inhibitory effect of hCG on LYM activity (French and Northey, 1983; Khil et al., 2007), as LYM numbers were actually greater in hCG-treated ewes in the current study. However, LYM numbers in ewes receiving hCG were not different from control numbers at d 11, indicating changing dynamics of the influence of hCG on immune response. Additionally, hCG appeared to have no effect on circulating MON, either in total numbers or as a percentage of WBC at d 7 or d 11; a contrast to previous findings in mice (Khil et al., 2007). Indeed, increases in total WBC and LYM numbers at d 7 and a lack of difference in any measured immune component at d 11, does not suggest systemic-immune depression by hCG directly (Khil et al., 2007) or through increased progesterone (Ramadan et al., 1997; Wulster-Radcliffe et al., 2003). Data from the current study do, however, support previous observations of a differential effect of pregnancy on activity of LYM and other immune components (Fabris et al., 1977), as both total LYM and LYM fraction of total WBC were reduced in pregnant ewes compared to non-pregnant ewes in the current study, independent of treatment.

Decreased RBC, Hgb, and Hct recorded at d 11 in hCG-treated ewes contrast to findings in human males treated with hCG, which revealed increased levels of these blood components (Tsujimura et al., 2005). Additionally, Plotka et al. (1988) observed a positive correlation between progesterone concentrations and RBC, Hgb, and Hct levels in wild horses, while in the current study, progesterone concentrations in hCG-treated ewes were

Table 6. Number of ewes with single or multiple lambs at parturition in animals conceiving at the estrus just before treatment, the first estrus after treatment, or the second estrus after treatment and introduction of rams, and number of lambs born per ewe lambing. Ewes were administered (i.v.) hCG or saline on days 4, 7, 10, and 13 after estrus detection of 1st cycle only.[1]

		Treatment[3]		
Estrus[2]	Lambs at term	Control	hCG	*P* - value[4]
Before treatment	Single	6 (6)	4 (4)	0.420
	Multiple	7 (14)	9 (20)	
	Lambs/ewe	1.5 ± 0.16	1.8 ± 0.16	0.174
1st after treatment	Single	0 (0)	1 (1)	0.63
	Multiple	4 (10)	6 (14)	
	Lambs/ewe	2.5 ± 0.28	2.1 ± 0.21	0.321
2nd after treatment	Single	0 (0)	0 (0)	1.000
	Multiple	4 (9)	3 (7)	
	Lambs/ewe	2.3 ± 0.28	2.3 ± 0.33	0.849

[1] Cycle was determined by lambing date.
[2] Treatment was applied on day 4, 7, 10, and 3 of the 1st cycle only.
[3] Total number of lambs per group shown in parenthesis.
[4] Fisher's exact test was used to calculate *P* - value when more than 25 % of cells contained expected values less than 5.

greater than CON ewes at the time that decreased RBC, Hgb, and Hct were observed. The link between hCG administration and RBC, Hgb, and Hct levels is poorly understood, but does appear to be temporal.

Greater frequencies of multiple fetuses in ewes treated with hCG were previously reported (Nephew et al., 1994; Zamiri and Hosseini, 1998; Cam and Kuran, 2004), however, comparable birth frequencies in the current study disagree with these accounts and instead support other findings in which hCG did not improve prolificacy (Gómez-Brunet et al., 2007). Additionally, hCG did not appear to affect prolificacy or pregnancy rate in ewes conceiving on the estrus immediately following treatment, a contrast to previous findings of increased pregnancy rate in heifers treated with hCG before breeding (Breuel et al., 1989). Data from the current study indicate that hCG administration did not improve pregnancy rate, lambs per ewe, or total lambs at parturition, in contrast to results by Kleemann et al. (1991), who reported increasing progesterone concentration resulted in increased embryo survival.

Conclusions

Human chorionic gonadotropin was shown to increase serum-progesterone concentration in both pregnant and non-pregnant ewes. This effect was delayed after initial injection in both groups, but the delay was greater in non-pregnant ewes. Ewes did not appear to become desensitized to hCG despite repeated administration. White blood cells, lymphocytes, mean corpuscular volume, and eosinophil percentage of white blood cells were increased and neutrophils were decreased by hCG at day 7 after estrus, but all returned to control levels by day 11. Red blood cells, hemoglobin, and hematocrit levels were not affected by hCG treatment at day 7, but were decreased at day 11 despite increased progesterone concentrations. Progesterone and specific immune components were altered by administration of hCG, but these changes did not seem to translate to superior lambing data at parturition. This study assumed a certain amount of natural embryonic loss in controls ewes based on previous research, and that this loss would be alleviated by administration of hCG. However, if natural loss did not occur or was overestimated in the ewes used as controls for this study, lambing data could not have been different due to treatment. Future studies must include an account of ovulation rates in order to establish the exact amount of natural embryonic loss, if any.

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Feedlot Performance, Carcass Characteristics, and Muscle CLA Concentration of Lambs Fed Diets Supplemented with Safflower Seeds and Vitamin E

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Summary

Sixty-eight Rambouillet ram lambs were used to evaluate the effects of safflower seed and vitamin-E supplementation on feedlot performance, carcass characteristics, and muscle-conjugated, linoleic-acid concentration. Lambs were fed finishing diets that were iso N and similar in estimated TDN containing safflower seed (SAFF) or no safflower seed (NOSAFF) in combination with 0 IU/d or 400 IU/d of supplemental vitamin E (NOVITE and VITE, respectively). Safflower seeds contained 43 percent oil with 79 percent linoleic acid, therefore SAFF diets were considered to contain 6 percent safflower oil. Final BW, DMI, ADG, and longissimus muscle area as measured by ultrasound did not differ ($P > 0.10$) between lambs fed SAFF and NOSAFF, or VITE and NOVITE. Gain:feed was lower ($P = 0.06$) for lambs fed SAFF compared to lambs fed NOSAFF (16.3 vs 14.3), but there was no difference ($P = 0.45$) in G:F between lambs fed VITE and NOVITE. Final weight, HCW, and fat thickness of slaughtered lambs were greater ($P < 0.05$) for lambs fed SAFF compared to NOSAFF (63.7 kg vs 59.7 kg final weight, 31.2 kg vs 29.0 kg HCW, and 0.3 cm vs 0.2 cm fat thickness); however, there were no differences ($P > 0.16$) in longissimus muscle area as measured by acetate traces, cooking loss, or Warner-Bratzler shear force between lambs fed SAFF versus NOSAFF. Final weight, HCW, fat thickness, longissimus muscle area, cooking loss, and Warner-Bratzler shear force did not differ ($P > 0.19$) between lambs fed VITE versus NOVITE. Dressing percentage was lower ($P < 0.10$) for lambs fed SAFF and VITE compared to lambs fed SAFF and NOVITE (47.6 percent vs 50.4 percent). Muscle from lambs fed SAFF had greater ($P \leq 0.001$) concentrations of conjugated linoleic acid (CLA; 4.9 percent vs 0.6 percent), total polyunsaturated fatty acid (17.8 percent vs 7.2 percent), and total unsaturated fatty acids (57.7 percent vs 50.7 percent); and lower ($P < 0.001$) concentrations of total SFA (35.7 percent vs 40.7 percent) and total monounsaturated fatty acid (39.9 percent vs 43.5 percent) than muscle from lambs fed NOSAFF. Lambs fed VITE had greater (P = 0.02) concentrations of total saturated fatty acids in muscle than lambs fed NOVITE (39.7 percent vs 36.7 percent). Lipid oxidation of muscle did not differ ($P > 0.32$) between lambs fed SAFF and NOSAFF or VITE and NOVITE. Vitamin E had few effects on feedlot performance, carcass characteristics, or muscle-fatty-acid concentrations; however, safflower-seed supplementation increased muscle concentration of CLA, linoleic acid, and polyunsaturated fatty acid to saturated-fatty-acid ratio resulting in a meat product that may be more beneficial to human health.

Key words: Conjugated Linoleic Acid, Fatty Acids, Lamb, Lipid Oxidation, Meat

Introduction

American consumers have become interested in the quality and health benefits of their food beyond that of basic nutrient content. The functional food market has focused on the health benefits of nutraceutical compounds found in plant products; however, milk and meat from ruminant animals contain conjugated linoleic acid (CLA), which have been shown to reduce carcinogenesis, atherosclerosis, the onset of diabetes, and body fat mass (Belury, 2002). Therefore, meat and milk products containing naturally high levels of CLA have the potential to be classified as functional foods (McGuire and McGuire, 2000). Conjugated linoleic acid is a long-chain, polyunsaturated fatty acid (PUFA), that is formed from linoleic acid via the process of biohydrogenation in the rumen or by the animal's tissues from trans-11 C18:1, another intermediate in the biohydrogenation of unsaturated fatty acids (Bauman et al., 2000). Safflower seed is high in linoleic acid (78 percent; Enig, 2000) and the concentration of CLA in lamb muscle has been increased through supplementation of safflower seeds or oil (Bolte et al., 2002; Kott et al., 2003; Boles et al., 2005).

Incorporating PUFA into the diet of ruminants also increases the concentration of unsaturated fatty acids in muscle tissue. Unsaturated fatty acids are more susceptible to lipid oxidation than saturated-fatty acids (SFA; Enig, 2000); therefore, it may be necessary to supplement animals with additional vitamin E (a potent antioxidant) in order to prevent flavor deterioration due to lipid oxidation (Wulf et al., 1995; Wood and Enser, 1997). Few researchers have investigated the effects of supplementing polyunsaturated acids in conjunction with vitamin E; therefore, the objectives of this study were to determine the effects of safflower-seed and vitamin-E supplementation on feedlot performance, carcass characteristics, and fatty acid concentration in muscle of ram lambs.

Materials and Methods

Sixty-eight Rambouillet ram lambs born in April and May at the Red Bluff Experimental Ranch grazed native range with their dams until weaning in late August. After weaning, lambs were moved to the Fort Ellis Sheep facilities near Bozeman, Mont. where a feedlot study was conducted. Ram lambs (average BW 32.4 kg ± 0.47 kg) were assigned to 1 of 12 feedlot pens in a manner that equalized average lamb BW across pens. Each pen was randomly assigned to 1 of 4 diets in 2 x 2 factorial arrangement with 3 pens of 5 to 6 lambs per diet. Diets included or did not include safflower seed (SAFF and NOSAFF) with 0 IU/d or 400 IU/d of vitamin E (NOVITE and VITE, respectively). Diets were formulated on a DM basis to be isocaloric, isonitrogenous, and to meet or exceed NRC (1985) requirements for Ca, P, and other nutrients (Table 1). Barley was rolled and safflower seeds were coarsely cracked. Safflower diets were assumed to contain 6 percent safflower oil based on the oil concentration of safflower seeds (43 percent oil with 79 percent linoleic acid). Lambs had ad libitum access to water and their respective diets, which were offered in self-feeders. Lambs were adjusted to their experimental diets during a 17-d step-up period and were then fed the finishing diets for 61 d. Feed bunks were monitored daily, and more feed was added to keep the bunks full. Feed refusals were removed, weighed, and recorded weekly throughout the trial. Random grab samples of the diets and feed refusals were collected, weighed, and dried in order to calculate DMI.

Feed samples were ground through a 1-mm screen and analyzed for DM, CP, and ether extract (AOAC, 2000). Weights were measured at the beginning and end of the finishing period after an 18-h fast from food and water. Real-time ultrasound was conducted to estimate longissimus muscle area at the end of the finishing period. Accuracy of ultrasound measurements was determined by comparing them to acetate traces of longissimus muscle area obtained at slaughter. Technician bias was -0.16 cm2 and the standard error of prediction was 0.37. The standard error of repeatability for this technician was not estimated during the current study, but was 0.22 in another research trial (unpublished data from our lab). All these values meet the requirements for accuracy suggested by Tait et al. (2005). Montana State University Institutional Animal Care and Use Committee approved all animal procedures.

Following the feeding period, two lambs from each pen (n = 24) weighing closest to the target final weight of 61 kg

Table 1. Ingredient and nutrient composition of finishing diets fed to ram lambs.

	Diets[1]			
	NOSAFF		SAFF	
Item	NOVITE	VITE	NOVITE	VITE
Ingredient, % DM basis				
Alfalfa pellets	29.4	29.4	38.3	38.3
Barley	49.3	49.4	24.4	24.4
Safflower seeds	-	-	16.2	16.2
Vitamin premix (control)	21.3	-	21.1	-
Vitamin E premix	-	21.2	-	21.1
Nutrient composition, % DM basis				
TDN	78.2	78.3	83.2	83.4
Crude protein	17.6	17.6	19.2	19.2
Ether extract	1.6	1.6	8.4	8.4
Vitamin E2, mg/kg	63	225	91	250
Calcium[2]	0.5	0.5	0.7	0.7
Phosphorus[2]	0.3	0.3	0.3	0.3
Fatty acid profile, % of total fatty acids				
Palmitic acid (C16:0)	15.2	15.4	12.1	12.4
Stearic acid (C18:0)	1.5	1.5	1.6	1.6
Oleic acid (C18:1)	16.2	16.6	14.3	14.7
Linoleic acid (C18:2)	36.1	36.7	39.5	40.2

[1] SAFF = supplemented with safflower seed, NOSAFF = not supplemented with safflower seed, VITE = supplemented with 400IU/d vitamin E, NOVITE = not supplemented with vitamin E.
[2] Calculated based on NRC tabular values of ingredients (NRC, 1982).

were selected and sent to a commercial slaughter facility approximately 100 miles west of Bozeman. Hot-carcass weights were collected at slaughter. Carcasses were chilled for 24 h at 2°C and then transported to the Montana State University Meat Laboratory. After a 5-d chill, carcasses were ribbed between the 12th and 13th ribs to allow for measurement of fat thickness and longissimus muscle area using traces drawn on acetate paper and measured with a planimeter. The carcasses were then fabricated according to the Institutional Meat Purchase Specifications (NAMP, 1988). Two loin chops 2.54 cm thick were removed from the right loin, deboned, vacuum-packaged, frozen, and stored at -77°C for future analysis of cooking loss, Warner-Bratzler shear force, lipid oxidation, and fatty acid concentration. Cooking loss and Warner-Bratzler shear force were estimated after cooking the meat in a plastic cook bag in an 80°C water bath to an internal temperature of 75°C. After cooking, the chops were placed in ice water and cooled to room temperature in order to prevent additional cooking once the desired temperature of 75°C was achieved. Chops were weighed before and after cooking in order to calculate cooking loss. Multiple 1.27 cm2 core samples were taken from each chop parallel to the muscle fibers, sheared with a TMS 30 Food Texturometer (Food Technology Corp., Rockville, Md.) fitted with Warner-Bratzler shear-force attachment. The shear-force values from all the cores from each animal were averaged and used for data analysis. Lipid oxidation was determined on loin chops thawed overnight at 4°C using the 2-thiobarbituric acid (TBARS) method described by Witte et al. (1970) and modified by Bedinghaus and Ockerman (1995).

Fatty acid methyl esters (FAME) of feed and muscle tissue were prepared according to the procedure described by Murrieta et al. (2003) and optimized to identify the different CLA methyl esters. Cross sections of the longissimus muscle, including any residual intramuscular fat, were freeze-dried and ground in an electric coffee grinder. Gas liquid chromatography was used to analyze for FAME using a Hewlett Packard 5890 GLC equipped with a flame ionization detector and an auto-sampler (Hewlett Packard, Avondale, Penn.). The column used for the chromatographic separations was a 100 m x 0.25 mm x 0.2 µm film thickness, fused-silica column (SP-2560; Sigma-Aldrich, Co., St. Louis, Mo.). Helium was used as the carrier gas, with a split ratio of 30:1 and 0.9 mL•m-1 column flow. Column temperature was programmed to be a constant temperature of 175°C for 65 min. Standard samples from Nu-Chek-Prep (Elysian, Minn.) were used to identify the various fatty acids, and the internal method of Murrieta et al. (2003) was used to determine fatty acid concentrations, with tridecanoic acid methyl ester added as an internal standard before extraction.

Data were analyzed as a completely randomized design using the GLM procedure of SAS (SAS Inst., Inc., Cary, N.C.) with pen as the experimental unit for feedlot data and individual animal as the experimental unit for carcass and fatty acid data. The effects of safflower seed, vitamin E, and their interaction were included in the model. Hot-carcass weight was included as a covariate for analysis of dressing percent, LMA, fat thickness and cooking loss. Least square means and associated standard errors are reported.

Results and Discussion

Growth and feed intake

There were no interactions ($P > 0.31$) between safflower and vitamin E supplementation for any feedlot-performance characteristics (Table 2). Initial BW, final BW, DMI, ADG, and longissimus muscle (LM) area as measured by ultrasound did not differ ($P > 0.10$) between lambs fed SAFF and NOSAFF; however, G:F was lower ($P = 0.06$) for lambs fed SAFF compared to lambs fed NOSAFF (.16 vs .14 ± .006). Initial weight, final weight, DMI, ADG, G:F, and longissimus muscle area as measured by ultrasound did not differ ($P > 0.24$) between lambs fed VITE compared to NOVITE.

In agreement with our results, no differences in final weight, DMI (Kott et al., 2003; Boles et al., 2005), or ADG (Mir et al., 2000; Bolte et al., 2002; Boles et al., 2005) were observed due to supplementation with safflower seed or

Table 2. Effect of safflower seed and vitamin E supplementation on growth, feed intake, and ultrasonic longissimus muscle area of ram lambs.

	Diets[1]					P-value[2]		
	NOSAFF		SAFF					
Item	NOVITE	VITE	NOVITE	VITE	SE[3]	S	V	S x V
N, pens	3	3	3	3				
Initial weight, kg	38.2	38.4	39.9	41.0	1.18	0.11	0.58	0.76
Final weight, kg	57.5	55.9	56.1	56.9	1.13	0.86	0.74	0.32
DM intake, kg/d	1.88	1.84	1.86	1.84	0.045	0.85	0.59	0.90
Daily gain, kg	0.32	0.29	0.27	0.26	0.021	0.11	0.45	0.53
G:F, g/kg	168.80	155.6	143.0	142.40	8.75	0.06	0.45	0.49
LMA[4], cm2	16.9	16.1	16.3	15.6	0.57	0.39	0.25	0.94

[1] SAFF = supplemented with safflower seed, NOSAFF = not supplemented with safflower seed, VITE = supplemented with vitamin E, NOVITE = not supplemented with vitamin E.
[2] S = effect of safflower seed supplementation, V = effect of vitamin E supplementation.
[3] SE for the four means.
[4] Longissimus muscle area (LMA) measured by ultrasound on all lambs (technician bias = -0.16 and standard error of prediction = 0.37).

oil. In contrast to our data, Mir et al. (2000) reported decreased DMI when lambs were supplemented with safflower oil and Kott et al. (2003) reported greater ADG when lambs were fed diets containing safflower seeds. We were the only researchers to observe lower G:F with safflower supplementation. Other researchers reported similar feed efficiencies when lambs were fed safflower oil or seeds (Mir et al. 2000; Bolte et al., 2002; Boles et al., 2005), but Kott et al. (2003) observed higher G:F when lambs were supplemented with safflower seeds. In the current study, initial weight tended to be lower and ADG tended to be higher for lambs fed NOSAFF ($P < 0.11$), which probably explains higher G:F by lambs on this diet. Oil may have more energy potential; however, its utilization and efficiency can be limited by negative effects on ruminal digestion, decreased intestinal absorption at high intakes, low contribution to total nutrient oxidation, or nutrient imbalance (Palmquist, 1994).

Similar to our findings, vitamin E supplementation had no affect on final weight or ADG in some other experiments (Macit et al., 2003b; Aksu et al., 2004). Wulf et al. (1995) also reported similar ADG and final weights between lambs supplemented with 0 or 500 IU vitamin E per day, but lower ADG for lambs supplemented with 1000 IU/d. Final weight, DMI, and ADG were also similar among lambs fed control diets, diets supplemented with soybean oil, and diets supplemented with soybean oil and vitamin E (Chen et al., 2008). In contrast to these and our results, vitamin E supplementation increased ADG (Macit et al., 2003a) and improved feed efficiency of lambs (Macit et al., 2003a,b; Aksu et al., 2004). These studies were conducted using different breeds of sheep (Awassi and Morkaraman) with no statistical analysis of DMI, which could explain differences between their results and ours.

Carcass Characteristics

Final weight and HCW of slaughtered lambs were greater ($P < 0.05$) for lambs fed SAFF compared to NOSAFF; however, there were no differences ($P > 0.16$) in longissimus muscle area measured by acetate traces, cooking loss, or Warner-Bratzler shear force between lambs fed SAFF compared to NOSAFF (Table 3). Final weight, HCW, longissimus muscle area, fat thickness, cooking loss, and Warner-Bratzler shear force of slaughtered lambs did not differ ($P > 0.19$) between lambs fed VITE versus NOVITE. There was an interaction ($P = 0.10$) between safflower-seed and vitamin-E supplementation for dressing percentage. Vitamin E supplementation decreased ($P < 0.10$) dressing percentage of carcasses from lambs fed SAFF (47.6 percent vs 50.4 percent ± .9 percent), but dressing percentage did not differ ($P > 0.10$) between VITE and NOVITE for lambs fed NOSAFF diets (averaging 48.6 percent).

Lambs were slightly under-finished at the termination of the study. Fat thickness was ≤ 0.3 cm and the industry standard is 0.5 cm of fat thickness. Therefore, heavier lambs in each pen were chosen for slaughter, which explains higher final weights and differences in results between Tables 2 and 3. Similar to our results, other researchers reported no differences in carcass characteristics when feedlot lambs were supplemented with safflower oil (Boles et al., 2005), safflower seeds (Bolte et al., 2002; Kott et al., 2003), or vitamin E (Macit et al., 2003a,b). We are not sure why vitamin E appeared to decrease dressing percentage when fed with SAFF in the current study. In contrast to our data, there were no differences in dressing percentage due to safflower (Bolte et al., 2002; Kott et al., 2003; Boles et al., 2005) or vitamin E supplementation (Wulf et al., 1995; Macit et al., 2003b). Similar to our results, supplementation with a combination of soybean and linseed oil had no affect on cooking loss or shear-force values of lamb (Radnz et al., 2009). Although vitamin E supplementation increased cooking loss in beef (Mitsumoto et al., 1995), Macit et al. (2003b) found no affect on cooking loss or shear-force values of lamb.

Muscle fatty acid concentration

There were interactions ($P < 0.10$)

Table 3. Effect of safflower seed and vitamin E supplementation on carcass characteristics of ram lambs selected for slaughter.

	Diets[1]					P-value[2]		
	NOSAFF		SAFF					
Item	NOVITE	VITE	NOVITE	VITE	SE[3]	S	V	S x V
N, lambs	6	5	5	6				
Final weight, kg	59.0	60.3	63.0	64.3	1.39	0.01	0.38	0.99
HCW, kg	28.6	29.4	31.8	30.5	0.89	0.03	0.83	0.27
Dressing percent	48.4	48.7	50.4	47.6	0.93	0.63	0.20	0.10
LMA[4], cm^2	18.0	16.8	17.7	17.8	0.97	0.75	0.59	0.51
Fat thickness, cm	0.23	0.23	0.33	0.34	0.045	0.03	0.96	0.89
Cooking loss, %	13.4	15.0	15.8	16.7	1.47	0.17	0.41	0.81
Shear force[5], kg	5.4	5.0	5.7	6.3	0.76	0.30	0.90	0.53

[1] SAFF = supplemented with safflower seed, NOSAFF = not supplemented with safflower seed, VITE = supplemented with vitamin E, NOVITE = not supplemented with vitamin E.
[2] S = effect of safflower seed supplementation, V = effect of vitamin E supplementation.
[3] SE for the four means.
[4] Longissimus muscle area measured by acetate traces.
[5] Warner-Bratzler shear force.

between safflower and vitamin-E supplementation for concentrations of total-fatty acids, myristic (C14:0), stearic (C18:0), arachidic (C20:0), palmitoleic (C16:1), linoleic (C18:2), and arachidonic (C20:4) acid in lamb muscle tissue (Table 4). Total fatty acid concentration in lamb muscle was lower ($P < 0.10$) in SAFF-fed lambs supplemented with VITE than in lambs fed NOSAFF and supplemented with VITE (7.7 percent vs 12.5 percent ± 1.8 percent). Linoleic-acid concentration was lowest ($P < 0.10$) in muscle from lambs fed NOSAFF diets (5 percent ± .8 percent), intermediate in muscle from lambs fed SAFF with NOVITE (9.7 percent ± .9 percent), and highest in muscle from lambs fed SAFF with VITE (12.3 percent ± .8 percent). Interactions between safflower seed and vitamin E supplementation for the minor fatty acids may not be of any practical importance and will not be detailed here.

Muscle from lambs fed SAFF had higher ($P \leq 0.001$) concentrations of the C18:1,trans-10 isomer, CLA, total PUFA, total unsaturated fatty acids, and PUFA:SFA than lambs fed NOSAFF. Muscle from lambs fed SAFF had lower ($P \leq 0.001$) concentrations of palmitic acid, oleic acid (C18:1,cis-9), vaccenic acid (C18:1,cis-11), total SFA, and total monounsaturated fatty acid (MUFA) than lambs fed NOSAFF. Lipid oxidation did not differ (P = 0.75) between SAFF and NOSAFF. Muscle from lambs supplemented fed VITE had higher ($P = 0.02$) concentrations of total SFA than muscle from lambs fed NOVITE (39.7 percent versus 36.7 percent ± .8 percent). Lipid oxidation and PUFA:SFA did not differ ($P > 0.32$) between VITE and NOVITE supplemented lambs.

The effect of safflower supplementation on total fatty acid concentration in lamb muscle has been variable. Bolte et al. (2002) and Boles et al. (2005) reported no difference in total fatty acid concentration from muscle of lambs supplemented with safflower seeds or oil. In contrast, Mir et al. (2000) reported that safflower oil supplementation tended to decrease lipid concentration in lamb muscle (Mir et al., 2000), and Kott et al. (2003) observed an increase in total fatty acid concentration in muscle from lambs fed safflower seeds. In contrast to our results, no difference in total-muscle-lipid concentration was observed, when vitamin E was fed in combination with other PUFA (Demirel et al., 2004; Chen et al., 2008). Differences in results between studies could be due to degree of finish or how closely the cuts were trimmed.

Similar to our research, Boles et al. (2005) reported that concentrations of palmitic and stearic acid in muscle decreased as level of safflower oil in the diet increased; however, Kott et al. (2003) and Mir et al. (2000) reported no differences in palmitic- and stearic-acid concentration in rib muscle of lambs supplemented with safflower. The SAFF diets in our study were lower in palmitic and oleic acids due to a higher concentration of these fatty acids in barley than safflower seed, which may partially explain lower concentrations of these fatty acids

Table 4. Effect of safflower seed and vitamin E supplementation on fatty acid profile of muscle sample extracts in ram lambs.

	Diets[1]							
	NOSAFF		SAFF			P-value[2]		
Item	NOVITE	VITE	NOVITE	VITE	SE	S	V	S x V
N, lambs	6	5	5	6				
Total fat content[3]	9.6^{ab}	12.5^{b}	11.2^{ab}	7.7a	1.79	0.37	0.88	0.09
Myristic (C14:0)[4]	4.7^{b}	3.9^{a}	4.5^{ab}	5.0^{b}	0.33	0.22	0.63	0.07
Palmitic (C16:0)[4]	25.1	27.1	22.5	22.7	0.66	0.0001	0.13	0.19
Stearic (C18:0)[4]	7.3^{a}	11.1^{b}	7.0^{a}	7.5^{a}	0.70	0.01	0.007	0.03
Arachidic (C20:0)[4]	1.2^{b}	0.9^{a}	1.0^{a}	1.2^{b}	0.07	0.68	0.70	0.009
Palmitoleic (C16:1)[4]	3.3^{b}	2.0^{a}	2.5^{a}	2.2^{a}	0.24	0.34	0.004	0.05
Oleic (C18:1, *cis*-9)[4]	36.6	38.6	27.2	26.6	1.23	0.001	0.60	0.30
Oleic (C18:1, *trans*-10)[4]	1.9	2.0	10.0	8.9	0.67	0.001	0.48	0.38
Oleic (C18:1, *cis*-11)[4]	1.4	1.3	1.1	1.1	0.07	0.002	0.59	0.29
Linoleic (C18:2)[4]	5.8^{a}	4.2^{a}	9.7^{b}	12.3^{c}	0.86	0.001	0.54	0.03
CLA (C18:2, *cis*-9,*trans*-11)[4]	0.6	0.6	5.3	4.5	0.34	0.001	0.26	0.33
Arachadonic (C20:4)[4]	2.1^{b}	1.1^{a}	1.7^{ab}	2.1^{b}	0.33	0.33	0.36	0.04
Total SFA[4]	38.3	43.0	35.0	36.3	1.14	0.003	0.02	0.15
Total MUFA[4]	43.2	43.8	40.9	38.9	1.21	0.007	0.57	0.29
Total PUFA[4]	8.5^{a}	5.9^{a}	16.6^{b}	18.9^{b}	1.26	0.0001	0.90	0.07
Total unsaturated fatty acids[4]	51.7	49.7	57.5	57.8	1.48	0.0002	0.57	0.45
PUFA:SFA	0.22	0.14	0.48	0.52	0.043	0.0001	0.59	0.15
Lipid oxidation[5]	0.20	0.32	0.24	0.35	0.111	0.75	0.33	0.97

[1] SAFF = supplemented with safflower seed, NOSAFF = not supplemented with safflower seed, VITE = supplemented with vitamin E, NOVITE = not supplemented with vitamin E.
[2] S = effect of safflower seed supplementation, V = effect of vitamin E supplementation.
[3] % on dry weight basis.
[4] % of total fatty acids.
[5] mg malonaldehyde/kg fresh meat.

in the muscle of lambs fed SAFF diets. Most previous researchers concluded that supplementation with safflower oil or seeds increased the concentration of linoleic acid, CLA, and C18:1 isomers in lamb muscle, while concentrations of oleic acid were decreased (Mir et al., 2000; Bolte et al., 2002; Kott et al., 2003; Boles et al., 2005). The primary product of fatty acid metabolism in growing sheep is palmitic acid, which can be further elongated to stearic acid and then desaturated to oleic acid by stearoyl-CoA desaturase (Sinclair, 2007). Starch diets promote greater activity of stearoyl-CoA desaturase (Daniel et al., 2004), and PUFA (including linoleic acid) also inhibited stearoyl-CoA desaturase gene 1 expression in mouse liver (Ntambi, 1992) and adipocytes (Sessler et al., 1996). These observations could explain why lambs supplemented with safflower seed had lower concentrations of MUFA and oleic acid in muscle compared to lambs fed NOSAFF (higher starch) diets. Our values for CLA in muscle of safflower supplemented lambs (4.9 percent) were higher than the concentrations reported by others (0.84 percent to 1.45 percent; Mir et al., 2000; Bolte et al., 2002; Kott et al., 2003; Boles et al., 2005); however, the concentrations of linoleic acid in our study (11 percent) were within the range reported by these authors (4.5 percent to 16 percent) and were similar to concentrations found in olive oil (10 percent; Enig, 2000).

In agreement with our data, Bolte et al. (2002) observed a decrease in total MUFA concentration in muscle of lambs supplemented with safflower seeds; however in contrast to our data, Bolte et al. (2002) and Kott et al. (2003) reported no difference in total SFA concentrations in lamb muscle due to safflower-seed supplementation. Differences in results between studies could be due to differences in the number and type of individual fatty acids used to calculate total fatty acid content or differences in biohydrogenation due to the composition of the basal diet. Lamb is generally considered a high-fat food (>5 percent) with a low PUFA:SFA (Wood and Enser, 1997; Sinclair, 2007). In the current experiment, total fatty acid concentration of muscle was 7.7 percent to 12.5 percent and the PUFA:SFA of safflower supplemented lamb meat was 0.48 and 0.52, which is higher than the recommended PUFA:SFA of 0.45 (Wood and Enser, 1997).

Few researchers have supplemented vitamin E in combination with different oils, and our study may be the first to report the effects of feeding safflower seed (oil) in conjunction with vitamin E in feedlot diets. Unsal et al. (2004) and Aksu et al. (2004) did not include a source of oil in the diet of lambs, but reported almost no differences in fatty acid composition of intra- or intermuscular fat due to Vitamin E supplementation. Chen et al. (2008) reported that lambs receiving vitamin E in addition to soybean oil had higher concentrations of PUFA and decreased concentrations of total SFA in lamb muscle than lambs supplemented with only soybean meal; however, vitamin E had no effect on concentrations of linoleic, palmitic, stearic, CLA, or total MUFA in lamb muscle. Demirel et al. (2004) reported no effects of supplemental vitamin E on total fat, CLA, or trans18:1 in muscle tissue, when fed with three different fat sources (Megalac, linseed oil, and fish oil) and observed no interactions between fat source and vitamin E.

Wulf et al. (1995) reported that supplemental vitamin E reduced the effect of storage time on lipid oxidation. We observed no difference in lipid oxidation among diets in the current study; however, we only estimated lipid oxidation at one time point: 24 h after thawing frozen muscle samples. In agreement with our data, Kerry et al (2000) and Yaprak et al. (2002) reported no differences in lipid oxidation on d 0 or d 2 to d4, respectively, between lambs supplemented or not supplemented with vitamin E with values ranging from 0.2 to 0.5. However, in agreement with Wulf et al. (1995) most researchers observed decreased lipid oxidation with vitamin-E supplementation as time progressed (Kerry et al., 2000; Yaprak et al., 2002).

Implications

This may be the first study to evaluate the effects of feeding safflower seeds in combination with Vitamin E on feedlot performance, carcass characteristics, and fatty acid concentration of lamb muscle. Muscle from lambs fed safflower seeds had higher percentages of linoleic acid, CLA, PUFA, total unsaturated fatty acids, and PUFA:SFA. Vitamin E supplementation decreased total fatty acid concentration of muscle from lambs fed safflower seeds and increased total SFA in lamb muscle. Our data suggests that lambs fed safflower seeds produced muscle with a fatty acid composition that may be more desirable to the modern consumer and more beneficial to human health.

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Pregnancy Rates after Ewes were Treated with Estradiol-17β and Oxytocin

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Summary

Ewes were assigned to the following treatments to determine whether estradiol-17β-oxytocin treatment affects luteal function and pregnancy rates on d 25: 1) diluent + saline (n = 26); 2) diluent + oxytocin (n = 25); 3) estradiol-17β + saline (n = 22); and 4) estradiol-17β + oxytocin (n = 24). On d 6 after expected estrus and mating, ewes received either i.v. injection of diluent or i.v. injection of 100 μg of estradiol-17β. Ten hours later, ewes received either i.v. injection of saline or i.v. injection of 400 USP units of oxytocin. Blood samples for progesterone assay were collected on d 6, d 7, d 8 (Period 1), d 16, d 18, d 20, d 22, and d 25 (Period 2). Transrectal ultrasonography on d 25 and progesterone concentrations were used to diagnose pregnancy. Neither estradiol-17β nor oxytocin affected pregnancy rates, and the estradiol-17β × oxytocin interaction was not significant. The pregnancy rate for diluent + saline was 61.5 percent; diluent + oxytocin, 76 percent; estradiol-17β + saline, 77.2 percent; and estradiol-17β + oxytocin, 62.5 percent. Progesterone concentration was greater ($P < 0.05$) in pregnant than in nonpregnant ewes (5.2 ± 0.3 ng/mL vs. 2.0 ± 0.6 ng/mL); the pregnancy status × period interaction was significant ($P < 0.01$); but estradiol-17β, oxytocin, and their interaction were not significant ($P > 0.05$). Treatment with estradiol-17β on d 6 after the expected onset of estrus and oxytocin 10 h later did not induce luteolysis or disrupt pregnancy in ewes.

Key Words: Sheep; Embryo Transfer; Estradiol; Oxytocin; Transcervical

Introduction

Surgical procedures have been used for at least 55 years to collect and transfer sheep embryos (Hunter et al., 1955; McKelvey et al., 1986; Nellenschulte and Niemann, 1992; Li et al., 2008). Sheep cervical anatomy makes nonsurgical embryo collection and transfer exceedingly difficult and considerably less effective than surgical procedures (McKelvey et al., 1986; Flohr et al., 1999; Wulster-Radcliffe et al., 1999; Anel et al., 2006; Candappa et al., 2009). Even though some of the surgical embryo transfer (ET) procedures are minimally invasive, they include analgesics, anesthetics, incisions, and sutures, and they require specialized training to perform.

Effective transcervical AI, embryo collection, and ET procedures for sheep may eliminate the need for surgical procedures. Sheep-specific transcervical procedures have been reported, but pregnancy rates after the procedures have been disappointing (Sayre and Lewis, 1997; Wulster-Radcliffe et al., 1999, 2004; Anel et al., 2006). Some of the procedures have included treatments to dilate the cervix and reduce the difficulty of manipulating a catheter through the cervix and into the uterus (Flohr et al., 1999; Wulster-Radcliffe et al., 2004; Anel et al., 2006; Candappa et al., 2009).

In one study, an i.v. injection of 100 µg of estradiol-17β on d 5 of an estrous cycle and an i.v. injection of 400 USP units of oxytocin 10 h later were used to dilate the cervix and permit atraumatic transcervical ET (Wulster-Radcliffe et al., 1999). Without the estradiol-17β-oxytocin treatment, the atraumatic transcervical ET procedure described in Wulster-Radcliffe et al. (1999) would not have been possible. Based on postmortem evaluations on d 14, the estradiol-17β-oxytocin treatment did not seem to reduce embryonic development, but the effects of the estradiol-17β-oxytocin treatment per se on later pregnancy rates were not determined (Wulster-Radcliffe et al., 1999). Thus, this experiment was conducted to determine whether treating ewes with estradiol-17β and oxytocin on d 6 and d 7, respectively, after the expected day of onset of estrus and mating would affect pregnancy rates on d 25.

Materials and Methods

Animals and Treatments

Estrus was synchronized using established procedures (Wulster-Radcliffe et al., 1999, 2004) during the autumn breeding season in a group of crossbred ewes (n = 97) that contained various combinations of white-faced and black-faced genetics. Ewes were mated naturally with rams immediately after progestogen withdrawal and i.m. injection of 400 IU of eCG (Sioux Biochemical, Sioux City, Iowa). Ewes were not checked for signs of estrus during any part of the experiment.

Treatments were in a 2 × 2 factorial array. Ewes were assigned to one of four randomized treatments: 1) diluent + saline (n = 26); 2) diluent + oxytocin (n = 25); 3) estradiol-17β + saline (n = 22); and 4) estradiol-17β + oxytocin (n = 24). During the afternoon of d 6 after the expected onset of estrus and mating (i.e., 48 h after progestogen withdrawal; Cline et al., 2001), approximately half of the ewes received a 5-mL i.v. injection of diluent (50 percent ethanol:50 percent sterile, isotonic saline), and the remaining ewes received an i.v. injection of 100 µg of estradiol-17β (Sigma Chemical Co., St. Louis, Mo.) in 5 mL of diluent. At 10 h after the initial injections (i.e., morning of d 7), approximately half of the diluent-treated and half of the estradiol-17β-treated ewes received 20-mL i.v. injections of sterile, isotonic saline, and the remaining diluent-treated and estradiol-17β-treated ewes received i.v. injections of 400 USP units of oxytocin (20 mL; Vedco, St. Joseph, Mo.; Khalifa et al., 1992; Wulster-Radcliffe et al., 1999). Injections were via a jugular vein. Estradiol-17β and oxytocin were administered on d 6 and d 7, respectively, because sheep embryos are commonly transferred during this portion of the estrous cycle (Li et al., 2008).

Blood Sampling

On d 6, d 7, d 8, d 16, d 18, d 20, d 22, and d 25 after the expected onset of estrus and mating, heparinized Vacutainer tubes (Becton Dickinson, Franklin Lakes, N.J.) were used to collect approximately 10 mL of jugular venous blood from each ewe. Blood samples were placed in an ice bath immediately after collection and stored there until approximately 1 h after the end of each collection period. Plasma was then harvested after centrifugation and stored in polypropylene tubes at -20°C.

Blood samples were collected just before injections were administered on d 6 and d 7. Blood samples were not collected between d 9 and d 15 because progesterone concentrations were expected to be similar between pregnant and nonpregnant ewes during those days (deNicolo et al., 2009).

Pregnancy Diagnosis

Transrectal ultrasonography 25 d after the expected onset of estrus and progesterone concentrations was used to diagnose pregnancy. An Aloka 500 V instrument equipped with a 5.0 MHz linear array transducer (Corometrics Medical Systems, Inc., Wallingford, Conn.) was used. Ewes were restrained in dorsal recumbency in a Poldenvale Commodore cradle (Premier Sheep Supplies, Washington, Iowa) during the ultrasound procedure. A ewe was considered pregnant if 1) an embryo was visible in an ultrasound image, 2) a heartbeat could be visualized, and 3) progesterone concentrations on d 16 through d 25 were greater than 2.0 ng/mL. A heartbeat could be detected in all embryos that were visualized. A ewe was considered nonpregnant if 1) an embryo was not visible in an ultrasound image and 2) progesterone concentrations on d 16 through d 25 were less than 2.0 ng/mL. Lambing data could not be collected, as the ewes in this study were scheduled to be sold as excess. However, the decision to sell a ewe was not based on the lack of reproductive soundness. In addition, approximately 20 percent of the ewes can be expected to lose embryos or fetuses after d 25, and these losses could compromise the ability to determine the short-term effects of the estradiol-17β and oxytocin treatments (Dixon et al., 2007).

Progesterone Assay

A solid-phase RIA [^{125}I] progesterone (Diagnostic Products, Los Angeles, Calif.) was used to quantify progesterone in all plasma samples (Seals et al., 2002; Lewis, 2003; Wulster-Radcliffe et al., 2003; Lewis and Wulster-Radcliffe, 2006).

Statistical Methods

Procedures for analyzing categorical

data were used to determine the effects of treatment on pregnancy rates, and general linear-model procedures were used to determine the effects of treatment on progesterone concentrations (SAS, Cary, N.C.). The model used to determine the effect of treatment on pregnancy rate included terms for estradiol-17β, oxytocin, and the estradiol-17β × oxytocin interaction.

The initial model used to analyze the progesterone data included terms for estradiol-17β, oxytocin, pregnancy status (i.e., pregnant or nonpregnant), period (i.e., Period 1 = d 6, d 7, and d 8; Period 2 = d 16, d 18, d 20, d 22, and d 25), all interactions, and error terms. Data were then sorted according to pregnancy status, and similar models were used to determine the effects of treatment and period on progesterone concentrations in pregnant ewes and nonpregnant ewes.

Results and Discussion

The results of this experiment indicate that neither estradiol-17 nor oxytocin administered on d 6 and d 7, respectively, after the expected onset of estrus and mating affected pregnancy rates on d 25, and the estradiol-17β × oxytocin interaction was not significant. The overall pregnancy rate was 69.1 percent (67/97), which was similar to the d 25-pregnancy rates in Dixon et al. (2007). The pregnancy rate for diluent + saline was 61.5 percent (16/26); diluent + oxytocin, 76 percent (19/25); estradiol-17β + saline, 77.2 percent (17/22); and estradiol-17β + oxytocin, 62.5 percent (15/24). Embryos that were transferred after estradiol-17β-oxytocin-induced cervical dilation on d 6 of the estrous cycle appeared to survive and develop until they were evaluated postmortem on d 14 of pregnancy (Wulster-Radcliffe et al., 1999). However, experiments must be conducted to determine whether embryos that are transferred transcervically after estradiol-17β and oxytocin treatment will survive to term.

Pregnancy status affected ($P < 0.05$) progesterone concentrations (pregnant, 5.2 ± 0.3 ng/mL of plasma vs. nonpregnant, 2.0 ± 0.6 ng/mL of plasma), and the pregnancy status × period interaction was significant ($P < 0.01$; Table 1). However, estradiol-17β treatment, oxytocin treatment, and the estradiol-17β × oxytocin interaction were not significant ($P > 0.05$; Table 1). In pregnant ewes, the concentration of progesterone during Period 1 (4.9 ± 0.3 ng/mL) was less ($P < 0.01$) than it was during Period 2 (5.4 ± 0.4 ng/mL). In nonpregnant ewes, progesterone concentrations were less ($P < 0.01$) during Period 2 than they were for all ewes during Period 1 and for pregnant ewes during Period 2. Progesterone concentrations in nonpregnant ewes had decreased ($P < 0.05$) to less than 1.0 ng/mL by d 16, and they remained less than 1.0 ng/mL through d 25. The differences in progesterone concentrations in pregnant and nonpregnant ewes and reduced progesterone concentrations in nonpregnant ewes on d 16 through d 25 after the expected onset of estrus and mating, compared with d 6 through d 8, are consistent with data from numerous other experiments (Lewis et al., 1981; Wulster-Radcliffe et al., 1999; deNicolo et al., 2009).

Considering nonpregnant ewes only, the estradiol-17β × oxytocin × period interaction was significant ($P < 0.01$; Table 2). During Period 2, progesterone concentrations were greater ($P < 0.05$) in ewes that were treated with estradiol-17β and oxytocin (1.7 ng/mL) than they were in ewes in the other groups (0.5 ng/mL).

The data indicating that use of estradiol-17β, oxytocin, or the combination of estradiol-17β and oxytocin do not reduce progesterone concentrations are consistent with the results of a previous experiment in which luteal-phase ewes were treated with estradiol-17β, oxytocin, or the combination of estradiol-17β and oxytocin (Wulster-Radcliffe et al., 1999). The results of the current experiment and Wulster-Radcliffe et al. (1999) are also consistent with the results of a study to determine whether exogenous oxytocin administered s.c or intraluteally at various times during the estrous cycle or pregnancy altered luteal function in ewes (Milvae et al., 1991). Milvae et al. (1991) found that exogenous oxytocin did not reduce the duration of the estrous cycle in nonpregnant ewes and did not interrupt pregnancy in

Table 1. Progesterone concentrations (ng/mL) in jugular plasma from ewes that had been diagnosed as pregnant or nonpregnant[a]

Day	Pregnant (n = 67)	Nonpregnant (n = 30)
6	4.7	3.7
7	4.6	3.7
8	5.5	4.2
16	6.5	0.9
18	5.9	0.5
20	5.0	0.5
22	4.5	0.8
25	5.0	0.9

[a] Ewes received one of four randomized treatments: 1) diluent + saline (n = 26); 2) diluent + oxytocin (n = 25); 3) estradiol-17β + saline (n = 22); and 4) estradiol-17β + oxytocin (n = 24). On d 6 after the expected onset of synchronized estrus and mating, each ewe received an i.v. injection of 5-mL of diluent or 100 µg of estradiol-17β in 5 mL of diluent. At 10 h after the initial injection, each ewe received an i.v. injection of either 20 mL of sterile, isotonic saline or 400 USP units (20 mL) of oxytocin. Jugular blood samples were collected from all ewes on d 6 d, d 7, d 8 (i.e., Period 1), d 16, d 18, d 20, d 22, and d 25 (i.e., Period 2) after the expected onset of estrus and mating. Transrectal ultrasonography 25 d after the expected onset of estrus and progesterone concentrations were used to diagnose pregnancy. Over all ewes, pregnancy status affected ($P < 0.01$) progesterone concentrations, and the pregnancy status × period interaction was significant ($P < 0.01$). The estradiol-17β treatment, oxytocin treatment, and estradiol-17β × oxytocin interaction were not significant. Considering pregnant ewes only, period affected ($P < 0.01$) progesterone concentrations (Period 1, 4.9 ± 0.3 ng/mL; Period 2, 5.4 ± 0.4 ng/mL), but neither the estradiol-17β treatment, oxytocin treatment, nor any of the interactions were significant.

Table 2. Progesterone concentrations (ng/mL) in jugular plasma from ewes that had been diagnosed as nonpregnant[a]

Day	Diluent + saline (n = 10)[b]	Diluent + oxytocin (n = 6)	Estradiol-17β + saline (n = 5)	Estradiol-17β + oxytocin (n = 9)
6	3.4	3.8	4.5	3.0
7	3.8	4.2	3.9	3.2
8	4.2	4.8	4.4	3.4
16	1.3	0.6	0.8	1.2
18	0.3	0.2	0.2	1.4
20	0.2	0.2	0.2	2.2
22	0.2	0.3	1.2	1.6
25	0.8	0.8	0.2	1.9

[a] Ewes received one of four randomized treatments: 1) diluent + saline (n = 26); 2) diluent + oxytocin (n = 25); 3) estradiol-17β + saline (n = 22); and 4) estradiol-17β + oxytocin (n = 24). On d 6 after the expected onset of synchronized estrus and mating, each ewe received an i.v. injection of 5-mL of diluent or 100 µg of estradiol-17β in 5 mL of diluent. At 10 h after the initial injection, each ewe received an i.v. injection of either 20 mL of sterile, isotonic saline or 400 USP units (20 mL) of oxytocin. Jugular blood samples were collected from all ewes on d 6, d 7, d 8 (i.e., Period 1), d 16, d 18, d 20, d 22, and d 25 (i.e., Period 2) after the expected onset of estrus and mating. Transrectal ultrasonography 25 d after the expected onset of estrus and progesterone concentrations were used to diagnose pregnancy. The estradiol-17β × oxytocin × period interaction was significant ($P < 0.01$), but none of the other interactions were significant.
[b] Number of ewes that were diagnosed as nonpregnant.

ewes that were mated before oxytocin treatments were commenced. The authors of one study asserted that exogenous oxytocin administered on d 1 to d 7 of the estrous cycle of ewes was luteolytic (Hatjiminaoglou et al., 1979), but this seems to be the only report of such an effect of oxytocin. Indeed, another study indicates that exogenous oxytocin may prolong luteal function in ewes (Sheldrick, 1992). Thus, one should not expect exogenous oxytocin on d 7 of the estrous cycle or pregnancy to induce luteolysis in ewes.

By contrast, exogenous estradiol-17β increased uterine secretion of $PGF_{2\alpha}$ in ewes (Ford et al., 1975), and exogenous estradiol-17β can be luteolytic in ewes. The luteolytic doses of estradiol-17β have ranged from 125 µg to 750 µg and have been administered i.m. in vegetable oil on d 9 and d 10 or d 11 and d 12 of the estrous cycle (Stormshak et al., 1969; Hawk and Bolt, 1970; Kittok and Britt, 1977; Sheldrick, 1992). Treatment of ewes i.m. with 250 µg or 750 µg of estradiol-17β in corn oil on d 1 and d 2, d 3 and d 4, or d 5 and d 6 of the estrous cycle did not affect luteal weight or morphology (Hawk and Bolt, 1970). Thus, a single i.v. injection of 100 µg of estradiol-17β on d 6 after the expected onset of estrus and mating should not be luteolytic, and this assertion is consistent with the results of the current experiment.

Conclusions

Treatment of ewes with 100 µg of estradiol-17β on d 6 and 400 USP units of oxytocin 10 h later, on d 7, after the expected onset of estrus, which should dilate the cervix and reduce the difficulty of manipulating a catheter through the cervix and into the uterus for ET, did not induce luteolysis or disrupt early pregnancy in this study. However, studies are needed to determine whether transcervical ET after estradiol-17β-oxytocin treatment on d 6 and d 7, respectively, will produce acceptable lambing rates.

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Can Sheep and Cattle Rumen Microorganisms be Conditioned to Invasive Weeds?[1]

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Summary

The invasive plant species, Spotted knapweed *(Centaurea maculosa* Lam.*)* and common tansy *(Tanacetum vulgare* L.*)*, are altering native rangeland communities in western North America (Tyser and Key, 1988; Jacobs, 2008). To increase our understanding of why sheep consume these species to a certain extent and cattle avoid them, *in vitro* dry matter digestibility (IVDMD), microbial gas production (MGP), and microbial purine concentrations (MPC) of *C. maculosa* or *T. vulgare* leaves or stems incubated in sheep or cattle rumen fluid were measured. Rumen microbes were not conditioned to these plants in Trials 1a and 1b, but were conditioned in Trials 2a and 2b. Total MGP of *C. maculosa* leaves or stems (Trial 1a; $P < 0.004$) and *T. vulgare* leaves (Trial 1b; $P < 0.07$) was less, but IVDMD of these plant parts were greater ($P < 0.05$) with cattle than sheep-rumen fluid. Conditioning ewe or cow rumen microbes to *C. maculosa* did not enhance 24-h MGP, IVDMD, or MPC of *A. arundinaceus* grass hay or *C. maculosa* leaves or stems (Trial 2a; $P > 0.10$). Conversely, conditioning ewe rumen microbes to *T. vulgare* increased (Trial 2b; $P < 0.04$) IVDMD of *A. arundinaceus* hay and *T. vulgare* leaves or stems. *Centaurea maculosa* leaves and stems and *T. vulgare* leaves were used by rumen microbes as a nutritious feedstuff and nutrient characteristics and overall low IVDMD and MPC suggest that *T. vulgare* stems represent a poor quality forage. To increase consumption, further research is warranted to determine species composition and physiological differences between sheep- and cattle-adapted rumen microbes.

Key Words: *Centaurea Maculosa*, Conditioning, Nutrient-Toxin Interactions, Rumen Microorganisms, *Tanacetum Vulgare*

Introduction

Although considered grazers, sheep and cattle select different proportions and species of grasses and forbs. For example, at times, sheep consume the invasive, perennial forbs common tansy (*Tanacetum vulgare* L.) and spotted knapweed (*Centaurea maculosa* Lam.), whereas cattle generally avoid them (Hein and Miller, 1992; Kelsey and Mihalovich, 1987; Lym and Kirby, 1987; Jacobs, 2008). An animal's ability to tolerate and continue to consume certain aversive plants may depend on its ability to digest its fiber and neutralize toxic compounds before body tissues are affected (Smith, 1992).

The complex rumen ecosystem contains general and specialized microorganisms, which allow ruminants to detoxify certain plant compounds (Weimer, 1998; Asanuma et al., 2002). Further, specialized bacteria present in some herbivores are not present in others, or at least not in significant quantities. These specialized bacteria may adapt to allow the host to consume plant materials that are toxic to non-adapted animals (Jones and Megarrity, 1986). Ruminants can tolerate higher quantities of toxins if the toxic plant is introduced gradually into their diet (Allison and Cook, 1981). In addition, extent and rate of digestion may also affect the ability of an animal to tolerate certain toxins (Kronberg and Walker, 1993), and cattle digest more fiber than sheep (Playne, 1978). Thus, greater microbial digestibility of toxic plant fiber in cattle may increase the release of chemicals associated with structural plant tissues (Hagerman and Butler, 1991). The objectives were to determine IVDMD, rumen microbial gas production (MGP), and microbial purine concentrations (MPC; microbial biomass indicator) from invasive plants incubated with non-conditioned or conditioned rumen fluid collected from sheep or cattle.

Materials and Methods

Experimental Design

The experimental protocol was approved by Montana State University's Institute for Animal Care and Use Committee. Two fistulated crossbred beef cows (mean BW = 816 kg) and two fistulated white-faced yearling ewes (mean BW, 63 kg) were housed in individual pens and used as donors of fresh rumen fluid. Trial 1 was used to determine inherent differences in MGP, MPC, and IVDMD of Garrison creeping foxtail (*Alopecurus arundinaceus* Poir) hay, *C. maculosa*, or *T. vulgare* between sheep and cows fed only a grass hay diet (2 percent of BW, DM-basis) at 1100 h mainly consisting of *A. arundinaceus* hay.; (Table 1). During Trial 1, fresh rumen fluid was collected from each sheep and cow, kept separate for each animal, and immediately incubated in flasks containing a buffer and either dried *A. arundinaceus* hay, or leaves or stems of *C. maculosa* (Trial 1a) or *T. vulgare* (Trial 1b).

Olson and Kelsey (1997) describe the *in vitro* system used to measure MGP, MPC, and IVDMD. Each plant species within each trial included three runs, with duplicates (n = 2 baths) in each run. Gas production was measured by water displacement in inverted burettes at 2 h, 3 h, 4 h, 6 h, 8 h, 12 h, 18 h, and

Table 1. Trials 1a, 2a (*Centaurea maculosa* Lam.) and 1b, 2b (*Tanacetum vulgare* L.): nutritive value of *A. arundinaceus* hay, *Centaurea maculosa,* and *Tanacetum vulgare*[1]

Item, %	CP	NDF	ADF	ASH
Ground forage material[2]				
A. arundinaceus hay	8.1	57.7	36.6	9.4
C. maculosa leaves	14.4	23.0	16.5	10.0
C. maculosa stems	7.5	53.0	44.6	6.1
T. vulgare leaves	17.6	25.2	17.6	12.2
T. vulgare stems	3.4	75.1	65.9	4.6
Chopped forage material fed to the animals				
Trial 1a: *C. maculosa*				
Sept. 27				
A. arundinaceus hay	6.8	53.9	35.5	9.5
Oct. 9				
A. arundinaceus hay	6.7	58.6	34.3	9.5
Trial 1b: *T. vulgare*				
Oct. 21				
A. arundinaceus hay	6.5	59.5	36.6	11.4
Trial 2a: *C. maculosa*				
Oct. 26				
A. arundinaceus hay, chopped	8.6	63.0	39.1	9.8
C. maculosa, chopped	10.6	40.2	33.7	7.5
Oct. 29				
A. arundinaceus hay, chopped	9.4	59.0	37.1	10.4
C. maculosa, chopped	10.7	38.9	32.3	8.0
Nov. 4				
A. arundinaceus hay, chopped	9.6	61.3	39.4	10.4
C. maculosa, chopped	12.1	31.3	25.4	9.2
Trial 2b: *T. vulgare*				
Nov. 10				
A. arundinaceus hay, chopped	9.3	59.5	37.5	11.4
Nov. 13				
T. vulgare, chopped	6.3	56.2	48.1	6.6
Nov. 16				
A. arundinaceus hay, chopped	9.2	58.7	37.9	11.4
T. vulgare, chopped	6.3	53.3	44.9	7.2

[1] Trial 1a: Sept. 27 to Oct. 15, 2004; Trial 1b: Oct. 16 to Oct. 22, 2004; Trial 2a: Oct. 26 to Nov. 5, 2004; Trial 2b: Nov. 6 to 21, 2004.
[2] Forage was ground to pass a 1-mm screen and placed into the flask during the *in vitro* trials.

24 h according to procedures of Roberts and Olson (1999) to determine MGP. After the 24-hr reading, flask contents were filtered to separate residue from the fluid fraction. Residues were dried at 60 ºC for 48 hr and weighed to determine IVDMD, and then analyzed for MPC (Zinn and Owens, 1986) as an indicator of microbial biomass.

For Trial 2a, one ewe and one cow were randomly selected to be conditioned over 10 d to *C. maculosa*, whereas the other ewe and cow were only fed *A. arundinaceus* hay at 2 percent BW (DM-basis). For the conditioned ewe and cow, quantity of *C. maculosa* in the diet was increased every other day in 5 percent increments until it comprised 25 percent of the diet. After Trial 2a, all ewes and cows were only fed *A. arundinaceus* hay for 7 d before Trial 2b. Protocol for Trials 2a and 2b was the same, except that *T. vulgare* was fed and the ewe and cow to be conditioned were switched.

Plant Material and Forage Analysis

During the summer of 2004, *C. maculosa* (early-maturity stage) and *T. vulgare* (late-maturity growth stage) were cut separately about 4 cm above the ground. Plants were separated into leaves and stems, dried at 45°C for 48 h, and ground in a Wiley Mill to pass a 1-mm screen; this material was placed into the flasks during the *in vitro* trials. To condition rumen microbes, *C. maculosa* (Trial 2a) and *T. vulgare* (Trial 2b) plants were chopped (less than 5 cm), air-dried, and then fed. Subsamples of *A. arundinaceus* hay, *C. maculosa*, and *T. vulgare* were ground and analyzed separately for ash and N by standard methods (AOAC, 1990); CP was calculated as 6.25 × N. The NDF and ADF were analyzed using Van Soest et al. (1991) procedures modified for an Ankom 2000 Fiber Analyzer (Ankom Technol. Corp., Fairport, N.Y.).

Statistical Analyses

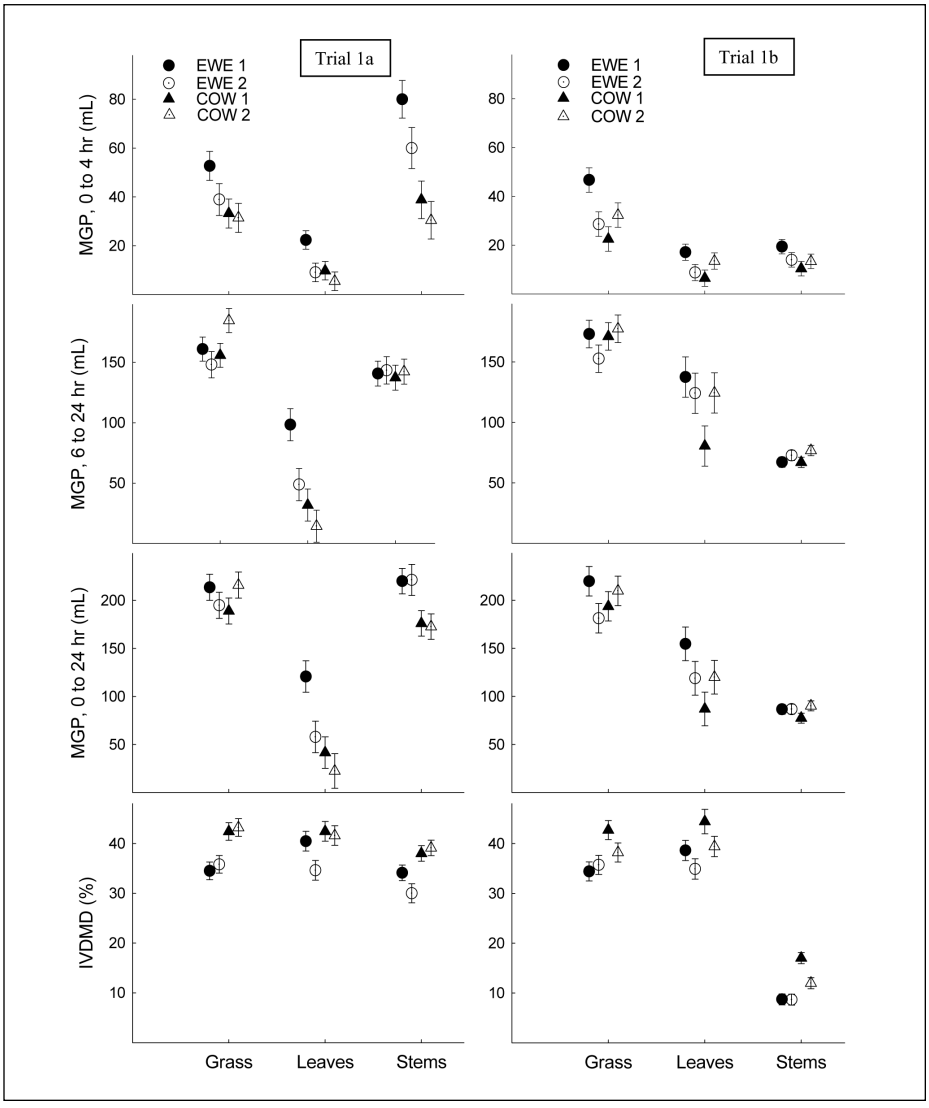
In both trials, the dependent variables MGP, MPC, and IVDMD of *A. arundinaceus* hay, and leaves or stems of the invasive species were analyzed using the Mixed procedure of SAS (SAS Inst. Inc., Cary, N.C.) and a model that included animal (treatment) as the fixed effect and flask as the experimental unit. Each trial was repeated three times (3 d per trial) and flasks were replicated in two water baths within each day; thus, six replications (flasks) per animal/trial were evaluated for each forage type (hay, leaves, or stems). A compound symmetry covariance structure was used for all variables in each model. For Trials 1a and 1b, orthogonal contrasts included: 1) ewe1 vs. ewe2, 2) cow1 vs. cow2, and 3) average of both ewes vs. average of both cows. For Trials 2a and 2b, contrasts included: 1) conditioned ewe vs. non-conditioned ewe, 2) conditioned cow vs. non-conditioned cow, and 3) conditioned ewe vs. conditioned cow. Spearman correlation coefficients were used to determine correlations of MGP, MPC, and IVDMD among ewes and cows (Trials 1a, b) and among the conditioned ewe and cow (Trials 2a, b).

Results and Discussion

Chemical Composition of *A. arundinaceus* Hay, *C. maculosa*, and *T. vulgare*

Chemical composition of the forages (Table 1) was not statistically analyzed, but differences were observed, which was probably due to sampling collection date, slight differences in soil and microclimate, or both. Quality of *C. maculosa* leaves and stems was comparable to previous reports (Table 1; Kelsey and Mihalovich, 1987; Olson and

Fig. 1. *In vitro* microbial gas production (MGP: 0 to 4 hr, 6 to 24 hr, and 0 to 24 hr) and dry matter digestibility (IVDMD) of grass hay, *C. maculosa* (Trial 1a) and *T. vulgare* (Trial 1b) leaves or stems from ambient rumen fluid. Data are presented as least squares means ± SE.



Kelsey, 1997). Ground *C. maculosa* and *T. vulgare* leaves, which were used in the flasks, had greater CP and less NDF and ADF than *A. arundinaceus* hay. The *T. vulgare* stems were considered a poor quality forage due to low CP and high fiber (NDF and ADF) concentrations. Chopped *C. maculosa* and *T. vulgare* plants, which were fed to the animals, contained a mixture of leaves and stems; thus, quality of the mixture was between leaf and stem qualities.

Centaurea maculosa

The IVDMD of *A. arundinaceus* hay and *C. maculosa* leaves and stems was greater with cow than with sheep rumen fluid ($P < 0.04$; Figure 1, Trial 1a). Cattle digest certain plant materials more than sheep (Playne, 1978), but cattle avoid *C. maculosa* in the field whereas sheep graze it. Our *in vitro* approach bypassed the animals' sensory capacity; thus, further research is warranted to determine if the bitter-tasting sesquiterpene lactone (cnicin) located in glandular trichomes on leaves of *C. maculosa* is more taste-aversive to cattle than sheep.

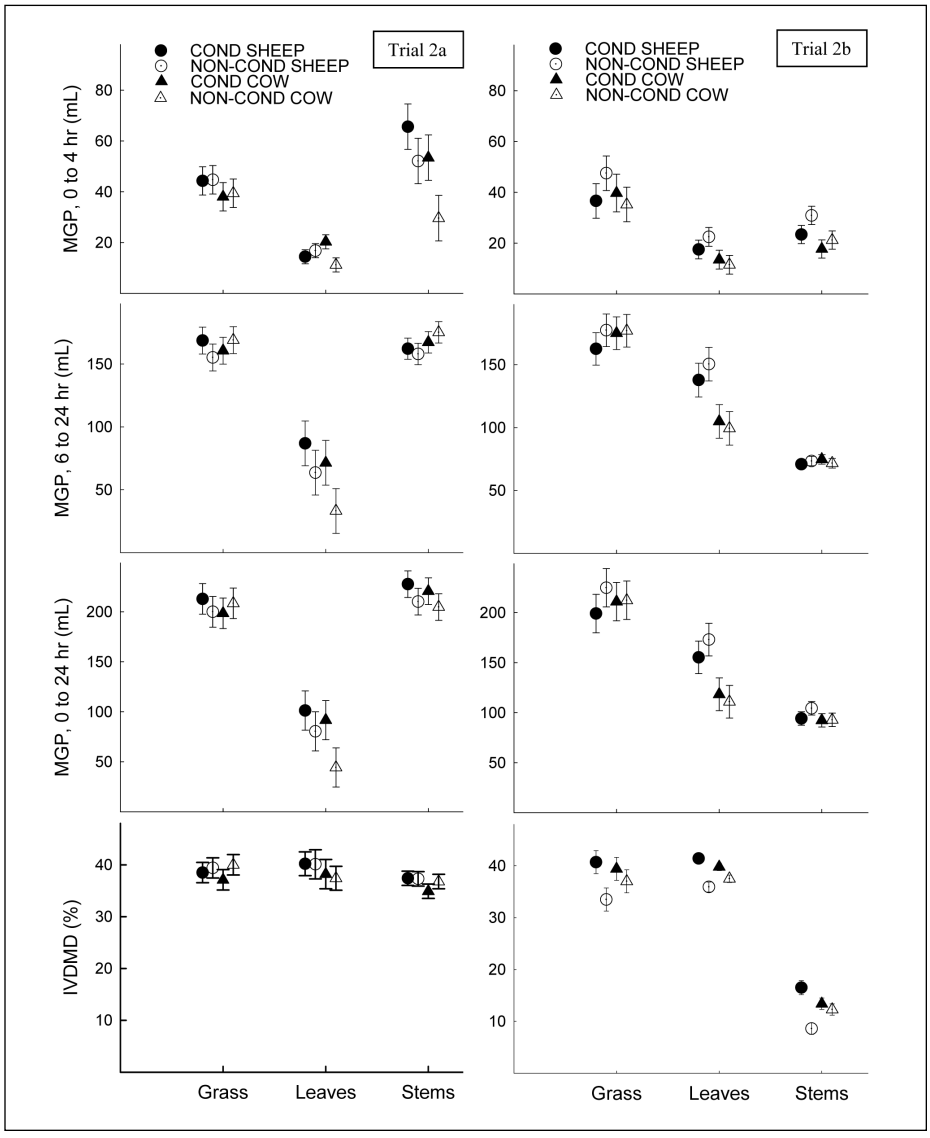
Total 24-h MGP from *C. maculosa* leaves and stems was low, but greater with sheep than cow rumen fluid ($P < 0.004$; Figure 1), even though MPC were similar ($P > 0.25$; 1.88 µg/mL to 3.57 µg/mL for leaves, 3.06 µg/mL to 4.73 µg/mL stems. Furthermore, MGP from *C. maculosa* leaves with sheep and cow rumen fluid was correlated ($r > 0.77$; $P < 0.05$) with MPC. Gas production from *C. maculosa* stems in sheep rumen fluid was not correlated with MPC, but was correlated ($r > 0.71$; $P < 0.05$) with MPC in cow rumen fluid.

Greater bacterial efficiency has been defined as less gas produced per unit of degraded organic matter (Blummel et al., 1997; Mlambo et al., 2008); thus, results indicated that rumen microbial populations from cattle rumen fluid were more efficient than from sheep rumen fluid. Greater efficiency may reflect that the secondary metabolites in *C. maculosa* differentially affect ambient sheep and cow rumen microbial populations. Soluble phenolics can be negatively correlated with MGP, but positively correlated with digestion (Mlambo et al., 2008). Cnicin concentrations in *C. maculosa* leaves may have been high enough to reduce MGP in cow rumen fluid only, without negatively affecting IVDMD. For example, a low saponin supply enhanced rumen microbial population growth, but reduced it when the dose was excessive (Sliwinski, et al., 2002). Leaves of *C. maculosa* collected in the same region at the same growth stage contained 3.6 percent cnicin concentrations (DM basis). Cnicin depresses MGP (Olson and Kelsey, 1997) and can inhibit rumen fermentation (Lowery and Kennedy, 1996).

Cnicin may have killed more cow than sheep rumen microbes, which would have reduced MGP, but would not have been detected in MPC; the technique used in the current study does not differentiate between alive and dead microbes. Even so, direct effects of toxicity may be more important than digestion inhibition when assessing deterrence by secondary metabolites (Bryant, et al., 1992). Thus, primary differences between non-conditioned sheep and cattle consuming *C. maculosa* may occur past the rumen, i.e., gut tissue or liver detoxification and excretion mechanisms (Foley et al., 1995; Provenza, 1995).

Conditioning rumen microbial population to *C. maculosa* (Figure 2, Trial 2a) was expected to increase IVDMD

Figure 2. *In vitro* microbial gas production (MGP: 0 to 4 hr, 6 to 24 hr, and 0 to 24 hr) and dry matter digestibility (IVDMD) of grass hay, *C. maculosa* (Trial 2a) and *T. vulgare* (Trial 2b) leaves or stems from rumen fluid conditioned with either *C. maculosa* (Trial 2a) or *T. vulgare* (Trial 2b). Data are presented as least squares means ± SE.



and MPC from leaves and stems with cow, and especially, sheep rumen fluid, because sheep graze the plant. However, conditioning did not enhance ($P > 0.10$) IVDMD, MGP, or MPC with rumen fluid from either animal species, which may have been due to the length of conditioning period not being long enough to allow microbial species to fully adapt. In the current study, nutrients were not limited, e.g., CP concentration in *C. maculosa* leaves was greater than 14 percent. Thus, conditioning with *C. maculosa* may not have been effective because anti-nutritional compounds in plants primarily affect microbial efficiency and production when nutrient availability is limiting (Lanyasunya et al., 2008). However, it should also be noted that some of the CP in *C. maculosa* leaves was from cnicin and not totally available to the animal.

With fluid from the conditioned cow, MPC and 24-h MGP from leaves were negatively correlated with IVDMD ($r = -0.97$; $P < 0.01$ and $r = -0.92$; $P < 0.05$, respectively), but positively correlated with IVDMD in the non-conditioned cow ($r = 0.61$ and $r = 0.70$; $P < 0.05$, respectively). A negative correlation between IVDMD, and MPC and 24-h MGP with conditioned cow rumen fluid indicates that microbial protein synthesis was favored over short-chain fatty acid production (Blummel and Orskov, 1993) and that the cow conditioned to *C. maculosa* was more efficient than the non-conditioned cow.

Unexpectedly, IVDMD, MGP, or MPC of the *A. arundinaceus* hay (control) did not differ between *C. maculosa*-conditioned and non-conditioned animals, indicating *in vitro* microbial populations were not negatively affected by cnicin. Adding *C. maculosa* to the basal diet could have increased bacterial utilization of the *A. arundinaceus* hay because of the added nutrients from *C. maculosa* leaves, or decreased bacterial utilization, because of secondary metabolites, e.g., cnicin, inhibiting MPC or MPG. Any negative effect associated with this secondary metabolite in *C. maculosa* may have been mitigated by its relatively high quality.

Tanacetum vulgare

The IVDMD and MPC of *T. vulgare* leaves and stems were expected to be less with non-conditioned sheep and cow rumen fluid, because they do not readily consume the plant. *Tanacetum vulgare* contains tanecetin, a sesquiterpene lactone that has antimicrobial properties (Hein, 1952); however, IVDMD of leaves was high (Figure 1, Trial 1b). Livestock may also avoid mature *T. vulgare*, because of its hardened stems resembling thin, mature corn stalks with low CP, and high NDF and ADF (Table 1). The nutritive value of the stems indicates that they contain minor amounts of cell solubles with considerable non-digestible fiber, i.e. ADF (Table 1). Presumably, the tanecetin and high fiber concentrations resulted in the low IVDMD, 24-h MGP, and MPC (1.45 µg/mL to 1.73 µg/mL) of stems with sheep- and cow-rumen fluid. In contrast to *T. vulgare* stems, leaves could be classified as a high-quality forage, based on CP, NDF, and ADF concentrations, while ignoring palatability or toxin issues.

The IVDMD of the *A. arundinaceus* hay and *T. vulgare* leaves and stems was greater with cow- than sheep-rumen fluid ($P < 0.03$; Figure 1, Trial 1b). Total 24-h MGP and MPC were greater with sheep- than with cattle-rumen fluid ($P = 0.07$, $P = 0.003$, respectively). Further, MGP from *T. vulgare* leaves with sheep-rumen fluid was correlated with IVDMD ($r = 0.67$, respectively; $P < 0.04$), but not correlated ($P > 0.54$) with IVDMD with cow-rumen fluid. Similar to *C. maculosa*, cattle-rumen microbes seemed more efficient than sheep-rumen microbes digesting *T. vulgare* leaves, i.e., greater digestion with less 24-h MGP and MPC.

Conditioning rumen microbial populations to *T. vulgare* was expected to increase IVDMD and MPC from leaves and stems, with the increase being greater for sheep than cow rumen fluid. Conditioning ewe rumen microbes to *T. vulgare* resulted in 7.2 percent, 5.4 percent and 7.9 percent greater ($P > 0.04$; Figure 2, Trial 2b) IVDMD of *A. arundinaceus* hay and *T. vulgare* leaves and stems, respectively, compared with fluid from the non-conditioned ewe, but 24-h MGP was similar ($P > 0.30$). Conditioning cow rumen microbes to *T. vulgare* did not enhance or decrease ($P > 0.12$; Figure 2, Trial 2b) IVDMD or 24-h MGP of *A. arundinaceus* hay or *T. vulgare* leaves or stems relative to fluid from the non-conditioned cow. The MPC for both animal species and *A. arundinaceus* hay, *T. vulgare* leaves or stems were similar ($P > 0.14$). In addition, IVDMD of *T. vulgare* stems was greater ($P = 0.09$) with fluid from the conditioned ewe than the conditioned cow. Results indicated that conditioning sheep, but not the cow, to *T. vulgare* increased microbial efficiency and was effective. Sheep may have specialized bacteria, allowing them to adapt to certain plant chemicals, which are not tolerated by cow bacteria (Jones and Megarrity, 1983, 1986).

Conclusions

By traditional measures of forage quality, *C. maculosa* leaves and stems, and *T. vulgare* leaves used in this study seemed to be nutritious forages, at least to rumen microbial populations. Utilization of *T. vulgare* by sheep may be increased if they are conditioned to *T. vulgare* before grazing. In fact, depending on secondary metabolite concentrations and quantity and quality of other available forages, sheep grazing *T. vulgare* may actually have greater IVDMD of grasses, which may increase growth rate or milk production. Grazing "value" and definition of any weed can change if negative consequences of consuming that weed are countered by positive responses from nutrients it offers. Based on size, cattle could consume much more *T. vulgare* than sheep if their microbial populations could be conditioned as effectively. Therefore, additional research is needed to evaluate differences between conditioning sheep and cattle on microbial species concentrations and physiological functions. Furthermore, because IVDMD of *C. maculosa* and *T. vulgare* leaves were high but their intake in the field is generally low, effects of secondary metabolites on gut tissue and liver metabolism should be evaluated.

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Manipulating Sheep Browsing Levels on Coyote Willow *(Salix exigua)* with Supplements[1]

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Summary

Macronutrients and additives have been used to suppress or promote livestock intake of upland tannin-containing browse species, but to our knowledge this technique has not been applied to sheep that feed on tannin-rich species in riparian areas. The objective of this study was to determine the effect of four supplement regimes on coyote willow (*Salix exigua*) intake by sheep during the dormant and growing seasons. Twelve Western White Face lambs (48 kg ± 4.5 kg) were placed in individual pens and assigned to one of four treatments which consisted of a basal diet of sudangrass and supplements predicted to either suppress (whole corn or quebracho tannin) or promote (cottonseed meal or polyethylene glycol, PEG) willow intake. Each of the four supplements was tested with dormant and growing willow in a Latin rectangle design with three periods and six lambs per group. Basal diet (sudangrass) intake was not affected by either promoter nor suppressor treatments in either season. Cottonseed meal effectively promoted intake of willow compared to the control and PEG treatments ($P< 0.05$) in the dormant season. No difference was detected between the control, quebracho-tannin, and whole-corn treatments, although the latter tended to depress dormant-willow intake of lambs. None of the treatments altered intake of coyote willow in the growing-season trials. Protein and possibly corn-based supplements may be effective tools to manipulate sheep browsing levels of *Salix exigua* but need to be tested in a field setting before management strategies with supplementation can be applied.

Key Words: Targeted Grazing, Tannins, Cottonseed Meal, Whole Corn, Polyethylene Glycol, Quebracho Tannin

Introduction

Willows (*Salix spp.*) are generally considered an important component of riparian areas because they influence biological diversity, water quality/quantity, and aquatic/terrestrial food webs and habitats of these ecosystems (Kauffman et al. 1983a, b; Mitsch and Gosselink 1986; Elmore and Bescheta 1987; Case and Kauffman 1997; Clary and Kruse 2004). Consequently, protection of willow populations from excessive browsing by domestic livestock is a common riparian management objective on rangelands of western North America. Nonetheless, in irrigated valleys or in areas where production of meadow grass hay is important, willow thicket encroachment from watercourse margins onto stream floodplains and terraces is considered undesirable. In such situations, hay farmers seek to control the spread of this species because it hinders the hay harvesting process and competes aggressively for water and other resources with the herbaceous understory (Gulickx et al. 2007). Thus, depending on site-specific management objectives, ranchers could manage livestock grazing to either promote or contain the growth of willow populations. This study investigated the feasibility of manipulating sheep preference for coyote willow by feeding supplements that were predicted to either suppress or stimulate intake levels of this woody plant.

Feed-supplementation strategies have been used repeatedly to modify livestock preference for woody species that contain secondary compounds (Silanikove et al. 1994, 1996; Gilboa et al. 2000; Provenza et al. 2000; Titus et al. 2000, 2001; Landau et al. 2002; Villalba et al. 2002a, b, c; Provenza et al. 2003; Villalba and Provenza 2005; Dziba et al. 2007). For example, concentrate supplements rich in starch, such as corn, can decrease intake and digestibility (Hanna et al. 1989; Pordomingo et al. 1991) and reduce preference for low-digestible forage, while a small quantity of condensed tannins added to the diet as supplement can induce animals to eat less of foods containing tannins (such as willows) when overall dietary protein is low (Villalba et al. 2002a, c). On the other hand, additives, such as polyethylene glycol (PEG) or supplemental protein, can induce goats and lambs to increase intake of tannin-rich species (Petersen and Hill 1991; Silanikove et al. 1996; Gilboa et al. 2000; Landau et al. 2002; Villalba et al. 2002a, c). To our knowledge, no prior studies have examined the effects of feed supplementation on intake levels of sheep that have access to willow, a plant genus, which usually contains high levels of tannins (Palo 1984; McWilliam et al. 2005).

The objective of this study was to determine the feasibility of: a) promoting sheep browsing on willow by offering supplements high in protein or containing a compound that binds tannins (i.e., PEG); and b) suppressing sheep browsing on willow by offering supplements high in energy or containing an external source of tannins. Specifically, we hypothesized that willow intake by sheep would be stimulated with cottonseed meal or PEG supplements and suppressed with corn or quebracho tannin.

Materials and Methods

Experimental Design

Our experiment was conducted in February and July 2006 at the New Mexico State University Campus Farm in Las Cruces, N.M. The Institutional Animal Care and Use Committee at New Mexico State University approved all procedures used in this experiment. Twelve Western White Face ewe lambs (48.1 kg ± 4.5 kg BW) were housed in individual pens (2 cm x 5m) with access to shelter and fresh water and were randomly divided into two groups. One group received supplements expected to suppress willow intake (SUP), while the other group received supplements predicted to enhance willow intake (PRO). A 3 x 6 Latin Rectangle experimental design was used to allow each animal within a group to consume both supplements and the control diet (n = 2 animals per treatment group per period). The three SUP treatments consisted of either: basal diet + whole corn; basal diet + quebracho tannin; or basal diet alone (control). A small amount of soybean meal was added to the ration of animals in the control (basal diet alone) and tannin (basal diet + quebracho tannin) treatments to ensure that all SUP diets were isonitrogenous (Table 1). The three PRO treatments consisted of either: basal diet + cottonseed meal; basal diet + PEG; or and basal diet alone (control).

Table 1. Percent composition of experimental diets predicted to suppress (SUP) or promote (PRO) willow browsing (*Salix exigua*) by sheep.

	SUP diets			PRO diets		
	Control	Energy	Tannin	Control	Protein	PEG[a]
	DM basis					
Sudangrass hay	97.49	64.83	91.03	83.56	76.66	76.04
Ground corn	0.00	0.00	0.00	15.39	0.00	20.07
Whole corn	0.00	34.11	0.00	0.00	0.00	0.00
Cottonseed cake	0.00	0.00	0.00	0.00	22.30	0.00
Soybean meal	1.47	0.00	2.43	0.00	0.00	0.00
PEG	0.00	0.00	0.00	0.00	0.00	2.84
Quebracho tannin (80%)	0.00	0.00	5.50	0.00	0.00	0.00
Mineral vitamin premix[b]	1.04	1.06	1.04	1.05	1.04	1.05
Total	100.00	100.00	100.00	100.00	100.00	100.00
DM%	92.00	91.00	93.00	91.00	92.00	92.00
CP%[c]	6.87	6.87	6.87	6.61	15.00	6.61
ME (Mcal/kg)[c]	1.73	2.22	1.73	2.00	2.00	2.00

[a] Polyethylene Glycol, Molecular weight 6000 g/mol
[b] Mineral oil 1.5%; limestone 33.74%; dicalcium phosphate 33.23%; salt 12.70%; ammonium sulfate 4.06%; EDDI 13.51%; selenium 0.54%; vitamin A 0.26%; vitamin E 0.46%.
[c] Estimated from NRC (1985)

A reduced amount of ground corn was added to the ration of animals in the control (basal diet alone) and PEG (basal diet + PEG) treatments to ensure that all PRO treatments were isoenergetic (Table 1). Treatment order was randomly determined for each animal in both the SUP and PRO groups. The study was conducted during the dormant season (February 2006) and repeated during the growing season (July 2006). Twelve different lambs from the same flock were used in each test.

Treatments

Ground sudangrass (*Sorghum vulgare var. sudanense*) hay was offered as the basal diet during the February 2006 trials to simulate the quality of dormant-season, herbaceous-rangeland forage. The sudangrass offered during the growing-season trials was current season growth harvested from a local farm and expected to have a higher nutritional value (up to 17-percent CP; NRC 1985) than that used for the dormant-season trials. In both trials, sudangrass hay was offered at 1.7-percent BW.

The SUP diets were isonitrogenous (Table 1) and contained either 5-percent quebracho tannins or corn at 0.4 percent BW mixed with ground sudangrass hay (control animals received hay only). The PRO diets were isoenergetic (Table 1) and consisted of sudangrass hay plus 20 g of polyethylene glycol 6000 g/mol (Sigma Aldrich, St. Louis, Mo.) or cottonseed meal (15-percent CP).

The SUP and PRO supplements were mixed with the basal sudangrass diet (Table1), which was offered in the evening (7:00 p.m.) and removed two hours before feeding willow (8:00 a.m.) the following morning. Amount of feed rejected was recorded daily to the nearest gram. Each period included a 5-day, diet-adaptation period followed by a 7-day trial period. Adaptation time was assumed to serve as a washout period to avoid carry over effects.

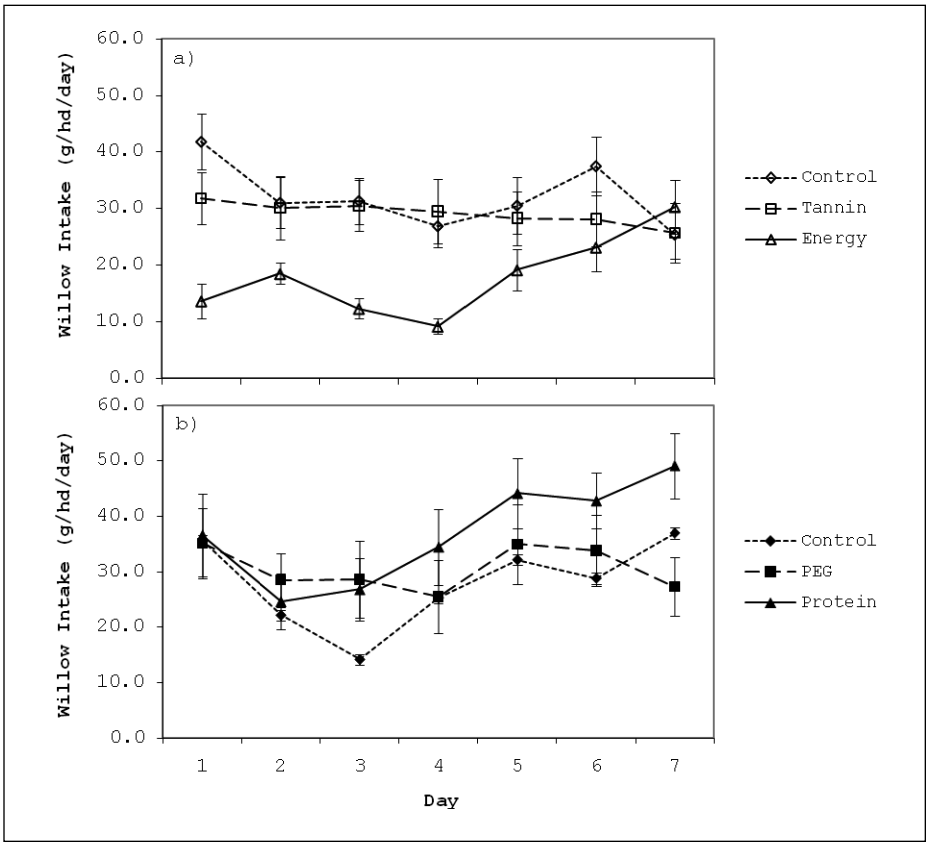
Willow Intake

Coyote willow (*Salix exigua*) branches were harvested from a designated site in the Rio Grande Valley about 8.5 km north of our experiment site and stored in a walk-in refrigerator at 4°C. Stems were clipped between 55 cm to 80 cm in length and collected from the grazeable height range of sheep (<1.5m) every 10m along the 100m-long, designated-harvest site.

Approximately 1200 grams of willow were removed from the refrigerator one hour prior to feeding tests each day. Each animal was offered 100 grams of willow per day in each of the three 7-day periods. Willow biomass offered was assumed to be sufficient to allow *ad libitum* intake during the 15-minute feeding trial period. Willow stems were stapled to a 1.5 m wooden stand at a 45-degree angle 50 cm to 95 cm from the ground. Stands were secured in each pen for a 15-minute feeding bout and then removed. The weight of the willow, plus the stand, was recorded before and after each individual feeding bout to the nearest gram. The difference in weight was calculated as willow consumed by each animal. A subset of willow stems (30 g) placed outside the pens was used to monitor water loss from willow stems and adjust intake estimates.

Twelve willow samples (55 cm to 80 cm, 20 g to 35 g) were collected during the dormancy (stems only) and growing season (stems and leaves), ground, and analyzed for condensed tannins using the vanillin-HCl method of Burns (1971) as modified by Price et al. (1978) at the end of each trial. Leaf-to-stem weight ratio of the growing-season willow samples was determined using 30 samples from 55 cm to 80 cm in length collected at the same site. Leaves were removed from stems and samples and dried at 50°C for 72 hours. Percent contribution to total sample weights of leaves and stems was calculated.

Figure 1a. Mean willow intake of lambs receiving diets that were predicted to suppress (a) or promote (b) willow intake (SUP and PRO treatments) across days during the dormant season. SUP diets consisted of sudangrass alone (Control), sudangrass + quebracho tannins (Tannins), or sudangrass + whole corn (Energy). Tannin and Control diets contained small amounts of soybean meal to ensure that all SUP diets were isonitrogenous. PRO diets consisted of sudangrass alone (Control), sudangrass + polyethylene glycol (PEG), or sudangrass + cotton seed meal (Protein). PEG and Control diets contained small amounts of ground corn to ensure all PRO diets were isoenergetic. Error bars indicate SEM.



Statistical Analyses

Willow- and basal-diet intake were analyzed separately for dormant and growing-season trials and for PRO and SUP groups. The effects of treatments during each season and within each group were analyzed in a 3 by 6 Latin Rectangle. The two blocking factors were time period (3 levels), and lamb (6 levels). A repeated measures analysis of days (7 levels) was conducted for each animal-time period combination. An autoregressive covariance AR(1) structure was assumed (Littell et al. 2006). The model allowed for heterogeneous variance for each lamb. The analyses were performed using Proc Mixed in SAS version 8.2 (SAS Institute Inc., 1999, Cary, N.C.). Statistical significance was considered at the $P = 0.05$ level.

Results and Discussion

During dormancy, willow offered consisted of bare twigs without leaves and contained 7.5 percent ± 1.2 SD condensed tannin. The sudangrass offered contained 51-percent TDN, 1.80 Mcal ME, and 6.3-percent CP (Table 1).

Intake of dormant willow by lambs was not suppressed when supplemented with whole corn or quebracho tannin ($F = 1.20$; $P = 0.35$). A day effect was observed ($P = 0.03$, Figure 1a) along with a day by treatment interaction ($P < 0.01$, Figure 1a) that reflected initial suppression of willow intake by diets containing corn, which tended to decrease over time within periods. No difference among treatments was observed on basal-diet consumption ($P = 0.21$) (Table 2). On average, lambs consumed 87-percent ± 5 SD of their offered diet (1.7-percent BW) (Table 2).

Our results differ from other studies that reported whole corn decreased forage intake, digestibility, and grazing time because animals met energy requirements sooner (Hanna et al. 1989; Pordomingo et al. 1991). Villalba et al. (2002c) reported a decrease in consumption of tannin-rich forage with corn supplementation, although their high-energy treatment contained 2.75 Mcal/kg of ME, which was somewhat higher than the 2.22 Mcal/kg of ME in high energy diets in this study. Suppressor diets containing higher ME content may lead to stronger willow intake depression than what was observed in this study.

Supplements predicted to promote willow intake affected the levels of dormant willow consumed by lambs ($P = 0.05$, Table 2). Cottonseed meal increased dormant-willow intake over the control treatment ($F = 4.59$, $P = 0.03$, Table 2). A day effect was observed ($P = 0.01$, Figure 1b), which reflected the tendency of lambs receiving cottonseed meal to consume increasing amounts of willow as trial days progressed. Animals receiving PEG exhibited intermediate levels of willow intake, which was not significantly higher than the control treatment (Table 2). No difference was observed in basal-diet consumption among treatments ($P = 0.62$, Table 2). On average, lambs consumed 93-percent ± 4 SD of their offered diet (1.7-percent BW, Table 2).

These results agree with previous research, demonstrating that lambs are better able to cope with foods containing high concentration of tannins if they are fed supplements that help offset nutrient losses incurred during metabolic detoxification processes (Freeland and Janzen 1974; Silanikove et al. 1997; Dziba et al. 2007). In addition to offsetting detoxification costs, supplemental protein may stimulate rumen-microbial activity and consequently increase digestibility, thereby reducing the negative feedback associated with ingestion of low-quality feeds containing tannins (Villalba et al. 2002c; Provenza et al. 2003).

Our results differ, however, from previous studies that observed a significant increase in the intake of tannin-rich foods when receiving PEG (Silanikove et al. 1994, 1996; Gilboa et al. 2000; Provenza et al. 2000; Titus et al. 2001; Villalba et al. 2002a, c). Our results also disagree with an earlier study conducted by Villalba et al. (2002c) who

Table 2. Mean daily intake (%) of willow and basal sudangrass ration for lambs receiving diets predicted to either suppress (SUP) or promote (PRO) consumption of dormant and growing coyote willow.

	Dormant Season Experiment				Growing Season Experiment			
	Willow[c]		Forage		Willow		Forage	
Treatment	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control[a]	32.0	21.3	92.2	14.8	74.9	31.1	99.7	1.2
Tannin	29.2	22.9	83.0	21.6	82.0	24.5	99.4	2.2
Energy	18.0	15.9	87.5	17.7	78.7	29.6	100.0	0.0
Control[b]	27.9[a]	26.5	89.5	23.9	74.8	30.4	100.0	0.0
PEG	30.6[ab]	28.6	91.1	19.1	74.8	29.1	100.0	0.0
Protein	37.0[b]	28.9	97.6	7.6	72.4	30.5	100.0	0.0

[a] SUP diets: control, quebracho tannin, whole corn
[b] PRO diets: control, Polyethylene glycol (PEG 6000 g/mol), cottonseed meal
[c] Column means followed by the same letter or without letters were not significantly different, $P < 0.05$ (Least Significant Difference Test).

determined that PEG was more effective than macronutrients for promoting intake of foods containing tannins. This apparent inconsistency may be due to the fact that tannin content in coyote willow during the dormant season was about half (7.5 percent) of that in the tannin-rich (15 percent) diets used by Villaba et al (2002 a, c). Alternatively (although unlikely), ground corn used in the PEG and control rations, to ensure that all PRO diets were isoenergetic, could have altered rumen pH (Murphy et. al 1994) thus inducing a slight decrease in willow intake, which may have neutralized the predicted effects of PEG. Our results suggest that nitrogen-rich supplements hold the most promise as promoters of dormant-willow intake.

Willow leaves and stems during the growing season trials contained an average of 15.2- percent ± 1.91 SD and 4.0-percent ± 1.34 SD condensed tannins, respectively, and accounted for 55 percent and 45 percent of average-sample DM weights. Thus, the average concentration of condensed tannins in the growing-season willow (stems and leaves) was 10.16 percent.

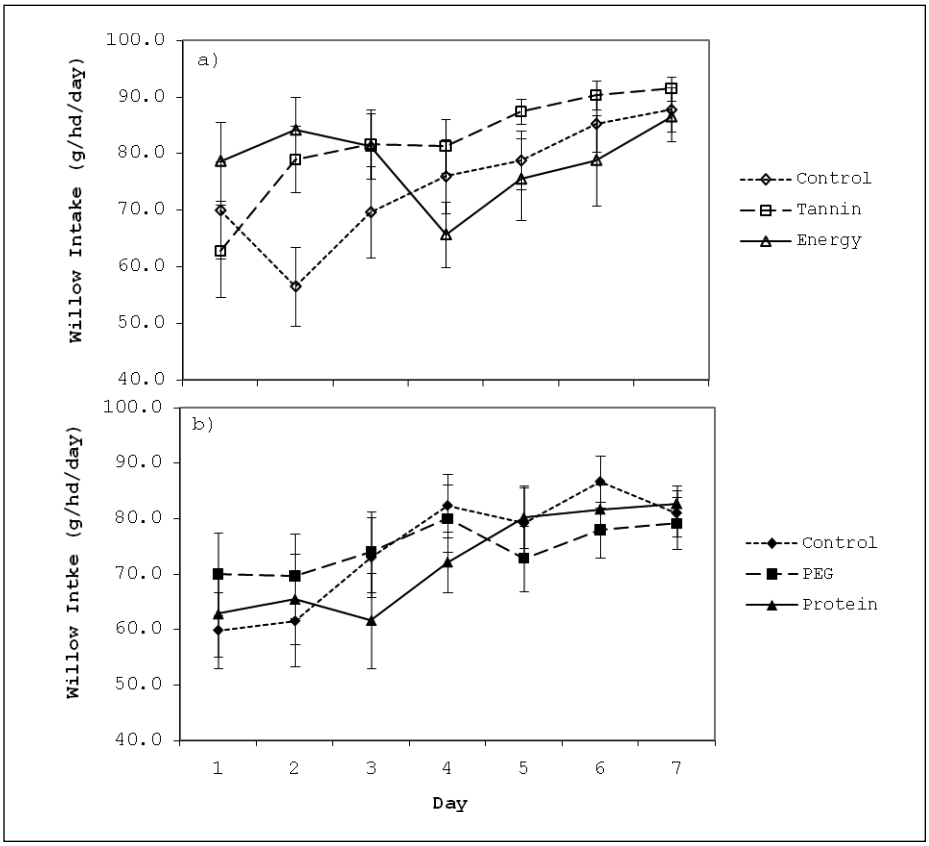
Willow intake did not differ among SUP treatments ($F = 0.23$, $P = 0.8$, Table 2) in the growing-season trials. A day effect was observed ($P = 0.04$, Figure 2a), which apparently reflected a transient, willow-intake suppression on day 4 for lambs fed the corn supplement. No difference among treatments was observed in basal-diet consumption ($P = 0.18$, Table 2).

Not surprisingly, lambs consumed almost 2.5 times more willow in summer than in winter (Table 2), which possibly reflected differences in nutrient concentration and palatability of green leafy material vs. dry stems. Treatments predicted to suppress willow intake (SUP) were not effective in altering sheep preference for willow in summer, although a transient depression in intake of lambs receiving corn appeared to occur on day 4. Difficulties in delivering quebracho tannins in the basal-diet mixture may have influenced our results. Further research is needed to test the effects of added tannins in supplements that conceal their astringency and preclude animal sorting.

Willow intake did not differ among PRO treatments ($F = 0.69$, $P = 0.53$, Table 2) during the growing season. A day effect was observed ($P < 0.01$, Figure 2b), which apparently reflected a decrease in willow intake in lambs receiving PEG supplement on days 5 to 7. Basal-diet consumption did not differ among treatments and was almost 100 percent each day (Table 2).

Increased nitrogen content of green willow and sudangrass may have improved willow palatability and possibly offset the negative effects of increased tannin concentrations, overshadowing any effects of PRO supplements in the growing-season study. Coyote willow contains up to 16.5-percent CP in the growing season (D.Cram, unpublished data) and recently harvested sudangrass hay can contain up to 17-percent CP (NRC 1985). The high levels of willow intake during the summer trials (animals avidly consumed most of the branches offered) strongly suggest that the use of supplements to boost willow intake during the growing season may be unnecessary.

Figure 1b. Mean willow intake of lambs receiving diets that were predicted to suppress (a) or promote (b) willow intake (SUP and PRO treatments) across days during the growing season. SUP diets consisted of sudangrass alone (Control), sudangrass + quebracho tannins (Tannins), or sudangrass + whole corn (Energy). Tannin and Control diets contained small amounts of soybean meal to ensure that all SUP diets were isonitrogenous. PRO diets consisted of sudangrass alone (Control), sudangrass + polyethylene glycol (PEG), or sudangrass + cotton seed meal (Protein). PEG and Control diets contained small amounts of ground corn to ensure all PRO diets were isoenergetic. Error bars indicate SEM.



Conclusions

Protein supplementation with cottonseed meal was effective at increasing intake of dormant-coyote willow by sheep when the quality of the alternative forage was low. More research is needed to identify opportunities to suppress sheep consumption of dormant-coyote willow with whole corn. Feasibility of manipulating coyote-willow preference of sheep using supplements during the growing season appears much less likely. Sheep in this study consumed almost all willow branches offered during the growing season, regardless of the supplement fed.

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Effects of Physical Isolation on Serum and Salivary Cortisol and Components of Complete Blood Counts in Yearling Ewes[1]

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Summary

Isolation is often stressful to herd animals. The objective of this study was to compare changes in serum and salivary cortisol concentrations and complete blood count (CBC) components in physically isolated sheep. Twelve Suffolk x Hampshire yearling ewes (64 kg ± 1.2 kg) were held indoors in either a common pen or individual pens for 10 consecutive days. Individual pens did not allow physical contact, but did not obstruct vision or sound of adjacent sheep. Serum and whole blood samples (venipuncture) and salivary samples (oral swab) were collected at 0700 (AM) and 1300 (PM) on each day with the exception of day 1 (no AM sample). Additionally, intensive samples were taken in 15-min intervals over a 2-h period on days 1, 5, and 10 for correlation purposes. Serum and salivary cortisol concentrations (RIA) and specific blood components (CBC) were determined. Serum cortisol concentrations did not differ between treatments in AM ($P = 0.452$) or PM samples ($P = 0.827$). Similarly, salivary cortisol concentrations did not differ between treatments for either period ($P > 0.768$). Serum and salivary cortisol concentrations were closely correlated among all samples ($r = 0.83$, $P < 0.001$). White blood cells were reduced ($P < 0.022$) by isolation on days 1, 2, 4, 5, and 6 in PM samples, but AM samples were not affected ($P = 0.594$) by treatment. Isolation also reduced neutrophils ($P < 0.037$) and increased lymphocytes ($P < 0.049$) on days 1, 2, and 5 in PM samples only. Mean corpuscular volume was reduced ($P < 0.001$) by isolation on all days in both AM and PM samples. Conversely, mean corpuscular hemoglobin concentrations were increased ($P < 0.009$) in all samples. Hematocrit was reduced in isolated sheep from day 2 to day 6 in AM samples ($P < 0.037$) and on all but day 10 in PM samples ($P < 0.050$). Physical isolation did not appear to influence other CBC components, including red blood cells and hemoglobin concentration. In general, stress components were greater during the first two days of isolation, regardless of treatment. This was likely due to the unfamiliar environment. Data from this study indicate physical isolation of yearling ewes for 10 days without visual and auditory isolation did not elicit noticeable changes in cortisol concentrations, while alterations in immune components of CBC were generally mild and inconsistent. Although certain non-immune components were substantially affected by physical isolation, causes or physiological significance of these changes are unclear.

Key Words: Complete Blood Counts, Isolation Stress, Salivary Cortisol, Serum Cortisol

Introduction

Although sheep have evolved as herd animals, many non-traditional production conditions, such as club-lambing, research settings, and maintenance of superior breeding stock require individual penning of sheep for extended periods of time. However, ramifications of physiological stress associated with unfamiliar and unnatural conditions have been documented (Morrison, 1983). Currently, reports of physiological stress levels in sheep facing seclusion are varied. Stackpole et al. (2003) and Tilbrook et al. (2008) reported no increase in serum concentrations of the stress hormone, cortisol, in sheep isolated for brief periods of time, while others have described consistent increases in blood cortisol concentrations (Apple et al., 1995; Degabriele and Fell, 2001; Wagenmaker et al., 2009), especially when isolation includes visual and auditory obstruction from other sheep. In addition to increased cortisol production, some have reported changes in immune components in stressed sheep, including increased numbers of white blood cells and neutrophils and decreased numbers of lymphocytes (Minton and Blecha, 1990; Minton et al., 1992). Less understood are changes in non-immune blood components as a response to stress in sheep, including reductions in hematocrit (Ali et al. 2001) and increased hemoglobin (Al-Qarawi and Ali, 2005). The objective of the present study was to evaluate changes in traditional stress markers, including serum cortisol concentration, salivary cortisol concentration, and components of complete blood counts in sheep exposed to physical, but not visual or auditory, isolation for 10 days and to quantify physiological stress levels in these isolated sheep.

MATERIALS AND METHODS

Animal care and facilities

The New Mexico State University Institutional Animal Care and Use Committee approved all procedures. Before use, ewes were weighed (64 kg ± 1.2 kg) and examined for health. Animals were held indoors under artificial cooling (25° C ± 4° C) and light (approximately 12 h of light and 12 h of dark each day) for the duration of the experimental period. All ewes had free access to water and were fed chopped alfalfa hay (approximately 1.25 kg/ewe) once daily at 0600. Approximately 7 cm of wood shavings were used as bedding over concrete floors. Bedding was changed every 72 h. Experimental procedures were conducted at New Mexico State University, Las Cruces, N.M. (32° 19' 11" N, 106° 45' 55" W; elevation 1,219 m) beginning on March 5, 2008. All ewes were of mixed Suffolk x Hampshire breeding and were produced at New Mexico State University. Ewes ranged from 10 mo to 12 mo of age and averaged from moderate to good body condition. Following the experimental period, all ewes received a cautionary dose of liquamysin (LA-200, 5 mL, s.c.).

Isolation model

Six ewes were randomly assigned to a common 6-m x 5-m indoor control pen with no divisions, while six ewes were penned in individual 2-m x 5-m indoor pens. All pens were constructed of galvanized-mesh, cattle panels (6 cm x 12 cm openings), thus, ewes in individual pens were physically isolated but not visually or audibly obstructed from other ewes. Individual pens were spatially separated by no less than 2 m to avoid across-fence physical contact. Ewes were placed in assigned pens for 10 d, beginning at 1200 on d 1. Blood and salivary samples were collected at 0700 and 1300 each day with the exception of d 1, on which no morning sample was collected. On d 1, d 5, and d 10, samples were collected intensively at 15-min intervals for 2 h, beginning at 1300 for use in correlation comparisons.

Sample collection and analysis

Blood samples were collected via jugular venipuncture into 10 mL vacuum tubes (Corvac serum-separator, Kendall Health Care, St. Louis, Mo.). Blood samples were kept at room temperature for 30 min to 60 min and were centrifuged (1,500 x g at 4° C for 15 min). After centrifugation, serum was stored in plastic vials at -80° C until assayed. For each blood sample, a simultaneous saliva sample was collected via 30 to 45 second swab of the mouth with a 1 by 2 cm cotton strip held with surgical forceps as previously described (Yates et al., 2010). Saliva samples were placed in salivette tubes (Sarstedt AG and Co., Numbrecht, Germany) and cooled in ice immediately after collection, then centrifuged (1,500 x g at 4° C for 15 min) and stored at -80° C until assayed. Serum cortisol was quantified by solid phase RIA using components of a com-

Figure 1. Serum cortisol concentrations in samples collected at 0700 from young ewes housed in a common pen (control) or those maintained in individual pens (isolated) for 10 days. Housing effect, *P* = 0.452, day effect, *P* < 0.001, isolation x day, *P* = 0.110.

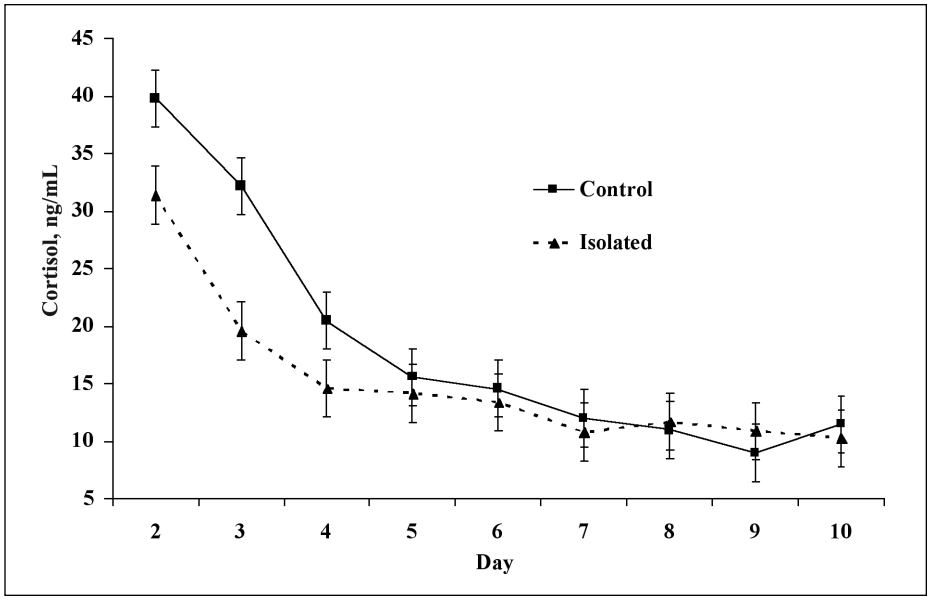


Figure 2. Serum cortisol concentrations in samples collected at 1300 from young ewes housed in a common pen (control) or those maintained in individual pens (isolated) for 10 days. Housing effect, *P = 0.827*, day effect, *P < 0.001*, isolation x day, *P = 0.657*.

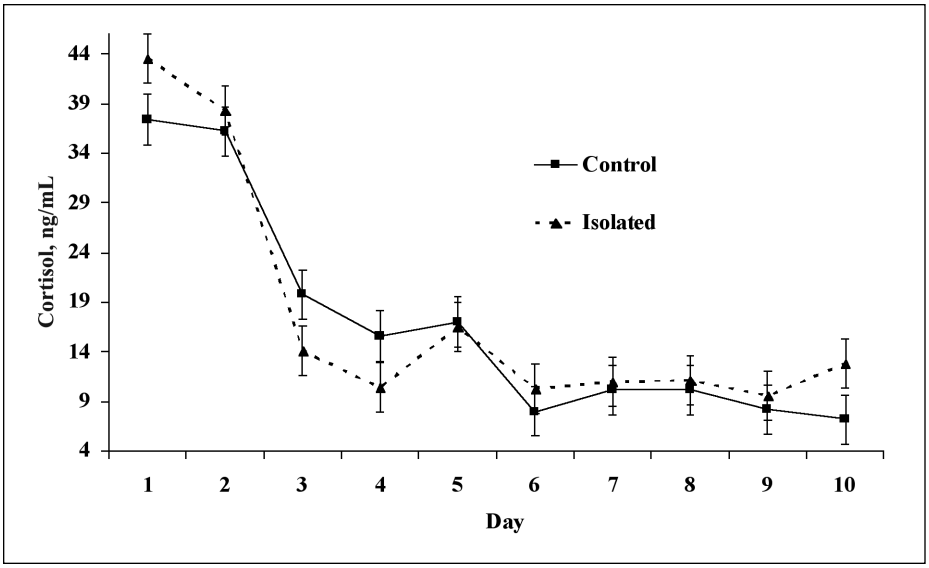
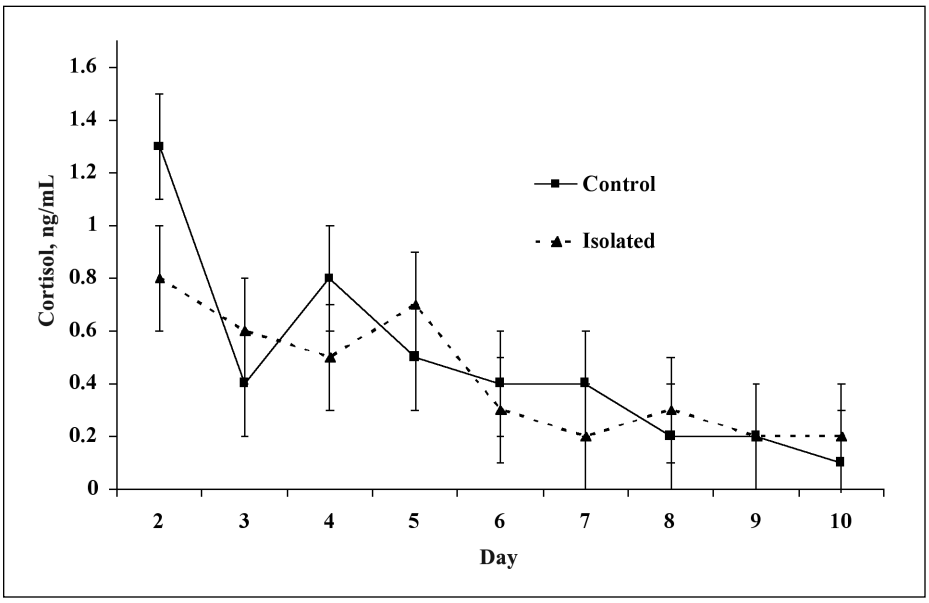


Figure 3. Salivary cortisol concentrations in samples collected at 0700 from young ewes housed in a common pen (control) or those maintained in individual pens (isolated) for 10 days. Housing effect, *P = 0.722*, day effect, *P = 0.002*, isolation x day, *P = 0.590*.



mercial kit (Coat-A-Count, Siemens Medical Solutions Diagnostics, Los Angeles, Calif.) with modifications described by Kiyma et al. (2004). Within-assay and between-assays CV were 2.5 percent and 1.4 percent, respectively, for serum determinations. Salivary cortisol concentrations were determined using the same commercial kit with modifications described by Yates et al. (2010). Within-assay and between-assays CV were 3.6 percent and 16.8 percent, respectively, for salivary determinations. Whole-blood samples were obtained by jugular venipuncture (EDTA-containing, whole-blood vacuum tubes, Kendall Health Care, St. Louis, Mo.). Immediately after sampling, whole-blood samples were cooled on ice and shipped (refrigerated) overnight to the New Mexico Department of Agriculture Veterinary Diagnostics Services, Albuquerque, N.M., for analysis of specific immune and non-immune components of complete blood counts.

Statistical analysis

Ewe was the experimental unit, and data were grouped for analysis into AM (0700) or PM (1300) samples. Experimental design was a split plot with isolation treatment in the main plot and day and treatment x day interaction in the sub plot. When treatment x day interactions were observed, data were examined for treatment effects within each day. Split-plot data were analyzed using the mixed procedure of SAS with the repeated measures function (SAS Inst. Inc., Cary, N.C.). Correlation coefficients were determined using the correlation procedure of SAS. For correlation determination, AM, PM, and intensively collected samples were included.

Results and Discussion

Serum and salivary cortisol concentrations

Serum cortisol concentrations did not differ ($P > 0.452$) between treatments in AM samples (Figure 1) or PM samples (Figure 2). Additionally, salivary cortisol concentrations did not differ ($P > 0.722$) between treatments for the AM (Figure 3) or PM period (Figure 4). The lack of induced change in serum or salivary cortisol concentrations in the present study indicates that physical isolation for 10 d without visual or auditory isolation did not elicit a typical stress response, as the hypothalamic-pituitary-adrenal axis did not appear more active in isolated sheep. This finding compliments previous work in which isolation did not result in increased serum cortisol concentration (Tilbrook et al., 2008). Stackpole et al. (2003) also reported no difference in cortisol concentrations in sheep that were both isolated and restrained. However, inclusion of restraint with isolation in other studies increased circulating cortisol (Apple et al., 1995; Minton et al., 1995; Rivalland et al., 2007), even when stress was applied for short durations only. Additionally, Wagenmaker et al. (2009) reported increased serum cortisol con-

Figure 4. Salivary cortisol concentrations in samples collected at 1300 from young ewes housed in a common pen (control) or those maintained in individual pens (isolated) for 10 days. Housing effect, *P = 0.449*, day effect, *P < 0.001*, isolation x day, *P = 0.092*.

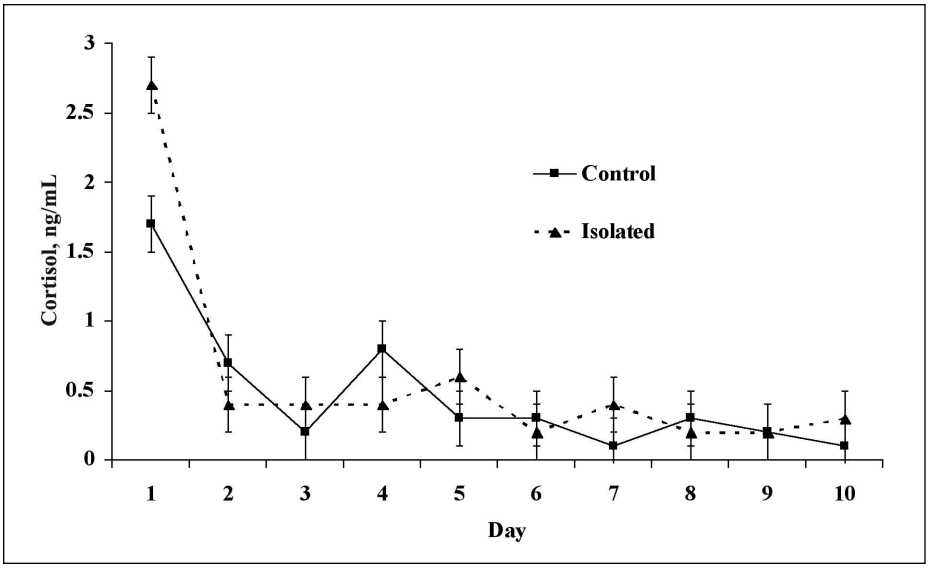
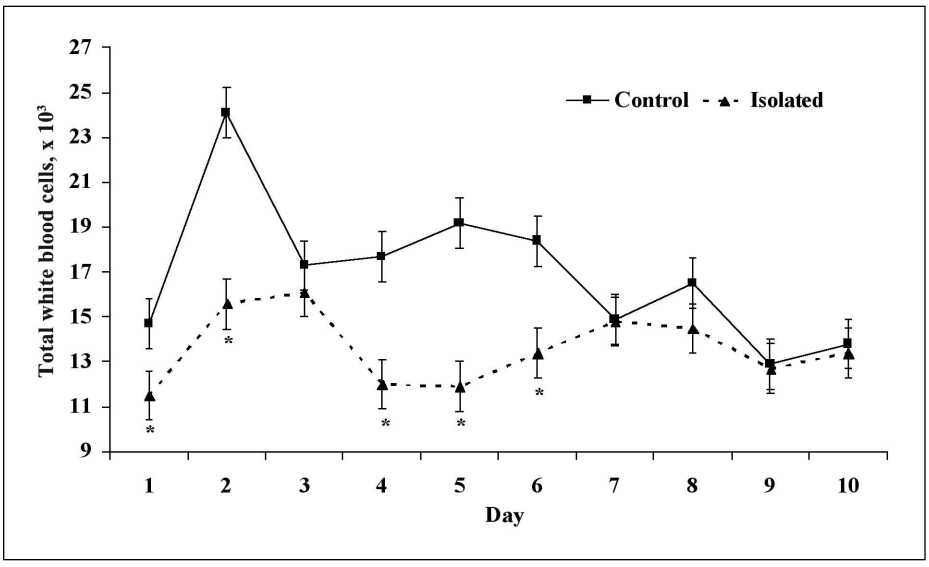


Figure 5. Total white blood cells in samples collected at 1300 from young ewes housed in a common pen (control) or those maintained in individual pens (isolated) for 10 days. Isolation x day, *P < 0.001*. Treatment differences (*P < 0.05*) within each day denoted by '*'.



centrations when isolation was coupled with blindfolding and predator cues. In the present study, serum and salivary cortisol concentrations differed among days (*P < 0.001*). In both biological fluids, cortisol concentrations were elevated on d 1 and 2, but fell and stabilized thereafter, suggesting acclimation to the new surroundings. Importantly, salivary cortisol concentrations were correlated with contemporary serum cortisol concentrations (r = 0.83, *P < 0.001*), which supports previous findings indicating that salivary cortisol concentration is a suitable non-invasive indicator of serum cortisol concentration (Yates et al., 2010).

Immune components of complete blood counts

Treatment x day interactions were observed (*P < 0.021*) in PM samples for total white blood cells, neutrophils, and lymphocytes. For these variables, treatment effects were examined within each day. In PM samples taken from isolated sheep, total white blood cells were reduced (*P < 0.022*; Figure 5) on d 1, d 2, d 4, d 5, and d 6, neutrophils were reduced (*P < 0.037*; Figure 6) on d 1, d 2, and d 5, and lymphocytes were increased (*P < 0.049*; Figure 7) on d 1, d 2, and d 5. However, monocyte, eosinophil, and basophil concentrations did not differ (*P > 0.250*) between treatments in PM samples, and no differences were observed between treatments (*P > 0.629*) in AM samples for any immune component of CBC. In general, immune components were not greatly or consistently influenced by physical isolation in the present study. Increased lymphocyte numbers in the present study were in contrast to previous findings in which seclusion actually decreased total lymphocytes (Minton and Blecha, 1990; Minton et al., 1992; Al-Qarawi and Ali, 2005). However, Degabriele and Fell (2001) reported that increased or decreased lymphocyte numbers in response to three weeks of isolation were specific to lymphocyte type. Decreases in total white blood cells and neutrophils in the present study were also different from previously reported increases in these immune components in isolated and restrained sheep (Minton and Blecha, 1990), although other types of stress have reportedly decreased total white blood cells in birds (Voslarova et al., 2006). Basophils, monocytes, and eosinophils did not differ due to treatment in any samples in the present study, which is consistent with some previous findings (Minton and Blecha, 1990), but is contrary to others (Gupta and Flora, 2005).

Non-immune components of complete blood counts

Treatment x day interactions were observed in both AM and PM samples (*P < 0.020*) for hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin concentrations. For these variables, treatment effects were examined within each day. Mean corpuscular volume was reduced (*P < 0.001*) by isolation on all days in both AM and PM samples. Conversely, mean corpuscular hemoglobin concentrations were

Figure 6. Neutrophils in samples collected at 1300 from young ewes housed in a common pen (control) or those maintained in individual pens (isolated) for 10 days. Isolation x day, $P < 0.001$. Treatment differences ($P < 0.05$) within each day denoted by '*'.

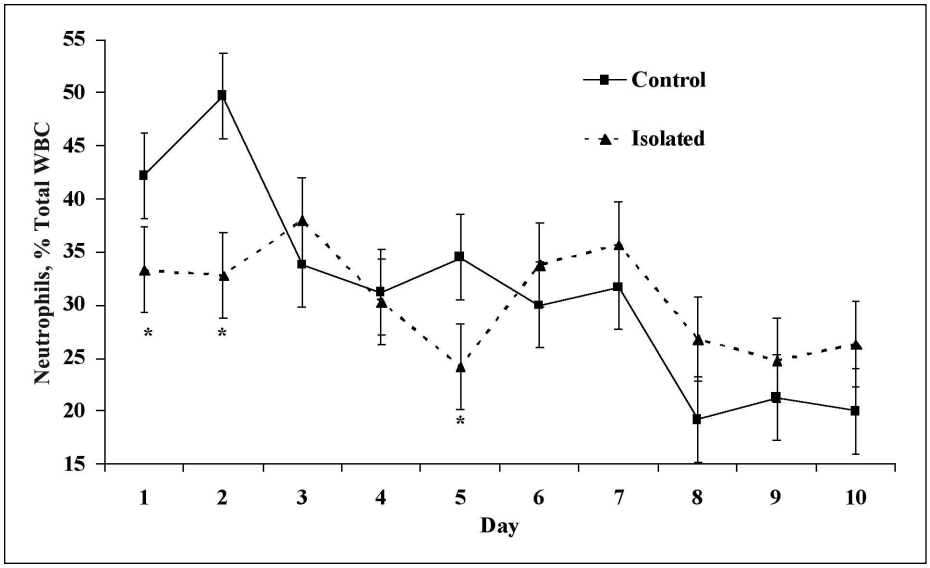
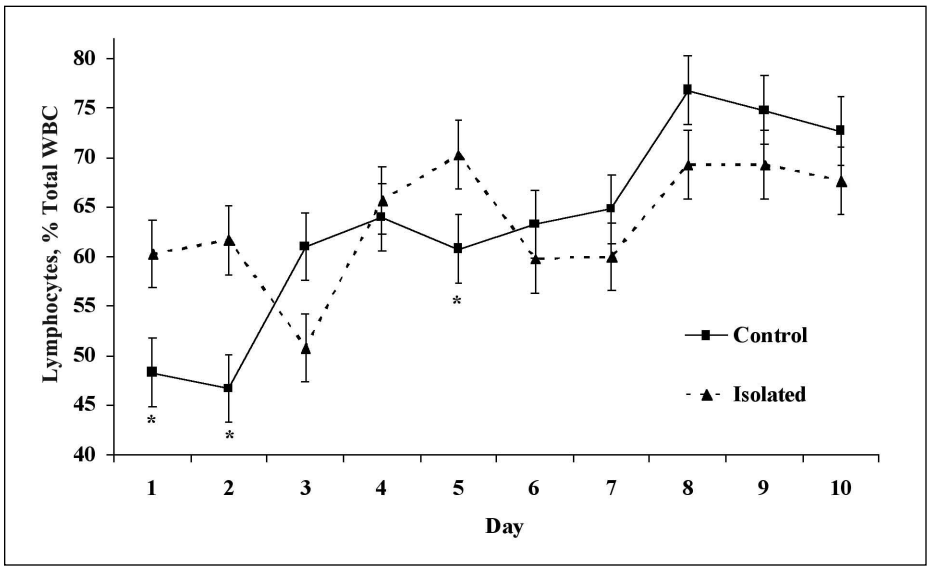


Figure 7. Lymphocytes in samples collected at 1300 from young ewes housed in a common pen (control) or those maintained in individual pens (isolated) for 10 days. Isolation x day, $P = 0.021$. Treatment differences ($P < 0.05$) within each day denoted by '*'.



increased ($P < 0.009$) in all samples. Hematocrit was reduced in isolated sheep from d 2 to d 6 in AM samples ($P < 0.037$) and on all but d 10 in PM samples ($P < 0.050$). Total red blood cell numbers did not differ ($P > 0.442$) between treatments in AM or PM samples. Physical isolation in the present study appeared to elicit the greatest response from non-immune components of CBC. Similar results for hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin concentrations have been found in stressed chickens (Bedánová et al., 2006) and stressed pheasants (Voslarova et al., 2006). Hematocrit reduction in isolated sheep also supports previous findings in sheep exposed to both isolation stress (Al-Qarawi and Ali, 2005) and transportation stress (Ali et al., 2001). Hemoglobin tended to be reduced in PM samples in isolated sheep but was unaltered in AM samples; a contrast to previous work that found increased hemoglobin in response to stress in sheep (Ali et al., 2001; Al-Qarawi and Ali, 2005) and other animals (Bedánová et al., 2006; Voslarova et al., 2006). Total red blood cell numbers did not differ, although stress has been shown to decrease erythrocyte counts in other species (Bedánová et al., 2006; Voslarova et al., 2006). Causes of altered non-immune components in this and other studies are unclear, although changes in both hematocrit and red blood cell numbers may be related more to dehydration associated with physical exertion (Averós et al., 2008) and, thus, might be more representative of changes elicited by physical activity than by actual physiological stress response.

Conclusions

Physical isolation without visual or auditory impairment for 10 d did not appear to stimulate the physiological stress response axis in young ewes, as neither serum nor salivary cortisol concentrations were affected by isolation treatment. Cortisol in both biological fluids was greatest on d 1 and d 2, but fell to stable levels by d 3, indicating acclimation to the unfamiliar indoor environment. Serum cortisol concentration was reflected in salivary cortisol concentration, indicating measurement of salivary cortisol might represent a suitable non-invasive alternative to measurement of cortisol in blood samples. Changes in immune components in response to the isolation model used in this study were largely inconsistent and contradicted previous reports in some cases. Although effects of isolation on non-immune blood components were more consistent and reflective of previous research, mechanisms for and physiological significance of these effects are unclear. Data from this study indicate that ewes were capable of coping with physical isolation for moderate periods of time, when allowed visual and auditory contact with other sheep.

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Research Notes are non-peer-reviewed articles that the Sheep & Goat Research Journal *editor feels may be of interest to the industry, and, in his opinion, are worthy of publishing as a service for our readers.*

Research Note: Sheep Antiserum as an Antibody Supplement in Newborn Lambs

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One of the greatest management challenges facing lamb producers today is keeping newborn lambs alive and healthy. More than 20 percent of lambs do not reach weaning, with 80 percent of those losses occurring in the first 3 days of life (Held, nonreference summary of professional observation). Starvation, hypothermia and scours account for most of those death losses. It has been estimated that 45 percent of all lambs that die during the first few days of life can be contributed to inadequate colostrum intake (McGuire et al., 1983). This can be contributed to ewe's colostrum being poor quantity or quality; bad udders (mastitis, hardbag); dysfunctional teats; multiple births (triplets, quads); neglect from the ewe; orphaned or weak lambs; and diseases, such as ovine progressive pneumonia (OPPV) and Johnes.

The lack of or reduced colostrum intake leaves the newborn lamb without adequate antibody protection to certain infectious diseases causing the newborn lamb to become sick and possibly die (Sawyer et al., 1977). The most common infectious agents causing death in lambs are *Clostridium perfringens* (type C & D), *Salmonella* spp., *Escherichia coli* (*E.coli*), and Mannheima hemolytica ((Rook et al, 1990). Navel ill, septicemias, *E.coli* enteritis and peracute pneumonia are more common in 2- to 3-day old lambs that lack passive immunity. *E.coli* endotoxemia tends to show up at 7 to 10 days of age. A study at the U.S. Sheep Experiment Station showed that 46 percent of lamb mortality was caused by scours and 8 percent by pneumonia, both of which are likely related to the lack of immune protection in the newborn lambs (Gates et al., 2000).

An important management tool for protection to infectious diseases is to ensure that newborn lambs receive adequate antibody intake within the first hours of life (Vihan, 1988). Colostrum provides energy, protein, minerals, vitamins, water and, most importantly, antibody protection against the infectious diseases mentioned above. Lambs are born antibody deficient and have comprised immune systems until they ingest colostrums. Adequate antibody intake is important for all lambs to ensure good health, survivability, and performance (Rook et al, 1990).

The purpose of this research project is to determine if a sterile, irradiated, hyper-immune serum product derived from healthy, hyper-immunized sheep can be used as an antibody supplement in newborn lambs.

Sterile, Irradiated-Antiserum Product

Two healthy Cheviot male (wether) adult sheep were vaccinated using commercial vaccines multiple times (4x at 21-day intervals) against specific *E.coli* endotoxin and *Cl. perfringens* enterotoxins, that are produced by major bacteria that cause illness in newborn lambs. The sheep were monitored for antibodies titers to *E.coli* endotoxin by a qualified, enzyme-linked immunoassay (ELISA, see below). Once adequate titers were achieved (1:32,000), 500 ml of whole blood was collected 21 days following the final vaccination. The serum, which contains the protective antibodies, was harvested by centrifugation (3,000 rpms), pooled, sterile-filtered (.45ul), bottled (100 ml), and sterile-irradiated to ensure product quality. The antiserum product was frozen (-20°C) until ready for use. The product was tested on an *E.coli* endotoxin-ELISA and had a sheep antibody (IgG) titer of 1:32,000.

Ewe's Colostrum

Colostrum was collected from three Cheviot ewes from the 2008 lambing season. The collected colostrum was pooled, mixed and aliquoted into 4 oz feedings and was frozen (-20°C) until

ready for use. The colostrum product was tested on an *E.coli* endotoxin-ELISA and had a sheep antibody (IgG) titer of 1:6,400.

Lamb-Milk Replacer

Lamb-milk replacer (Merrick, Inc, Middleton, Wisc.) was purchased and mixed according to label instructions. The milk was also tested on an *E.coli* endotoxin-ELISA and had a bovine (IgG) titer of 1:800.

Lamb Care and Use

Twelve (12) newborn Cheviot lambs were collected at birth from ewes that had multiple births (triplets, twins), ewes with bad udders, or older ewes. Lambs were not allowed to nurse the ewe. Lambs were given an ID tags, and navels were disinfected with 7-percent iodine. Blood was drawn from lambs prior to receiving their first designated feeding, approximately 30 minutes following birth.

Blood samples (3 ml) were collected from the jugular vein using 18-ga x ½-inch needle and 3cc syringe. Collected blood was transferred to 5 ml vacutainer blood tubes, which were labeled with the lamb's ID and collection date. Collected blood tubes were refrigerated (4°C) overnight, then centrifuged (15 min at 3,000 rpm) for serum collection. Serum was transferred to a small tube, labeled, dated and frozen (-20°C).

The newborn lambs were assigned into four (4) groups with three (n=3) lambs per group. Group A received one, 4 oz feeding of pooled colostrum. Group B received orally one feeding of 20 cc of antiserum in 4 oz of milk replacer. Group C received 10 cc dose subcutaneous (two, a 5 cc dose per upper shoulder) and oral feeding of 4 oz of milk replacer. Group D received oral feeding of 4 oz of milk replacer only. Following the first feedings, all lambs received lamb milk-replacer (Merrick) according to label instructions.

All lambs were evaluated at the time of feedings for appetite, activity, and illness. Appetite and activity were scored 0 to 5 with 0 = none, 2.5 = moderate, and 5 = strong. Possible illness was determined by lack of appetite and activity and by body temperature (102.4°F = normal). Lambs were evaluated for diseases of the respiratory, digestive, muscular and skeletal, and central nervous systems. Illness was scored 0 to 5 with 0 = severe, 2.5 = moderate, 5 = none. All evaluation scores, temperatures and treatments were recorded.

Lambs that appeared to be ill (lack of activity and appetite with temperature) were treated with supportive medicine (antibiotics and anti-inflammatories). Lambs that died during the study were submitted to South Dakota State University Animal Diagnostic Research and Diagnostic Laboratory, Brookings, S.D. for diagnostic evaluation.

Lambs also had blood drawn at 24 hrs, 1 week, 2 weeks and 4 weeks of age after receiving their first designated feeding. Blood samples (3 ml) were collected, processed and frozen as stated above. All collected serum samples were evaluated for endotoxin antibody titer levels using a qualified endotoxin ELISA assay that measured sheep IgG.

Lambs had access to fresh water and lamb creep starter (18-percent protein). All lambs had tails docked 1 week after birth, and male lambs were castrated after 4 weeks of age. All lambs were off study at 4 weeks of age (28 days) and were weaned after 5 weeks of age. All lambs were vaccinated at 28 days for clostridial diseases (type C and D).

The Institutional Animal Care and Use Committee (IACUC) at Ovis LLC approved the protocol for this experiment, according to guidelines provided by the *Guide For the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (1998)

Anti-Endotoxin Enzyme-Linked Immunosorbent Assay (ELISA)

An ELISA assay described by American Research Products was used to test collected lamb serums for the presence of specific antibodies to lipopolysaccharide (LPS) from *E.coli* J5 (Sigma-Aldrich, St. Louis, Mo.). Briefly, ninety-six well MaxiSorp ELISA plates (Nunc) were used to coat LPS to the plates. A commercial sheep serum (American Research Products, Belmont, Mass.) was used as the endotoxin positive control, with a titer of 1:4,000. Newborn-lamb serum (Ovis LLC, Canton, S.D.) was collected after birth to be used as a negative control (<1:100). Test serums and controls were diluted in phosphate-buffered saline, pH 7.4, Tween 20 (nonionic detergent) (PBST) (Sigma Aldrich, St. Louis, Mo.) starting at 1:100 dilution and serial diluted on the plates to have 100 uL per well. Test-and controls-serum samples were replicated twice on each plate. The plates were incubated at 37°C for one hour in a humidified incubator and then washed three times with PBST wash buffer. Rabbit anti-sheep IgG (H and L) alkaline phosphate conjugate (Kirkegaard and Perry Laboratories, Gaithersburg, Md.) was added at a concentration of 1:2000 by diluting in PBST and adding 100 uL per well. The plates were incubated for 30 minutes as stated above. The plates were washed three times with PBST and color was developed by adding 100uL per well of TMB substrate solution (Kirkegaard and Perry Laboratories, Gaithersburg, Md.). The plates were read 20 minutes after substrate addition at an optical density (OD) of 630nm with a microplate reader (ELx800 Automated Microplate Reader, DiaLab, Austria). The data reduction was performed using Gen 5 software with the microplate reader.

Group A: Ewe's Colostrum

Three lambs (A-500, A-575, and A-576) that received 4 oz of ewe's colostrum orally had strong appetites (score = 5) and activity (score = 5) with no illnesses (score = 5) seen during the 28-day trial (Table 1). All three lambs had negative (<1:100) endotoxin titers at birth (Table 2). Endotoxin titers (933.3) were detected at 24 hrs after birth with a slight decrease (466.7) at 1 week of life. Endotoxin titers did decrease slightly (333.3) at 2 weeks of life but increased (933.3) at 4 weeks of life.

Group B: Antiserum Product Oral

Three lambs (B-494, B-495, and B-513) that received 20cc of antiserum product orally in 4 oz of lamb milk replacer had strong appetites (score = 5) and activity (score = 5) with no illnesses (score = 5) seen during the 28-day trial (Table 1). All three lambs had negative (<1:100) endotoxin titers at birth (Table 2). Endotoxin titers (666.7) were detected at 24 hrs after birth with an increase (800) at 1 week of life. Endotoxin titers did decrease (233.3) at 2 weeks of life but increased (466.7) at 4 weeks of life.

Table 1. Individual Lamb Health Scores

Group & Lamb #	Activity*	Appetite*	Illness**	Comment:
Group A: Ewe's Colostrum - 4 oz orally				
500	5	5	5	healthy
575	5	5	5	healthy
576	5	5	5	healthy
Group B: Antiserum orally - 20 ml in 4 oz Lamb-milk replacer				
494	5	5	5	healthy
495	5	5	5	healthy
513	5	5	5	healthy
Group C: Antiserum sq - 10 ml				
493	5	5	5	healthy
519	5	5	5	healthy
580	NA	NA	NA	removed from study
Group D: Lamb-milk Replacer - 4 oz orally				
487	5	5	5	healthy
526	1	2	0	died; septicemia
570	2	3	2	joint ill; treated with antibotics

* score 0-5 (0 = none, 2.5 = moderate, 5 = strong)
** score 0-5 (0 = severe, 2.5 moderate, 5 = none)
sq = subcutaneous NA (not available)

Table 2. Individual Lamb Endotoxin Titers

Group & Lamb #	Birth	24 hrs	1 week	2 weeks	4 weeks	Comment:
Group A: Ewe's Colostrum – 4 oz Oral						
500	<100	800	800	200	800	healthy
575	<100	1600	400	400	400	healthy
576	<100	400	200	400	1600	healthy
Group B: Antiserum orally - 20 ml in 4 oz Lamb-milk replacer						
494	<100	800	400	400	800	healthy
495	<100	800	1600	200	400	healthy
513	<100	400	400	100	200	healthy
Group C: Antiserum sq - 10 mls						
493	<100	3200	400	200	400	healthy
519	<100	3200	1600	800	800	healthy
580	<100	NA	NA	NA	NA	removed from study
Group D: Lamb-milk Replacer - 4 oz orally						
487	<100	<100	200	200	400	healthy
526	<100	<100	100	NA	NA	died, septicemia
570	<100	<100	100	400	400	joint ill, treated

sq = subcutaneous NA = not available < = less than

Group C: Antiserum Product Subcutaneous

Two lambs (C-493 and C-519) that received 10cc of antiserum product subcutaneous had strong appetites (score = 5) and activity (score = 5) with no illnesses (score = 5) seen during the 28-day trial (Table 1). Both lambs had negative (<1:100) endotoxin titers at birth (Table 2). Endotoxin titers (3200) were detected at 24 hrs after birth with a decrease (1000) at 1 week of life. Endotoxin titers did decrease slightly (500) at 2 weeks of life but increased (600) at 4 weeks of life. One lamb in this group (C-580) escaped the lambing pen and was reunited with its mother and therefore removed from the study.

Group D: Milk Replacer

Two of the three lambs (D-526, and D-570), that received 4 oz of lamb-milk replacer, had health-related issues. Lamb D-487 remained healthy throughout the

study (Table 1). One lamb (D-526) died at 9 days of age due to a bacterial septicemia (*Klebsiella* sp). Lamb D-570 developed a joint-ill condition starting at 2 days of age, which continued throughout the 28-day study. The lamb was given antibiotics to treat the condition. Lamb D-487 had a strong appetite (score = 5) and activity (score = 5) with no illnesses (score = 5) seen during the 28-day trial. All three lambs had negative (<1:100) endotoxin titers at birth (Table 2.). Endotoxin titers remained negative (<1:100) at 24 hrs after birth with a slight increase (133.3) at 1 week of life. Endotoxin titers did increase slightly (300) at 2 weeks and (400) at 4 weeks of life.

All the lambs in Groups A, B and C remained healthy throughout the 28-day trial compared to Group D. When evaluating endotoxin titers, both Groups A and B had comparable endotoxin titers throughout the 28-day study. Group C had much higher titers at 24 hrs when compared to Group B, which may have to do with gut absorption vs subcutaneous receipt of the antiserum product. The lambs in Group D had negative endotoxin titers (< 1:100) at 24 hrs, which probably contributed to the two lambs health problems.

Lamb D-526 was noticed ill at 2 days of age with lack of appetite and activity. The lamb's temperature was normal (102.4°F). The lamb was treated with antibiotic (penicillin) with a noticed improvement but became ill several days later. The lamb was thin and had a temperature (103.1°F), and central nervous symptoms (occasional seizure). The lamb died overnight. The carcass was submitted to SDSU ADRDL for diagnostic findings. The diagnostic report indicated a severe septicemia (*Klebsiella* sp.), probably due to the lamb being colostrum-deprived.

Lamb D-570 was noticed ill at 3 days of age with lack of appetite and activity. The lamb's temperature was slightly elevated (103°F) and it had difficulty walking and inflamed front joints. The lamb was treated with penicillin and banamine with no improvement. The attending veterinary prescribed tetracycline and continued banamine treatment. The lamb improved in appetite but continued to have a stiff gate throughout the 28-day study.

Even though lamb D-487 had a negative endotoxin titer at 24 hrs, the lamb remained healthy throughout the 28-day trial.

This study demonstrated that newborn lambs that received antibody supplement orally and subcutaneously remained just as healthy during the 28-day study, as newborn lambs that received 4 ounces of pooled ewe's colostrum. The use of the antibody supplement by subcutaneous delivery gave a much higher level of antibody response and should have further research completed to determine its use in lamb-production operations. Control lambs that did not receive any antibody supplement or colostrum, were not as healthy and came down with illnesses associated with the lack of passive immunity.

This sterile-irradiated-antibody supplement could be used as a management tool for newborn lambs less than 24 hours of age, when adequate ewe's colostrum is not available or for newborn lambs that have difficulty nursing. Further research in lamb-production operations should be conducted to confirm efficacy.

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Substituting Corn Dried Distillers Grains for Cottonseed Meal in Lamb Finishing Diets: Carcass Characteristics, Meat Fatty Acid Profiles, and Sensory Panel Traits[1]

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Summary

The effects of replacing cottonseed meal (CSM) with corn dried distillers grains (DDG) on carcass characteristics, meat fatty acid profiles, and sensory panel traits were investigated in Rambouillet wether lambs. Lambs (n = 44) were individually fed ad libitum diets for 84 d containing DDG that replaced 0 percent (0DDG), 33 percent (33DDG), 66 percent (66DDG), or 100 percent (100DDG) of the CSM in a completely randomized design. Carcass characteristics, fatty acid profiles (weight percentage), and sensory panel traits from the LM were determined on 8 randomly selected wethers per diet. Carcass characteristics were not affected ($P > 0.14$) by diet. As DDG increased in the diet, extracted fat from the LM linearly increased ($P = 0.004$). The *trans*-9, 10, and 11 isomers of 18:1 and *cis*-vaccenic (18:1 *cis*-11) acid linearly increased ($P < 0.09$) in the LM, and linoleic (18:2 *cis*-9, *cis*-12) and arachidonic (20:4) acids linearly decreased ($P < 0.02$) as DDG increased in the diet. The CLA *cis*-9, *trans*-11 isomer quadratically increased ($P = 0.07$) in the LM as percentage of DDG increased in the diet. Increasing DDG in the diets quadratically affected ($P < 0.05$) cook-loss, initial and sustained juiciness, sustained tenderness, and flavor intensity. Meat from lambs fed 100DDG had less ($P = 0.01$) cook-loss and greater ($P < 0.04$) initial and sustained juiciness than meat from lambs fed 0DDG diet. Results indicated that partially or totally substituting DDG for CSM in lamb-finishing diets is acceptable and may enhance sensory traits.

Key Words: Carcass, Dried Distillers Grains, Feedlot, Lamb, Meat Fatty Acids, Sensory Panel Traits Introduction

Introduction

As quantity and availability of dried distillers grains (DDG) continues to increase (FAPRI, 2009), the livestock industry has responded by exploring more feed formulation uses for this nutrient-dense co-product. However, research to evaluate effects of feeding DDG on lamb-carcass characteristics, meat fatty acids (FA), and sensory panel traits is limited. Huls et al. (2006) reported that lamb-backfat (BF) thickness decreased, but other carcass characteristics remained similar when DDG with solubles replaced soybean meal and a portion of the corn in 90-percent concentrate diets. Schauer et al. (2008) fed lambs up to 60 percent DDG with solubles, which replaced all of the soybean meal and a portion of the barley, and reported only flank streaking and quality grade being affected.

Feeding DDG with or without solubles has resulted in variable affects on carcass characteristics, meat FA profiles, and sensory attributes in cattle (Koger et al., 2004; Gill et al., 2008; Depenbusch et al., 2009a; Depenbusch et al., 2009b). Evaluating FA profiles in ruminant-derived muscle tissue is important because some saturated FA in the human diet are directly related to elevated-blood cholesterol, which has been related coronary heart disease (Fletcher et al., 2005), and anti-carcinogenic effects of CLA have been reported (Belury, 1995). In addition, meat FA composition can affect sensory panel traits (Ponnampalam et al., 2001; Chung et al., 2006). For example, Crouse and Ferrell (1982) reported that flavor is highly correlated to 18:1 and 18:3 (r = -0.33 and 0.33, respectively) FA concentrations. Therefore, objectives were to evaluate effects of replacing cottonseed meal (CSM) with DDG on lamb-carcass characteristics, meat FA profiles, and sensory panel traits.

Materials and Methods

Animals and Management

Texas A&M University Institutional Animal Care and Use Committee approved the experimental protocol was approved (#2007-92). A detailed description of animals and management has been reported in a companion paper (McEachern et al., 2010). Briefly, Rambouillet wether lambs (n = 44; approximate age = 4 mo; initial BW = 28.8 kg ± 3.5 kg) were weighed at the beginning of the adaptation period 28 d before study initiation, stratified by BW, and randomly assigned to diets (n = 11 wethers per diet) and individual pens. Pelleted and isonitrogenous diets contained corn DDG that replaced 0 percent (0DDG), 33 percent (33DDG), 66 percent (66DDG), or 100 percent (100DDG) of the CSM (Table 1); Table 1 was previously reported in the companion paper (McEachern et al., 2010), but also included here for convenience. Lambs were individually fed *ad libitum* and once per day at 0800; daily feed offered to each lamb was the previous day's intake plus approximately 15 percent on a DM-basis.

Lambs were shorn 5 d before study initiation and on d 82. Lamb BW was recorded, and serum was collected from centrifuged blood on d 0, d 14, d 28, d 56, and d 84. Lamb growth, wool characteristics, and serum NEFA, urea N, and IGF-1 concentrations are presented in a companion paper (McEachern et al., 2010). On d 85, 8 lambs per diet were randomly selected, transported 0.5 km to the Angelo State University Food Safety and Product Development Laboratory (San Angelo, Texas), and slaughtered to evaluate carcass and sensory characteristics and meat FA profiles.

Sample Collection and Measurements

Feeds: Samples of diets were randomly collected on d 0, d 19, d 41, and d 69, dried at 55°C, ground to pass a 1-mm screen, and stored at -20°C. Samples of each diet were combined for d 0 and d 19 and for d 41 and d 69, thus chemical analyses were evaluated for two pooled sets of samples, averaged, and presented in Table 1. Nitrogen was analyzed by a standard method (AOAC, 2006) and CP calculated as 6.25 × N. Sodium borate-Na phosphate buffer and enzymatic digestion procedures were used to analyze soluble and degradable feed protein, respectively (Roe et al., 1990). Crude fat was measured by ether extraction (AOAC, 2006). The NDF and ADF were analyzed using Van Soest et al. (1991) procedures modified for an Ankom 2000 Fiber Analyzer (Ankom Technol. Corp., Fairport, N.Y.) without correcting for residual ash and using α-amylase. Sulfur was evaluated using a Leco (model SC-432, St. Joseph, Mich.) analyzer, and all other minerals were analyzed by a Thermo Jarrell Ash IRIS Advantage HX Inductively Coupled Plasma Radial Spectrometer (Thermo Instrument Systems, Inc., Waltham, Mass.). Corn DDG and diets were also evaluated for individual fatty acids as described in the following section.

Carcass Characteristics and Feed and Meat Fatty Acid Profiles: Detailed descriptions of carcass characteristics, feed and meat FA profiles, and sensory panel trait analyses have been previously reported (Whitney and Lupton, 2010). Following harvest, HCW was recorded and carcasses were chilled at 2°C ± 1°C. At 48 h postmortem, each carcass was ribbed between the 12th and 13th ribs for carcass evaluation. Carcasses were analyzed to determine BF thickness at the 12th rib, LM area, and circumference of both legs across the stifle joints on the intact carcass, maturity, and flank streaking (USDA, 1997). At 48-h postmortem, the loin (NAMP #232a) was removed according to procedures of NAMP (1997). Five chops, 2.54-cm thick, were cut from starting at the posterior end and stored 4 months at -80°C until analyzed; the first chop was designated for FA methyl ester analysis, and the other four chops were designated for sensory analysis.

Fatty acid methyl esters were prepared by direct esterification with methanolic KOH of muscle tissue and feed samples according to procedures of Murrieta et al. (2003). Fatty acid profiles of feed and meat samples were analyzed according to procedures reported by Boles et al. (2005). For each loin chop, 2 sub-samples were collected from cross-sections of the LM, including any residual intermuscular fat, freeze-dried, and ground in an electric coffee grinder. Meat moisture was determined before and after freeze-drying. From each of these samples, 2 sub-samples were again collected, analyzed, and averaged. The resulting FA methyl esters were then analyzed by GLC as described by Boles et al. (2005); FA concentrations were determined using the methods of Murri-

Table 1. Ingredient, chemical composition (% DM basis), and cost of corn dried distillers grains (DDG), cottonseed meal (CSM) and diets.

Item	DDG[2]	CSM[2]	Diet (% of CSM replaced by DDG) 0DDG	33DDG	66DDG	100DDG
Cottonseed hulls			25.0	25.0	25.0	25.0
DDG			–	6.6	13.2	20.0
CSM			20.0	13.4	6.8	–
Grain sorghum, crushed			47.40	46.95	46.51	46.04
Molasses			3.0	3.0	3.0	3.0
Limestone			2.0	1.85	1.69	1.54
Ammonium chloride			0.75	0.75	0.75	0.75
Salt			0.85	0.85	0.85	0.85
Urea			–	0.6	1.2	1.82
Mineral premix[1]			1.0	1.0	1.0	1.0
CP, %	22.5	50.8	18.8	17.9	18.7	19.0
Soluble protein, %	35.0	21.0	29.5	30.5	44.5	47.0
Degradable protein, %	49.0	49.0	57.5	45.5	60.5	68.0
Crude fat, %	4.4	5.3	4.6	5.0	4.6	5.2
NDF, %	41.8	17.0	25.4	26.6	25.2	27.1
ADF, %	14.5	14.0	14.9	17.5	14.3	15.0
TDN, %	71.0	76.0	85.0	85.0	85.5	85.0
Ca, %	0.10	0.34	0.83	1.02	0.86	1.00
P, %	0.80	1.66	0.44	0.48	0.41	0.44
Ca:P	0.13	0.21	1.89	2.13	2.10	2.27
Mg, %	0.30	0.86	0.25	0.26	0.22	0.22
K, %	1.13	1.76	0.89	0.91	0.84	0.88
Na, %	0.48	0.27	0.51	0.44	0.52	0.52
S, %	0.40	0.58	0.28	0.29	0.28	0.30
Fe, mg/kg	171	145	424	504	325	284
Zn, mg/kg	90.0	72.0	59.5	59.0	57.5	59.5
Cu, mg/kg	5.0	15.0	4.0	5.0	3.5	4.0
Mn, mg/kg	53.0	22.0	48.0	55.5	50.0	54.5
Mo, mg/kg	1.0	2.4	0.60	0.85	0.70	0.80
Cost $/metric ton	180.78	254.63	221.46	219.07	216.68	214.22
Cost $/kg of gain			1.14	1.23	1.21	1.13

[1] Mineral premix ingredients: sodium chloride, potassium chloride, sulfur, manganous oxide, zinc oxide, vitamins A, D, and E, calcium carbonate, CSM, cane molasses, and animal fat. Soluble and degradable protein fractions = % of CP. Cost/metric ton of feed estimated using information from local markets and current Feedstuffs magazines: cottonseed hulls ($116), DDG ($181), CSM ($255), milo ($240), molasses ($265), limestone ($198), ammonium Cl ($1086), salt ($243), urea ($695), mineral premix ($591). Cost of feed kg-1 gain = ([Cost/metric ton of feed/1000] × [feed/gain]).
[2] The random sample of DDG that was used in the diets was collected when feed was pelleted; the random CSM samples were from a different source than that used in the diets.

eta et. al. (2003) with tridecanoic acid (C13:0) methyl ester added before extraction as an internal standard. The FA percent was calculated on dry-weight basis by using the recovery of an internal standard as follows: [(1 mg of internal standard × total area under curve for all peaks)/(area under standard peak/sample weight)] × 100, and then converted to fresh-tissue basis. Known FA averaged 94.6 percent of total extracted fat (CV = 1.5 percent).

Sensory Panel Evaluation: A trained sensory panel (6 to 7 members; Cross et al., 1978) evaluated chops cut from the loin section (AMSA, 1995). Randomly selected chops were allowed to thaw for 24 h at 2°C ± 1°C and cooked on a clam-shell-style grill (Kerth et al., 2003) for 7 min. Samples were trimmed to less than 0.64 cm of outside fat and connective tissue, cut into 1.27-cm × 1.27-cm portions, and placed in warming pans until served to panelists. Chop samples were evaluated for initial and sustained juiciness, initial and sustained tenderness, and flavor intensity on a scale of 1 to 8, where 1 = extremely dry, tough, and bland, and 8 = extremely juicy, tender, and intense, respectively. Additionally, chops were evaluated for off-flavor, where 1 = extreme off-flavor and 4 = no off-flavor. Samples from each chop were evaluated by panelists who were secluded in partitioned booths with a controlled level of red

incandescent light. A "warm-up" sample chop was served at initiation of each sensory session, followed by 6 to 8 chop samples per session. Panelists were instructed to cleanse their palate with a salt-free saltine cracker and water before each sample.

Statistical Analyses

Data were analyzed using the PROC MIXED procedure (SAS Inst. Inc., Cary, N.C.). Lamb carcass characteristics, meat moisture, and sensory panel traits were analyzed using a model that included diet with lamb as the experimental unit. Fatty acid data were analyzed using a model that included diet with meat subsample (n = 2; using lamb within diet as the subject) as the random effect and lamb as the experimental unit. Data are reported as least-square means with greatest standard errors. Diet effects were tested using the following single degree of freedom non-orthogonal contrasts: 1) linear and 2) quadratic effects of replacing CSM with DDG, and 3) 0DDG vs. 100DDG. PROC IML was used to generate coefficients for contrasts with unequal spacing (DDG replacing 0 percent, 33 percent, 66 percent, 100 percent of the CSM). Only the highest order contrast that was significant ($P < 0.10$) was discussed. Correlations were evaluated using the Spearman correlation procedure.

Results and Discussion

Chemical and Fatty Acid Composition of Diets

Chemical composition of DDG, CSM, and diets are presented in Table 1 and have previously been reported (McEachern et al., 2010); FA profiles of DDG and diets are presented in Table 2. Diets contained relatively similar CP, but soluble and degradable CP increased with increasing concentration of DDG in the diet. All diets were formulated to be isonitrogenous and for this purpose, feed urea was added to the diets. In addition, crude fat was 0.6 percentage units greater in 100DDG vs. 0DDG. Percentage of oil increased (except for 66DDG) and percentages of 16:0 and total saturates decreased as percentage of DDG increased in the diet. The DDG contained a high concentration of 18:2, which agrees with values reported for DDG with solubles (Harfoot, 1981); however, this did not result in 18:2 greatly increasing in the diets as percentage of DDG increased.

Table 2. Fatty acid (FA) profile of corn dried distillers grains (DDG) and diets.

		Diet (% of CSM replaced by DDG)[2]			
Item[1]	DDG	0DDG	33DDG	66DDG	100DDG
Oil, % of DM	3.3	4.2	4.6	4.2	4.7
FA, % of oil					
C16:0	11.9	16.5	15.9	15.7	14.8
C18:0	2.3	2.1	2.2	2.1	2.3
C18:1	25.3	26.2	26.1	26.7	26.2
C18:2	53.6	48.7	49.5	49.3	49.3
C18:3	1.9	1.5	2.0	1.5	2.1
C20:0	0.5	0.3	0.4	0.3	0.4
C20:1	0.4	0.3	0.3	0.3	0.3
C22:1	0.2	0.2	0.4	0.2	0.3
C24:0	0.4	0.2	0.2	0.2	0.2
Total SFA, %	15.2	19.4	19.1	18.5	17.9

[1] The FA expressed as a percentage of total FA.

Carcass Characteristics

The use of DDG as an alternative for CSM in Rambouillet wether diets had no affect ($P > 0.13$) on carcass characteristics (Table 3). Lambs could have been harvested earlier because slaughter live weight (SLW) and BF thickness of all lambs were greater than what has been suggested for the sheep industry (Snowder et al., 1994). Huls et al. (2006) reported that BF thickness declined, but all other carcass characteristics were similar for lambs fed diets containing 23 percent DDGS vs. no DDGS. Schauer et al. (2008) showed that increasing DDGS in diets up to 60 percent did not affect lamb carcass characteristics, except for increased flank streaking and USDA quality grade. Similar results have been reported in cattle (Uwituze, 2008; Depenbusch et al. 2009b), but Depenbusch et al. (2009a) and Gordon et al. (2002a) reported that BF thickness declined as distillers grains increased in the diet. Research investigating the inclusion of DDG in lamb-finishing diets has shown no negative effect on carcass characteristics, thus it could serve as a plausible substitute for CSM in lamb-finishing diets.

Meat Fatty Acid Profiles

Concentrations of the weight percentages of FA methyl esters are presented in Table 3. Weight percentages of 14:0, 16:1, 18:0, and 20:0 acids were not affected by diet ($P > 0.19$), but 16:0 was greater ($P = 0.07$) in meat from lambs fed 0DDG vs. 100DDG. Feeding DDG to cattle has resulted in variable meat stearic FA responses (Gill et al., 2008; Depenbusch et al., 2009a). Total percentage of fat in LM tissue linearly increased ($P = 0.004$) as DDG increased in the diet, and LM from lambs fed 100DDG had 1.4 times greater fat than LM from lambs fed 0DDG.

Total-saturated FA concentrations were not different ($P > 0.22$) among diets, which agrees with others who reported similar meat saturated FA concentrations in cattle fed DDG with solubles. For humans, dietary intake of some saturated FA are directly linked to elevated concentrations of blood cholesterol, which can increase the risk for coronary heart disease (Fletcher et al., 2005), but 18:0 has been reported to be neutral in relation to human health (Grundy, 1989; Grundy, 1994; Pariza, 2004). Cobb (1992) suggested that stearic acid should be considered separately from other saturated FA when discussing dietary control of cholesterol. Therefore, saturated FA concentration was also evaluated in the current study without including 18:0. When 18:0 was excluded, saturated FA was less ($P = 0.09$) in meat from lambs fed 100DDG vs. 0DDG.

The 18:1 FA were the most abun-

Table 3. Effects of substituting corn dried distillers grains (DDG) for cottonseed meal (CSM) on carcass characteristics and meat fatty acid profile (FA; %, weight basis).

	Diet (% of CSM replaced by DDG)					*P*-value[1]		
Item[2]	0 DDG	33 DDG	66 DDG	100 DDG	SEM	Linear	Quad	0DDG vs. 100DDG
n	8	8	8	8				
Carcass characteristics								
Slaughter live wt., kg	49.0	50.3	50.7	49.4	1.4	0.83	0.37	0.87
HCW, kg	29.3	29.7	29.9	29.2	0.9	0.97	0.54	0.95
LM area, cm^2	15.8	15.4	14.8	16.4	0.7	0.69	0.14	0.54
Backfat, cm	0.76	0.6	0.6	0.5	0.1	0.23	0.90	0.22
Body wall, cm	2.16	2.31	2.18	1.93	0.15	0.21	0.19	0.27
Leg circumference, cm	67.19	67.75	67.88	67.63	0.86	0.71	0.64	0.72
Meat moisture, %	67.59	66.43	66.42	67.20	1.27	0.84	0.45	0.83
Meat fatty acids, %								
Myristic acid (14:0)	5.99	5.51	5.63	5.55	0.28	0.34	0.49	0.27
Palmitic acid (16:0)	26.87	25.76	26.34	25.90	0.39	0.17	0.38	0.07
Palmitoleic acid (16:1)	3.20	3.18	3.05	3.45	0.16	0.42	0.15	0.36
Stearic acid (18:0)	7.27	7.94	7.87	7.61	0.35	0.65	0.20	0.58
Oleic acid (18:1, *cis*-9)	33.24	35.26	33.61	34.89	0.72	0.30	0.62	0.10
trans-9,10,11 isomers of 18:1	2.55	2.94	4.20	4.24	0.27	<0.001	0.23	<0.001
cis-Vaccenic acid (18:1, *cis*-11)	1.41	1.45	1.45	1.51	0.04	0.09	0.81	0.07
CLA (18:2 *cis*-9 *trans*-11)	0.29	0.31	0.34	0.30	0.02	0.50	0.06	0.86
Linoleic (18:2, *cis*-9 *cis*-12)	11.47	10.10	9.89	8.67	0.64	0.006	0.94	0.005
Arachidic acid (20:0)	0.35	0.32	0.34	0.34	0.01	0.89	0.20	0.67
Arachidonic acid (20:4)	2.40	2.01	1.77	1.53	0.21	0.001	0.66	0.01
Fat, %	4.70	5.77	6.76	6.65	0.67	0.004	0.42	0.007
Saturated FA, %[3]	40.49	39.54	40.19	39.40	0.65	0.36	0.91	0.23
Saturated FA, %; no 18:0[4]	33.22	31.60	32.32	31.79	0.58	0.17	0.36	0.09

[1] Linear and quadratic (Quad) orthogonal polynomial contrasts.
[2] FA % was calculated on dry-weight basis by using the recovery of an internal standard as follows: [(1 mg of internal standard × total area under curve for all peaks)/(area under standard peak/sample weight)] × 100, and then converted to fresh-tissue basis.
[3] Saturated FA = all saturated FA (C14:0 to C20:0)
[4] Saturated FA, no 18:0 = all saturated FA excluding stearic acid (18:0).

dant of all FA, which agrees with previous reports (Kott et al., 2003; Boles et al., 2005; Juarez et al., 2008). Weight percentage of 18:1, *cis*-9 was greater ($P = 0.09$) in meat from lambs fed 100DDG vs. 0DDG. Or-Rashid et al. (2008) suggested that greater 18:1 *cis*-9 in ruminal fluid may indicate partial protection of 18:1 *cis*-9 from ruminal biohydrogenation. Even though ruminal fluid was not evaluated in the current study, FA composition of intramuscular fat can reflect ruminal FA profile (Vasta et al., 2009).

Weight percentage of 18:1 *cis*-11 isomer linearly increased ($P < 0.08$) as percentage of DDG increased in the diet and was greater ($P < 0.07$) in meat from lambs fed 100DDG vs. 0DDG. Total 18:1 *trans* isomers linearly increased ($P < 0.001$) as percentage of DDG increased in the diet and were greater ($P < 0.001$) in meat from lambs fed 100DDG vs. 0DDG. Greater dietary fat has resulted in greater total 18:1 *trans*-10 isomer in LM of sheep (Wynn et al., 2006). Crouse and Ferrell (1982) reported a negative relationship between muscle 18:1 and carcass fat, but no correlation ($P > 0.35$) was observed in the current study.

Increasing percentage of DDG in the diet quadratically increased ($P = 0.07$) *cis*-9, *trans*-11 CLA isomer and linearly decreased ($P = 0.007$) *cis*-9, *cis*-12 isomer of 18:2. Meat from lambs fed 0DDG had greater ($P = 0.005$) weight percentage of *cis*-9, *cis*-12 isomer of 18:2 than lambs fed 100DDG. Viapond et al. (1995) reported that feeding lambs malt distillers grains increased meat *cis*-9, *cis*-12 isomer of 18:2, but did not affect 16:0, 16:1, 18:0, or 18:1 FA. Feeding distillers grains to cattle increased meat n-6 FA concentrations (Gill et al., 2008), which is opposite of what was observed in the current study for *cis*-9, *cis*-12 isomer of 18:2 and 20:4.

The *cis*-9, *trans*-11 CLA isomer originates exclusively through the biohydrogenation pathway (BH) (Griinari et al., 2000), and BH can be reduced by increasing ruminal acidity (Harfoot and Hazelwood, 1997). Kim et al. (2007) suggested that greater *cis*-9, *cis*-12 isomer of 18:2 in lamb muscle tissue resulted from greater *cis*-9, *cis*-12 isomer of 18:2 escaping a more acidic ruminal environment. In addition, ruminal BH proceeds to a greater extent as NDF concentration increases (Sackman et al., 2003), which is related to cellulolytic microbes

being the primary microbes involved in BH (Kelper and Tove, 1967). Depenbush et al. (2009a) reported that feeding DDG with solubles may favor cellulolytic bacteria in the rumen. Even though NDF did not linearly increase as percentage of DDG increased in the diet, ruminal pH may have increased, resulting in BH proceeding to a greater extent. However, DDG small particle size may reduce its effective fiber (Schingoethe, 2006) and feeding DDG with solubles has reduced ruminal pH (May, 2007; Uwituze, 2008). Furthermore, greater *cis*-9, *cis*-12 isomer of 18:2 escaping ruminal BH would result in less 18:0 in muscle tissue and lead to less 18:1 *cis*-9 (Kim et al., 2007), and greater 18:0 would be expected if more complete BH did occur (Vasta et al., 2009), which is in direct contrast to the current study. Additional research evaluating effects of feeding DDG on BH and other factors, i.e. ruminal-particle-passage rate, on muscle-tissue FA profile is warranted.

Sensory Panel Traits

Sensorial attributes for lamb chops are presented in Table 4. Incorporating DDG in the diet resulted in less cooking loss ($P < 0.004$). Initial- and sustained-juiciness scores quadratically increased ($P < 0.05$) in unison with greater DDG in the diet, and meat from lambs fed 100DDG had greater ($P < 0.04$) initial and sustained juiciness than meat from lambs fed 0DDG. Greater juiciness was also reported by Leupp et al. (2009), but not by Roeber et al. (2005) when analyzing sensory attributes of steaks from steers fed DDG. In addition, initial- and sustained-juiciness scores were not correlated ($P > 0.11$) to lamb BF or percentage of fat in LM, but was negatively correlated (r = -0.34, -0.56; $P = 0.06$, <0.001, respectively) to cook-loss. Cook-loss was also correlated (r = -0.35, $P = 0.04$) to sustained tenderness. Interestingly, initial-tenderness scores showed no effect due to DDG replacement ($P > 0.15$), but sustained-tenderness scores exhibited a quadratic response ($P = 0.04$) to DDG replacement.

Considering the linear increase in initial and sustained juiciness scores, tenderness would also be expected to linearly increase due to a halo effect as described by Roeber et al. (2000); increased sustained juiciness creating a generalized notion of increased tenderness by the panelists. Roeber et al. (2000) documented that consumers generalized displeasure in tenderness based on decreased juiciness of the sample. Initial (r = 0.74, 0.70, $P < 0.001$) and sustained (r = 0.68, 0.66; $P < 0.001$) juiciness was correlated to initial and sustained tenderness, respectively.

Flavor intensity of chops quadratically increased ($P = 0.003$), but off-flavor ratings were not affected by diet ($P = 0.14$). This mirrors results reported by Leupp et al. (2009) and Gordon et al. (2002b) who also observed increased flavor intensity with no effect on off-flavors. Perception of flavor intensity may also be a function of increased percentage of fat in the meat and greater initial and sustained juiciness. Flavor intensity was correlated to cook loss, initial and sustained juiciness, tenderness scores, off-flavor, and 18:1 *cis*-9 isomer (r = -0.43, 0.52, 0.60, 0.58, 0.56, -0.45, 0.43, respectively; $P < 0.02$). In contrast to the current study, a positive correlation between beef flavor and off-flavor intensity has been reported (Calkins and Hodgen, 2007).

Conclusions

Substituting DDG for CSM in lamb-finishing diets did not affect carcass characteristics. However, as DDG increased in the diet, percentage of total fat and fatty acid profile in muscle were affected; most notably an increase in the *trans* isomers of 18:1 and a decrease in *cis*-9, *cis*-12 isomer of 18:2. Results indicate that partially or totally substituting DDG for CSM in lamb finishing diets is acceptable and enhances juiciness, tenderness, and flavor intensity without affecting off-flavor of lamb meat.

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Table 4. Effects of substituting dried distillers grains (DDG) for cottonseed meal (CSM) on sensory panel traits of lambs chops.

	Diet (% of CSM replaced by DDG)					*P*-value[1]		
Item[2]	0DDG	33DDG	66DDG	100DDG	SEM	Linear	Quadratic	0DDG vs. 100DDG
cook-loss	23.3	18.3	19.6	20.0	0.9	0.03	0.004	0.01
initial juiciness	4.7	5.5	5.4	5.4	0.2	0.04	0.04	0.02
sustained juiciness	4.8	5.8	5.5	5.2	0.2	0.11	<0.001	0.03
initial tenderness	5.5	6.1	5.8	5.6	0.3	0.93	0.15	0.87
sustained tenderness	5.4	6.2	5.9	5.6	0.3	0.70	0.04	0.53
flavor intensity	4.9	5.6	5.3	5.0	0.1	0.98	0.003	0.60
off-flavor	3.9	3.8	3.9	3.9	0.1	0.59	0.14	0.86

[1] Linear and quadratic orthogonal polynomial contrasts.
[2] Cook-loss expressed as the % of weight loss from raw weight; initial and sustained juiciness and tenderness and flavor intensity scored on an 8-point scale (1 = extremely dry, tough, and bland, and 8 = extremely juicy, tender, and intense, respectively); off-flavor scored on a 4-point scale (4 = no off-flavor, 1 = extreme off-flavor).

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Effect of Finishing Crossbred Meat Goats with a Similar Total Quantity of Finisher Ration Over Variable Duration

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Summary

The objective of this research was to assess the effect of finishing weaned crossbred meat goats with a similar total quantity of finisher ration over variable duration on meat goat production performance measures. Thirty weaned crossbred kids were blocked by body weight and genotype and assigned to three different lengths of finishing periods (45 days, 90 days or 135 days). Each finishing period treatment was replicated in two 0.4 ha Joy chicory (*Cichorium intybus* L.) plots with 5 kids in each and supplemented with 138 kg of commercial finisher ration over 45 days, 90 days or 135 days. While total finisher-ration consumption (27.30 kg, 27.49 kg and 28.26 kg/head) and cost ($9.90, $9.83 and $9.76 for the 45-day, 90-day and 135-day-finishing durations, respectively) did not differ statistically, finisher ration cost-per-kg gain ($1.89, $1.54 and $1.39, respectively) decreased linearly ($P < 0.05$) and total live-weight gain (5.18 kg, 6.42 kg and 7.23 kg) and return-over-finisher ration ($4.42, $7.79 and $19.82, respectively) increased linearly ($P < 0.05$) with increase in length-of-finishing period. Finishing weaned meat goats over a longer duration with the same quantity of finisher ration was economically beneficial if labor costs are not included. When labor cost was factored into the equation, cost per kg gain increased and return-over-feed cost and labor decreased linearly ($P < 0.05$) with increase in length-of-finishing period from 45 days to 135 days of finishing. Finishing over a longer period resulted in negative return, which decreased linearly from -$0.08 for the 45 days, to -$1.21 for the 90 days and -$3.36 for the 135-day finished groups. It was not economically beneficial to finish crossbred meat goats using paid labor. Boneless-retail cut from the leg, loin, shoulder and rack increased linearly ($P < 0.05$) from 45 days to 135 days of finishing. No significant difference was observed in back-fat thickness while kidney, pelvic and heart fat tended to be higher for the 45-day-finished group

Key Words: Meat Goat, Finishing Period, Chevon Production, Return Over Finisher Ration Cost

Introduction

With the ever-increasing ethnic population in the United States, the demand for goat meat is on the rise. Forages/browse constitute the major feed resources for meat-goat producers in Tennessee during much of the year (Ball et al., 1991). Considering the high-nutrient need of weaned meat goats in their active stage of growth, it may be practically impossible to meet their nutrient needs for finishing on a forage diet alone. This is more so during the winter and summer seasons when native forages precipitously decline in nutrient quality and quantity. As a consequence, growth rate of weaned kids remains sub-optimal, and they take longer to attain market weight (Lema et al., 2008).

Any finishing diet for meat goats should be carefully assessed in terms of its cost effectiveness and ability to meet the animal's nutrient needs. Provision of supplemental commercial grower/finisher ration for meat goats is widely practiced in Tennessee, often without any guideline or standard. This condition can result in under feeding or over feeding of animals, which may indirectly affect the return from a meat-goat enterprise.

To ameliorate this situation, evaluation of the economics of alternative-finishing methods and their effect on other performance variables is of paramount significance. The general belief held by livestock producers is that finishing on a high plane of nutrition over a short duration is more economical in contrast to finishing over a longer period of time. This is because the longer the animal is finished, the higher the feed and labor costs. To date, there is no research information on the influence of finishing meat goats using the same total quantity of finisher ration over variable duration on meat-goat-performance measures.

The objective of this study was to evaluate the effect of finishing crossbred meat goats with a similar total quantity of finisher ration for 45 days, 90 days or 135 days on growth rate, chevon production (boneless-retail cut), carcass characteristics and return-over-feed cost.

Materials and Methods

Joy forage chicory (*Cichorium intybus* L.) was planted in six 0.4 ha plots on Arlington silt loam (Fine-silty, mixed, thermic cumulic hapludoll) soil at Tennessee State University, Agricultural Research and Education Center (36° 7' N, 86° 41' W; Nashville, Tenn.) at the rate of 23 kg per ha according to vendor recommendation. Before planting, soil samples were taken and plots were fertilized with 30 kg/ ha of ammonium nitrate according to soil test.

In the summer of 2009, thirty weaned F1 crossbred does (Kiko x Boer, Kiko x Spanish and Spanish x Boer) 120 days ± 8 days old were dewormed with Ivomec at 1ml per 21 kg body weight for internal parasite, blocked by body weight and genotype and randomly assigned to the six plots with 5 kids per plot. Subsequently, replicated plots with five kids in each were randomly assigned to 45-day, 90-day or 135-day-finishing-period treatments and group fed a total of 138 kg of commercial-finisher ration at the rate of 3.1 kg, 1.52 kg and 1.03 kg per day/per group, respectively over the entire finishing duration. The total of 138 kg finisher ration used was determined based on previous related experiments.

Animals in each plot were fed daily at 9:00 AM CST with their respective quantity of finisher ration and refusals recorded at 9:00 AM CST the next morning. Finisher-ration feeding was started as soon as the does were placed in their respective plots. Animals were continuously grazed throughout the finishing period. Based on visual observation, all the plots were comparable in pre-grazing herbage mass at the start of grazing. Stocking rate in each plot was deliberately kept low so that animals on the longer finishing durations would still have sufficient forage until the end of the finishing period, and forage availability would not influence the difference between the short- and long-finishing durations. Finisher-ration consumption was closely monitored and adjusted to keep total consumption for the finishing durations similar.

Water and a mineral and vitamin supplement (20 percent Na Cl, 16 percent Ca, 7 percent P, 1 percent Mg, 1 percent K, 1.25 percent S, 600 ppm CO, 70 ppm I, 2000 ppm Fe, 3000 ppm Mn, 60 ppm Se, 5000 ppm Zn, 225,000 IU/lb vitamin A, 40,000 IU/lb vitamin D-3 and 210 IU/lb vitamin E; Zinpro Corporation, Chaska, Minn.) was available free choice in each plot throughout the finishing period.

Does were weighed at two-week intervals and daily, biweekly and season-long average-weight gain estimated by regressing body weight gain over time. Finisher-ration samples were composited daily dried at 60 C° in a forced draft oven ground to pass 1-mm screen in a Wiley Mill and stored in air-tight Ziploc bags for chemical analysis at the end of the experiment. Feed samples were analyzed for dry matter (DM) (AOAC, 1995). Crude protein (CP), acid-detergent fiber (ADF) and neutral-detergent fiber (NDF) were determined using NIRS. Net energy (NE) was calculated according to Moore and Undersander (2002). The macro- and micro-mineral elements (P, K, Ca and Mg) were assayed using Perkin-Elmer SCIEXELAN600 Inductively Coupled Plasma Mass Spectrometry (Applera Corporation, Norwalk, Conn.).

At the end of the finishing period, final body weight was recorded, and does were kept off finisher ration over the weekend before being transported to an abattoir in Chapel Hill, Tenn. and slaughtered for carcass-characteristic determination. Slaughter weight and hot-carcass weight (HCW) were recorded, and carcasses chilled for 24 h before recording chilled-carcass weight (CCW). Hot-carcass weight and CCW were used to calculate hot-dressing percentage (HDP), and chilled- dressing percentages (CDP), respectively. Kidney, pelvic and heart fat (KPH) was removed and recorded. Carcasses were ribbed between the 12th and 13th ribs and back-fat thickness measured in mm. Subsequently, carcasses were separated into the four, major retail cuts (leg, shoulder, loin and the rack), and the leg and shoulder were de-boned and weights recorded.

Growth rate, chevon production (boneless-retail cut), carcass characteristics, feed-cost-per-kg gain and return-over-finisher-ration cost were used as major criteria for evaluating treatment effects. The data generated from the experiment was analyzed via ANOVA for a randomized complete block design (SAS, 1995) with the model including duration of finishing, block, genotype and interaction effects. Treatment means were separated using Least Square Means procedure.

Results and Discussion

Table 1 shows the chemical composition of the commercial finisher ration used in the study.

Total finisher-ration intake for the entire finishing period for the three finishing durations was similar (Table 2), as planned. Table 3 shows live-weight gain, cost-of-finisher-ration consumed, finisher-ration cost per gain and return-over-finisher ration and labor cost associated with the three finishing durations.

Table 1. Chemical composition of commercial finisher ration used in the study (DM basis)

Nutrient	
Crude protein, %	15.50
Ether extract, %	3.46
Acid -detergent fiber, %	22.00
TDN, %	80.50
Ash, %	8.00
Net Energy[1], Mcal/kg	1.60
Ca, mg/kg	12,400.00
P, mg/kg	5820.00

[1] Calculated

Slaughter weight, though not statistically different among the three finishing durations, showed an increasing trend with increase in finishing duration. Average-daily gain decreased linearly ($P < 0.05$) with increase in length of finishing period. The opposite was true for total live-weight gain. Finisher-ration efficiency (kg weight gain divided by kg finisher-ration consumption) was lowest for the 45-day duration and increased linearly ($P < 0.05$) with increase in length-of-finishing period. In spite of similar total finisher-ration consumption, dollar value of live weight increased linearly ($P < 0.05$) with length of finishing period.

Table 3. Effect of finishing crossbred meat goats with similar total quantity of finisher ration for 45, 90 or 135 days while grazing Joy chicory.

	Duration of finishing, Days			
	45	90	135	SEM
Initial weight, kg	21.06	21.50	21.03	1.01
Slaughter weight, kg	26.24	27.92	28.26	1.22
Finisher ration intake, kg	27.30	27.47	28.00	0.16
Live weight gain				
Start to slaughter, kg	5.18[a]	6.42[b]	7.24[c]	0.85
Daily, g	115.22[a]	71.33[b]	53.60[c]	23.14
Finisher ration efficiency[1]	0.19[c]	0.23[b]	0.26[a]	0.06
Dollar value of live weight gain[2]	14.25[c]	17.68[b]	19.90[a]	0.11
Cost of finisher ration[3], $	9.83	9.89	10.08	-
Finisher ration cost / kg weight gain, $	1.89[a]	1.54[b]	1.39[c]	0.10
Return over finisher ration, $	4.42[c]	7.79[b]	9.82[a]	0.02
Labor cost[4], $	4..50[c]	9.00[b]	13.50[a]	-
Return over grower ration and labor, $	-0.08	-1.21	-3.36	-

[1] Kg weight gain / kg finisher ration consumed
[2] at $2.75/kg,
[3] At $0.36/kg,
[4] At $8.00/hr,
[a,b,c] Means in the same row with different superscripts are different ($P < 0.05$)

To date, there is a dearth of research data and information regarding the effect of finishing meat goats on similiar quantity of finisher ration with variable finishing period on meat-goat performance. Hence no direct comparison can be made between our results and those of other workers on the subject.

Holding total finisher-ration intake constant for the different finishing durations in the present study was essential to avoid confounding effects of finisher-ration intake, thus enabling us to measure the effect of length of finishing period independent of feed intake with respect to finisher-ration consumption. Although keeping total finisher ration constant resulted in lower daily finisher-ration allowance and consumption per animal as finishing duration increased, animals on the longer durations (90 days and 135 days) appeared to still get their daily nutrient needs as evidenced by the available post grazing herbage mass and quality.

The increase in total weight gain observed with increased length of finishing may be partly explained by the normal physiological increase in organ growth and development as the animals increase in age. Correspondingly, increased digesta-retention time typically observed at lower feeding levels might have also positively contributed to the observed improvement in total weight gain with longer finishing duration, since daily supplemental feed intake decreased as finishing duration increased.

Table 2. Finisher ration intake of crossbred meat goats.

	Duration of Finishing, Days		
	45	90	135
Total, kg	27.30	27.49	28.00
Per day, g	606.70	305.40	208.00
Per kg live weight, g/day	28.22	14.50	9.95
Net energy intake, Mcal/day	0.97	0.49	0.33

Regardless of finishing duration, average-daily gains of animals under the three finishing treatments were relatively low. This might suggest that the amount of nutrient offered or available to the animals might have been insufficient for maximal weight gain, or as reported by Lema et al. (2008) that meat goats generally do not respond well in growth rate to concentrate supplementation similar to beef cattle, and it may be more economical to raise meat goats on good quality browse/forage alone (Wildeus et al., 2004).

While cost-of-finisher-ration consumed remained similar for the three finishing periods, cost per kg of weight gain decreased and return-over-finisher ration increased ($P < 0.05$) linearly with length of finishing. However, when labor cost, which included number of hours needed to feed, water and care for the animals under each finishing duration was factored into the equations, the extra return obtained as a result of prolonging finishing period was not sufficient to offset the increased labor cost associated with longer finishing period. With labor cost included, none of the three finishing periods were economically beneficial. The data suggest that, if unpaid, family labor is utilized it is beneficial to finish crossbred meat goats over a longer period of time with the same quantity of finisher ration rather than to attempt to finish them in a short duration.

Both chilled- and hot-carcass-dressing percentages increased linearly ($P < 0.05$) with increasing length of the finishing period (Table 4). Because the animals were off feed (concentrate feed) over the weekend before they were slaughtered, dressing percentages for all the three finishing durations were relatively low. Boneless-retail cut from the leg, loin, shoulder and rack also showed a similar trend (Table 4). Likewise, boneless leg, loin weight, rack and boneless shoulder weights tended to increase with length of finishing. Back-fat thickness and carcass-fat cover were similar among the three finishing durations. On the other hand, kidney, pelvic and heart fat was highest ($P < 0.05$) for the 45 day than for the 90-day and 135-day finished groups, suggesting that when meat goats are finished within a short duration on a high plane of nutrition, excess consumed energy is preferentially deposited as fat in these regions of the carcass

Conclusions

Based on the results of this research, we conclude that provided unpaid, family labor is available and other conditions are not limiting, it is economically advantageous and provides enhanced return from meat-goat enterprises by finishing crossbred meat goats using the same quantity of finisher ration over a longer finishing period rather than attempting to finish them fast over a short period. Finishing meat goats using paid labor is not cost effective. If family labor is not available, it may be more economically sound to finish meat goats on good quality pasture or browse alone.

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Table 4. Effect of finishing crossbred meat goats while grazing Joy chicory on major retail cuts and carcass characteristics.

	Duration of Finishing, Days			
	45	90	135	SEM
Slaughter wt. kg	26.24	27.92	28.26	1.01
Hot carcass weight, kg	9.32[a]	9.36[a]	13.11[b]	0.40
Chilled carcass weight, kg	8.84[a]	9.12[a]	12.79[b]	0.41
Hot carcass dressing percentage	31.59[a]	34.05[b]	46.39[c]	0.91
Chilled carcass dressing percentage	29.97[a]	33.18[b]	45.26[c]	0.11
Boneless shoulder, kg	1.32[a]	1.39[a]	2.18[b]	0.05
Boneless leg, kg	2.03[a]	2.07[a]	2.83[b]	0.02
Loin weight, kg	0.52[a]	0.62[a]	0.99[b]	0.52
Rack, kg	2.29[a]	2.99[a]	4.19[b]	0.34
Boneless retail cut[2], kg	6.18[a]	7.07[b]	10.20[c]	0.71
Back-fat thickness,mm	0.18	0.18	0.16	0.01
Kidney, pelvic and heart fat, g	0.17[a]	0.04[b]	0.05[b]	0.01

[1] Boneless shoulder + Boneless leg + Loin weight + Rack
[a,b,c] Means in the same row with different superscripts are different ($P < 0.05$)

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Evaluation of Ultrasonography to Measure Fetal Size and Heart Rate as Predictors of Fetal Age in Hair Sheep

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Summary

There is little information available on methods to estimate fetal age of hair sheep. The objective of this study was to determine whether crown-rump length (CRL) and/or fetal heart rate (FHR) could be used to predict fetal age in hair sheep using transrectal B-mode and Doppler ultrasonography. St Croix White, Barbados Blackbelly and Dorper X St. Croix White ewes (n = 54) were scanned weekly beginning 28 d after a successful mating. Linear array B-mode ultrasonography (5 MHz) was used to measure CRL and visual FHR, and Doppler ultrasonography was used to measure audible FHR. Due to the size of the fetus CRL was not measurable after 42 d of gestation, and visual FHR was not measurable after 70 d. Audible FHR was not consistently measurable before 35 d but could be measured through 140 d of gestation. Single fetuses had greater CRL ($P < 0.01$) than multiple fetuses at d 35 and d 42 of gestation. There was no difference ($P > 0.10$) in visual or audible FHR between single and multiple fetuses. Visual FHR was higher ($P < 0.0001$) than audible FHR at 49 d of gestation. Both CRL and audible FHR had a linear relationship with days of gestation for single and multiple fetuses. The relationship between days of gestation and visual FHR was best described by a cubic equation for single fetuses and a quadratic equation for multiple fetuses. Accuracy of the regression equations and the software in the ultrasound machine was evaluated by scanning a set of ewes (n = 51) also with known breeding dates. Both the equations and the software underestimated actual age of single fetuses by 2.5 d ($P < 0.01$). For multiple fetuses the equation overestimated the age by 1 day, and the software underestimated age by over 2 d ($P < 0.01$). Overall, the regression equations underestimated fetal age by 1 d and the software underestimated fetal age by more than 2 d ($P < 0.004$). Fetal age can be estimated with acceptable accuracy in hair sheep breeds, regardless of fetal number, using existing methods that were developed using other breeds of sheep.

Key Words: Sheep; Fetus; Ultrasonography; Fetal Heart Rate

Introduction

Pregnancy diagnosis in sheep usually is done using some form of ultrasonography, since rectal palpation is impractical due to the size of the animals. Linear-array ultrasonography is a common method because it allows the operator to view the fetus, determine fetal number and collect measurements of the fetus to determine fetal age (Griffin and Ginther, 1992; Karen et al. 2004; Romano and Christians, 2008). One measurement that is commonly used to estimate fetal age is crown-rump length (CRL). In addition to CRL, other measurements used to estimate fetal age in sheep include head width and thoracic depth (Sergeev, et al., 1990) and biparietal skull and body trunk diameter (Aiumlamai, et al., 1992). Neither of these studies indicated that there was an effect of fetal number on the estimated age of the fetus based on the measurements collected. Audible Doppler ultrasonography also has been used for pregnancy detection in the ewe (Lindahl, 1971), sow (Too et al., 1974; Pierce et al., 1976), mare (Mitchell, 1973) and cow (Mitchell, 1973).

Because hair sheep breeds in the tropics are smaller than most wool breeds in temperate areas and lamb birth weights are smaller (Godfrey et al. 2004; Godfrey, 2005), it is unknown if CRL measurements can accurately predict fetal age in these breeds using existing criteria. In addition, there is little information available on the effect of multiple fetuses on the accuracy of fetal age predictions. The objectives of this study were to compare measurements of CRL and fetal heart rate (FHR) throughout gestation using transrectal B-mode and Doppler ultrasonography in hair sheep. The effect of multiple fetuses on the accuracy of the measurements was evaluated.

Materials and Methods

The Institutional Animal Care and Use Committee of the Agricultural Experiment Station of the University of the Virgin Islands approved all procedures prior to commencement of this study (FASS, 1999). Hair sheep ewes (n = 54) were bred during a 35-d period in October 2003, and the number of ewes of each breed type that was carrying sin-

Table 1. Number of ewes of each breed type with either single, twin or triplet fetuses.

	Breed type			
Sire breed	Barbados Blackbelly	Dorper	Dorper x St. Croix White	St. Croix White
Dam breed	Barbados Blackbelly	St. Croix White	Dorper x St. Croix White	St. Croix White
Fetal number	Number of ewes			
1	12	2	5	1
2	10	4	4	10
3	0	6	0	0
Number of fetuses	32	28	13	21

Figure 1. Crown-rump length (CRL) of single and twin hair sheep fetuses measured between d 28 and 42 of gestation.

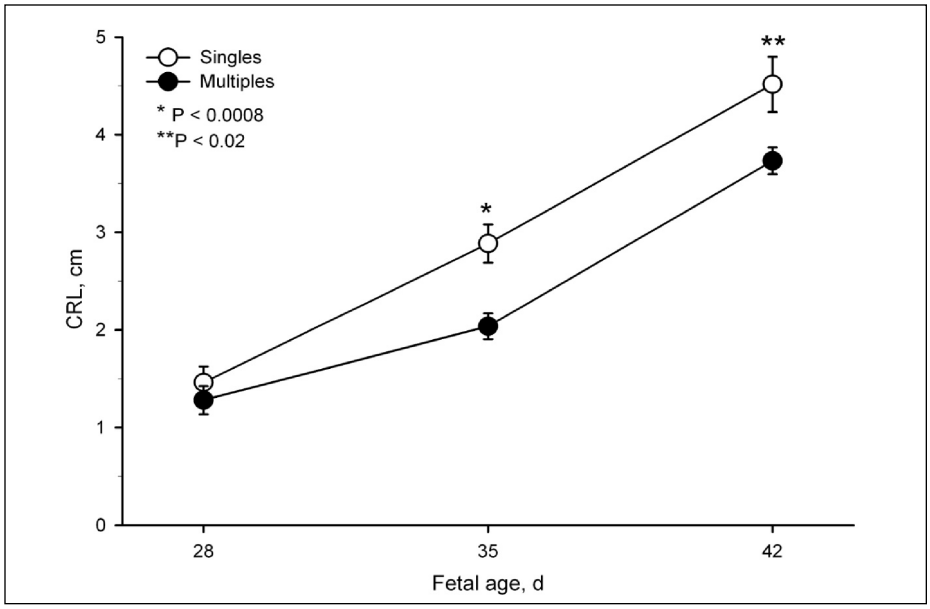
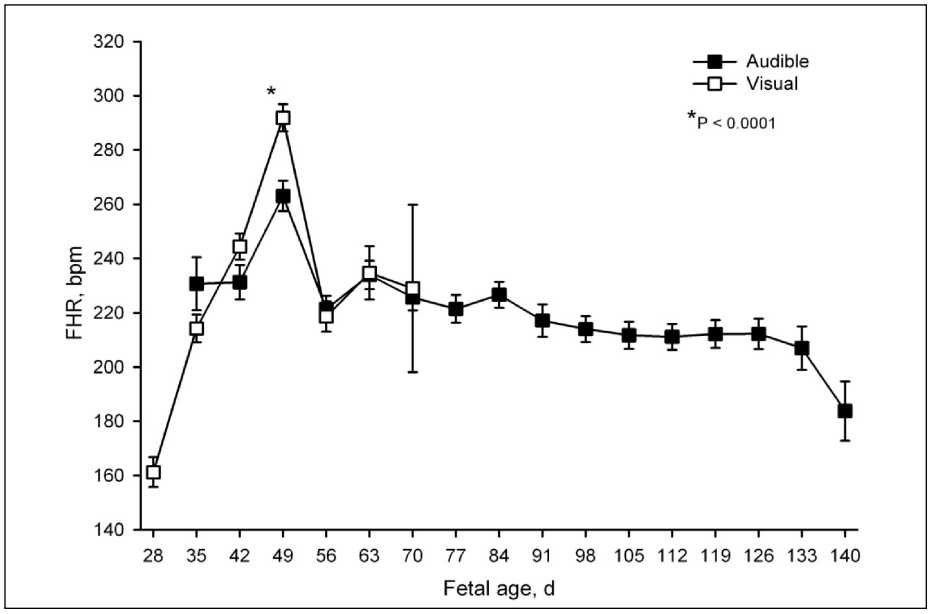


Figure 2. Audible and visual fetal heart rate (FHR) of hair sheep fetuses measured using Doppler or B-mode ultrasonography, respectively.



gle, twin or triplet fetuses is shown in Table 1. Rams were equipped with marking harnesses during the breeding period, and ewes were checked twice daily for crayon marks to determine actual breeding dates of the ewes.

Fetal measurements were collected weekly beginning on d 28 after a successful mating (d 0), based on crayon marks and non-return to estrus. Ewes were placed in dorsal recumbency in a flip cradle for the transrectal ultrasound examinations. Linear-array, B-mode ultrasonography was used to measure CRL and visually measure FHR using a Pie Medical Scanner 480 with a 5/7.5 MHz linear-array, switchable transducer set to 5 MHz for all examinations or a 5 MHz linear-array intracavity probe (Classic Medical, Tequesta, Fla., USA). Doppler ultrasound coupled to a battery-operated headset was used to measure audible FHR using a rectal probe (Medata Systems Ltd., West Sussex, UK). Two trained technicians conducted all ultrasound exams distributed uniformly across days and breeds.

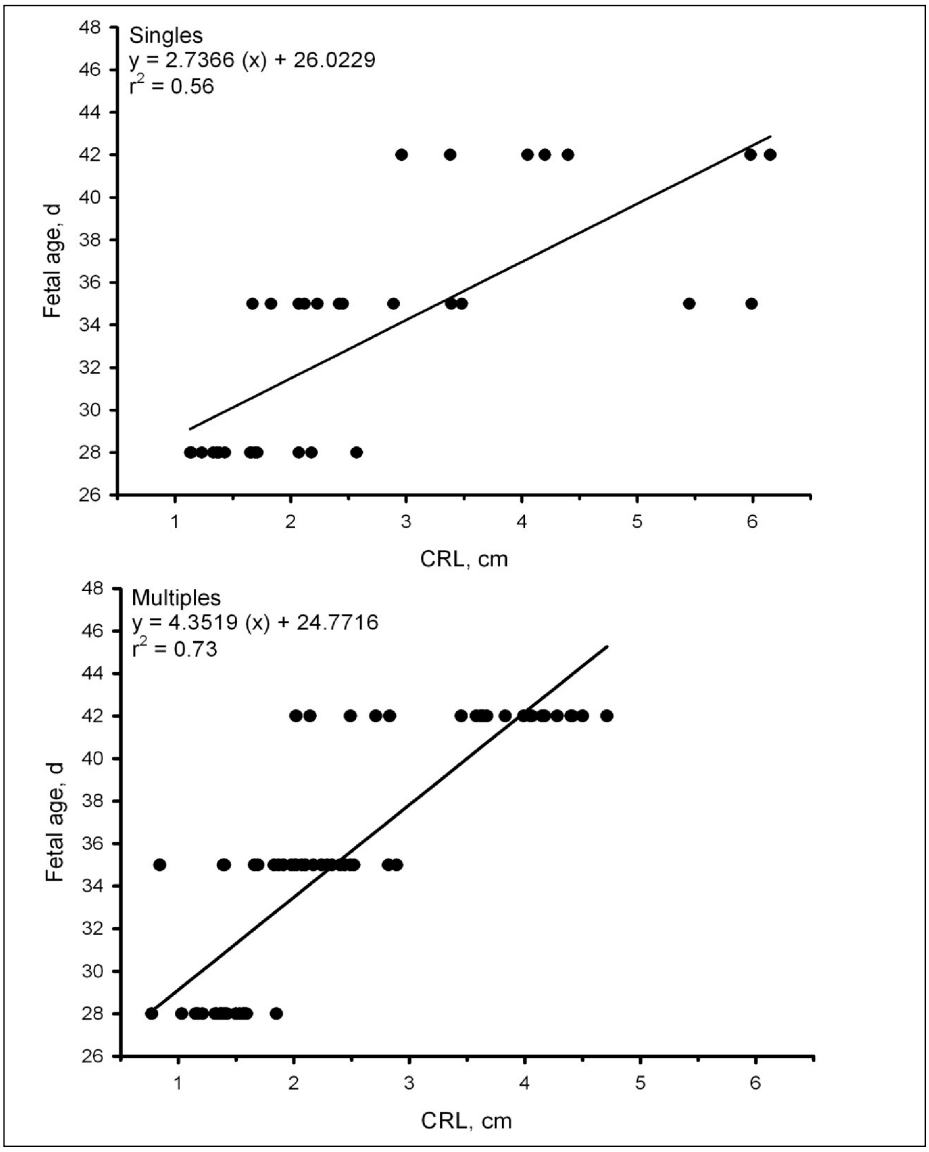
Visual FHR was measured on the image of the fetus using the linear-array ultrasound by counting the heartbeats on the screen for 10 s. The CRL was measured on a still image of the fetus on the screen using electronic calipers provided through the software on the machine. The cursor was placed on the top of the head and the second point was placed at the most distant point over the rump of the fetus.

Audible FHR was measured using the Doppler ultrasound probe by counting the heartbeats through the earphones for 10 s. All FHR were adjusted to beats/min (BPM). In ewes with multiple fetuses, measurements were collected on each fetus at each time point. This was accomplished by rotating the head of the transducer and monitoring the sound as one fetal heartbeat faded out and the transducer picked up the next fetal heartbeat. Because there was no way to determine which fetal heartbeat was being measured using the Doppler ultrasound, the results were coded by fetus number (1, 2 or 3). Repeatability of fetal-heart-rate measurements was not determined in this study, so the error associated with making the FHR measurements cannot be partitioned from the variance associated with the trait.

Data were analyzed using GLM procedures for repeated measures (SAS, 1999). Fetal breed type, number of fetuses and day of gestation were the main effects used in the model for CRL and audible and visual FHR. Because there were only two sets of triplets, fetuses were classified as either singles or multiples for the final analysis. There was no effect ($P > 0.10$) of fetal breed type on CRL or FHR (audible and visual), so all data were pooled across breed types for subsequent analysis. Relationships between fetal age and CRL and FHR measurements were determined using stepwise regression analysis with linear, quadratic and cubic terms included in the models within litter size.

To compare fetal ages produced by the machine software and the generated regression equations for CRL and fetal age, a second set of St. Croix White, Barbados Blackbelly and Dorper x St. Croix White ewes (n = 51) with known breeding dates, carrying single (n = 21) or multiple (n=30) fetuses were scanned for CRL between 26 d and 45 d of gestation (validation group). The age of the fetus determined by the software in the ultrasound machine and the regression equations developed for single or multiple fetuses were compared to the actual fetal age based on breeding dates. The

Figure 3. Regression of crown-rump length (CRL) on days of gestation in single or multiple hair sheep fetuses. The dots represent a measurement of CRL on day 28, 35 or 42 of gestation.



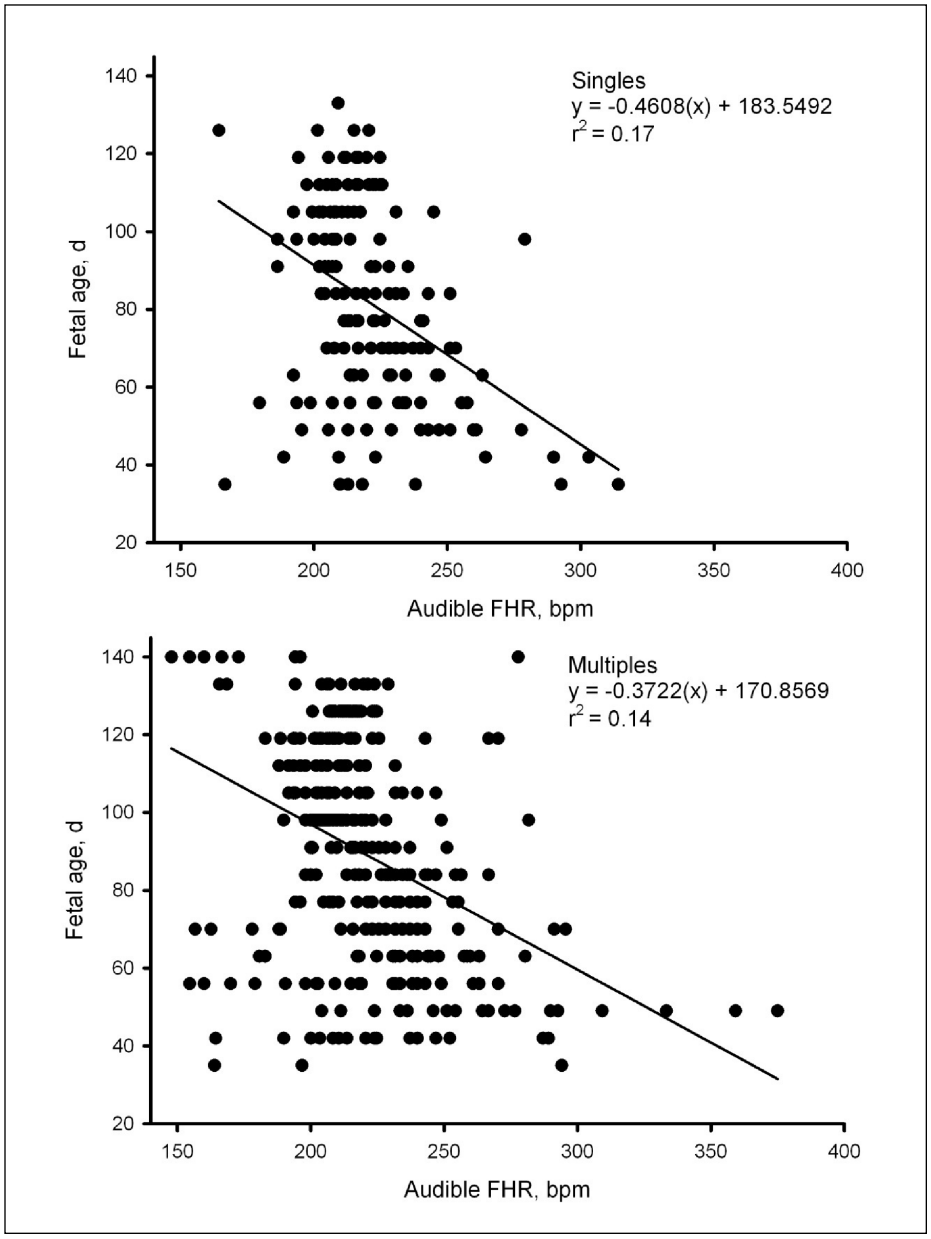
specific algorithm used in the ultrasound machine to estimate fetal age was not specified in the operators' manual. The differences were calculated as (actual age – estimated age), where estimated age was produced either by the software in the ultrasound machine or by the regression equations generated in this study. General Linear Models procedures of SAS were used to compare the differences using number of fetuses (single or multiple) in the model as the main effect. The Student's t-test was used to determine if the differences were equal to zero within fetal number groups.

Results and Discussion

Due to the size of the fetus and 5 MHz transducer depth of penetration, CRL was not consistently measurable before 28 d and after 42 d of age, and visual FHR was not consistently detectable before 28 d and after 70 d of age. Audible FHR was not consistently detectable before 35 d of age but could be measured through 140 d of age using the Doppler ultrasound. Romano and Christians (2008) reported that pregnancy could be detected in ewes as early as d 16 using a 7.5 MHz transducer, but the authors did not conduct any measurements of the fetus in the study. Other studies have shown that fetal measurements, such as biparietal-skull diameter and thoracic diameter, can be measured between 49 d and 140 d of gestation (Sergeev et al., 1990, Aiumlamai et al., 1992). In both of these studies the ultrasound was conducted transabdominally using either a 7.5, 5 or 3.5 MHz transducer, as opposed to transrectally using a 5 MHz transducer as in the present study.

There was no difference ($P > 0.10$) in CRL between single and multiple fetuses at 28 d of age, but single fetuses had a greater CRL than multiple fetuses at 35 d ($P < 0.0008$) and 42 d ($P < 0.02$) (Figure 1). In contrast, Sergeev et al. (1990) reported that there were no differences in head length among litter sizes of 1, 2 or 3 lambs. The difference in results between the two studies may be due to the type of fetal measurements being collected. This highlights the need to validate whatever method is chosen to estimate fetal age in sheep, whether it is CRL or head length or diameter. Visual FHR was similar to audible FHR at all times except for 49 d

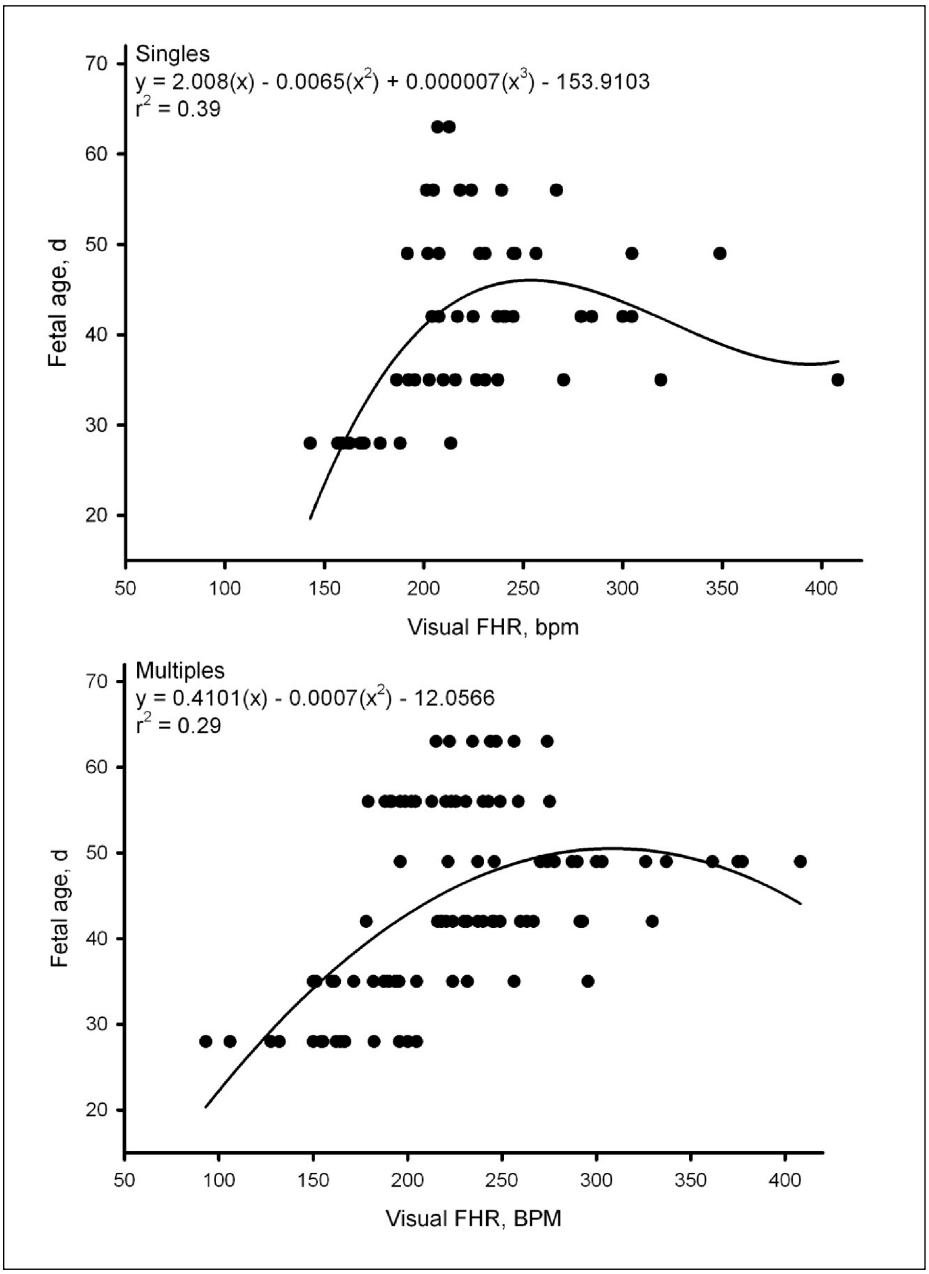
Figure 4. Regression of audible fetal heart rate (FHR) on days of gestation in single or multiple hair sheep fetuses. The dots represent a measurement of audible FHR between days 35 and 140 of gestation.



of age ($P < 0.0001$, Figure 2). There was an increase in FHR using both methods on d 49 but it was unclear what might have caused this. The values reported for FHR in the current study were higher than those reported by Aiumlamai et al. (1992) during the entire gestation. The decrease in FHR as fetal age increases was consistent with reports in sheep (Aiumlamai et al., 1992) and cattle (Breukelman et al., 2004).

Crown-rump length had a linear relationship ($P < 0.0001$) with age (Figure 3) in single and multiple fetuses. The brief time period (14 d) when CRL could be measured in the current study limits the amount of information that can be obtained about fetal age using the methods described. The use of other fetal measurements, such as skull or thoracic diameter, may expand the portion of gestation during which fetal age can be estimated in hair sheep (Sergeev et al., 1990; Aiumlamai et al., 1992). In cattle biparietal diameter of the cranium was measureable between d 35 and d 100 of gestation, and the relationship was linear (Breukelman et al., 2004). In rein-

Figure 5. Regression of visual fetal heart rate (FHR) on days of gestation in single or multiple hair sheep fetuses. The dots represent a measurement of visual FHR between days 28 and 70 of gestation.



deer CRL was measurable in week 3 through week 17 of gestation, and chest width and depth was measurable through week 19 of gestation, after which the fetus became too large to measure (Vahtiala et al., 2004). The authors also reported that CRL and chest width and depth had quadratic relationships with gestational age in reindeer.

Audible FHR had a linear relationship ($P < 0.0001$) with day of age (Figure 4) in single and multiple fetuses. Visual FHR had a curvilinear relationship ($P < 0.0001$) with day of age, that was best described by a cubic equation in single fetuses and a quadratic equation in multiple fetuses (Figure 5). In contrast, Aiumlamai et al. (1992) reported that visual FHR had a linear relationship with fetal age during the second half of gestation, but they did not distinguish between single or twin fetuses. In the current study the visual FHR is lowest at d 28, before audible FHR was consistently measureable, and this may contribute to the curvilinear relationship being reported.

The fetal age of the validation group that was scanned to evaluate the accuracy of the machine software and the generated regression equations for CRL ranged from 26 d to 45 d of age, based on breeding dates. On d 26, d 29 and d 38 fetal ages estimated by measuring CRL and using the regression equations developed in the current study (Figure 3) were greater ($P < 0.05$) than those estimated using CRL and the software in the ultrasound machine (Figure 6). For single fetuses, both the equation and the software underestimated actual age by 2 d to 3 d based on CRL measurements (Table 2). For multiple fetuses, the equation overestimated the age by 1 d and the software underestimated age by more than 2 d ($P < 0.0001$). Overall, the equations generated in the current study underestimated fetal age by 1 d, and the software underestimated fetal age by more than 2 d ($P < 0.04$). This small discrepancy between actual age and estimated age may not be important enough to impact decisions regarding management or predicting lambing time of ewes carrying single or multiple fetuses.

Conclusions

Fetal age can be estimated with acceptable accuracy in hair sheep breeds, regardless of fetal number, using existing methods that were developed using other breeds of sheep. It may be beneficial to develop systems that utilize measurements besides crown-rump length, such as cranial or thoracic diameter, to expand the number of days during gestation during which fetal age can be estimated. Because audible and visible fetal heart rate can be monitored for more days during gestation, it has the potential to be developed more fully as a tool for estimating fetal age.

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Figure 6. Relationship between actual fetal age and fetal age estimated by software included with the ultrasound equipment and the equations generated in this study (Figure 3).

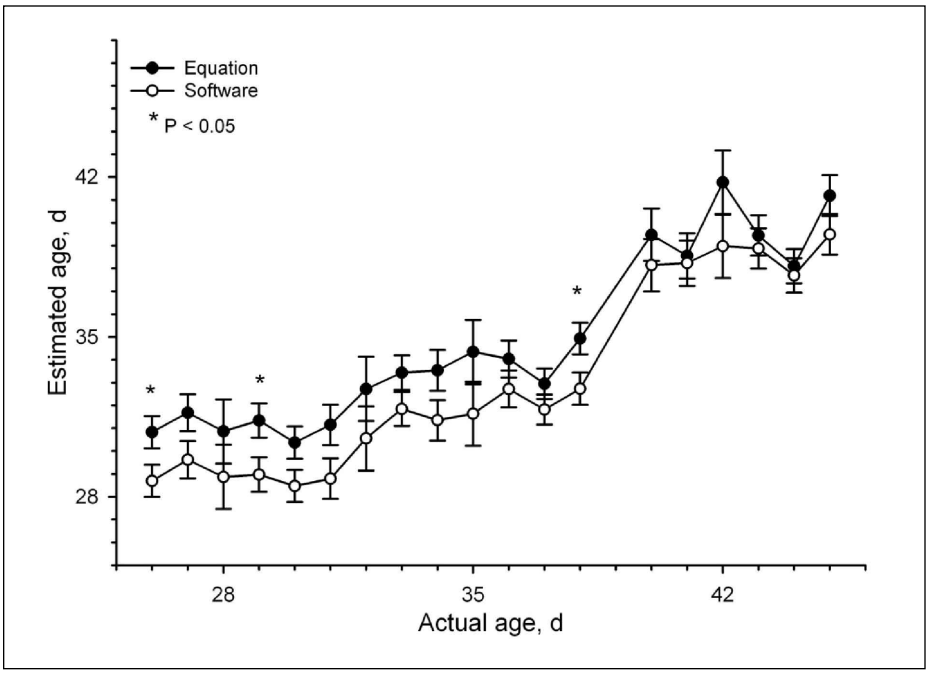

Table 2. Differences between actual and estimated fetal ages using CRL measurements based on fetal number in the validation group of ewes.

Number of fetuses	Comparison	Difference[a]
Single	Actual - Equation	2.6 ± 0.5[b]
	Actual - Software	2.4 ± 0.5[b]
Multiple	Actual - Equation	-0.4 ± 0.5[c]
	Actual - Software	2.4 ± 0.5[b]
Pooled	Actual - Equation	1.1 ± 0.4[d]
	Actual - Software	2.4 ± 0.4[e]

[a] Difference was calculated by subtracting fetal age estimated by equations generated in this study or fetal age estimated by ultrasound machine software from the actual fetal age, based on day of breeding. The equation used for a single fetus was y = 2.7366(x) + 26.0229 and for multiple fetuses was y = 4.3519(x) + 24.7716 (Fig. 3).
[b,c] Values within fetal number group or within comparison are different ($P < 0.0001$).
[d,e] Pooled values are different ($P < 0.02$).

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Protein Supplementation of Low-quality Forage: Influence of Frequency of Supplementation on Ewe Performance and Lamb Nutrient Utilization[1]

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Summary

Supplementation frequency (SF) of CP for ruminants consuming low-quality forage can be decreased to once every 7 d; however, no data are available describing the effects of decreasing SF to once every 10 d. Our objectives were to evaluate the influence of length of SF on forage intake, digestibility, N balance, digested N retained, and plasma concentration of urea-N in lambs and reproductive performance in pregnant ewes. Supplementation frequency included daily (D), once every 5 d (5D), or once every 10 d (10D) supplementation, and an unsupplemented control (CON). Sixteen wethers (31 ± 1 kg BW) were individually fed in a digestibility study (n = 4 wethers / treatment). The amount of CP supplied by each supplement was approximately 0.15 percent of BW/d (averaged over a 10-d period) and formulated to meet CP requirements. Sixty pregnant Rambouillet ewes (75 ± 0.4 kg BW) in the last third of gestation were used in a performance study. The amount of CP supplied by each supplement was approximately 0.11 percent of BW/d (averaged over a 10-d period) and formulated to meet CP requirements, not including CON. Basal diets consisted of low-quality (5 percent CP) barley straw. Total DMI and OM intake were not affected ($P \geq 0.93$) by supplementation. However, forage DMI, OM intake, and N intake by lambs decreased ($P \leq 0.06$) linearly as SF decreased. Apparent total tract digestibility of N for supplemented lambs was approximately 300 percent greater ($P < 0.001$) than the CON, with no difference ($P = 0.57$) as SF decreased. Digested N retained and N balance were greater ($P \leq 0.01$) for supplemented wethers than for CON, with no difference ($P > 0.31$) due to SF. Plasma urea (PU; mM) was measured over the10-d period. Supplemented lambs had increased ($P < 0.001$) PU compared with CON, but was not effected ($P = 0.32$) by SF. Crude protein SF had no affect ($P > 0.21$) on post-lambing weight change, pre- and post-lambing BCS change, lambing date, and average lamb birth weight. Results suggest ruminants consuming low-quality forage can be supplemented with protein as infrequently as once every 10 d, while not negatively affecting nutrient digestibility or ewe performance.

Key Words: Crude Protein, Lamb, Reproduction, Sheep, Supplementation Frequency

Introduction

In the northern Great Plains, calculated winter feed costs are often $20 to $50 per ewe per year. Management and nutritional practices that decrease winter feed costs, while maintaining rangeland health, may increase profitability for livestock producers. One management alternative that may decrease winter feed costs is to extend the grazing season through the winter months of December, January, and February. Protein supplementation may be necessary during this time period (Schauer et al., 2001), and the non-feed costs associated with providing supplemental protein can be substantial (labor, fuel, hours, etc.). Current research suggests that the frequency of protein supplementation may be decreased to once every 7 days while maintaining livestock performance (Huston et al., 1999; Bohnert et al., 2002; Schauer et al., 2005). However, as supplementation frequency decreases, N retention decreases and can alter rumen microbial populations (Farmer et al., 2004b). If supplementation frequency (SF) can be decreased from daily to once every 10 d, labor and fuel costs can be further reduced. Therefore, our objectives were to evaluate the influence of length of supplementation frequency on forage intake, digestibility, N balance, digested N retained, and plasma concentration of urea-N in lambs (as a model for ewes) and reproductive performance in pregnant ewes.

Materials and Methods

All animal care and handling procedures were approved by the North Dakota State University Institutional Animal Care and Use Committee before the initiation of the research.

Digestion Study

Sixteen wethers (31 ± 1 kg BW; approximately 90 d of age) were used in a completely randomized design (Cochran and Cox, 1957) to evaluate the efficacy of N use in lambs fed low-quality forage (5 percent CP) and supplemented with soybean meal (SBM) daily or infrequently. Wethers were randomly allotted to SF treatments (n = 4 wethers/treatment) and housed in individual metabolism crates within an enclosed barn with continuous lighting.

Wethers had continuous access to fresh water and chopped barley straw (4 cm to 8 cm length). Barley straw was provided (in 2 equal portions; 0700 h and 1700 h) daily at 120 percent of the average intake for the previous 5 d, with feed refusals from the previous day determined before feeding. A trace mineral salt mix was available free choice (22 percent Ca, 8 percent P, 16.5 percent Na, 0.25 percent Mg, 1 percent S, 5 ppm Co, 35 ppm I, 1350 ppm Mn, 2.5 ppm Se, 1890 ppm Zn, and 90,909, 12,727, and 227 IU/kg vitamins A, D, and E, respectively; DM basis). In addition, an intramuscular injection of vitamins A and D (500,000 IU and 75,000 IU of vitamins A and D, respectively) was administered to each wether at the onset of the trial. Supplementation frequency included daily (D), once every 5 d (5D), or once every 10 d (10D) supplementation, and an unsupplemented control (CON). All supplemented wethers received the same total amount of supplement over a 10-d period; therefore, the 5D and 10D treatments received fivefold and tenfold the amount of supplement (N basis) on their respective supplementation day compared with D treatments. The amount of CP supplied by each supplement was 0.15 percent of initial BW/d (averaged over 10-d) based on intake and protein requirements (NRC, 1985). To prevent bias due to weight change due to treatment during each period, the quantity of supplement provided in each period was based on initial BW. Ingredient and nutrient content of the barley straw and supplement are described in Table 1.

The experimental period was 30 d. Forage intake was determined on d 19 to d 28. Samples of barley straw, SBM, and orts were collected on d 19 to d 28 and dried at 55°C for 48 h. On d 21 to d 30, total fecal and urine output were collected. Urine was composited daily by wether (25 percent of total; weight basis) and stored at 4°C. Sufficient 6 N HCl (150 mL) was added to urinals daily to maintain urine pH < 3. A sub-sample of each daily fecal sample (7.5 percent; weight basis) was dried at 55°C for 96 h to calculate fecal DM. On d 21 to d 30, 12 mL of blood was collected from the jugular vein at 4 h after feeding using a heparinized syringe. Blood samples were immediately transferred to vacutainers (Fisher Scientific, catalog no. 0268360), placed on ice, centrifuged (5000 x g, 15 min), and plasma harvested and stored (-20°C).

Dried feed and fecal samples were ground through a Wiley mill (1-mm screen). Daily samples of barley straw, SBM, and orts were composited by lamb on an equal weight basis (20 percent as-fed). Feed, orts, and fecal samples were analyzed for DM and OM (AOAC, 1997). Concentrations of NDF (Van Soest et al, 1991, as modified by Ankom Technology, Fairport, NY) and ADF (Goering and Van Soest, 1970, as modified by Ankom Technology) were determined using an Ankom 200 Fiber Analyzer (Ankom Technology) without sodium sulfite, with amylase, and without ash corrections as sequentials. Feed, orts, fecal, and urine samples were analyzed for Kjeldahl N (AOAC, 1997) and plasma samples were assayed for urea-N using a colorimetric diacetyl monoxine procedure (Urea Nitrogen, Procedure No. 535, Sigma Diagnostics, St. Louis, Mo.)

Ewe Performance Study

Sixty pregnant Rambouillet ewes (75 ± 0.4 kg BW; 3.1 ± 0.1 BCS) in the last third of gestation were stratified by age and BCS (1 = emaciated, 5 = obese)

Table 1. Dietary ingredient and nutrient composition of lamb and ewe diets (DM basis).

Item	Barley Straw	Soybean Meal
Supplement composition		
Soybean meal, %	-	100
Nutrient composition, %		
CP	5.0	52.6
OM	90.9	92.7
NDF	71.8	18.2
ADF	43.7	4.9

and assigned randomly within stratification to 1 of 3 SF treatments (as described in the digestion study, but not including CON) in a completely randomized design to evaluate ewe performance and lamb birth weight when consuming low quality forage (5 percent CP) and supplemented with SBM daily or infrequently. Ewes were sorted by treatment and allotted randomly to 1 of 12 pens (n = 4 pens/treatment). Protein supplements were offered as D, 5D, or 10D at 0800 h to provide approximately 0.11 percent of BW/d of CP (averaged over a 10-d period; 145 g/d) until lambing based on intake and protein requirements (NRC, 1985). Ewes had continuous access to fresh water and chopped barley straw (4 cm to 8 cm length). A trace-mineralized-salt mix was available free choice (22 percent Ca, 8 percent P, 16.5 percent Na, 0.25 percent Mg, 1 percent S, 5 ppm Co, 35 ppm I, 1350 ppm Mn, 2.5 ppm Se, 1890 ppm Zn, and 90,909, 12,727, and 227 IU/kg vitamins A, D, and E, respectively; DM basis). Ingredient and nutrient content of the barley straw and supplement are described in Table 1.

Ewe BW and BCS (both visual appraisal and palpation of subcutaneous fat cover) were measured every 14 d until lambing and within 14 d following lambing for approximately 57 d. All weights were consecutive 2-d unshrunk weights. Ewe BCS was evaluated independently by the same two observers throughout the experiment. Forage and supplement samples (approximately 200 g) were collected weekly, dried at 55°C for 48 h, ground through a Wiley mill (1-mm screen), and composited by month for analysis of ADF and NDF, N, and OM as described in the digestion study.

Statistical Analysis

Nitrogen balance data (excluding plasma-urea-N data) were analyzed as a completely randomized design (Cochran and Cox, 1957) using the GLM procedure of SAS (SAS Inst. Inc., Cary, N.C.) with animal serving as experimental unit. The model included lamb and treatment. Plasma urea-N was analyzed using the Repeated statement with the Mixed procedure of SAS. The model included treatment, day, and treatment x day. Additionally, lamb x treatment was used to specify variation between lambs (using the RANDOM statement). Lamb x treatment was used as the SUBJECT and autoregression used as the covariance structure. Response variables included: 1) DM, OM, NDF, and N intake; 2) total tract digestibility of DM, OM, NDF, and N; 3) N balance; 4) digested N retained; and 5) plasma concentration of urea N. Orthogonal contrasts included: 1) CON vs. protein supplementation and 2) linear effect of supplementation frequency. Significance was declared at $P < 0.10$.

Ewe and lamb performance data were analyzed as a completely randomized design using the GLM procedure of SAS with pen serving as experimental unit. The model included treatment. Response variables included: 1) ewe weight change; 2) ewe BCS change; and 3) lamb birth date and average lamb weight. Orthogonal contrasts included: 1) D vs. infrequent supplementation; and 2) linear effect of supplementation frequency. Significance was declared at $P < 0.10$.

Results and Discussion

Digestion Study

The effect of supplementation frequency on lamb intake, diet digestibility, and nitrogen balance is reported in Table 2. Total DMI and OM intake were not affected ($P \geq 0.93$) by CP supplementation; however, intake of hay DM and OM was affected by CP supplementation ($P = 0.06$; Table 2), with 5D and 10D linearly decreasing ($P < 0.06$) hay DM and OM intake. Total DM and OM intake responded similarly, with total DM and OM intake exhibiting a linear decrease ($P = 0.06$) as SF decreased. Also, daily NDF and N intake decreased linearly ($P = 0.06$) as SF decreased; but all supplemented treatments had higher N intake than CON ($P < 0.001$; Table 2). Apparent total tract digestibility of DM and OM was greater ($P = 0.001$) for lambs fed supplements, with no difference ($P > 0.34$) resulting from SF. Additionally, no difference ($P = 0.18$) was observed for NDF digestibility. However, apparent total tract digestibility of N for supplemented lambs was greater ($P < 0.001$) than the CON, with no difference ($P = 0.57$) because of SF (Table 2). Daily fecal N excretion decreased ($P < 0.001$) and urinary N excretion increased ($P < 0.001$; Table 2) due to CP supplementation. As SF decreased, fecal N excretion exhibited a linear decrease ($P < 0.001$) from D to 5D; however, no difference was noted due to CP SF for urinary N excretion ($P = 0.95$). Daily N balance and digested N retained were greater ($P < 0.01$) with CP supplementation, with no difference observed for SF ($P \geq 0.31$).

Treatment x day interactions ($P < 0.001$; Figure 1) were observed for plasma concentration of urea-N. However, after considering the nature of the interactions, we concluded that discussing treatment means while providing the treatment x day figure would aid in interpretation and discussion of the data. Lamb plasma urea-N was greater ($P < 0.001$) in CP-supplemented lambs than in CON (Table 2). No difference was observed due to CP SF ($P = 0.32$) for lamb plasma urea-N concentrations.

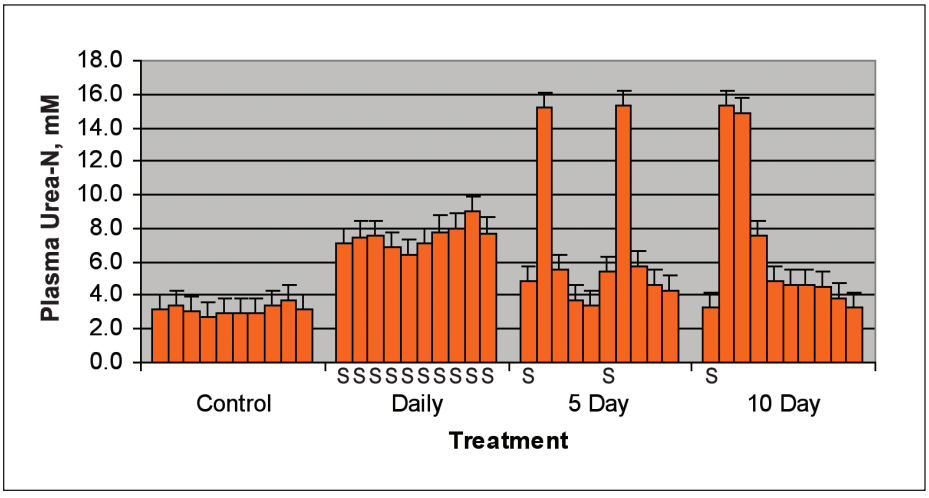
While lamb forage intake data were similar to those reported by some researchers (Ferrell et al., 1999; Bohnert et al., 2002), they contrasted with other studies evaluating protein supplementation of low-quality forage (DelCurto et al., 1990; Köster et al., 1996; Bandyk et al., 2001). Bohnert et al. (2002) reported no affect of CP supplementation on hay DM and OM intake, with total DM, OM, and N intake increasing with supplementation. Our results support the conclusions of Bohnert et al. (2002) and Mertens (1985, 1994) who all concluded that, when daily NDF intake is greater than 12.5 g/kg BW per d dry matter intake is maximized, with no expected increase in DMI when supplemental CP is supplied. This conclusion is further supported by Ferrell et al. (1999) who reported no increase in forage intake when lambs supplemented with CP were already consuming 13.0 g NDF / kg BW per d. In the current study, daily NDF intake by control lambs was 13.1 g/kg BW per d with a range of 10.8 g/kg to 13.1 g/kg for supplemented lambs. In experiments which showed that supplemental CP elicited an increase in forage intake, NDF intake of unsupplemented controls was 6.4 g/kg , 5.1 g/kg , and 8.2 g/kg BW per d (DelCurto et al., 1990; Köster et al., 1996; Bandyk et al., 2001, respectively). Based on our results and the results of others, it appears that protein supplementation to lambs consuming at least 12.5 g/kg BW per d of NDF will not result in an increase in forage intake.

Table 2. Effect of supplementation frequency on lamb intake, diet digestibility, and nitrogen balance.

	Supplementation frequency[a]					*P*-value[c]	
Item	CON	D	5D	10D	SEM[b]	CON vs. supp.	Linear SF
Daily DMI, g/kg BW							
Hay	18.3	17.7	15.0	14.4	1.1	0.06	0.06
Supplement[d]	0.0	2.8	2.8	2.8			
Total	18.3	20.4	17.8	17.1	1.07	0.94	0.06
Daily OM intake, g/kg BW							
Hay	16.7	16.1	13.7	13.1	1.0	0.06	0.05
Supplement[e]	0.0	2.5	2.5	2.5			
Total	16.7	18.7	16.2	15.6	1.0	0.93	0.06
Daily NDF intake, g/kg BW	13.1	13.1	11.3	10.8	0.7	0.14	0.06
Daily N intake, g/kg BW	0.147	0.373	0.344	0.335	0.012	<0.001	0.06
Total tract digestibility, %							
DM	43.8	50.7	51.6	52.1	0.01	0.001	0.43
OM	45.0	52.3	53.0	54.1	0.01	0.001	0.34
NDF	43.9	46.0	46.2	47.9	0.02	0.18	0.45
N	16.1	64.1	66.6	65.7	0.02	<0.001	0.57
Daily N excretion, g/kg BW							
Fecal	0.123	0.134	0.115	0.116	0.009	<0.001	<0.001
Urinary	0.096	0.247	0.256	0.248	0.014	<0.001	0.95
Daily N balance, g/kg BW	-0.072	-0.008	-0.027	-0.029	0.014	0.01	0.31
Daily digested N retained, %[f]	-308.7	-3.4	-11.7	-13.8	9.0	<0.001	0.43
Plasma urea-N, mM	3.12	7.49	6.80	6.69	0.55	<0.001	0.32

[a] CON = no supplement; D = soybean meal every day; 5D = soybean meal every 5th day; 10D = soybean meal every 10th day.
[b] n = 4 wethers / treatment.
[c] CON vs. supp. = control vs. supplemented treatments; Linear SF = linear effect of supplementation frequency.
[d] D received 2.8 g/kg BW daily; 5D received 14 g/kg BW once every 5 d; 10D received 28 g/kg BW once every 10 d;
[e] D received 2.5 g/kg BW daily; 5D received 12.5 g/kg BW every 5th d; 10D received 25 g/kg BW every 10th d.
[f] Calculated as (Daily N retention, g/kg BW/Daily N digested, g/kg BW) x 100.

Figure 1. Effect of crude protein supplementation frequency on plasma urea-N (mM) of lambs. Columns from left to right for each treatment represent d 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 of a 10-d supplementation window, respectively. Treatments were as follows: Control = no supplement; Daily = soybean meal every day; 5 Day = soybean meal every 5th day; 10 Day = soybean meal every 10th day. Each column with an S below represents a supplementation day. Treatment x day interaction (*P* < 0.001). SEM = 0.91.



The effect of supplementation frequency on forage intake has been reported as both positive and negative (Beaty et al., 1994; Huston et al., 1999; Krehbiel et al., 1998; Bohnert et al., 2002). Beaty et al. (1994) observed that when steers consuming wheat straw were supplemented daily or three times weekly, three times weekly supplemented steers consumed 17 percent less straw and 12 percent less total DM compared to daily supplemented steers. Bohnert et al. (2002) reported that as SF decreased from daily to once every 6 d, forage intake decreased by approximately 13.5 percent (average of degradable and undegradable intake protein treatments) and total DM intake decreased by approximately 12 percent. As SF decreased in the current study from D to 5D and 10D, hay DM intake decreased by 12 percent and 19 percent, respectively, and total DM intake decreased by 13 percent and 16 percent, respectively.

Similar to the report by Bohnert et al. (2002), this decrease in forage and total DM intake can be partially explained by depressions in intake in the days following supplementation (data not shown). However, these responses are not similar to results reported by Huston et al. (1999) and Krehbiel et al. (1998). Huston et al. (1999) reported that hay and total DM intake was not affected when ewes consuming wheat straw were supplemented with cottonseed meal daily or once every 7 d. In a similar fashion, Krehbiel et al. (1998) reported an increase in total intake of bromegrass hay by ewes supplemented with soybean meal every 24 or 72 h, compared to unsupplemented controls.

Our results suggest that apparent total tract digestibility of DM and OM was increased by CP supplementation, with no differences due to SF. These results support conclusions reached by other researchers (DelCurto et al., 1990; Beaty et al., 1994; Bandyk et al., 2001; Bohnert et al., 2002). Wickersham et al. (2008a) observed increases in both OM and NDF total tract digestibility as the amount of DIP was increased in daily supplementation. Research by Petersen (1987) suggests that increases in DM and OM digestibility following CP supplementation are largely the result of increased N availability for ruminal microflora. It is not readily apparent why NDF apparent total tract digestibility did not increase in the current study. Other researchers (DelCurto et al., 1990; Beaty et al., 1994; Bandyk et al., 2001; Bohnert et al., 2002) have demonstrated that NDF digestibility usually follows DM and OM digestibility, increasing with CP supplementation.

Apparent total tract N digestibility for supplemented lambs was approximately 309 percent greater than the CON. While greater in magnitude, this increase in N digestibility is similar to responses reported by Bohnert et al. (2002). In both the current trial and in the experiment by Bohnert et al. (2002), the low N digestibility can largely be attributed to the high fiber content and low N content of the low-quality forage utilized. Additionally, results from Bohnert et al. (2002) and Ferrell et al. (1999) indicated that, for diets containing low-quality forage, a large portion of the fecal N is metabolic fecal N. In fact, Ferrell et al. (1999) reported that up to 105 percent of observed fecal N loss may be the result of metabolic fecal N. This large excretion of fecal N in studies utilizing low-quality forage fed to ruminants led Ferrell et al. (1999) to the conclusion that caution should be used when interpreting apparent total tract N digestibility.

In the current study, daily N excretion behaved differently than previously reported (Coleman and Wyatt, 1982; Bohnert et al., 2002). Coleman and Wyatt (1982) reported that steers consuming low-quality forage and supplemented with cottonseed meal every d, every 2 d, or every 3 d had no difference in daily fecal N excretion compared with a control. In contrast, Bohnert et al. (2002) supplemented CP daily, once every 3 d, or once every 6 d to lambs consuming low-quality forage and reported an increase in daily fecal N excretion. Similar results were reported by Wickersham et al. (2008a) where steers fed low-quality forage had an increase in both fecal and urinary N excretion as the amount of daily supplemented DIP increased. In the current study, we observed a 2 percent decrease in daily fecal N excretion for supplemented lambs compared to the CON, and a linear decrease as SF decreased. However, urinary N excretion in our study behaved in a similar fashion to Coleman and Wyatt (1982) and Bohnert et al. (2002), increasing with CP supplementation with no affect because of SF. Schmidt-Nielson and Osaki (1958) reported that, when fed a 3 percent digestible CP diet, ewes had a decrease in urea N excreted by the kidney compared to ewes fed a 7.5 percent digestible CP diet. In our trial, the lambs on the 5D and 10D treatments were fed a N deficient diet for 4 d or 9 d, respectively. The results observed in the current trial were similar to those of Schmidt-Nielson et al. (1957) when a N-deficient diet was fed to camels and a reduction in urea-N excretion was observed compared with a N sufficient diet. Daily urinary N excretion was similar across all supplementation frequencies, which would indicate that lambs have the ability to regulate renal N excretion.

In the current trial daily N balance was improved (less negative) with CP supplementation compared to the CON lambs; however, as SF became less frequent N balance decreased numerically, though not significantly. Bohnert et al. (2002) reported similar results. This is partly explained by the reduction in N retained as supplementation frequency decreased. However, both N balance and N retained of the lambs supplemented less frequently were greater than that of the lambs that received CP supplemented daily. In contrast, Wickersham et al. (2008b) observed a tendency for increased N retained in steers fed low-quality forage as supplementation frequency of DIP decreased. This suggests that N efficiency was similar between lambs across the SF treatments. The results of the current trial and Bohnert et al. (2002) imply that lambs fed low-quality forage (5 percent CP) can conserve N efficiently when CP SF is as infrequent as 10 d.

Bohnert et al. (2002) reported that lambs supplemented CP as infrequently as once every 6 d had similar digested N retained to daily supplementation, even though in their trial N balance was decreasing as SF decreased. In our trial, the negative values for N balance and daily digested N retained indicate that the lambs were losing weight (data not shown); however, the values for 5D and 10D supplemented treatments were similar in magnitude to D, and in all cases were less negative than CON. These results suggest that ruminants consuming low-quality forage are capable of efficiently conserving N when supplemented with CP as infrequently as once every 10 d. Daily plasma urea N concentrations shown in Figure 1 support this conclusion. For the 10D supplemented-treatment, plasma-urea-N appeared to maintain a peak concentration for 2 d, whereas the peak for 5D was restrained to a single day. Maintenance of the plasma-urea-N peak for an additional day indicates that N may have been recycled longer for lambs with SF of 10D than for lambs with SF of 5D, resulting in similar N balance for the two treatments. Bimodal peaks were observed in the 5D treatment following supplementation by experimental design. The peaks of plasma-Urea-N concentrations in the current trial were similar to those observed in Huston et al. (1999), Krehbiel et al. (1998), and Bohnert et al. (2002). Wickersham et al. (2008b) also noted an increase in plasma-urea-N concentrations of steers on the day of supplementation when DIP was supplemented every third day, whereas plasma-

urea-N concentrations remained steady when steers were supplemented daily. Similarly, Wickersham et al. (2008a) noted an increase in plasma-urea-N concentrations in steers as the amount of daily supplemented DIP in the diet increased linearly.

Low-protein diets and(or) restricted feeding can alter gastrointestinal tract permeability to urea and renal urea excretion regulation (Harmeyer and Martens, 1980 and Kennedy and Milligan, 1980). Harmeyer and Martens (1980) concluded that three factors influence urea excretion via the kidneys: 1) changes in plasma urea concentrations correspond to changes in filtered urea loads, 2) glomerular filtration rate changes, and 3) tubular resorption of urea changes. Urea-N removal via the portal drained viscera increased when supplementation frequency increased to every 3 d compared with every day supplementation (Krehbiel et al., 1998). Wickersham et al. (2008a) reported that as the amount of DIP increased in the supplement, urea production, gut entry, return to the ornithine cycle, fecal excretion, and anabolic use increased. This suggests that as DIP increased in the diet, steers were more efficient in N recycling. Although not significant, as DIP was supplemented at 183 mg of N/kg of BW every third day to steers, urea production, urea-gut entry, urea returned to the ornithine cycle, and anabolic use of urea decreased compared with daily supplementation of 61 mg of N/kg of BW (Wickersham et al., 2008b). However, when steers were supplemented with 549 mg of N/kg of BW every third day, increased urea production, gut entry, urea returned to the ornithine cycle, and anabolic use were observed compared with steers supplemented with 183 mg of N / kg of BW (Wickersham et al., 2008b). The results from Wickersham et al. (2008a, 2008b) would suggest that the lambs in the current trial were able to more efficiently conserve and recycle urea-N as supplementation frequency decreased.

Urea transporters are tightly regulated by dietary components, such as concentrates and silage (Simmons et al., 2009). Simmons et al. (2009) observed an increase in urea transporter, bUT-B, in all epithelial layers of the rumen in a concentrate-based diet compared with a silage-based diet. The urea transporter in the silage-based diet was mainly localized to the stratum basale layer (Simmons et al., 2009). Ludden et al. (2009) observed two distinct urea transporters in the gastrointestinal tract and related tissues of lambs fed low-quality forage and supplemented with both RDP and RUP. Urea-transporter-B expression was observed in the liver, which is significant because in mammals, the liver is the site of ureagenesis (Ludden et al., 2009). This would indicate that UT-B may be a major factor in transporting urea out of the liver in the N recycling process (Ludden et al., 2009). Ludden et al. (2009) reported there were no differences in UT-B expression due to RUP supplementation. However, in the same trial, N-glycosylated UT-B expression was increased in the ventral rumen of the lambs fed RDP daily. Ludden et al. (2009) suggests that N-glycosylated UT-B expression in the ventral rumen was increased due to the daily supplementation of RDP and may play a major role in urea excretion and not urea recycling. The increased daily urinary N excretion and plasma-urea-N concentrations of the CP-supplemented lambs would suggest that a urea transporter is playing a vital role in urea excretion. However, the increased daily N balance of the CP-supplemented lambs would suggest that urea transporters would play a vital role in urea recycling, especially in the 5D and 10D lambs.

Ewe Performance Study

Post-lambing (within 14-d post-lambing) weight change and pre-lambing (within 14-d pre-lambing) and post-lambing weight and BCS changes were not affected ($P > 0.90$) by CP SF (Table 3). However, as SF decreased pre-lambing-weight change trended towards

Table 3. Effect of supplementation frequency on ewe performance and lamb birth weight.

	Supplementation frequency[a]				P-value[c]
Item	D	5D	10D	SEM[b]	Linear SF
Supplement DMI, g/d[d]	145	145	145		
Initial weight, kg	75	75	75	0.4	
Initial body condition score	3.0	3.3	3.0	0.1	
Weight change, kg					
Prelambing[e]	1.7	1.5	5.6	1.3	0.06
Postlambing[f]	-3.2	-1.7	-3.3	1.4	0.96
Body condition score change					
Prelambing[e]	-0.1	-0.1	-0.1	0.1	0.95
Postlambing[f]	-0.2	-0.3	-0.2	0.2	0.90
Lamb birth date, Gregorian d	265	267	264	2	0.85
Lamb birth weight, kg	5	5	5	0.2	0.21

[a] D = soybean meal every day; 5D = soybean meal every 5th day; 10D = soybean meal every 10th day.
[b] n = 4 pens / treatment.
[c] Linear SF = linear effect of supplementation frequency.
[d] D received 145 g daily; 5D received 725 g once every 5 d; 10D received 1,450 g once every 10 d.
[e] Within 14 d prior of lambing.
[f] Within 14 d following lambing.

increasing linearly ($P = 0.06$). Crude protein SF had no affect ($P > 0.21$) on lambing date or average lamb birth weight (Table 3).

Results for the performance study support results derived from the digestion study, indicating that CP SF can be decreased to once every 10 d for ruminants consuming low-quality forages. Our performance results are similar to results for once every 6 d or 7 d CP supplementation observed by Bohnert et al. (2002) and Huston et al. (1999), respectively. However, Farmer et al. (2004a) observed an increase in BW loss when cows were supplemented with increasing amounts of urea (0 percent to 45 percent) 3 d/wk compared with beef cows supplemented with urea daily at increasing levels. Similar results were reported that when cows were supplemented daily with CP, BW loss was reduced compared with cows that were supplemented only 2 d/wk (Farmer et al., 2001). The results of the current trial are the first data, that we are aware of, suggesting that CP SF can be decreased to once every 10 d for ruminants consuming low-quality forage.

Conclusions

No negative effects on N balance, BW and BCS, lambing date, and birth weight were observed for once every 10-d supplementation of CP when compared to daily and once every 5-d supplementation. Sheep producers in the northern Great Plains may consider crude-protein supplementation with soybean meal once every 10 d as a management alternative for reducing dormant-season supplementation costs to ewes in the last trimester of pregnancy.

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Effects of Supplemental Cobalt on Nutrient Digestion and Nitrogen Balance in Lambs Fed Forage-based Diets

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Summary

The objective of this study was to determine the effects of supplemental cobalt on nutrient digestion and nitrogen balance in lambs fed a forage-based diet. Sixteen wether lambs (initial BW = 28.6 ± 1.3 kg) were used in a two-period crossover design and randomly allotted to one of two treatments being ad libitum grass hay (7.1 percent CP, 68.5 percent NDF, DM basis) plus 45.0 g of dried distillers grains with a commercial mineral formulated to provide 1.1 mg/d of cobalt (CONTROL) in the form of cobalt carbonate or a commercial mineral containing supranutritional levels of cobalt carbonate providing 7.1 mg/d of Cobalt (COBALT). Experimental periods were 21 d in length and consisted of 15 d for diet adaptation and 6 d of total fecal and urine collection. Forage DM, OM, and NDF intake tended to increase ($P = 0.091$) when lambs consumed COBALT. Despite increased forage intake; fecal DM, OM, and NDF flow ($P \geq 0.654$) did not differ between cobalt levels. Total tract DM, OM, and NDF digestibility (percent of total intake) did not differ ($P \geq 0.591$) between CONTROL and COBALT. No differences were observed between cobalt levels for total N intake ($P = 0.129$), total tract N digested (g/d; $P = 0.135$), or urine N output ($P = 0.812$). The provision of additional cobalt to lambs did not increase ($P = 0.251$) N retention. Providing lambs a forage-based diet containing 7.1 mg/d of cobalt tended to increase forage intake but did not affect total tract digestibility or N balance.

Key Words: Cobalt; Digestion; Lamb; Nitrogen Balance

Introduction

Supplementing a forage-based diet with Co may be useful due to the higher ruminal B12 production observed with high-forage diets (forage:concentrate ratio = 100:1) compared to a relatively higher concentrate (40:60) diets (Sutton and Elliot, 1972). Adding supplemental Co to a high-concentrate diet formulated to be deficient in Co increased ruminal propionate production in beef steers (Tiffany et al., 2002). Eskeland et al. (1974) reported an improvement in lamb N balance when propionate was intravenously infused.

Gall et al. (1949) suggested that the digestibility of a diet decreases through bacterial changes when Co is deficient. Lopez-Guisa and Satter (1992) reported that supplementing Co and Cu above the NRC (1989) recommendations increased *in situ* digestibility of low-quality forages. However, literature on the effects of high levels of supplemental organic Co *in vivo* is currently unavailable. Therefore, our hypothesis was that nutrient digestibility and N balance would be altered when supplemental organic cobalt was fed to growing lambs consuming low-quality forages. The objectives of this study were to evaluate the effects of supplemental Co on diet digestibility and N balance in lambs consuming chopped grass hay.

Materials and Methods

Sixteen western, white-face Rambouillet wether lambs (initial body weight = 28.6 kg ± 1.3 kg) were used to evaluate the effect of supplemental organic Co on N balance and total tract digestibility of a forage-based diet. Lambs were randomly allotted to treatment and were placed in individual stainless steel crates housed in a temperature controlled barn (22 °C) with continuous lighting. All lambs were offered chopped (5.1 cm) grass hay (7.1 percent CP and 68.5 percent Neutral Detergent Fiber, DM basis) at 105 percent of the previous days forage intake. In addition, lambs received either 45.0 g (as fed) dried distillers grains that contained a commercial mineral (Bullseye all purpose mineral, Ralco Nutrition Inc., Marshall, Minn., USA) formulated to provide 1.1 mg/d of Co (CONTROL) or the same commercial mineral, which included a highly digestible proprietary form of organic Co (Bullseye all purpose mineral plus CoMax 100, Ralco Nutrition Inc., Marshall, Minn., USA) formulated to provide 7.1 mg/d of Co (COBALT). The experiment was set up as a two-period, switch-back design so that each lamb received each treatment. Each experimental period lasted 21 days with 15 days for diet adaptation and 6 days for sample collection. Lambs were removed from crates and allowed to exercise for four days between experimental periods. All procedures were approved by the Northern Great Plains Research Laboratory Animal Care and Use Committee.

Total urine and fecal output was collected at 0800 on days16 through day 21 of each period. Fecal samples were weighed and a 10-percent subsample was collected each day and composited per animal for each collection period and immediately placed in a 55°C oven. Urine collection vessels contained 30 mL 50 percent HCl (16 M) to maintain urine pH < 3.0 in order to minimize microbial growth and NH3 volatilization. Dried feed, orts, and feces were ground through a 1 mm screen (Wiley mill; Thomas Hill and Sons, Philadelphia, Pa.) and analyzed for DM, ash (AOAC, 1990) and neutral detergent fiber (ANKOM Technologies, Fairport, N.Y.) as described by Vogel et al. (1999). Feed, feces, and urine were analyzed for N by combustion (CE Elantech, Lakewood, N.J.). Cobalt concentrations in feeds were determined by digesting samples in nitric acid and assaying for Co content by inductively coupled plasma optical emission spectrometry (Ultima 2, Horiba Scientific, Kyoto, Japan). Nutrient analysis of hay and supplements are presented in Table 1.

All data were analyzed as a two-period, crossover design using the MIXED model of SAS (SAS, Inst., Cary, N.C.). Level of significance set at $P \leq 0.05$. The model included period and cobalt level, with animal as the random variable. Due to reasons unrelated to treatment, one lamb was removed from the experiment during period 1 and one lamb was removed from the experiment during period 2.

Results and Discussion

Forage DM, OM, and NDF intake increased ($P \leq 0.091$) when lambs consumed 7.1 mg/d of Co (Table 2). Likewise total dietary intake was greater ($P = 0.093$) for lambs fed Co. The provision of additional Co to lambs did not ($P \geq 0.654$) influence fecal excretion of DM, OM, or NDF. Total tract DM, OM, and NDF digestion increased for lambs fed COBALT ($P \leq 0.098$). Nevertheless, total tract digestibility of DM, OM, and NDF (percent of intake) did not differ ($P \geq 0.591$) between cobalt levels (Table 2).

Forage N intake tended ($P = 0.107$) to increase when lambs were fed COBALT. However, total N intake ($P = 0.129$) did not differ across treatments. In addition, fecal N excretion was not different ($P = 0.901$) between cobalt levels. Therefore, no differences were observed between cobalt levels for total tract N digested (g/day; $P = 0.135$) or digestibility (percent of N intake; $P = 0.596$). Urine N output was not influenced ($P = 0.812$) by additional Co and averaged 4.03 g/day.

Table 1. Nutrient composition of ingredients.

		Supplements[a]	
Item	**Hay**	**CONTROL**	**COBALT**
DM	90.0	86.9	86.1
OM (percent of DM)	90.2	94.1	93.4
CP (percent of DM)	7.1	32.5	32.4
NDF (percent of DM)	67.9	55.1	55.8
Cobalt (mg/kg DM)	< 0.2	29.2	183.0

[a] CONTROL = Ad libitum intake of grass hay (7.1 percent CP, 68.5 percent NDF, DM basis) plus 45 g (as fed) dried distillers grains plus Bullseye mineral added in order to provide 1.1 mg/d of cobalt; COBALT = Ad libitum intake of grass hay plus 45 g (as fed) dried distillers grains containing Bullseye mineral™ plus CoMax 100™ added to provide 7.1 mg/d of Cobalt.

Table 2. Influence of supplemental organic cobalt on intake, fecal flow, and digestion of DM, OM, and NDF when wethers were fed forage-based diets.

Item	Cobalt levels[a] Control	Cobalt	SEM[b]	*P* < 0.05
DM Intake (g/day)				
Forage	844	883	42.6	0.091
Total	882	922	42.6	0.093
OM Intake (g/day)				
Forage	761	798	38.5	0.086
Total	798	834	38.5	0.091
NDF Intake (g/day)				
Forage	577	606	29.3	0.079
Total	599	628	29.3	0.078
Fecal excretion (g/day)				
DM	198	200	8.7	0.818
OM	163	164	7.2	0.819
NDF	140	142	6.5	0.654
Total tract digested (g/day)				
DM	684	721	36.3	0.098
OM	634	670	33.3	0.091
NDF	459	486	24.6	0.097
Total tract digestibility (percent of intake)				
DM	77.5	78.0	0.60	0.591
OM	79.6	80.0	0.58	0.602
NDF	77.1	76.8	0.68	0.732

[a] CONTROL = Ad libitum intake of grass hay (7.1 percent CP, 68.5 percent NDF, DM basis) plus 45 g (as fed) dried distillers grains plus Bullseye mineral added in order to provide 1.1 mg/d of cobalt; COBALT = Ad libitum intake of grass hay plus 45 g (as fed) dried distillers grains containing Bullseye mineral™ plus CoMax 100™ added to provide 7.1 mg/d of Cobalt.
[b] Control n = 14; Cobalt n = 15.

/kg of DM, respectively. The Co requirements for lambs, as suggested by the NRC (2007), are 0.1 mg to 0.2 mg Co/kg of DM to meet Co requirements and 0.5 mg to 1.0 mg Co/ kg of DM for optimal microbial growth. Previous work in beef cattle (Tiffany and Spears, 2005) fed high-concentrate diets that were marginally deficient in Co noted an increase in DM intake when Co was added to the diet at 0 mg, 0.05 mg, and 0.15 mg/kg of DM. Furthermore, Schwarz et al. (2000) reported an increase in intake when beef bulls were fed diets that exceeded NRC (1996) requirements for Co.

Although there was an increase in dietary DM intake with Co supplementation, the concomitant increase in total tract DM, OM, and NDF digested (g/d) and lack of differences in fecal nutrient flow (g/d) led to no differences being observed in total tract digestibility of DM, OM or NDF. An increase in DM intake accompanied with no change in total tract digestibility, as reported herein, has been well documented previously (reviewed by Galyean and Owens, 1991). This response was likely due to changes in rumen kinetics. Specifically, the tendency for forage intake to increase may have been due to an improvement in ruminal digestibility with Co supplementation (Lopez-Guisa,

Nitrogen retention expressed as g/day, percent of N intake, or percent of N digested was not different ($P \geq 0.251$) when lambs fed chopped hay were supplemented with Co.

In normal production systems, lambs of this weight would likely not receive low-quality forage. However, it was of interest to determine ways to improve the digestibility of medium- to low-quality hay (7.1 percent CP and 68.5 percent NDF, DM basis). This is important because of the increased cost of finishing lambs due to high grain prices and the desire of some segments of the lamb finishing industry to grow lambs for a longer period of time for backgrounding on grass and/or for grass-fed markets (Held, 2005). Dried distiller's grains were used only as a carrier for the mineral supplements. Based on post-experiment laboratory analysis, CONTROL and COBALT diets provided lambs with 1.2 mg and 8.0 mg Co

Table 3. Influence of supplemental organic cobalt on N intake, N excretion, and N balance in wethers fed forage-based diets.

Item	Cobalt levels[a] Control	Cobalt	SEM[b]	*P* < 0.05
N Intake (g/day)				
Forage	9.54	9.95	0.48	0.107
Total	11.60	12.00	0.48	0.129
Fecal N excretion (g/day)	2.76	2.77	0.11	0.901
Total tract N digested (g/day)	8.80	9.19	0.40	0.135
Total tract N digestibility (percent of intake)	76.20	76.60	0.64	0.596
Urine N (g/day)	4.01	4.06	0.17	0.812
Total N excreted (g/day)	6.70	6.80	0.24	0.782
Total N retention (g/day)	4.79	5.13	0.34	0.251
N retention (percent N intake)	41.20	41.70	1.60	0.818
N retention (percent N digested)	54.20	54.30	2.00	0.948

[a] CONTROL = Ad libitum intake of grass hay (7.1 percent CP, 68.5 percent NDF, DM basis) plus 45 g (as fed) dried distillers grains plus Bullseye mineral added in order to provide 1.1 mg/d of cobalt; COBALT = Ad libitum intake of grass hay plus 45 g (as fed) dried distillers grains containing Bullseye mineral™ plus CoMax 100™ added to provide 7.1 mg/d of Cobalt.
[b] Control n = 14; Cobalt n = 15.

1992; Zelenák et al., 1992) and therefore increased ruminal kp (rate of passage; Owens and Goetsch, 1986) and subsequently increased passage rate of digesta out of the rumen.

Supplemental Co can improve blood concentration of glucose via succinate production (Underwood and Sutter, 2001) especially in Co-adequate diets (Tiffany et al., 2002). Supplemental glucose or propionate has been shown to improve N balance in lambs (Eskeland et al., 1974); therefore it was surprising that supplemental Co in the current study had little effect on N metabolism. This was in spite of a numerical increase in total tract N digested. Similarly, supplementing sheep fed Mulga leaves (Acacia aneura) with added Co had no effect on N digestibility but did lower N balance compared to unsupplemented controls (McMeniman et al., 1981).

Conclusions

Dietary Co increased forage intake in lambs although the mechanism of action is not clear. The increase in intake may have been due to improvements in ruminal fermentation related to alterations in the ruminal microbial population, specifically the cellulolytic bacteria, which appear to be most sensitive to additional Co. Further, due to the nature of this experiment, accurate evaluation of BW gain was not possible. Hence, it is unclear at this time whether or not the intake differences translate to an improvement in BW gain. Therefore, a site and extent of digestion and growth performance trial is warranted to more completely evaluate the impact of supranutritional levels of Co in growing lambs.

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Effect of Feeding System on Meat Goat Growth Performance and Carcass Traits[1]

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Summary

This research sought to evaluate the effect of diet on meat-goat-growth performance, carcass traits, and fatty acid profiles of the meat product. Fifty-six meat-goat kids were allocated to one of two feeding systems. The control treatment (FORAGE; n = 27) was a forage-only system that was composed of grazing and chopped hay. The treatment group (GRAIN; n = 28) was fed one percent of their BW grain mix in addition to the FORAGE diet. Animals were fed to a target end weight of 36.4 kg. Animals on the GRAIN treatment had higher ADG and fewer days on feed ($P < 0.05$). Dietary treatment did not impact ($P > 0.10$) dressing percent, tenderness, or fat-cover score. Animals on GRAIN had more desirable carcass-selection scores ($P < 0.01$). The percentage of saturated fatty acids, unsaturated fatty acids, MUFA, PUFA, omega-6, and omega-3 fatty acids in longissmus muscle ($P > 0.10$) was not impacted by diet. Animals on GRAIN tended to have a higher omega-6: omega-3 ratio ($P = 0.06$). Feeding low levels of concentrates to meat goats increased ADG and reduced days on feed without impacting dressing percentage, fat- cover score, tenderness and fatty acid composition of the meat product. When evaluating the production costs of both systems, the benefits of increased rate of gain and fewer days on feed may not offset the added cost of production.

Key Words: Meat Goat, Growth Performance, Carcass Traits, Fatty Acids

Introduction

Meat-goat inventory in the United States has been increasing over the past decade (USDA, 2007). As the inventory of meat goats increase in the United States, so does the need for more information regarding basic production principles. Meat-goat producers are challenged to ensure packers have a consistent supply of animals. Many producers are working together to form marketing cooperatives to develop a consistent, reliable meat-goat supply. Consistent product quality is needed for a marketing cooperative to be successful.

Feeding system impacts growth and subsequent carcass traits. Increasing concentrates in meat-goat diets results in increased live-harvest weights, as well as increased carcass weights (Ryan et al., 2007; Haddad, 2005; Urge et al., 2004). Goats fed high-concentrate diets appear to have more muscling (Johnson and McGowan, 1998). However, the cost benefit of feeding additional concentrates has not been fully explored in goat-production systems and needs to be evaluated.

Recent literature suggests that the ability to replace saturated fatty acid (SFA) content with monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) has beneficial effects on human health (Williams, 2000). Little is known about the nutritional value of goat meat for human consumption. Banskalieva et al. (2000) summarized literature addressing fatty acid composition of goat meat. In a limited dataset, these authors demonstrated goat meat possesses desirable fatty acids compared with meat of other ruminant livestock. More research is needed to explore the various factors that may influence fatty acid profiles of goat-meat products.

In building a market for a product, it is imperative to evaluate that product thoroughly and have a clear vision of what that product provides. Diet can have a major impact on growth and carcass traits. Therefore, the objective of this research was to evaluate the effect of feeding systems on meat-goat-kid growth performance, carcass traits, and fatty acid profiles of the meat product.

Materials and Methods

Fifty-six meat-goat kids were allocated to one of two feeding systems. Seventeen producers provided goats; each producer's goats were split across the two finishing systems. Goats were predominantly Boer breeding. Twenty-five goats were fullblood or high-percentage Boer breeding, and 31 goats were Boer F1 crosses, as defined by the breeder. Diet treatment was stratified across breed composition and gender. The control treatment was a forage-only system (FORAGE; n = 27), that was composed of grazing irrigated pasture during the summer through early fall and then chopped hay during the late fall and winter. Irrigated pasture was comprised of primarily orchard grass (*Dactylis glomerata*), ryegrass (*Lolium perenne*), tall fescue (*Schedonorus phoenix* (Scop.) Holub), and white clover (*Trifolium repens*). The test treatment was the same as the FORAGE system; however, animals were fed one percent of their BW a pelleted, commercially available, goat grain mix (GRAIN; n = 28). The grain contained the following minimum nutrient concentrations: 16 percent CP; 2.5 percent crude fat; and 6.0 percent CF (Bar Ale, Inc., Williams, Calif.). Grain was offered to animals once per day, in the morning. Both treatments had ad libitum access to water and a trace-mineral supplement. Average-initial weight for FORAGE was 22.92 kg with a range in weight from 15.87 kg to 28.57 kg. Average-initial weight for GRAIN was 23.04 kg with a range of 19.05 kg to 29.02 kg. All animals were dewormed and vaccinated for *Clostridial* diseases upon initiation of the trial. During the grazing period, four animals in the FORAGE group died due to heavy parasite infestation. All anthelmintics (albendazole, moxidectin, and ivermectin) were administered as per the consulting veterinarian's recommendation.

During the grazing period, both treatments grazed the same pasture, separated with temporary fencing. Dry matter availability was estimated weekly using the GrassMaster probe (Novel Ways Electronic Product Development; Hamilton, NZ). Animals were rotated when approximately 50 percent of the forage DM was consumed. Forage DM availability did not drop below 2016 kg DM/ha. Pasture-clip samples were collected prior to grazing for forage-quality analysis. Samples were collected each time animals were moved (approximately 7d to 10 d). Pasture samples were composited by month for analysis. When the animals moved from pasture to a chopped-hay diet, a grab sample was collected each time hay was chopped for the animals. Four different hay mixes (Table 1) were used during the chopped-hay portion of the project. Hay mixes 1 and 4 were 50-percent-alfalfa hay and 50-percent-grass hay; hay mixes 2 and 3 were 50-percent-alfalfa hay and 50-percent-oat hay. Forage-chemical composition (Table 1) was evaluated using near infrared reflectance spectroscopy (FOSS in North America, Eden Prairie, Minn.).

Animals were fed to a target ending weight of 36.4 kg. All goats were weighed

Table 1. Forage quality (DM basis).

Nutrients, % forage DM	July Pasture	August Pasture	Hay Mix 1[a]	Hay Mix 2[b]	Hay Mix 3[b]	Hay Mix 4[a]
CP	12.90	13.27	17.10	20.01	17.88	20.39
ADF	39.41	38.01	36.30	29.99	31.95	31.06
NDF	57.83	60.33	51.66	44.82	44.61	42.12
Ca	0.621	0.497	0.956	1.140	1.088	1.336
P	0.323	0.340	0.338	0.350	0.327	0.313
RFV[c]	94	91	109	136	133	143

[a] Hay mix was 50:50 alfalfa hay and grass hay. Mix 1 was fed September/October and mix 4 was fed February/March.
[b] Hay mix was 50:50 alfalfa hay and oat hay. Mix 2 was fed October/November and mix 3 was fed December/January.
[c] Relative Feed Value – calculated from ADF and NDF values

every two weeks to monitor for desired ending weight. Due to harvest facility management, animals had to go to harvest in lots. When a group of animals averaged the desired ending weight, that group of animals was sent to harvest. Carcass measurements included hot-carcass weight (kg), dressing percentage (%), subcutaneous fat-cover score, and carcass-selection score. Subcutaneous fat-cover score was evaluated on a scale of one to three (1.00 to 1.99 = minimal fat cover; 2.00 to 2.99 = moderate fat cover; 3.00 to 3.99 = excessive fat cover; McMillin and Pinkerton, 2006). Carcass-selection score was also evaluated on a scale of one to three (1.00 to 1.99 = heavy muscling; 2.00 to 2.99 = moderate muscling; 3.00 to 3.99 = light muscling; McMillin and Pinkerton, 2006).

Longissimus muscle samples were collected from a subset (approximately 40 percent) of each treatment group. Samples were obtained from matching locations (post-12th rib) and then frozen until the lipid and Warner-Bratzler shear force analyses (Savell et al., 1994). Lipid samples were extracted from goat-meat samples in triplicate using the chloroform-methanol procedure of Folch et al. (1957), as modified by Realini et al. (2005). Extract was converted to fatty acid methyl esters (Duckett et al., 2002). Fatty acids were quantified by incorporation of an internal standard, methyl nonadecanoate ($C_{19:0}$) into each sample before methylation. The fatty acid methyl esters were analyzed using a Varian 3800 gas chromatograph (Agilent Technologies, Santa Clara, Calif.), and separated using a 100-m capillary column (0.25-mm i.d. and 0.20-µm film thickness, SP 2560; Supleco, Bellefonte, Pa.). Individual fatty acids were identified by comparison of retention times with standards (obtained from Sigma-Aldrich, St. Louis, Mo.).

Growth data, hot-carcass weight, and Warner Bratzler Shear Force data were analyzed as a completely randomized design (PROC MIXED; SAS Inst. Inc., Cary, N.C.); the model included the fixed effect of dietary treatment. A one-sample proportion t-test was used to determine if ending weight differed from the desired end weight of 36.4 kg. Carcass-selection score and fat cover were analyzed using hot-carcass weight as a covariate (PROC MIXED; SAS Inst. Inc., Cary, N.C.). Hot-carcass weight was not significant ($P = 0.08$) in the model for carcass-selection score and was removed from the model. However, hot-carcass weight was a significant covariate ($P < 0.01$) for carcass-fat-cover score; therefore adjusted least squares means are reported. Fatty acid data were analyzed as a split plot using PROC MIXED (SAS Inst. Inc., Cary, N.C.). Fixed effects in the model included diet, preparation, and the subsequent interaction. Random effects in the model included goat (diet) and goat*preparation (diet).

Results and Discussion

Animal Performance

Animals started the trial weighing 23 kg (Table 2; $P = 0.86$). Goats were fed to the target end weight of 36.4 kg; however, ending weight differed due to diet. Goats on the GRAIN diet were approximately 4 kg heavier at harvest (Table 2; $P < 0.001$). Neither FORAGE nor GRAIN differed from the desired end weight of 36.4 kg ($P > 0.05$). The GRAIN group had fewer days on feed and a greater ADG (Table 2; $P < 0.05$) compared with the FORAGE treatment.

Average-daily-gain (g) values for animals on test were low compared to some research (Haddad, 2005; Cameron et al., 2001), yet in line with others (Mushi et al., 2009; Turner et al., 2005; Urge et al., 2004). However, the increase in ADG with the addition of grain to the diet is supported by the literature. Haddad (2005) showed increasing rate of gain in growing kids fed diets with increasing levels of concentrate. Numerous other researchers show increasing rates of gain with increasing level of concentrate in the diet (Mushi et al., 2009; Turner et al., 2005; Cameron et al., 2001).

The lower-than-desired rates of gain can likely be attributed to health problems during beginning stages of the trial. During the adaptation to the grazing component of the project, numerous animals were treated for coccidiosis and parasite infestations. In the FORAGE group, eight animals were treated; 11 animals were treated in the GRAIN group.

The majority of published literature investigated higher level of concentrates in treatment diets compared to the current project. Even small amounts of concentrates in the diet may improve rate of gain and reduce days on feed if feeding to a desired end weight.

Carcass

The GRAIN group had heavier carcasses (Table 2; $P = 0.001$), which correlates to the heavier ending weights observed in the live performance of the goats. There was no difference in fat cover between the FORAGE and GRAIN group (Table2; $P = 0.75$). How-

Table 2. Effect of feeding system on meat goat growth performance and carcass traits.

	FORAGE	GRAIN	SEM	P-value
Beginning weight, kg	22.92	23.05	0.58	0.863
Ending weight, kg	34.98	39.15	0.62	<0.001
Days on feed	246	211	11.01	0.022
ADG, g/d	49.61	81.86	4.47	<0.001
Hot carcass weight, kg	17.39	19.34	0.42	0.001
Dressing percentage	49.90	49.40	0.96	0.699
Selection score[a]	2.72	2.38	0.07	0.001
Fat cover score[b,c]	2.25	2.29	0.08	0.754
Warner Bratzler Shear Force, kg	3.48	3.13	0.45	0.559

[a] Selection score: 1.00 to 1.99 = heavy muscling; 2.00 to 2.99 = moderate muscling; 3.00 to 3.99 = light muscling (McMillin and Pinkerton, 2006).
[b] Fat cover score: 1.00 to 1.99 = minimal fat cover; 2.00 to 2.99 = moderate fat cover; 3.00 to 3.99 = excessive fat cover (McMillin and Pinkerton, 2006).
[c] Adjusted least squares means are reported, due to significant ($P = 0.008$) covariate of hot carcass weight.

ever, goats on GRAIN had a more desirable carcass-selection score, indicative of increased muscling throughout the carcass (Table 2; $P < 0.001$). There was no difference between the two diets for tenderness (Table 2; $P = 0.56$).

Mushi et al. (2009) observed increased-carcass fatness and conformation score with increased levels of concentrate in the diets of goats. Shear force in the aforementioned study tended to decline with increasing concentrate levels in the diet; however, they did not differ ($P > 0.1$; Mushi et al., 2009). When comparing Boer x Spanish and Spanish goats fed on feedlot diets to those fed on range diets, Osman et al. (1999) reported increased fat thickness and improved carcass-conformation score for goats fed in a feedlot setting compared to a range diet. The goats on a feedlot diet from Osman et al. (2009) also had heavier carcasses (38.17 kg) compared to the range-fed goats (20.51 kg; $P < 0.05$). Ryan et al. (2007) fed Boer-crossbred goats diets that consisted of range (0 percent), and 50 percent, 70 percent, and 90 percent concentrate. There was no difference in Warner Bratzler Shear Force (average 5.65 kg; $P > 0.05$) due to increased levels of concentrate in diet, which corresponds to this study. Increasing level of concentrate in the diet improved carcass-confirmation score and had no effect on shear force of the meat product. The goats in this study were fed to similar ending weights, as opposed to a specified date, thus potentially masking the difference in fat thickness observed in previously published research.

Fatty Acid

Diet did not affect the percentage of saturated fatty acids, unsaturated fatty acids, MUFA, PUFA, omega-6, and omega-3 fatty acids in *longissmus* muscle of meat goats (Table 3; $P > 0.10$). Animals on GRAIN tended to have a higher omega-6: omega-3 ratio (Table 3; $P = 0.06$). Paired *longissmus* samples were used to evaluate the effect cooking had on the fatty acid profiles. There was a trend for cooking to increase the percentage of PUFA (Table3; $P = 0.07$), omega-3 (Table 3; $P = 0.10$) and omega-6 fatty acids (Table 3; $P = 0.12$).

Webb et al. (2005) compared the mean-molar percentages of various fatty acids in freeze-dried meat of goats and sheep. Relative to saturated and unsaturated fatty acids, the data for Boer goats in this study is comparable with Webb's published data. Johnson and McGowan (1998) compared production-system impact (intensive vs. semi intensive) on carcass characteristics and meat quality of young goats. The intensive system involved creep feeding the kids and maintaining concentrate feeding through slaughter. In contrast, the semi-intensive system involved concentrate supplementation of a grazing system. They reported composite samples from intensively raised goats had higher saturated and PUFA, whereas semi-intensively raised goats had higher MUFA and a higher ratio of unsaturated to saturated. Ryan et al. (2007) found that feeding concentrate diets compared with range diets increased total fatty acids. Additionally, by increasing level of concentrate in the diet, the omega-6: omega-3 ratio was increased (Ryan et al., 2007), which is in agreement with our observations. It has also been reported that feeding high levels of concentrate increases the omega-6: omega-3 ratio in lamb (Demirel at al., 2006). Increasing the amount of concentrate in goat diets does affect the omega-6: omega-3 ratio, potentially impacting human nutrition. Raes et al. (2004) stated that human nutritionists recommend a higher intake of PUFA, particularly omega-3 fatty acids. Ideally, the increased intake of omega-3 fatty acids would be at the expense of omega-6 fatty acids. Therefore, high levels of concentrate feeding of goats may negatively impact the omega-6: omega-3 fatty acid ratio from a human nutrition perspective.

Conclusions

Feeding low levels of concentrates to meat goats did increase rate of gain, and subsequently, lowered the days on feed when animals were fed to a targeted ending weight. Additionally, feeding low levels of concentrates did improve carcass muscling without impacting dressing percentage, fat-cover score, or tenderness of the meat product. There was minimal impact on fatty acid composition of the meat product with grain-fed goats. However, the resulting higher omega-6: omega-3 ratio was a shift in an undesirable direction. Research is needed to further explore the impact of feeding systems on meat-goat production and the associated costs.

Table 3. Effect of feeding system on goat meat lipid profiles.

	FORAGE		GRAIN		P-value		
	Raw	Cooked	Raw	Cooked	Diet	Prep	Diet*Prep
Saturated fatty acids, %	55.38 ± 2.22	55.14 ± 2.22	52.50 ± 2.00	53.85 ± 2.00	0.484	0.978	0.525
Unsaturated fatty acids, %	44.62 ± 2.22	45.86 ± 2.22	47.50 ± 2.00	46.15 ± 2.00	0.484	0.978	0.525
MUFA, %	40.67 ± 2.08	40.70 ± 2.08	43.31 ± 1.89	40.64 ± 1.89	0.568	0.449	0.439
PUFA, %	4.26 ± 0.66	5.16 ± 0.66	4.26 ± 0.60	5.51 ± 0.60	0.808	0.067	0.757
omega-6 fatty acids, %	3.07 ± 0.55	3.53 ± 0.55	3.30 ± 0.50	4.31 ± 0.50	0.409	0.123	0.548
omega-3 fatty acids, %	1.34 ± 0.20	1.63 ± 0.20	1.09 ± 0.18	1.27 ± 0.18	0.207	0.100	0.710
omega-6: omega-3	2.66 ± 0.45	2.35 ± 0.45	3.28 ± 0.41	3.88 ± 0.41	0.059	0.708	0.184
PUFA:SFA	0.081 ± 0.015	0.099 ± 0.015	0.087 ± 0.014	0.105 ± 0.014	0.708	0.202	0.991

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